

Transfusion Medicine Self-Assessment and Review, 4th Edition



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Transfusion Medicine

Self-Assessment and Review,

4th Edition

Edited by

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Preface

The editors and authors of this book are pleased to have participated in the preparation of this valuable resource. The first three editions of *Transfusion Medicine Self-Assessment and Review* were quite successful, most importantly, because they filled an educational niche in the field. In particular, residents and fellows training in the field of transfusion medicine have come to regard this book as an important educational resource, both for learning the field and for board examination preparation.

Although there are additional educational resources in transfusion medicine that now meet this same need (eg, the Web-based Transfusion Medicine Question of the Day), we are confident that this fourth edition of the text will continue to be useful to the educator and the student. For some of us, there is no adequate replacement for a well-worn and dog-eared book!

In this edition, three new chapters have been added: Massive Transfusion and Extracorporeal Membrane Oxygenation, Neonatal and Pediatric Transfusion, and Laboratory Management. Importantly, the chapter on Hematopoietic Progenitor Cells, Cord Blood, and Growth Factors has been replaced by a chapter on Hematopoietic Cell Transplantation and Cellular Processing. As an added feature this edition includes a bonus test on a mix of the topics presented to provide a real-life testing simulation.

All chapters have 50 questions created anew for this edition—900 opportunities to hone your skills. In addition, the questions and explanations have been reviewed specifically to reflect the new Food and Drug Administration donor deferral guidelines and the new evidence-based indications for therapeutic apheresis from the American Society for Apheresis.



The authorship of this text has changed over the years, but the commitment to providing a vibrant educational resource that accurately reflects current transfusion medicine understanding has not. As with the prior editions, the authors anticipate that the fourth edition of *Transfusion Medicine Self-Assessment and Review* will be a resource benefitting all who desire an improved understanding of transfusion medicine. We hope that our text is an effective and enjoyable tool that will enhance the reader's understanding of the field for years to come.

Amy E. Schmidt, MD, PhD
H. Cliff Sullivan, MD, F(ACHI)
Editors

About the Editors

Amy E. Schmidt, MD, PhD, is a Medical Director at CSL Plasma. Prior to this, she was an Assistant Professor at the University of Rochester Medical Center (URMC), where she was the Associate Laboratory Director of Coagulation as well as the Assistant Laboratory Director of Transfusion Medicine/Blood Bank at Strong Hospital. She also started and served as the first fellowship director of the Transfusion Medicine fellowship at URMC. Preceding URMC, Schmidt was an Assistant Professor at Indiana University/IUPUI, where she served as the Director of Apheresis. She was also the Director of Transfusion Medicine and Blood Bank at the VA Hospital in Indianapolis as well as at Eskenazi Medical Center in Indianapolis.

Schmidt obtained a BA from Washington University in St. Louis in both Biochemistry and Anthropology. She then attended medical school at Saint Louis University Medical Center where she earned both her MD and PhD. Schmidt's thesis, under S. P. Bajaj, PhD, was on the structure and function of blood clotting proteins and understanding how they interact. She then did a pediatric internship at Lucille Packard Childrens Hospital/Stanford in Palo Alto, CA. Following this, she completed a Clinical Pathology residency at Barnes Jewish Hospital/Washington University in St. Louis, MO, a fellowship in Transfusion Medicine and Blood Banking at the University of Alabama at Birmingham, and another fellowship in Hematopathology at SUNY-Upstate in Syracuse, NY.

Schmidt is active in several committees related to Transfusion Medicine/Blood Banking, apheresis, and HLA. Moreover, she enjoys teaching medical students, residents, fellows, medical technologists, and nurses whenever the opportunity arises. She continues to have a special interest in coagulation and the specificity with which the various coagulation factors interact.



H. Cliff Sullivan, MD, F(ACHI), serves as an Associate Professor at Emory School of Medicine in Atlanta, Georgia. His clinical appointments include being the Laboratory Director of the Stem Cell and Cellular Therapy Laboratory, Co-Director of the Histocompatibility and Molecular Immunogenetics Laboratory, and Director of the Point-of-Care Laboratory at Emory Rehabilitation Hospital. Additionally, he assists in staffing the Transfusion Medicine Service at Emory University Hospital.

Sullivan's training journey includes a BA from Emory University's College of Arts and Sciences in 2006 and an MD from Emory University School of Medicine in 2011. His postgraduate training encompasses an Anatomic and Clinical Pathology residency, a Transfusion Medicine fellowship led by Cassandra Josephson, MD, and a Histocompatibility (HLA) fellowship guided by Drs. Howard M. Gebel, PhD, and Robert A. Bray, PhD.

Sullivan is actively involved in numerous committees, both nationally and institutionally, contributing to advancements in pathology and laboratory medicine. He has held leadership roles in the American Society for Clinical Pathology, American Medical Association, and College of American Pathologists. Furthermore, he has demonstrated a passion for education by actively engaging in teaching medical students, residents, and fellows. He hopes that this edition serves as a valuable educational tool for those interested in learning the various facets of transfusion medicine.

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Quality and Compliance

H. Cliff Sullivan, MD, F(ACHI)

Key Points from the *Technical Manual*

- A defined organizational structure in addition to senior management's support of, and commitment to, the quality policy, goals, and objectives is key to ensuring the success of a quality management system.
- The fields of transfusion medicine and biotherapies are highly regulated, involving multiple regulatory agencies and accreditation organizations.
- In addition to legally binding regulations, the Food and Drug Administration (FDA) periodically publishes recommendations in guidance documents.
- Blood establishments that manufacture or participate in the manufacturing of blood and blood components are inspected by the FDA to determine compliance with requirements.
- The Centers for Medicare and Medicaid Services regulates all US medical laboratories under CLIA regulations, which establish requirements for certification.
- Deviations from requirements, standards, and regulations must be addressed by identifying, documenting, and classifying occurrences and assessing effects on quality.

H. Cliff Sullivan, MD, F(ACHI), Associate Professor, Emory University School of Medicine; Laboratory Director, Cellular Therapy Laboratory, Emory University Hospital; and Co-Director, HLA Laboratory, Emory University Hospital, Atlanta, Georgia
The author has disclosed no conflicts of interest.



QUESTIONS

Question 1: Which of the following organizations is considered a regulatory agency?

- A. The Joint Commission (TJC).
 - B. Centers for Medicare and Medicaid Services (CMS).
 - C. Foundation for the Accreditation of Cellular Therapy (FACT).
 - D. Association for the Advancement of Blood & Biotherapies (AABB).
 - E. Clinical Laboratory Improvement Amendments of 1988 (CLIA).
-

Question 2: Regarding FDA licensure and FDA registration, which of the following statements is true?

- A. Licensed facilities engage in the manufacture and manipulation of blood and blood products but not in interstate commerce.
 - B. Unregistered facilities do not manufacture or manipulate blood or blood products, but they are still regularly inspected.
 - C. Registered facilities manufacture blood and blood products and do engage in interstate commerce.
 - D. All licensed facilities are also registered facilities.
 - E. Registered and unregistered facilities are inspected at regular intervals.
-

Question 3: Which of the following is the federal statute that addresses public health and contains regulations for CMS such as CLIA requirements for laboratories that perform tests on human samples?

- A. Title 21 of the Code of Federal Regulations (21 CFR).
- B. Title 29 CFR.
- C. Title 42 CFR.
- D. Federal Food, Drug, and Cosmetic Act (FFDCA).
- E. Public Health Services Act.



Question 4: Select the applicable part of Title 21 of the Code of Federal Regulations (21 CFR) that addresses licensed biological products regulated by the Center for Biologics Evaluation and Research (CBER).

- A. Part 210.
 - B. Part 600.
 - C. Part 864.
 - D. Part 1270.
 - E. Part 1271.
-

Question 5: A condition or element that is needed to ensure effectiveness, safety, or quality would best describe which of the following terms?

- A. Standard.
 - B. Regulation.
 - C. Specification.
 - D. Requirement.
 - E. Recommendation.
-

Question 6: Which of the following statements is true of current good manufacturing practice (cGMP) regulations?

- A. If personnel have had adequate experience in the laboratory and have received proper training, they do not necessarily need to have all the educational requirements to perform their assigned functions.
 - B. Laboratory facilities must have adequate lighting and ventilation for all functions performed.
 - C. If reagents do not have expiration dates, then the order of use does not matter.
 - D. A and B.
 - E. A, B, and C.
-

Question 7: Select the statement that is true regarding drugs and biological products as defined by federal agencies.



- A. A biological product is a product that is applicable to the prevention, treatment, or cure of a human disease or condition.
 - B. Blood and blood products are considered biological products but not drugs.
 - C. Biological products are articles recognized in the National Formulary.
 - D. Drugs are subject to the Public Health Services Act.
 - E. Biologic products are subject to the federal Food, Drug, and Cosmetic Act.
-

Question 8: Which of the following statements best describes quality assurance?

- A. A concept with measures to ensure that product manufacturing is carried out in a consistent manner that results in high-quality products.
 - B. An organized structure consisting of interrelated and interconnected processes and components that cooperate to guarantee quality.
 - C. An orderly process that converts quality policies into measurable outcomes and criteria and sets forth a process to achieve and meet them in a given timeframe.
 - D. An integral part of quality assurance involves sampling, testing, and observation to determine if a process is performing as anticipated.
 - E. An entity that addresses organizational leadership in fostering a commitment to quality in part by understanding the management of resources and quality planning.
-

Question 9: Performing white cell counts on leukocyte-reduced Red Blood Cells (RBCs) is an example of which quality concept?

- A. Quality assurance.
- B. Quality control.
- C. Quality management.
- D. Quality systems.
- E. Quality planning.



Question 10: Developing a standard operating procedure detailing the steps for process and test method validation would fall under which quality concept?

- A. Quality assurance.
 - B. Quality control.
 - C. Quality management.
 - D. Quality systems.
 - E. Quality planning.
-

Question 11: Which of the following are considered elements of a quality management system (QMS) in the setting of transfusion medicine?

- I. Leadership and customers.
 - II. Documents and records.
 - III. Human resources.
 - IV. Process improvement.
 - V. Process control.
-
- A. I, II, IV, V.
 - B. I, III, IV, V.
 - C. II, III, IV.
 - D. III, IV, V.
 - E. All the above.
-

Question 12: The blood bank management hires an individual to supervise and oversee the quality system. Which of the following duties would fall underneath this individual's responsibilities?

- A. Review and approve validation plans and results.
- B. Supply resources to perform different aspects of the quality management system.
- C. Establish and implement the overall quality policy including goals and objectives.
- D. Assign designees and delineate their roles and responsibilities.



- E. Oversee the overall operations of the blood bank and ensure compliance with regulatory agencies and accrediting organizations.
-

Question 13: A blood center is looking to contract out the distribution and packaging of the blood products that they collect and process. In reviewing various options, the blood center administration carefully evaluates each distributor and their ability to meet predetermined specifications. Select the answer choices that most accurately describe the blood center's evaluation process.

- A. Assessment.
 - B. Supplier agreement.
 - C. Supplier qualification.
 - D. Final inspection.
 - E. All the above.
-

Question 14: During hospital renovations, the blood bank is moved from the first floor to the third floor to be closer to the operating rooms. Before performing type and screens on the relocated instruments, what process must be performed?

- A. Retraining of employees.
 - B. Revalidating analytic sensitivity and specificity.
 - C. Calibration.
 - D. Running quality controls.
 - E. If the instruments sustained no damage during the move, testing can be performed.
-

Question 15: The blood bank has decided to switch from a solid-phase antibody identification method to a gel-based method, which has been selected after a thorough evaluation process. Before validation of the new method can take place, which of the following activities must be completed?

- A. Change control.
- B. Assessment.



- C. Process control.
 - D. Supplier qualification.
 - E. Quality control.
-

Question 16: In test method validation, what is the term used to define the ability of a test or assay to detect the intended analyte?

- A. Analytic sensitivity.
 - B. Analytic specificity.
 - C. Accuracy.
 - D. Precision.
 - E. Reportable range.
-

Question 17: Which of the following answer choices would be an example of a quality indicator?

- A. Process improvement.
 - B. Proficiency testing.
 - C. Turnaround time.
 - D. Blood utilization.
 - E. Customer focus.
-

Question 18: New personnel must be trained to carry out their duties according to policies and procedures. To ensure that employees maintain their ability to carry out their duties, competency assessments should be undertaken to evaluate their competence. Which of the following is one of the minimal regulatory competency assessment requirements?

- A. Assessment of problem-solving skills.
- B. Evaluation of time and self-management.
- C. Testing memorization of standard operating procedures.
- D. Monitoring response to nonconformances.
- E. Direct observation of teamwork and cooperation with customers.



Question 19: How often is it suggested that quality control be performed on AB and Rh antisera?

- A. With each test run.
 - B. Every 8 hours.
 - C. Every 12 hours.
 - D. Daily.
 - E. Weekly.
-

Question 20: How often is it recommended to perform quality control performance on a freezer's alarm activation?

- A. Every 4 hours.
 - B. Every 8 hours.
 - C. Daily.
 - D. Weekly.
 - E. Quarterly.
-

Question 21: Select the answer choice that fills in the blanks accurately: In the storage of blood and blood components, the temperature is to be monitored _____ and recorded _____.

- A. Every 4 hours; every 4 hours.
 - B. Every 8 hours; every 8 hours.
 - C. Continuously; continuously.
 - D. Every 8 hours; twice a day.
 - E. Continuously; every 4 hours.
-

Question 22: For gamma-emitting irradiation of blood products, the intended minimum dose is 25 Gy and 15 Gy delivered to the central portion of product and any portion of the product, respectively. To ensure that the intended dose is being delivered, how often should dose verification be performed on a cesium-137 radiation source?

- A. Quarterly.
- B. Semiannually.



- C. Annually.
 - D. Every 2 years.
 - E. Every 5 years.
-

Question 23: For immunohematology tests, such as those performed in the blood bank, how many times a year should proficiency testing (PT) be performed and how many samples should be tested at each assessment (ie, PT event)?

- A. 2 times per year; 4 samples per PT event.
 - B. 2 times per year; 5 samples per PT event.
 - C. 3 times per year; 4 samples per PT event.
 - D. 3 times per year; 5 samples per PT event.
 - E. 4 times per year; 4 samples per PT event.
-

Question 24: Select the answer choice that correctly pairs the immunohematology test with the corresponding criteria for acceptable proficiency testing.

- A. ABO group – 80%.
 - B. RhD type – 80%.
 - C. Unexpected antibody detection – 100%.
 - D. Compatibility testing – 80%.
 - E. Antibody identification – 80%.
-

Question 25: A blood bank fails to meet criteria for PT acceptance performance on two consecutive PT events for ABO typing. Which of the following statements best describes this scenario?

- A. This is considered unsuccessful PT performance, but the laboratory can continue to perform ABO typing at least until the next PT event.
- B. This is considered unsatisfactory PT performance, but the laboratory can continue to perform ABO typing at least until the next PT event.



- C. This is considered repeated unsuccessful PT performance, and the laboratory will have to cease performing ABO typing for at least 6 months.
 - D. This is considered unsatisfactory PT performance, and the laboratory will have to cease performing ABO typing for at least 6 months.
 - E. This is considered unsuccessful PT performance, and the laboratory will have to cease performing ABO typing for at least 6 months.
-

Question 26: A blood specimen is collected for pretransfusion testing. The phlebotomist who drew the sample places the label (see label below) on the tube in front of the blood bank medical laboratory staff. The patient identifying information on the specimen label matches the identifying information on the blood product request form except the request does not have the patient's middle initial. Which of the following answers accurately describes the specimen acceptability?

Name: John E. Doe
Medical Record Number: 123456789
Date of collection: 01/01/2023
Phlebotomist: Jane Doe

- A. The specimen should be rejected because the label was not placed on the tube while in the presence of the patient.
 - B. The specimen should be rejected because the label is missing the time of collection.
 - C. The specimen should be rejected because the patient identifying information on the specimen label and the request form do not match exactly.
 - D. A and B.
 - E. A, B, and C.
-

Question 27: Labeling of blood products and components should be in accordance with the most updated version of the US Industry Consensus Standard for the Uniform Labeling of Blood and Blood Components using the ISBT 128 (Information Standard for Blood



and Transplant). Which of the following label bar codes are considered mandatory by ISBT 128?

- A. Collection date.
 - B. Product code.
 - C. Dimensions.
 - D. Special testing performed.
 - E. Collection time.
-

Question 28: In addition to ISBT 128 requirements for bar code labeling, which of the following is a required labeling element for all allogeneic blood products and blood components per AABB standards?

- A. Anticoagulant used.
 - B. Rh type.
 - C. Red cell antibody specificities.
 - D. Date of donation.
 - E. Storage temperature.
-

Question 29: What are the maximum donor identification numbers (DINs) that can be affixed to a blood product?

- A. One.
 - B. Two.
 - C. Three.
 - D. Four.
 - E. There is no limit to the number of DINs, it just depends on how many facilities the product passes through before transfusion.
-

Question 30: How often must blood bank and transfusion medicine policies, processes, and procedures be reviewed?

- A. Every 6 months.
- B. Every year.
- C. Every 2 years.



- D. Every 5 years.
 - E. Only when policies, processes, and procedures are updated.
-

Question 31: A group of associated tasks and activities used to achieve an intended work objective best defines which of the following terms?

- A. Document.
 - B. Policy.
 - C. Process.
 - D. Procedure.
 - E. Record.
-

Question 32: Regarding donor record retention requirements, which record must be indefinitely retained?

- A. Records of traceability of blood, components, derivatives, and critical materials.
 - B. Donor notification records of abnormal testing results.
 - C. Records of donor identification numbers and final disposition of blood, components, or derivatives.
 - D. Records of donors who are placed on permanent deferral from donation secondary to risk factor.
 - E. Donor consent records.
-

Question 33: Regarding patient record retention requirements, which record must be indefinitely retained?

- A. Documentation of two recipient ABO determinations.
- B. Records of blood product final disposition and of transfusion recipient identification.
- C. Records of clinically significant red cell antibodies.
- D. Emergency release documentation with physician signature attesting that clinical need for transfusion necessitates the release of blood/blood products before completion of appropriate compatibility or infectious disease testing.
- E. Recipient consent records.



Question 34: What is the record retention requirement for the validation of new blood bank software and hardware systems?

- A. 2 years.
 - B. 2 years after the retirement of the system.
 - C. 5 years.
 - D. 5 years after the retirement of the system.
 - E. 10 years after the retirement of the system.
-

Question 35: How long must records related to proficiency testing be retained per AABB standards?

- A. 1 year.
 - B. 2 years.
 - C. 5 years.
 - D. 10 years.
 - E. Indefinitely.
-

Question 36: What is the minimum record retention time for the investigation of adverse events to tissues (eg, cornea, bone, cartilage, dura mater, etc)?

- A. 1 year.
 - B. 2 years.
 - C. 5 years.
 - D. 10 years.
 - E. Indefinitely.
-

Question 37: Upon receipt, blood derivatives, such as Rh Immune Globulin and recombinant coagulation factors, should be inspected to ensure they meet criteria for clinical use. How long should these records be retained?

- A. 1 year.
- B. 2 years.
- C. 5 years.



- D. 10 years.
 - E. Indefinitely.
-

Question 38: Per AABB standards, how long are patient samples to be stored in the blood bank?

- A. 3 days from receipt of sample.
 - B. At least 3 days after transfusion.
 - C. 7 days from receipt of sample.
 - D. At least 7 days after transfusion.
 - E. Until the patient is discharged from the hospital or 7 days after transfusion, whichever is longest.
-

Question 39: A patient's blood type is incorrectly recorded into the medical record as group A, Rh-negative, when it should be group O, Rh-positive. In order to correct the blood type, what elements need to be documented?

- A. Identity of the person who made the change.
 - B. Time that change is made.
 - C. Original recorded result should be deleted.
 - D. A and B.
 - E. All of the above.
-

Question 40: If a death is determined to be caused by blood donation or transfusion of blood or blood products, by when must a written report be submitted to the Centers of Biologics Evaluation and Research (CBER) and what mode(s) of delivery are acceptable?

- A. 24 hours by facsimile or email.
- B. 24 hours by telephone, facsimile, express mail, or e-mail.
- C. 7 days by mail alone.
- D. 7 days by mail or facsimile.
- E. 7 days by mail, facsimile, or e-mail.



Question 41: A blood center contracts out the packing, labeling, and distribution to another facility. The contracted facility alerts the blood center that the labeling protocol for two units of apheresis pathogen-reduced platelets deviated from GMP requirements and there are concerns about the units' safety. Both units were subsequently distributed to one of the blood center's clients. The client was notified, and the units were placed in quarantine. Which of the following statements is true regarding the reporting of product deviations?

- A. The report can be submitted only by mail.
 - B. CBER must be notified as soon as possible but the facility has up to 45 days from the date that deviation was discovered.
 - C. No report needs to be filed as the units were apheresis pathogen-reduced platelets and not RBCs, which is a product derived from a whole blood unit.
 - D. No report needs to be filed as the unit was placed in quarantine before the units were transfused.
 - E. No report needs to be filed by the blood center as it was the contracted facility who made the error.
-

Question 42: Which of the following examples accurately describes a proper assessment of quality metrics?

- A. Clinician transfusion practices should be peer-reviewed.
 - B. Proficiency testing provides an internal means of assessment.
 - C. Quality control is an example of external assessment.
 - D. Findings of assessments need only be reviewed by the personnel conducting the assessment.
 - E. Routine assessments are nonessential elements of quality systems, but they are highly recommended.
-

Question 43: Laboratory inspections are a part of external assessments performed by voluntary accrediting organizations on a regular basis. Which of the following accrediting agencies performs inspections on a 2-year cycle (ie, every 2 years)?



- I. AABB.
 - II. College of American Pathologists (CAP).
 - III. The Joint Commission (TJC).
 - IV. Foundation for Accreditation of Cellular Therapy (FACT).
-
- A. I and II.
 - B. II.
 - C. II and III.
 - D. I, III, IV.
 - E. All of the above.
-

Question 44: The process of taking appropriate measures to eliminate the cause of a deviation, nonconformance, or adverse event and prevent recurrence can best be defined as which of the following terms?

- A. Investigation.
 - B. Correction.
 - C. Corrective action.
 - D. Preventive action.
 - E. Implementation.
-

Question 45: A 35-year-old female, group O, Rh-negative, with gastrointestinal bleeding and a hemoglobin level of 6.5 g/dL gave her consent for blood transfusion. The physician explained that the blood would help treat the patient's symptoms of anemia by improving oxygen-carrying capacity. The risk of infectious disease transmission was discussed as were the mitigations taken to prevent disease transmission. The patient's questions were answered, and the patient was given the choice to sign consent or to sign the refusal of blood products. Which of the elements in the consent of the patient are missing?

- A. The infectious risks were explained but the noninfectious risks (eg, transfusion reactions) were not.
- B. Although the risks and benefits were explained, the alternatives to treatment were not.



- C. It is not necessary to give the patient the opportunity to sign for refusal of blood products, the choice to accept transfusion is sufficient.
 - D. A and B.
 - E. B and C.
-

Question 46: Before transfusion of an RBC unit to the patient in question #45, the transfusionist verifies the two independent identifiers and ABO/Rh typing of the recipient and donor along with the crossmatch interpretation, special transfusion requirements, and unit expiration date. Along with the assistance of an electronic identification system located at the nurse's station, the transfusionist positively identifies the recipient and matches the recipient to the donor blood product using two independent identifiers. Which of the following statement about the pretransfusion verification process is correct?

- A. There is no need to verify Rh type between recipients and donors.
 - B. The transfusion should not proceed because there was not a two-person verification (ie, the transfusionist and another individual).
 - C. The transfusion should not proceed because the recipient donor verification process was not performed in the presence of the recipient.
 - D. B and C.
 - E. All required elements of the pretransfusion verification process were fulfilled; the transfusion should proceed.
-

Question 47: The blood bank is getting ready to issue heart valve tissue for a patient undergoing valve repair in the operating room. The laboratory staff verifies that the product name and quantity match the order requisition. The name and medical record number of the intended recipient are also verified. Lastly, the dates and times of expiration and release were checked. Which of the following statements is accurate about the issue verification process?

- A. A final inspection of the valve tissue was not performed.



- B. The valve should not be issued without the package insert documents.
- C. The quantity of the tissue need not be verified against the order requisition.
- D. A and B.
- E. All required elements of the verification process were fulfilled; the tissue should be released.
-

Question 48: During an AABB inspection, the inspector audits the medical record documentation of a representative number of patients transfused in the hospital since the previous inspection. In addition to the transfusion order and the patient consent, the following document is one of the audited records. Which of the following statements correctly describes the audited record?

Component: RBC DIN: 123456789 Donor ABO/Rh: A, Rh-positive Transfusion date: 01/01/2023 Transfusion start time: 14:00 Transfusion end time: 15:45	Pretransfusion Vital Signs* Temperature: 36.5 C Heart rate: 75 bpm Blood pressure: 120/80 mmHg	Vital Signs 15 Minutes into the Transfusion Temperature: 37.0 C Heart rate: 78 bpm Blood pressure: 125/85 mmHg	Posttransfusion Vital Signs Temperature: 36.8 C Heart rate: 80 bpm Blood pressure: 130/80 mmHg
<hr/> <p>Total volume transfused: 300 mL Transfusionist: Nurse Jane Doe Transfusion-related adverse event: None</p> <hr/>			

*Per institutional protocol, the vital signs should be taken before transfusion, 15 minutes into the transfusion, and after the transfusion.

- A. The record is missing the expiration date.
- B. The record is missing the lot number of the unit.
- C. The total volume transfused is not required.



- D. A and C.
 - E. All required documentation elements are present.
-

Question 49: Select the answer choice that correctly fills in the blanks. Sterility testing was inadvertently not performed on a platelet unit before distribution. This unit represents a _____ as not all testing requirements were performed per protocol and the unit should be _____.

- A. Nonconformance; quarantined.
 - B. Deviation; detained.
 - C. Laboratory error; destroyed.
 - D. Adverse event; segregated.
 - E. Variance; deferred.
-

Question 50: Which of the following statements is true regarding a hospital transfusion committee?

- A. The chair of the transfusion committee should ideally be the hospital blood bank director.
- B. A hospital transfusion committee is a requirement of both the AABB and TJC.
- C. Nursing representation should be limited to a representative of nursing education and an operative room nurse representative.
- D. Peer-review of blood utilization should be performed by physicians from various clinical services.
- E. It is considered a conflict of interest for the blood center to participate in the hospital transfusion committee, as the blood bank is usually a blood center customer.

ANSWERS

Question 1: B. Centers for Medicare and Medicaid Services (CMS).



Explanation:

- Regulatory agencies and accrediting organizations require that blood banks and transfusion services follow certain standards and guidelines including those that address quality assurance of the blood products and services they supply.
- Regulatory agencies are independent, autonomous governmental bodies that require no direct executive oversight. These agencies are generally established by legislative acts and function to create and enforce standards that regulate different markets or specialty fields.
- Examples of regulatory agencies that regulate blood banks and transfusion services are:
 - FDA.
 - CMS.
 - State Departments of Health.
 - Health and Human Services (HHS).
 - Occupational Safety and Health Administration.
- Accreditation organizations are usually private institutions that develop criteria to evaluate and inspect peer organizations to determine if the set criteria are met. Organizations or institutions that meet criteria are deemed “accredited,” indicating that these institutions are compliant with set standards.
- Examples of accrediting organizations that regulate blood banks and transfusion services are:
 - TJC.
 - FACT.
 - AABB.
 - College of American Pathologists (CAP).
- CLIA is a set of regulations set forth by CMS that address laboratory quality, including quality assurance and quality control, with which US laboratories must comply. CLIA grants deemed status to accrediting organizations such as AABB, CAP, and TJC, specifying that the standards of these organizations either meet or exceed those set forth by federal agencies.
- Many of the regulatory agencies and accrediting organizations also include standards that satisfy international agencies such as the International Organization for Standardization (ISO) and the Clinical and Laboratory Standards Institute (CLSI).



Question 2: D. All licensed facilities are also registered facilities.

Explanation:

- FDA licensure is required for facilities that engage in interstate commerce. Most blood centers, plasmapheresis facilities, and product testing laboratories are licensed.
 - FDA registration is required for facilities that manufacture and/or manipulate blood or blood products but do not engage in interstate commerce. Most hospital-based blood banks and transfusion services are registered.
 - Unregistered facilities do not manufacture blood or blood products. Unregistered facilities would include transfusion services that do not manipulate blood or blood products except for thawing.
 - Facilities that are licensed are also registered and can have blood products that are regulated only under the registration and not the licensure.
 - All facilities that are licensed and/or registered must be inspected on a regular cycle at predetermined intervals. Unregistered facilities are not regularly inspected but can be inspected if issues are reported.
-

Question 3: C. Title 42 CFR.

Explanation:

- Regulations are statements issued by a board, commission, or agency that have the force of the law. Rules are statements that are published to interpret and enforce the law.
- The Code of Federal Regulations (CFR) is a collection of rules that have been previously published in the *Federal Register*.
- Title 21 of CFR contains rules of the Food and Drug Administration (FDA) including those pertaining to blood, blood products, tissue (including hematopoietic stem cells), and drugs.
- Title 29 of CFR contains rules pertaining to labor and safety, including OSHA regulations surrounding bloodborne pathogens and chemical hygiene.
- Title 42 of CFR contains rules concerning public health and contains regulations for CMS such as requirements for test-level com-



plexity and personnel. In particular, Part 493 of Title 42 specifies that all laboratories performing tests on human samples must be certified under the CLIA.

- The FFDCA is a set of laws passed by Congress in 1938 granting the FDA the authority to regulate the safety of food, drugs, and cosmetics.
 - The Public Health Services Act addresses the prevention of the spread of communicable diseases and the licensure of biologic products, including the suspension of licenses of products determined to be a danger to public health.
-

Question 4: B. Part 600.

Explanation:

- The Code of Federal Regulations (CFR) Title 21 contains a codification of rules of the various executive departments and federal government agencies including the FDA. This includes several regulations that are applicable to blood banks, transfusion services, and cellular therapy.
 - 21 CFR 210 – *Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs* – This code addresses the minimum requirements for the facilities and methods used to manufacture, process, and store a drug to uphold safety and quality standards.
 - 21 CFR 600 – *Biological Products: General* – This code addresses both licensed biological products regulated by the Center for Biologics Evaluation and Research (CBER) and products regulated by the Center for Drug Evaluation and Research (CDER). These regulatory bodies process biologics license applications (BLAs), fatality reports, deviation reports, and other relevant communications. Cellular therapy products are subject to this code.
 - 21 CFR 864 – *Hematology and Pathology Devices* – This code addresses the classification of devices used in hematology and pathology that are intended for human use. This includes the regulation of Blood Establishment Computer System (BECS), information systems that are used in the manufacturing, testing, and distribution of blood and blood components.



- 21 CFR 1270 – *Human Tissue Intended for Transplantation* – This was a code that was removed by the FDA in 2022 in conjunction with 21 CFR 882.5875 (*Human dura mater*), as these regulations applied to specific tissues recovered before May 25, 2005. As the tissues from this time were no longer believed to remain in inventory for transplantation, this code was believed to be obsolete and was removed. Tissues recovered after May 25, 2005 are covered in 21 CFR 1271.
 - 21 CFR 1271 – *Human Cells, Tissues, and Cellular and Tissue-Based Products* – This code addresses cellular therapy products, including HPCs, and covers general provisions; procedures for registration and licensing; donor eligibility; current good tissue practice requirements; inspection; and enforcement of establishments or institutions collecting, processing, and/or receiving cellular therapy products.
-

Question 5: D. Requirement.

Explanation:

- A requirement is a condition or element that is necessary to ensure effectiveness, safety, and/or quality. Requirements can be objectives that a process or product must execute, attributes a process or product needs for proper function, and performance measures a process or product must achieve.
- Standards are requirements that represent core principles meant to uphold safety, efficacy, and quality. Standards are often used as the criteria to obtain accreditation.
- Regulations are statements issued by a board, commission, or agency that have the force of the law. Rules are statements that are published to interpret and enforce the law.
- Specifications are sets of requirements that processes or procedures should meet. Specifications can be provided as descriptive references, standards, drawings, and/or instructions.
- Regarding laboratory quality, recommendations are suggestions for process improvements that address potential problems identified during an internal or external assessment. During laboratory inspections, recommendations will be made for issues that do not meet the criteria for deficiencies but are still cause for concern.



Question 6: B. Laboratory facilities must have adequate lighting and ventilation for all functions performed.

Explanation:

- cGMP requirements address various elements and functions of a laboratory as well as provide a quality framework to run the laboratory effectively.
- Regarding personnel, cGMP regulations outline the following:
 - Personnel should be adequate in number, training, experience, and education to ensure competency to perform their assigned duties.
 - Personnel should have the capabilities commensurate with their assigned duties, have a thorough understanding of the procedures that they perform, and have adequate information of the regulations applicable to their assigned duties.
 - Personnel who could adversely affect the safety and purity of products should be excluded from the laboratory.
- Insofar as facilities, cGMP mandates cleanliness of facilities and that facilities have adequate space, lighting, and ventilation to perform all functions.
- Per cGMP, standardization and calibration of all equipment, supplies, and reagents should follow manufacturer's instructions and be performed on a regularly scheduled basis. Supplies and reagents should be used before the expiration date, and for those without expiration dates, they should be used in order of receipt (ie, first in/first out).
- To meet cGMP requirements, production and process controls should be defined, documented, and implemented per procedures, policies, and/or controls. Standard operating procedures should include the minimum requirements outlined by Title 21 CFR Part 606.100.
- cGMP also outlines labeling standards for controls, blood, and blood components.



Question 7: A. A biological product is a product that is applicable to the prevention, treatment, or cure of a human disease or condition.



Explanation:

- Blood and blood products are considered drugs and biological products.
 - A drug is intended for the use in the diagnosis, mitigation, treatment, cure, or prevention of a disease; it is intended to affect the structure or any function of the human body.
 - Drugs are articles that are recognized by the National Formulary, US Pharmacopoeia, and/or the Homoeopathic Pharmacopoeia of the US and are subject to the federal Food, Drug, and Cosmetic Act.
 - A biological product is a product applicable to the prevention, treatment, or cure of a human disease or condition and can be composed of sugars, proteins, nucleic acids, living tissues and cells, or a combination of these substances.
 - Examples of biological products include:
 - Blood, blood component, or derivative.
 - Therapeutic serum.
 - Allergenic product.
 - Virus.
 - Toxin.
 - Antitoxin.
 - Vaccine.
 - Protein (not including chemically synthesized polypeptides).
 - Biological products are subject to the Public Health Services Act.
-

Question 8: A. A concept with measures to ensure that product manufacturing is carried out in a consistent manner that results in high-quality products.

Explanation:

- Quality assurance (QA) can be described as a concept with measures to ensure that product manufacturing is carried out in a consistent manner that results in high-quality products. Its aims include ensuring accurate and reliable results, minimizing errors, and developing efficacious processes. QA programs should include detection, investigation, assessment, correction, and prevention of errors through reviews of process performance.



- Quality systems (QS) can be defined as organized structures consisting of interrelated and interconnected processes and components that cooperate to guarantee quality. Each process consists of inputs that are converted to outputs through a series of steps and components.
- Quality planning (QP) is an orderly process that converts quality policies into measurable outcomes and criteria and sets forth a process to achieve and meet them in a given timeframe. QP supplies the foundation and framework for developing and operating a QS.
- Quality control (QC) is an integral part of quality assurance that involves sampling, testing, and observation to determine if a process is performing as anticipated. Examples of QC include testing and monitoring reagents, products, and instruments to verify they are performing as expected. Examples of QC include product QC, reagent QC, visual inspection, clerical checks, temperature monitoring, and cell counts of blood products. QC failure to meet expectations or specifications can be indicative of a problem with a process or its execution.
- Quality management (QM) can be described as an entity that addresses organizational leadership in fostering a commitment to quality in part by understanding the management of resources and quality planning. QM should assess an organization's inter-dependent processes in the context of suppliers and customers and generate controls that are geared to maintain quality of the processes and the products.

**Question 9: B. Quality control.****Explanation:**

- See answer to question #8.
- Performing WBC counts on leukocyte-reduced blood products helps ensure that the number of remaining WBCs in a unit meet the leukocyte reduction standards.

**Question 10: A. Quality assurance.**

**Explanation:**

- See answer to question #8.
 - Planning and developing procedures that aim to make sure that processes and products meet specifications, requirements, and/or standards are important aspects of quality assurance along with assessments/reviews, reporting, and improvement undertaken with the same aim of adherence to quality.
-

Question 11: E. All the above.

Explanation:

- QMS encompasses aspects of QA, QS, QP, QC, and QM (see answer #8).
- Thus, basic elements of QMS include the following:
 - Organization.
 - Leadership and management.
 - Human resources.
 - Facilities, work environment.
 - Safety.
 - Documents.
 - Records.
 - Monitoring.
 - Evaluation.
 - Process control and improvement.
 - Equipment management.
 - Information management.
 - Suppliers and materials management.
 - Management of nonconforming events.

Question 12: A. Review and approve validation plans and results.

Explanation:

- An organization with a QMS needs structured leadership with well-defined roles.



- All the answers provided except for answer choice “A” are responsibilities that should be under the top or executive management.
- The top or executive management’s role in QMS should include the following:
 - Supply resources to perform different aspects of the quality management system.
 - Establish and implement the overall quality policy including goals and objectives.
 - Assign designees and delineate their roles and responsibilities. This includes designating an individual with oversight of the quality operations often referred to as a quality officer or quality manager.
 - Oversee the overall operations of the blood bank and ensure compliance with regulatory and accrediting agencies.
 - Partake in review and approval of quality documents (eg, policies, procedures, processes).
 - Review and assess the effectiveness and efficacy of the QMS.
 - Enforce compliance with standard operating procedures as well as with quality assurance programs.
- The quality officer or quality manager’s responsibilities should include the following:
 - Review and approve validation plans and results, standard operating procedures, training plans, and supplier and maintenance agreements.
 - Monitor employee training and compliance.
 - Review deviations, nonconformances, adverse reactions, and customer complaints.
 - Review and determine product specifications and suitability.
 - Perform audits and self-inspections.
 - Oversee and manage external inspections.
 - Report to regulatory agencies and accrediting organizations when needed.



Question 13: C. Supplier qualification.

Explanation:

- Supplier qualification is the evaluation and selection of suppliers of services, materials, and equipment based on the supplier’s



capability to meet a customer's specific requirements. Any supplier that performs laboratory testing is to be accredited by an accrediting organization such as the AABB.

- Other types of qualifications include:
 - Employee – ensuring that an employee's education, experience, and training are sufficient to perform the requirements of a position.
 - Equipment – verifying that specifications required to perform the expected tasks or tests are met.
- An agreement should define the expectations between the supplier and the customer and carefully detail the responsibilities to be covered by each party. An agreement is generally made after supplier qualification and should be reviewed regularly.
- Assessments are independent and systematic evaluations or examinations that are performed at regular intervals to ensure that laboratory activities are implemented accurately and efficiently, are compliant with expected specifications, and are meeting objectives. Assessments are usually performed by comparing actual results and outcomes to expected results and outcomes. There are various types of assessments including internal assessments, external assessments, management assessments, quality assessments, and utilization assessments. Utilization assessments include peer-review processes that monitor transfusion practices, specimen collection, specimen labeling, blood usage, relevant laboratory results, and adverse events, among others.
- Final inspection is the review or evaluation of services, blood, blood components, derivatives, and tissues to ensure specified requirements are met before distribution or issue. Inspection can include measurement, examination, testing, and/or visual appearance. Similar inspection should occur during receipt of services, blood, blood components, derivatives, and tissues.



Question 14: C. Calibration.

Explanation:

- Calibration is the process of ensuring that a test, instrument, or equipment is producing results with accuracy and precision. Calibration is often performed by running a test and comparing



results to a certified reference standard, which is a sample containing analyte of known value or amount.

- Calibration should be performed at minimum before the initial implementation, after activities that could affect calibration (eg, moving instruments, failing quality controls), and at regular intervals, which are generally prescribed by the manufacturer.
- Calibration should follow the manufacturer's instructions on performance, acceptance criteria, and steps taken when results are unsatisfactory.
- There should be preventive measures in place to safeguard against elements or actions that would invalidate calibration.
- If employees have been trained and deemed competent, they do no need to be retrained unless the method and/or procedure has changed.
- Validation of a test or instrument is a process undertaken to establish that results are consistent, accurate, and precise. Validation has many steps that include determining the limits of detection, limits of linearity, reproducibility of results, and accuracy against a standard or through comparison studies. The process of validation takes time and is very important in ensuring that a test meets all predetermined specifications.
- Although limited validation or verification may be performed after a move, a full validation in which analytic sensitivity and specificity are re-established is not necessary. See answer to question #16 for additional information on validation.
- Quality control is routine testing of control reagents to monitor whether equipment and/or tests are properly functioning. When quality controls fail, the validity of results, methods, products, or services need to be evaluated and investigated before release of the test results, methods, products, or services.



Question 15: A. Change control.

Explanation:

- Change control is a process that addresses the development of new processes and procedures or the change of existing processes and procedures. The change control should detail the specifications of the new process or change, the effect it will have on current processes, the resources required to implement the



change, and the steps required to initiate the change. Change control should precede validation and implementation of the new process or change.

- Process control refers to the policies, processes, and procedures that are set in place to ensure quality of services, blood, blood products, derivatives, and tissue. Elements of process control include but are not limited to:
 - Change control.
 - Proficiency testing.
 - Quality control.
 - Identification.
 - Traceability.
 - Inspection.
 - Inventory management.
 - Storage.
 - Transportation.
 - Supplier qualification should be completed before selection of the vendor.
 - See the answer to question #13 for details on supplier qualification and assessment. The answer to question #8 defines quality control.
-

Question 16: B. Analytic specificity.

Explanation:

- Test method validation must be completed before implementation of a nonwaived test to ensure performance specifications are met.
- If a lab is bringing on an FDA-approved method, then at minimum, the following test attributes must demonstrate comparable results to those reported by the manufacturer:
 - Accuracy: the ability of the test or assay to precisely detect and/or quantify an analyte (ie, ability to obtain the right result).
 - Precision: a test or assay's reproducibility, which is usually expressed in coefficient of variation (CV) (ie, ability to reproduce the same result on a given specimen). The lower the CV, the more precise the assay.



- Reportable range: the lower and upper limit of values that a test or assay can reliably measure. This range will extend below and above the reference range.
 - Reference intervals: the observed ranges in a healthy population. This range should be within the reportable range of the assay.
 - If a lab is bringing on a non-FDA-approved method, then at minimum, in addition to the requirements for an FDA-approved method, the following test attributes should be established:
 - Analytic sensitivity: the absolute lowest value that a test or assay can reliably detect. This is also termed the limit of detection.
 - Analytic specificity: the ability of a test or assay to detect the intended analyte in lieu of other analytes. This step usually involves ensuring that the analyte can be detected in the presence of interfering substances, which in medical laboratories include icterus, lipemia, and hemolysis.
 - Other test performance characteristics such as specimen stability.
-

Question 17: C. Turnaround time.**Explanation:**

- Quality indicators are objective metrics that can be reviewed to evaluate the quality of processes or outputs, such as products and services. Quality indicators can give a laboratory an indication of how it is meeting its goals regarding production, processes, quality metrics, and customer service. Examples of quality indicators include productivity (eg, number of tests completed or products manufactured) measures, tracking the number of errors made, turnaround time, and customer satisfaction surveys.
- Process improvement should be part of quality programs to permit continuous improvement and growth. Investigation of non-conformances and determination of their root causes provides an opportunity to implement process improvements to prevent their recurrence. Internal audits, tracing quality indicators, external assessments (eg, laboratory inspection), and customer feedback all provide opportunities for process improvements.



- Proficiency testing (PT) is an external assessment designed to ensure laboratories are producing expected test results. PT usually involves testing unknown samples provided by an accredited PT program. Although PT itself is not a quality indicator, monitoring PT performance to evaluate the number of PT failures could be a quality indicator. PT failures also provide opportunities for process improvements.
 - Monitoring blood utilization facilitates the evaluation of an institution's blood product ordering practices, adverse reactions, and appropriateness of transfusion among other facets of blood usage. Quality indicators can be used to assess blood utilization and can include blood wastage rates, crossmatch-to-transfusion ratios, and expiration rates.
 - Customer focus is a tenet of QMS ensuring that customer needs are met. In transfusion medicine and blood banking, customers can be hospitals, physicians, and patients. Quality indicators of customer service include customer satisfaction, on-time delivery times, and preventable adverse reactions.
-

Question 18: A. Assessment of problem-solving skills.**Explanation:**

- There are six minimal regulatory competency assessment requirements that are designed to ensure that employees can perform their duties per organizational policies and procedures:
 1. Direct observation of the employee performing routine patient testing including specimen handling, processing, and testing.
 2. Monitoring of how an employee records and reports the results of tests.
 3. Review of how employees handle intermediate and/or indeterminate test results, quality control records, proficiency testing, worksheets, and maintenance records.
 4. Direct observation of the employee performing maintenance and function checks of the equipment.
 5. Assessment of the employee's test performance by testing internal blind test specimens, external proficiency testing, or previously analyzed test specimens.
 6. Assessment of the employee's problem-solving skills.



Question 19: D. Daily.

Explanation:

- Quality control should be performed on all testing reagents to ensure that they are providing the expected results.
- QC may be performed at more frequent intervals when reagents and/or equipment are first implemented. Once an established in-range QC record is obtained, QC intervals may be reduced, but should never fall below the manufacturer's recommended intervals.
- The following frequencies are commonly suggested and accepted QC intervals for blood bank reagents (ie, not regulations), especially when no guidance is offered by the manufacturer.
 - QC on antisera, antihuman globulin sera, and red cells should be tested daily.
 - QC on reagents used to perform transfusion-transmissible disease marking testing should be performed with each test.



Question 20: E. Quarterly.

Explanation:

- The following table supplies the suggested quality performance intervals for refrigerators, freezers, and platelet incubators in the blood bank.

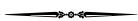
	Refrigerators	Freezers	Platelet Incubator
Recorder	Daily	Daily	Daily
Manual temperature	Daily	Daily	Daily
Temperature charts	Daily review and change weekly	Daily review and change weekly	Daily review and change weekly
Alarm system board	Daily	Daily	NA
Alarm activation	Quarterly	Quarterly	Quarterly
Ambient platelet storage	NA	NA	Every 4 hours



Question 21: E. Continuously; every 4 hours.

Explanation:

- Temperature monitoring of storage areas, refrigerators, freezers, and open storage is an important part of quality control and ensures that reagents and products are maintained at temperatures that correlate with expected performance.
- Per the AABB standards, the storage temperature of blood and blood components must be monitored continuously and recorded at least every 4 hours.
- Standards stipulated that the ambient temperature of open storage areas is to be monitored at least every 4 hours and recorded at least every 4 hours.



Question 22: C. Annually.

Explanation:

- Dose verification should be performed with the canister fully loaded.
- Dose verification for an irradiator with cesium-137 radiation source should be performed annually.
- Dose verification for an irradiator with cobalt-60 radiation source should be performed semiannually.
- Dose verification for an irradiator with an alternate radiation source should be performed per the manufacturer's recommendation.
- Dose verification should also be performed after:
 - Installation.
 - Maintenance/repairs.
 - Moving/relocating the irradiator.



Question 23: D. 3 times per year; 5 samples per PT event.

**Explanation:**

- Per Title 42 of the Code of Federal Regulations, part 493 Subpart I - *Proficiency Testing Programs for Nonwaived Testing*, laboratories must participate in PT from a proficiency testing program approved by the Department of Health and Human Services for each analyte they test. If there is no PT available for a given analyte, another method of external assessment must be used.
 - Per 42 CFR 493.959, which outlines PT requirements for immunohematology tests performed in the blood bank. The tests regulated under this code are ABO grouping, RhD typing, unexpected antibody detection, compatibility testing, and antibody identification.
 - Per the code, proficiency testing is to be performed at 3 evenly spaced intervals per year with 5 samples tested at each PT event for a total of 15 tests per year.
-

Question 24: E. Antibody identification – 80%.**Explanation:**

- For each test or analyte, a preset acceptance criteria must be met to pass PT successfully. Passing generally means that the laboratory obtained the consensus result with the other laboratories participating in the proficiency test program. Consensus is often set at 90%. Obtaining the consensus result is considered acceptable, and failing to get the consensus result is considered unacceptable.
- For many of the tests in other laboratory disciplines the acceptable passing rate is 80%, which would mean for an assessment of five samples, the laboratory must get acceptable results on four of the samples.
- Due to the critical clinical consequences that errors in the blood bank could cause, some of the immunohematology tests must achieve a passing rate of 100%:
 - ABO group – 100%.
 - RhD type – 100%.
 - Compatibility testing – 100%.
 - Unexpected antibody detection – 80%.
 - Antibody identification – 80%.



Question 25: A. This is considered unsuccessful PT performance, but the laboratory can continue to perform ABO typing at least until the next PT event.

Explanation:

- Unsatisfactory PT performance is the failure to meet criteria for acceptable PT performance, which for ABO grouping would be 100% (ie, acceptable results for all five samples per PT event). Receiving one unsatisfactory PT performance does not require a laboratory to stop performing the test for this analyte.
- Unsuccessful PT performance means not meeting acceptable PT performance criteria (ie, unsatisfactory PT performance) on two out of three PT events. For ABO grouping, for which PT is performed three times per year, an example of unsuccessful PT performance would be receiving unsatisfactory PT performance on the first and third PT performance of the year. Receiving one unsuccessful PT performance does not require a laboratory to stop performing the test for this analyte; they can continue testing at least until the next PT event.
- Repeat unsuccessful PT performance is receiving another unsuccessful PT performance inside of six or fewer PT events. Thus, repeat unsuccessful PT performance could be receiving three consecutive unsatisfactory PT performances or receiving unsatisfactory PT performance on three out of four PT events. Since PT for immunohematology analytes is performed three times per year, six PT events would span 2 years; thus, repeat unsuccessful PT performance could be receiving unsuccessful PT performance two years in a row or three unsatisfactory performances in one year. Receiving repeat unsuccessful PT performance does require a laboratory to cease performing the test for the analyte for at least 6 months.
- After 6 months, a test can be reinstated if the laboratory performs a detailed root-cause analysis, provides corrective actions to prevent reoccurrence, performs impact analysis that PT failure has on patient testing, supplies personnel training or retraining documentation, attains satisfactory PT performance on two PT events (ie, reinstatement PT) for the analyte in question, and demonstrates that they were compliant with directive to cease testing.

**Question 26: E. A, B, and C.****Explanation:**

- For patient safety, patient samples must be identified with a properly affixed label containing the requisite information for unique patient identification.
 - Label requirements as outlined by the AABB standards include:
 - Specimens should be labeled in the presence of the recipient.
 - Specimens should have at least two patient identifiers (ie, name and medical record number).
 - There should be a method to identify the person who collected the sample, as well as the date and time of collection.
 - The specimen label must match the identification information on the request.
 - Any samples with incomplete, inaccurate, or illegible labels should be rejected.
-

Question 27: B. Product code.**Explanation:**

- As a part of process control, AABB standards requires identification and traceability of blood products and components from collection to infusion.
- An integral step in fulfilling this requirement is proper labeling. Labeling of blood products and components should be in accordance with the most updated version of the US Industry Consensus Standard for the Uniform Labeling of Blood and Blood Components using ISBT 128 (Information Standard for Blood and Transplant).
- ISBT 128 requires the following bar codes in the following positions:
 - Donor identification number (DIN) – Upper left quadrant.
 - Product code – Lower left quadrant.
 - ABO/Rh typing – Upper right quadrant.
 - Expiration date and time – Lower right quadrant.
- Optional bar codes include special testing, collection date (or date and time), dimensions, and data matrix symbol.



- Required bar codes should be accompanied by eye-readable text corresponding to the data contained in the bar code. Text is below and left justified to the bar code.
 - Beneath the DIN, the collection/processing facility name and address can be found.
-

Question 28: E. Storage temperature.**Explanation:**

- Storage temperature is a required label element for all blood and blood components.
- Anticoagulant, Rh type, and red cell antibody specificities (if detected) are required for some blood components. These elements are not required for cryoprecipitate or pathogen-reduced cryoprecipitated fibrinogen complex. The anticoagulant is also not required for frozen, deglycerolized, washed, or rejuvenated RBCs.
- The date of donation is an additional labeling requirement for autologous blood and blood components.
- Other labeling requirements for blood and blood components include:
 - Blood component name, must be machine-readable and eye-readable.
 - Donor identification number, must be machine-readable and eye-readable.
 - Collection facility, must be machine-readable and eye-readable.
 - Facility performing modification (eg, irradiation), only if it leaves that facility.
 - Volume.
 - Expiration date.
 - ABO group, must be machine-readable and eye-readable.
 - “Volunteer Donor” if applicable.
 - “Paid Donor” if applicable.
 - “Autologous Donor” if applicable.
 - CMV seronegative, if applicable.
 - Indication of low volume, if applicable.
 - Number of units in a pool.



- In addition to date of donation, other labeling requirements for autologous blood and blood components include:
 - “For autologous use only.”
 - Recipient name, ID number, and name of facility where transfusion will occur (if available).
 - Biohazard label if infectious disease testing is positive.
 - “Donor untested” if applicable.
 - “Donor tested within the last 30 days” if applicable.
 - Additional labeling requirements for dedicated/directed donor blood or blood components include:
 - Recipient name, ID number, and name of facility where transfusion will occur (if available).
 - Biohazard label if infectious disease testing is positive.
 - “Donor tested within the last 30 days” if applicable.
-

Question 29: B. Two.

Explanation:

- A unique DIN should be placed on the blood product and attached container at the collection facility or pooling facility. This DIN should not be concealed, changed, or removed by subsequent facilities that handle the product.
 - If a facility receives a product with a non-ISBT 128 DIN, an ISBT 128 DIN will be assigned and placed on the product container. The assigned label should also identify the facility that assigned the ISBT 128 DIN.
 - Including the collection facility DIN, a maximum of 2 DINs may be affixed to the product or container. Other DINs should be concealed, changed, or removed. A patient identification number may be affixed in addition to the 2 DINs.
-

Question 30: C. Every 2 years.

Explanation:

- Blood banks and transfusion services must have specific policies, processes, and procedures to address the maintenance of documents and records.



- There must be a master list or table of contents of all documents such as policies, processes, procedures, and labels.
 - Qualified and authorized personnel must review all newly created and revised/updated documents before they are put into use. Review must also occur at least every 2 years.
 - All documents are to be easily accessible at the location where the described activities are performed. Retired or obsolete documents are to be archived as per record retention policies.
-

Question 31: C. Process.

Explanation:

- A document is written or electronic information and/or work instructions. Procedures, policies, process, manuals, and forms are examples of documents.
 - A policy is a documented prevailing principle guiding laboratory decisions and workflow.
 - A process is a group of associated tasks and activities used to achieve an intended work objective. In a process, inputs contribute to action that results in outputs.
 - A procedure is a step of instructions performed by an individual to accomplish a work task.
 - A record is captured written or electronic information that provides evidence of results or performed activities. Records exist only after an activity or test is performed and documented.
-

Question 32: D. Records of donors who are placed on permanent deferral from donation secondary to risk factor.

Explanation:

- The only donor-related records that require indefinite retention are those records for donors who are placed on permanent or indefinite deferral from donation secondary to identified risk factor (eg, reactive infectious disease marker, positive travel history, etc) to possible recipients.



- All the other answer choices have a 10-year retention requirement. In fact, except for donors with permanent/indefinite referrals, all other donor-related documents have a 10-year retention requirement. Thus, if a question is posed related to donor-related record retention, 10 years is likely the answer.
-

Question 33: C. Records of clinically significant red cell antibodies.

Explanation:

- Recipient record retention requirements vary but are usually 5 or 10 years depending on the record type. However, there are some records that require indefinite storage. The following table provides examples of records and their corresponding retention requirements.

Retention Requirement (years)	Record Type
Indefinite	<ul style="list-style-type: none">• Clinically significant antibodies• Significant adverse event to transfusion• Special transfusion requirements
5	<ul style="list-style-type: none">• Therapeutic apheresis-related records• Therapeutic phlebotomy-related records• Blood and blood component requests• Blood and blood component orders• Recipient consent• Patient identification verification before transfusion• Potential adverse events during or shortly after transfusion• Proper identification verification of recipient and blood/blood component before transfusion



- | | |
|----|--|
| 10 | <ul style="list-style-type: none">• Final disposition of blood/blood component and identification of recipient if transfused by facility• ABO/Rh type results and interpretation• Patient testing for detection of antibodies• Two recipient ABO determinations• Serologic crossmatch results and interpretation• Irradiation of cellular blood components• Final inspection of blood/blood component before release from the blood bank• Medical director approval for release of blood/blood products that are abnormal in appearance• Proper identification verification of recipient and blood/blood component before issue/release from blood bank• Statement with physician signature attesting that clinical need for transfusion necessitates the release of blood/blood products before completion of appropriate compatibility or infectious disease testing• Abnormal test results notification• Records related to neonatal transfusion |
|----|--|
-

Question 34: B. 2 years after the retirement of the system.

Explanation:

- In addition to donor and recipient record retention requirements, there are record retention requirements for documents, policies, procedures, etc relating to quality. The following table provides examples of quality-related records and their corresponding retention requirements.

Retention Requirement (years)	Record Type
2 after the retirement of system	<ul style="list-style-type: none">• Implementation/modification of hardware, software, databases• Validation of hardware, software, databases, user-defined tables, electronic data transfer or receipt• Documentation of system versions and the dates used



5	<ul style="list-style-type: none">• Supplier evaluations• Agreements and agreement reviews with suppliers• Validation of newly implemented or updated processes/procedures• Proficiency testing program participation• Review/approval of new and updated documents before implementation• Documentation of biennial policy/process/procedure reviews• Assessment result review• Blood utilization peer-review assessment• Implementation of modifications of policies/processes/procedures secondary to corrective/preventative actions• Corrective action plans• Preventive action plans• Investigation of alarms• Biological, radiation, chemical safety monitoring
10	<ul style="list-style-type: none">• Incoming critical materials inspection• Quality control for methods, reagents, and equipment• Review of quality control results for methods, reagents, and equipment• Blood product temperature storage records• Ambient temperature storage records• Inspection before shipping• Validation of shipping containers and of processes• Description of blood/blood components nonconformances• Evaluation of blood/blood components nonconformances• Disposition of nonconforming blood/blood components• Proper disposal/discard of blood/blood components• Reports of fatality• Evaluation of transmissible disease(s)• Reporting of transmissible disease(s)



Question 35: C. 5 years.

Explanation:

- See table in answer #34.



Question 36: D. 10 years.

Explanation:

- Most tissue-related records are to be retained for a minimum of 10 years.
 - One caveat to the standard 10-year retention requirement is the tissue recipient's medical record documenting the receipt of tissue including details such as the tissue type, quantity, identification number, expiration date, and date of issue/use among other data. These records are to be retained 10 years beyond the final disposition date.
-

Question 37: D. 10 years.

Explanation:

- Blood derivatives are generally derived from whole blood or can be manufactured. They are used as drugs or treatments for specific conditions and are usually injectable.
 - Examples of blood derivatives include coagulation factor concentrates (eg, NovoSeven), immunoglobulin preparations [Rh immunoglobulin, intravenous immunoglobulin (IVIG)], and albumin.
 - Like tissue-related records, most derivative-related records are to be retained for a minimum of 10 years.
 - Also, like tissue-related records, one caveat to the standard 10-year retention requirement is the derivative recipient's medical record documenting the receipt of the derivative including details such as the product name, lot number, quantity, and date and time of administration among other data. These records are to be retained 10 years beyond the date of distribution, infusion, disposition, or expiration, whichever date is latest.
-

Question 38: D. At least 7 days after transfusion.

Explanation:

- Patient specimens are to be stored at 1-6 C for at least 7 days after transfusion.



- A segment from transfused red-cell-containing blood products should also be stored at 1-6 C for at least 7 days after transfusion.
 - The retention of patient specimens and segments of transfused products permits follow-up testing in case of adverse events, such as transfusion reactions.
 - Three days is the expiration time for type and screen and type and crossmatch samples. After 72 hours, a new sample must be sent to the blood bank for pretransfusion testing. This time limit is in place to account for patients who have been transfused or pregnant in the last 3 months or in whom transfusion and pregnancy history are unknown.
 - Institutions can have their own policy for the expiration of samples from patients who have not been pregnant or transfused in the last 3 months. However, most institutions use the 72-hour rule for all patients for practical purposes and to ensure that the sample tested reflects the patient's current immunologic status.
-

Question 39: A. Identity of the person who made the change.

Explanation:

- Processes and procedures addressing record changes should delineate that the following be documented:
 - The date that the changes are made.
 - The identity of the person who made the changes.
 - The original record should remain and not be concealed by the changed record.
 - All elements of the changed record should be retained for the entirety of the original record's retention period.
-

Question 40: E. 7 days by mail, facsimile, or e-mail.

Explanation:

- Title 21 of the Code of Federal Regulations (CFR) Part 606 section 170 (b) regulates the reporting of fatalities attributed to blood donation or blood transfusion.



- Notifications of such fatalities are to be addressed to the Director of Office of Compliance and Biologics Quality at CBER, which is a Center within the FDA that regulates biologic products, including blood, that are intended for human use.
- CBER must be notified of the fatality as soon as possible by one of the following methods of delivery:
 - Telephone.
 - Facsimile.
 - Express mail.
 - Electronically transmitted mail.
- CBER must receive a formal written report of the investigation of the fatality within 7 days of the event by one of the following methods of delivery:
 - Mail.
 - Facsimile.
 - Electronically transmitted mail.



Question 41: B. CBER must be notified as soon as possible but the facility has up to 45 days from date that deviation was discovered.

Explanation:

- Title 21 CFR part 606.171 is “Reporting of product deviations by licensed manufacturers, unlicensed registered blood establishments, and transfusion services.” This code mandates the reporting of any event associated with deviations from good manufacturing practices, standards, regulations, or any other established specifications that could affect the potency, purity, or safety of blood or any blood product.
- Proper report forms (Form FDA-3486) must be submitted to CBER either by mail or electronically via CBER’s web-based application. If submitted by mail, the envelope should indicate a biological product deviation report is enclosed.
- Notification is to be made as soon as possible but no later than 45 days from the date that the deviation is discovered.
- Reports are to be submitted if the blood or blood product is distributed even if the deviation is caught before transfusion of the product or the product is returned for other reasons.



Question 42: A. Clinician transfusion practices should be peer-reviewed.

Explanation:

- Routine assessments of policies and procedures are essential elements of quality systems.
- Internal assessments include quality monitors such as quality controls (eg, low and high controls for a given assay), validation, and calibration among others.
- Proficiency testing is an example of an external quality assessment designed to verify that laboratory tests deliver the expected results. Inspections, audits, and surveys are other examples of external assessment.
- Utilization review such as blood and blood product utilization as well as test utilization are assessments that evaluate the appropriateness and efficiency of the use of procedures, services, and resources.
- Required assessments include peer review of clinical transfusion practices including transfusion indications, adherence to transfusion guidelines, clinical response to transfusions, and transfusion reactions.
- Results and findings of assessment should be reviewed by management, quality representative, or personnel delegated to assessment review.



Question 43: A. I and II.

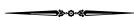
Explanation:

- AABB is an accreditation organization that provides a set of standards covering the various aspects of blood banks and transfusion services including immunohematology, administration, blood collection, molecular testing, cellular therapy services, and patient blood management among others. Inspections are performed on a 2-year cycle by volunteer inspectors with the assistance of some AABB staff inspectors.
- CAP accredits clinical laboratories and anatomic pathology laboratories. The organization provides checklists of standards with which laboratories must comply in order to be granted and main-



tain accreditation. Inspections are performed on a 2-year cycle by volunteer inspectors with the assistance of some CAP staff inspectors.

- The Joint Commission is an accreditation organization that has a broader health-care focus extending beyond the laboratory, delving into hospital safety and clinical practice. TJC also publishes a set of standards, which have a heavy focus on patient safety with goals of improving accuracy of patient identification, effectiveness of communication, and prevention of disease. Inspections are performed over a 3-year cycle solely by TJC staff inspectors.
- FACT is the accreditation arm of two professional societies that are dedicated to the improvement and progress of cellular therapy:
 - American Society of Blood and Marrow Transplantation (ASBMT).
 - International Society of Cellular Therapy (ISCT).
- FACT publishes international standards for cellular therapy and cord blood banks, which cover both laboratory and clinical facets of cellular therapy. The standards are established in conjunction with the Joint Accreditation Committee of ISCT and the European Society for Blood and Marrow Transplantation (EMBT) known as JACIE. Inspections are performed over a 3-year cycle by volunteer inspectors.



Question 44: C. Corrective action.

Explanation:

- When a deviation, nonconformance, or adverse event occurs, implementation of process improvement is required to maintain and/or re-establish quality.
- Process improvement begins with investigation of the deviation, nonconformance, or adverse event to determine the root cause(s).
- Once the cause(s) are discovered, actions must be set in place to correct the deviation, nonconformance, or adverse event and prevent recurrence. The 9000 series of the International Organization for Standardization (ISO) provide definitions of the following terms:
 - Correction: Action to eliminate a detected nonconformity.



- Corrective action: Action to eliminate the cause of a nonconformity and prevent recurrence.
 - Preventive action: Action to eliminate the cause of a potential nonconformity or other potential undesirable situation.
 - After corrective/preventive actions have been established, the new and/or improved process is implemented.
 - The newly implemented process(es) should be monitored to evaluate their effectiveness and efficacy.
-

Question 45: D. A and B.

Explanation:

- The AABB standards require that the blood bank/transfusion service medical director participate in developing the policies and procedures of transfusion recipient consent.
 - The minimum elements of consent include:
 - Description of the risks.
 - Description of the benefits.
 - Alternatives to transfusion, inclusive of nontreatment.
 - Opportunity to ask questions.
 - Right to accept or refuse transfusion.
-

Question 46: C. The transfusion should not proceed because the recipient donor verification process was not performed in the presence of the recipient.

Explanation:

- After the issue/release of blood but before transfusion of the blood or blood product, the transfusionist should verify the following:
 - Two independent recipient identifiers in the presence of the recipient.
 - Recipient ABO group and Rh type.
 - Donation identification number.
 - Donor ABO group and donor Rh type, if required.
 - If performed, the crossmatch interpretation.



- If applicable, the special transfusion requirements.
 - Unit expiration date.
 - These elements should also be verified at the time of issue/release of blood or blood product from the blood bank along with the date and time of issue as well as the final visual inspection.
-

Question 47: D. A and B.

Explanation:

- Before the issue/release of tissue from the blood bank and/or tissue bank, the following elements should be verified.
 - Two recipient independent identifiers, if tissue is intended for a specific patient.
 - Product name and quantity should match the order requisition.
 - Product should be issued with the package insert documents or recorded on the product contents list.
 - Date and time of expiration.
 - Date and time of issue/release.
 - Product final visual inspection.
-

Question 48: E. All required documentation elements are present.

Explanation:

- Medical record documentation of transfusion is very important in terms of traceability and patient safety. Proper documentation should include the following elements:
 - Transfusion order.
 - Patient consent.
 - Name of component.
 - DIN.
 - Donor ABO/Rh type.
 - Transfusion date and time.
 - Vital signs to be taken before, during, and after transfusion per facility protocol.
 - Volume transfused.



- Transfusionist identification.
 - Transfusion-related adverse events, if applicable.
 - Expiration date and time are elements required for the medical record documentation in the use of tissue. Other required elements include the quantity, identification number, person applying the tissue, and any related adverse events.
 - Lot number is an element required for the medical record documentation in the use of derivatives. Other required elements include the name of product, the quantity, administration date and time, person dispensing the derivative, and any related adverse events.
-

Question 49: A. Nonconformance; quarantined.

Explanation:

- Nonconformance is a failure to fulfill requirements, whether they be mandated by standards, federal law, or regulatory agencies. As the platelet in this question did not undergo all testing requirements, it would be considered a nonconforming unit. Such units should either be quarantined and/or destroyed.
 - Deviations are departures from regulations, standards, policies, processes, and procedures. Not performing testing would be a deviation, but the product would be a nonconforming unit. The unit should be quarantined or destroyed, not detained.
 - Segregate is to physically isolate or separate blood or blood products to prevent their unintentional distribution/issue or use.
 - Quarantine refers to the physical isolation of a nonconforming product to prevent their unintentional distribution/issue or use. It can be thought of as segregation of a nonconforming product.
 - An adverse event can be divided into two types: a complication involving a donor as a consequence of donation or involving a patient who received a transfusion or other medical procedure.
 - A deferral refers to the ineligibility of a donor to donate due to failing to meet donation criteria.
-

Question 50: D. Peer-review of blood utilization should be performed by physicians from various clinical services.



Explanation:

- A hospital transfusion committee is not a requirement in and of itself. However, it can serve to comply with peer-review requirements set forth by the AABB and patient safety goals set by TJC.
- The transfusion committee can also serve additional functions including monitoring ordering practices, blood usage, adverse events, sample collection and labeling, blood wastage, and near-miss events. Moreover, the committee can develop and improve processes and policies related to blood and transfusion.
- Although it is recommended that the hospital blood bank director be a part of the transfusion committee, it is preferable for the committee chair to be another physician with transfusion expertise. By separating the roles, conflicts of interest may be avoided when discussing and changing various clinical services and blood bank policies and procedures.
- Although there should be nursing representation from nursing education and the operating room, there should also be a nurse and a physician from clinical services that utilize blood and transfusion services (eg, anesthesia, emergency medicine, cardiothoracic surgery, critical care, trauma, medicine, and neonatology/pediatrics).
- It is recommended that a blood center physician serve as a member of the transfusion committee, at least in some capacity. Having a blood center representative can be beneficial to forge stronger hospital relationships, open provider-customer communication, provide regulatory and quality expertise, promote continuing education, and supply optimal look-back assistance.
- Other recommended transfusion committee membership includes the blood bank manager, pathology department director and residents, pharmacist, biomedical engineer, and risk management representation.
- During meetings, the different members should present reports to the rest of the transfusion committee and chair.
 - The blood bank manager should report data on:
 - Transfusion reactions.
 - Blood usage.
 - Blood wastage.
 - Blood product deviations.



- The transfusion medicine physician/blood bank director can detail:
 - Critical events (eg, mistransfusions).
 - Transfusion-transmitted disease monitoring.
 - Near misses.
- Physicians from diverse services who perform blood product utilization review can report on clinical appropriateness of transfusions.
- Nursing should provide updates on blood administration practices and assessments on the floors and in the operating room.
- Biomedical engineers can report on equipment installation and maintenance (eg, blood warmers and refrigerators).

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2

Medical Assessment of Blood Donors, Blood Collection (Autologous and Allogeneic), and Donor Complications

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- To be accepted for allogeneic blood donation, individuals must feel healthy and well on the day of donation and must meet all AABB and Food and Drug Administration requirements, as well as medical criteria defined by the blood collection facility and its medical director.
- Potential donors must be provided with predonation education about the blood donation process and an opportunity to have their questions answered before every blood donation.
- Adverse events related to donation must be assessed, investigated, and monitored.
- All units of blood collected should be immediately placed in quarantine in a designated area until: donor information and donation records have been reviewed; the current donor formation has been compared to the previous information; the donor's previous deferrals have been examined; and all laboratory testing has been completed.



QUESTIONS

Question 1: For whole blood donation, what is the deferral period for a male who has sex with another male?

- A. No deferral.
 - B. 3 months.
 - C. 1 year.
 - D. 3 years.
 - E. Permanent deferral.
-

Question 2: A 20-year-old female wants to donate whole blood. She had a new tattoo 4 months ago. When can she donate?

- A. Now.
 - B. She can donate in 2 months.
 - C. She can donate in 8 months.
 - D. She can donate in 20 months.
 - E. She is permanently deferred.
-

Question 3: On the donor history questionnaire, a young man says that he recently traveled to Africa. Upon further questioning, he says he traveled to Cameroon 2 months ago. When can he donate?

- A. Now.
 - B. He can donate in 1 month.
 - C. He can donate in 4 months.
 - D. He can donate in 10 months.
 - E. He is permanently deferred.
-

Question 4: A man who served in the US military but was stationed at a base in Germany for 5 years between 1985-1990 wants to donate blood. He fills out the donor history questionnaire. When can he donate blood?

- A. Now.



- B. Now, but only if the products are pathogen-reduced.
 - C. In 2030.
 - D. He can only donate platelets and they must be pathogen-reduced.
 - E. He is permanently deferred.
-

Question 5: A 25-year-old female filled out the donor questionnaire and marked that she had sex with a man who had sex with another man. Upon further questioning, she says that this occurred 2 days ago. When can the donor donate?

- A. Now.
 - B. 1 month.
 - C. 3 months.
 - D. 6 months.
 - E. 1 year.
-

Question 6: Which of the following donors is eligible to donate whole blood?

- A. A 58-year-old female, blood pressure 175/108 mmHg, pulse 72 bpm, hemoglobin 13 g/dL, temperature 36.9 C.
 - B. A 19-year-old male, blood pressure 184/97 mmHg, pulse 106 bpm, hematocrit 49%, temperature 36.7 C.
 - C. A 79-year-old female, blood pressure 120/76 mmHg, pulse 72 bpm, hemoglobin 12.6 g/dL, temperature 37.2 C.
 - D. A 53-year-old female, blood pressure 153/81 mmHg, pulse 90 bpm, hemoglobin 11 g/dL, temperature 37.3 C.
 - E. A 16-year-old male, blood pressure 123/67 mmHg, pulse 61 bpm, hematocrit 46%, temperature 37.8 C.
-

Question 7: Which of the following donors can donate whole blood?

- A. A male with a hemoglobin of 12.5 g/dL by finger stick.
- B. A female with a hemoglobin of 11.0 g/dL by finger stick.
- C. A male with a hemoglobin of 13.6 g/dL by finger stick.



- D. A female with a hemoglobin of 11.5 g/dL by finger stick.
 - E. A male with a hemoglobin of 13.9 g/dL by earlobe puncture.
-

Question 8: After reviewing donor health history questionnaires for the day, a supervisor notices that a donor marked “yes” to recent travel outside the country. Unfortunately, there is no documentation as to where. No one remembers where the donor said that they traveled. How long does the blood collection facility have to obtain this information?

- A. The information cannot be obtained after donation.
 - B. 12 hours following donation.
 - C. 24 hours following donation.
 - D. Before product labeling of the unit(s).
 - E. Before transfusion of the unit(s).
-

Question 9: A 23-year-old female wants to donate platelets. She recently had a positive ANA and is being evaluated for lupus. Which of the following infectious disease screening tests is likely to be positive?

- A. Anti-HCV.
 - B. Anti-HIV-1/2.
 - C. Anti-HBc.
 - D. Rapid plasma reagin (RPR) nontreponemal test.
 - E. HCV nucleic acid test (NAT).
-

Question 10: A whole blood donor had the Hepatitis B vaccine last week and asks if this could affect any of the infectious disease testing. What can you tell them?

- A. The vaccine never causes a positive test result.
- B. The vaccine can cause a positive result for 3 days after given.
- C. The vaccine can cause a positive result for 18 days after given.
- D. The vaccine can cause a positive result for 30 days after given.
- E. The vaccine can cause a positive result for 90 days after given.



Question 11: How long does the blood donor center have to make reasonable attempts to notify a donor if he or she has a positive test result that would disqualify the individual as a donor?

- A. 3 days.
 - B. 7 days.
 - C. 4 weeks.
 - D. 8 weeks.
 - E. 6 months.
-

Question 12: Which donor can donate today?

- A. A woman who donated 1 unit of whole blood 6 weeks ago.
 - B. A man who donated a double RBC product 8 weeks ago.
 - C. A man who donated a triple unit of platelets 3 days ago.
 - D. A woman who donated granulocytes 1 day ago.
 - E. A man who donated a single unit of platelets 3 days ago.
-

Question 13: Which donor is acceptable to donate whole blood today?

- A. A 15-year-old male who weighs 170 lb and has parental permission.
 - B. An 18-year-old female who weighs 105 lbs.
 - C. A 23-year-old male who weighs 204 lb and received hepatitis B immunoglobulin 6 months ago.
 - D. A 25-year-old female who weighs 140 lb and had a healthy baby 4 weeks ago by vaginal delivery.
 - E. A 27-year-old male who weighs 120 lb and had an FDA-approved COVID-19 vaccine 2 days ago.
-

Question 14: Which donor is eligible to donate today?

- A. A 43-year-old man who had sex with a female prostitute 1 month ago.
- B. A 25-year-old female who had sex with a male partner who was HIV positive 2 months ago but she has tested negative.



- C. A 32-year-old man who had sex with a male partner who was HBV positive 6 months ago but has tested negative.
 - D. A 45-year-old man who was in jail for 48 hours last week.
 - E. A 56-year-old male who had sex 1 month ago with a female who uses heroin via needle.
-

Question 15: A 25-year-old female comes to donate whole blood. On the health history questionnaire, she says that she was recently pregnant and also recently had a transfusion. Upon further questioning, she said that she had a healthy infant via vaginal delivery 8 weeks ago and that she also had two units of RBCs transfused after delivery due to blood loss. When is she eligible to donate?

- A. Now.
 - B. She can donate now as long as she provides negative infectious disease testing.
 - C. She can donate in 4 weeks.
 - D. She can donate in 8 weeks.
 - E. She can donate in 10 months.
-

Question 16: Which person is eligible to donate today?

- A. A 67-year-old male who had surgery 2 months ago to repair a ruptured Achilles tendon and required allogeneic tendon insertion.
 - B. A 54-year-old male who was burned in a campfire accident and received autologous skin grafts 1 month ago.
 - C. A 76-year-old who fractured their right femur and right radius and ulna in a car accident and required multiple surgeries and allogeneic bone transplants 1 month ago.
 - D. A 33-year-old female who was in a house fire and required multiple allogeneic skin grafts 6 weeks ago.
 - E. A 67-year-old male who was in a car accident and required massive transfusion of multiple blood products 2 months ago.
-

Question 17: Which donor meets Food and Drug Administration (FDA) criteria to donate platelets by plateletpheresis?



- A. A 43-year-old man who takes aspirin daily.
 - B. A 37-year-old male who last took clopidogrel 3 days ago.
 - C. A 62-year-old female who last took ticlopidine 7 days ago.
 - D. A 47-year-old female who last took aspirin for a headache 24 hours ago, but only took 2 doses.
 - E. A 51-year-old male who last took clopidogrel and aspirin 15 days ago.
-

Question 18: A 23-year-old male is taking isotretinoin for acne. When can he donate blood components?

- A. He is permanently deferred.
 - B. He is deferred 3 years from his last dose.
 - C. He is deferred 1 year from his last dose.
 - D. He is deferred 1 month from his last dose.
 - E. He is deferred 3 months from his last dose.
-

Question 19: Which donor is permanently deferred from donating?

- A. A 23-year-old male who takes isotretinoin for acne.
 - B. A 45-year-old male who takes finasteride for hair growth.
 - C. A 69-year-old male who takes dutasteride for benign prostatic hyperplasia.
 - D. A 33-year-old female who takes acitretin for psoriasis.
 - E. A 39-year-old male who takes etretinate for psoriasis.
-

Question 20: Which donor is acceptable to donate blood today?

- A. A 23-year-old male who had brain surgery as an infant and received a dura mater graft.
- B. A 54-year-old male who received cadaveric pituitary human growth hormone for 3 years in 1976-1979.
- C. A 37-year-old male who received human growth hormone for 4 years in 1990-1994.
- D. A 27-year-old male who had acute lymphocytic leukemia (ALL) when he was 2 years old.
- E. A 63-year-old female treated for syphilis 2 months ago.



Question 21: What is the deferral period for vaccination with measles, mumps, and rubella (MMR)?

- A. No deferral.
- B. 1 day.
- C. 1 week.
- D. 2 weeks.
- E. 4 weeks.



Question 22: A 25-year-old male wants to donate blood. On his donor health questionnaire, he answers that he recently received a vaccine. When asked for details, he says he was exposed to mpox and received the Jynneos vaccine. When can he donate?

- A. Now.
- B. 2 weeks after the vaccine.
- C. 21 days after the mpox exposure.
- D. 4 weeks after the vaccine.
- E. 8 weeks after the mpox exposure.



Question 23: A donor received the ACAM2000 vaccine for smallpox 2 weeks ago. When can she donate?

- A. Now.
- B. 4 weeks after receiving the vaccine as long as she has no complications.
- C. 56 days after receiving the vaccine as long as she has no complications.
- D. 12 weeks after receiving the vaccine as long as she has no complications.
- E. Any time after the site has developed a scab.



Question 24: A donor contracted malaria on a recent trip to Africa. How long must he wait to donate?

- A. After he completes treatment, he can donate.
- B. He can donate 3 months after treatment completion.



- C. He can donate 12 months after treatment completion.
 - D. He can donate 24 months after treatment completion.
 - E. He can donate 36 months after treatment completion.
-

Question 25: Which donor is eligible to donate platelets by plateletpheresis today?

- A. A 43-year-old male who took the last dose of vorapaxar 2 weeks ago.
 - B. A 39-year-old female who took the last dose of piroxicam 3 days ago.
 - C. A 54-year-old male who took the last dose of prasugrel 2 days ago.
 - D. A 46-year-old female who took the last dose of ticagrelor 5 days ago.
 - E. A 61-year-old male who takes aspirin every other day.
-

Question 26: Which donor is eligible to donate blood the soonest?

- A. A 62-year-old male who just had the last dose of Tegison (etretinate)
 - B. A 43-year-old female who just had her last dose of Arava (leflunomide).
 - C. A 53-year-old female who just had the last dose of Aubagio (teriflunomide).
 - D. A 46-year-old male who just had the last dose of Rinvoq (upadacitinib).
 - E. A 32-year-old female who just had the last dose of Cellcept (mycophenolate mofetil).
-

Question 27: How long should a donor wait after being diagnosed with Zika virus to donate?

- A. There is no deferral.
- B. Wait 7 days after symptoms resolve.
- C. Wait 14 days after symptoms resolve.
- D. Wait 30 days after symptoms resolve.
- E. Wait 120 days after symptoms resolve.



Question 28: The donor history questionnaire that is used by most blood centers in the United States was _____ by the AABB and is _____ by the Food and Drug Administration (FDA).

- A. Endorsed; mandated.
 - B. Developed; recognized.
 - C. Mandated; developed.
 - D. Mandated; endorsed.
 - E. Endorsed; developed.
-

Question 29: Which of the following donors is eligible to donate?

- A. Donor immunized against German measles (rubella) 2 weeks ago.
 - B. Donor received varicella zoster immunization 3 weeks ago.
 - C. Donor immunized against rubeola 4 weeks ago.
 - D. Donor received an experimental, unlicensed vaccine as part of a research project 12 weeks ago.
 - E. None of the above.
-

Question 30: A 25-year-old woman with acute myeloid leukemia and an antibody to HPA-1a would like her sister, who is HPA-1a negative, to donate platelets throughout her treatment. Which requirement is different for allogeneic and directed donors?

- A. Directed donors may be eligible with hemoglobin as low as 11 g/dL.
 - B. Directed donors may be tested for infectious diseases once every 30 days.
 - C. Directed donations may be omitted from the irradiation requirement.
 - D. Directed donations may have an extended outdate relative to allogeneic donations.
 - E. Directed donors may donate more frequently.
-

Question 31: What is the minimum platelet count a donor must have to donate platelets by apheresis?



- A. 100,000/ μ L.
 - B. 150,000/ μ L.
 - C. 200,000/ μ L
 - D. 250,000/ μ L.
 - E. 300,000/ μ L.
-

Question 32: A frequent plasma donor is defined as an individual donating plasma more frequently than every 4 weeks. Which of the following statements concerning this type of donor is true?

- A. They must have a total protein greater than 10 g/dL.
 - B. Females may donate with a hematocrit \geq 35%.
 - C. Males may donate with a hematocrit \geq 38%.
 - D. Total protein and protein electrophoresis must be done every 4 months.
 - E. A health assessment must be performed at their initial donation and not done again as long as they continue to donate.
-

Question 33: Which of the following individuals expecting surgery is eligible to donate autologous units?

- A. A 75-year-old male with prostate cancer whose hemoglobin level is 10.0 g/dL. He is otherwise healthy and is scheduled for a prostatectomy in 3 weeks.
- B. A 35-year-old paraplegic with infected decubitus ulcers over the sacrum and a hemoglobin level of 13.0 g/dL. Debridement and skin grafting are scheduled in 14 days.
- C. A 40-year-old female with degenerative joint disease. Her hemoglobin level is 11.0 g/dL. She will undergo bilateral knee replacement in 24 hours.
- D. A 16-year-old with chronic tonsillitis and a hemoglobin level of 15.0 g/dL, who will undergo tonsillectomy in 2 weeks. There is no physician order, but the patient's mother gives approval and insists upon autologous donation.
- E. None of the above.



Question 34: If only 400 mL of whole blood is collected because the donor developed a vasovagal reaction, which component(s) of that donation may be used for transfusion?

- A. Red cells, plasma, and platelets.
 - B. Red cells and plasma.
 - C. Red cells and platelets.
 - D. Plasma and platelets.
 - E. Red cells only.
-

Question 35: A 58-year-old male blood donor has a “slow” collection. What is the maximum time for whole blood donation that ensures the platelets and plasma will still be suitable for transfusion?

- A. 10 minutes.
 - B. 20 minutes.
 - C. 25 minutes.
 - D. 28 minutes.
 - E. 30 minutes.
-

Question 36: Which of the following statements concerning vasovagal donor reactions is true?

- A. They are characterized by hypotension and tachycardia.
 - B. They are most common in repeat donors.
 - C. They are most common in individuals with heavier weight.
 - D. The pathophysiology is excessive sympathetic activity.
 - E. They are more common in young donors.
-

Question 37: Which of the following is a true statement concerning the use of blood collected from individuals with hereditary hemochromatosis?

- A. Blood from therapeutic phlebotomies of hereditary hemochromatosis patients cannot be used for transfusion.
- B. Eligibility criteria for hereditary hemochromatosis patients are stricter than those for whole blood donors.



- C. Blood collected more frequently than every 56 days cannot be used for transfusion.
 - D. A prescription from the physician treating the hereditary hemochromatosis patient is required.
 - E. None of the above.
-

Question 38: Which of the following is an appropriate treatment for a vasovagal reaction?

- A. Elevate the donor's legs.
 - B. Talk to the donor and distract them.
 - C. Apply an ice pack to the donor.
 - D. Discontinue the donation.
 - E. All of the above.
-

Question 39: Which of the following is the most common donor adverse reaction to whole blood donation?

- A. Bruise or hematoma.
 - B. Vasovagal reaction.
 - C. Nerve injury.
 - D. Arterial puncture.
 - E. Allergic reaction.
-

Question 40: A 24-year-old male is donating platelets via apheresis. There is a machine failure and he has an estimated blood loss of 250 mL. Two weeks ago, he also had a blood loss of less than 200 mL. When can he return to donate platelets?

- A. He can return in 2 days.
- B. He can return in 6 weeks.
- C. He can return in 8 weeks.
- D. He can return in 14 weeks.
- E. He can return in 16 weeks.



Question 41: Infrequent plasma donors cannot donate plasma more frequently than once every how often?

- A. Once a week.
 - B. Once every 2 weeks.
 - C. Once every 3 weeks.
 - D. Once every 4 weeks.
 - E. Once every 8 weeks.
-

Question 42: The most common sign that one has placed the phlebotomy needle in an artery is?

- A. Bright red blood.
 - B. Difficulty keeping the needle in place.
 - C. Clotting in the tubing.
 - D. Blood leaking around the needle.
 - E. None of the above.
-

Question 43: A 35-year-old male was in a car accident and required massive transfusion. He subsequently developed antibodies to D, Jk^a, Jk^b, C, and E. He donated 2 units of autologous blood for his upcoming surgery. These units were both used during surgery yesterday. He had additional bleeding and went back to the OR. A frozen unit was thawed and deglycerolized and sent to the OR. The patient did not require the unit. What should be done with this unit?

- A. Tell the patient's doctor that it is rare and should be transfused.
 - B. Add glycerol and freeze again because it is rare.
 - C. Release the unit for use in another patient.
 - D. Keep the unit for the remainder of the 24 hours in case the patient needs it.
 - E. Post the unit's availability on the rare unit site for blood banks. Maybe someone close by needs it.
-

Question 44: A patient is having orthopedic surgery today and has a hematocrit of 45%. The patient is adamant that they do not want a blood transfusion or any allogeneic blood products. The decision is



made to perform acute normovolemic hemodilution. At what temperature and for how long can the donor/patient's blood be stored?

- A. At 1-6 C for up to 48 hours.
 - B. At room temperature for up to 4 hours.
 - C. At room temperature for up to 8 hours.
 - D. At room temperature for up to 12 hours.
 - E. At room temperature for up to 2 hours.
-

Question 45: Blood collected perioperatively for transfusion should be labeled with all of the following except?

- A. Donor/patient name.
 - B. Medical record number.
 - C. For autologous use only.
 - D. Blood type.
 - E. Date and time of collection.
-

Question 46: How long can intraoperative blood recovered with processing be kept at room temperature?

- A. 4 hours from the time of processing.
 - B. 6 hours from the time of processing.
 - C. 8 hours from the time of processing.
 - D. 12 hours from the time of processing.
 - E. 24 hours from the time of processing.
-

Question 47: What is the longest length of time that blood collected for acute normovolemic hemodilution (ANH) be kept at 1-6 C?

- A. 4 hours.
- B. 6 hours.
- C. 8 hours.
- D. 12 hours.
- E. 24 hours.



Question 48: The minimum hemoglobin to donate autologous blood is?

- A. 10.0 g/dL.
 - B. 11.0 g/dL.
 - C. 12.0 g/dL.
 - D. 12.5 g/dL.
 - E. 13.0 g/dL.
-

Question 49: A donor received an unlicensed vaccine for Ebola as part of a study at a local medical school today. When can the donor donate whole blood?

- A. 2 weeks.
 - B. 4 weeks.
 - C. 8 weeks.
 - D. 6 months.
 - E. 12 months.
-

Question 50: The maximum amount of blood that a person can donate per donation is?

- A. 5 mL/kg.
- B. 7.5 mL/kg.
- C. 10.5 mL/kg.
- D. 12.5 mL/kg.
- E. 15 mL/kg.

ANSWERS

Question 1: B. 3 months.

**Explanation:**

- To increase blood supply, the FDA revised the guidelines for reducing the risk of human immunodeficiency virus by blood and blood products in response to the COVID-19 pandemic. Thus, in 2020, the deferral changed from 12 months to 3 months.
-

Question 2: A. Now.

Explanation:

- The donor can donate now. The FDA changed the guidance for deferral following piercing or tattoos from 12 months to 3 months in 2020 in response to the COVID-19 pandemic.
 - The donor had her tattoo 4 months ago which meets the 3-month deferral period.
 - One exception is that if the tattoo was done by a state-regulated entity with sterile needles and new unused ink, there is no deferral.
-

Question 3: B. He can donate in 1 month.

Explanation:

- In 2020, the FDA revised the recommendations to reduce the risk of transfusion-transmitted malaria to increase the blood supply during the COVID-19 pandemic. The deferral changed from 12 months to 3 months.
- The donor returned from Cameroon 2 months ago, so he can donate after waiting 1 more month.
- Per FDA guidance, the donor could donate today with no deferral period for travel to a malarial endemic region if the blood components are pathogen-reduced using an FDA-approved pathogen reduction method.

**Question 4: A.** Now.**Explanation:**

- The man is able to donate today with no deferral. He can donate any type of product and it does not have to be pathogen reduced.
- The FDA revised their recommendations to reduce possible risk of transmission of Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease by blood and blood components in response to the COVID-19 pandemic.
- Before 2020, people who had spent time in certain European countries or on military bases in Europe who were deemed to have been exposed to a potential risk of transmission of Creutzfeldt-Jakob or variant Creutzfeldt-Jakob disease were permanently deferred from donating. These donors have been allowed to re-enter as donors.

**Question 5: C.** 3 months.**Explanation:**

- The donor can donate in 3 months. The FDA revised the criteria for deferrals in 2020 in response to the COVID-19 pandemic and urgent need to increase the blood supply. The deferral was changed from 12 months to 3 months.

**Question 6: C.** A 79-year-old female, blood pressure 120/76 mmHg, pulse 72 bpm, hemoglobin 12.6 g/dL, temperature 37.2 C.

**Explanation:**

Criteria	Requirement
Age	>16 years old as appropriate by applicable state law
Blood volume collected	Maximum of 10.5 mL/kg of donor weight, including samples; blood collection container must be approved for volume collected
Blood pressure	90-180 mm Hg systolic 50-100 mm Hg diastolic
Pulse	50-100 beats per minute <50 beats per minute acceptable if athlete*
Temperature	<37.5 C (99.5 F) if measured orally, or equivalent if measured by another method
Hemoglobin/ hematocrit	Males: Hgb 13.0 g/dL or Hct 39% Females: Hgb 12.5 g/dL or Hct 38%

*The blood center physician may approve donation if the pulse is <50 bpm. This is typically done for donors who are athletic.



Question 7: C. A male with a hemoglobin of 13.6 g/dL by finger stick.

Explanation:

- The FDA has stated that earlobe hemoglobin measurements are unacceptable because of substantial variation in hemoglobin values compared with concurrent values from venipuncture samples.
- The FDA's May 2015 final rule [21 CFR 630.10(f)(3)(i)(B)] defines the minimum hemoglobin level for males as 13.0 g/dL and the minimum level for females as 12.5 g/dL.



Question 8: C. 24 hours following donation.

Explanation:

- The blood collection facility should determine donor eligibility before collection (same day). If additional information is needed, 24 hours is permitted to prevent wastage of the donation. If the donor cannot be reached for clarification, then the unit should be discarded.



Question 9: D. RPR nontreponemal test.

Explanation:

- Nontreponemal tests such as the rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL) are used in screening for syphilis. Positive results are then confirmed using a more specific treponemal test.
- False-positive nontreponemal test results are seen in various medical conditions unrelated to syphilis, including autoimmune disorders, older age, and injection drug use.



Question 10: C. The vaccine can cause a positive result for 18 days after given.

Explanation:

- The hepatitis B vaccine can cause a positive hepatitis B surface antigen (HBsAg) test result in blood donors for up to 18 days after vaccination. When a donor tests repeatedly positive for HBsAg, which confirms positive by neutralization, he or she may be able to donate again if confirmed that the donor received the vaccine for routine reasons and within 28 days prior to donation.
- If the vaccination was given in response to an exposure incident, then the donor must wait 12 months from the date of exposure before being tested for donor reentry.



Question 11: D. 8 weeks.

Explanation:

- Blood donor centers have 8 weeks to make reasonable attempts to notify donors of positive infectious disease results that disqualify the donor from future donation.
-

Question 12: E. A man who donated a single unit of platelets 3 days ago.

Explanation:

Donation Interval	Procedure
16 weeks	2-unit erythrocytapheresis
8 weeks	Whole blood donation
4 weeks	Infrequent plasmapheresis
2 days	Leukocytapheresis
2 days	Single apheresis platelet donation
7 days	Double or triple apheresis platelet donation

Question 13: E. A 27-year-old male who weighs 120 lbs and had an FDA-approved COVID-19 vaccine 2 days ago.

Explanation:

- Donors are deferred for 6 weeks after the delivery of a healthy infant. Donors are deferred for 12 months after receiving hepatitis B immunoglobulin. All donors must weigh a minimum of 110 lbs.
- The lower age limit for blood donation is 16 years and there is no upper limit. Individuals who are 16 or 17 years old are permitted to donate blood according to state laws.



- There is no deferral period for COVID-19 vaccination if a nonreplicating, inactivated, or RNA-based COVID-19 vaccine was used.
-

Question 14: D. A 45-year-old man who was in jail for 48 hours last week.

Explanation:

- Sexual contact with an individual with hepatitis B, symptomatic hepatitis C, or unknown hepatitis is a 12-month deferral. Residing with or sexual contact with a person with asymptomatic hepatitis C is not cause for deferral.
 - Sexual contact with an individual with HIV infection or a positive test for the HIV/AIDS virus is a 3-month deferral.
 - Sexual contact with a prostitute or anyone else who takes money, drugs, or other payment for sex is a 3-month deferral.
 - Sexual contact with anyone who has ever used needles to take drugs or steroids or anything not prescribed by their doctor is a 3-month deferral. A person who has used needles to take drugs not prescribed by their doctor is deferred 3 months.
 - Incarceration in jail, juvenile detention, lockup, or prison for more than 72 consecutive hours is a 12-month deferral.
-

Question 15: C. She can donate in 4 weeks.

Explanation:

- Transfusion of allogeneic blood products such as RBCs, plasma, platelets, or whole blood is associated with a 3-month deferral as this was changed by the FDA during the COVID-19 pandemic.
-

Question 16: B. A 54-year-old male who was burned in a campfire accident and received autologous skin grafts 1 month ago.



Explanation:

- Transfusion with any allogeneic blood product is associated with a 3-month deferral.
 - Transplant of allogeneic tendon, skin, or bone is also a 3-month deferral.
 - Transplant with autologous skin or bone is not associated with any deferral.
-

Question 17: E. A 51-year-old male who last took clopidogrel and aspirin 15 days ago.

Explanation:

- In addition to donor eligibility requirements at 21 CFR 640.3 and recommendations applicable to donors of whole blood, the FDA's December 2007 Guidance for Industry and FDA Review Staff, "Collection of Platelets by Automated Methods" recommends:
 - Deferral of donors taking aspirin within 48 hours before collection because aspirin blocks the generation of thromboxane A2, which inhibits the platelet release reaction and renders the platelets inactive. The guidance recommends donor deferral of 14 days for Plavix (clopidogrel) or Ticlid (ticlopidine).
 - Ingestion of aspirin or aspirin-containing medications within "2 full days" preceding donation or ingestion of medications that irreversibly inhibit platelet function is cause for deferral of a plateletpheresis donor because a plateletpheresis donor serves as the sole source of platelets for a patient.
 - However, under the same circumstances, a donor is eligible to donate when taking aspirin within 48 hours of collection if the donor is *not* the sole source of platelets because the collection is pooled with collections from other donors.
-

Question 18: D. He is deferred 1 month from his last dose.



Explanation:

- Isotretinoin (Accutane, Amnesteem, Absorica, Claravis, Myorsan, Sotret, and Zenatane) is a teratogen and has the potential to cause birth defects in a developing fetus. Therefore, donors taking teratogenic medications are deferred from donating for various periods of time to allow the teratogenic medication to clear their bodies.



Question 19: E. A 39-year-old male who takes etretinate for psoriasis.

Explanation:

- All of these medications are teratogenic:
 - Finasteride (Proscar or Propecia): 1-month deferral after last dose.
 - Isotretinoin (Accutane, Amnesteem, Absorica, Claravis, Myorsan, Sotret, and Zenatane): 1-month deferral after last dose.
 - Dutasteride (Avodart or Jalyn): 6-month deferral after last dose.
 - Acitretin (Soriatane): 3-year deferral.
 - Etretinate (Tegison): permanent deferral.



Question 20: C. A 37-year-old male who received human growth hormone for 4 years in 1990-1994.

Explanation:

- Donors who have had blood cancers such as leukemia and lymphoma are permanently deferred from donating at most blood and plasma donation centers across the United States. Receipt of an allogeneic dura mater graft is a permanent deferral due to risk of Creutzfeldt-Jakob disease.
- Similarly, receipt of human growth hormone before 1985 is a permanent deferral as it was derived from the pituitary of cadavers and had a risk of Creutzfeldt-Jakob disease.



- Human growth hormone available after 1985 is primarily produced recombinantly and receipt is not associated with a deferral.
 - Diagnosis and treatment for gonorrhea and/or syphilis is associated with a 3-month deferral following treatment.
-

Question 21: E. 4 weeks.

Explanation:

- Live vaccines such as MMR (specifically, rubella) are associated with a 4-week deferral. The chicken pox and Zostavax shingles vaccine are also 4-week deferrals.
 - Yellow fever vaccine, oral polio vaccine, and oral typhoid vaccine are associated with 2-week deferrals. Mumps and rubeola vaccine separate from MMR is also a 2-week deferral.
 - There is no deferral for influenza vaccine, pneumonia vaccine, tetanus vaccine, HPV vaccine, or meningitis vaccines.
-

Question 22: C. 21 days after the mpox exposure.

Explanation:

- The Jynneos vaccine is not associated with any deferral. However, following exposure to mpox, donors are deferred for 21 days after the last exposure.
-

Question 23: C. 56 days after receiving the vaccine as long as she has no complications.

Explanation:

- If the donor has complications such as skin reactions beyond the vaccination site or general illness related to the vaccine, she should wait an additional 14 days after all the vaccine complications resolve or 8 weeks from the date of the vaccine (whichever is longer).



Question 24: E. He can donate 36 months after treatment completion.

Explanation:

- A donor must wait 3 years after treatment for malaria to donate. If a donor returns from vacationing in a malaria-endemic region can donate in 3 months. A donor must wait 3 years after living more than 5 years in a malaria-endemic region.



Question 25: B. A 39-year-old female who took the last dose of piroxicam 3 days ago.

Explanation:

- For all of these medications, there is no deferral to donate whole blood.
- But there is a deferral for donating platelets by apheresis where the platelets from the donor would be the sole source of platelets. When taking aspirin, a donor must stop for 2 full days before donating platelets. A donor must wait 7 days after taking Brilinta (ticagrelor) before donating platelets and 3 days after taking Effient (prasugrel) before donating platelets. After taking Feldene (piroxicam), a donor must wait 2 full days to donate platelets. Donors must wait 14 days after taking Plavix (clopidogrel) or Ticlid (ticlopidine) to donate platelets. Lastly, if a donor is taking Zontivity (vorapaxar), they must wait 1 month before donating platelets.



Question 26: D. A 46-year-old male who just had the last dose of Rinvoq (upadacitinib).

Explanation:

- The deferrals associated with various medications are given below:
 - Rinvoq (upadacitinib) – 1 month.
 - Thalidomide (thalidomide) – 1 month.



- Cellcept (mycophenolate mofetil) – 6 weeks.
 - Aubagio (teriflunomide) – 2 years.
 - Arava (leflunomide) – 2 years.
 - Erivedge (vismodegib) – 2 years.
 - Odomzo (sonidegib) – 2 years.
 - Tegison (etretinate) – permanent deferral.
 - Soriatane (acitretin) – 3 years.
 - Avodart, Jalyn (dutasteride) – 6 months.
 - Accutane, Amnesteem, Absorica, Claravis, Myorisan, Sotret, Zenatane (isotretinoin) – 1 month.
 - Proscar, Propecia (finasteride) – 1 month.
-

Question 27: E. Wait 120 days after symptoms resolve.

Explanation:

- Following diagnosis with Zika virus, a donor should wait more than 120 days after symptoms resolve prior to donating.
-

Question 28: B. Developed; recognized.

Explanation:

- The DHQ was developed by the AABB and is formally recognized by the FDA guidance. The AABB DHQ is not mandated. A blood donor center can develop a questionnaire and submit it to the FDA in a prior approval supplement under 21 CFR 601.12(b).
-

Question 29: C. Donor immunized against rubeola 4 weeks ago.

Explanation:

- Live vaccines such as MMR (specifically, rubella) are associated with a 4-week deferral. The chicken pox and Zostavax shingles vaccine are also 4-week deferrals.



- Yellow fever vaccine, oral polio vaccine, and oral typhoid vaccine are associated with 2-week deferrals. Mumps and rubeola vaccine separate from MMR is also a 2-week deferral.
 - There is no deferral for influenza vaccine, pneumonia vaccine, tetanus vaccine, HPV vaccine, or meningitis vaccines.
 - Experimental vaccines are a 12-month deferral.
-

Question 30: B. Directed donors may be tested for infectious diseases once every 30 days.

Explanation:

- Recipient-specific, designated, or directed blood donors must meet the same criteria as allogeneic blood donors.
 - The one exception is that for frequent directed donors, FDA regulations permit infectious disease testing with only the first donation in each 30-day period.
 - Due to the increased risk of transfusion-associated graft-vs-host disease, donations from relatives are an absolute indication for irradiation.
 - Units collected from directed donors have the same expiration date as allogeneic units.
-

Question 31: B. 150,000/ μ L.

Explanation:

- Donors must have a minimum platelet count of 150,000/ μ L to be eligible to donate.
- FDA guidance requires that a sample for a platelet count be drawn before collection. This count should be used for eligibility determination (current collection or future collections) and to set the yield parameters. The count may be completed before the procedure starts or immediately following initiation. If the platelet count cannot be obtained (eg, during collections on a mobile drive), an average of previous counts from the donor or a default count can be used to program the instrument. If one of these surrogate counts is used, a triple platelet collection cannot be performed.



- Plateletpheresis donors may donate as frequently as twice per week. They are not allowed to donate more than 24 times per year. This restriction arises from the fact that early apheresis equipment removed significant numbers of lymphocytes during plateletpheresis. This resulted in concerns that this could, with time, lead to immunodeficiency. Studies of these donors did demonstrate decreased T-lymphocyte counts and immunoglobulin G levels 8 months after donation. However, current apheresis instruments remove significantly fewer lymphocytes, and more recent studies have failed to demonstrate immunologic changes.
 - The total plasma volume (including plasma collected on the platelet product and concurrent plasma) must not exceed 500 mL (or 600 mL if the donor is 175 lbs or greater) or the volume described in the instrument's labeling.
-

Question 32: D. Total protein and protein electrophoresis must be performed every 4 months.

Explanation:

- Plasma donations can also be categorized by the frequency with which the donor donates:
 - Infrequent plasma donors: the interval between donations is more than 4 weeks. These donors must fulfill whole blood donor requirements. For exceptions, see regulations at 21 CFR 630.25.
 - Frequent plasma donors: the interval between donations is less than 4 weeks. These donors must fulfill criteria outlined in 21 CFR 640.65.
 - These requirements are similar to those for whole blood donation but differ in the following respects:
 - Malaria risk is not a cause for deferral.
 - The total serum protein must be at least 6.0 g/dL but no more than 9.0 g/dL, assessed before each plasmapheresis procedure
 - Either a serum protein electrophoresis or quantitative immunodiffusion assay must be performed every 4 months. The plasma protein fractionation must be within normal limits.
 - The donors must undergo a physical examination once a year.



- Frequent plasma donors must not have red cell losses greater than 200 mL within 8 weeks. If they do, they are deferred for 8 weeks from the last red cell loss.
 - As with plateletpheresis, there must be at least 2 days between donations and no more than two donations within a 7-day period.
-

Question 33: E. None of the above.

Explanation:

- Refer to FDA Guidance for industry: “Determining donor eligibility for autologous donors of blood and blood components intended solely for autologous use—compliance policy (August 2016).” Autologous donations *must* be ordered by the patient’s physician.
 - Blood should not be collected when the donor/patient has a bacterial infection that could be associated with bacteremia because this places him or her at risk for septic transfusion reactions at the time of transfusion of the blood component.
 - There are additional patient issues that should be considered as contraindications to autologous blood donation, including the following:
 - Presence of diseases with which the donor/patient has a fixed cardiac output (eg, aortic stenosis) or has a cardiac output that is volume-dependent (eg, hypertrophic cardiomyopathy or subaortic stenosis).
 - Presence of unstable angina or severe coronary artery disease.
 - Recent myocardial infarction or stroke.
 - Cyanotic heart disease.
 - A minimum hemoglobin level of 11.0 g/dL (hematocrit of 33%) is acceptable for autologous donation (AABB *Standards*).
 - Deferral based on responses to questions on the donor history questionnaire differ for autologous donors. If more than 1 unit is required, the phlebotomy schedule is established by the blood collection facility consistent with the physician’s order. The last phlebotomy must be scheduled ≥ 72 hours before the anticipated surgery. This is to avoid hypo-



volemia and attendant negative effects for the patient (*AABB Standards*). The donor/patient's physician and the collection facility medical director decide the interval between donations.

- Autologous units are for autologous use only and must not be used (crossed over) for other patients.
-

Question 34: E. Red cells only.

Explanation:

- When low-volume allogeneic collections occur, the Red Blood Cell (RBC) component should be separated and labeled as "RBCs Low Volume." The plasma and platelet components should be discarded. This is, in part, due to the higher citrate concentration in these low-volume units. Research has shown that over- and undercollected units do not adversely affect red cell storage for 21 to 35 days.
-

Question 35: B. 20 minutes.

Explanation:

- The average whole blood collection takes 10 minutes. If the collection takes longer than 15 to 20 minutes, then the platelets and plasma may not be suitable for transfusion. The concern is that a slow collection suggests that the coagulation system may be activated and, as in active bleeding, the platelets are activated and the coagulation profile is hyperfibrinolytic. Thus, the platelets prepared from whole blood could be adversely affected.
-

Question 36: E. They are more common in young donors.

Explanation:

- Vasovagal reactions consist of pallor, lightheadedness, anxiety, diaphoresis, hyperventilation, irregular breathing, weakness, nau-



sea, vomiting, hypotension, and bradycardia. Risk factors include:

- Young age.
 - Low weight.
 - First-time donation.
 - Epidemic fainting.
 - Inattentive or noncommunicative phlebotomist.
 - The pathophysiology of vasovagal reactions represents excessive parasympathetic outflow. During blood donation, hypovolemia leads to sympathetic activation. The parasympathetic response to counteract this, however, is excessive, leading to bradycardia and vasodilatation with hypotension.
 - Reactions can occur anytime during the donation process (even before phlebotomy) but most will occur within 15 minutes of the completion of donation. Vasovagal reactions with syncope occur in 0.1% to 0.3% of donations.
-

Question 37: D. A prescription from the physician treating the hereditary hemochromatosis patient is required.

Explanation:

- To transfuse blood collected from patients with hereditary hemochromatosis without labeling the unit of blood with the donor's disorder, the following criteria must be met:
 - The patient must meet the same eligibility requirements as other whole blood donors.
 - To collect blood more frequently than every 8 weeks, a physician's prescription, including instructions on the frequency of phlebotomy and hematocrit/hemoglobin limits, is required or a physician must examine the patient and certify that he or she is in good health on the day of each donation.
 - There must be *no* fees charged to *any* hereditary hemochromatosis patients undergoing phlebotomy, including those who do not meet eligibility requirements for whole blood donation. The rationale behind not charging for phlebotomies for both eligible and ineligible donors is to eliminate any incentives (ie, the cost of the phlebotomy) that may encourage an individual to withhold information concerning risk factors on the donor history questionnaire.



- Establishments do not need an exception or approval of alternative procedure under 21 CFR 640.120 if the requirements set forth in 21 CFR 630.15(a)(2) are met.
 - One study estimated that if *only* the units from eligible hemochromatosis patients undergoing maintenance phlebotomy were used for transfusion, this would represent a 16% increase in the US RBC supply.
-

Question 38: E. All of the above.

Explanation:

- All of the answers are treatments for vasovagal reactions.
-

Question 39: A. Bruise or hematoma.

Explanation:

- The most common donor reaction is bruising or hematoma followed by vasovagal reactions. Less common are allergic reactions to tape and antiseptic cleansers such as betadine or iodine and vasovagal reactions associated with syncope.
-

Question 40: C. He can return in 8 weeks.

Explanation:

- The donor had a less than 200 mL blood loss 2 weeks ago and a blood loss of 250 mL today. Thus, he is deferred for 8 weeks from the second loss (today).



Initial Red Cell Loss	Second Red Cell Loss within 8 Weeks	Deferral
<200 mL	<200 mL	No deferral
<200 mL	>200 mL but <300 mL	Defer for 8 weeks from second loss
>200 mL but <300 mL	NA	Defer for 8 weeks from initial loss
<200 mL	Total loss from first and second losses >300 mL	Defer for 16 weeks from second loss
≥300 mL	NA	Defer for 16 weeks from initial loss



Question 41: D. Once every 4 weeks.

Explanation:

- Infrequent plasma donors: the interval between donations is more than 4 weeks. These donors must fulfill whole blood donor requirements. For exceptions, see regulations at 21 CFR 630.25.
- Frequent plasma donors: the interval between donations is less than 4 weeks. These donors must fulfill criteria outlined in 21 CFR 640.65.



Question 42: A. Bright red blood.

Explanation:

- Arterial puncture occurs when the needle passes through the vein and enters the artery underlying the vein. It is characterized by any of the following:
 - Severe or unusual pain.
 - Rapid filling of the bag in 92%.
 - Bright red blood in 75%.



- Movement of the needle with each heart beat in 33%.
 - Inexperience in the phlebotomist is a risk factor.
 - Treatment consists of immediate discontinuation of donation and firm pressure applied to the site for at least 10-20 minutes. A pressure bandage should be applied and left in place for 3 to 4 hours.
 - Arterial puncture can rarely be complicated by one or more of the following:
 - Arterial pseudoaneurysm.
 - Arterial-venous fistula formation.
 - Compartment syndrome.
-

Question 43: B. Add glycerol and freeze again because it is rare.

Explanation:

- The patient has antibodies to D, Jk^a, Jk^b, K, Fy^a, and Fy^b. Based upon the chart below, the frequency of finding a unit of RBCs negative for these antigens would be:

$$0.15 \times 0.23 \times 0.28 \times 0.91 \times 0.34 \times 0.15 = \\ 0.00045 \text{ or } 0.05\%.$$

Blood Group System	Antigen	Antigen Frequency %*	% Donor Blood Compatible (Antigen-Negative)
Rh	D	85	15
	C	70	30
	E	30	70
	c	80	20
	e	98	2
Kell	K	9	91
	Kp ^a	2	98
	Js ^a	0.1 Whites 19.5 Blacks	99.9 Whites 80 Blacks
	k, Kp ^b , Js ^b	99.9	0.1



Duffy	Fy ^a	*66	34
	Fy ^b	*85	15
		*Note: 68% of Blacks are Fy(a-b-)	
Kidd	Jk ^a	77	23
	Jk ^b	72	28
MNS	M	78	22
	N	72	28
	S	55	45
	s	89	11
P	P ₁	80	20
Lewis	Le ^a	22	78
	Le ^b	72	28
	*Note: 22% of Blacks are Le(a-b-)		
Lutheran	Lu ^a	8	92
	Lu ^b	99.9	0.1

*Frequencies are for people in the United States who are of European ancestry, unless otherwise indicated. Frequencies may differ in people of African ancestry, Asian ancestry, and other groups.

- This is a very, very rare RBC unit. It should be refrozen immediately. This patient should be encouraged to donate blood as soon as he is recovered.
-

Question 44: C. At room temperature for up to 8 hours.

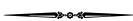
Explanation:

- Acute normovolemic hemodilution (ANH) is the removal of blood from a donor/patient and restoration of the volume with



the infusion of an acellular fluid. The collected whole blood can then be used during the surgical procedure. ANH minimizes blood loss because the blood hemorrhaged during the procedure is of lower hematocrit because of the dilution.

- The solutions used are either crystalloid (infused at a ratio of 3 mL:1 mL whole blood removed) or colloid (infused at a ratio of 1 mL:1 mL of whole blood removed).
- The blood is collected into standard blood bags containing anticoagulant. It is stored at room temperature in the operating room and reinfused according to standard fluid management. To maximize effect, the blood is reinfused in the reverse order that it was drawn. In this way, the lower concentrations of red cells are reinfused first.
- The total amount of blood collected depends upon the patient's ability to tolerate the anemia induced by the procedure. The patient's circulating volume and perfusion status must be monitored. The blood is stored at room temperature to preserve platelet function. As a result, storage should not exceed 8 hours from the start of collection. If blood is not used within 8 hours, they can be stored at 1-6 C for up to 24 hours.
- The blood must be appropriately labeled with the following information: patient's full name, medical record number, date and time of collection, and "For autologous use only."
- ANH units collected by operating room staff minimize procurement and administration costs. Because the blood does not leave the operating room, the dangers of clerical errors and their results (eg, ABO-incompatible transfusion) are minimized.



Question 45: D. Blood type.

Explanation:

- The bag should be labeled with donor/patient name, medical record number, date and time of collection, and "For autologous use only."
 - Bacteria are frequently cultured from recovered and processed autologous blood. Reports have failed to identify adverse consequences of this. Nevertheless, intraoperative blood should not be used when the surgical field is grossly contaminated or frankly infected.



- Intraoperative blood, whether processed or not, should be transfused through a microaggregate filter. Processing can occur either in the operating room or at another site. If the unit of blood is processed outside of the operating room, processes and procedures must be in place to verify donor/patient and product identity before infusion.
- Blood is usually washed using normal saline. The use of other solutions, just as during routine red cell transfusions, could result in clotting of the product or hemolysis.



Question 46: C. 8 hours from the time of processing.

Explanation:

Collection	Storage Temperature	Shelf Life
Acute normovolemic hemodilution	Room temperature	8 hours from start of collection
	1-6 C	24 hours from start of collection
Intraoperative blood recovered with processing	Room temperature	8 hours from time of processing
	1-6 C	24 hours from time of processing
Intraoperative blood recovered without processing	Room temperature or 1-6 C	8 hours from start of collection
Shed blood with or without processing	N/A	8 hours from start of collection



Question 47: E. 24 hours.

Explanation:

- See table above in question #46.



Question 48: B. 11.0 g/dL.

Explanation:

- A minimum hemoglobin level of 11.0 g/dL (hematocrit of 33%) is acceptable for autologous donation.
 - The minimum hemoglobin for females to donate allogeneic blood is 12.5 g/dL and for males is 13.0 g/dL (*AABB Standards*).
-

Question 49: E. 12 months.

Explanation:

- Live vaccines such as MMR (specifically, rubella) are associated with a 4-week deferral. The chicken pox and Zostavax shingles vaccine are also 4-week deferrals.
 - Yellow fever vaccine, oral polio vaccine, and oral typhoid vaccine are associated with 2- week deferrals. Mumps and rubeola vaccine separate from MMR is also a 2-week deferral.
 - There is no deferral for influenza vaccine, pneumonia vaccine, tetanus vaccine, HPV vaccine, or meningitis vaccines.
 - Experimental vaccines are a 12-month deferral.
-

Question 50: C. 10.5 mL/kg.

Explanation:

- See table in answer #6.

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3

Blood Components: Preparation, Storage, and Characteristics

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- The blood component identification process uniformly uses both a bar-coded and eye-readable unique identification number that is assigned to each sample tube and each component prepared from the donation.
- Processing methods for components from whole blood are typically defined based on the method used to separate the platelets from the whole blood.
- Plasma preparations are defined and regulated through extensive combinations of differences in collection methods, storage temperatures, freezing methods, secondary processing, timing, and storage after thawing.
- Postcollection modification of blood components may include prestorage leukocyte reduction by filtration, pooling, cryopreservation, or pathogen inactivation.
- Because the safety, purity, potency, and quality of the blood components may be affected by improper storage, alarm setting should be configured to notify necessary personnel *before* the upper or lower limits of acceptable storage temperature are exceeded.



QUESTIONS

Question 1: A unit of Red Blood Cells (RBCs) collected and stored in citrate-phosphate-dextrose-adenine (CPDA-1) has a maximum shelf-life of how many days?

- A. 14 days.
 - B. 21 days.
 - C. 35 days.
 - D. 42 days.
 - E. 49 days.
-

Question 2: A unit of RBCs collected and stored in citrate-phosphate-dextrose (CPD) has a maximum shelf-life of how many days?

- A. 14 days.
 - B. 21 days.
 - C. 35 days.
 - D. 42 days.
 - E. 49 days.
-

Question 3: A unit of RBCs collected and stored in which solution has the lowest hematocrit?

- A. ACD.
 - B. CPD.
 - C. Additive solution (AS).
 - D. CP2D.
 - E. CPDA-1.
-

Question 4: A unit of RBCs is washed in an open system with normal saline due to a patient having IgA deficiency. What is the new shelf-life of the washed RBC unit if stored at 1-6 C?

- A. 1 hour.
- B. 4 hours.



- C. 8 hours.
 - D. 24 hours.
 - E. The same as the original unit.
-

Question 5: Which additive solution has the highest amount of mannitol?

- A. AS-1.
 - B. AS-3.
 - C. AS-5.
 - D. AS-1 and AS-3.
 - E. All have the same amount of mannitol.
-

Question 6: A shipper is being prepared for transport of RBCs to local hospital blood bank. A total of 45 units of RBCs are being transported. At what temperature must the RBCs remain while being transported?

- A. 0-10 C.
 - B. 1-6 C.
 - C. 1-10 C.
 - D. 4-10 C.
 - E. 1-15 C.
-

Question 7: A unit of RBCs was returned from the operating room because it was not used. The blood bank staff would like to put it back into storage for reissue. All of the following conditions must be met except?

- A. The unit must have an intact seal.
- B. The unit must have been maintained at a temperature of 1-10 C.
- C. At least one sealed segment of the integral donor tubing must be attached.
- D. A tag must be attached indicating that it has been issued once before.
- E. The technologist must inspect it and deem it acceptable before reissue and document this in the records.



Question 8: Leukocyte-reduced RBC units must contain less than which number of leukocytes?

- A. $<1.0 \times 10^6$ leukocytes per unit.
 - B. $<3.0 \times 10^6$ leukocytes per unit.
 - C. $<5.0 \times 10^6$ leukocytes per unit.
 - D. $<7.0 \times 10^6$ leukocytes per unit.
 - E. $<9.0 \times 10^6$ leukocytes per unit.
-

Question 9: All of the following are advantages of leukocyte reduction of RBC units as compared to irradiation of RBC units except?

- A. Prevention of graft-vs-host disease (GVHD).
 - B. Prevention of HLA alloimmunization.
 - C. Decrease in febrile transfusion reactions.
 - D. Prevention of cytomegalovirus (CMV) transmission.
 - E. All of these are advantages of leukocyte reduction over irradiation.
-

Question 10: A unit of RBCs in CPD plus AS-3 was collected 10 days ago. It was irradiated today so it can be transfused to a marrow transplant recipient. What is the expiry date of this unit?

- A. 32 days from now.
 - B. 28 days from now.
 - C. 18 days from now.
 - D. 24 hours from now.
 - E. 25 days from now.
-

Question 11: What is the most common percentage of glycerol used in freezing RBC units in the United States?

- A. 5%.
- B. 10%.
- C. 30%.
- D. 40%.
- E. 50%.



Question 12: A frozen unit of RBCs was thawed and deglycerolized. It currently looks like a dark colored mass of jelly. What is the most likely cause?

- A. The unit is bacterially contaminated.
 - B. Normal saline was used in the deglycerolization process in error.
 - C. It was frozen for too long.
 - D. The unit was thawed at 37 C.
 - E. The unit was from someone with sickle trait.
-

Question 13: Following deglycerolization, what % of the red cells must be recovered according to standards?

- A. $\geq 95\%$.
 - B. $\geq 90\%$.
 - C. $\geq 85\%$.
 - D. $\geq 80\%$.
 - E. $\geq 75\%$.
-

Question 14: A rare unit of RBCs was thawed and deglycerolized in an open system. What is the expiration date of the product now?

- A. 14 days.
 - B. 7 days.
 - C. 24 hours.
 - D. 4 hours.
 - E. 2 hours.
-

Question 15: A 29-year-old female with an anti-U is scheduled for cardiac surgery tomorrow. Two S-, s-, U- units are deglycerolized in preparation for possible transfusion. The patient successfully had surgery today and required only one unit to be transfused. What should be done with the other rare unit?

- A. Refrigerate the unit until the expiration time and discard if not clinically needed.
- B. Advise medical staff to transfuse the unit because it is rare.



- C. Extend the shelf-life of the unit by 24 hours.
 - D. Release the rare unit so it can be made available for another patient.
 - E. Document the value of the antigen-negative unit and refreeze for up to another 10 years.
-

Question 16: According to AABB *Standards*, 90% of whole-blood-derived (WBD) platelet units should contain how many platelets per unit as a minimum?

- A. 3×10^{10} .
 - B. 3×10^{11} .
 - C. 5.5×10^9 .
 - D. 5.5×10^{10} .
 - E. 5.5×10^{11} .
-

Question 17: The minimum acceptable pH of a unit of apheresis platelets at the end of the storage period is?

- A. 5.0.
 - B. 5.2.
 - C. 6.0.
 - D. 6.2.
 - E. 7.0.
-

Question 18: What is the shelf-life of a unit of platelets without agitation?

- A. 4 hours.
- B. 8 hours.
- C. 24 hours.
- D. 3 days.
- E. 5 days.



Question 19: Which of the following is associated with platelet storage?

- A. Increased H⁺ concentration.
 - B. Platelet activation.
 - C. Change in shape from discoid to spherical.
 - D. Decreased expression of glycoprotein Ib.
 - E. All of the above.
-

Question 20: Transfusion of 1 platelet concentrate unit (ie, the platelets present in one whole blood donation) into a hematologically stable adult of average size with no history of transfusion and/or pregnancy is expected to increase the platelet count by:

- A. 1000 to 5000/ μ L.
 - B. 3000 to 5000/ μ L.
 - C. 3000 to 12,000/ μ L.
 - D. 5000 to 10,000/ μ L.
 - E. 30,000 to 40,000/ μ L.
-

Question 21: For Fresh Frozen Plasma (FFP) preparation and labeling, the plasma must be separated from the RBCs and frozen within how many hours?

- A. 2 hours.
 - B. 4 hours.
 - C. 8 hours.
 - D. 12 hours.
 - E. 24 hours.
-

Question 22: Which of the following statements are true when comparing whole-blood-derived platelets (ie, pooled platelets) to apheresis platelets?

- A. Apheresis platelets decrease the donor exposure for infectious disease transmission as they are from 1 donor.



- B. There is a lower risk of transfusion-related acute lung injury (TRALI) when using whole-blood-derived platelets from male-only donors.
 - C. Apheresis platelets decrease the risk of septic transfusion reaction.
 - D. All of the above.
 - E. None of the above.
-

Question 23: In the preparation of cryoprecipitate, FFP is thawed at what temperature?

- A. 0-4 C.
 - B. 1-6 C.
 - C. 1-10 C.
 - D. 20-25 C.
 - E. 37 C.
-

Question 24: Which statement(s) is true regarding the shelf-life of FFP?

- A. 12 months at ≤ -18 C.
 - B. 24 months at ≤ -18 C.
 - C. 7 years at ≤ -65 C.
 - D. 10 years at ≤ -65 C.
 - E. A and C are both true.
-

Question 25: Which statement or statements are true regarding cryosupernatant (cryoprecipitate-reduced plasma or CRP; the fraction of plasma remaining after the removal of cryoprecipitable proteins)?

- A. CRP is equivalent to FFP.
- B. CRP can be used to manufacture albumin solutions.
- C. CRP can be used to make immunoglobulin preparations.
- D. CRP contains half the fibrinogen of FFP.
- E. B and C are true.



Question 26: Following preparation from FFP, cryoprecipitate must be frozen within what time?

- A. 30 minutes.
 - B. 1 hour.
 - C. 2 hours.
 - D. 4 hours.
 - E. 8 hours.
-

Question 27: Thawed cryoprecipitate that is pooled in an open system can be stored at 20-24 C for what time?

- A. 1 hour.
 - B. 2 hours.
 - C. 4 hours.
 - D. 6 hours.
 - E. 8 hours.
-

Question 28: According to AABB *Standards*, each bag of cryoprecipitate must contain a minimum of how many international units of Factor VIII and how many milligrams of fibrinogen?

- A. 70 IU of Factor VIII and 100 mg of fibrinogen.
 - B. 80 IU of Factor VIII and 100 mg of fibrinogen.
 - C. 80 IU of Factor VIII and 150 mg of fibrinogen.
 - D. 150 IU of Factor VIII and 100 mg of fibrinogen.
 - E. 150 IU of Factor VIII and 150 mg of fibrinogen.
-

Question 29: A single unit of thawed cryoprecipitate can be stored at 20-24 C for how long?

- A. 1 hour.
- B. 2 hours.
- C. 4 hours.
- D. 6 hours.
- E. 8 hours.



Question 30: How many bags of cryoprecipitate are needed to increase the fibrinogen level of a 65-kg male with a hematocrit of 40% from 75 mg/dL to 200 mg/dL? Assume a blood volume of 70 mL/kg.

- A. 17 bags.
 - B. 20 bags.
 - C. 23 bags.
 - D. 25 bags.
 - E. 30 bags.
-

Question 31: How are granulocytes stored that are collected for transfusion?

- A. At 1-6 C without agitation.
 - B. At 1-6 C with agitation.
 - C. At 20-24 C without agitation.
 - D. At 20-24 C with agitation.
 - E. At 37 C without agitation.
-

Question 32: What is the shelf-life of granulocytes?

- A. 8 hours.
 - B. 24 hours.
 - C. 48 hours.
 - D. 72 hours.
 - E. 5 days.
-

Question 33: For which patient would a granulocyte transfusion be most indicated?

- A. A 34-year-old male with systemic lupus erythematosus (SLE) who was just diagnosed with osteomyelitis.
- B. A 23-year-old female with aplastic anemia who has systemic histoplasmosis that was diagnosed 2 days ago.



- C. A 56-year-old male with acute myelogenous leukemia (AML) who has an absolute neutrophil count (ANC) of 340/ μ L and a temperature of 38.5 C.
 - D. A 62-year-old female with a history of AML who received an allogeneic stem cell transplant 8 days ago, has an ANC of 2/ μ L, and has *Enterobacter* growing from blood culture bottles despite being on adequate antibiotic therapy for the last 5 days.
 - E. A 67-year-old male with chronic lymphocytic leukemia (CLL) who has an ANC of 790/ μ L, *Staphylococcus aureus* growing from blood culture bottles, and was just started receiving intravenous vancomycin.
-

Question 34: Which statement is true of granulocytes?

- A. Each product must be ABO compatible with the recipient and crossmatched.
 - B. Each bag contains $\sim 3 \times 10^{11}$ platelets.
 - C. Each bag contains 20-50 mL of RBCs.
 - D. Each bag must contain $\geq 1 \times 10^{10}$ granulocytes in 75% of units tested.
 - E. All of the above are true.
-

Question 35: What is/are the most common side effect(s) of granulocyte colony-stimulating factor (G-CSF) administration?

- A. Shortness of breath.
 - B. Weight gain.
 - C. Bleeding.
 - D. Back pain and headache.
 - E. Pruritis.
-

Question 36: What is/are side effect(s) of using hydroxyethyl starch (HES) to collect granulocytes?

- A. Anaphylactoid reactions.
- B. Weight gain.



- C. Pruritis.
 - D. Decreased von Willebrand factor (vWF) levels.
 - E. All of the above.
-

Question 37: Which statement is true regarding granulocyte transfusions?

- A. Patients receiving granulocyte transfusions are not at risk for transfusion-associated GVHD.
 - B. Granulocytes should be irradiated to prevent transfusion-associated GVHD.
 - C. Granulocytes should not be irradiated as their function will decrease.
 - D. Irradiation decreases the shelf-life of granulocytes and is rarely performed.
 - E. Irradiation can prevent HLA alloimmunization.
-

Question 38: What is the shelf-life of thawed Plasma Frozen within 24 Hours After Phlebotomy (PF24) at 1-6 C?

- A. 4 hours.
 - B. 8 hours.
 - C. 12 hours.
 - D. 24 hours.
 - E. 3 days.
-

Question 39: Pathogen inactivation is effective at eliminating and reducing the risk of transmission of which pathogen(s)?

- A. Parvovirus B19.
- B. Hepatitis A.
- C. Human immunodeficiency virus.
- D. Bacterial spores.
- E. Hepatitis E.



Question 40: AABB *Standards* and FDA guidance documents permit the collection of which blood product combinations by automated apheresis?

- A. A double unit of RBCs and a double unit of platelets.
 - B. A triple unit of RBCs.
 - C. A single unit of RBCs and a double unit of platelets.
 - D. A single unit of RBCs, platelets, and plasma.
 - E. All of the above.
-

Question 41: What is the shelf-life of apheresis platelets that have been volume reduced in an open system?

- A. 2 hours.
 - B. 4 hours.
 - C. 8 hours.
 - D. 12 hours.
 - E. 24 hours.
-

Question 42: A single RBC unit that is set to expire in 37 days is split and an aliquot is placed in a syringe for a neonate. What is the expiration of the aliquoted product in the syringe?

- A. 4 hours.
 - B. 8 hours.
 - C. 24 hours.
 - D. 7 days.
 - E. 37 days.
-

Question 43: Which items below are required to extend the shelf-life of a platelet from 5 days to 7 days?

- A. Bacterial culture followed by secondary testing using a culture method or FDA-approved rapid test.
- B. A platelet storage container cleared or approved by FDA for 7-day storage.
- C. Update the label to reflect the new expiration date.



- D. A and C.
 - E. A, B, and C.
-

Question 44: What is the mechanism of action of FDA-approved pathogen-reduction technology for single-donor platelets?

- A. Amotosalen and UVA light.
 - B. Amotosalen and UVB light.
 - C. Riboflavin and UV light.
 - D. Amotosalen and visible light.
 - E. Riboflavin and visible light.
-

Question 45: According to several studies, what are differences between untreated platelets and platelets treated with pathogen-reduction technologies (PRT)?

- A. Patients treated with PRT platelets had lower corrected count increments (CCIs).
 - B. Patients treated with PRT platelets had less frequent platelet transfusions.
 - C. Patients treated with untreated platelets had more minor bleeding events.
 - D. Patients treated with PRT platelets had less higher-grade bleeding events.
 - E. Patients treated with untreated platelets had more platelet refractoriness.
-

Question 46: What is the shelf-life of Thawed Plasma?

- A. 24 hours.
- B. 3 days.
- C. 5 days.
- D. 7 days.
- E. 10 days.



Question 47: What is the minimum number of platelets that must be in a single unit of apheresis platelets according to the AABB Standards and FDA?

- A. 5.5×10^{10} .
- B. 3.0×10^{11} .
- C. 5.0×10^6 .
- D. 3.0×10^{10} .
- E. 5.5×10^{11} .



Question 48: What is the shelf-life of cryoprecipitate frozen at ≤ -18 C?

- A. 6 months.
- B. 1 year.
- C. 2 years.
- D. 3 years.
- E. 7 years.



Question 49: At what temperature must RBCs frozen in 40% glycerol be stored?

- A. ≤ -20 C.
- B. ≤ -65 C.
- C. ≤ -100 C.
- D. ≤ -120 C.
- E. ≤ -150 C.



Question 50: What is the shelf-life of RBCs that are stored in CPD with AS?

- A. 21 days.
- B. 28 days.
- C. 35 days.
- D. 42 days.
- E. 49 days.



ANSWERS

Question 1: C. 35 days.

	Anticoagulant/ Preservative		
	ACD/CPD/CP2D	CPDA-1	AS
Storage/shelf life (days)	21	35	42
Hematocrit	$\leq 80\%$	$\leq 80\%$	52 to 60%

ACD = acid-citrate-dextrose; AS = additive solution; CPD = citrate-phosphate-dextrose; CP2D = citrate-phosphate-dextrose-dextrose; CPDA-1 = citrate-phosphate-dextrose-adenine.

Explanation:

- Shelf-life of RBCs in an open system: 24 hours at 1-6 C.
- Shelf-life of frozen RBCs: 10 years.
- Volume varies according to anticoagulant/preservative with ACD, CPD, CP2D, and CPDA-1 having volumes of 250 to 300 mL and AS having volumes of 350 to 450 mL.
- Additive solution is added to the concentrated RBCs after the removal of platelet-rich plasma and provides optimal red cell preservation. The composition of additive solutions varies (see table in answer #5).



Question 2: B. 21 days.

Explanation:

See table in answer #1.



Question 3: C. AS.

**Explanation:**

- A unit of RBCs collected in AS has the lowest hematocrit of 52-60% as compared to RBCs stored in CPDA-1, CP2D, CPD, and ACD which have an approximate hematocrit of 80%.
-

Question 4: D. 24 hours.**Explanation:**

- The shelf-life of a unit of RBCs that was in an open system is 24 hours at 1-6 C.
-

Question 5: A. AS-1.

Element	AS-1	AS-3	AS-5
Dextrose (mg/mL)	2200	1100	900
Adenine (mg/mL)	27	30	30
Monobasic sodium phosphate (mg/mL)	0	276	0
Mannitol (mg/mL)	750	0	525
Sodium chloride (mg/mL)	900	410	877
Sodium citrate (mg/mL)	0	588	0
Citric acid (mg/mL)	0	42	0

Explanation:

- Additive solutions extend the expiration date to 42 days.
- Hemolysis at the end of storage should be <1% (in the United States) or <0.8% (in the European Union).

**Question 6: C.** 1-10 C.**Explanation:**

- For RBC units, the storage temperature must be at 1-6 C and for transport, the temperature must be at 1-10 C.
-

Question 7: D. A tag must be attached indicating that it has been issued once before.**Explanation:**

- Blood banks/transfusion services must have policies, processes, and procedures to ensure that the integrity of blood, blood components, derivatives, or tissue that have been returned to the blood bank/transfusion service are safe to return to inventory and potentially be issued to the same or another patient at a later time.
 - Examples of integrity checks for RBC units may include visual inspection of the unit and checking the unit for acceptable temperature (1-10 C if not issued in a cooler; 1-6 C if issued in a cooler).
 - Acceptability for return of blood, blood components, derivatives, or tissues to inventory should be documented and that documentation retained for an appropriate length of time.
-

Question 8: C. $<5.0 \times 10^6$ leukocytes per unit.**Explanation:**

- Leukocyte-reduced RBC components (LR-RBCs) must contain less than 5.0×10^6 leukocytes per unit. LR-RBCs must contain at least 85% of the original RBCs.
- The shelf-life of LR-RBCs is the same as the original RBC unit.
- Irradiation affects the proliferative activity of lymphocytes through DNA damage. Irradiation prevents GVHD. It does not provide the benefits of leukocyte reduction such as prevention of



HLA alloimmunization, avoidance of febrile reactions, and prevention of cytomegalovirus (CMV) transmission.

- Theoretically, leukocyte reduction could prevent GVHD and has done so in animal models. The minimum number of leukocytes required for the prevention of GVHD is not known. Hence, LR-RBCs cannot be used for prevention of GVHD.
 - Thawing and deglycerolization results in LR-RBCs. However, this is labor intensive and results in significant loss of the red cell component.
 - Washing does not result in significant leukocyte reduction.
-

Question 9: A. Prevention of GVHD.

Explanation:

- See answer to Question 8. Irradiation is superior to leukocyte reduction in terms of GVHD prevention.
-

Question 10: B. 28 days from now.

Explanation:

- The maximum shelf-life of irradiated RBCs is 28 days, even if the original expiration would exceed this period. If an irradiated unit's shelf-life is less than 28 days, then that remains the shelf-life of the unit following irradiation. In other words, the shelf-life of an irradiated unit is 28 days or its original shelf-life, whichever comes first.
- Irradiation is associated with unacceptable 24-hour red cell survival (<75% recovery) after 28 days. Irradiation is also associated with an increase in supernatant potassium. At 48 hours after irradiation, the supernatant potassium doubles.
- Irradiation does not affect the shelf-life of platelets.



Question 11: D. 40%.

Explanation:

- The most common percentage of glycerol used is 40%. For differences between the use of 20% and 40% glycerol, see table below.

Concentra-tion of Glycerol	Speed of Freezing	Freezing Tempera-ture	Storage Tempera-ture	Glyceroli-zation	Deglycero-lization
20% (low glycerol method)	Rapid	≤−196 °C	≤−120 °C	Complex	Simple
40% (high glycerol method)	Slow	≤−80 °C	≤−65 °C	Simple	Complex



Question 12: E. The unit was from someone with sickle trait.

Explanation:

- When thawed and deglycerolized without using additional wash fluid, frozen red cells from a donor with sickle-cell trait form a semi-solid dark gel. In some cryopreservation programs, donations are screened for hemoglobin S before freezing.
- Insufficient anticoagulation would result in the presence of clots within the unit.
- Use of hypotonic solutions for washing would result in hemolysis. Normal saline is a standard solution for deglycerolization and washing RBCs. Inadequate deglycerolization causes in-vivo or in-vitro hemolysis.
- Bacterial contamination changes the color and appearance of the unit and may cause hemolysis.
- Valuable rare RBC units can remain frozen for up to 10 years and be successfully thawed and deglycerolized.



Question 13: D. $\geq 80\%$ of RBCs.

Explanation:

- Following the deglycerolization process, $\geq 80\%$ of the red cells must be recovered. It is unknown whether glycerol may cause renal toxicity, anaphylaxis, or thrombocytopenia.
- Inadequately removed glycerol can cause in-vivo or in-vitro hemolysis. The presence of glycerol in the red cells renders them hypertonic relative to solutions with physiologic osmolalities. If the cells are placed in these solutions, there is movement of water into the cells, resulting in their rupture.
- During the process of deglycerolization, solutions of progressively lower osmolality are used to wash the red cells. This allows for the removal of the glycerol from the cells without excess hemolysis.



Question 14: C. 24 hours.

Explanation:

- A unit of RBCs that is thawed and deglycerolized in an FDA-approved closed system is good for 14 days.
- A unit that is thawed and deglycerolized in an open system is good for 24 hours.



Question 15: E. Document the value of the antigen-negative unit and refreeze for up to another 10 years.

Explanation:

- S-, s-, U- units are quite rare. If unused, this unit should be refrozen before the 24-hour expiration. After refreezing, the unit can be stored for up to 10 years but when thawed, the amount of time before expiration will be the amount of time left from the original thaw/deglycerolization process (the clock does not start over; it only pauses).



- The rare unit should not be released into general inventory because of its rarity.
- The cells could also be rejuvenated. Rejuvenation restores the adenosine triphosphate and 2,3 diphosphoglycerate (2,3 DPG) levels in the red cells. RBCs in a closed system can be rejuvenated up to 3 days after expiration. Such cells can then be frozen. Rejuvenated RBCs must be washed to remove the rejuvenation solution because the inosine in the solution is toxic.
- The patient should be encouraged to donate blood in the future (autologous or allogeneic) because of the extremely rare blood type.



Question 16: D. 5.5×10^{10} .

Explanation:

- See table below.

Product	Platelet Count	Volume	Leukocytes
Platelets	5.5×10^{10}	~50 mL	$>10^9$
Platelets Leukocytes Reduced	5.5×10^{10}	~50 mL	$<8.3 \times 10^5$
Pooled Platelets Leukocytes Reduced	Depends upon pool size	Depends upon pool size	$<5.0 \times 10^6$
Apheresis Platelets	3.0×10^{11}	~300 mL	$>10^9$
Apheresis Platelets Leukocytes Reduced	3.0×10^{11}	300 mL	$<5.0 \times 10^6$



Question 17: D. 6.2.

Explanation:

- The minimum acceptable pH of platelet units at the end of the storage period is ≥ 6.2 . A pH <6.2 could indicate inadequate gas



exchange through the bag during storage or bacterial contamination and growth. A pH <6.2 results in platelet activation and loss of function.



Question 18: C. 24 hours.

Explanation:

- The shelf-life of platelets without gentle agitation is 24 hours. Gentle agitation is necessary to allow for adequate oxygen exchange through the platelet bag. Failure to do so could result in a decrease in pH below acceptable levels and activation of the platelets.



Question 19: E. All of the above.

Explanation:

- During storage, platelets activate and the pH decreases (increased H⁺ concentration). When platelets activate, the glycoprotein Ib levels decrease due to shedding and internalization; the platelets also change from discoid to spherical in shape.
- Some facilities visually inspect platelet products for the “swirling effect.” If a platelet product is backlit, a shimmering effect can be seen as the tumbling discoid platelets reflect the light at different angles. When the platelets activate and become spheres, the light is reflected at the same angle, resulting in the loss of the swirling effect.
- It is important to note that loss of the swirling effect can be a result of anything that causes platelet activation (including pH <6.2, temperature <20 C, and age of the product) and may not always be a surrogate marker for bacterial contamination.



Question 20: D. 5000 to 10,000/ μ L.

**Explanation:**

- In a non-alloimmunized, hematologically stable adult, the transfusion of 1 unit of platelet concentrate is expected to increase the platelet count by 5000 to 10,000/ μ L. The transfusion of 1 unit of apheresis platelets or 1 unit of pooled platelets (usually a pool of six) is expected to increase the platelet count by 30,000 to 60,000/ μ L.
-

Question 21: C. 8 hours.**Explanation:**

- For the preparation of FFP, plasma must be separated from red cells and placed in the freezer within 8 hours of whole blood collection, or within the time frame specified in the manufacturer's directions for use for the blood collection system.
-

Question 22: D. All of the above.**Explanation:**

- Because apheresis platelets are derived from a single donor, the provision of apheresis platelets reduces donor exposure in comparison to pooled platelet concentrates, thereby reducing the risk of disease transmission.
- Male donors are less likely to have HLA or granulocyte antibodies. If an HLA or granulocyte antibody is present in a donor, pooling the donation will reduce the concentration of the antibody. This may result in a greater reduction of the risk of TRALI as compared to using an apheresis unit from one donor.
- The incidence of bacterial contamination in pooled platelet units derived from six whole blood donations has been found to be five times greater than that of apheresis platelets. This is because six venipunctures are performed with the pooled component, with the possibility of contamination with skin flora from each. Only one venipuncture is needed for apheresis components.

**Question 23: B. 1-6 C.****Explanation:**

- To prepare cryoprecipitate, FFP is thawed at 1-6 C.
-

Question 24: E. A and C are both true.**Explanation:**

- FFP characteristics:
 - Volume: 200-250 mL/unit.
 - Contents: all coagulation factors at physiologic concentrations.
 - Shelf-life: 12 months at ≤ -18 C or 7 years at ≤ -65 C.
 - Transport temperature: maintain in the frozen state.
 - Thawing temperature: 30-37 C.
 - Storage/shelf-life after thawing: 24 hours at 1-6 C (preserves Factors V and VIII levels).
 - Transport temperature after thawing: 1-10 C.
 - Dose: 10-15 mL/kg with further therapy guided by clinical response and prothrombin time (PT)/activated partial thromboplastin time (aPTT) measurements.

**Question 25: E. B and C are true.****Explanation:**

- CRP is not the therapeutic equivalent of FFP because it lacks certain coagulation factors.
- CRP is routinely used as a raw material in manufacturing the following:
 - Albumin solutions.
 - Immunoglobulin preparations.
 - Non-Factor VIII coagulation factor concentrates.
- CRP contains approximately half of the fibrinogen, Factor VIII, and fibronectin present in FFP. CRP is depleted of the largest multimers of vWF. CRP is *not* deficient in ADAMTS13, a vWF-cleaving protease, the deficiency of which has been implicated in throm-



botic thrombocytopenic purpura (TTP). Refractory TTP is the only FDA-approved indication for CRP.



Question 26: B. 1 hour.

Explanation:

- Cryoprecipitate is prepared by thawing FFP at 1-6 C. Cryoprecipitate must be frozen within 1 hour.



Question 27: C. 4 hours.

Explanation:

Component	Storage	Transport	Expiration
Cryoprecipitated AHF	≤–18 C	Maintenance of the frozen state	1 year
Cryoprecipitated AHF thawed at 37 C	20 to 24 C	20 to 24 C	Pooled open system: 4 hours Pooled closed system: 6 hours Single thawed unit: 6 hours



Question 28: C. 80 IU of Factor VIII and 150 mg of fibrinogen.

Explanation:

Element	Quantity in 1 Bag of Cryoprecipitate
Fibrinogen	>150 mg
von Willebrand factor	~50% present in the original unit
Factor VIII	≥80 units
Factor XIII	~20% to 30% present in the original unit



Question 29: D. 6 hours.

Explanation:

- See table in answer #27.



Question 30: C. 23 bags.

Explanation:

- The calculation to determine the dose of cryoprecipitate to reach a desired fibrinogen level is as follows:
 - Weight (kg) × 70 mL/kg = blood volume (mL)
 $65 \times 70 = 4550 \text{ mL}$
 - Blood volume (mL) × (1.0 – hematocrit) = plasma volume (mL)
 $4550 \times (1 - 0.4) = 2730 \text{ mL}$
 - (Desired fibrinogen level in mg/dL – initial level) × plasma volume/100 mg/dL = mg of fibrinogen needed
 $(200 - 75) \times 2730 / 100 = 3413$
 - mg fibrinogen needed/150 mg fibrinogen per bag = number of bags required
 $3413 / 150 = 22.75$



Question 31: C. At 20-24 C without agitation.

Explanation:

- Granulocytes are stored at 20-24 C without agitation. Agitation results in granulocyte activation and degranulation. Storage below room temperature results in loss of function.



Question 32: B. 24 hours.

Explanation:

- The shelf-life of granulocytes according to the AABB *Standards* is 24 hours. The bactericidal function of granulocytes rapidly



decreases after granulocyte collection. Granulocytes should be transfused as soon as possible.

Question 33: D. A 62-year-old female with a history of AML who received an allogeneic stem cell transplant 8 days ago, has an ANC of $2/\mu\text{L}$, and has *Enterobacter* growing from blood culture bottles despite receiving adequate antibiotic therapy for the last 5 days.

Explanation:

- Indications for granulocyte transfusions in adult patients:
 - Infection unresponsive to appropriate antimicrobial therapy.
 - Transient (reversible) marrow depression.
 - Absolute neutropenia ($<500/\mu\text{L}$).
- Indications for granulocyte transfusion in neonatal and pediatric patients:
 - Bacterial sepsis.
 - Transient (reversible) marrow depression.
 - Neutropenia with absolute neutrophil count of $<3000/\mu\text{L}$ and decreased marrow neutrophil stores as indicated by $<7\%$ of marrow nucleated cells being metamyelocytes or more mature forms.
- Controversial indications:
 - Systemic fungal infection.
 - Prophylaxis during cancer chemotherapy.



Question 34: E. All of the above are true.

Explanation:

- Characteristics of granulocytes are given in the table below. ABO compatibility and crossmatching are required because the red cell content is $>2 \text{ mL}$. Microaggregate and/or leukocyte reduction filters are not used during granulocyte transfusion.



Characteristic	Quantity
Granulocyte content	$\geq 1 \times 10^{10}$ in 75% units tested
Red cell content	20-50 mL (hematocrit 10%)
Platelet content	3×10^{11} (equivalent to 1 plateletpheresis unit)
Total volume	250-300 mL

**Question 35: D. Back pain and headache.****Explanation:**

- Back pain and headache are common side effects of G-CSF administration. They are thought to result from stretching of pain receptors in the marrow space by the active growth and proliferation of the neutrophils and their precursors. G-CSF may also be associated with nausea.
- Citrate is used as an anticoagulant for granulocyte collections. It anticoagulates blood by chelating calcium so it is not available to participate in the coagulation cascade. Hypocalcemia is a manifestation of citrate toxicity. The effects of hypocalcemia range from minor circumoral paresthesias and muscle tremor to more severe complications such as tetany and cardiac effects.
- Weight gain occurs with HES. As a slowly excreted colloid, it can expand the donor's intravascular volume. It also can leave the intravascular space, resulting in tissue build-up with the movement of water into the third space. Expanded circulatory volume may also cause headaches. Additional reactions to HES include:
 - Anaphylactoid reactions caused by the generation of complement fragments by the alternate complement pathway.
 - Intractable pruritis caused by skin deposition.
 - Prolongation of the aPTT as well as decreases in fibrinogen levels. This is thought to result from the dilutional effects from volume expansion, as well as decreases in Factor VIII activity and Factor VIII antigen levels. HES increases vWF antigen turnover, resulting in decreased antigen levels. As Factor VIII is stabilized by vWF, Factor VIII levels decline as well. Bleeding time may also be prolonged because of decreased vWF.
 - These dose-dependent risks are usually not of concern in the setting of granulocyte collection where exposure is limited.



- Corticosteroid administration can also complicate underlying diseases in the donor, such as hypertension, diabetes mellitus, and peptic ulcer disease.
-

Question 36: E. All of the above.

Explanation:

- See answer #34.
-

Question 37: B. Granulocytes should be irradiated to prevent transfusion-associated GVHD.

Explanation:

- Complications of granulocyte transfusion include:
 - Acute pulmonary insufficiency and distress.
 - Transfusion-associated GVHD.
 - HLA alloimmunization.
 - Irradiation prevents transfusion-associated GVHD, which has a high mortality rate (approximately 95%). Irradiation prevents replication of DNA in lymphocytes in the component and thereby prevents lymphocyte proliferation.
 - Patients receiving granulocytes are usually profoundly immunosuppressed and at risk of GVHD. Numerous cases of transfusion-associated GVHD caused by granulocytes have been reported and, therefore, all granulocyte products should be irradiated.
 - Irradiation does not damage granulocytes in bactericidal function and, therefore, is not contraindicated.
 - Irradiation has no effect on the expiration time of granulocytes. They should not be stored for more than 24 hours and should be transfused as soon as possible after preparation.
 - Irradiation does not inhibit CMV and does not prevent HLA alloimmunization.
-

Question 38: D. 24 hours.

**Explanation:**

- Once thawed, PF24 has a shelf-life of 24 hours at 1-6 C. Because there is up to a 24-hour delay in separating the plasma from the red cells in a unit of whole blood from which PF24 is destined to be made, there is a concern that levels of the heat-labile coagulation Factors V and VIII, could be decreased significantly.
 - Most studies have found that the level of Factor V in PF24 is normal (100%), while the level of Factor VIII is decreased significantly to about 70%. Fortunately, 70% of any individual coagulation factor is sufficient to provide for adequate hemostasis.
-

Question 39: C. HIV.**Explanation:**

- Pathogens for which inactivation is highly effective include gram-negative and gram-positive bacteria, and viruses including HIV, hepatitis B and C, and West Nile virus.
 - Pathogen inactivation is not as effective for non-enveloped viruses such as human parvovirus B19, hepatitis A, and hepatitis E and is ineffective against bacterial spores.
 - An additional benefit of pathogen-inactivation technology is the inactivation of T cells, which reduces the risk of transfusion-associated GVHD.
 - In December 2014, the FDA approved the first pathogen-inactivation system in the United States. Pathogen inactivation is now widely available and used within the United States and other countries.
-

Question 40: D. A single unit of RBCs, platelets, and plasma.**Explanation:**

- Both AABB *Standards* and FDA guidance documents address the removal of red cells by automated apheresis methods and



include collection protocols for the following product combinations:

- A single unit of RBCs and plasma.
 - A single unit of RBCs and platelets.
 - A single unit of RBCs, platelets, and plasma.
 - A double unit of RBCs only.
-

Question 41: B. 4 hours.

Explanation:

- Decreasing the supernatant substances of platelets (whole-blood-derived or apheresis platelets) is occasionally required for two clinical reasons:
 - To reduce the total transfusion volume to minimize the possibility of transfusion-associated circulatory overload (TACO).
 - To partially remove incompatible ABO alloantibodies (to minimize the risk of hemolysis).
 - Volume reduction using an open procedure reduces the expiration date of the platelets to 4 hours. Volume reduction using a closed procedure (with a sterile connecting device) does not change the original expiration date of the platelet product.
 - Regardless of whether an open or closed procedure is utilized, volume-reduced platelets should be transfused as soon as possible because of the minimal amount of plasma remaining in the component.
 - Volume-reduced platelets, like any platelet product, are stored at 20-24 C.
-

Question 42: A. 4 hours.

Explanation:

- Certain transfusion recipients (neonates, most commonly) require very low volume transfusions (eg, 10-30 mL). This makes aliquotting from a “mother” unit a desirable practice. If the aliquotting procedure is performed in a closed manner, using a sterile con-



necting device, the “mother” unit retains its original expiration date.

- The expiration date of the aliquoted unit is dependent on the storage container that is utilized:
 - Cellular components stored in syringes have an expiration of 4 hours.
 - Cellular components stored in an FDA-approved transfer bag have the same expiration as the “mother” unit.
- Units of blood (RBCs, plasma, apheresis platelets) in primary blood containers are also sometimes split into smaller transfer bags for larger recipients to minimize the risk of TACO. Platelets should always be stored at 20-24 C to maximize their effectiveness.
- Aliquoting multiple times from the same primary container may be appropriate to minimize waste and reduce donor exposure for the recipient. Facilities should have policies, processes, and procedures in place that define the minimum acceptable volume of the primary container for which the component may still be transfused as a “whole” unit.



Question 43: E, A, B, and C.

Explanation:

- In October 2021, the FDA required implementation of “bacterial risk control strategies” to mitigate the risk of septic transfusion reactions caused by bacterial contamination of platelets stored at room temperature. Options for compliance and to extend platelet shelf-life to day 6 or 7 are below.
 - Large-volume delayed sampling (LVDS) at ≥ 36 (5-day expiration) or LVDS at ≥ 48 hours (7-day expiration) or primary culture at ≥ 24 (to support transfusion through day 3).
 - Primary culture at ≥ 24 hours + Secondary testing with culture or Rapid Bacterial Testing (RT) (5-, 6-, and 7-day expiration).
 - LVDS at ≥ 36 + Secondary testing with culture or RT (6- and 7-day expiration).
 - Pathogen reduction technology (PRT) (5-day expiration).
- In addition to requirements to support a 5-day expiration, extending the expiration from day 5 to day 7:



- Requires culture followed by secondary testing using a culture method or rapid test that is an FDA-approved “safety measure.”
- Requires a platelet storage container cleared or approved by FDA for 7-day storage.
- The blood center or transfusion service that performs the secondary testing must update the container label to reflect the new expiration date as required by 21 CFR 606.121(c)(4)(i). Extending expiration beyond 5 days is a manufacturing procedure requiring FDA registration and blood product listing, as defined in 21 CFR 607.3(d).



Question 44: A. Amotosalen and UVA light.

Explanation:

- The only FDA-approved pathogen-reduction technologies for apheresis platelets stored in plasma and platelet additive solution (PAS) uses amotosalen (a psoralen) and UVA light to cross-link nucleic acids irreversibly. This inactivates a broad spectrum of viruses, bacteria, and parasites, as well as white cells that can cause transfusion-associated graft-vs-host disease (TA-GVHD).
- Riboflavin and UV light is another PRT but it is not yet FDA approved for use in apheresis platelets.



Question 45: A. Patients treated with PRT platelets had lower CCIs.

Explanation:

- Large randomized clinical trials have shown lower corrected count increments, decreased transfusion intervals, more platelet refractoriness, and/or more bleeding events with PRT platelets.



Question 46: C. 5 days.

**Explanation:**

- The two most common plasma products used in the United States are Fresh Frozen Plasma (FFP) and Plasma Frozen within 24 Hours of After Phlebotomy (PF24).
 - When either FFP or PF24 is thawed for transfusion, it must be stored at 1-6 C. According to the FDA, these two products only have a 24-hour shelf-life after thawing.
 - After 24 hours, these products can be relabeled as Thawed Plasma and stored at 1-6 C for up to 5 days.
-

Question 47: B. 3.0×10^{11} .**Explanation:**

- See table in answer #16.
-

Question 48: B. 1 year.**Explanation:**

- See table in answer #27.
-

Question 49: B. ≤ -65 C.**Explanation:**

- See table in answer #11.
-

Question 50: D. 42 days.**Explanation:**

- See table in answer #1.



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4

Carbohydrate Blood Group Antigens

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Key Points from the *Technical Manual*

- The antigens of the ABO, H, LE, I, P1PK, GLOB, FORS, and SID blood systems are defined by carbohydrate epitopes on glycoproteins and glycosphingolipids.
- The H antigen is the precursor to both the A and B antigens and is expressed on all red cells except in the rare Bombay phenotype.
- An inverse reciprocal relationship exists between the presence of group A and B antigens on red cells and the presence of anti-A, anti-B, or both, in sera.
- ABO grouping requires both antigen typing of red cells for A and B antigens (forward grouping) and typing of serum or plasma for the presence of anti-A and anti-B iso agglutinins (reverse grouping).
- ABO discrepancies occur when forward grouping (red cells) and reverse grouping (serum/plasma) do not agree.
- Discrepancies can be resolved with additional testing methods to enhance missing reactivity or eliminate spurious reactivity.



QUESTIONS

Question 1: Select the answer choice that consists exclusively of carbohydrate blood group antigens.

- A. B, I, K, P.
 - B. A, S, Le^a, Sd^a.
 - C. O, i, Le^b, Fy^a.
 - D. B, Le^b, I, P.
 - E. K, P, i, A.
-

Question 2: The H antigen is characterized by which terminal sugar moiety?

- A. Galactose.
 - B. N-acetylglucosamine.
 - C. Fucose.
 - D. N-acetylgalactosamine.
 - E. Sucrose.
-

Question 3: At what time does ABO expression reach adulthood levels?

- A. 5-6 weeks of gestation.
 - B. 3-6 months.
 - C. 1 year.
 - D. 2-4 years.
 - E. 5-10 years.
-

Question 4: On which chromosome does the ABO gene reside?

- A. 9q.
- B. 4q.
- C. 19p.
- D. 19q.
- E. 6p.



Question 5: Compared to individuals of European ancestry, those of African ancestry have lower prevalence of which ABO blood group?

- A. O.
 - B. A.
 - C. B.
 - D. AB.
 - E. H.
-

Question 6: A donor center in western United States reports that a large majority of its donor population is of Asian ancestry. Given this donor demographic, the blood center would be expected to collect slightly more blood from which blood groups compared to donor centers collecting predominantly from donors of European or African ancestry?

- A. Groups O and A.
 - B. Groups A and B.
 - C. Groups B and AB.
 - D. Groups AB and O.
 - E. Groups A and AB.
-

Question 7: In the table below, forward typing (red cell grouping) and reverse typing (serum grouping) demonstrate the expected reactivities for which ABO blood group?

Forward Type		Reverse Type	
Anti-A	Anti-B	A1 Cells	B Cells
0	4+	4+	0

- A. Group O.
- B. Group A.
- C. Group B.
- D. Group AB.
- E. Group H.



Question 8: ABO antibodies are unique among antibodies targeting carbohydrate antigens in that they are:

- A. IgM antibodies.
 - B. Naturally occurring.
 - C. Clinically insignificant.
 - D. Associated with hemolytic disease of the fetus and newborn.
 - E. Detected on indirect antibody screen.
-

Question 9: In the setting for Red Blood Cell (RBC) transfusion, which of the following recipient-donor pairs would be expected to be compatible?

Answer Choice	Recipient ABO Group	Donor ABO Group
A.	O	A
B.	O	B
C.	AB	A
D.	A	B
E.	B	AB

Question 10: In the setting of plasma transfusion, which of the following recipient-donor pairs would be expected to be compatible?

Answer Choice	Recipient ABO Group	Donor ABO Group
A.	O	A
B.	A	B
C.	AB	A
D.	A	O
E.	B	O



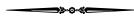
Question 11: In the setting of platelet (suspended in plasma) transfusions, which of the following ABO recipient-donor pairs would be most worrisome for hemolysis?

Answer Choice	Recipient ABO Group	Donor ABO Group
A.	O	A
B.	B	A
C.	AB	A
D.	A	O
E.	B	O



Question 12: In the setting of cryoprecipitate transfusion, which of the following is true regarding ABO compatibility?

- A. Must be ABO identical.
- B. A type and crossmatch must be performed before release.
- C. A type and screen must be performed before release.
- D. There must be compatibility in the minor crossmatch direction (no donor antibodies directed against recipient).
- E. There are no ABO requirements (recipients can receive cryoprecipitate from any donor regardless of ABO group).



Question 13: For which type of transplantation is ABO incompatibility not a contraindication?

- A. Heart.
- B. Kidney.
- C. Lung.
- D. Pancreas.
- E. Stem cell.



Question 14: A laboratory staffer grabs the typing reagent with a yellow label on the work bench. What antibody does this reagent contain?



- A. Anti-D.
 - B. Anti-A.
 - C. Anti-B.
 - D. Anti-AB.
 - E. Anti-O.
-

Question 15: In the mating of a blood group B mother and a blood group AB father, which of the following genotypes could be excluded as a possible offspring?

- A. A/O.
 - B. B/O.
 - C. B/B.
 - D. A/A.
 - E. A/B.
-

Question 16: Which of the following reagents could be used to resolve the ABO discrepancy shown below?

	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
Patient result	4+	0	1+	4+

- A. *Ulex europaeus*.
 - B. *Dolichos biflorus*.
 - C. *Arachis hypogaea*.
 - D. *Bandeiraea simplicifolia*.
 - E. *Lotus tetragonolobus*.
-

Question 17: Select the answer choice in which the neutralizing substance is correctly paired with the antibody it neutralizes.



Answer Choice	Neutralizing Substance	Antibody
A.	Plasma	Sd ^a
B.	Guinea pig urine	Chido
C.	Saliva	I
D.	Hydatid cyst fluid	P1
E.	Breast milk	Rodgers



Question 18: In which clinical setting could you see the following ABO discrepancy?

	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
Patient Result	4+	1+	0	4+

- A. Gastrointestinal infection.
- B. Mycoplasma pneumonia.
- C. Hematologic malignancy.
- D. Intravenous immunoglobulin infusion.
- E. Refractory migraines.



Question 19: The predominant isohemagglutinin(s) in group A and B serum/sera is/are:

- A. IgM anti-A,B.
- B. IgM anti-A in group B serum and anti-B in group A serum.
- C. IgM anti-H.
- D. IgG anti-A,B.
- E. IgG anti-A in group B serum and anti-B in group A serum.



Question 20: Reverse typing (serum grouping) is not required in which of the two situations below?

- A. Donor and recipient typing.



- B. Confirmatory typing of donor units and infants younger than 1 year of age.
 - C. Confirmatory typing of donor units and infants younger than 4 months of age.
 - D. Infants younger than 1 year of age and donor typing.
 - E. Infants younger than 1 year of age and autologous blood donors.
-

Question 21: Which of the following transfusion scenarios could account for the forward and reverse typing results shown below?

	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
Patient Result	3+ MF	2+ MF	0	4+

- A. Transfusion of a group B recipient with group O RBCs.
 - B. Transfusion of a group A recipient with group B RBCs.
 - C. Transfusion of a group A recipient with group O RBCs.
 - D. Transfusion of a group B recipient with group A RBCs.
 - E. Transfusion of a group O recipient with group A RBCs.
-

Question 22: Which patient's typing results may display the pattern of reactivity shown below?

	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
Patient Result	0	4+	WP	0

- A. 40-year-old male with diabetes.
- B. 28-year-old female with hepatitis C.
- C. 85-year-old male with coronary artery disease.
- D. 3-year-old child with a hernia.
- E. 26-year-old female after an ABO-mismatched hematopoietic stem cell transplantation (group O recipient, group A donor).



Question 23: Given its reactivity with the H antigen, *Ulex europaeus* can provide approximation of the amount of H antigen on red cells. What would be the expected order of blood group reactivity from highest to lowest?

- A. O>A₂>B>A₁>A₁B.
 - B. O> A₁>A₂>A₁B>B.
 - C. O>B>A₂>A₁B>A₁.
 - D. O>A₁>A₁B>B>A₂.
 - E. O>A₁>A₁B>A₂B>B.
-

Question 24: A 65-year-old male recently diagnosed with myelodysplastic syndrome was scheduled to undergo an ABO-compatible stem cell transplant procedure (group O recipient and donor). Although the major crossmatch was negative (donor cells against recipient plasma), the minor crossmatch was incompatible (donor plasma against recipient cells). However, donor indirect antibody screen was negative. Surrogate crossmatches with plasma from random donors were all incompatible with the recipient cells. Which of the following is likely responsible for the patient's polyagglutination?

- A. T polyagglutination.
 - B. Sd(a++) polyagglutination.
 - C. HEMPAS polyagglutination.
 - D. Hyde Park polyagglutination.
 - E. Tn polyagglutination.
-

Question 25: The H antigen, the precursor to A and B antigens, can be synthesized by two different fucosyltransferase (FUT) enzymes, encoded by two different FUT genes. Pick the correct pairing of the FUT gene with the H chain type synthesized by its gene product.

- A. *FUT1* (H gene) with Type 1 chain H antigen in secretions.
- B. *FUT2* (secretor gene) with Type 1 chain H antigen on red cells.
- C. *FUT1* (H gene) with Type 2 chain H antigen on red cells.
- D. *FUT2* (secretor gene) with Type 2 chain H antigen in secretions.
- E. *FUT 3* (secretor gene) with Type 3 chain H antigen in secretions.



Question 26: D-galactose is the terminal sugar that defines which blood group?

- A. A.
- B. B.
- C. C.
- D. O.
- E. Le^a.



Question 27: A nonfunctional FUT2 fucosyltransferase, but functional FUT1 fucosyltransferase and N-acetylgalactosamine transferase would result in which of the following phenotypes?

- A. Group A expressed on red cells but not in secretions.
- B. Group A expressed in secretions but not on red cells.
- C. Group A not expressed on red cells nor in secretions.
- D. Group A expressed on red cells and in secretions.
- E. Group A expressed on red cells and H antigen expressed in secretions.



Question 28: A patient with the following ABO testing results was found to be crossmatch incompatible with all cells tested, including O-negative red cells. What are the most likely phenotype and genotype?

Blood Group	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
0	0	0	4+	4+

- A. O; *Hh, Sese*.
- B. Para-bombay; *hh, sese*.
- C. Para-bombay; *Hh, Sese*.
- D. Bombay; *hh, sese*.
- E. Bombay; *hh, Sese*.



Question 29: Autoantibodies to the H antigen are characterized by which of the following?

- A. IgG antibodies reactive at room temperature that are most commonly found in individuals with Bombay or para-Bombay phenotype.
 - B. IgM antibodies reactive at broad thermal range (4-37 C) that are most commonly found in individuals with Bombay or para-Bombay phenotype.
 - C. IgM antibodies reactive at room temperature that are most commonly found in group O individuals.
 - D. IgG antibodies reactive at room temperature that are most commonly found in group A₁ individuals.
 - E. IgM antibodies reactive at room temperature that are most commonly found in group A₁ individuals.
-

Question 30: Which explanation best accounts for the ABO discrepancy shown below?

	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
Patient Result	1+	4+	4+	0

- A. Autosomal dominant phenotype in which the A glycosyltransferase has increased capacity to incorporate UDP-D-galactose.
- B. Autosomal recessive phenotype in which B glycosyltransferase has increased capacity to incorporate UDP-D-galactose.
- C. Autosomal dominant phenotype in which A glycosyltransferase has increased capacity to incorporate to incorporate UDP-N-acetylgalactosamine.
- D. Autosomal dominant phenotype in which the B glycosyltransferase has increased capacity to incorporate UDP-N-acetylgalactosamine.
- E. Autosomal recessive phenotype in which the B glycosyltransferase has increased capacity to incorporate UDP-N-acetylgalactosamine.



Question 31: Transient decreases in red cell expression of which blood group can be seen during pregnancy?

- A. ABO.
 - B. Lewis.
 - C. Rh.
 - D. P.
 - E. Lutheran.
-

Question 32: Which of the following statements is correct concerning the synthesis of Lewis antigens?

- A. Expression of the Le^a and Le^b antigen is dependent on the fucosyltransferases encoded by the H (FUT1) and secretor (FUT2) genes.
 - B. Lewis enzyme fucosylates Type 2 chains.
 - C. Lewis enzyme will fucosylate the penultimate N-acetylglucosamine of the precursor Type 1 chain to form the Le^a antigen.
 - D. Lewis enzyme transfers a second fucose to the terminal galactose of the H antigen to form the Le^b antigen.
 - E. In general, the Le^b antigen is generated directly from the Le^a antigen.
-

Question 33: What would be the Lewis and Secretor genotype of an individual with the Le(a+b-) phenotype?

Answer Choice	Lewis Genotype	Secretor Genotype
A.	<i>lele</i>	<i>sese</i>
B.	<i>lele</i>	<i>SeSe</i> or <i>Sese</i>
C.	<i>LeLe</i> or <i>Lele</i>	<i>sese</i>
D.	<i>LeLe</i> or <i>Lele</i>	<i>SeSe</i> or <i>Sese</i>
E.	<i>LeLe</i> or <i>Lele</i>	<i>Se^wSe^w</i>



Question 34: Pretransfusion testing for a preoperative patient reveals the presence of anti-Le^a and anti-Le^b antibodies. Which of the following is a possible genotype for this patient?

- A. *lele Sese.*
 - B. *LeLe Sese.*
 - C. *LeLe sese.*
 - D. *Lele Sese.*
 - E. *Lele sese.*
-

Question 35: Fill in the blanks: The _____ Lewis phenotype can be transiently seen in children as the _____ enzyme activity is still developing.

- A. Le(a+b+); FUT2.
 - B. Le(a+b+); FUT3.
 - C. Le(a-b-); FUT2.
 - D. Le(a-b-); FUT3.
 - E. Le(a-b+); FUT1.
-

Question 36: Le^{bH} antibodies would react most strongly with red cells bearing which phenotype?

- A. Le^a, group A1 cells.
 - B. Le^b, group B cells.
 - C. Le^a, group O cells.
 - D. Le^b, group O cells.
 - E. Le^c, group A1 cells.
-

Question 37: The Le^b antigen is a known receptor for what pathogen?

- A. *Plasmodium vivax.*
- B. *Plasmodium falciparum.*
- C. *Parvovirus B19.*
- D. *Enterobacteriaceae.*
- E. *Norovirus.*



Question 38: Which of the following statements is true concerning the I and i antigens?

- A. I is a nonbranched structure that is present on adult cells.
 - B. Children develop the I+ phenotype by the time they are 10 years old.
 - C. Autosomal recessive mutations in the FUT1 gene can result in the i_{adult} phenotype (I-i+).
 - D. I and i can serve as the precursors to ABO antigens and other type 1 chain antigens.
 - E. Congenital cataracts can be associated with i_{adult} phenotype.
-

Question 39: Select the scenario in which the reaction temperature and cell type are correctly paired with the expected anti-I and anti-i reactivity.

Answer Choice	Temperature	Cell Type	Anti-I	Anti-i
A.	4 C	Adult	0	4+
B.	22 C	Adult	1-2+	0
C.	4 C	Cord	4+	0
D.	22 C	Cord	4+	0
E.	37 C	Adult	4+	4+

Question 40: How would you expect an anti-IH to react when crossmatched with donors based on ABO blood group?

- A. Positive with group A₁ and O donors.
- B. Positive with group A₁ donors but negative with group O donors.
- C. Negative with group A₁ and O donors.
- D. Negative with group A₁ donors but positive with group O donors.
- E. Positive with group A₁, B, AB, and O donors.



Question 41: A 20-year-old female presents to the emergency department with a 1- to 2-week history of fatigue, fever, chills, and cough. She has noted a progressive shortness of breath; and this morning she noted dark-colored urine. On visual inspection, she is notably pale and has labored breathing. Auscultation reveals crackling/rumbling sounds bilaterally with inhalation. A chest x-ray shows peribronchial and perivasculär interstitial infiltrates. Laboratory values are significant for:

- Hemoglobin: 8.0 g/dL
- Direct antiglobulin test: 4+
- Bilirubin: 2.9 mg/dL
- Haptoglobin: 5 mg/dL
- Lactose dehydrogenase: 700 units/L

Which of the following antibodies and pathogens could be responsible for her signs and symptoms:

- A. Autoanti-i and Epstein-Barr virus (EBV).
 - B. Alloanti-I and EBV.
 - C. Autoanti-I and *Mycoplasma pneumoniae*.
 - D. Alloanti-I and *Mycoplasma pneumoniae*.
 - E. Autoanti-i and *Mycoplasma pneumoniae*.
-

Question 42: Select the answer choice in which the phenotype and corresponding antigen expression are correct.

- A. P₂ phenotype – cells express P, p, and P₁.
 - B. P₁ phenotype – cells express P₁ and P but not P^K.
 - C. p phenotype – cells express p not P₁ or P^K.
 - D. P₁^K phenotype – cells express P₁, P, and P^K.
 - E. P phenotype – cells express P, P₁, but not P^K.
-

Question 43: Of the P1PK and GLOB group phenotypes, which has the highest prevalence in the populations of European and African ancestries?

- A. P₁.



- B. P₂.
- C. p.
- D. P₁^K.
- E. P₂^k.



Question 44: The p phenotype is thought to be due to inactivating mutations in this gene product:

- A. Beta 1,3 N-acetylgalactosaminyl transferase 1.
- B. Alpha 1,4 galactosyltransferase 1.
- C. Beta 1,6 N-acetylgalactosaminyl transferase.
- D. Alpha 1,3/4 fucosyltransferase.
- E. Group A glycosyltransferase.



Question 45: Which of the following is true concerning the P1 antibody?

- A. It is an anti-IgG that reacts at body temperature.
- B. Usually found in the plasma of patients with P1 phenotype.
- C. Its reactivity can be inhibited by guinea pig urine.
- D. Generally, it is not necessary to honor anti-P1 with provision of P1-negative cells.
- E. Anti-P1 activity is diminished when tested against enzyme-treated red cells.



Question 46: An otherwise healthy 29-year-old female is being seen in the infertility clinic due to a history of multiple early-term spontaneous abortions. Which of the following antibody(ies) could fit this clinical presentation?

- A. Anti-p.
- B. Anti-P.
- C. Anti-PK.
- D. Anti-P1.
- E. Anti-PP1Pk.



Question 47: A 4-year-old patient, who was recently sick with an upper respiratory virus, is found to have evidence of intravascular hemolysis on his hematology lab test results and peripheral blood smear. Given his presentation, paroxysmal cold hemoglobinuria is suspected, and a Donath-Landsteiner test is ordered. Given the phenotype of the cells tested (see table below), which answer choice shows the correct reaction patterns that would be expected when incubating with the patient's plasma?

Answer Choice	Cell Phenotype	Incubation at 4 C	Incubation at 37 C	Incubation at 4 C Followed by Incubation at 37 C
A.	Patient's own cells	No hemolysis	No hemolysis	No hemolysis
B.	P ₂	No hemolysis	Hemolysis	Hemolysis
C.	p	No hemolysis	No hemolysis	Hemolysis
D.	P ₂ ^k	No hemolysis	No hemolysis	No hemolysis
E.	P ₁	Hemolysis	No hemolysis	Hemolysis



Question 48: The _____ antigen is the receptor for Shiga toxins seen in shigella dysentery.

- A. P^k.
- B. P.
- C. P₁.
- D. P.
- E. LKE.



Question 49: A sample from a 36-year-old female patient is pan-reactive with all cells on the indirect antibody screen as well as on the subsequent panel, but the auto-control is negative. An antibody to a high-prevalence antigen is suspected. Pretreatment of panel cells with proteolytic enzymes and reducing agents do not have any effect on the reactivity. However, when guinea pig urine is added to



the plasma, all reactivity is greatly reduced or eliminated. Which of the following antibodies would exhibit this reactivity pattern?

- A. Anti-AnWj.
- B. Anti-Sd^a.
- C. Anti-L^b.
- D. Anti-L^a.
- E. Anti-P.



Question 50: A routine type and screen was ordered and the following typing discrepancy was noted.

Testing Reagent	Forward Typing		Reverse Typing	
	Poly Anti-A	Poly Anti-B	A Cells	B Cells
Patient Result	1+	0	4+	3+

Typing was repeated and results were the same as in the initial testing. No reactivity was seen when A1 lectin was used on the patient's red cells. Genotyping revealed two copies of common group O alleles. Which blood group could account for these results?

- A. Group A subgroup.
- B. Sd^a.
- C. FORS.
- D. I.
- E. P1PK.

ANSWERS

Question 1: D. B, Le^b, I, P.

**Explanation:**

- The following table lists common carbohydrate and protein blood group antigens.

Carbohydrate Blood Group Antigens	Protein Blood Group Antigens
ABO	Rh (D, C, c, E, e)
Le ^a and Le ^b	K and k
I and i	Jk ^a and Jk ^b
P	Fy ^a and Fy ^b
Sd ^a	S and s

**Question 2: C. Fucose.****Explanation:**

- The H antigen is the requisite biosynthetic precursor for the A and B antigens. Glycosyltransferases encoded by the A and B genes add different sugars to the H antigen's subterminal galactose. It is characterized by a terminal fucose.
 - Galactose is the terminal sugar that characterizes the B antigen.
 - N-acetylglucosamine is a sugar that is central to the H antigen and is in linkage with the subterminal galactose.
 - N-acetylgalactosamine is the terminal sugar that characterizes the A antigen.
 - The absence of galactose and N-acetylgalactosamine characterizes group O red cells, which express only the H antigen.

**Question 3: D. 2-4 years.****Explanation:**

- ABO expression can be detected starting at 5-6 weeks of gestation and expression continues to increase until adult levels are reached by the age of 2-4 years.



- ABO antibodies generally begin developing at 3-6 months of age with most children displaying titers by the age of 1. Adult level titers are generally reached by ages 5-10.
-

Question 4: A. 9q.**Explanation:**

- The ABO gene resides at 9q34.2 and encodes for the glycosyltransferases responsible for the addition of the respective terminal sugar moieties. The gene consists of 7 exons (18 kb) with the protein's open reading frame mostly in exons 6 and 7. A and B alleles are autosomal codominant, whereas the O phenotype is an autosomal recessive trait, resulting from the inheritance of two ABO genes encoding nonfunctional glycosyltransferases.
 - 4q31.21 is the location for the MNS genes (*GYPB*, *GYPB*, *GYPE*) which encode glycophorin A and glycophorin B, where the M, N, S, s, and U antigens reside.
 - 19p13.3 is the location of the LE (*FUT3*) gene which encodes the fucosyltransferase that is integral to the synthesis of the Lewis antigens (eg, Le^a , Le^b).
 - 19q13.33 is the location of the H (*FUT1*) gene, which encodes the fucosyltransferase that is integral to the synthesis of the H antigen, the base structure of the A and B antigens. When no H gene is present (H null), no A or B antigens are expressed even if the ABO gene is present. The absence of the H gene results in the Bombay phenotype.
 - 6p24.2 is the location of the *GCNT2* (*IGNT*) gene that encodes the glucosaminyl transferase responsible for the synthesis of the I antigen.
-

Question 5: B. Group A.**Explanation:**

- The following table depicts the prevalence of ABO blood groups in different ethnic populations in the US.



ABO Group	Population of European Ancestry(%)	Population of African Ancestry (%)	Population of Asian Ancestry (%)
O	45	49	43
A	40	27	27
B	11	20	25
AB	4	4	5

- The only blood group with a lower frequency in those of African ancestry than in those of European ancestry is blood group A.

**Question 6: C. Groups B and AB.****Explanation:**

- Given that the donor pool described is mostly of Asian ancestry, we would expect the blood center to collect more group B and AB blood compared to donor centers that collect from donors of predominantly European and African ancestries. The Asian population has a higher prevalence of group B and slightly higher prevalence of group AB compared to populations of European and African ancestries. (See table in answer #5).

**Question 7: C. Group B.****Explanation:**

- Forward typing or red cell grouping is a reaction between sample red cells and reagent anti-A and anti-B antisera to characterize the ABO phenotype of the red cells.
- Reverse typing or serum grouping is a reaction between sample plasma and reagent A and B cells to characterize the isoantibodies contained in the sample.
- In general, an individual should not contain isoantibodies that target antigens on their red cells.



- The following table demonstrates the expected reactivities of the ABO blood groups:

Blood Group	Forward Type		Reverse Type	
	Anti-A	Anti-B	A1 Cells	B Cells
O	0	0	4+	4+
A	4+	0	0	4+
B	0	4+	4+	0
AB	4+	4+	0	0



Question 8: D. Associated with hemolytic disease of the fetus and newborn.

Explanation:

- ABO antibodies are predominantly IgM although blood group O individuals do produce anti-A,B which is IgG. However, antibodies to other carbohydrate antigens are also IgM, so this does not make ABO antibodies unique.
- ABO antibodies are naturally occurring, as are antibodies to other carbohydrate antigens. As such, this feature is not unique to ABO antibodies.
- Though antibodies to some of the other carbohydrate antigens are not clinically significant, ABO antibodies are clinically significant, and are able to cause intravascular hemolysis in the setting of ABO-incompatible transfusions.
- Given that A,B antibodies are IgG, they can cross the placenta and cause hemolytic disease of the fetus and newborn (HDFN). In fact, since the advent of Rh immune globulin (RhIG), ABO HDFN has become the most common cause of HDFN, although it is generally mild in severity.
- ABO antibodies are not commonly detected on routine indirect antibody screens, because cells on screening panels are group O, to permit identification of non-ABO antibodies. Antibodies to other carbohydrate antigens (eg, Le, P1) can be detected on indirect antibody screens.



Question 9: C. Recipient group AB; donor group A.

Explanation:

- An individual will possess naturally occurring ABO antibodies to ABO blood group antigens they do not possess.
- The following table demonstrates the expected ABO antibodies for each ABO blood group:

ABO Blood Group	Expected ABO Antibodies
O	Anti-A, anti-B, anti-A,B
A	Anti-B
B	Anti-A
AB	None

- These antibodies are generally IgM antibodies (exception: anti-A,B can be IgG), and are clinically significant, able to induce acute intravascular hemolysis.
- Regarding RBC transfusion, ABO compatibility is determined by the major crossmatch – recipient plasma and donor red cells. In other words, does the recipient possess naturally occurring ABO antibodies to the donor red cell antigens?
- Of the answer choices provided, only choice C shows an ABO-compatible recipient-donor pair. The group AB recipient has no ABO antibodies, and thus would be considered a universal recipient of RBC units.
- The following table shows compatible ABO selection for RBC transfusion for recipients based on ABO group.

Recipient ABO Blood Group	First Choice Donor	Second Choice Donor	Third Choice Donor	Fourth Choice Donor
O	O			
B	B	O		
A	A	O		
AB	AB	A	B	O



- As the table demonstrates, blood group O donors are the universal donors, able to donate to any recipient. The red cells do not express antigen targets for recipient ABO antibodies.



Question 10: A. Recipient group type O; donor group type A.

Explanation:

- Regarding plasma transfusion, ABO compatibility is determined by the minor crossmatch – donor plasma and recipient red cells. In other words, does the donor possess naturally occurring ABO antibodies to the recipient red cell antigens?
- Of the answer choices provided, only choice A shows an ABO-compatible recipient-donor pair. The group O recipient has no antigens to be targeted by donor ABO antibodies, and thus would be considered a universal recipient of plasma.
- The following table shows compatible ABO selection for plasma transfusion for recipients based on ABO group.

Recipient ABO Blood Group	First Choice Donor	Second Choice Donor	Third Choice Donor	Fourth Choice Donor
O	O	A	B	AB
B	B	AB		
A	A	AB		
AB	AB			

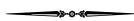
- As the table demonstrates, group AB donors are the universal donors, able to donate to any recipient, because the plasma does not contain naturally occurring antibodies to target recipient ABO antigens.



Question 11: D. Recipient group A; Donor group O.

**Explanation:**

- Due to the limited platelet supply, it is not uncommon to supply out-of-group platelets to a recipient. As with plasma transfusion, for platelets suspended in plasma, ABO compatibility is determined by the minor crossmatch – donor plasma and recipient red cells. In other words, does the donor possess naturally occurring ABO antibodies to the recipient red cell antigens?
- Answer choice 'A' is ABO compatible as the recipient O red cells do not have any target for the group A donor's B antibodies.
- For answer choices 'B,' 'C,' and 'E,' the donor has anti-B directed toward the recipient red cells and could induce some hemolysis. In general, anti-B titers are lower than anti-A titers, and the risk of significant hemolysis from the amount of anti-B in an apheresis unit of platelets is low.
- The recipient-donor pair for answer choice 'D' poses the highest risk of hemolysis because anti-A titers, especially in blood group O individuals, can be high. Of note, with the advent of platelet additive solution and pathogen reduction, the amount of residual plasma and ABO antibodies in platelet products is low, as is the risk of hemolysis in ABO-incompatible platelet transfusions.
- If an ABO-incompatible platelet transfusion was needed, ABO titers could be performed, and low-titer units could be preferentially given.
- As platelets express ABO antigens, exposure to corresponding ABO antibodies can lead to increased clearance; therefore, post-transfusion count increments may be lower than expected.



Question 12: E. There are no ABO requirements (recipients can receive cryoprecipitate from any donor regardless of ABO group).

Explanation:

- Although ABO compatibility is often preferred, there are no ABO requirements when it comes to transfusion of cryoprecipitate. Any recipient can receive cryoprecipitate from any donor, regardless of ABO group.
- The volume of cryoprecipitate is small. Even when 2 pools (10 units) are transfused, the amount of plasma is only 100 mL ± 20 mL. As such, the risk of hemolysis is low, and reports of signif-



ificant hemolysis in the setting of ABO-incompatible cryoprecipitate transfusions are lacking.

- Furthermore, antibody titers in cryoprecipitate are low, and it has been extrapolated that the chance of 2 pools (10 units) of cryoprecipitate containing a titer of >100 is <1:3 million.



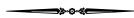
Question 13: E. Stem cell.

Explanation:

- As ABO antigens are expressed on the endothelium and on most solid organs, ABO incompatibility is considered a contraindication to solid organ transplantation. Indeed, anti-ABO can lead to hyperacute and acute allograft antibody mediated rejection. Recipient-donor pairs are required to be ABO compatible in the host-vs-graft direction.
- Of note, there are a couple of circumstances in which ABO-incompatible solid organ transplants can take place. First, there are so-called desensitization protocols that aim to reduce recipient ABO antibody titers through a variety of means including plasma exchange, rituximab, IVIG, and other medications and biologics aimed at decreasing antibody production. Second, the United Network of Organ Sharing (UNOS) permits the transplantation of non-A1 kidneys to group B recipients, provided that the recipient's anti-A titers are low. Remember, non-A1 antigen expression is lower than A1; hence, there is not as much target for recipient anti-A. Allograft outcomes for non-A1 kidney to group B recipient transplants have been shown to be comparable to ABO-compatible transplants.
- ABO compatibility is not required for stem cell transplantation; in fact, approximately 50% of stem cell transplants are between recipients and donors who are ABO mismatched. Even so, ABO mismatch transplants are at increased risk for hemolysis (in cases of major and minor crossmatch incompatibility), pure red cell aplasia (in cases of major crossmatch incompatibility), and passenger lymphocyte syndrome (in cases of minor crossmatch incompatibility).
- Eventually, once fully engrafted, the recipient will take on the ABO phenotype of the donor. However, while awaiting engraft-



ment, transfusion support should be limited to products that are compatible with recipient and donor ABO blood groups.

**Question 14: C. Anti-B.****Explanation:**

- Blood grouping reagents are color-coded depending on the antibody in the container. This color-coding system is universal, regardless of the manufacturer, and is important to avoid blood grouping errors.
- The anti-B reagent has a yellow label while the anti-A reagent has a blue label. (Just remember this is the opposite of what would be expected, as anti-B does *not* correlate with the blue label).
- The antihuman globulin reagent has a green label.
- The anti-D reagent has a gray label.

**Question 15: D. A/A.****Explanation:**

- ABO phenotype is dependent on ABO genotype. ABO genes are co-dominantly expressed, so both maternal and paternal glycosyltransferases will be expressed along with the resultant ABO blood group antigens.
- The following table demonstrates the possible genotypes for each ABO group phenotype.

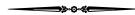
ABO Phenotype	Possible ABO Genotypes
A	<i>A/A or A/O</i>
B	<i>B/B or B/O</i>
AB	<i>A/B</i>
O	<i>O/O</i>

- A mother who is group B could pass along a *B* or *O* allele, while a group AB father would pass along either an *A* or *B* allele. Thus,



the possible genotypes of the offspring would be A/B , B/O , B/B , or A/O .

- The A/A genotype would be possible with a group A and A mating pair, a group A and AB mating pair, or a group AB and AB mating pair.



Question 16: B. *Dolichos biflorus*.

Explanation:

- Lectins are plant-derived substances that react with red cell antigens and can help in antigen detection.
- The following table lists common lectins used in blood bank work-up and their corresponding cognate antigens.

Lectin	Binds to
<i>Dolichos biflorus</i>	A1
<i>Ulex europaeus</i>	H
<i>Vicia graminea</i>	N
<i>Bandeiraea simplicifolia</i>	B
<i>Arachis hypogaea</i>	T
<i>Lotus tetragonolobus</i>	H

- A_1 and A_2 are the two most common A subgroups with A_1 representing approximately 80% of group A individuals and A_2 constituting the remaining 20%.
- Dolichos biflorus* will bind A_1 cells but not A_2 and thus can be used to differentiate between the two subgroups.
- Anti- A_1 can be present in 1-8% of group A_2 individuals and 22-35% of group A_2B individuals, and can result in apparent ABO discrepancies, with individuals typing as group A on the forward typing yet showing anti- A_1 reactivity on the reverse typing.
- Group A_1 individuals, who possess more glycosyltransferase activity, express up to five times the number of A antigens on red cells than group A_2 individuals. Given the decreased A expression, A_2 red cells express more H antigen and will therefore bind *Ulex europaeus*.



Question 17: D. Neutralizing substance: hydatid cyst fluid; P1 antibody.

Explanation:

- Neutralizing substances can be used to bind antibodies of certain specificity.
- The use of neutralizing substances can be useful in pretransfusion testing when more than one antibody is suspected. In these instances, neutralizing substances can be employed to eliminate the corresponding reactivity and permit identification of underlying specificities.
- The following table is a list of more commonly used neutralizing substances and the antibodies they bind.

Neutralizing Substance	Antibody
Guinea pig urine	Anti-Sd ^a
Hydatid cyst fluid	Anti-P1
Pigeon eggs	Anti-P1
Breast milk	Anti-I
Plasma	Anti-Chido, anti-Rogers
Saliva	Anti-H, anti-Le ^a



Question 18: A. Gastrointestinal infection.

Explanation:

- Acquired B phenotype arises when weak B expression occurs on group A cells.
- Forward typing will demonstrate strong agglutination with anti-A reagents and weak agglutination (<2+) with anti-B reagents. Reverse typing should show reactivity only with group B cells.
- This phenomenon results from deacetylation of N-acetylgalactosamine (GalNAc), which characterizes the A antigen, converting the structure into galactosamine, resembling the group B galactose and able to react with anti-B reagents.



- Given that some enteric bacteria produce deacetylase enzymes, acquired B phenotype is often seen in the clinical setting of gastrointestinal infection and/or gastrointestinal malignancy.
 - The forward typing discrepancy can be resolved by:
 - Incubating patient's own serum with autologous red cells (should not agglutinate).
 - Using a different monoclonal anti-B reagent (different clones will have varying reactivity with acquired B antigen).
 - Acidifying anti-B to a pH of 6.0 (acidified anti-B should not react with acquired B antigen).
 - Treating patient red cells with acetic anhydride to re-acetylate the A antigen.
-

Question 19: B. IgM anti-A in group B serum and anti-B in group A serum.

Explanation:

- The predominant immunoglobulin isotype found in the serum of group A and group B individuals is IgM.
 - The group O individuals also have IgM (anti-A and anti-B); however, group O individuals also have IgG anti-A,B, which agglutinates both A and B cells. It is believed that this antibody targets a common epitope found on both A and B antigens, as reactivity cannot be differentially absorbed.
 - Given that IgG can cross the placenta, the IgG isotype in the sera is likely the reason that hemolytic disease of the fetus and newborn most often occurs in offspring of group O mothers.
 - IgM anti-H is found in the sera of individuals with Bombay phenotype (ie, H-null).
-

Question 20: C. Confirmatory typing of donor units and infants younger than 4 months of age.

Explanation:

- Forward and reverse typing are required for all blood donors and recipients.



- Upon receipt of blood units from blood supply, blood banks are required to perform confirmatory typing; however, reverse typing in this scenario is not required.
 - Because infants do not start producing isoantibodies until 3-6 months of age, reverse typing does not need to be performed on infants less than 4 months of age.
-

Question 21: B. Transfusion of a group A recipient with group B RBCs.

Explanation:

- Mixed-field reactions can present as ABO discrepancies with unexpected results seen on forward typing.
 - Mixed-field reactions occur when red cells from more than one blood group are detected on forward typing.
 - Mixed-field reactions are most often observed after transfusion with out-of-group red cells or after an ABO-mismatched hematopoietic stem cell transplantation (ie, ABO mismatch between recipient and donor).
 - In the present case, there is a mixed-field reaction demonstrating presence of A and B cells. On reverse typing, only B antibodies are present, indicative of a blood group A individual. The most likely explanation is a group A recipient transfused with group B red cells (ie, ABO-incompatible transfusion).
 - Less commonly, mixed-field reactions can be seen in some ABO subgroups (eg, A3) and blood chimerism of twins.
-

Question 22: C. 85-year-old male with coronary artery disease.

Explanation:

- Some ABO discrepancies result from weak or missing serum reactivity, which can be detected on reverse typing.
- Infants less than 4-6 months of age who have yet to form their own isoantibodies have weak or missing serum reactivity. However, by 1 year of age, they start to display adequate reactivity on reverse typing, consistent with their blood group.
- Serum from the elderly can also result in weak or missing reverse typing, as immunoglobulin titers tend to decrease with increasing age.



- Patients who have undergone hematopoietic stem cell transplantation may not display reactivity on reverse typing consistent with donor blood group due to induction of tolerance. For example, a blood group B recipient receiving a transplant from a group O donor may still form anti-A but may never form anti-B isohemagglutinins.
 - ABO subgroups and hypogammaglobulinemia, either congenital or acquired, can result in weak or missing serum reactivity.
-

Question 23: A. O>A₂>B>A₁>A₁B.

Explanation:

- The H antigen is a precursor to the A and B antigens.
 - In the absence of A and B genes, the H antigen remains unmodified and characterizes the O group. As such, group O red cells express the largest amount of H antigen.
 - As A₂ red cells express less A antigen than A₁ cells, A₂ cells express more H antigen.
 - The presence of both A and B genes will lead to conversion of more H antigen to A and B antigens, respectively, resulting in fewer unmodified H antigens.
-

Question 24: E. Tn polyagglutination.

Explanation:

- Polyagglutination is the red cell agglutination by all or many sera due to an inherited or acquired defect of the red cell.
- The Tn antigen is a precursor in the O-glycan pathway and is generally not exposed in most normal tissues, a so-called cryptic antigen. T-synthase converts the Tn antigen to the T antigen.
- Tn polyagglutination or Tn syndrome is characterized by acquired somatic mutations in the C1GALT1C1 (COSMC) gene, resulting in a nonfunctional T-synthase and exposed Tn antigen. Most healthy individuals contain naturally occurring anti-Tn, resulting in agglutination of affected red cells in patients with Tn syndrome.
- Tn syndrome is often described in patients with hematologic malignancies.



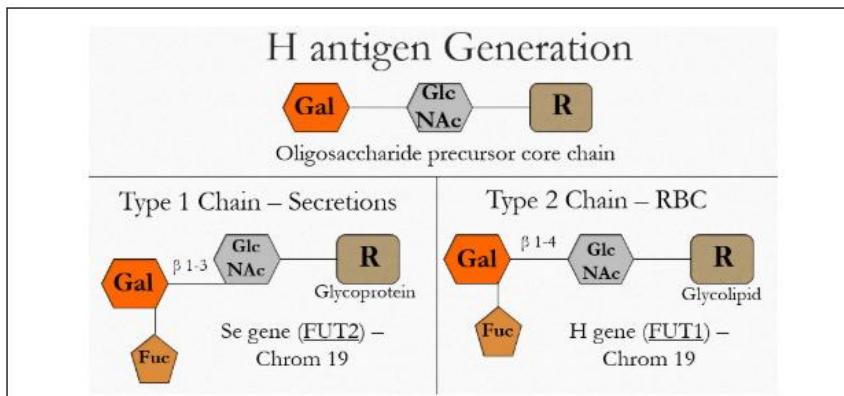
- T polyagglutination or T activation is an acquired form of polyagglutination where bacterial enzymes cleave glycophorin A and/or B oligosaccharides, thus exposing normally cryptic T antigens. This is more common in the setting of clinical infection with polyagglutination abating upon resolution of the infection.
- Sd(a++), HEMPAS, and Hyde Park are all inherited forms of polyagglutination, which all result in expression of immunodominant antigenic structures.
- The many forms of polyagglutination can be distinguished based on differential reactivity with lectins.



Question 25: C. FUT1 (H gene) with Type 2 chain H antigen on red cells.

Explanation:

- The H antigen can be synthesized on red cells or in secretions depending on the fucosyltransferase enzyme that adds the terminal fucose to the base chain.
- Fucosyltransferase can be generated by two genes: FUT1 (H gene) and FUT2 (secretor gene) (see figure below).



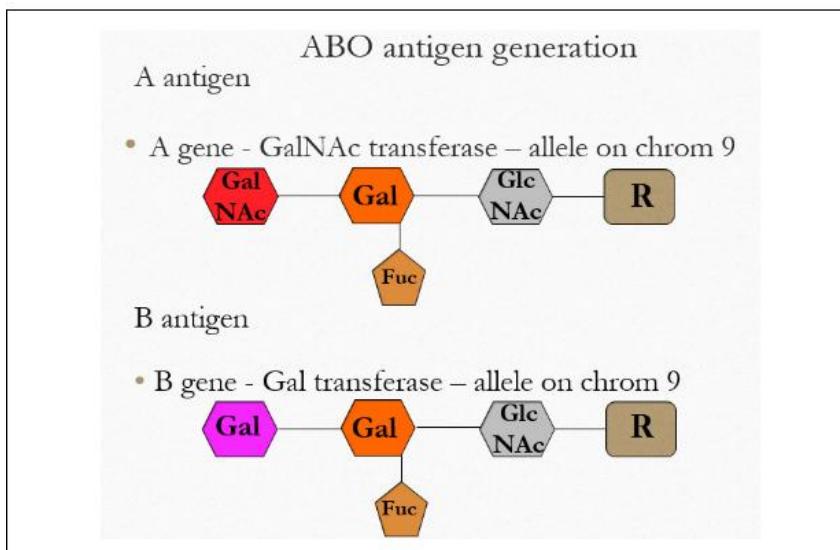
- The FUT1 enzyme will add fucose to Type 2 chains on red cells, while the FUT2 enzyme will add fucose to Type 1 chains in secretions (salivary, respiratory, gastrointestinal, genitourinary).
- FUT3 (LE gene) encodes for a fucosyltransferase that synthesizes the Le^a antigen.



Question 26: B. Group B.

Explanation:

- The ABO blood groups are encoded by ABO genes that do not transplant the surface antigen per se but encode glycosyltransferases that modify the carbohydrate base H antigen by the addition of a terminal sugar.
- The group A glycosyltransferase adds a terminal N-acetylgalactosamine, which defines the group A antigen (see figure below).



- Group B glycosyltransferase adds D-galactose, defining the group B antigen.
- There is no blood group C in the ABO system.
- Genes that result in group O do not encode a functional glycosyltransferase, and so, the H antigen is left with its terminal L-fucose unmodified by the addition of another sugar.
- The Lewis transferase will add an L-fucose to the penultimate N-acetylglucosamine of the precursor Type 1 chain thus forming the Le^a antigen.



Question 27: A. Group A expressed on red cells but not in secretions.



Explanation:

- The FUT2 fucosyltransferase is responsible for generating the H antigen in secretions. If it is dysfunctional (ie, nonsecretor status), then ABO blood groups will not be expressed in secretions even if the A and/or B transferases are functional.
 - The FUT1 fucosyltransferase is responsible for generating the H antigen on red cell membranes.
 - The N-acetylgalactosamine transferase generates the A antigen from the H antigen.
 - See answers #25 and #26 for more details.
-

Question 28: D. Bombay; hh, sese.

Explanation:

- The forward and reverse typing for the Bombay phenotype appear identical to those of group O. One key distinction is that sera from individuals with the Bombay phenotype will react with group O cells in addition to group A1 and B cells. Additionally, they will be crossmatch incompatible with all donors except other individuals with Bombay phenotype.
- Bombay phenotype is an autosomal-recessive phenotype, defined by absence of H, A, and B antigens due to the inability to synthesize H. It results from dysfunctional H (FUT1) genes (homozygous hh) and secretor (FUT2) genes (homozygous sese). Thus, neither Type 1 nor Type 2 chains can be used as precursors for H antigen synthesis, and individuals will not have the antigen in their secretions nor on their red cells (see figure in answer #25).
- As there is no H antigen on Bombay red cells, there is no reactivity with *Ulex europaeus*, an anti-H lectin.
- Para-Bombay phenotype is generally characterized by nonfunctional H (FUT1) gene (hh), however, there is at least one functional secretor gene (eg, Sese); these individuals can express ABH antigens in their secretions.
- Interestingly, although Type 2 chains cannot be used as precursors for ABH synthesis in para-Bombay phenotype, red cells can passively absorb Type 1 ABH antigens on their membranes, resulting in some ABH expression.



Question 29: E. IgM antibodies reactive at room temperature that are most commonly found in group A₁ individuals.

Explanation:

- H antibodies can present as alloantibodies or autoantibodies.
- When present as auto-H antibodies, they are of the IgM isotype and typically reactive at room temperature. They are generally found in group A₁ individuals who have low H expression. A₁ individuals can also form auto-HI antibodies.
- Auto-H antibodies are considered to be clinically insignificant and rarely associated with hemolysis; however, hemolysis has been described in the setting of auto-HI after the transfusion of group O red cells, which has the highest expression of the H antigen.
- Allo-H antibodies are also of the IgM isotype and are reactive at a broad thermal range (4-37 C). They are found in individuals with Bombay and para-Bombay phenotype.
- Unlike auto-H antibodies, allo-H antibodies are clinically significant and able to fix complement and induce hemolysis. Transfusion must be from other H-negative individuals.



Question 30: D. Autosomal dominant phenotype in which the B glycosyltransferase has increased capacity to incorporate UDP-N-acetylgalactosamine.

Explanation:

- B(A) phenotype results from an amino acid polymorphism (Pro234Ala or Ser235Gly) in which the B gene product has increased capacity to add some N-acetylgalactosamine to the H antigen. This results in weak A expression on group B red cells.
- The anti-A reactivity on the forward typing using monoclonal anti-A reagents is usually weak with easily dispersed agglutination.
- Using polyclonal anti-A reagents can help resolve forward typing discrepancy.
- A(B) phenotype has been described whereby increased H antigen expression may allow some generation of B antigen by the A glycosyltransferase.

**Question 31: B. Lewis.****Explanation:**

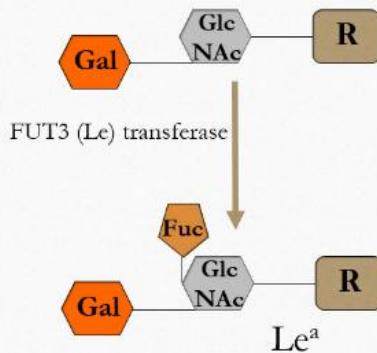
- Lewis antigens are expressed on red cells and platelets as well as the gastrointestinal and genitourinary epithelium. The gastrointestinal tract is the purported source of most Lewis glycolipids found in plasma.
- Unlike other red cell antigens, Lewis antigens are not produced by red cells. Instead, they are absorbed passively by the red cell membrane from plasma.
- During pregnancy, it is not uncommon for Lewis-positive women—Le(a+b−) or Le(a−b+)—to transiently type as Le(a−b−). This is thought to occur through elution of the Lewis antigen by plasma lipoprotein, which can absorb Lewis antigen and which increases during pregnancy, along with the gestational increase in plasma volume.

**Question 32: C. Lewis enzyme will fucosylate the penultimate N-acetyl-glucosamine of the precursor Type 1 chain to form the Le^a antigen.****Explanation:**

- Expression of the Lewis antigen is dependent on the fucosyltransferases encoded by the Lewis (FUT3) and secretor (FUT2) genes. Both enzymes tend to fucosylate Type I chains in secretions.
- Recall that the secretor transferase transfers a fucose to the terminal galactose of the precursor Type 1 chain to form the H antigen.
- The Lewis transferase will add a fucose to the penultimate N-acetyl-glucosamine of the precursor Type 1 chain thus forming the Le^a antigen (see figure below).
- The Lewis transferase can also transfer a second fucose to Type 1 chain H antigen thus forming the Le^b antigen. Due to steric hindrance of the subterminal fucose on the Le^a antigen, the Le^b is not formed directly from Le^a.

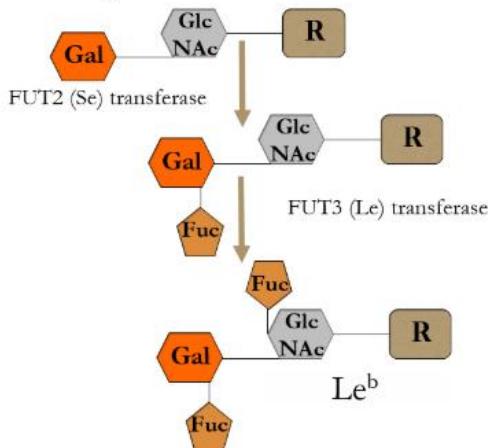


Le^a Generation



Le^b Generation

Type 1 Chain – Secretions



Question 33: C. LeLe or Lele; sese.

Explanation:

- Lewis phenotype is determined by the Lewis and secretor gene status and the resulting presence and/or absence of the Lewis and secretor fucosyltransferases. The table shown below provides the



Lewis and secretor genotypes along with the corresponding phenotype.

Lewis Genotype	Secretor Genotype	Phenotype
<i>lele</i>	<i>sese</i>	Le (a–b–)
<i>lele</i>	<i>SeSe</i> or <i>Sese</i>	Le (a–b–)
<i>LeLe</i> or <i>Lele</i>	<i>sese</i>	Le (a+b–)
<i>LeLe</i> or <i>Lele</i>	<i>SeSe</i> or <i>Sese</i>	Le (a–b+)*
<i>LeLe</i> or <i>Lele</i>	<i>Se^w/Se^w</i>	Le (a+b+) [†]

*In the presence of both the secretor and Lewis transferases, Type 1 chain H antigen is preferentially synthesized over the Le^a antigen. Consequently, more Leb antigen is generated, and individuals appear to be Le^a antigen-negative.
† *Se^w* is a gene that encodes a weak secretor fucosyltransferase.



Question 34: A. *lele Sese*.

Explanation:

- Individuals with anti-Le^a and anti-Le^b usually have the Le(a–b–) phenotype and have the *lele* genotype.
- The following table demonstrates the Lewis genotype, secretor genotype, the corresponding Lewis phenotype, and the secretor status:

Lewis Genotype	Secretor Genotype	Lewis Phenotype	Secretor Status
<i>lele</i>	<i>sese</i>	Le(a–b–)	Nonsecretor
<i>lele</i>	<i>SeSe</i> or <i>Sese</i>	Le(a–b–)	Secretor
<i>LeLe</i> or <i>Lele</i>	<i>sese</i>	Le(a+b–)	Nonsecretor
<i>LeLe</i> or <i>Lele</i>	<i>SeSe</i> or <i>Sese</i>	Le(a–b+)	Secretor



Question 35: A. Le(a+b+); FUT2.

**Explanation:**

- Newborns tend to type as Le(a-b-), but 50% will type as Le(a+b-) after ficin treatment, which enhances reactivity with the Le antigen.
 - FUT2 levels are not fully developed in children, and thus the H antigen is not highly expressed in secretions. Since Le^b is synthesized from H antigen, it is also not highly expressed in young children.
 - As the level of FUT2 enzyme activity increases with age, Le^b antigen expression increases, and children can transiently type as Le(a+b+).
 - It is not until age 5 or 6 that a reliable Lewis phenotype can be obtained.
-

Question 36: D. Le^b, group O cells.

Explanation:

- Lewis antibodies are generally naturally occurring IgM, and thus not usually considered clinically significant, rarely implicated in hemolytic transfusion reactions or hemolytic disease of the fetus and newborn.
 - Lewis antibodies are most commonly seen in Le(a-b-) individuals or during pregnancy when women may transiently type as Le(a-b-) (see answer #31).
 - Anti-Le^b antibodies can sometimes exhibit ABO specificity, such as with the Le^{bH} antibody, which reacts with Le^b antigen and the H antigen. Since group O and A₂ cells express the most H antigen, the L^{bH} antibody reacts most strongly with Le^{b+} group O and A₂ cells.
 - Other Le^b antibodies with ABO specificity include anti-ALe^b and anti-BLe^b.
-

Question 37: E. Norovirus.



Explanation:

- Some blood group antigens can act as receptors for different pathogens.
- Le^b is a known receptor for noroviruses and *Helicobacter pylori*. The H antigen can also bind both noroviruses and *H. pylori*. Of note, patients who type as Le(a–b–) may have increased susceptibility to *Escherichia coli* and *Candida*.
- The Duffy antigen is the receptor for *Plasmodium vivax*, and individuals who type as Fy(a–b–) have increased protection against *P. vivax* infection and subsequent malarial disease. Glycophorin A and B, sialoglycoproteins that contain the M and N antigens, have been shown to bind to *P. falciparum*, and thus, facilitate entry into the red cell.
- The P antigen has been purported as a receptor for parvovirus B19.
- *Enterobacteriaceae* have been shown to be structurally similar to ABO antigens and have been implicated as the potential immune sensitizers leading to the development of naturally occurring ABO antibodies.



Question 38: E. Congenital cataracts can be associated with i_{adult} phenotype.

Explanation:

- I and i are antigens that are found on all cell membranes and are characterized by repeating terminal lactosamine. These antigens can serve as precursors to ABO antigens and other Type 2 chains, which explains their presence on red cell membranes.
- The i antigen is a nonbranched, linear structure that is found on neonatal red cells, while the I antigen is typically a branched structure, synthesized from the i antigen. As children age, more I is synthesized at the expense of i. By 2 years of age, children type as I+.
- *GCNT2* is the I gene, which encodes the N-acetylglucosaminyl-transferase that is responsible for synthesizing the branched I antigen from the linear i antigen.
- Autosomal recessive mutations in the *GCNT2* gene can lead to the i_{adult} phenotype, which is encountered in Asian populations and can be associated with congenital cataracts, depending on where in the gene the mutation resides.



Question 39: B.

Explanation:

Temperature	Cell Type	Anti-I	Anti-i
22 C	Adult	1-2+	0

- Anti-I and anti-i are of the IgM isotype. Anti-I is usually not clinically significant and is found in the serum of many individuals. Anti-i is less commonly found in healthy individuals. Of note, those with i_{adult} phenotype can develop alloanti-I.
- Given that adult cells express the I antigen, anti-I is more reactive with adult cells. By contrast, anti-i reacts more strongly with cord cells, as cord cells from neonates express only i antigen.
- Both anti-I and i reactivity will be enhanced at 4 C compared to 22 C. Neither antibody is usually reactive at 37 C except in some cases of cold agglutinin disease.
- The following table demonstrates the expected reactivity patterns of anti-I and anti-i based on temperature and cell type:

Temperature	Cell Type	Anti-I	Anti-i
4 C	Adult	4+	0-1+
4 C	Cord	0-2+	3+
22 C	Adult	2+	0
22 C	Cord	0	2-3+



Question 40: D. Negative with group A1 donors but positive with group O donors.

Explanation:

- Anti-HI are most commonly found in A_1 individuals, who express the least amount of H antigen compared to the other blood groups.
- Given the increased expression in H antigen, group O and A_2 red cells will react the strongest with anti-IH. Suspicion of an HI antibody may arise when crossmatches with O cells, which express high levels of H antigen, are positive, and when crossmatches with A_1 cells, which express little H antigen, are negative.



- Positive crossmatches with all A₁, B, AB, and O donors would be more consistent with an anti-H, which can be seen in individuals with Bombay phenotype.
-

Question 41: C. Autoanti-I and *Mycoplasma pneumoniae*.

Explanation:

- Although auto-I and auto-i can be present in healthy individuals, in the setting of cold agglutinin syndrome, they can be clinically significant and cause hemolysis.
 - The patient presented in the question demonstrates classic signs and symptoms of walking pneumonia, which is generally caused by *Mycoplasma pneumoniae*.
 - In the setting of *Mycoplasma pneumoniae*, individuals can develop a strong autoanti-I, which can lead to brisk autoimmune hemolysis, as this patient appears to be experiencing (low hemoglobin, high bilirubin, low haptoglobin, high lactose dehydrogenase, and a positive direct antiglobulin test).
 - Primary cold agglutinin syndrome can also be seen in patients with chronic lymphocytic leukemia, Waldenström macroglobulinemia, and other lymphoproliferative conditions.
 - A transient anti-i can be seen in individuals with EBV infections, primarily infectious mononucleosis.
-

Question 42: B. P1 phenotype – cells express P₁ and P but not P^K.

Explanation:

- The P1PK (P₁, P^K), GLOB (P), and 209 collection (LKE) consist of glycosphingolipid antigens that constitute the P blood system.
- The predominant antigens in the system are P^K, P, and P₁, with the former two being found on a wide variety of cells (red cells, lymphocytes, kidney, endothelium, synovium) and the latter being restricted to red cells.
- P denotes an antigen, not a phenotype. Conversely, p denotes a phenotype not an antigen. Individuals with the p phenotype do not express P₁, P, or P^K.



- P^K phenotypes do not express P, and the P₁ and P₂ phenotypes refer specifically to the presence or absence of the P₁ antigen, respectively.
- The following table shows the phenotypes and corresponding antigen expression:

Phenotype	P ₁ expression	P expression	P ^K
P ₁	+	+	-*
P ₂	-	+	-*
p	-	-	-
P ₁ ^K	+	-	+
P ₂ ^K	-	-	+

*Although P1 and P2 phenotypes synthesize P^K, in these phenotypes, P^K serves as a precursor to the P antigen. Thus, the P antigen is expressed but the P^K antigen is not.



Question 43: A. P₁.

Explanation:

- The following table provides the prevalence of the P₁PK and GLOB group phenotypes among different populations.

Phenotype*	Population of European Ancestry	Population of African Ancestry	Population of Asian Ancestry
P ₁	79%	94%	20%
P ₂	21%	6%	80%
p	Rare	Rare	Rare
P ₁ ^K	Rare	Rare	Rare
P ₂ ^K	Rare	Rare	Rare

*Note: Capital "P" denotes an antigen not a phenotype.

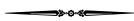




Question 44: B. Alpha 1,4 galactosyltransferase 1.

Explanation:

- The p phenotype is characterized by the absence of the P1, P, and P^K antigens. Genetically, the phenotype is due to inactivating mutations in the exons of the alpha-1,4-galactosyltransferase (A4GALT) gene. Such mutations prevent the first step in both the globo-glycosphingolipids (eg, PK, P) and the paragloboside or neolacto-glycosphingolipids (eg, P1) synthesis, in which the precursor lactosylceramide (ceramide dihexose) is acted upon by A4GALT to form the P^K antigen in the globoside pathway and Lc3 in the paragloboside pathway.
- Beta-1,3-N-acetylgalactosaminyl transferase 1 is the enzyme that converts the P^K antigen to the P antigen.
- Beta-1,6-N-acetylgalactosaminyl transferase converts the linear i antigen into the branched I antigen.
- Alpha-1,3/4-fucosyltransferase is the Lewis gene product that adds a fucose to the penultimate N-acetylglucosamine of the precursor of Type 2 chains in the formation of Le^a antigen and adds a second fucose to the H antigen in the formation of Le^b antigen.
- Group A glycosyltransferase adds N-acetylgalactosamine to the precursor H antigen.



Question 45: D. You do not generally need to honor anti-P1 with provision of P1-negative cells.

Explanation:

- Anti-P1 is of the IgM isotype that generally reacts only weakly at room temperature.
- Anti-P1 can be naturally occurring in individuals with P2 phenotype. Recall that individuals with P1 phenotype express P1, whereas those with P2 phenotype do not. In general, individuals generate antibodies to nonself targets.
- Anti-P1 reactivity can be inhibited by hydatid cyst fluid and pigeon eggs, both of which contain a P1-like substance. These inhibiting substances can be used in serologic workups to investigate underlying antibodies when there is P1 antibody that may be masking other reactivity.



- Because anti-P1 usually reacts only at room temperature, P1-positive cells generally have normal survival even when transfused to patients with P1 antibodies. Of note, P1 expression is thought to decrease in storage. Thus, P1 antibodies may not react as strongly with P1-positive red cells.
- Anti-P1 activity is enhanced when tested against enzyme-treated red cells.



Question 46: E. Anti-PP1Pk.

Explanation:

- Anti-PP1Pk is found in the plasma of individuals with the p phenotype.
- Early spontaneous abortion is associated with the anti-PP1Pk, or the anti-Tj^a. This antibody is actually a mixture of each of the constituent antibodies (anti-P, anti-P1, anti-Pk).
- Anti-PP1Pk can be of the IgG subtype and can cross the placenta, which has high expression of the P and PK antigens and, thus, is a prime target for the IgG anti-PP1Pk.
- Recall that p is a phenotype, not an antigen. Thus, there is no anti-p.



Question 47: D.

Cell Phenotype	Incubation at 4 C	Incubation at 37 C	Incubation at 4 C Followed by Incubation at 37 C
P ₂ ^k	No hemolysis	No hemolysis	No hemolysis

Explanation:

- Patients with paroxysmal cold hemoglobinuria form autoantibody with P-like specificity, which is of the IgG isotype.
- This auto-P is characterized as biphasic, given that it binds red cells at colder temperatures in the peripheral blood system and binds complement at warmer temperatures in the more central



body system. Binding of complement leads to intravascular hemolysis.

- A Donath-Landsteiner test can be performed in which the patient's plasma is tested against red cells at different incubation temperatures: 4 C, 37 C, and one at 4 C that is then warmed to 37 C. Hemolysis should only be noted in the biphasic condition.
 - The auto-P antibody will react with most cells including the patient's own cells. However, the rare p phenotype and P₁^k/P₂^k phenotypes do not express the P antigen; therefore, auto-P will not react with these cells and no hemolysis will be noted at any incubation temperature.
-

Question 48: A. P^k.**Explanation:**

- P^k is the receptor for Shiga toxins seen in shigella dysentery. Shiga toxin is also the causative agent in *Escherichia coli*-associated hemolytic uremic syndrome (HUS). P^k can also bind *Streptococcus suis*.
 - P is the receptor for parvovirus B19, which causes fifth disease (erythema infectiosum).
 - P1 can bind pigeon egg and hydatid cyst fluid.
 - Again, p is not an antigen but rather a phenotype. Thus, p is not a receptor.
 - LKE is present on pluripotent embryonic cells and on tumors, and thus, serves as an oncofetal marker.
-

Question 49: B. Anti-Sd^a.**Explanation:**

- The Sd^a antigen is a carbohydrate antigen that belongs to the Sid system; however, it formerly belonged to the 901 Series of antigens, of which AnWj is also a member.
- The antigens with the 901 Series do not fit in with a specific blood group system or blood group collection. Antigens with the 901 series generally have a high prevalence of >99%. Given this



high prevalence, antibodies to these antigens will appear pan-reactive on antibody panels.

- Sd^a is expressed on adult red cells but not cord red cells.
- Sd^a is resistant to proteolytic enzymes (eg, papain, trypsin) and reducing agents (eg, DTT, AET). Therefore, reactivity before and after treatment will be unchanged.
- Sd^a antibodies are inhibited by guinea pig urine (or urine from Sd^a-positive individuals), which acts as an antibody-inhibiting substance. Consequently, adding guinea pig urine to the plasma should eliminate, or greatly reduce, reactivity seen on the antibody panel.
- When Sd^a antibodies agglutinate cells, the reaction appears to be mixed field and the agglutinates appear refractile under the microscope.
- Sd^a antibodies are not considered clinically significant.



Question 50: C. FORS.

Explanation:

- The FORS system consists of a single low-prevalence glycosphingolipid antigen, FORS1.
- It is generated by the addition of an N-acetylgalactosamine to the P antigen. This terminal sugar lends it a resemblance to group A. Consequently, it can react with some polyclonal anti-A reagents. The patient's group O red cells are likely FORS1-positive, which is reacting with the anti-A reagent.
- Given this reactivity profile, FORS1 was initially reported as an A subgroup.
- FORS1 will not react with A1 lectin (*Dolichos biflorus*), but it will react with *Helix pomatia*, which binds alpha N-acetylgalactosamine residues.
- Anti-FORS1 can be naturally occurring and is found in the plasma of most individuals. Although it can cause in-vitro hemolysis, its clinical significance is unknown.



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5

Protein-Based Blood Group Antigens

H. Cliff Sullivan, MD, F(ACHI)

Key Points from the *Technical Manual*

- Of 384 antigen specificities currently recognized, 354 belong to one of 44 blood group systems representing either a single gene or two or more closely linked homologous genes.
- Antigens not classified in a system or collection have either low or high prevalence and make up the 700 and 901 series, respectively.
- The Rh blood group system is very immunogenic, complex, and polymorphic.
- Weak D phenotypes are defined as having a reduced amount of D antigen and may require an indirect antiglobulin test for detection.
- Anti-K is the most common immune red cell antibody not in the ABO or Rh systems; it can cause severe hemolytic disease of the fetus and newborn as well as hemolytic transfusion reactions.
- The FY glycoprotein consists of five antigens. Fy3, Fy5, and Fy6 are high-prevalence antigens.
- JK antibodies can cause severe acute hemolytic transfusion reactions.



QUESTIONS

Question 1: The RHD and RHCE genes are located on which chromosome?

- A. 1.
 - B. 6.
 - C. 15.
 - D. 19.
 - E. 24.
-

Question 2: Which of the following modified Wiener haplotypes is correctly paired with the corresponding Fisher-Race haplotype?

- A. R1 – Ce.
 - B. R2 – Dce.
 - C. R0 – ce.
 - D. r – ce.
 - E. r' – Rce.
-

Question 3: A 47-year-old male with a remote history of multiple transfusions while undergoing hematopoietic stem cell transplant for acute lymphoblastic leukemia in childhood, presents to the hospital for acute gastrointestinal bleeding. He is blood group O, Rh-negative and has a historical anti-D. Given the severity of the bleeding, there is no time to wait for a current type and screen study. The treatment team requires emergency release O, Rh-negative blood. What will be the most likely genotype of this unit in modified Wiener nomenclature?

- A. R0r''.
- B. rr.
- C. r'r.
- D. r"r.
- E. ryr'.



Question 4: A group O, Rh-positive patient is phenotyped for Rh. Select the answer choice that displays a possible Fisher-Race nomenclature of the patient's genotype based on the following Rh phenotyping results.

Anti-C	Anti-E	Anti-c	Anti-e
0	4+	4+	4+

- A. *Dce/cE.*
 - B. *Ce/DCe.*
 - C. *ce/cE.*
 - D. *cE/Ce.*
 - E. *cE/DcE.*
-

Question 5: A patient with a history of cirrhosis is admitted for bleeding varices. The patient's hemoglobin is 6.8 g/dL, and a type-and-crossmatch test is ordered for one unit of Red Blood Cells (RBCs). An antibody to an antigen within the Rh system is found. For which of the following antibodies would it be most difficult to obtain an antigen-negative unit?

- A. D.
 - B. C.
 - C. E.
 - D. c.
 - E. e.
-

Question 6: What kind of mutation most commonly results in the Rh-negative phenotype in individuals of European ancestry?

- A. Frame-shift.
- B. Point mutations.
- C. Base substitutions.
- D. Insertion.
- E. Deletion.



Question 7: Which of the following genotypes would be expected to express the most amount of D antigen?

- A. $R2R2$.
- B. $R1R1$.
- C. $R1R2$.
- D. $R1r$.
- E. rr .



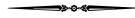
Question 8: Select the RhD phenotype expected to have increased D antigen expression.

- A. Weak D.
- B. Partial D.
- C. DEL.
- D. D--.
- E. DHAR.



Question 9: In which of the following scenarios is weak D testing considered necessary?

- A. Rh-negative female who just gave birth to an Rh-positive neonate.
- B. Female neonate born to an Rh-negative female.
- C. Confirmation of Rh status of an Rh-negative RBC unit upon receipt.
- D. Male patient requiring transfusion secondary to bleeding.
- E. Confirmation of Rh-positive RBC unit.



Question 10: A person with which of the following RhD phenotypes is not at risk for forming a D alloantibody when transfused with D-positive blood?

- A. RhD-negative.
- B. Weak D type 15.
- C. Weak D type 1.
- D. Weak D type 11.
- E. Partial DIIIa D.



Question 11: A 50-year-old, Rh-negative (rr) female experienced significant blood loss during a hip replacement. Postoperatively, her hemoglobin was 6.0 g/dL and she was transfused with a Rh-negative (r'r') RBC unit. Weeks later, she now presents to the follow-up clinic with symptomatic anemia and a hemoglobin of 6.8 g/dL. A new type-and-screen test is ordered and new reactivity with D and C specificities are detected on the antibody panel. Which of the following could explain this reactivity?

- A. Anti-f.
 - B. Anti-D and anti-C.
 - C. Anti-G.
 - D. Anti-D with some C reactivity.
 - E. Anti-Ce.
-

Question 12: A patient who is R1R2 would be at highest risk of developing an antibody to which compound antigen?

- A. G.
 - B. Rh7 (Ce).
 - C. f (ce).
 - D. Rh27 (cE).
 - E. Rh22 (CE).
-

Question 13: Select the answer choice that correctly fills in the blanks for the following statement: The Rh_{null} phenotype is most often due to mutations in the _____ gene and results in _____ of the red cells.

- A. *RhD*; stomatocytosis.
- B. *RHAG*; stomatocytosis.
- C. *RhCe*; microcytosis.
- D. *RhD*; acanthocytosis.
- E. *RHAG*; acanthocytosis.



Question 14: After anti-D, which of the following Rh antibodies is the most clinically significant in causing HDFN?

- A. Anti-C.
 - B. Anti-c.
 - C. Anti-E.
 - D. Anti-e.
 - E. Anti-M.
-

Question 15: A 34-year-old male presents to the emergency department with severe internal bleeding after a motor vehicle accident. The patient is group O, Rh-positive but has a historic anti-C. Emergency release blood is requested given the severity of the bleeding. Which transfusion strategy would be the best option while awaiting the results of updated serologic testing and provision of appropriate antigen-negative units?

- A. Group O, Rh-positive.
 - B. Group O, Rh-negative.
 - C. Group O, Rh-positive, then transition to group O, Rh-negative if bleeding continues after administration of 10 RBC units.
 - D. Group O, Rh-negative or Rh-positive, whichever is available in the blood bank general inventory.
 - E. Group O, Rh_{null}.
-

Question 16: A 29-year-old Asian multiparous female experiences postpartum hemorrhage, necessitating blood transfusion. On type and screen, the patient types as group O, Rh-positive and the antibody screen demonstrates an anti-E. Given this history, what other antibody could also be present or considered?

- A. Anti-D.
- B. Anti-G.
- C. Anti-e.
- D. Anti-C.
- E. Anti-c.



Question 17: As part of routine perinatal testing, a type-and-screen test is ordered for a 29-year-old female, G0P1. The indirect antibody screen is positive, and a subsequent antibody panel reveals the presence of an anti-C. An antibody titer is performed at the request of the ordering obstetrician. According to the test results, what is the titer of the anti-C?

Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64
Anti-C	3+	2+	1+	m+*	0	0	0

*m+ = microscopically positive.

- A. 4.
- B. 1:4.
- C. 8.
- D. 1:8.
- E. 16.



Question 18: Select the answer choice that correctly fills in the blanks: A partial D individual should be considered Rh-_____ as a transfusion recipient and Rh-_____ as a blood donor.

- A. Positive; positive.
- B. Negative; negative.
- C. Positive, negative.
- D. Negative; positive.
- E. Null; null.



Question 19: A 39-year-old male comes into the emergency department with brisk bleeding from an open leg wound sustained during a fall from a ladder. The patient is pale, tachycardic, and short of breath. His hemoglobin is 7.0 g/dL. The emergency department physician orders a type and crossmatch for RBCs for pending transfusion. The pretransfusion antibody testing reveals the patient is group O, Rh-positive, as well as the presence of a C^w antibody. The blood bank does not have C^w antisera on hand. Which of the fol-



lowing options would be most appropriate for providing suitable units to the patient?

- A. Provide emergency release group O-positive units.
- B. Delay transfusion until C^w-negative units are obtained from the donor center, which could take up to 4-6 hours.
- C. Provide C-negative units that are crossmatch compatible.
- D. Provide emergency release group O-negative units.
- E. Provide immediate-spin, crossmatch-compatible units.



Question 20: A 20-year-old female with sickle cell disease presents with an acute stroke. In preparation for a red cell exchange, a type-and-crossmatch test is ordered. The patient types as group O, Rh-positive. She has a newly identified anti-e, as well as a historic anti-K. The autocontrol is negative. The patient's red cell phenotype is:

Anti-C	Anti-e	Anti-c	Anti-E	Anti-K	Anti-Fy ^α	Anti-Fy ^β	Anti-Jk ^α	Anti-Jk ^β	Anti-S
0	4+	4+	0	0	0	0	4+	4+	0

What is the most likely explanation that accounts for the presence of an anti-e in a patient who phenotypes as e-positive?

- A. There was a mistake in the phenotyping results.
- B. Antibody identification is incorrect as the plasma used was from a different patient.
- C. The antibody is an autoantibody.
- D. The patient expresses a weak e variant.
- E. The patient expresses a partial e variant.



Question 21: Which of the following statements is true concerning the MNS system?

- A. The system consists of antigens located on three glycoproteins—glycophorin A, glycophorin B, and glycophorin X.
- B. Glycophorin B is more abundant than glycophorin A on the cell surface.



- C. The glycoproteins in the MNS system are multi-pass proteins in the cell membrane.
 - D. Glycophorin B is a potential receptor for *Plasmodium vivax*.
 - E. Glycophorin A is N-glycosylated, whereas glycophorin B is not.
-

Question 22: Which of the following phenotypes could be explained by a homozygous complete deletion of the coding region of the GYPB gene?

- A. M-, N-, S-, s-, U-.
 - B. M+, N+, S-, s-, U+.
 - C. M+, N+, S-, s-, U-.
 - D. M-, N-, S+, s+, U-.
 - E. M+, N+, S+, s+, U-.
-

Question 23: The antigens in the MNS system can be differentially affected depending on the enzyme employed. In the table below, select the answer choice that accurately depicts the expected effect on MNS expression based on the enzyme employed.

Answer Choice	Ficin or Papain	Trypsin	Alpha-chymotrypsin
A.	MN cleaved/ Ss cleaved	MN cleaved/Ss resis- tant	MN partially cleaved/ Ss cleaved
B.	MN cleaved/ Ss cleaved	MN cleaved/Ss resis- tant	MN cleaved/Ss resis- tant
C.	MN cleaved/ Ss cleaved	MN partially cleaved/ Ss cleaved	MN cleaved/Ss resis- tant
D.	MN resistant/ Ss resistant	MN cleaved/Ss resis- tant	MN partially cleaved/ Ss cleaved
E.	MN resistant/Ss resistant	MN cleaved/Ss resis- tant	MN cleaved/Ss resis- tant



Question 24: Which of the following choices in the table below accurately characterizes the antibodies to the MNS antigens?

Answer Choice	Antibody	Isotype	Clinically Significant Y/N
A.	Anti-M	IgM	Y
B.	Anti-N	IgG	N
C.	Anti-S	IgM	N
D.	Anti-s	IgG	N
E.	Anti-U	IgG	Y



Question 25: Glycophorin B carries which of the following antigens?

- A. M.
- B. N.
- C. U.
- D. Gerbich.
- E. Sialic acid.



Question 26: Select the answer choice that is true concerning antibodies to Lutheran antigens.

- A. Lu^b antibodies are more common than anti-Lu^a.
- B. Most Lutheran antibodies are of the IgM isotype.
- C. Anti-Lu^a can be naturally occurring and can be of the IgG and IgA isotypes.
- D. Anti-Lu^a are strongly reactive, often detected at immediate spin.
- E. Lutheran antibodies generally cause severe hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN).



Question 27: The Kell and Kx systems consist of approximately 36 antigens. Which of the following phenotypes has the lowest frequency in the general population?

- A. K-k+.
- B. K+k-.
- C. K+k+.
- D. Kp(a-b+).
- E. Js(a-b+).



Question 28: The patient in question #5 returns to the hospital months later for recurrent variceal bleeding. Again, the patient's hemoglobin is low at 6.5 g/dL and another type and crossmatch is sent for the transfusion of one RBC unit. In addition to an anti-e, an anti-K is also identified. Approximately how many donor RBC units would have to be crossmatched to find one unit that is e-negative and K-negative?

- A. 10-11 units.
- B. 25-26 units.
- C. 44-45 units.
- D. 54-55 units.
- E. 60-61 units.



Question 29: Which of the following scenarios would be associated with the highest K expression?

- A. Mutations that lead to K_0 phenotype.
- B. K+ red cell treatment with DTT.
- C. Kp^a/Kp^a homozygosity.
- D. K+ red cell treatment with papain.
- E. Absence of Kx protein.



Question 30: HDFN due to anti-K is most prominently characterized by which of the following?

- A. Hyperbilirubinemia.



- B. Reticulocytosis.
 - C. Erythroblastosis.
 - D. Hyperhemolysis.
 - E. Anemia.
-

Question 31: McLeod syndrome is due to deletions/inactivation mutations of the gene encoding the Kx protein. Which of the following is most characteristic of McLeod syndrome?

- A. Stomatocytosis of red cells.
 - B. Causative deletions and inactivating mutations are located on chromosome 7q.
 - C. Development of anti-Ku.
 - D. Development of anti-Kx.
 - E. Development of anti-Km.
-

Question 32: What would the expected red cell phenotype be of an individual who is homozygous for a mutation of the erythroid-specific GATA-1 transcription factor binding site on the *DARC* promoter region?

- A. Fy(a+b+).
 - B. Fy(a+b-).
 - C. Fy(a-b+).
 - D. Fy(a-b-).
 - E. Fy^x.
-

Question 33: Which of the following statements accurately characterizes Duffy antibodies?

- A. Anti-Fy^a and anti-Fy^b have decreased reactivity with enzyme-treated cells.
- B. Duffy antibodies are generally naturally occurring IgM.
- C. Fy^b antibodies are more commonly encountered than Fy^a antibodies.



- D. Fy^a and Fy^b antibodies are generally associated with severe acute and delayed hemolytic transfusion reactions.
- E. Anti-Fy3 reacts with all red cells even with Fy(a–b–) phenotype cells.
-

Question 34: A patient with sickle cell disease is admitted for acute chest syndrome. The team orders a type and crossmatch in preparation for red cell exchange. The antibody screen is positive, and after an initial antibody identification panel, the laboratory staffer suspects the presence of anti-K, anti-C, and anti-Fy^a. Next, the antibody identification panel is repeated with ficin-treated cells. Assuming that the prediction is correct, which of the following answer choices correctly summarizes the expected reactivities of the antibodies with the ficin-treated cells?

Answer Choice	Anti-K Reactivity with Ficin-Treated Cells	Anti-C Reactivity with Ficin-Treated Cells	Anti-Fy ^a Reactivity with Ficin-Treated Cells
A.	Increased	Increased	Decreased
B.	Decreased	Increased	Decreased
C.	Unchanged	Decreased	Increased
D.	Unchanged	Increased	Decreased
E.	Unchanged	Decreased	Increased



Question 35: Select the answer choice that correctly fills in the blanks: Individuals who can produce anti-Jk3 would be expected to have the _____ phenotype. Red cells from these individuals would be _____ to lysis by 2M urea.

- A. Jk(a–b–); resistant.
- B. Jk(a+b–); sensitive.



- C. Jk(a–b+); resistant (shriveled).
 - D. Jk(a+b+); sensitive.
 - E. Jk(a–b–); sensitive (shriveled).
-

Question 36: Anti-Jk^a and anti-Jk^b are very common causes of delayed hemolytic transfusion reactions (DHTR), likely due to the fact that titers can wane over time and go undetected during pre-transfusion antibody testing. However, anti-Jk^a and anti-Jk^b are also known to cause severe acute HTRs. What characteristic could help explain the pathogenicity of anti-Jk^a and anti-Jk^b in acute HTR?

- A. Anti-Jk^a and anti-Jk^b cause extravascular hemolysis.
 - B. Anti-Jk^a and anti-Jk^b are predominantly IgG2.
 - C. Anti-Jk^a and anti-Jk^b generally require antiglobulin test for detection.
 - D. Anti-Jk^a and anti-Jk^b are predominantly IgG4.
 - E. Approximately half of anti-Jk^a and anti-Jk^b bind complement.
-

Question 37: Band 3 is an important glycoprotein membrane with approximately 1 million copies per red cell. It functions as an ion exchanger, permitting exchange of Cl⁻ and HCO₃⁻ ions across the red cell membrane, and as a tether between the membrane and cytoskeleton proteins, such as ankyrin and protein 4.2. Which blood group system is found on the extracellular loops of Band 3?

- A. YT system.
 - B. Diego system.
 - C. Dombrock system.
 - D. Colton system.
 - E. Cromer system.
-

Question 38: To which blood group system does the Wr^a antigen belong?

- A. Rh system.
- B. Kell system.
- C. Vel system.



- D. Diego system.
 - E. MNS system.
-

Question 39: Within the Dombrock system, which phenotype defines the Dombrock-null phenotype?

- A. Jo(a-).
 - B. Do(a-b+).
 - C. Gy(a-).
 - D. Hy-.
 - E. Do(a-b-).
-

Question 40: Of the following red cells, which one would be expected to have the highest expression of LW antigens?

- A. Rh-positive cord red cells treated with ficin.
 - B. Rh-positive adult red blood cells.
 - C. Rh-negative red cells.
 - D. Rh_{null} red cells.
 - E. Rh-positive adult red cells treated with pronase.
-

Question 41: There is a case in which one of your specialists in the blood bank suspects antibodies directed towards Chido/Rodgers antigens. Which method is correctly matched to the reaction that would be expected with anti-Chido/Rodgers?

- A. Incubation with breast milk – reactivity increased.
 - B. Indirect antibody testing (IAT) with Chido-positive red cell – reactivity inhibited.
 - C. Treatment with papain – reactivity increased.
 - D. Incubation with plasma – reactivity inhibited.
 - E. IAT with Rodgers-positive red cell – reactivity inhibited.
-

Question 42: What Gerbich system phenotype is associated with elliptocytosis?



- A. GE:5.
 - B. GE:6.
 - C. GE:-2,3,4.
 - D. GE:-2,-3,4.
 - E. GE: -2,-3,-4.
-

Question 43: Antigens of the Cromer system are situated on the decay accelerating factor (DAF or CD55), a complement-regulatory protein, which inhibits C3-convertases thereby inhibiting autologous complement and protecting red cells from lysis. Which of the following statement is true of the Cromer system?

- A. Individuals with the Cromer-null phenotype can undergo spontaneous hemolysis due to lack of protection from CD55.
 - B. Individuals with the Cromer-null phenotype can produce anti-IFC which are generally nonreactive with most panel cells.
 - C. Cromer antigens are sensitive to ficin and papain.
 - D. Cromer antibodies are associated with severe hemolytic disease of the fetus and newborn.
 - E. Cromer antibodies are considered clinically insignificant.
-

Question 44: Like the Colton system antigens, antigens on this system are also located on aquaporin protein, forming a channel allowing the passage of water and glycerol.

- A. Gil system.
 - B. Raph system.
 - C. Knops system.
 - D. Indian system.
 - E. Vel system.
-

Question 45: Antibodies to antigens in this blood group system can be acquired and are most commonly seen in elderly patients who present with weakly positive DAT results.

- A. Lan system.
- B. JR system.



- C. YT system.
 - D. John Milton Hagen system.
 - E. OK system.
-

Question 46: Which of the following statements is true regarding blood group collections?

- A. Blood group collections are groups of antigens that are serologically dissimilar.
 - B. Antigens within blood group collections are restricted to low-prevalence antigens.
 - C. The genetic basis of antigens within blood group collections is usually uncharacterized.
 - D. Blood group collections are defined as an antigen or group of antigens that are mapped to a gene or a group of related genes.
 - E. Antigens within a blood group collection are those that have had a gene identified and sequenced, and the effect on the phenotype has been confirmed.
-

Question 47: The 700 series of antigens are accurately characterized by which of the following statements?

- A. Antigens within the 700 series are of very high prevalence, generally found in >99% of most populations.
 - B. Antigens within the 700 series are of very low prevalence, generally found in <1% of most populations.
 - C. When an individual develops an antibody to an antigen within the 700 series, it is generally very difficult to find antigen-negative units.
 - D. Antibodies to the antigens within the 700 series are clinically insignificant.
 - E. There are only six antigens that belong to the 700 series.
-

Question 48: Which of the following shows the Class I HLA antigen correctly matched to its corresponding Bg (Bennet-Goodspeed) antigen name.



- A. HLA-B17 – Bg^a.
 - B. HLA-B7 – Bg^a.
 - C. HLA-A28 – Bg.^b
 - D. HLA-B1 – Bg^b.
 - E. HLA-B17 – Bg^c.
-

Question 49: The Indian blood group is the microbial receptor to which of the following pathogens?

- A. Parvovirus B19.
 - B. *E. coli*.
 - C. *Haemophilus influenzae*.
 - D. Poliovirus.
 - E. Plasmodium vivax.
-

Question 50: A 35-year-old male with no significant medical history is admitted after a motor vehicle accident. His hemoglobin is 7.0 g/dL, and he is pale with tachycardia. An order for type and crossmatch was placed, as internal bleeding was suspected. The patient's typing results are provided here:

Forward Typing		Reverse Typing	
Anti-A	Anti-B	A Cells	B Cells
0	4+	4+	2+

The antibody screen is positive, with two of three screen cells reacting. A subsequent antibody panel reveals reactivity that is stronger at immediate spin than at the indirect antibody test (IAT) phase. The autocontrol is negative. Prewarming the serum eliminates all reactivity. Which of the following antibodies could explain these findings?

- A. Anti-i.
- B. Anti-Jk^a.
- C. Anti-M.
- D. A cold autoantibody.
- E. Antibody to a group B subtype.



ANSWERS

Question 1: A. 1.

Explanation:

- Both the *RHD* and *RHCE* genes are located on chromosome 1. *RHD* encodes the D antigen while *RHCE* encodes the C/c and E/e antigens in different pairings (CE, Ce, cE, ce).
- The major histocompatibility complex (MHC) is located on the short arm of chromosome 6. The MHC contains the loci for the human leukocyte antigen (HLA) genes (*A*, *B*, *C*, *DR*, *DQ*, *DP*).
- Beta-2 microglobulin, which complexes with HLA Class I molecules, is encoded by a gene on chromosome 15.
- Chromosome 19 contains the H (*FUT1*), Se (*FUT2*), and LE (*FUT3*) genes.
- Normally, there are only 23 pairs of chromosomes, so there is no chromosome 24.



Question 2: D. r – ce.

Explanation:

- The Wiener and Fisher-Race nomenclatures describe the possible combinations of Rh antigens that are expressed on a single chromosome (either the paternal chromosome 1 or the maternal chromosome 1). In other words, they provide shorthand for the possible Rh haplotypes.
- The following table provides the Fisher-Race haplotypes with the corresponding modified Wiener haplotypes:

Fisher-Race Haplotype	Modified Wiener Haplotype
Rh Positive	
DCe	R1
DcE	R2
Dce	R0



Fisher-Race Haplotype	Modified Wiener Haplotype
DCE	RZ
Rh Negative	
ce	r
Ce	r'
cE	r''
CE	ry



Question 3: B. rr.

Explanation:

- Wiener haplotypes with R are found in Rh-positive individuals, and this patient needs Rh-negative blood (excluding answer choice 'A').
- The most common Wiener haplotype without RhD antigen expression in the general population is the r haplotype. Most Rh-negative individuals are of the rr (or ce/ce) genotype.
- Prevalence of Rh haplotypes (descending order) in a population of European ancestry: R1 > r > R2 > R0.
- Prevalence of Rh haplotypes (descending order) in a population of African ancestry: R0 > r > R1 > R2.
- Prevalence of Rh haplotypes (descending order) in a population of Asian ancestry: R1 > R2 > R0 > r.
- Rz, r', r'', and ry all have low prevalence.



Question 4: A. Dce/cE.

Explanation:

- The Fisher-Race nomenclature provides a way to describe the possible combinations of Rh antigens that are expressed by a single chromosome.



- Given that the patient is RhD positive, at least one haplotype must be expressed starting with D (excluding answer choices 'C' and 'D').
- The patient is homozygous for c. Thus, haplotypes with C can be excluded (excluding answer choices 'B' and 'D').
- The patient is heterozygous for E and e; thus, E and e must be represented in the haplotypes (excluding answer choices 'B' and 'E')
- Answer choice 'A' accurately describes the patient's Rh genotype in Fisher-Race nomenclature. Other possibilities include ce/DcE and Dce/DcE.

**Question 5: E. e.****Explanation:**

- In general, the US donor pool consists mainly of individuals of European ancestry.
- Knowing the frequencies of the blood group antigens can help determine the probability of finding antigen-negative units when a recipient expresses corresponding antibodies. In this case, the frequency of the e antigen among donors of European ancestry is 98%; finding antigen-negative units will be difficult, as only 2% of donors will be antigen negative.
- The following table provides the frequencies of the common Rh antigens in two populations.

Rh Antigen	Frequency in Population of European Ancestry (%)*	Frequency in Population of African Ancestry (%)*
D	86	92
C	70	33
E	30	21
c	80	97
e	98	99

*Percentages are approximate.

**Question 6: E. Deletion.****Explanation:**

- The RhD phenotype is most commonly the result of a complete deletion of the RhD gene.
 - Its complete absence in RhD-negative individuals may explain, at least in part, the immunogenicity of the antigen, given that there is no baseline similarity in RhD protein structure between RhD-negative and -positive individuals.
 - In some individuals of African ancestry, the RhD phenotype is due to a premature stop codon secondary to a 37-basepair insertion.
 - Point mutations and base substitutions are synonymous.
-

Question 7: A. R2R2.**Explanation:**

- The Ceppellini effect describes a phenomenon of decreased D antigen expression caused by the inheritance of the C antigen in a *trans* position to the D antigen. In other words, the C antigen is expressed on one chromosome, while the D antigen is expressed on the other homologous chromosome.
- Thus, the Ceppellini effect would be expected when at least one haplotype expresses D and the other haplotype expresses C. Answer choices 'B' (R1R1 - DCe/DCe) and 'C' (R1R2 - DCe/DcE) display the Ceppellini effect with decreased D expression. Answer choice 'B' would have a two-way suppression of the D antigen, given that D and C are in *trans* twice.
- R2R2 (DcE/DcE) does not result in C expression, and so, would not induce a Ceppellini effect. Thus, D antigen expression will be higher compared to the other answer choices, including E (rr), which would correlate with an RhD-negative phenotype.
- To note, the genotype in answer choice D (*R1r – DCe/ce*) will also *not* induce a Ceppellini effect (D and C are present only in the *cis* position). Furthermore, only one copy of the D gene is present, so expression will be lower as compared to R2R2 (2 copies).

**Question 8: D. D--.****Explanation:**

- Of the answer choices, only D-- would be expected to have increased D antigen expression.
- D--, along with Dc- and DCw-, have elevated D phenotype with increased D expression and concurrent absent or weak expression of C/c and E/e, secondary to replacement of RHCE gene with portions of RHD gene.
- Weak D phenotypes have decreased RhD expression due to single nucleotide polymorphisms (SNPs) resulting in transmembrane or intracellular single amino acid (AA) changes. It is postulated that these AA sequences can prevent proper cellular membrane insertion, leading to decreased number of D antigens on the cell surface.
- Partial D phenotypes are due to replacement of the RHD gene with portions of RHCE gene, generally resulting in single amino acid changes located on the extracellular portion of the D antigen.
- DEL (D-elution) phenotype is usually found in individuals of Asian ancestry and characterized by a severe reduction in D antigens due to a variety of different mutations. RhD expression is so low that routine serologic methods will not detect it; however, levels are sufficient for absorption and elution of anti-D.
- DHAR is a phenotype that is found in individuals of German ancestry. These individuals lack the RHD gene. However, the RHCE gene encodes some proteins with D epitopes, which can react with monoclonal anti-D reagents. Despite this anti-D reactivity, the absence of the D antigen means that DHAR individuals can be alloimmunized to the D antigen.

**Question 9: B. Female neonate born to an Rh-negative female.****Explanation:**

- An RhD method capable of detecting weak D and partial D is required for all donors to ensure RhD-negative status and prevent sensitization of an RhD-negative recipient.



- Although a segment from donor units must be typed at the blood bank upon receipt from distributors to confirm blood type, RhD-negative units need not be tested for the presence of weak D (weak-D testing of units is required only at the level of the manufacturer).
- Patients do not require weak-D testing, as misclassification of a weak-D-positive patient as RhD-negative would result in transfusion of RhD-negative RBC units with no serious clinical implications.
- The only exception to weak-D testing in patients is in newborns of D-negative mothers to determine risk of alloimmunization. If the newborn is weak D positive, then the mother would be a candidate for prophylactic Rh Immune Globulin.



Question 10: C. Weak D type 1.

Explanation:

- Given the absence of D antigen in D-negative individuals, exposure to D-positive blood is considered very immunogenic.
- Most weak D types (common types 1, 2, and 3) contain similar extracellular protein structure to wild type D; thus, weak D individuals do not form alloanti-D. However, individuals with weak D types 11 and 15 have been reported to form alloanti-D upon exposure to RhD-positive RBC units.
- With slightly different extracellular epitopes, partial D individuals can be allosensitized to wild type D and form alloanti-D. Additionally, since many partial D individuals will type as D with common anti-D reagents, partial D status may be established only after the development of alloanti-D.



Question 11: C. Anti-G.

Explanation:

- This patient received Rh-D negative blood and subsequently developed what appeared to be an anti-D and anti-C. This may at first seem like a discrepancy. However, there is a G antigen (103 serine residue) that is found within both D and C antigens. So, it



is expressed with all haplotypes except r (ce) and r'' (cE). This Rh-negative patient was rr (ce/ce) and received r'r' (Ce/Ce) blood. Thus, she was exposed to the G antigen and most likely developed an anti-G, which appears as an anti-D and anti -C on a standard antibody workup (since the G antigen is contained on both C and D).

- The G antibody could be absorbed using D+C- or D-C+ red cells. However, determining G specificity is clinically relevant only in pregnant women to determine if Rh Immune Globulin (RhIG) therapy is indicated. If reactivity is determined to be G alone, then the patient should receive RhIG to prevent anti-D alloimmunization. In other patients, distinguishing anti-G from anti-D and -C is not necessary, as provision of D- and C-negative units will suffice.
- Anti-f and anti-Ce are antibodies to compound antigens in which two antigens are expressed on a single protein.
- This patient's reactivity is unlikely to be explained by anti-D and anti-C, or anti-D with some C reactivity as she received Rh-negative units.



Question 12: C. f (ce).

Explanation:

- Compound antigens are defined by epitopes that are formed by conformational arrangement of amino acids expressed on the C/c and E/e antigens encoded by genes on the same chromosome (ie, *cis* position).
- Recall that the RHCE gene encodes for a single protein that bears C/c and E/e antigens. The f antigen is present when the c and e antigens are inherited in *cis*.
- The f antigen (ce antigen) is present on the R0 (Dce) and r (ce) haplotypes. This patient is R1R2 (Ce/cE) and would be at risk of developing an f antibody, the patient does not have the ce antigen. (It is true that both e and c are expressed in this individual; however, the antigens are located on different proteins, so they do not form a compound ce antigen.)
- Rh7 antigen (Ce) antigen is present on R1 (DCe) and r' (Ce) haplotypes. This patient is an R1 and would not be at risk of developing a Rh7 antibody.



- Rh27 antigen (cE) antigen is present on R2 (DcE) and r[“] (cE) haplotypes. This patient is an R2 and would not be at risk of developing an Rh27 antibody.
- Rh22 antigen (CE) is present on RZ (DCE) and ry (CE) haplotypes. Although this patient could develop an Rh22 antibody (because there is no CE), the RZ and ry haplotypes are so rare that the chances of being exposed to Rh22-positive blood would be far less than being exposed to f-positive blood.



Question 13: B. RHAG; stomatocytosis.

Explanation:

- The RHAG gene is structurally like the RHD and RHCE genes and is located on chromosome 6.
- The RHAG blood group consists of the RHAG1 (Duclos) antigen, the RHAG2 [OI(a)] antigen, and the RHAG3 (DSLK) antigen.
- Given that mutations in the RHAG gene can lead to the Rh_{null} phenotype, it is thought that the encoded protein's function in tethering the RhD and RhCE proteins to the cell membrane.
- The Rh_{null} phenotype can result from RHAG gene mutations, which are the most common form and are termed "regulator" type. Less commonly, the Rh_{null} phenotype results from a combination of the common RHD gene deletion and RHCE gene mutations. This form is termed "amorph."
- On peripheral smear, Rh_{null} red cells appear as stomatocytes – red cells with a slit-like, or mouth-like (stoma), area of central pallor. Acanthocytosis (red cells of varying shapes and sizes) can be seen in McLeod syndrome. Microcytosis (small red cells) can be seen in a variety of etiologies, most notably anemia.



Question 14: B. Anti-c.

Explanation:

- With the advent of RhIG, hemolytic disease of the fetus and newborn (HDFN) due to anti-RhD has become dramatically less prevalent.



- Anti-c is generally IgG and has the potential to cause severe HDFN. However, even though anti-C, anti-E, and anti-e are also of the IgG isotype, they generally do not cause severe disease.
 - Anti-M is not an Rh antibody and is generally of the IgM isotype. IgM antibodies generally do not cross the placenta; hence, they have only rarely been implicated in HDFN.
-

Question 15: B. Group O, Rh-negative.**Explanation:**

- Since this patient is a male and group O, Rh-positive, it may be tempting to choose group O, Rh-positive units. However, this patient has a historic anti-C; thus, provision of C-negative RBCs would be preferred. So, we would be looking for c-positive RBC units.
 - If you recall, the most common Wiener haplotype that is c-positive is r (ce), and most Rh-negative units will have a rr (ce/ce) genotype. So, providing this patient with group O, Rh-negative RBCs would increase the chance of providing C-negative units.
 - Answer choice C would be a more viable option if you started with the provision of group O, Rh-negative blood and then switched to group O, Rh-positive if bleeding were to persist, especially if group O, Rh-negative inventory prohibited continued issuance. Once bleeding subsided, provision of group O, Rh-negative blood, or phenotyped C-negative blood, could be issued.
 - Although the Rh_{null} blood would technically meet the C-negative requirement, this is a rare blood type, and it would not be a practical approach.
-

Question 16: E. Anti-c.**Explanation:**

- This patient is group O, Rh-positive and thus would not make an anti-D, unless she were a partial D, which is not indicated in the



question stem. Given that the G epitope is present on the D antigen, the patient would also not be likely to form an anti-G.

- Because the patient is Rh-positive and has an anti-E, she should be homozygous for the e antigen. The possible genotypes are R1/R1 (DCe/DCe), R1/R0 (DCe/Dce), or R0/R0 (Dce/Dce). R1 is the most common D-positive haplotype in persons of Asian ancestry (also in those of European ancestry, see table below). Therefore, the patient is most likely R1R1 (DCe/DCe).

Rh Haplotype	Frequency in Population of European Ancestry (%) [*]	Frequency in Population of African Ancestry (%) [*]	Frequency in Population of Asian Ancestry (%) [*]
DCe (R1)	42	17	70
DcE (R2)	14	11	21
Dce (R0)	4	44	3
DCE (Rz)	rare	rare	1
ce (r)	37	26	3
Ce (r')	2	2	2
cE (r'')	1	rare	rare
CE (ry)	rare	rare	rare

*Percentages are approximate.

- In persons of African ancestry, the most common haplotype is R0, and the most likely genotype in an E-negative individual would be R0/R0 (Dce/Dce).
- To form the anti-E, the patient had to be exposed to the E antigen (potentially through pregnancy) which would mean she was likely exposed to the R2 (DcE) phenotype, which is by far the most common haplotype bearing the E allele across all populations. If this is the case, then she was simultaneously exposed to the c antigen and would be at risk of having an anti-c. Because many E-negative units are c-positive units [r (ce)], it is prudent to rule out a potential accompanying anti-c.

**Question 17: A. 4.****Explanation:**

- Titration is a serologic method most often employed to determine the strength or concentration of an antibody in a serum sample. To perform the test, the serum is serially diluted and tested against a reagent red cell with a known phenotype. The same cell is used for all dilutions. As such, the antigen concentration remains constant.
- The titer is the reciprocal of the last dilution that produces visible, macroscopic agglutination. In this case, the last dilution to produce macroscopic agglutination is 1:4, so the titer would be 4. The higher the titer, the stronger or more antibody there is in the serum.
- Titration can also be used to partially quantitate the strength of an antigen on the red cell surface. In this case, the testing cell is diluted, and the serum remains constant at all dilutions. The higher the titer, the stronger the amount of antigen on the red cell surface.
- In pregnancy, titers are performed to assess risk of hemolytic disease of the fetus and newborn (HDFN). When titers reach threshold, commonly set at 1:16 for most antibodies (common exception is 1:8 for anti-K), additional measures such as intracranial Doppler ultrasound will be employed to further assess evidence of HDFN.

**Question 18: D. Negative; positive.****Explanation:**

- Given the potential for a partial D individual to be sensitized against wild-type D, the person would be best treated as a Rh-negative transfusion recipient to receive Rh-negative blood.
- Because blood from a partial D donor has the potential to sensitize an Rh-negative individual, the donor would be best treated as Rh-positive to avoid transfusion to an Rh-negative individual.



Question 19: C. Provide C-negative units that are crossmatch compatible.

Explanation:

- The C^w antigen is part of the Rh system and has a low prevalence of approximately 2%. Most C^w-positive units are also C-positive. Conversely, most C-negative units will also be C^w-negative. Therefore, crossmatching C-negative units and providing compatible units would be a suitable solution in this scenario.
- This patient's condition is deteriorating, and he needs blood. Delaying transfusion by 4-6 hours while waiting for antigen-negative units may place the patient at risk.
- Although the need is urgent, the patient is not likely, at this point, to require emergency blood. There is likely time to perform a crossmatch. Additionally, given that most C-negative units will be C^w-negative, limiting crossmatches to C-negative units will also save time.
- Providing immediate-spin, crossmatch-compatible units would not be appropriate, as the identification of a clinically significant antibody requires an IAT crossmatch.
- Another option would be to provide group O-negative units. Most group O-negative units will be rr (ce/ce), and thus, they would be C-negative, which in turn would likely mean that they are C^w-negative. However, it would be most appropriate if these units were crossmatched, not released emergently.



Question 20: E. The patient expresses a partial e variant.

Explanation:

- This patient most likely has a partial e variant resulting from a mutation that leads to a slightly varied antigenic structure with altered or missing epitopes when compared to the wild type. The resultant antigen can be similar enough to wild type to react with reagent antisera, and thus, the individual will type as e-positive. However, the structure is dissimilar enough to permit development of an anti-e when exposed to the altered or missing epitopes found on the wild-type variant. In this manner partial e variants are like partial D variants.



- Partial e variants are rather common in patients of African ancestry and those with sickle cell disease. In patients who type as e-positive and develop an anti-e, it is helpful to arrange for red cell genotyping to determine if a variant is present.
 - Although phenotyping results and antibody identification errors are plausible, the partial e variant is more likely, as it is often encountered in patients with sickle cell disease.
 - Autoantibodies with anti-e specificity have historically been described. However, with the advent of molecular testing and detection of e variants, it is hypothesized that many of the e auto-antibodies actually represent cases of partial e variants. The auto-control in this case was negative as well.
 - Weak e variants have been described. However, they are not as commonly encountered as partial e variants. Weak e variants would unlikely lead to anti-e antibody formation.
-

Question 21: E. Glycophorin A is N-glycosylated, whereas glycophorin B is not.

Explanation:

- The antigens of the MNS system are contained on two glycoprotein structures – glycophorin A (GPA) and glycophorin B (GPB).
 - GPA and GPB pass through the cellular membrane only once with an extracellular N-terminal domain and an intracellular C-terminal domain.
 - The external domains of GPA and GPB are rich in sialic acid O-glycans with GPA being N-glycosylated while GPB is nonglycosylated.
 - In terms of cell expression, GPA is more abundant than GPB by fivefold (1,000,000 vs 200,000, respectively)
 - GPA and GPB are thought to act as cellular receptors for *Plasmodium falciparum*.
-

Question 22: C. M+, N+, S-, s-, U-.



Explanation:

- The M and N antigens are located on GPA, while the S, s, and U antigens are located on GPB.
- Accordingly, a complete deletion of the GYPB coding regions would lead to absence of the S, s, and U antigens. M and N expression should be unaffected by GYPB deletions, as the GYPA gene should be intact.
- The U-negative phenotype is not common, found only in about 2% of individuals of African ancestry.



Question 23: A. MN cleaved/Ss cleaved; MN cleaved/Ss resistant; MN partially cleaved/Ss cleaved.

Explanation:

Ficin or Papain	Trypsin	Alpha-chymotrypsin
MN cleaved/ Ss cleaved	MN cleaved/ Ss resistant	MN partially cleaved/ Ss cleaved

- The M, N, S, and s antigens are all destroyed by ficin, papain, and pronase. Thus, corresponding antibodies will be nonreactive with cells treated with these agents. Of note, the U antigen, which is present on GYB, is usually enzyme resistant.
- M and N antigens are cleaved by trypsin, whereas S and s are resistant to trypsin. Conversely, when alpha-chymotrypsin is used, M and N are only partially cleaved, whereas S and s are completely destroyed.



Question 24: E. Anti-U; IgG; Y.

Explanation:

Antibody	Isotype	Clinically Significant Y/N
Anti-U	IgG	Y



- Antibodies to M and N are generally of the IgM isotope. They can be naturally occurring, are not considered clinically significant, and are rarely implicated in hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN). Thus, antigen-negative units are not required unless the antibodies are reacting at body temperature (37 C).
 - Conversely, antibodies to the S, s, and U antigens are of the IgG isotope. They are considered clinically significant, causing hemolytic transfusion reactions and HDFN. Thus, these antibodies need to be honored with provision of antigen-negative units.
-

Question 25: C. U.

Explanation:

- Glycophorin B is one of 4 glycophorins, the others being glycophorin A, C, and D (Note: there is a glycophorin E gene, which does not encode a functional protein). These proteins are single-pass membrane structures.
- These molecules carry several blood group antigens:
 - Glycophorin A: M/N.
 - Glycophorin B: S/s, U (also carries the “N” antigen, which is a domain that has identical structure to the N antigen found on glycophorin A; this is why N-negative individuals do not form an alloanti-N).
 - Glycophorins C and D: Gerbich antigens.
- Although the glycophorins bear the majority of sialic acid on the red cell surface, sialic acid does not constitute a blood group antigen nor a blood group system. The sialic acid heavily contributes to the overall negative charge of red cells.
- It is thought that glycophorins A and B are used as receptors for *Plasmodium falciparum*; glycophorins C and D are thought to interact with cytoskeletal protein 4.1, thereby contributing to membrane integrity. (Note: absence of cytoskeletal protein 4.1 is associated with reduced glycophorin C and D and leads to hereditary elliptocytosis.)
- Antibodies to glycophorin-A-associated blood group antigens are generally IgM and clinically insignificant while antibodies to glycophorin B-, C-, and D-associated blood group antigens are generally IgG and clinically significant.



Question 26: C. Anti-Lu^a can be naturally occurring and can be of the IgG and IgA isotypes.

Explanation:

- Lu^b antigen is ubiquitous in most populations, whereas Lu^a is less common, with highest prevalence in those of European and African ancestry (approximately 8%). Consequently, antibodies to Lu^a are more frequently encountered than antibodies to Lu^b.
- Lutheran antibodies are usually of the IgG subtype; however, they are generally associated with mild, delayed hemolytic transfusion reactions and have not been reported to incite severe HDFN.
- Lu^a antibody can also be naturally occurring. When it is, it is usually of the IgM isotype, but can be IgG and/or IgA as well.
- Lutheran antibodies are usually best detected by indirect antibody testing.



Question 27: B. K+k-.

Explanation:

- The frequency of k, Kp^b, and Js^b is very high in the population; whereas, the frequency of K, Kp^a, and Js^a is generally low.
- The following table provides the frequencies of the more common Kell phenotypes.

Phenotype	Frequency in Population of European Ancestry (%) [*]	Frequency in Population of African Ancestry (%) [*]
K-k+	91	98
K+k+	8.8	2
K+k-	0.2	Rare
Kp(a-b+)	97.7	100
Kp(a+b+)	2.3	Rare
Kp(a+b-)	Rare	0



Phenotype	Frequency in Population of European Ancestry (%)*	Frequency in Population of African Ancestry (%)*
Js(a-b+)	100	80
Js(a+b+)	Rare	19
Js(a+b-)	0	1

*Percentages are approximate.



Question 28: D. 54-55 units.

Explanation:

- This patient has antibodies to anti-e and anti-K, and thus, he needs a unit that is both e-negative and K-negative.
- To determine the frequency of antigen-negative units, first you need to know the frequency of the antigen(s) of interest. In this case, e and K. In the donor population, e has a frequency of approximately 98% and K has a frequency of approximately 9%. (The table below provides frequencies of the common non-Rh blood group antigens in the donor population). The antigen-negative frequency = 1 minus the antigen frequency. Thus, the antigen-negative frequency for e = 1 – 0.98 (0.02), and the antigen-negative frequency for K = 1 – 0.09 (0.91).

Blood Group Antigen	Frequency in US Donor Population (%)*
K	9
K	98
Jk ^a	75
Jk ^b	75
Fy ^a	65 (10% in those of African ancestry)
Fy ^b	83 (20% in those of African ancestry)
M	80



Blood Group Antigen	Frequency in US Donor Population (%) [*]
N	72
S	55
s	90

^{*}Percentages are approximate.

- Next, you need to determine the frequency of finding a unit that is negative for both e and K. This is simply the product of the antigen-negative frequencies ($0.02 \times 0.91 = 0.0182$).
- Finally, in order to calculate the number of units that would need to be crossmatched in order to find compatible units, divide the number of compatible units desired by the product of the antigen-negative frequencies. In this case, only one unit is needed, so this would be $1/0.0182 = 54.9$. Thus, for this patient, approximately 55 random donor units would need to be crossmatched in order to find one compatible unit. In practice, crossmatches would generally be limited to prescreened units that had already been phenotyped.



Question 29: D. K+ red cell treatment with papain.

Explanation:

- The Kell antigens (present on the Kell glycoprotein) are resistant to enzymes including ficin, papain, trypsin, and alpha-chymotrypsin. However, due to the presence of the disulfide bonds, which are responsible for the folding of the extracellular domain, the protein is sensitive to reducing agents such as DTT and AET. The K antigen is also sensitive and destroyed by EDTA glycine.
- The K_0 null phenotype is due to a variety of mutations (eg, splice-site, missense, nonsense) and results in the complete absence of Kell antigens.
- Kp^a expression is rare and associated with mutations that appear to lead to decreased Kell antigen expression, which is notable in Kp^a/Kp^a homozygosity.



- The Kx protein, which is an integral membrane protein, is linked via a disulfide bond to the Kell glycoprotein. If the Kx protein is absent, Kell expression is reduced.
 - For unknown reasons, absence of the Gerbich (Ge2 and Ge3) antigens, located on glycophorin C and D, is associated with decreased Kell expression.
-

Question 30: E. Anemia.**Explanation:**

- Anti-K is predominantly IgG1 and capable of crossing the placenta and causing HDFN.
 - Unlike HDFN due to other blood group antibodies, anti-K is not characterized by the degree of hemolysis and the subsequent accumulation of bilirubin in the amniotic fluid.
 - Additionally, the degree of red cell turnover, as measured by reticulocytosis and erythroblastosis, is also not as prominent in anti-K HDFN compared to HDFN due to other blood group antibodies, such as anti-D.
 - The K antigen expression appears early in erythroid development and is present on red cell progenitors. It is thought that anti-K leads to the clearance of early red cell precursors, which have yet to produce hemoglobin. Thus, the products of hemolysis, such as bilirubin, are not prominent. Simultaneously, the removal of red cell precursors leads to a prominent anemia without accompanying reticulocytosis and erythroblastosis.
-

Question 31: E. Development of anti-Km.**Explanation:**

- The McLeod syndrome is X-linked and thus due to deletions or inactivation mutations of the X chromosome, specifically the XK gene, which encodes the Kx protein. Chromosome 7q is the location of the KEL gene, composed of 19 exons.
- McLeod syndrome can present with a wide range of neurologic, muscular, and psychiatric symptoms; and it can be associated



with spur cells (ie, acanthocytes). Stomatocytosis is a characteristic of the Rh_{null} phenotype.

- Individuals with the K₀ phenotype can produce anti-Ku (ie, anti-KEL5), which is reactive with all non-K₀ cells, even those with the McLeod phenotype.
- Anti-Km can be produced by all individuals with McLeod syndrome after transfusion. This antibody will be compatible with McLeod phenotype red cells and K0 phenotype red cells.
- Anti-Kx can be seen in those with McLeod syndrome but only when in conjunction with chronic granulomatous disease (CGD), which is due to mutations/deletions of the CYBB gene that encodes a subunit of NADPH oxidase. CYBB is also located on the X chromosome and can be co-deleted with the XK gene. Such co-deletion will lead to CGD individuals with McLeod syndrome, who can produce anti-Kx in addition to anti-Km.



Question 32: D. Fy(a–b–).

Explanation:

- Individuals of African ancestry have three possible alleles at the DARC locus (gene-encoding Duffy glycoprotein): Fy^a, Fy^b, and Fy.
- The Fy allele results in no red cell Duffy protein expression due to a mutation that disrupts the DARC gene promoter region binding site of the erythroid specific GATA-1 transcription factor. This mutation is usually found in genes with the Fy^b sequence.
- When homozygous, this mutation results in the Fy(a–b–) phenotype on red cells.
- Given that this mutation is erythroid specific, other cells (ie, non-red cells) in the body can still express the Duffy protein. Thus, these individuals are not at risk of producing anti-Fy^b.
- Fy^x is a weak variant of Fy^b with reduced protein expression on red cells.



Question 33: A. Anti-Fy^a and anti-Fy^b have decreased reactivity with enzyme-treated cells.



Explanation:

- Fy^a and Fy^b are sensitive to enzyme treatment. Antibodies directed against these antigens will be less reactive with red cells treated with enzymes. Interestingly, FY3 and FY5 are not sensitive to enzyme treatment; thus, no difference in reactivity would be expected between enzyme-treated and untreated red cells.
- Anti-Fy^a and anti-Fy^b are of the IgG1 subtype and generated after exposure; rarely are they naturally occurring.
- Anti-Fy^a are approximately 20 times more commonly encountered than anti-Fy^b.
- Although anti-Fy^a and anti-Fy^b are associated with hemolytic transfusion reactions, they are generally mild in severity. HDFN due to Duffy antibodies ranges from mild to severe.
- Anti-Fy3 reacts with all red cells except Fy(a–b–) cells.



Question 34: D. Unchanged; increased; decreased.

Explanation:

- Proteolytic enzymes can help identify antibody specificities, especially when more than one antibody is suspected.
- Proteolytic enzymes will generally cleave antigens with larger extracellular portions. Thus, the reactivity of these proteins with corresponding antibodies is diminished.
- Antigens that are more membrane bound are largely left intact. Thus, when larger extracellular components are cleaved from the protein, reactivity of the underlying membrane-bound antigens is increased with corresponding antibodies.
- Reactivity of some antigens is left unaffected as they are not cleaved by proteolytic enzymes, and their reactivity is not further increased by cleavage of the other enzyme-sensitive antigens. Such is the case with the K antigen. However, the K antigen is destroyed by reducing agents such as DTT and AET.
- The following table provides the expected reactivity of common red cell antigens when treated with typical proteolytic enzymes:



Enhanced by Enzyme	Diminished by Enzyme	Unaffected by Enzyme
ABO/H	MNS	Kell
Lewis	Duffy	Diego
I	Lutheran*	Colton
P	Chido	
Rh	Rodgers	
Kidd	Yt ^a	

*Lutheran antigens resist ficin and papain treatment but are sensitive to trypsin and alpha-chymotrypsin.

—————
Question 35: A. Jk(a–b–); resistant.

Explanation:

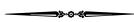
- There are three antigens within the Kidd system: Jk^a, Jk^b, and Jk3.
- The Kidd null phenotype, Jk(a–b–) is usually due to Jk3 homozygosity. This phenotype is very rare in most populations but is most prevalent in individuals of Polynesian ethnicity (1/400), especially those of Niuean ancestry (1.4%).
- Individuals with the Kidd-null phenotype can make anti-Jk3 after transfusion.
- Kidd antigens are part of the urea transporter (human urea transporter 11) on red cells and help take up urea to prevent crenation (shriveling/shrinking) in hypertonic solutions. When placed in 2M urea, the Kidd antigens will take up urea, leading to an osmotic influx and subsequent lysis of the cell. Contrarily, Jk(a–b–) will not take up urea and would be resistant to 2M urea.



Question 36: E. Approximately half of anti-Jk^a and anti-Jk^b bind complement.

Explanation:

- Anti-Jk^a and anti-Jk^b do cause extravascular hemolysis, however, this would not necessarily explain their ability to cause severe acute HTR.
- Anti-Jk^a and anti-Jk^b are predominantly IgG1 and IgG3, both of which are capable of binding complement. This can lead to intravascular hemolysis as is seen in acute HTR. Some anti-Jk^a and anti-Jk^b are IgM, which also readily binds complement. Together, approximately 50% of anti-Jk^a and anti-Jk^b can bind complement.
- Although some anti-Jk^a and anti-Jk^b are IgG2 and IgG4, these subtypes are not good binders of complement and would not lead to intravascular hemolysis.
- Anti-Jk^a and anti-Jk^b are capable of directly binding to Jk^a- and Jk^b-positive red cells, but they generally require antiglobulin test for detection due to low titers. This characteristic would not help explain the pathogenicity of the antibodies in severe acute HTR.



Question 37: B. Diego system.

Explanation:

- Band 3 is home to the Diego system, consisting of the two name-sake antigens—the low-prevalence Di^a and high-prevalence Di^b. Antibodies to Di^a and Di^b are primarily IgG1 and IgG3 antibodies. They are not generally associated with hemolytic transfusion reactions but can cause hemolytic disease of the fetus and newborn.
- Other antigens in the Diego system include Wr^a, Wr^b, Fr^a, BOW/NFLD, Wd^a, Vg^a, Wu/Disk, Hg^a/Mo^a, and Sw^a/SW1. Wr^a is low prevalence and Wr^b is high prevalence; however, Wr^b is not expressed when glycophorin A (GPA) is also absent, suggesting a close association between Band 3 and GPA.
- The YT system is located on acetylcholinesterase, a neurotransmitter enzyme with unknown function on red cells.
- The Dombrock protein has an adenosine diphosphate ribosyl-transferase structure.



- The Colton system is located on water channel Aquaporin-1.
 - The Cromer system is located on CD55 or decay accelerating factor (DAF), a complement-regulator protein.
-

Question 38: D. Diego system.

Explanation:

- The Wr^a antigen is part of the Diego system, which is located on Band 3. Wr^a is of low prevalence, while Wr^b is of high prevalence. Given its low prevalence, provision of antigen-negative units should not pose an issue, except that those antisera are not always readily available.
 - Antibodies to Wr^a can be clinically significant as they have been implicated in hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. Thus, an IAT crossmatch is required before transfusion.
 - Wr^a antibodies can demonstrate broad reactivity, as they can be reactive at immediate spin, IAT phase, and at 37 C.
 - Antigens in the Rh system include D, C, E, c, e, C^w, f, and G.
 - Antigens in the Kell system include K, k, Kp^a, Kp^b, and Kx.
 - Antigens in the Vel system include Vel.
 - Antigens in the MNS system include M, N, S, s, and U.
-

Question 39: C. Gy(a-).

Explanation:

- The Dombrock system is composed of the antithetical Do^a and Do^b antigens along with high-prevalence antigens Jo^a (Joseph), Hy (Holley), Gy^a (Gregory), DOLG, DOMR, and DOYA. These antigens are located on a protein with an adenosine-diphosphate-ribosyltransferase structure that is encoded by the ART4 gene.
- The Dombrock-null phenotype, Gy(a-), lacks not only the Gy^a antigen but also the Do^a, Do^b, Jo^a, and Hy antigens. Sensitized individuals with the Dombrock-null phenotype can generate anti-Gy^a, which would be reactive with most other Dombrock phenotypes.



- Hy- and Jo(a-) phenotypes can still express some Do^b, although weakly, and Jo(a-) can also weakly express Do^a.
- Although Do(a-b-) would appear to indicate absence of Do^a and Do^b, it is not a Dombrock phenotype. The DOYA- phenotype is Do^a- and Do^b-negative, but weakly expresses the Gy^a, Jo^a, and Hy antigens.



Question 40: A. Rh-positive cord red cells treated with ficin.

Explanation:

- The Landsteiner-Wiener (LW) system contains the antithetical LW^a (high prevalence) and LW^b (low prevalence) antigens, among others. The LW protein is also known as the intercellular adhesion molecule-4 (ICAM-4), which is part of Band3/Rh-cytoskeleton complex and is thought to function in red-cell-vascular endothelium interaction.
- Given its close relationship with Rh, LW expression is higher on Rh-positive red cells. LW expression is also high on cord red cells compared to adult red cells.
- LW expression is sensitive to pronase but is resistant to ficin, papain, trypsin, and alpha-chymotrypsin. DTT will destroy/reduce LW^a.
- Antibodies to LW antigens are not generally clinically significant, rarely implicated in hemolytic transfusion reactions or hemolytic disease of the fetus and newborn.



Question 41: D. Incubation with plasma – reactivity inhibited.

Explanation:

- Chido and Rodgers antigens are not produced on red cells, but rather are part of the C4d complement component. These antigens attach to the erythrocytes from plasma.
- Antibodies to Chido/Rodgers antigens are of the IgG subclass but are not considered clinically significant as they do not induce HTRs or HDFN.



- Chido/Rodgers antibodies are best detected by IAT, and reactivity is diminished by treatment with ficin or papain, as the antigens are proteolytic enzyme sensitive.
 - Chido/Rodgers antibodies can be inhibited by plasma, as they will bind to the complement fragments found in plasma. In this case, plasma acts as a neutralizing substance. Breast milk is a neutralizing substance that will inhibit anti-I reactivity.
-

Question 42: E. GE: -2,-3,-4.

Explanation:

- The Gerbich system antigens are located on glycophorin C (GPC) and/or glycophorin D (GPD), which are both encoded by the gene GYPC. Translation of GPC or GPD is dependent on the initiation site.
 - The cytoplasmic domains of GPC and GPD interact with the cytoskeleton of the red cell. The interaction that GPC and GPD provides between the membrane and skeleton may explain why cells that have the Gerbich-null phenotype (ie, negative for the high-prevalence Ge2, Ge3, and Ge4 antigens), have an elliptical shape.
 - GE:-2, 3, 4 and GE: -2,-3, 4 are two other phenotypes that are negative for high-prevalence antigens.
 - GE:5 and GE:6 are phenotypes that are positive for the low-prevalence antigens Wb and Ls^a, respectively.
 - Antibodies to most Gerbich antigens are not considered clinically significant, as they do not induce hemolytic transfusion reactions and rarely induce hemolytic disease of the fetus and newborn.
-

Question 43: E. Cromer antibodies are considered clinically insignificant.

Explanation:

- Absence of CD55 is insufficient to lead to spontaneous hemolysis due to redundancy of another complement-regulatory protein, CD59, which is closely linked to CD55 via a glycosylphosphati-



dylinositol (GPI) anchor. Recall that defects in GPI can lead to absence of CD55 and CD59, leading to paroxysmal nocturnal hemoglobinuria, in which pathologic auto-hemolysis is observed.

- Individuals with the Cromer-null phenotype can produce an anti-IFC, but this antibody is reactive with all cells minus other Cromer-null red cells.
- Cromer antigens are resistant to ficin, papain, and trypsin; however, they are sensitive to alpha-chymotrypsin. DTT only slightly reduces expression of Cromer antigens.
- Cromer antibodies are considered clinically insignificant as they have not been definitively demonstrated to cause hemolytic transfusion reactions. Protection against hemolytic disease of the fetus and newborn is attributed to antibody absorption/sequestration due to high placental CD55 expression.



Question 44: A. Gil system.

Explanation:

The table (pages 232-233) provides highlights on the blood group systems provided as answer choices in this question.



Question 45: D. John Milton Hagen system.

Explanation:

- The table (pages 232-233) provides highlights on the blood group systems provided as answer choices in this question.



Blood Group System	Location	Antigens	Enzyme and Reducing Agent Treatment	Antibody Clinically Significant (Y/N)	Additional Notes
Gil	Aquaporin 3 (AQP3)	Consists of one high-prevalence antigen: Gil	Resistant: proteolytic enzyme, DTT	N	
Raph	Tetraspanin (CD151)	MER2 (RAPH1) – 92% prevalence	Resistant: papain Sensitive: trypsin, pronase, DTT	N	CD151 deficiency: sensorineural deafness, ESRD, pretibial epidermolysis bullosa
KN	Complement receptor 1 (CR1 or CD35)	High prevalence: Kn ^a , McC ^a , S11, Yk ^a , S13, KCAM Low prevalence: Kn ^b , MCC ^b , S12	Resistant: papain, ficin Sensitive: trypsin, DTT	N	McC ^b and S12 may be protective against <i>P. falciparum</i>
IN	CD44 (receptor for hyaluronan)	High prevalence: In ^b , INF1, INJA Low prevalence: In ^a	Sensitive: ficin, papain, trypsin, DTT	N	AnWi, may be located on CD44, and is resistant to enzymes. Anti-AnWj is clinically significant
Vel	Small integral protein 1 (SMIM1)	High-prevalence antigen: Vel	Resistant: proteolytic enzyme Sensitive: DTT	Y (mild to severe HTR, rare HDFN)	Anti-Vel: often a mix of IgM and IgG



Blood Group System	Location	Antigens	Enzyme and Reducing Agent Treatment	Antibody Clinically Significant (Y/N)	Additional Notes
Lan	ABCB6 (ATP-binding cassette transporter)	High-prevalence antigen: Lan	Resistant: proteo-lytic enzyme, DTT	Y (mild to severe HTR, rare HDFN)	
JR	ABCG2 (ATP-binding cassette transporter)	High-prevalence antigen: J ^a	Resistant: proteo-lytic enzyme, DTT	Y (mild to severe HTR, rare HDFN)	Associated with resistance to cancer drugs and xenobiotics
YT	Acetylcholinesterase	High-prevalence antigen: Y ^a Low-prevalence antigen: Y ^b	Resistant: trypsin Sensitive: ficin, papain, DTT	N (but anti- Y ^a reported in HTR)	
John Milton Hagen	Semaphorin glycoprotein CD108 (Sema7A)	High prevalence: JM _H , JM _{MH} , JM _{HL} , JM _{MG} , JM _{MN} , JM _{MQ}	Sensitive: proteo-lytic enzyme, DTT	N	Elderly can have loss of CD108, can make anti-JMH associated with weak-positive DAT
OK	CD147 (Basigin)	High prevalence: Ok ^a , OKGV, OKVM	Resistant: proteo-lytic enzyme, DTT	Unknown	Antibodies have been detected only a few times (Basigin is a <i>P. falciparum</i> receptor)



Question 46: C. The genetic basis of antigens within blood group collections is usually uncharacterized.

Explanation:

- Blood group collections are groups of antigens that are serologically, biochemically, or genetically similar; however, the exact genetic underpinnings have yet to be elucidated.
 - Collections differ from systems in that blood group systems contain one or more antigens that are mapped to a gene or group of linked homologous genes. Not only have these genes been identified and sequenced, but they also have been demonstrated to affect phenotype.
 - Antigens within a blood group collection are generally either of very low or very high prevalence.
-

Question 47: B. Antigens within the 700 series are of very low prevalence, generally found in <1% of most populations.

Explanation:

- Antigens within the 700 series do not fit the criteria to belong to a blood group system or collection. The 700 series antigens all have very low prevalence, generally found in <1% of most populations.
- In contrast, antigens within the 901 series have very high prevalence, generally found in >99% of most populations.
- Antigens within the 700 series include: JVF, HJK, JONES, REIT, Kg, Chr^a, Bx^a, Bi, Li^a, Je^a, and To^a among others.
- Given their low prevalence, detecting antibodies may be difficult because most reagent red cells will lack the antigen. However, once detected, finding compatible units is not difficult, as the majority of donors will be negative for the corresponding antigen.
- Antibodies to the antigens within the 700 series can be clinically significant, as some have been reported to cause hemolytic disease of the fetus and newborn.
- The 700 series has several antigens, however, there are only a handful or so antigens to date that constitute the 901 series.

**Question 48: B.** HLA-B7 – Bg^a.**Explanation:**

- Although generally only nucleated cells express Class I HLA proteins, some mature red cells express Class I HLA proteins termed Bg antigens. These antigens are thought to be vestigial remnants of HLA antigens.
- It is important to note that just because an individual expresses the corresponding HLA proteins on other cells does not necessarily mean these will also be expressed on the red cells; but some HLA types are better expressed than others (see table below demonstrating Bg name with its corresponding HLA protein).

Bg	HLA
Bg ^a	B7
Bg ^b	B17 (B57 or B58)*
Bg ^c	A28 (A68 or A69) [†]

*B17 is the parent antigen of B57 and B58. In other words, B57 and B58 are splits of B17. A Bg^b antibody could be reactive with a cell that expresses a B57 or B58.
†A28 is the parent antigen of A68 and A69. In other words, A68 and A69 are splits of A28. A Bg^c antibody could be reactive with a cell that expresses either a A68 or A69. A68 and A69 are also cross-reactive with A2; thus, a Bg^c antibody could also react with a cell that expresses A2.

- Bg antigens are resistant to proteolytic enzymes and reducing agents such as DTT and AET; however, they can be removed from the red cell membrane by EDTA/glycine-HCL or chloroquine.
- Bg antibodies have been reported in cases of HTR.

**Question 49: D.** Poliovirus.



Explanation:

- Many blood group antigens are thought to function as receptors for microbial pathogens. The following table summarizes blood group antigens and the microbes for which they are the reputed receptor.

Blood Group Antigen	Microbial Pathogen
P	Parvovirus B19
Anton (AnWj)	<i>Haemophilus influenzae</i>
Cromer	<i>E. coli</i> , Coxsackievirus, ECHO virus
Indian	Poliovirus
Duffy	<i>Plasmodium vivax</i>
Glycophorin A/B (MNS)	<i>Plasmodium falciparum</i>



Question 50: C. Anti-M.

Explanation:

- Anti-M are generally IgM antibodies that are clinically insignificant, rarely implicated in hemolytic disease of the fetus and newborn or hemolytic transfusion reactions. As an IgM, anti-M will often react more strongly at immediate spin than at IAT. Additionally, warming the reaction to 37 C can eliminate reactivity.
- Anti-M can also cause weak reactivity on reverse typing, leading to ABO discrepancies. In the present case, the forward typing is consistent with group B, whereas the reverse typing is more consistent with group O. To resolve this discrepancy, the reverse typing can be carried out at 37 C or by using group A and B reagent cells that are also M negative.
- Although anti-i are IgM antibodies that would show up on immediate spin and diminish with prewarming, they would not likely cause ABO discrepancy or react with reagent cells, as the i-antigen expression is not normally found on adult red cells.



- Anti-Jk^a is a clinically significant antibody that is an IgG. As such, it would not be expected to react at immediate spin and would be stronger on IAT. Also, prewarming would not diminish reactivity, but could rather increase it. Moreover, anti-Jk^a would not lead to the ABO discrepancy detailed in this question.
- Cold autoantibodies are known to cause ABO discrepancies. They are also of the IgM subclass, which are reactive at immediate spin and usually nonreactive at 37 C. In cases of cold autoantibody, the autocontrol would likely be positive.
- Even though an antibody to a group B subtype could cause the ABO discrepancy in the question stem, these are rarer than antibodies to group A subtypes. Additionally, as all panel cells are group O, anti-B would not be detected.

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6

Pretransfusion Testing and Antibody Identification

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- A clinically significant red cell antibody is one that is frequently associated with hemolytic disease of the fetus and newborn, hemolytic transfusion reactions, or a noticeable decrease in the survival of transfused red cells.
- Before starting antibody identification testing, it is important to consider the patient's medical history including transfusions, pregnancies, transplantations, diagnoses, drugs, immunotherapies, and biologic therapies.
- Biologic therapies are expanding and use of novel therapies may affect serologic testing in the future.
- An antibody may be tentatively excluded or ruled out if an antigen is present on a reagent cell and the patient's serum or plasma is not reactive with it.
- The use of two reactive and two nonreactive red cell samples is the very minimum acceptable for antibody confirmation.
- DNA-based methods are used to resolve conflicting results in antibody identification or in serologic-based vs genotyping-based phenotype discrepancies.



QUESTIONS

Question 1: According to the AABB *Standards*, ABO grouping and Rh typing should be performed with each collection. If the initial test with anti-D is negative, what should be done?

- A. Label the unit as D negative.
 - B. Test the blood again with a method to detect weak D and if positive, label it weak D.
 - C. Test the blood again with a method to detect weak D and if positive, label it D positive.
 - D. Repeat the testing again with the same reagent and if negative, label it D negative.
 - E. Send the unit for molecular testing.
-

Question 2: Per AABB Standard 5.8.4, units may be labeled as antigen negative without testing the current donation under what conditions?

- A. One previous donation from the same donor was tested for the antigen(s).
 - B. Two previous donations from the same donor were tested for the antigen(s) and found to be concordant.
 - C. Three previous donations from the same donor were tested for the antigen(s) and found to be concordant.
 - D. Four previous donations from the same donor were tested for the antigen(s) and found to be concordant.
 - E. Five previous donations from the same donor were tested for the antigen(s) and found to be concordant.
-

Question 3: A sample of blood from each allogeneic donation must be tested for which of the following?

- A. HBV RNA.
- B. HBsAg.
- C. Anti-HCV.
- D. HCV DNA.
- E. B and C.



Question 4: If a cytapheresis donor is dedicated to the support of a particular patient, when is testing required?

- A. On each donation product.
 - B. On the first donation and at least every week thereafter.
 - C. On the first donation and at least every 90 days thereafter.
 - D. On the first donation and at least every 30 days thereafter.
 - E. On the first donation and at least every 6 months thereafter.
-

Question 5: Autologous blood components that will be transfused outside the collection facility have to undergo which tests?

- A. WNV RNA.
 - B. Anti-HBc.
 - C. Anti-HCV.
 - D. Anti-HbsAg.
 - E. A, B, and C.
-

Question 6: When drawing blood from a patient for a type and screen, what must be present?

- A. The patient's name on the bracelet must match the name on the collection tube label.
 - B. The patient's MRN must match the MRN on the collection tube label.
 - C. The patient's name and gender must match the information on the collection tube label.
 - D. The patient's name and birth date must match the information on the collection tube label.
 - E. None of the above.
-

Question 7: Which of the following is true?

- A. Two unique identifiers must be on any patient sample.
- B. The label must be attached to the sample container before leaving the patient bedside.



- C. A way to identify the date and time as well as who collected the sample must exist.
 - D. A and B.
 - E. A, B, and C.
-

Question 8: Samples from a patient type and screen as well as segments from a transfused blood product must be retained for how many days after a transfusion?

- A. 3 days.
 - B. 7 days.
 - C. 14 days.
 - D. 21 days.
 - E. 30 days.
-

Question 9: When whole blood, red cell components, and granulocyte components are received by the hospital transfusion service from the blood bank, what must be done before release for transfusion?

- A. The ABO grouping of each unit using an attached segment must be repeated to verify the ABO group of the product.
 - B. The Rh type of each unit using an attached segment must be repeated to verify the Rh type of the product.
 - C. The Rh type of only the Rh-negative units using an attached segment must be repeated to verify the Rh type of the product.
 - D. A and B.
 - E. A and C.
-

Question 10: What testing is required before routine transfusion of a patient?

- A. ABO grouping and Rh typing.
- B. Antibody screen.
- C. HIV, HCV, and HBV testing.
- D. A, B, and C.
- E. A and B.



Question 11: Select the correct answer choice to fill in the blank. If a patient has been transfused in the previous ____ days, a new sample must be obtained from that patient within 3 days of transfusion.

- A. 7 days.
 - B. 14 days.
 - C. 30 days.
 - D. 90 days.
 - E. 180 days.
-

Question 12: A new patient who has never been typed by the transfusion service has been admitted to the hospital and has a hemoglobin level of 5 g/dL. An order for 2 units of Red Blood Cells (RBCs) is received. What must be done before release of the RBCs?

- A. Type and screen.
 - B. Type and screen and an additional typing on a sample collected at a different time from the first sample.
 - C. Type and screen and retesting of the same sample for typing as long as the patient identification was verified at the time of sample collection using an electronic identification system.
 - D. Type and screen and asking the patient to declare the known blood type.
 - E. B and C.
-

Question 13: According to AABB Standard 5.15.5, red cells in apheresis granulocytes and platelets shall be crossmatched unless the component is prepared by a method that results in less than what volume of red cells?

- A. 10 mL.
- B. 5 mL.
- C. 3 mL.
- D. 2 mL.
- E. 1 mL.



Question 14: Which statement relating to initial pretransfusion testing of neonates is *false*?

- A. A sample from the neonate needs to be tested for ABO and Rh.
 - B. The serum of the neonate or mother can be tested for antibodies.
 - C. Only the serum or plasma of the neonate can be tested for antibodies.
 - D. The plasma of the neonate or mother can be tested for antibodies.
 - E. Repeat testing is not required during the same admission or until the neonate reaches 4 months.
-

Question 15: Which of the below are accepted methods to prevent transfusion-associated graft-vs-host disease?

- A. Leukocyte reduction.
 - B. Irradiation.
 - C. HLA matching.
 - D. ABO/Rh testing and antigen matching.
 - E. Use of units from a close family member.
-

Question 16: Which of the following should be on a blood unit label released from the transfusion service for patient infusion?

- A. Two unique identifiers for the patient.
 - B. Donation identification number or pool number.
 - C. Initials of the blood bank staff releasing the unit.
 - D. Interpretation of the compatibility tests.
 - E. A, B, and D.
-

Question 17: Before release of any blood component, the transfusion service staff should perform a final check of the records and the blood component. They should check all of the following except:

- A. The patient's two unique identifiers, plus ABO group and Rh type.



- B. The date and time the product was received from the blood supplier.
 - C. Special transfusion requirements.
 - D. Expiration date of the product.
 - E. The crossmatch interpretation (if performed).
-

Question 18: All of the following requirements must be met for reissue of a component returned to the transfusion service except:

- A. The RBC component shows signs of only minor hemolysis.
 - B. The component must have been maintained at an acceptable temperature.
 - C. The component was visually inspected and found to be acceptable for reissue.
 - D. At least one sealed segment is still available for RBC components.
 - E. The container must be intact and not accessed or opened in any way.
-

Question 19: If blood components are emergently released before compatibility testing, what must be done?

- A. This would not happen; blood would not be released without compatibility testing.
- B. A tag should be attached very conspicuously to the unit to indicate that compatibility testing has not been completed.
- C. The attending/ordering physician must sign a statement before release of the blood components indicating that the situation is urgent enough to require release of blood components before the completion of compatibility testing.
- D. The blood bank physician must call and speak with the requesting physician and explain the importance of waiting for the completion of compatibility testing.
- E. All of the above.



Question 20: Before nonemergent transfusion of blood products, consent must be obtained. All of the following are required elements of consent according to the AABB Standard 5.28.1.1 except:

- A. The right to accept or refuse transfusion.
 - B. A description of the risks and benefits of transfusion.
 - C. The right to select blood products from specific donors such as unvaccinated donors.
 - D. The opportunity to ask questions.
 - E. A description of alternatives to transfusion.
-

Question 21: Which of the following would be acceptable for patient identification when drawing samples for pretransfusion testing?

- A. Identification by a third party, if the patient is unresponsive.
 - B. Patient's name on a medical alert identification bracelet.
 - C. Patient's name outside of the hospital room.
 - D. Patient's name on a whiteboard in his or her room.
 - E. Hospital chart at the foot of the patient's bed.
-

Question 22: Which of the following sample/patient pairs is acceptable for crossmatching a unit of RBCs?

- A. Sample drawn 2 days before anticipated transfusion; patient transfused 2 months ago.
 - B. Sample drawn 10 days before anticipated transfusion; pregnancy history is unknown.
 - C. Sample drawn 4 days before anticipated transfusion; patient delivered an infant 1 month ago.
 - D. Sample drawn 7 days before anticipated transfusion; transfusion history is unknown.
 - E. Sample drawn 5 days before anticipated transfusion; patient received RBCs 5 days ago.
-

Question 23: A 19-year-old female of African ancestry with sickle cell disease presents with acute chest syndrome. She is treated with intravenous fluids, oxygen, and analgesics. Her hemoglobin level is



6 g/dL. A red cell exchange procedure is planned and 8 RBC units are ordered. The patient is group O, Rh-positive. Her antibody workup is given in the accompanying table. What is the frequency of compatible donors of European ancestry?

- A. 10%.
 - B. 5.7%.
 - C. 4.0%.
 - D. 3.4%.
 - E. 2.2%.
-

Question 24: A type-and-crossmatch order is indicated with which of the following surgical procedures?

- A. Cholecystectomy.
 - B. Hip replacement surgery.
 - C. Inguinal hernia repair.
 - D. Routine caesarean section.
 - E. Excision of a facial seborrheic keratosis.
-

Question 25: Which of the following antibodies is least likely to be a naturally occurring antibody?

- A. Anti-P₁.
 - B. Anti-I.
 - C. Anti-Le^a.
 - D. Anti-D.
 - E. Anti-N.
-

Question 26: Which of the following antibodies is most likely to be a naturally occurring antibody?

- A. Anti-e.
- B. Anti-K.
- C. Anti-Jk^b.
- D. Anti-Fy^a.
- E. Anti-M.



Antibody Identification Panel, Question 23



Question 27: The antibody identified by the accompanying panel is directed against an antigen that is destroyed by which of the following?

- A. Bromelin.
 - B. Papain.
 - C. Ficin.
 - D. Dithiothreitol (DTT).
 - E. *Ulex europaeus*.
-

Question 28: Which of the following samples is acceptable for pre-transfusion testing?

- A. Sample labeled at the patient's bedside. Label contains the date, patient's full name, and hospital number.
 - B. Sample labeled at the patient's bedside. Label contains the patient's full name and hospital number.
 - C. Sample labeled at the nursing station. Label contains the date, patient's full name, hospital number, and phlebotomist's initials.
 - D. Sample labeled in the hallway outside of the patient's room. Label contains the date, patient's full name, hospital number, and phlebotomist's initials.
 - E. Sample labeled at the patient's bedside. Label contains the date, patient's full name, and phlebotomist's initials.
-

Question 29: The reactivity of which of the following antibodies is usually enhanced by the enzyme treatment of red cells?

- A. Anti-Fy^a.
 - B. Anti-E.
 - C. Anti-JMH.
 - D. Anti-N.
 - E. Anti-Pr.
-

Question 30: A 38-year-old female presents for bilateral mastectomy for breast cancer. The patient's history is notable for the trans-



Antibody Identification Panel, Question 27



fusion of 4 RBC units approximately 15 years ago for postpartum hemorrhage. Preoperative testing reveals a positive antibody screen. The antibody identification panel is provided. The most likely explanation for the positive antibody screen is:

- A. An alloantibody to E.
 - B. An alloantibody to k.
 - C. An alloantibody to Jk^a, E, and k.
 - D. An alloantibody to Js^b.
 - E. An autoantibody.
-

Question 31: Which of the following antigens is resistant to denaturation when red cells undergo enzyme treatment?

- A. Fy^a.
 - B. S.
 - C. N.
 - D. Ch^a.
 - E. Jk^b.
-

Question 32: Which of the following statements about enhancement media and potentiators is *true*?

- A. Albumin enhances antibody uptake by reducing the net negative charge of the red cell.
- B. Low-ionic-strength saline (LISS) enhances antibody uptake by reducing the zeta potential and allows increased attraction between positively charged antibodies and negatively charged red cells.
- C. Enzyme treatment enhances antibody uptake by decreasing the net negative charge of the red cell by removing sialic acid residues.
- D. Polybrene enhances the uptake of antibodies by neutralizing the negative charges of the sialic acid residues on red cells.
- E. Polyethylene glycol (PEG) enhances agglutination by decreasing the negative charge (zeta potential) around red cells.



Antibody Identification Panel, Question 30



Question 33: What is the correct identification of the antibody in the accompanying panel and what is the correct description?

- A. The antigens are located on a creatinine transporter.
 - B. Over time, the antibodies frequently fall to undetectable levels.
 - C. Antibodies to these antigens are not associated with hemolysis.
 - D. The antibodies are the most common cause of hemolytic disease of the fetus and newborn (HDFN).
 - E. Red cells lacking both the "a" allele and the "b" allele are common in people of African ancestry.
-

Question 34: A patient with an antibody to a high-incidence antigen has an emergency need for blood. The only available antigen-negative compatible units are ones for which testing for transfusion-transmitted diseases has not been completed. Which of the following statements is true?

- A. Additional testing would not be necessary if these were apheresis units of the same component from the same donor transfused to the same recipient within 30 days of the original unit that was fully tested.
 - B. The pending testing need not be completed if the units are transfused because harm has already been done if the units are infected.
 - C. The units should not be labeled to indicate the pending tests because it might alarm the patient.
 - D. It is not required that the physician requesting the transfusion be notified of reactive test results because the units have already been transfused.
 - E. A statement signed by the ordering physician is not needed to document emergency release because it will increase legal liability.
-

Question 35: A type and screen is ordered for a 27-year-old pregnant female. This is her second pregnancy. She was in a car accident when she was 17 years old and received 2 units of RBCs.



Antibody Identification Panel, Question 33

Cell	D	C	c	E	e	f	V	C ^w	K	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	JK ^a	JK ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	Xg ^b	IS	AHG	CC
I	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	0	0	0	+			
II	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+				
1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	3+				
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+				
3	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+				
4	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+				
5	0	+	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+				
6	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	1+				
7	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	1+				
8	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	1+				
9	0	0	+	0	+	+	0	0	0	0	+	0	0	+	0	0	0	+	0	0	+	0	0	+	0	0	+				
10	0	0	+	0	+	+	0	0	0	0	+	0	0	+	0	0	0	+	0	0	+	0	0	0	0	0	+				

Patient's cells



Which statement is true of the antibody identified in the accompanying panel?

- A. The antibody is not associated with a dosage effect.
 - B. The antibody typically shows decreased reactivity with proteolytic enzyme-treated reagent red cells.
 - C. Thirty percent of people of European ancestry can form the antibody on exposure to an immunizing event.
 - D. The antibody is an IgG immune antibody implicated in HDFN and hemolytic transfusion reactions.
 - E. Between 40% and 50% of donor units will be crossmatch-compatible with the patient.
-

Question 36: What complement component does the broad-spectrum or polyspecific antihuman globulin (AHG) reagent bind to?

- A. C1.
 - B. C2.
 - C. C3d.
 - D. C4.
 - E. C5.
-

Question 37: Polyspecific or broad-spectrum AHG must contain which of the following?

- A. Anti-IgA.
 - B. Anti-IgD.
 - C. Anti-IgE.
 - D. Anti-IgG.
 - E. Anti-IgM.
-

Question 38: A type and screen is performed on a 72-year-old male who required a cardiac bypass surgery. He has never been transfused before. Which of the following statements is true concerning the antibody identified in the accompanying panel?



Antibody Identification Panel, Question 35



- A. The frequency of the antigen is much lower in people of African ancestry than in people of European ancestry.
 - B. Reactivity is enhanced with enzyme-treated red cells.
 - C. It is not associated with a dosage effect.
 - D. It is usually an IgM antibody.
 - E. Reactivity is enhanced by adjusting the pH to 8.0.
-

Question 39: Which of the following lectin-antigen pairs is correct?

- A. *Vicia graminea* and the A₁ antigen.
 - B. *Bandeiraea simplicifolia* and the B antigen.
 - C. *Dolichos biflorus* and the T antigen.
 - D. *Salvia horminum* and the Tk antigen.
 - E. *Arachis hypogaea* and the H antigen.
-

Question 40: Which of the following represents an appropriate use of the direct antiglobulin test (DAT)?

- A. Forward-typing a patient to determine the ABO group.
 - B. Reverse-typing a patient to determine the ABO group.
 - C. Looking for bound immunoglobulin in a patient with drug-induced hemolysis.
 - D. Performing an antibody screen.
 - E. Performing a crossmatch.
-

Question 41: Many drugs have been implicated as a cause of immune-mediated hemolysis. Which of the following drug-related mechanisms for a reactive DAT result is *least* likely to be associated with overt hemolysis?

- A. Antibody formation against a drug, with absorption of the immune complex to the red cell membrane.
- B. Antibody formation against a red cell membrane component in association with the drug.
- C. Non-immunologic protein adsorption onto the red cell membrane caused by the drug.



Antibody Identification Panel, Question 38



- D. Antibody formation against the drug and a red cell membrane component.
 - E. Autoantibody formation in association with a drug.
-

Question 42: A 56-year-old female is scheduled for elective surgery tomorrow; her type and screen results are shown in the accompanying panel. What antibodies are suspected and what should be done next?

- A. Anti-C; nothing more is needed.
 - B. Anti-C; prepare two antigen-negative units for tomorrow.
 - C. Anti-C and anti-Lu^a; test an additional cell that expresses only Lu^a and is negative for C and Lu^b.
 - D. Anti-C and anti-Lu^a; nothing more is needed.
 - E. Anti-C and anti-Lu^a; prepare two antigen-negative units for tomorrow.
-

Question 43: Which of the following could cause a false-positive DAT result?

- A. IgA coating of the red cells.
 - B. Use of an EDTA specimen for testing.
 - C. IgM coating of the red cells.
 - D. Incubating the cells after the addition of the antiglobulin reagent.
 - E. Saline contaminated with colloidal silica.
-

Question 44: All of the following are examples of indirect antiglobulin tests (IATs) except:

- A. Antibody elution.
- B. AHG crossmatch.
- C. Weak-D testing.
- D. Antibody identification panel.
- E. Most antibody titration.



Antibody identification Panel, Question 42



Question 45: Which of the following is associated with a false-positive IAT result?

- A. Improper incubation temperature or time.
 - B. Too many or too few red cells used.
 - C. Undercentrifugation.
 - D. Too little serum added.
 - E. Sensitized red cells.
-

Question 46: A 36-year-old African-American female has a type and screen ordered as a routine prenatal test. This is her third pregnancy. Which antibody(s) is/are present in the accompanying panel?

- A. A warm autoantibody.
 - B. An antibody to a high-frequency antigen.
 - C. Anti-Fy^a and anti-Fy^b.
 - D. A cold autoantibody.
 - E. Anti-Kp^b.
-

Question 47: A computer crossmatch is appropriate for which of the following patients?

- A. Patient has a history of anti-Jk^b. Historic blood type is group O, Rh positive. Typing of current sample is group O, Rh positive. The antibody screening panel is negative.
- B. Patient has no known previous blood type or antibody screen results. Current antibody screen is negative. Patient types as group O, Rh negative. Typing on a second sample confirms the patient is group O, Rh negative.
- C. Patient has a history of a negative antibody screen. Historic blood type is group A, Rh positive. Current antibody screen is positive for anti-E. Current blood type is group A, Rh positive.
- D. Patient has no known previous blood type or antibody screen results. Current antibody screen is negative. Patient types as group B, Rh negative.
- E. Patient has a history of a negative antibody screen. There is no known history of a previous ABO group or Rh type. Current antibody screen is positive for an autoantibody as all screening



Antibody Identification Panel, Question 46



cells and the autocontrol are positive. Patient types as group A, Rh positive. Typing on a second sample confirms the patient is group A, Rh positive.



Question 48: Which of the following statements regarding rare units of blood is true?

- A. Most allogeneic donors will be compatible.
- B. Family members represent a potential source of rare blood donors.
- C. Most donor centers will have access to rare donor units in their inventories.
- D. Units that are negative for high-prevalence antigens are the only rare units for which it is appropriate to contact a rare donor inventory.
- E. Rare units are most frequently required in the setting of warm-reactive autoantibodies.



Question 49: Which of the following antigens is associated with polyagglutinability?

- A. V antigen.
- B. K antigen.
- C. Kx antigen.
- D. C antigen.
- E. T antigen.



Question 50: Which of the following crossmatch techniques would most likely detect the combination of anti-Do^a in the recipient and the Do^a antigen on red cells from a donor?

- A. Computer crossmatch.
- B. Minor crossmatch
- C. Immediate-spin crossmatch.
- D. Complete crossmatch.
- E. Platelet crossmatch.



ANSWERS

Question 1: C. Test the blood again with a method to detect weak D and if positive, label it D positive.

Explanation:

- Per AABB Standard 5.8.2, the blood should be tested a second time with a reagent that can detect weak D and if positive labeled as D positive.
 - If this second test is negative, the blood product can be labeled as D negative.
-

Question 2: B. Two previous donations from the same donor were tested for the antigen(s) and found to be concordant.

Explanation:

- Per AABB Standard 5.8.4, the unit can be labeled as antigen negative without testing the current unit if two previous units from the same donor from separate donations were tested and found to be concordant.
-

Question 3: E. B and C.

Explanation:

- Per AABB Standard 5.8.5, samples from allogeneic blood donors must be tested for HBV DNA, HBsAg, anti-HCV, HCV RNA, anti-HIV-1/2, HIV-1 RNA, anti-HTLV-I/II, WNV RNA, and syphilis by a serologic test.
-

Question 4: D. On the first donation and at least every 30 days thereafter.

**Explanation:**

- Per AABB Standard 5.8.5, testing shall be performed on the first donation and then at least every 30 days thereafter for a cytapheresis donor dedicated to support a particular patient.
-

Question 5: E, A, B, and C.**Explanation:**

- Autologous blood components that will be transfused outside the collection facility must be tested for HBV DNA, HBsAg, anti-HBc, anti-HCV, HCV RNA, anti-HIV-1/2, HIV-1 RNA, anti-HTLV-I/II, WNV RNA, and syphilis serologic test.
 - Testing for anti-HBsAg is not required as this will detect people who have received hepatitis B vaccination as well.
-

Question 6: D. The patient's name and birth date must match the information on the collection tube label.**Explanation:**

- At least two unique identifiers must be used to correctly identify that the sample came from the intended patient.
-

Question 7: E, A, B, and C.**Explanation:**

- Per AABB Standard 5.11.2, patient samples must have two unique identifiers.
- Additionally, the label must be attached to the blood collection tube before medical personnel leave the patient's bedside.
- Lastly, there must be a mechanism to identify the date, time, and person who collected the blood sample. This could be as easy as writing them on the tube and using the nurse's initials.



- The transfusion lab can accept only properly labeled tubes with all of the information. If an unlabeled tube arrives, it must be discarded. A nurse cannot come to the lab and affix a label to the tube.
-

Question 8: B. 7 days.

Explanation:

- The type and screen and segments from the transfused product must be kept for 7 days.
-

Question 9: E. A and C.

Explanation:

- When whole blood, red cell components, or granulocyte products are received from the blood bank, the ABO typing of each unit using an attached segment must be repeated to verify the ABO group of the component.
 - Additionally, the Rh typing of only Rh-negative units using an attached segment must be repeated to verify the Rh type of the component.
-

Question 10: E. A and B.

Explanation:

- Before transfusion with any blood component containing red cells, typing for ABO and Rh as well as antibody detection is required. For transfusion of platelets and/or plasma, only ABO grouping and Rh typing is required.
 - Notably, a type and screen test is typically ordered as a singular entity.
-



Question 11: D. 90 days.

Explanation:

- A new sample must be obtained from a patient within 3 days of transfusion if the patient has been transfused in the previous 3 months with any blood component containing red cells.
 - Additionally, a new sample must also be obtained within 3 days of transfusion if a patient has been pregnant within the previous 3 months.
 - Lastly, a sample must be obtained within 3 days of transfusion if the history of transfusion is unknown or unclear.
-

Question 12: E. B and C.

Explanation:

- A patient who has never been typed in a hospital or lab system requires two ABO/Rh typings before product release. This can be done as described in options B and C.
 - Initially a type and screen can be done and followed by an additional typing on a sample collected at a different time from the first sample.
 - Another option is a type and screen followed by type retesting of the same sample as long as the patient identification was verified at the time of sample collection using an electronic identification system.
 - Using the patient's declared ABO/Rh is not acceptable as the patient could be wrong.
-

Question 13: D. 2 mL.

Explanation:

- A crossmatch is required for all products containing greater than 2 mL of red cells.



Question 14: C. Only the serum or plasma of the neonate can be tested for antibodies.

Explanation:

- In initial pretransfusion testing of a neonate, a sample from the neonate needs to be tested for ABO and Rh. A sample from the mother cannot be used to test for the ABO and Rh.
- The serum of the neonate or mother can be tested for antibodies. Many neonates are small with very little blood volume and thus, the mother's serum or plasma can be used to screen for antibodies.
- Neonates are not able to make antibodies until about 4 months of age. Thus, a repeat screening for antibodies is not needed until the neonate turns 4 months as long as the neonate stays admitted to the hospital.



Question 15: B. Irradiation.

Explanation:

- To prevent transfusion-associated graft-vs-host disease, the only acceptable methods are either irradiation or use of a pathogen-reduction system that has been proven to inactivate leukocytes and is FDA approved.
- Leukocyte reduction does not inactivate the white cells, it only reduces the number.
- HLA-matched components and units from a close family member actually increase the risk of transfusion-associated graft-vs-host disease and require irradiation of the units before transfusion.



Question 16: E. A, B, and D.

Explanation:

- A blood container being released from the transfusion service for infusion into a patient should contain:
 - Two unique identifiers for the patient.



- The donation identification number or pool number.
 - Interpretation of the compatibility tests.
 - The initials of the blood bank staff releasing the unit is not needed.
 - The blood bank staff should visually inspect all blood components immediately before release.
-

Question 17: B. The date and time the product was received from the blood supplier.

Explanation:

- The staff should check all of the following:
 - The patient's two unique identifiers, ABO group, and Rh type.
 - Special transfusion requirements.
 - Expiration date of the product.
 - The crossmatch interpretation (if performed).
 - The donation identification number, donor ABO group, and the Rh type (if required).
 - The date and time of issue.
 - Visual inspection of the blood product.
 - The staff is not required to check the date and time that the product was received from the blood supplier.
-

Question 18: A. The RBC component shows signs of only minor hemolysis.

Explanation:

- An RBC unit returned with signs of minor hemolysis cannot be reissued, it would not pass visual inspection.
 - The other criteria are all requirements for reissue of a returned blood component.
-

Question 19: B. A tag should be attached very conspicuously to the unit to indicate that compatibility testing has not been completed.



Explanation:

- The attending/ordering physician must sign a statement before or after the release of the blood components indicating that the situation is urgent enough to require release of blood components before the completion of compatibility testing.
 - The blood bank physician does not have to call and speak with the requesting physician and explain the importance of waiting for compatibility testing to be completed.
 - The transfusion service medical director and the patient's doctor will be notified immediately of any abnormal test results that could affect patient safety.
-

Question 20: C. The right to select blood products from specific donors such as unvaccinated donors.

Explanation:

- The requirements for consent are the right to accept or refuse transfusion, a description of the risks and benefits of transfusion, the opportunity to ask questions, and a description of alternatives to transfusion.
 - The right to choose blood products from specific donors is not included and it is not possible to select products from specific donors unless the patient has certain people provide directed donations.
-

Question 21: A. Identification by a third party, if the patient is unresponsive.

Explanation:

- The most common cause of ABO-incompatible transfusion reactions is a clerical error. At least two-thirds of these errors occur outside of the laboratory, at the time of pretransfusion sample collection, or at the time of transfusion.
- When collecting blood samples, positive identification is required. This consists of one or more of the following:



- An official hospital wristband and/or unique identification band such as a blood bank or emergency room identification band.
 - Identification by a third party (if the patient is unresponsive).
 - The patient stating his or her name.
 - The patient's chart, name on a whiteboard in the patient's room, or name on the outside of the hospital room does not represent adequate identification.
-

Question 22: A. Sample drawn 2 days before anticipated transfusion; patient transfused 2 months ago.

Explanation:

- AABB *Standards* requires that samples be drawn within 3 days of transfusion if the patient has been transfused or pregnant within the past 3 months or if the transfusion and/or pregnancy history is unknown.
 - Many institutions, because of difficulties obtaining this information, will use a 3-day rule for all patients.
 - The goal of this requirement is to prevent delayed hemolytic transfusion reactions. Individuals with recent transfusions or pregnancies may be forming blood group antibodies that would not be detectable if samples are drawn too far in advance of the anticipated time of transfusion.
-

Question 23: C. 4.0%.

Explanation:

- Three antibodies are present:
 - Anti-K reacting at immediate-spin only.
 - Anti-Fy^a demonstrating dosage (cells 1 and 2 are heterozygous and are 1+, whereas cell 4 is homozygous and is 2+) and destroyed by enzymes.
 - Anti-Jk^b.
- To calculate the frequency of compatible donors, the frequency of antigen-negative donors for each antigen must be multiplied



together and then multiplied by 100. In this case, the equation is as follows:

Frequency of compatible donors = 0.45 (frequency of group O) × 1 (frequency of Rh-positive and -negative donors) × 0.35 (frequency of Fy^a-negative donors) × 0.91 (frequency of K-negative donors) × 0.28 (frequency of Jk^b-negative donors) × 100% = 4.0%.

Question 24: B. Hip replacement surgery.

Explanation:

- With a type-and-screen order, the recipient's ABO and D antigen types are determined and the serum is screened for clinically significant antibodies. The screen is performed by testing the potential recipient's serum against two or three commercially prepared group O red cell samples with a known antigenic makeup.
- A type-and-crossmatch order includes all of the elements of a type-and-screen order (see above); in addition, however, one or more RBC units are crossmatched so that they may be available immediately for those situations that are more likely to require transfusion.
- A type-and-screen order is appropriate for procedures that may require a transfusion but for which transfusion is not likely (eg, cholecystectomy and routine caesarean section). In those procedures for which a significant blood loss is not expected (eg, excision of a facial seborrheic keratosis and inguinal herniorrhaphy), a type-and-screen order is not indicated.
- If the antibody screen is negative and a transfusion is required, compatible RBCs can be provided very quickly, generally within 10 to 15 minutes, depending on the method of crossmatching used. This is predicated on having a blood sample in the blood bank for which the type and screen has been performed and the sample is still suitable for crossmatching.
- If the antibody screen is positive, an antibody identification panel is performed. In such a case, crossmatch-compatible, antigen-negative units can be placed on hold in case they are needed urgently.



- A maximum surgical blood ordering schedule (MSBOS) or other institutional guidance for transfusion during surgery should be helpful.
-

Question 25: D. Anti-D.**Explanation:**

- A mnemonic device for remembering naturally occurring antibodies: LIPMAN (Lewis, Ii, P, M, ABH, N).
- Naturally occurring antibodies are usually IgM, cold-reactive, and present in the serum of individuals who have had no known exposure to red cells expressing the antigen (eg, no previous transfusions or pregnancies).
- Naturally occurring antibodies result from exposure to antigens within the environment, which are also present on red cells. For example, anti-A results from exposure to the same antigen present on microbes within the gastrointestinal (GI) tract or antigens present on pollen.
- Immune IgG antibodies are produced by exposure to red cells expressing the foreign antigens. Sources of exposure include pregnancy, transfusion, and organ or hematopoietic progenitor cell transplantation.
- As a rule of thumb, naturally occurring IgM antibodies are directed against carbohydrate epitopes, whereas immune IgG antibodies are directed against peptide epitopes. The most common naturally occurring and immune antibodies are listed in the table below:

Naturally Occurring Antibodies	Immune Antibodies
Anti-A, anti-B, anti-A,B, anti-H	Antibodies to Rh system antigens
Anti-I	Antibodies to Kell system antigens
Anti-Le ^a , anti-Le ^b	Antibodies to Duffy system antigens
Anti-M, anti-N	Anti-S, anti-s
Anti-P ₁	Antibodies to Kidd system antigens

**Question 26: E. Anti-M.****Explanation:**

See answer #25 above.

Question 27: D. Dithiothreitol (DTT).**Explanation:**

- The antibody identified in the panel is anti-K. DTT is a sulphydryl-reducing agent that can be used to destroy Kell system antigens. Other such agents, including 2-mercaptoethanol (2ME), β -mercaptoethylamine, and 2-aminoethylisothiouronium bromide (AET), also denature Kell system antigens.
 - Kell system antigens are not affected by treatment with papain, ficin, or bromelin.
 - Trypsin and chymotrypsin, when used in combination, can denature Kell system antigens but do not do so separately.
 - Sulphydryl-reducing agents also denature other antigens, including LW^a, Do^a, Do^b, and Yt. DTT also denatures IgM antibodies by reducing the disulfide bond present in the joining chain of the IgM molecule.
 - *Ulex europaeus* is a lectin that binds to and causes the agglutination of red cells expressing the H antigen.
-

Question 28: A. Sample labeled at the patient's bedside. Label contains the date, patient's full name, and hospital number.**Explanation:**

- To minimize clerical errors and the risk of ABO-incompatible transfusions, AABB *Standards* requires the following:
 - The intended recipient shall be identified positively at the time of sample collection.
 - Labels shall bear sufficient information for the unique identification of the recipient and include two independent identifiers and the date the sample was collected.



- Labels shall be attached to the sample before leaving the bedside of the intended recipient.
 - There shall be a mechanism to identify the individual who drew the blood from the patient. This may involve the phlebotomist's initialing the sample, signing paperwork, or entering an identifier into the computer. It does not have to be initialing of the sample.
 - Before testing is performed, the identifying information on the specimen label must match all identifying information on the request form.
 - Answer B is incorrect because the required date is missing.
 - Answer C and D are incorrect because the sample was not labeled at the patient's bedside.
 - Answer E is incorrect because a second unique identifier such as a hospital number is missing.
-

Question 29: B. Anti-E.**Explanation:**

- Enzymes that either enhance or destroy antigen-antbody reactions can be very useful to identify the specificity of alloantibodies, particularly in situations in which there are complex mixtures of multiple alloantibodies. Enzymes cleave membrane glycoproteins and sialic acid residues. This cleavage removes a steric barrier that may enhance the reactivity of certain antibodies for their corresponding antigens. However, glycoproteins and sialic acid residues are critical components of some blood-group antigens, and their removal results in the destruction of these antigens.
- The effects of enzyme treatment on blood group antigen-antibody interactions are described in the table below:

Antibodies Enhanced by Enzyme Treatment	Antigens Denatured by Enzyme Treatment
P ₁ Rh system	M, N, S Fy ^a , Fy ^b , Fy ⁶



Antibodies Enhanced by Enzyme Treatment	Antigens Denatured by Enzyme Treatment
Le ^a , Le ^b	Ch ^a , Rg ^b , JMH
Jk ^a , Jk ^b	In ^b
I, i	Yt ^a
ABH	Pr
	Tn
	Xg ^a
	Gerbich 2 and 4
	Cromer

- Enzymes do not readily destroy s and U antigens of the MNSs blood group system. Enzymes have no effect on Kell, Lutheran, and Gerbich 3. Commonly used enzymes and their sources are listed in the following table:

Enzyme	Source
Ficin	Figs
Papain	Papaya
Trypsin	Pig stomach
Bromelin	Pineapple



Question 30: E. An autoantibody.

Explanation:

- The autocontrol (patient red cells incubated with patient serum) is reactive and all of the panel cells are reactive at the AHC



phase. The most likely explanation for these findings is a warm-reactive autoantibody. The autocontrol is roughly equivalent to the DAT; this suggests the presence of an autoantibody in the patient's serum.

- Another diagnostic possibility in this case is an antibody with high titer and low avidity characteristics; however, this is less likely because:
 - The autocontrol may or may not be reactive.
 - The autocontrol is usually only reactive if the antibody is anti-JMH.
 - The reactivity of all panel cells at the AHG phase will be w+ to 1+. In this case, the reactivity is 3+.
- Js^b and k are high-incidence antigens. Antibodies directed against these antigens could give a similar pattern on the antibody identification panel. However, the autocontrol would be negative.
- Many of the cells as well as the autocontrol would be negative if there was an alloantibody to E.
- Lastly, the autocontrol would also be negative if there were antibodies to three antigens and variation in agglutination strength would likely also be seen.



Question 31: E. Jk^b.

Explanation:

See answer #29.



Question 32: B. Low-ionic-strength saline (LISS) enhances antibody uptake by reducing the zeta potential and allows increased attraction between positively charged antibodies and negatively charged red cells.

Explanation:

- The process of agglutination can be divided into two phases:
 - Binding of the antibody to its target antigen.
 - Formation of a linked lattice between red cells.



- Numerous enhancing media and potentiators are used to facilitate this process.
- LISS enhances antibody uptake by reducing the zeta potential and allows increased attraction between positively charged antibodies and negatively charged red cells.
- PEG enhances antibody-antigen binding by excluding water from around the red cells, effectively concentrating the antibody and favoring binding to its target. IgM antibodies, such as ABO and Lewis, are weaker or not detected with PEG. Warm autoantibodies are greatly enhanced by PEG.
- Overall, PEG is more sensitive than LISS with respect to alloantibody detection.
- The false-positive rate is higher for PEG in comparison to LISS: 1.3% vs 0.1%.
- Polybrene enhances formation of the linked lattice by neutralizing the negative charges of the sialic acid residues on the red cell, allowing red cells to come closer together. The addition of sodium citrate can disperse the spontaneous agglutination induced by Polybrene. If an antibody is present, the red cells will be crosslinked and will not disperse. If no antibody is present, the red cells will disperse. Polybrene detects ABO antibodies as well as clinically significant alloantibodies.
- Albumin enhances linked lattice formation by reducing the net negative charge of the red cells, allowing the cells to come closer together.
- Enzyme treatment enhances linked lattice formation by decreasing the net negative charge of the red cells by removing sialic acid residues. This allows the red cells to come closer together.



Question 33: B. Over time, the antibodies frequently fall to undetectable levels.

Explanation:

- The antibody is anti-Jk^a, and it demonstrates a dosage effect. The Kidd blood group antigens, Jk^a and Jk^b, are located on the human erythroid urea transporter protein HUT1. This is supported by the fact that Jk(a-b-) red cells resist lysis when placed in 2M urea.
- Kidd antibodies are associated with hemolysis. Most are IgG3, and the Kidd antigens are clustered on the red cell membrane.



This could account for the complement activation and intravascular hemolysis sometimes seen with Kidd antibodies.

- Kidd antibodies tend to fall to undetectable levels over time. Because of this, Kidd antibodies are the most commonly implicated antibodies in delayed hemolytic transfusion reactions.
 - Because these antibodies are IgG and the antigens are expressed on fetal red cells, Kidd antibodies can cause HDFN. However, this is uncommon. The antibody most frequently implicated in this disease process is anti-D.
 - The blood group system associated with a lack of both the "a" and "b" alleles in people of African ancestry is the Duffy system (Fy), not the Kidd system.
-

Question 34: A. Additional testing would not be necessary if these were apheresis units of the same component from the same donor transfused to the same recipient within 30 days of the original unit that was fully tested.

Explanation:

- The AABB *Standards* require that units of blood released before the completion of testing designed to prevent disease transmission must:
 - Be conspicuously labeled with the testing that is not complete.
 - Have the testing completed as soon as possible.
 - Be accompanied by a statement signed by the requesting physician indicating that the clinical situation was urgent enough to require release before the completion of testing.
 - In the event that a unit must be released before the completion of all required infectious disease testing, if a test proves to be reactive, the transfusion service and the ordering physician must be notified as soon as possible.
 - For a cytapheresis donor dedicated to support a specific patient, testing must be performed before the first apheresis component is released for transfusion and at least every 30 days thereafter.
-

Question 35: D. The antibody is an IgG immune antibody implicated in HDFN and hemolytic transfusion reactions.

**Explanation:**

- The antibody identified by the panel is anti-E. Screening cell II and panel cells 3 and 6 are positive for the E antigen. A positive reaction is observed between the patient's serum and these three reagent cells at the antiglobulin phase of testing. A negative reaction is observed between the patient's serum and the nine reagent cells negative for the E antigen.
- E antibodies are typically IgG and are always considered to be clinically significant with respect to hemolysis.
- E antibodies frequently demonstrate a dosage effect when comparing the relative strengths of reactivity of red cells homozygous for E antigen expression (ie, EE) with those heterozygous for expression (ie, Ee). This effect may be more obvious with weakly reactive antibodies.
- Rh antigens are not denatured by enzyme treatment. The reactivity of Rh system antibodies may be enhanced when they are tested against enzyme-treated red cells.
- E-negative blood components are indicated for this patient. The red cells of approximately 70% of people of European ancestry and almost 80% of people of African ancestry type negative for the E antigen. Between 70% and 80% of all donor units will be crossmatch-compatible with the patient's serum.

**Question 36: C. C3d.****Explanation:**

- Polyspecific or broad-spectrum AHG must contain antihuman IgG and anti-C3d.

**Question 37: D. Anti-IgG.****Explanation:**

- Polyspecific or broad-spectrum AHG must contain antihuman IgG and anti-C3d.
- Polyspecific AHG reagent is used predominantly in the DAT.



- Polyspecific AHG is not routinely used in antibody screening or crossmatching because antibodies that are detectable only by their ability to fix complement are rare. In these tests, monospecific AHG, containing only anti-IgG activity, is used.
-

Question 38: D. It is usually an IgM antibody.

Explanation:

- It demonstrates a dosage effect.
 - Characteristics of anti-M and the M antigen include the following:
 - The antigen is destroyed by enzymes.
 - Antibody reactivity is enhanced by lowering the pH to 6.2.
 - Anti-M is most commonly IgM and naturally occurring.
 - The antibody may be glucose dependent (ie, glucose in the preservative of the screening cells may inhibit activity).
 - Anti-M can be clinically significant if there is an IgG component, but this is very rare.
 - Most antibodies to the M antigen are IgM and react best at room temperature.
 - The M antigen is present in 78% of people of European ancestry and 74% of people of African ancestry.
-

Question 39: B. *Bandeiraea simplicifolia* and the B antigen.

Explanation:

- Lectins are proteins, usually extracted from plants, that bind to specific carbohydrate antigens. They are sometimes used as an alternative to reagent antibodies. The specificity of the lectin can depend on the dilution of the lectin.
- Examples of lectins and the antigens that they bind include the following:
 - *Arachis hypogaea*, the peanut lectin, which binds to the T, Th, and Tk antigens.
 - *Salvia horminum* recognizes the Tn and Cad antigens but not the T, Th, and Tk antigens.



- *Dolichos biflorus* recognizes either the A1 antigen or Cad antigen, depending on the dilution.
 - *Vicia graminiae* has specificity for the N antigen.
 - *Ulex europaeus* binds to and causes the agglutination of red cells expressing the H antigen.
 - *Bandeiraea simplicifolia* recognizes the B antigen.
-

Question 40: C. Looking for bound immunoglobulin in a patient with drug-induced hemolysis.

Explanation:

- The DAT is used to demonstrate the in-vivo coating of red cells with antibody or complement.
- All of the choices except E involve antibody coating of red cells in vitro (ie, in the test tube).
- The DAT is appropriately used in clinical situations associated with immune-mediated hemolysis, which include the following:
 - Hemolytic disease of the fetus and newborn (HDFN).
 - Autoimmune hemolytic anemia.
 - Transfusion reactions.
 - Drug-induced hemolysis.



Question 41: C. Non-immunologic protein adsorption onto the red cell membrane caused by the drug.

Explanation:

- A wide variety of drugs have been associated with reactive DAT results, with or without overt hemolysis, although the majority of implicated drugs are antibiotics.
- Several mechanisms have been postulated to explain reactive DATs caused by drugs and drug-induced immune hemolytic anemia.
 - Some drugs (eg, penicillin and acyclovir) initiate an immune response that can be detected only with drug-treated red cells.



- Some drugs (eg, piperacillin and ceftriaxone) initiate an immune response that can be detected only with untreated red cells in the presence of a solution of the drug.
 - Some drugs (eg, α -methyldopa and procainamide) induce antibodies serologically indistinguishable from those detected in patients with warm-reactive autoantibodies.
 - The reactive DAT associated with some drugs (eg, cephalosporins, most commonly cephalothin) is caused by a mechanism independent of antibody production. These drugs alter the red cell membrane so there is non-immunologic protein adsorption to the membrane. This may result in a weakly-reactive DAT, due to adsorption of IgG and/or C3, but hemolytic anemia rarely results.
-

Question 42: C. Anti-C and anti-Lu^a; test an additional cell that expresses only Lu^a and is negative for C and Lu^b.

Explanation:

- The Lutheran blood group system is also known as a B-cell adhesion molecule (B-CAM). It is rare for a person to be Lu(a–b–). The frequency of Lu(a+b–) in most populations is 0.2%. Lu(a+b+) occurs at 7.4% and Lu(a–b+) occurs with 92.4% frequency.
 - Lu^a antibodies do not frequently cause hemolysis or transfusion reactions, but cases of hemolysis have been described.
 - To confirm or rule out an antibody, typically laboratories use a rule of two or three—meaning two or three positive cells that react and at least two to three negative cells for the antigen that do not react with the antibody. Typically, cell lines that are homozygous for the antigen in question are used for confirmation of the antibody. In this case, only one cell line was homozygous and the other one with Lu^a was heterozygous. An additional homozygous Lu^a cell line is desirable to confirm the Lu^a antibody.
 - Finding Lu^a negative blood will be difficult. The surgery may need to be postponed in order to find RBC units to have on hand or the patient could donate units in advance of her surgery.
-

Question 43: E. Saline contaminated with colloidal silica.



Explanation:

- The DAT demonstrates in-vivo coating of RBCs by antibody or complement. RBCs from the patient are washed, AHG reagent is added, and the cells are immediately centrifuged and examined for agglutination.
- Causes of false-positive DATs include:
 - Coating of the red cells by complement in vitro (eg, complement being fixed at cold temperatures during specimen storage).
 - Aggregation of RBCs by the gel present in serum separator tubes.
 - Complement fixation by 5% or 10% dextrose in intravenous solutions.
 - Septicemia or bacterial contamination of stored blood samples leading to T activation.
 - Contamination of the saline with materials that can cause spontaneous aggregation of RBCs (eg, colloidal silica from glass bottles).
 - Contamination through improperly cleaned glassware.
 - Overcentrifugation.
 - Improperly prepared AHG reagent (eg, containing anti-species antibodies).
- Causes of false-negative DATs include:
 - IgA or IgM coating the red cells, not IgG or complement (most AHG reagents do not detect IgA or IgM).
 - Incubating the red cells after addition of the AHG reagent, because this may cause the antibodies in the AHG reagent to disassociate.



Question 44: A. Antibody elution.

Explanation:

- An IAT demonstrates in-vitro reactions between RBCs and antibodies. The antibody and its target antigen are incubated together in the test tube, they bind, and this is detected via the addition of an AHG reagent.
- Examples of IATs include:
 - Weak-D testing.



- Antibody screens.
 - Antibody identification.
 - Compatibility testing.
 - Some antigen typings.
 - Most antibody titrations.
 - Antibody elution involves the removal of antibodies bound to red cells either in the patient or test tube. It does not involve the detection of antibodies using an AHG reagent.
-

Question 45: E. Sensitized red cells.

Explanation:

- The IAT procedure is performed by incubating patient serum or a reagent with either donor or patient red cells, depending upon the type of IAT performed. The cells are then washed and an AHG reagent is added. The cells are centrifuged and examined for agglutination. The presence of agglutination indicates that antibody has bound to the red cells.
- Causes of false-negative IAT:
 - Failure to remove unbound globulin so that it neutralizes the AHG reagent (ie, improper or inadequate red cell washing).
 - Loss of bound antibody with time due to delayed testing.
 - Loss of AHG reagent activity due to improper storage (extreme temperature), bacterial contamination, or contamination with human serum.
 - Failure to add the AHG reagent.
 - Undercentrifugation.
 - Too many or too few red cells used.
 - Prozone reactions.
 - Loss of reactivity of the antigens on the red cells (eg, out-of-date reagent RBCs).
 - No active complement present to detect IgM antibodies (eg, use of plasma instead of serum).
 - Improper incubation temperature or time.
 - Too little serum added (too little antibody present on the red cells to be detected by the AHG reagent).
- Causes of false-positive IAT:
 - Sensitized red cells (red cells coated with antibody before incubation).



- Contamination of saline with materials that can cause spontaneous aggregation of the red cells (eg, colloidal silica from glass bottles).
 - Contamination through improperly cleaned glassware.
 - Overcentrifugation.
 - Improperly prepared AHG reagent (containing anti-species antibodies).
-

Question 46: C. Anti-Fy^a and anti-Fy^b

Explanation:

- A warm autoantibody is not present as one cell line is negative and the patient's cells are negative. A cold autoantibody is not present because the IS phase is negative for all cell lines. Anti-Kp^b is unlikely as one cell line is negative and dosage is seen with cells positive for both Fy^a and Fy^b showing stronger agglutination. An antibody to a high-frequency antigen is possible but unlikely as antibodies to the Duffy group are present.
 - Duffy is the receptor for *Plasmodium vivax* and *Plasmodium knowlesi*. Individuals negative for Fy^a and Fy^b are resistant to these forms of malaria. The Fy(a–b–) phenotype is rare in populations of European, Chinese, and Japanese ancestry but very common in individuals of African ancestry (68%).
-

Question 47: B. Patient has no known previous blood type or antibody screen results. Current antibody screen is negative. Patient types as group O, Rh negative. Typing on a second sample confirms the patient is group O, Rh negative.

Explanation:

- The AABB *Standards* requires that if there is no record of previous detection of an antibody, at minimum, testing to detect ABO incompatibility must be performed. This requirement could be fulfilled through a complete crossmatch, an immediate-spin crossmatch, or a computer crossmatch. If an antibody is detected



or if there is history of an antibody, then a complete crossmatch must be performed.

- To perform a computer crossmatch: the computer system must be validated on site to ensure that only ABO-compatible red-cell-containing components can be selected for transfusion, and two determinations of the recipient's ABO group must have been made. This requirement may be fulfilled by retesting the current sample, testing a second current sample, or comparison of current testing with previous records. The computer system must contain the following information for the component to be transfused:
 - Donor unit number.
 - Component name.
 - ABO group.
 - Confirmed ABO group and Rh type.
- The computer system must contain the following information for the recipient of the component:
 - Two unique recipient identifiers.
 - ABO group.
 - Rh type.
 - Antibody screen results.
- A method must exist to verify correct data entry before the component is released. The computer system must contain logic to alert the user to discrepancies between the recipient's and the component's ABO and Rh types (ie, incompatibilities).



Question 48: B. Family members represent a potential source of rare blood donors.

Explanation:

- Rare donor units are not readily identified in routine allogeneic blood donors, which is what makes them rare. Rare units include those units that are negative for high-prevalence antigens as well as units that are negative for a combination of common antigens.
- The American Rare Donor Program (ARDP), a rare donor inventory, can assist in the identification of both types of rare units. Most blood centers will not readily have appropriate rare donor units in their inventories. It is a given that most, if not all, units crossmatched in patients with warm-reactive autoantibodies will



be incompatible, but this itself is not an indication for contacting a rare donor inventory.

- Rare units are reserved for those patients who have known antibodies to high-prevalence antigens or have multiple antibodies to common antigens. Family members are a potential source of rare blood donors, particularly when a unit lacking a high-prevalence antigen is required. Siblings from the same parents are often the best source of serologically compatible blood, since the absence of a high-prevalence antigen is usually associated with the inheritance of the same recessive blood group gene from each heterozygote parent.
- Other sources of rare units include the mother of an infant with HDFN (resulting from either multiple antibodies or an antibody to a high-prevalence antigen) and autologous RBC units obtained from patients with rare phenotypes who are expected to need blood in the future.



Question 49: E. T antigen.

Explanation:

- Polyagglutination occurs when most human sera agglutinate red cells. This results from naturally occurring antibodies directed against antigens on the cells. In the majority of instances, these are acquired antigens that represent the exposure of cryptic antigens (eg, T, Tk, Tn, Tx). C, K, Kx, and V are inherited red cell antigens not associated with polyagglutination.
- Antigens associated with polyagglutination are shown in the table below:

Antigen	Inherited/Acquired	Association
T	Acquired	Infection with <i>Pneumococcus</i> , <i>Clostridium perfringens</i> , <i>Vibrio cholera</i> , influenza virus
Tn	Acquired	Infection with <i>Clostridia</i> , <i>Bacteroides</i> , <i>E. coli</i> , or <i>Proteus</i>



Antigen	Inherited/Acquired	Association
Tk	Acquired	Infection with <i>Bacteroides fragilis</i> and <i>Serratia marcescens</i>
Tx	Acquired	Infection with <i>Pneumococci</i>
Cad	Inherited	None

**Question 50: D.** Complete crossmatch.**Explanation:**

- Anti-Do^a is almost always an IgG antibody. The antigen is expressed on red cells, lymphocytes, marrow, spleen, lymph nodes, intestine, ovary, testes, and fetal heart. It is not expressed on platelets, so a platelet crossmatch would not make sense.
- A complete crossmatch consists of testing at immediate spin followed by testing using an AHG reagent. This is also referred to commonly as the AHG crossmatch. The complete crossmatch will detect IgM antibodies as well as IgG antibodies.
- In a minor crossmatch, the donor's plasma is tested with the recipient's red cells to identify antibodies in the donor that could react with recipient red cells. This form of testing was used before the advent of component therapy, when whole blood, containing large amounts of plasma, was transfused. This type of crossmatching is not frequently performed today and would not be effective in the situation described.
- The immediate-spin crossmatch (also called an incomplete crossmatch) detects IgM antibodies, most importantly ABO antibodies. It is a rapid test. In patients with negative antibody screens, this crossmatch actually represents a final confirmation of ABO compatibility. As a result, most institutions use the immediate-spin crossmatch only when the antibody screen is negative.
- A computer crossmatch uses an appropriately validated computer to compare the ABO group and Rh type of a patient with the ABO group and Rh type of a red cell component to determine compatibility. It does not detect incompatibility for other antigen systems.



- The platelet crossmatch detects incompatibilities between antibodies in patient serum (anti-HLA and anti-HPA) and antigens on donor platelets.

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7

Clinical Transfusion Practice and Effective Use of Blood Components

James Sikora, MD, MPH

Key Points from the *Technical Manual*

- Transfusionists must be educated on appropriate clinical indications for transfusion and proper safety steps involved in a successful transfusion process.
- Institutions must identify appropriate blood and blood component issue and delivery mechanisms, as well as requirements for returning components if transfusion is delayed.
- Before planned transfusion, the transfusionist must verify available, appropriate, and patent venous access; ensure informed consent is complete; administer any ordered prophylactic medicine; and gather required equipment (eg, blood warmer, infusion pump, pressure devices, and emergency equipment).
- There has not thus far been evidence that whole blood is superior to blood component therapy in any patient population. The use of whole blood in civilian trauma is increasing and remains an active area of research.



QUESTIONS

Question 1: Regarding the use of premedication to reduce the risk of allergic reactions or febrile nonhemolytic transfusion reactions (FNHTR), which of the following statements is true?

- A. Antipyretics are effective and should be given routinely.
 - B. Premedication must be given at the same time as the administration of blood components.
 - C. Nonpharmacologic methods have been shown to reduce the incidence.
 - D. Moderate to severe allergic reactions are not reduced with pre-medication.
 - E. Corticosteroids act immediately to treat allergic reactions.
-

Question 2: Blood warmers can be used in the transfusion of all of the following blood components except:

- A. Red cells.
 - B. Plasma.
 - C. Platelets.
 - D. Cryoprecipitate.
 - E. Granulocytes.
-

Question 3: The use of a blood warmer is beneficial in transfusion in the following settings except:

- A. Routine transfusions when clinical hypothermia is of concern.
 - B. Trauma.
 - C. Transfusion through a central venous device.
 - D. Transfusion in neonates.
 - E. Transfusion of platelets.
-

Question 4: Which is an acceptable way to warm blood components before transfusing?



- A. Microwave.
 - B. Hot plate.
 - C. Warm waterbath.
 - D. Device that is approved by the Food and Drug Administration (FDA) specifically for blood warming.
 - E. Allowing product to reach room temperature outside of storage.
-

Question 5: A surgical colleague calls to ask for guidance on using a pressure device to deliver blood at a faster rate than gravity. You say, yes, however:

- A. Pressure over 30 mmHg may compromise the seams of the blood component bag.
 - B. A small-gauge cannula is required to prevent hemolysis.
 - C. External pressure on the component bag significantly damages red cells.
 - D. External pressure devices greatly increase the speed of platelet transfusion.
 - E. An increase in intravenous (IV) catheter or cannula size may be as effective as external pressure.
-

Question 6: Which is an acceptable IV catheter size for use in transfusing cellular blood components?

- A. 10 gauge.
 - B. 12 gauge.
 - C. 18 gauge.
 - D. 26 gauge.
 - E. 28 gauge.
-

Question 7: Which of the following solutions is compatible with administering blood components?

- A. Lactated Ringer's solution.
- B. D5 (5% dextrose) normal saline.
- C. 0.9% sodium chloride (normal saline).



- D. D5 half normal saline.
 - E. D10 W (10% dextrose + water).
-

Question 8: Which is the appropriate way to start a transfusion?

- A. Transfuse at 2 mL/minute, observe for 30 minutes, increase to ordered rate.
 - B. Transfuse at 2 mL/minute, observe for 15 minutes, increase to ordered rate.
 - C. Transfuse at 50 mL/minute, observe for 30 minutes, increase to ordered rate.
 - D. Transfuse at 50 mL/minute, observe for 15 minutes, increase to ordered rate.
 - E. Begin at ordered transfusion rate, no observation needed.
-

Question 9: Which of the following is an advantage for transfusing blood products rapidly (as tolerated)?

- A. Correction of deficiency in a timely manner.
 - B. Decrease in severity of possible transfusion reaction.
 - C. Decreased potential to cause reactions.
 - D. Reduced incidence of allergic reactions.
 - E. Reduced incidence of septic reactions.
-

Question 10: What is the first step that should be taken if a transfusion reaction is suspected?

- A. Call the blood bank.
 - B. Slow the rate of transfusion.
 - C. Stop the transfusion.
 - D. Alert the code team.
 - E. Give appropriate medication.
-

Question 11: Which member of the care team is allowed to initiate a transfusion reaction workup?



- A. Medical student.
 - B. Resident.
 - C. Fellow.
 - D. Nurse.
 - E. Any member of the team.
-

Question 12: Which of the following statements regarding red cell transfusion is true?

- A. Restrictive transfusion strategies are generally not recommended.
 - B. The indication for red cell transfusion is to increase oxygen-carrying capacity in patients with anemia who are unable to compensate.
 - C. Transfusion of red cells may be used to increase volume status in a patient with low hematocrit.
 - D. Laboratory results (hemoglobin/hematocrit) may be used reliably in patients with active bleeding.
 - E. Transfusing two Red Blood Cell (RBC) units is the appropriate minimal dose.
-

Question 13: RBC units stored for extended periods will see which change?

- A. Extracellular potassium decreases.
 - B. 2,3-diphosphoglycerate (2,3-DPG) declines.
 - C. Free hemoglobin decreases.
 - D. Free iron decreases.
 - E. Red cells become more deformable and less fragile.
-

Question 14: Emergency transfusion of uncrossmatched group O, RhD-positive red cells is acceptable in all of the following cases, except:

- A. Male trauma patient with massive ongoing hemorrhage.
- B. 70-year-old female with massive ongoing hemorrhage due to injuries sustained from a motor vehicle crash.



- C. 25-year-old gunshot victim with massive ongoing hemorrhage with known non-ABO alloantibody.
 - D. 8-year-old male who was in a bicycle-vs-car accident; now has internal bleeding and minimally palpable blood pressure.
 - E. 25-year-old female with ongoing internal bleeding due to motor vehicle crash.
-

Question 15: Hemoglobin substitutes, or hemoglobin-based oxygen carriers (HBOCs) are:

- A. Widely available and may be used in any case where transfusion of red cells might be used.
 - B. May be used only in cases where a religious belief does not allow red cell transfusion.
 - C. Risk free and trending toward completely replacing red cell transfusion.
 - D. Currently available in the United States for use in pharmaceutical clinical trials or expanded access granted by FDA.
 - E. May be used only in patients with a rare blood type where a suitable RBC unit cannot be located.
-

Question 16: Which of the following is true when considering transfusion of red cells in a patient with sickle cell disease?

- A. Alloimmunization rates are lower in the sickle cell population compared to the general population.
 - B. Risk of iron overload in this population is not a concern.
 - C. Hyperhemolysis secondary to red cell transfusion is possible.
 - D. Antigens on the red cells of the donor population are similar to those of the patients with sickle cell disease.
 - E. Transfusion history is not an important factor in the decision.
-

Question 17: Which of the following are true regarding hyperhemolysis?

- A. Hemoglobin level following transfusion is equal to or higher than before the transfusion.



- B. May be acute or delayed.
 - C. Due to a previous antibody that was not detected at screening.
 - D. Only the transfused cells are hemolyzed.
 - E. Subsequent transfusions are likely to be free of this phenomenon.
-

Question 18: Which is *not* an indication for manual or automated exchange transfusion in a patient with sickle cell disease?

- A. Stroke.
 - B. Severe symptomatic acute chest syndrome.
 - C. Acute splenic sequestration.
 - D. Multisystem organ failure.
 - E. Vaso-occlusive pain crisis.
-

Question 19: How should transfusion of red cells be handled in a patient taking daratumumab treatment?

- A. Proceed as usual, daratumumab does not interact with red cells.
 - B. Use of low-ionic-strength saline in pretransfusion testing.
 - C. Testing with reagent cells pretreated with dithiothreitol (DTT).
 - D. Use of polyethylene glycol (PEG) in pretransfusion testing.
 - E. Heat samples prior to pretransfusion testing.
-

Question 20: What is the generally accepted threshold for prophylactic platelet transfusions in hospitalized patients?

- A. 5000/ μ L.
- B. 10,000/ μ L
- C. 15,000/ μ L.
- D. 20,000/ μ L.
- E. 25,000/ μ L.



Question 21: How is the evidence described to support prophylactic platelet transfusions for invasive procedures?

- A. Strong and based on high-quality evidence.
 - B. Strong and based on multiple large-scale randomized trials.
 - C. Strong, but further studies are needed.
 - D. Weak and based on low-quality evidence.
 - E. Weak and based on high-quality evidence.
-

Question 22: Before central venous catheter placement, what is the prophylactic platelet threshold that AABB suggests for transfusion?

- A. $<5000/\mu\text{L}$.
 - B. $<10,000/\mu\text{L}$.
 - C. $<15,000/\mu\text{L}$.
 - D. $<20,000 \mu\text{L}$.
 - E. $<25,000 \mu\text{L}$.
-

Question 23: What is the prophylactic platelet count that AABB suggests for lumbar puncture and other elective nonneuraxial surgery?

- A. $10,000/\mu\text{L}$.
 - B. $20,000/\mu\text{L}$.
 - C. $30,000/\mu\text{L}$.
 - D. $40,000/\mu\text{L}$.
 - E. $50,000/\mu\text{L}$.
-

Question 24: What is the common recommendation for platelet transfusion in thrombocytopenic patients who are actively bleeding?

- A. $20,000/\mu\text{L}$.
- B. $30,000/\mu\text{L}$.
- C. $40,000/\mu\text{L}$.
- D. $50,000/\mu\text{L}$.
- E. $60,000/\mu\text{L}$.



Question 25: What is the common recommendation for prophylactic platelet count before elective neuraxial surgery?

- A. 40,000/ μ L.
 - B. 60,000/ μ L.
 - C. 80,000/ μ L.
 - D. 100,000/ μ L.
 - E. 120,000/ μ L.
-

Question 26: In regard to ABO and RhD matching for platelets, which of the following is true?

- A. ABO matching for platelets is a requirement.
 - B. Platelets express ABH antigens.
 - C. ABO matching reduces mortality, bleeding, and transfusion-reaction rates.
 - D. ABO matching does not improve platelet count increment.
 - E. Platelets express Rh antigens.
-

Question 27: Large, multicenter retrospective reviews have shown that the frequency of alloimmunization from RhD-positive platelet units in both immunocompetent and immunocompromised patients is?

- A. Less than 2%.
 - B. Less than 10%.
 - C. Less than 30%.
 - D. Less than 50%.
 - E. Less than 75%.
-

Question 28: How can the risk of alloimmunization from a single RhD-positive unit be virtually eliminated?

- A. Washing the unit before transfusion.
- B. Decreasing the volume of the unit before transfusion.
- C. Administering intravenous immune globulin (IVIG) after transfusion.



- D. Administering Rh Immune Globulin (RhIG) within 72 hours of transfusion.
 - E. Administering a combination of intravenous immune globulin (IVIG) and Rh immune globulin (RhIG) within 1 week of transfusion.
-

Question 29: What is the expected 1-hour posttransfusion increment for a typical apheresis platelet ($>3 \times 10^{11}$ platelets) when transfused to an average-size, healthy recipient?

- A. 10,000-20,000/ μ L.
 - B. 30,000-60,000/ μ L.
 - C. 60,000-80,000/ μ L.
 - D. 80,000-100,000/ μ L.
 - E. 100,000-120,000/ μ L.
-

Question 30: Which of the following is the least likely cause for a consistent failure to achieve an appropriate platelet count increment following platelet transfusion?

- A. Sepsis.
 - B. Disseminated intravascular coagulation (DIC).
 - C. Bleeding.
 - D. Drug effects.
 - E. Immune etiology.
-

Question 31: What is the 1-hour corrected count increment (CCI) of a patient with a body surface area (BSA) of 2.0 m² and a platelet count of 5000/ μ L who receives a unit of apheresis platelets containing 4×10^{11} platelets with a posttransfusion platelet count of 25,000/ μ L?

- A. 5000.
- B. 10,000.
- C. 15,000.
- D. 20,000.
- E. 25,000.



Question 32: What amount of time should be given before evaluating a patient for immune-associated platelet refractoriness?

- A. 5 minutes after transfusion.
 - B. 10-60 minutes after transfusion.
 - C. 90-120 minutes after transfusion.
 - D. Within 24 hours of transfusion.
 - E. Any time following transfusion.
-

Question 33: What is the most common cause for immune-associated platelet refractoriness?

- A. Antibodies that target a human leukocyte antigen (HLA).
 - B. Antibodies that target human platelet antigens (HPAs).
 - C. Antibodies that target ABO antigens.
 - D. Antibodies that target Rh antigens.
 - E. Antibodies that target CD38 antigens.
-

Question 34: Regarding prophylactic transfusion of plasma for invasive procedures, which of the following statements is *not* true?

- A. Prophylactic transfusion of plasma exposes patients to risks associated with plasma.
- B. Mild-to-moderate coagulation marker abnormalities fail to predict bleeding.
- C. Modest elevations in prothrombin time (PT)/international normalized ratio (INR) are usually not corrected by plasma alone.
- D. Numerous studies have failed to demonstrate that prophylactic plasma transfusions affect bleeding outcomes.
- E. Prophylactic transfusion of plasma is an acceptable method of correcting a modestly increased activated partial thromboplastin time (aPTT).



Question 35: Which of the following is an acceptable scenario for plasma transfusion?

- A. Correction of PT/INR before an elective procedure.
 - B. Active central nervous system hemorrhage in the absence of coagulopathy or vitamin K antagonist therapy.
 - C. Plasma-protein deficiencies for which a licensed coagulation factor concentrate is available.
 - D. Correction of aPTT before an elective procedure.
 - E. Bleeding patient with multiple coagulation factor deficiencies.
-

Question 36: What is the best method to reverse vitamin K antagonist therapy urgently if there are no contraindications?

- A. Intravenous vitamin K.
 - B. Oral vitamin K.
 - C. Three-factor prothrombin complex concentrate and IV vitamin K.
 - D. Plasma.
 - E. Four-factor prothrombin complex concentrate and IV vitamin K.
-

Question 37: Cryoprecipitate contains all of the following except:

- A. Factor XII.
 - B. Fibrinogen.
 - C. Factor VIII.
 - D. Von Willebrand factor (vWF).
 - E. Factor XIII.
-

Question 38: All of the following are true regarding granulocyte transfusion except:

- A. Granulocyte components are stored at 4 C.
- B. Granulocyte components must be used within 24 hours of collection.



- C. Granulocyte components require crossmatching before transfusion.
 - D. Granulocyte components are matched to the recipient by the ABO-matching rules used for red cells.
 - E. Granulocyte components should always be irradiated.
-

Question 39: Which of the following is *not* a current practice in the massive transfusion protocol?

- A. Resuscitation with mainly crystalloid solutions.
 - B. Resuscitation with a balance of blood components.
 - C. Resuscitation supplemented with low-titer group O whole blood.
 - D. Resuscitation guided by coagulation laboratory values.
 - E. Resuscitation using warmed products.
-

Question 40: What does the 1:1:1 balance of products in a massive transfusion protocol represent?

- A. One unit of plasma: one unit of apheresis platelets: one unit of red cells.
 - B. One unit of albumin: one unit of apheresis platelets: one unit of red cells.
 - C. One liter of crystalloid: one unit of whole-blood-derived platelet concentrate: one unit of red cells.
 - D. One unit of plasma: one unit of whole-blood-derived platelet concentrate: one unit of red cells.
 - E. One unit of plasma: one unit of whole-blood-derived platelet concentrate: one unit of low-titer group O whole blood.
-

Question 41: A 70-year-old female presents to the emergency department with complaints of fatigue and dyspnea for the last week. The patient has a medical history of atrial fibrillation for which she takes warfarin. Pertinent positives on the physical exam include pale skin and mucosa, and tachycardia with a normal blood pressure. She shows no signs of active bleeding. Pertinent laboratory testing results include: hematocrit of 20%, platelet count of 40,000/



μL , white cell count of 8,000/ μL , and an INR of 4. What is the most appropriate blood component for this patient?

- A. Red cells.
 - B. Platelets.
 - C. Plasma.
 - D. Cryoprecipitate.
 - E. No blood components needed currently.
-

Question 42: A 23-year-old male with history of sickle cell disease (hemoglobin SS) is admitted to the hospital with symptoms that include: dyspnea, hypoxemia, and pulmonary infiltrates on chest X-ray. Notable laboratory test results in this patient include: hemoglobin level of 7.1 g/dL, Hgb S of 45%, and white cell count of 20,000/ μL . The patient's oxygen saturation is 90% on 2L nasal cannula. The transfusion medicine team is consulted on treatment. What is the best recommendation for initial treatment in this patient?

- A. Perform an automated red cell exchange with goal of 30% hematocrit and less than 30% Hgb S.
 - B. Give treatment for symptoms, but avoid transfusion of red cells due to risk of alloimmunization.
 - C. Give treatment for symptoms, but avoid transfusion of red cells due to risk of hyperhemolysis syndrome.
 - D. Transfuse red cells until a hemoglobin level of at least 14 g/dL is reached.
 - E. Transfuse a unit of RBCs and observe for clinical improvement.
-

Question 43: A middle-aged male is admitted to the trauma center following a motor vehicle crash. Medical history is unknown. On arrival, he is combative and confused. Vital signs on arrival include tachycardia to 140 bpm and a blood pressure of 80/30 mmHg. Initial lab results include: hematocrit 15%, white cells 13,000/ μL , platelets 80,000/ μL , and INR of 3.0. The patient received approximately 500 mL of crystalloid and one unit of RBCs en route to the hospital. What is the best strategy to resuscitate the patient and control coagulopathy?



- A. Transfuse platelets until a platelet count of 100,000/ μ L is reached.
 - B. Transfuse plasma until an INR of 2.0 is reached.
 - C. Transfuse red cells until a hematocrit of 30% is reached.
 - D. Transfuse red cells, plasma, and platelets in a 1:1:1 ratio.
 - E. Transfuse crystalloid and red cells to maintain blood pressure of 120/80 mmHg.
-

Question 44: Which of the following scenarios is an appropriate use of plasma?

- A. Patient with minor esophageal varices bleeding has an INR of 1.8 and is not taking anticoagulation medication.
 - B. Patient with HIV and malnutrition has an INR of 3.0 and is not bleeding.
 - C. Patient with suspected thrombocytopenic purpura (TTP).
 - D. Patient who needs an invasive biopsy and has an INR of 1.9.
 - E. Patient taking warfarin who needs emergency surgery.
-

Question 45: A 25-year-old female has just given birth and she is experiencing postpartum bleeding that is higher than expected. Her laboratory coagulation parameters are all significantly increased above the reference range. Her fibrinogen level is 50 mg/dL and platelet count is 40,000/ μ L. Which of the following blood products or factor concentrates should be considered for her initial treatment?

- A. Cryoprecipitate.
 - B. Cryoprecipitate-reduced plasma.
 - C. 4-factor prothrombin complex concentrate.
 - D. Factor VIII concentrate.
 - E. Recombinant Factor VIIa.
-

Question 46: A 50-year-old male with a recent stem cell transplant has the following lab results: PT/ aPTT within the reference range, hemoglobin level 9 g/dL, platelet count 8000/ μ L. How many units



of apheresis platelets should be transfused to prophylactically prevent spontaneous bleeding?

- A. 0.
 - B. 1.
 - C. 2.
 - D. 3.
 - E. 4.
-

Question 47: What is the percentage of blood donors who are considered to have universal plasma?

- A. 1.
 - B. 3.
 - C. 10.
 - D. 15.
 - E. 20.
-

Question 48: A 55-year-old multiparous woman has a platelet count of 30,000/ μL and is scheduled to have an invasive procedure. She receives a transfusion of one unit of apheresis platelets. A blood draw 45 minutes following the transfusion reveals a platelet count of 30,000/ μL . What is the most likely cause of the patient's lack of response to the platelet transfusion?

- A. Active bleeding.
 - B. HLA antibodies.
 - C. Splenomegaly.
 - D. Septicemia.
 - E. Drug interaction.
-

Question 49: What is the appropriate test to determine if platelet refractoriness is due to an immune or nonimmune etiology?

- A. HLA antibody screen.
- B. Antibody crossmatching.
- C. Imaging of the spleen.



- D. Posttransfusion platelet count at 1 hour.
 - E. HLA of the patient.
-

Question 50: Which of the following is a common adverse event associated with granulocyte transfusion?

- A. HLA alloimmunization.
- B. Transfusion-associated graft-vs-host disease.
- C. Immune thrombocytopenia.
- D. Thrombocytopenic purpura (TTP).
- E. Chronic granulomatous disease.

ANSWERS

Question 1: C. Nonpharmacologic methods have been shown to reduce the incidence.

Explanation:

- Nonpharmacologic methods (leukocyte reduction, washing, volume reduction, platelet additive solution, etc) are effective ways to reduce the incidence of common transfusion reactions.
 - Premedication (and corticosteroids) take time to be effective and need to be given before transfusion.
 - Antipyretic effectiveness is limited and may mask common reactions.
 - Pretreatment with corticosteroids may be effective for moderate to severe allergic reactions, particularly in future transfusions.
-

Question 2: C. Platelets.

Explanation:

- Blood warmers are contraindicated for platelet transfusions.



- Other blood components may be used with a warmer; however, the manufacturer's suggestions should be followed.
-

Question 3: E. Transfusion of platelets.

Explanation:

- Blood warmers are contraindicated for platelet transfusions.
 - Other choices listed are all instances where hypothermia is of concern and use of a blood warmer may be beneficial.
-

Question 4: D. Device that is approved by the Food and Drug Administration (FDA) specifically for blood warming.

Explanation:

- Any device used to warm blood components must be approved by the Food and Drug Administration for that purpose.
-

Question 5: E. An increase in IV catheter or cannula size may be as effective as external pressure.

Explanation:

- Increasing the IV catheter or cannula size is the best way to increase flow rates.
 - Pressure greater than 300 mmHg would compromise the seams of the component bag.
 - External pressure does not damage red cells, but gives only a modest increase in flow rates.
 - Use of a small cannula could cause hemolysis with external pressure.
-

Question 6: C. 18 gauge.

**Explanation:**

- 25 to 14 gauge is an acceptable size for transfusion of cellular blood components.
-

Question 7: C. 0.9% sodium chloride (normal saline).

Explanation:

- No medications or solutions other than 0.9% sodium chloride injection must be administered with blood components through the same tubing at the same time.
 - The AABB *Standards* allow for few exceptions to this restriction.
-

Question 8: B. Transfuse at 2 mL/minute, observe for 15 minutes, increase to ordered rate.

Explanation:

- The infusion for all nonemergent blood components must start slowly (approximately 2 mL/minute) for the first 15 minutes with direct observation for adverse events.
 - Following this observation period, the rate may be increased as ordered.
-

Question 9: A. Correction of deficiency in a timely manner.

Explanation:

- Transfusing at a rapid rate (eg, 240 mL/hour) has the advantage of correcting a deficiency quickly, as well as reducing the amount of time needed by the recipient and nursing staff.
- However, a rapid rate may increase the incidence or severity of transfusion reactions.



Question 10: C. Stop the transfusion.

Explanation:

- The first step to investigating a possible transfusion reaction is to immediately stop the transfusion.
- The severity of a reaction can be dependent on the amount of product transfused.



Question 11: E. Any member of the team.

Explanation:

- Any member of the medical team should feel empowered to report a possible transfusion reaction.



Question 12: B. The indication for red cell transfusion is to increase oxygen-carrying capacity in patients with anemia who are unable to compensate.

Explanation:

- Red cell transfusion is used to increase the oxygen-carrying capacity in those who cannot compensate to maintain adequate tissue oxygenation.



Question 13: B. 2,3-diphosphoglycerate (2,3-DPG) declines.

Explanation:

- The red cell “storage lesion” is characterized by red cells that are less deformable/more fragile and increases in extracellular potassium, free hemoglobin, and iron.



Question 14: E. 25-year-old female with ongoing internal bleeding due to motor vehicle crash.

**Explanation:**

- Group O, Rh-positive red cells are routinely used in emergency situations for males and postmenopausal women (age may vary by institution).
 - The supply of group O, Rh-negative blood is not adequate to support all emergency needs and should be reserved for women of childbearing potential.
-

Question 15: D. Currently available in the United States for use in pharmaceutical clinical trials or expanded access granted by the Food and Drug Administration.

Explanation:

- Hemoglobin substitutes are currently available in the US only for pharmaceutical clinical trials or expanded access granted by the FDA.
-

Question 16: C. Hyperhemolysis secondary to red cell transfusion is possible.

Explanation:

- Hyperhemolysis occurs when the hemoglobin level following transfusion for severe anemia is lower than before the transfusion. It may be acute or delayed. It may be associated with a new antibody or past antibody. The transfused cells and the patient's own cells are hemolyzed
 - Alloimmunization rates are higher in the sickle cell population.
 - The general donor population is mostly a different ethnic background than the sickle cell population and has a difference of occurrence of antigens on red cells.
 - Transfusion history is extremely important to consider in the sickle cell population.
-

Question 17: B. May be acute or delayed.

**Explanation:**

- See discussion of hyperhemolysis in answer #16.
-

Question 18: E. Vaso-occlusive pain crisis.**Explanation:**

- All of the choices are an indication for exchange in a sickle cell patient except a vaso-occlusive pain crisis.
 - A pain crisis should be addressed with adequate pain control and intravenous fluid.
-

Question 19: C. Testing with reagent cells pretreated with dithiothreitol (DTT).**Explanation:**

- DTT is a reducing agent that causes denaturation of CD38 but not CD47 on the red cell surface. This is achieved by destroying the disulfide bonds. DTT also destroys antigens in the Kell system.
 - PEG, LISS, and heating will not eliminate daratumumab binding.
-

Question 20: B. 10,000/ μ L.**Explanation:**

- Although some studies have shown levels as low as 5000/ μ L, 10,000/ μ L is most commonly used and is recommended by many clinical guidelines.
-

Question 21: D. Weak and based on low-quality evidence.

**Explanation:**

- There is not much evidence currently in support of the prophylactic transfusion of platelets for minor or major procedures.
 - The decision should be based on discussion between the procedural team and the transfusion team along with published guidelines specific to the procedure.
-

Question 22: D. <20,000/ μ L.**Explanation:**

- AABB suggests platelet transfusion for central line placement if the platelet count is below 20,000/ μ L.
-

Question 23: E. 50,000/ μ L.**Explanation:**

- AABB suggests that a platelet count of 50,000/ μ L is adequate to perform a lumbar puncture and any other elective surgery that is not neuraxial in nature.
-

Question 24: D. 50,000/ μ L.**Explanation:**

- Attempting to keep a platelet count of 50,000/ μ L is thought to be ideal.
 - However, if a bleeding patient with a qualitative platelet dysfunction is known, platelet transfusion even at normal levels may be necessary.
-

Question 25: D. 100,000/ μ L.

**Explanation:**

- A platelet level of 100,000/ μ L is needed for neuraxial surgery due to the low tolerance of even a minor bleeding incident.
-

Question 26: B. Platelets express ABH antigens.

Explanation:

- Platelets express ABH antigens, although they are not required to be ABO matched before transfusion.
 - No definitive evidence exists for reduction in mortality, bleeding, and/or transfusion reaction rates with ABO matching.
 - ABO matching has been shown to increase the platelet count increment following transfusion.
 - Platelets do not express Rh antigens, but red cells present in the component may, and have to be addressed in some patients who are Rh negative.
-

Question 27: A. Less than 2%.

Explanation:

- A large, multicenter retrospective study showed an alloimmunization rate of less than 2%.
-

Question 28: D. Administering Rh Immune Globulin (RhIG) within 72 hours of giving.

Explanation:

- Giving RhIG within 72 hours of an Rh-positive platelet transfusion mostly eliminates the chance of alloimmunization in an Rh-negative patient.
- Washing and volume reduction help with allergens that may be present in plasma, but not alloimmunization.



- IVIG is not commonly used in this scenario to prevent alloimmunization.
-

Question 29: **B.** 30,000-60,000/ μ L.

Explanation:

- The transfusion of one unit of apheresis platelets should increase the platelet count by 30-60,000/ μ L.
-

Question 30: **E.** Immune etiology.

Explanation:

- Platelet refractoriness is most often due to nonimmune causes (choices A-D).
-

Question 31: **B.** 10,000.

Explanation:

$$\text{CCI} = \text{Platelet increment} \times \text{BSA}(\text{m}^2)$$

$$\text{CCI} = (20,000) \times 2.0/4.0$$

$$\text{CCI} = 10,000$$

Question 32: **B.** 10-60 minutes after transfusion.

Explanation:

- Platelet counts should be obtained between 10 and 60 minutes to evaluate for immune refractoriness.



Question 33: A. Antibodies that target a human leukocyte antigen (HLA).

Explanation:

- Antibodies that target HLA are the most common cause of platelet immune refractoriness.
-

Question 34: E. Prophylactic transfusion of plasma is an acceptable method of correcting a modestly increased activated partial thromboplastin time (aPTT).

Explanation:

- Prophylactic transfusion of plasma is usually unnecessary for the following reasons:
 - There are risks associated with plasma transfusions.
 - Mild-to-moderate abnormalities in coagulation parameters has failed to predict bleeding.
 - Plasma does not work well to correct mild PT/INR abnormalities.
 - No evidence to support prophylactic plasma transfusion and bleeding outcomes.
-

Question 35: E. Bleeding patient with multiple coagulation factor deficiencies.

Explanation:

- Plasma transfusion is an acceptable way to replenish a deficit of multiple coagulation factors in a bleeding patient.
 - Deficit in a single coagulation factor with an available concentrate available should be treated with the specific concentrate rather than plasma.
-

Question 36: E. Four-factor prothrombin complex concentrate and IV vitamin K.

**Explanation:**

- Urgent reversal of vitamin K antagonists is best achieved by using a four-factor prothrombin complex concentrate (PCC). Four-factor PCC contains Factors II, VII, IX, and X in the nonactivated state as well as proteins C and S.
 - Four-factor PCCs are superior to three-factor PCCs.
 - Vitamin K administration does not immediately reverse vitamin K antagonism, but is important to give in addition to PCCs for prolonged reversal.
-

Question 37: A. Factor XII.**Explanation:**

- Cryoprecipitate contains fibrinogen, Factor VIII, vWF, fibronectin, and Factor XIII.
 - It does not contain Factor XII.
-

Question 38: A. Granulocyte components are stored at 4 C is incorrect.**Explanation:**

- Granulocytes are stored at room temperature (>20 C), must be used within 24 hours.
 - They require crossmatching due to red cells in the component and are matched to recipients by ABO rules.
 - Granulocytes must be irradiated.
-

Question 39: A. Resuscitation with mainly crystalloid solutions.**Explanation:**

- The practice of heavy resuscitation with crystalloid solution was used in the past, but after discovering many adverse outcomes from the use (dilutional coagulopathy, abdominal compartment



syndrome, pulmonary complications, etc) treatment has shifted to balanced, blood-centric methods.

Question 40: D. One unit of plasma: one unit of whole-blood-derived platelet concentrate: one unit of red cells.

Explanation:

- Because apheresis platelets are widely used, a common misconception of the 1:1:1 ratio is that the platelet component is one apheresis platelet.
 - However, it is a whole-blood-derived platelet that is used in the ratio.
-

Question 41: A. Red cells.

Explanation:

- The patient has signs and symptoms of anemia. Transfusion of red blood cells should be considered to increase the oxygen-carrying capacity and increase oxygen delivery to her organs.
 - Her platelet count is low, but she is not actively bleeding. The INR is increased, but is explained by the warfarin therapy.
-

Question 42: E. Transfuse a unit of RBCs and observe for clinical improvement.

Explanation:

- Acute chest syndrome is a serious complication associated with sickle cell disease.
- Causes may include infection, pulmonary infarction, fat embolism, or an unknown etiology.
- Treatment includes hydration, antibiotics, respiratory support, and reduction of hemoglobin S. Common practice is to initiate treatment with simple transfusion and follow for improvement.
- More severe cases may require red cell exchange.



Question 43: D. Transfuse red cells, plasma, and platelets in a 1:1:1 ratio.

Explanation:

- Current practice in resuscitation with rapid bleeding is to transfuse red cells, plasma, and platelets in a 1:1:1 ratio or as close to it as possible.
-

Question 44: C. Patient with suspected thrombocytopenic purpura (TTP).

Explanation:

- Treatment of thrombocytopenic purpura (TTP) requires the use of plasma, which contains ADAMTS13, a metalloprotease that breaks down von Willebrand multimers into smaller fragments.
 - When ADAMTS13 is lacking, large von Willebrand multimers circulate along with platelet activation that cause microthrombi formation.
 - TTP is a medical emergency and requires immediate therapeutic plasma exchange using plasma. If a delay is expected in performing an exchange, plasma may be transfused until exchange can be performed.
-

Question 45: A. Cryoprecipitate.

Explanation:

- Obstetric patients experiencing postpartum bleeding are at an increased risk of disseminated intravascular coagulopathy (DIC).
 - DIC lowers the fibrinogen levels disproportionately to other coagulation factors, necessitating additional cryoprecipitate to be transfused.
-

Question 46: B. 1.

**Explanation:**

- The generally acceptable platelet count to prevent spontaneous bleeding is 10,000/ μ L, although some studies hint that it may be lower.
 - One unit of apheresis platelets should increase the platelet count by 30,000-60,000/ μ L, giving this patient a level high enough to prevent spontaneous bleeding.
-

Question 47: B. 3.**Explanation:**

- Within the population, there are approximately 2.5-3% of people that are blood group AB.
 - Group AB plasma is considered the universal plasma.
-

Question 48: B. HLA antibodies.**Explanation:**

- Because this patient showed no response at 45 minutes after platelet transfusion, this scenario is likely immune-mediated destruction. The most likely immune etiology is antibodies to HLA, especially in someone that has had multiple pregnancies.
 - A nonimmune etiology would likely result in an increase in the count but not to the extent expected.
-

Question 49: D. Posttransfusion platelet count at 1 hour.**Explanation:**

- A cost-effective and relatively fast test to determine immune or nonimmune etiology for platelet refractoriness is the posttransfusion platelet count at 1 hour.
- No increase in platelet count points to an immune etiology.



- An increase in platelet count (modest, but not to the expected level) points to nonimmune etiology.
-

Question 50: A. HLA alloimmunization.**Explanation:**

- Patients who require granulocyte transfusion typically require several doses. Each dose exposes the recipient to a different HLA, increasing the risk of alloimmunization. Transfusion-associated graft-vs-host disease is prevented by the irradiation of granulocyte products.
- The other answers are not associated with granulocyte transfusion.

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8

Hemolytic Disease of the Fetus and Newborn, and Rh Immune Globulin

Deanna C. Fang, MD

Key Points from the *Technical Manual*

- Hemolytic disease of the fetus and newborn (HDFN) is caused by maternal red cell antibodies that are specific to a paternally derived red cell antigen. The maternal IgG antibody is transported across the placenta, where it destroys fetal red cells, causing fetal anemia and neonatal hyperbilirubinemia.
- ABO HDFN is common but usually causes only mild to moderate symptoms.
- The most common clinically significant antibodies that cause HDFN are anti-D and anti-K; anti-C, -c, and -E, along with some others are significant but not common.
- The Kleihauer-Betke test is used to quantify fetomaternal hemorrhage (FMH) levels.
- The calculated Rh Immune Globulin (RhIG) dose should be rounded up if the number to the right of the decimal point is ≥ 0.5 or rounded down if the number to the right of the decimal point is <0.5 . In either case, an additional vial should be added to the result.

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QUESTIONS

Question 1: A neonate is born in the United States with ascites, pleural effusions, pericardial effusions, and generalized dermal edema, consistent with the presentation of hydrops fetalis. What is the most likely cause for the neonate's condition?

- A. Nonimmune cause.
 - B. Anti-A.
 - C. Anti-B.
 - D. Anti-D.
 - E. Anti-K.
-

Question 2: In hemolytic disease of the fetus and newborn (HDFN), which choice is the correct statement about the subclasses of IgG that are more likely to cause severe and/or early hemolytic disease?

- A. IgG1 and IgG2 are more likely than IgG3 and IgG4.
 - B. IgG1 and IgG3 are more likely than IgG2 and IgG4.
 - C. IgG2 and IgG3 are more likely than IgG1 and IgG4.
 - D. IgG2 and IgG4 are more likely than IgG1 and IgG3.
 - E. IgG3 and IgG4 are more likely than IgG1 and IgG2.
-

Question 3: What is the most common cause of HDFN in developed countries?

- A. Antibodies to the ABO antigen system.
 - B. Antibodies to the Rh antigen system.
 - C. Antibodies to the Kell antigen system.
 - D. Antibodies to the Kidd antigen system.
 - E. Antibodies to the Duffy antigen system.
-

Question 4: The antibodies below are typically associated with reactivity at colder temperatures. Which antibody should still be considered a potential risk factor for HDFN?



- A. Anti-I.
 - B. Anti-Le(a).
 - C. Anti-Le(b).
 - D. Anti-M.
 - E. Anti-P1.
-

Question 5: Which statement is true regarding ABO incompatibility as a cause of HDFN?

- A. ABO incompatibility is not a cause of HDFN because anti-A and anti-B are IgM antibodies that do not cross the placenta.
 - B. It can occur in the first pregnancy without any history of a sensitizing event.
 - C. It tends to have a more severe clinical presentation than Rh(D)-related HDFN.
 - D. All expectant mothers of group O, A, or B typing must be monitored during pregnancy for risk of HDFN.
 - E. It has an incidence of 15% of pregnancies.
-

Question 6: Which scenario is most likely for an occurrence of HDFN due to ABO incompatibility?

- A. In European ancestry: Group A mother, group B infant.
 - B. In European ancestry: Group O mother, group AB infant.
 - C. In Asian ancestry: Group O mother, group B infant.
 - D. In African populations: Group O mother, group A infant.
 - E. In African populations: Group O mother, group B infant.
-

Question 7: HDFN related to a first-known pregnancy is most likely to involve antibodies against which antigen group?

- A. ABO.
- B. Rh (DCE).
- C. KEL (Kell).
- D. FY (Duffy).
- E. JK (Kidd).



Question 8: What answer best describes the incidence of D-negative phenotype in the United States?

- A. 15% European ancestry, 8% Asian ancestry, <0.1% African ancestry.
 - B. 15% European ancestry, 8% African ancestry, <0.1% Asian ancestry.
 - C. 15% Asian ancestry, 8% European ancestry, <0.1% African ancestry.
 - D. 15% Asian ancestry, 8% African ancestry, <0.1% European ancestry.
 - E. 15% African ancestry, 8% European ancestry, <0.1% Asian ancestry.
-

Question 9: Which statement is true about maternal alloimmunization?

- A. The risk of fetomaternal hemorrhage (FMH) increases as gestational age increases, with the greatest risk occurring at time of delivery.
 - B. Although anti-A and anti-B IgM are naturally occurring antibodies, class switching to IgG antibodies does not occur until the mother has been alloimmunized to group A and/or B antigens (eg, through transfusion, prior pregnancy, etc).
 - C. The risk of alloimmunization to RhD is nonexistent until the mother is exposed to at least 2 mL of D-positive red cells.
 - D. The rate of alloimmunization to RhD is increased in the setting of ABO-incompatible mothers.
 - E. All of the above.
-

Question 10: There are many *RHD* genotypes that can result in a serologic weak-D phenotype. For which of the following genotypes can a female of childbearing potential be managed as a D-positive patient?

- A. Weak D1 only.
- B. Weak D2 only.
- C. Weak D3 only.
- D. Weak D1 and D2.
- E. Weak D1, D2, and D3.



Question 11: What is the approximate risk of a D-negative mother being alloimmunized to a D-positive fetus if no intervention occurs?

- A. 2%.
 - B. 7%.
 - C. 16%.
 - D. 38%.
 - E. 52%.
-

Question 12: What statement is true about RhIG?

- A. It is used to treat alloimmunization to the RhD antigen.
 - B. It can be given only after delivery to avoid exacerbating fetal hemolysis during pregnancy.
 - C. It is available in doses of 50 µL, 300 µL, and 600 µL.
 - D. The mechanism of efficacy is not known.
 - E. RhIG is contraindicated for partial-D mothers due to risk of severe hemolytic transfusion reactions for the mother.
-

Question 13: What is the approximate risk of a D-negative mother being alloimmunized to a D-positive fetus with RhIG prophylaxis?

- A. <0.1%.
 - B. 0.5%.
 - C. 1%.
 - D. 2%.
 - E. 3%.
-

Question 14: In which scenario should a mother who has just delivered a neonate be considered a candidate for RhIG prophylaxis?

- A. D-negative mother who makes anti-Di(a); neonate types as D positive.
- B. D-positive mother with negative antibody screens; neonate types as D negative.
- C. Partial-D mother who makes anti-D; neonate types as D positive.
- D. Weak-D2 mother who does not make anti-D; neonate types as D positive.
- E. All of the above.



Question 15: The 33rd edition of *Standards for Blood Banks and Transfusion Services* states that women who are pregnant or who have been pregnant recently shall be considered for Rh Immune Globulin administration in which of the following situations?

- A. The woman types as D-negative (with optional weak-D testing).
 - B. The woman is not known to be actively alloimmunized to the D antigen.
 - C. The fetus' Rh typing is either unknown or is positive (with weak-D typing being required when testing for D is negative).
 - D. A and B.
 - E. All of the above.
-

Question 16: A woman in her first pregnancy with no significant medical history and getting routine care had an unremarkable obstetric course until presenting to the emergency department after being in a car accident at 35 weeks of gestation. A sample for type and screen is sent to the blood bank. The patient is group O, RhD-negative. The three-cell antibody screen is positive and is reflexed to the 10-cell panel for antibody identification workup (see accompanying panel). The identified antibody has a titer of one. Which statement is the *best* interpretation of the results and action to take?

- A. This patient should receive RhIG; she is D negative and all D-negative pregnant women should automatically get RhIG.
- B. The weak antibody findings indicate that the patient's red cells express either weak D or partial D, and the laboratory should send the sample to a reference laboratory for *RHD* genotyping. While the results of genotyping are pending, the patient should be considered a candidate for RhIG administration.
- C. The patient does not need RhIG because she has already been alloimmunized and makes alloanti-D. It is too late for RhIG.
- D. The antibody results are likely due to previous RhIG administration. The patient should be interviewed and/or any available medical records for her should be checked for previous RhIG use. In the meantime, she should be considered a candidate for RhIG.
- E. It does not matter if the antibodies are due to alloimmunization vs passively acquired RhIG. The presence of antibodies in any form are already protective; she does not need RhIG at this time.

**Antibody Identification Panel, Question 16**

Cell	D	C	c	E	e	f	V	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	IS	AHG	CC
I	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	0	+	0	1+		
II	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	1+		
1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	+	0	+	+	+	0	+	0	+	0	0	1+		
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	+	0	0	0	1+		
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	1+		
4	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	1+		
5	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+		
6	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+		
7	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	+		
8	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+		
9	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	+	0	0	0	+		
10	0	0	+	0	+	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+		
Patient's cells																											0	0	+	



Question 17: A woman presents to the emergency department for acute abdominal pain and vaginal bleeding. Beta-HCG results show that the patient is pregnant, and she experiences a spontaneous abortion while still in the emergency room. She reports that she had another incident of vaginal bleeding 4 days ago but did not seek medical attention at the time because she did not know she was pregnant. Her typing is D negative. Which is the *best* statement regarding when and whether she should receive RhIG?

- A. Within 72 hours of event. This patient would still benefit from RhIG.
 - B. Within 24 hours of event. This patient would no longer benefit from RhIG.
 - C. Within 48 hours of event. This patient would no longer benefit from RhIG.
 - D. Within 72 hours of event. This patient would no longer benefit from RhIG.
 - E. None of the above.
-

Question 18: A pregnant D-negative woman has the following results seen on the accompanying 10-cell antibody identification panel. Which of the following statements represents the best interpretation of results and/or course of action, if any?

- A. The patient makes anti-D and is not a candidate for RhIG.
- B. The patient makes multiple antibodies and is not a candidate for RhIG.
- C. Further workup is needed for antibody identification. In the meantime, the patient should be considered a candidate for RhIG until workup and identification is complete.
- D. Further workup is needed for antibody identification, but one can already determine that the patient would not benefit from RhIG prophylaxis.
- E. This antibody profile does not indicate any risk for hemolytic disease of the fetus and newborn.



Antibody Identification Panel, Question 18

Antibody Identification Panel, Question 18																															
Cell	D	C	c	E	e	f	V	C ^w	K	K	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	IS	AHG	CC
I	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	0	0	0	4+		
II	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	0	4+			
1	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	0	4+			
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	4+				
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	4+				
4	+	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	4+				
5	0	+	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	4+				
6	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+				
7	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+				
8	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+				
9	0	0	+	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	0	+				
10	0	0	+	0	+	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+				



Question 19: For mothers who are candidates for RhIG prophylaxis, in which circumstance is RhIG prophylaxis *not* automatically indicated?

- A. Amniocentesis.
 - B. First-time mother at first prenatal visit.
 - C. 28th week of gestation.
 - D. After delivery if neonate is typed as D positive.
 - E. Ectopic pregnancy.
-

Question 20: Which best fills in the blanks of the following sentence: ____ suppresses alloimmunization of up to ____ of D-positive ____.

- A. 50 µg RhIG; 10 mL; fetal red cells.
 - B. 50 µg RhIG; 2.5 mL; fetal whole blood.
 - C. 50 µg RhIG; 5 mL; fetal whole blood.
 - D. 300 µg RhIG; 30 mL; fetal red cells.
 - E. 300 µg RhIG; 25 mL; fetal red cells.
-

Question 21: A mother presents in her second pregnancy to an obstetric clinic. Chart review of her first pregnancy shows that she typed as group AB, D negative with negative antibody screens throughout. Antenatal history was unremarkable until delivery, when she received an RBC unit and a 300-µg dose of RhIG after delivery. The neonate was D positive. Now, 1 year later, the lab is seeing a positive antibody screen with the following antibody pattern at a titer of 4 (see accompanying panel). Which of the following is the *most* likely explanation?

- A. Prior RhIG administration is causing testing interference.
- B. The mother received an inadequate dose of RhIG.
- C. The mother is alloimmunized due to receiving a D-positive RBC unit.
- D. The lab is seeing passively acquired antibody from the mother receiving a D-negative RBC unit.
- E. None of the above.



Antibody Identification Panel, Question 21

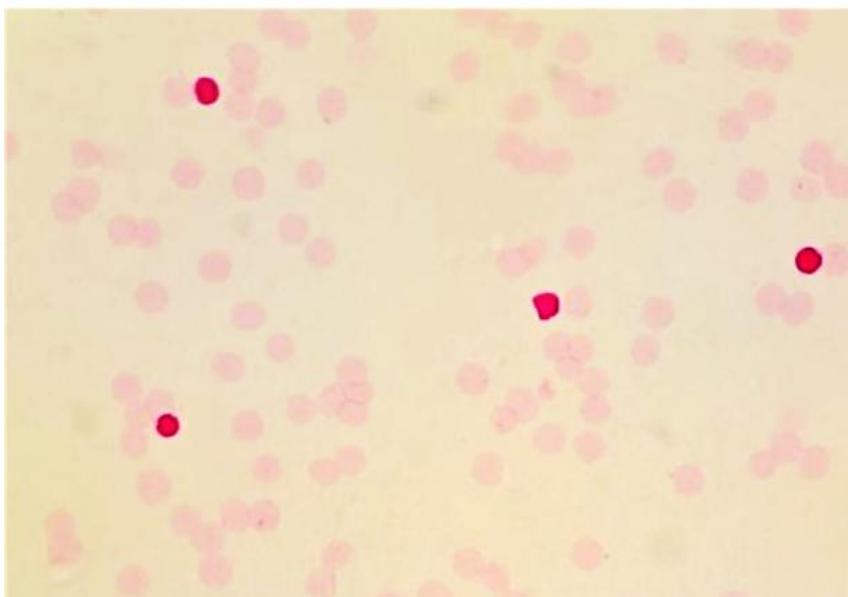


Question 22: Which statement is *most* correct about the sensitivity of a rosette test: A positive rosette test indicates the presence of at least how many fetal red cells in the maternal circulation?

- A. 1 mL.
 - B. 5 mL.
 - C. 10 mL.
 - D. 15 mL.
 - E. 20 mL.
-

Question 23: A D-negative mother has a complicated delivery. Maternal antibody screens are historically negative. The neonate is D-positive. Assuming that this is a 70-kg mother with an estimated 5000 mL blood volume, and based on the image seen in the figure below, how many vials of 300- μ g RhIG should be administered to the mother?

- A. 2.
- B. 3.
- C. 4.
- D. 5.
- E. 6.





Question 24: All D-negative mothers who give birth to D-positive neonates (and who do not already make anti-D) should get additional testing and, if indicated, quantification of fetomaternal hemorrhage (FMH).

- A. True.
 - B. False.
-

Question 25: When should a female of child-bearing potential who anticipates being pregnant get tested for red cell antibodies?

- A. Before attempting pregnancy.
 - B. First prenatal visit.
 - C. 20 weeks of gestation.
 - D. 24 weeks of gestation.
 - E. 28 weeks of gestation.
-

Question 26: A pregnant patient with positive red cell antibodies has been getting regular Doppler ultrasounds of the fetal middle cerebral artery to monitor the peak systolic velocity (MCA-PSV). Other than testing and monitoring, there have been no interventions up to this point. Today, at 32 weeks gestation, the MCA-PSV is measured at 1.36 multiples of the median (MoM) for gestational age. Which of the following statements is correct regarding her future management?

- A. No interventions necessary, plan for delivery at term (40 weeks of gestation).
- B. Plan to proceed with delivery at 37-38 weeks of gestation.
- C. Plan for intrauterine transfusion.
- D. Administer steroids to promote lung maturity and plan for urgent delivery with subsequent neonatal admission to intensive care unit (NICU) for management of neonatal complications of HDFN.
- E. None of the above.



Question 27: A pregnant mother comes in for her first prenatal visit and is typed for ABO/RhD and undergoes antibody screening. The antibody screen is positive (see accompanying panel). Which of the following options is the next most appropriate step for determining the risk for HDFN?

- A. Testing for antibody titers.
 - B. Paternal (geno)typing.
 - C. Intrauterine cord blood sampling to type fetal red cells.
 - D. Amniocentesis for fetal typing on amniocytes (PCR).
 - E. Chorionic villus biopsy.
-

Question 28: What is notable about the antibody specificity seen in the panel for Question 27 with regards to HDFN?

- A. It has a higher critical titer compared to other antibody specificities.
 - B. HDFN with this antibody is generally less severe compared to HDFN associated with anti-D.
 - C. Most cases of alloimmunization are the result of prior pregnancies.
 - D. Neonates affected with HDFN will have lower levels of reticulocytes and amniotic bilirubin compared to neonates affected by anti-D.
 - E. None of the above.
-

Question 29: What is the current preferred test/method for assessing fetal anemia?

- A. 450 nm ($\Delta OD450$) spectral analysis of amniotic fluid bilirubin levels.
- B. Cord blood direct antiglobulin test (DAT).
- C. Cordocentesis for fetal hematocrit testing.
- D. Doppler monitoring of peak systolic velocity (PSV) of middle cerebral artery (MCA).
- E. Unconjugated bilirubin levels in maternal serum/plasma.

**Antibody Identification panel, Questions 27 and 28**

Cell	D	C	c	E	e	f	V	G ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	IS	AHG	CC
1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	0	3+			
11	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+			
1	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	0	0	0	3+			
2	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	+			
3	+	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	0	+			
4	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	3+			
5	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+			
6	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	0	+			
7	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	3+				
8	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0	0	+				
9	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	+	0	0	0	+				
10	0	0	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	0	+	0	0	0	0	0	0	+				
Patient's cells																											0	0	+		



Question 30: What is the formula to calculate the volume of RBC units to transfuse for an IUT, given the following?

Hct = hematocrit

EFW = estimated fetal weight (g)

0.14 mL/g = conversion factor for EFW to fetal blood volume

A.

$$\frac{[EFW \text{ (g)}] \times [0.14 \text{ (mL/g)}] \times [\text{target Hct} - \text{pretransfusion Hct}]}{[\text{RBC unit Hct}]}$$

B.

$$\frac{[EFW \text{ (g)}] \times [\text{target Hct} - \text{pretransfusion Hct}]}{[\text{RBC unit Hct}] \times [0.14 \text{ (mL/g)}]}$$

C.

$$\frac{[\text{RBC unit Hct}]/}{[EFW \text{ (g)}] \times [0.14 \text{ (mL/g)}] \times [\text{target Hct} - \text{pretransfusion Hct}]}$$

D.

$$\frac{[\text{RBC unit Hct}] \times [0.14 \text{ (mL/g)}]}{[EFW \text{ (g)}] \times [\text{target Hct} - \text{pretransfusion Hct}]}$$

E.

$$\frac{[EFW \text{ (g)}] \times [0.14 \text{ (mL/g)}]}{[\text{target Hct} - \text{pretransfusion Hct} + \text{RBC unit Hct}]}$$



Question 31: A pregnant female whose previous pregnancy was complicated by HDFN now presents at 10 weeks of gestation with an anti-c titer of 128 and anti-S titer of 16. Which one of the following is correct?

- A. It is too early to do anything at this stage; must wait until 20 weeks of gestation before the fetus is large enough to benefit from any intervention.
- B. In cases of recurrent HDFN, it is critical to serially monitor antibody titers to track the risk for severe HDFN.



- C. IVIG with therapeutic plasma exchange (TPE) can delay development of severe anemia in the fetus.
 - D. The course of HDFN in this pregnancy is expected to progress similarly to the course of HDFN in the prior pregnancy.
 - E. None of the above.
-

Question 32: According to the 2023 ASFA guidelines, for the indication in question #31 scenario, TPE treatment would be considered which category?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. TPE for this indication is not addressed in the ASFA guidelines.
-

Question 33: The clinical team calls the blood bank to request a unit of antigen-negative RBCs for intrauterine transfusion (IUT) to manage HDFN. Which statement is correct regarding selection and preparation of RBC units for intrauterine transfusion in this scenario?

- A. Irradiation is indicated only if the RBC unit is from a related donor or HLA-selected donor.
 - B. There is no benefit to using an HbS-negative unit unless the fetus is expected to have sickle cell disease (ie, family history of sickle cell disease).
 - C. RBC units >7 days old are not eligible for use in intrauterine transfusions.
 - D. Crossmatching is not usually needed because it is prohibitive to obtain fetal serum for crossmatching.
 - E. If it is a challenge to get antigen-negative units (eg, high-frequency antigen or multiple antigens to avoid) and the mother is used as the RBC donor, the maternal red cells should be washed.
-

Question 34: What best describes the general strategy used for planning intrauterine fetal transfusions?



- A. Transfusion threshold <20%; target hematocrit >35%.
 - B. Transfusion threshold <20%; target hematocrit >40%.
 - C. Transfusion threshold <30%; target hematocrit >40%.
 - D. Transfusion threshold <30%; target hematocrit = 50%.
 - E. Transfusion threshold <40%; target hematocrit = 50%.
-

Question 35: Which statement is correct about the timing for clinical concern one should have for hyperbilirubinemia seen in HDFN?

- A. The greatest concern for complications of hyperbilirubinemia is for the fetus/neonate in the second trimester of pregnancy.
 - B. The greatest concern for complications of hyperbilirubinemia is for the fetus/neonate in the third trimester of pregnancy.
 - C. The greatest concern for complications of hyperbilirubinemia is for the fetus/neonate after delivery.
 - D. The greatest concern for complications of hyperbilirubinemia is for the mother during pregnancy.
 - E. The greatest concern for complications of hyperbilirubinemia is for the mother after delivery.
-

Question 36: A neonate born to a first-time mother develops jaundice and mild unconjugated hyperbilirubinemia. HDFN is suspected, but maternal antibody screens were negative during pregnancy. Cord blood DAT result was positive, but eluate was negative against the three-cell screening panel. What statement is *most* correct?

- A. This is a case of HDFN caused by autoantibodies.
 - B. Symptoms are expected to be mild.
 - C. This is an example of mechanical hemolysis due to manipulations performed during delivery.
 - D. The positive cord blood DAT result and signs of hemolysis are due to maternal administration of RhIG.
 - E. None of the above.
-

Question 37: Which statement is true regarding options for treating neonatal HDFN?



- A. Because HDFN involves neonatal anemia as well as antibodies and bilirubin in the plasma, exchange transfusion requires the use of whole blood unit(s) to allow exchange of both blood components in the neonate.
 - B. It takes several days of multiple phototherapy treatments before an improvement in bilirubin is typically seen.
 - C. IVIG is indicated if exchange transfusions are not successful.
 - D. IVIG is associated with increased risk for necrotizing enterocolitis (NEC) and hemolysis.
 - E. There are no contraindications for transfusion products for neonates undergoing phototherapy.
-

Question 38: A double-volume exchange transfusion is expected to remove approximately what ___% of circulating red cells and what ___% of total serum bilirubin?

- A. 85% red cells; 75% total bilirubin.
 - B. 85% red cells; 50% total bilirubin.
 - C. 65% red cells; 70% total bilirubin.
 - D. 60% red cells; 80% total bilirubin.
 - E. 50% red cells; 50% total bilirubin.
-

Question 39: Fetal neonatal alloimmune thrombocytopenia (FNAIT) can include which of the following presentations?

- A. Maternal thrombocytopenia.
 - B. Fetal/neonatal thrombocytopenia.
 - C. Neonatal ecchymosis and petechiae.
 - D. Fetal/neonatal intracranial hemorrhage.
 - E. B, C, and D.
-

Question 40: Which of the following represents the *best* approximate incidence of FNAIT in pregnancies?

- A. 1 in 50 (2%).
- B. 1 in 100 (1%).
- C. 1 in 500 (0.2%).



- D. 1 in 1000 (0.1%).
 - E. 1 in 10,000 (0.01%).
-

Question 41: FNAIT occurring in the United States is most likely caused maternal antibodies with specificity against which HPA antigen?

- A. HPA-1a.
 - B. HPA-1b.
 - C. HPA-3a.
 - D. HPA-3b.
 - E. HPA-5a.
-

Question 42: Approximately what percentage (%) of the population is HPA-1a negative in the United States?

- A. 0.02%.
 - B. 0.5%.
 - C. 2%.
 - D. 5%.
 - E. 10%.
-

Question 43: Which statement is true regarding FNAIT-associated intracranial hemorrhage (ICH)?

- A. ICH is fatal in approximately 35% of cases.
- B. Although clinically severe, ICH due to FNAIT affects only 1 in 100,000 live births.
- C. If fatality is averted, nonfatal cases of ICH generally have a good prognosis without neurologic/developmental sequelae.
- D. The majority of cases of ICH occur during delivery because of the associated physical manipulation that occurs during delivery.
- E. ICH cannot be prevented; therefore, interventions can address only the management of ICH after it has already occurred.



Question 44: FNAIT occurs only in a second or subsequent pregnancy, because it requires a first pregnancy to cause alloimmunization to HPA.

- A. True.
 - B. False.
-

Question 45: When should a pregnant patient be considered at risk for FNAIT and, therefore, more closely monitored for FNAIT during pregnancy?

- A. When she has a history of FNAIT affecting a previous pregnancy.
 - B. When she has a sister with a history of pregnancy affected by FNAIT.
 - C. When she has an absence of the *HLA-DRB3*01:01* allele (of the HLA-DR52 antigen).
 - D. A and B.
 - E. A, B, and C.
-

Question 46: A mother presents to the clinic with her second pregnancy. She has a positive history of having her first pregnancy affected by FNAIT and is referred to a fetal medical center that has experience in dealing with FNAIT-associated pregnancies. She was previously typed as HPA-1b/1b. Which of the following statements regarding perinatal evaluation for FNAIT is true?

- A. The rate of FNAIT recurrence in a subsequent pregnancy is approximately 15%.
- B. Because the mother has been typed as HPA-1b/1b homozygous and already has a history of FNAIT, maternal antibody testing is not required.
- C. If the father serologically types as HPA-1a positive, then the fetus/neonate must also be positive for HPA-1a.
- D. If the father is not known and/or unavailable for HPA typing, fetal platelet genotyping is recommended.
- E. None of the above.



Question 47: What statement is true regarding perinatal management of FNAIT?

- A. A neonate requires a platelet transfusion and the blood bank should issue only units negative for the HPA-antigen of interest (“HPA-selected” unit), so as not to give an “incompatible” platelet unit. If an HPA-selected unit is not available, the mother must be used for directed donation of platelets.
- B. Intrauterine IVIG administration to the fetus is preferable to IVIG administration to the mother.
- C. There is no utility in antenatal interventions before 24 weeks of gestation because fetal platelets are not affected by maternal alloantibodies until after 24 weeks of gestation.
- D. A platelet count of 30,000/ μ L is recommended as a transfusion threshold in nonbleeding neonates. For neonates with life-threatening bleeding (ICH, gastrointestinal), a threshold of 100,00/ μ L should be used, followed by a threshold of 50,000/ μ L for at least 7 days.
- E. The platelet count obtained from cord blood at the time of delivery should represent the lowest platelet level of the neonate, as platelet levels are only expected to increase only after birth once the maternal source of alloantibodies has been removed.



Question 48: Which of the following statements are true for *both* FNAIT and primary immune thrombocytopenia (ITP) in pregnancy?

- A. Maternal platelet levels do not correlate with fetal/neonatal platelets levels.
- B. The diagnosis for both FNAIT and ITP are defined by a combination of HPA typing and HPA antibody testing.
- C. Both FNAIT and ITP are associated with a high risk of in-utero ICH.
- D. In the setting of (suspected) ITP and FNAIT, umbilical cord blood should be tested for baseline platelet levels. Neonates should be monitored as platelet levels can decrease after birth, with a nadir typically seen within the first week.
- E. A and D.



Question 49: What is the role of laboratory testing for ITP-associated pregnancies?

- A. Fetal platelet levels must be closely monitored during pregnancy.
 - B. HPA antibody testing should be performed to determine when additional interventions or management need to be initiated.
 - C. If the neonate presents with severe thrombocytopenia (platelets <50,000/ μ L), parental testing should be performed to exclude NAIT.
 - D. Platelet crossmatches are recommended to identify compatible platelet units for transfusion.
 - E. None of the above.
-

Question 50: Which of the following is true regarding the treatment and management options with ITP-associated pregnancies?

- A. The primary goal of management of ITP during pregnancy is to minimize bleeding complications of the fetus/neonate.
- B. In the absence of any bleeding or symptoms, management should be aimed at keeping the mother's platelet levels above 10,000/ μ L.
- C. Similar to FNAIT, IVIG or steroids administered to the mother are considered first-line management options for ITP.
- D. Cranial ultrasounds should be performed on neonates with a platelet count of <100,000/ μ L to assess for ICH.
- E. None of the above.

ANSWERS

Question 1: A. Nonimmune cause.

Explanation:

- Hydrops fetalis, literally meaning "edema of the fetus," can be defined by the presence of an abnormal fluid accumulation in at least two extravascular compartments, which could include



ascites, pleural effusions, pericardial effusion, placental edema, subcutaneous edema, and anasarca.

- This question is intended to highlight that hydrops fetalis (also known as fetal hydrops) is a condition that can have multiple etiologies.
- Hydrops fetalis can be categorized as immune hydrops or nonimmune hydrops.
- Immune hydrops is hydrops fetalis that is due to hemolytic disease of the newborn and fetus (HDFN).
 - In HDFN, the pregnant mother has been alloimmunized to paternally inherited antigens that are expressed on the fetal red blood cells (RBCs) and/or erythroid precursors. IgG antibodies are transported across the placenta by FcRn transporter and cause immune-mediated destruction of fetal RBCs and/or erythroid precursors.
 - IgM is not transported across the placenta. Therefore, IgM antibodies do not cause HDFN.
 - In response to the immune-mediated anemia, the fetal compensatory response includes increased extramedullary hematopoiesis in the liver and spleen, which can result in hepatomegaly and splenomegaly. “Erythroblastosis fetalis” is actually an older term for HDFN, derived from the observation of increased erythroblasts (immature RBCs) seen in the peripheral blood smears of newborns.
 - A proposed etiology of hydrops in HDFN is that the affected liver decreases protein (albumin) production, causing decreased oncotic pressure. Fetal cardiac output also increases to compensate for the compromised oxygen delivery and may result in high-output cardiac failure, leading to fetal demise.
 - Not all cases of HDFN will result in hydrops fetalis. The presentation of HDFN can range from a purely serologic finding (clinically asymptomatic neonate with a positive DAT corresponding with positive alloantibodies identified in mother), to anemia (mild to severe), to hydrops fetalis and/or fetal death.
- Historically, HDFN due to RhD incompatibility (where mother made anti-D with a D-positive fetus) was the predominant cause of hydrops fetalis. With the advent of Rh Immune Globulin (RhIG) prophylaxis (discussed in more detail later in this chapter), 90% of hydrops fetalis cases in developed countries are now nonimmune cases.



- Nonimmune causes of hydrops fetalis are beyond the scope of this book, but include a broad range of underlying etiologies that include, but are not limited to, conditions of the following categories: cardiovascular, chromosomal/aneuploidies, infections, metabolic, hematologic, twin-pregnancies, chondrodysplasias, genetic, urologic, etc.
-

Question 2: B. IgG1 and IgG3 are more likely than IgG2 and IgG4.

Explanation:

- There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4. IgG1 and IgG3 are more effective at fixing complement than IgG2 and IgG4.
 - They are more likely than IgG2 and IgG4 to be associated with clinically significant hemolytic disease of the fetus and newborn.
-

Question 3: A. Antibodies to the ABO antigen system.

Explanation:

- ABO incompatibility is the most common cause of HDFN in countries with RhIG prophylaxis.
 - Before the implementation of RhIG prophylaxis, Rh(D) incompatibility was historically the most common cause of HDFN.
 - Antibodies to antigens in the Rh, KEL (Kell), Jk (Kidd), Fy (Duffy), and MNS blood groups can all cause HDFN.
 - Antibodies to Le (Lewis) do not cause HDFN. These antibodies are primarily IgM which do not cross the placenta. Furthermore, Lewis antigens are poorly expressed fetal RBCs and do not reach full expression on red cells until 5-6 years of age.
-

Question 4: D. Anti-M.



Explanation:

- Anti-I, Le(a), Le(b), M, and P1 antibodies are antibodies that are associated with reactivity at room (22 C) and/or cold (4 C) temperatures.
 - Anti-I, Le(a), Le(b), and P1 antibodies are *not* associated with HDFN. These antibodies are predominantly IgM. Furthermore, I, Le, P antigens are poorly expressed on fetal/neonatal red cells.
 - Anti-M, on the other hand, can comprise IgG and has, on rare occasions, been associated with HDFN. If warm-reacting IgG antibodies of anti-M specificity are identified, they should be considered a potential risk factor for hemolytic transfusion reactions and for HDFN.
-

Question 5: B. It can occur in the first pregnancy without any history of a sensitizing event.

Explanation:

- It is true that anti-A and anti-B are predominantly IgM in A and B individuals. However, in O individuals, the predominant isotype is IgG, which can cause ABO-incompatibility-related HDFN.
- ABO-incompatible HDFN has a milder clinical presentation compared to RhD-associated HDFN. It typically presents in neonatal period with mild to moderate jaundice. Any anemia seen tends to be mild.
- Currently there is not a well-established role for anti-A or anti-B monitoring during pregnancy and it is not standard of care in obstetric practice. A previous history of ABO-incompatible HDFN can be a predictor of recurrent (and more significant) ABO-incompatible HDFN in the subsequent pregnancy(ies). However, ABO serologies do not correlate well with clinical presentation.
- While ABO incompatibility occurs in approximately 15% or more pregnancies (depending on the ethnic population), the incidence of HDFN due to ABO incompatibility is low (1-4%, depending on the ethnic population).
- HDFN due to ABO incompatibility is usually mild and self-limiting. Phototherapy is usually adequate for managing cases that require intervention.



Question 6: E. In African populations: Group O mother, group B infant.

Explanation:

- The most common scenario of ABO-incompatibility-associated HDFN, based on the ancestry, is as follows:
 - European: Group O mother, group A neonate.
 - Asian: Group O mother, group A neonate.
 - African: Group O mother, group B neonate.
-

Question 7: A. ABO.

Explanation:

- Anti-A and anti-B are considered “naturally occurring” antibodies, meaning that they are produced by an individual without requiring previous exposure (alloimmunization) to A and/or B antigens.
 - Antibodies to all of the antigen groups in the other answer options are considered “unexpected” antibodies, meaning that they are not expected to be encountered in a nonalloimmunized (nonsensitized) individual.
-

Question 8: B. 15% European ancestry, 8% African ancestry, <0.1% Asian ancestry

Explanation:

- The incidence of the D-negative phenotype is approximately 15% for persons of European ancestry, 8% for African ancestry, and <0.1% for Asian ancestry.
-

Question 9: A. The risk of fetomaternal hemorrhage (FMH) increases as gestational age increases, with the greatest risk being at time of delivery.



Explanation:

- Alloimmunization to the D antigen can occur with exposure to as little as 0.1 mL D-positive red cells.
- Alloimmunization can occur through pregnancy, transfusion, transplantation, contaminated needlestick exposure, etc.
- Most transfusion services have a practice of reserving D-negative units for females of potential child-bearing potential to avoid alloimmunization, given the potentially severe consequences of HDFN.
- During pregnancy risk factors for intrauterine hemorrhage include:
 - Abdominal trauma.
 - Placental previa.
 - Abruptio placentae.
 - Ectopic pregnancy.
 - Threatened termination.
 - Fetal death.
 - Amniocentesis.
 - Intrauterine manipulation.
 - Abortion.
- Alloimmunization is not required for an individual to make IgG anti-A and anti-B.
- The incidence of alloimmunization to RhD is increased in ABO-compatible pregnancies compared to ABO-incompatible pregnancies.



Question 10: E. Weak D1, D2, and D3.

Explanation:

- Serologic weak D phenotype can be defined as a red cell reactivity of 2+ or less with anti-D typing reagent. *RHD* genotyping is used to further determine whether the serologic weak D phenotype is due to “weak D” or “partial D.”
- Studies evaluating patients of primarily European ancestry have shown that approximately 95% of those with serologic weak D phenotype are due to weak D1, D2, and D3 genotypes and that these weak D genotypes do not appear to be at risk for making alloanti-D.



- Potential mothers who demonstrate a weak D1, D2, or D3 genotype can be considered as D-positive and be spared the additional costs and steps of preventing alloimmunization to RhD because they are not at risk of making antibodies to RhD antigen.
 - Patients who are not D-positive (or Weak D1, D2, or D3) should be presumed capable of being alloimmunized to the RhD antigen.
-

Question 11: C. 16%.

Explanation:

- Although reports vary, the risk of a D-negative mother being alloimmunized to a D-positive fetus is approximately 16% if there is no intervention.
-

Question 12: D. The mechanism of efficacy is not known.

Explanation:

- RhIG is polyclonal anti-D that is derived from human plasma and is used to prevent alloimmunization to the RhD antigen after or in anticipation of exposure to D antigen.
- Therefore, once a patient has made anti-D, there is no further role for RhIG with regards to preventing alloimmunization; alloimmunization has already happened.
- Formulations can be intramuscular (IM) or intravenous (IV).
- RhIG is commonly available in doses of 50 µg, 120 µg, and 300 µg.
- Although there are theories as to how RhIG helps to prevent alloimmunization, the exact mechanism of action is not known.
- Interestingly, although RhIG can bind fetal D-positive RBCs and be a cause of positive direct antiglobulin test (DAT) result, it does not pose any significantly increased risk for fetal hemolysis.
- Mothers who are genotyped as partial D should be considered candidates for RhIG.
- RhIG is also FDA approved for treatment of immune thrombocytopenia (ITP) and can be an alternative to IVIG. Although RhIG is available in for IM or IV administration, IV administration should be used for ITP. ITP patients should be D-positive.

**Question 13: A. <0.1%.****Explanation:**

- The practice of RhIG prophylaxis decreased the rate of alloimmunization to D-antigen to <0.1%.
 - It is because of this that ABO is now the primary cause of HDFN where RhIG prophylaxis is implemented.
 - However, RhIG does not prevent alloimmunization to other RBC antigens.
-

Question 14: A. D-negative mother who makes anti-Di(a), neonate types as D positive.**Explanation:**

- Di(a) is an antigen in the DI (Diego) group and should not be confused with Rh(D).
- RhIG only address suppression of alloimmunization to Rh(D) and its effect is unrelated to the presence or absence of non-D alloantibodies.
- D-positive (including weak D1, D2, and D2) mothers will not benefit from RhIG, since they should not make alloanti-D.
- Mothers who already make alloanti-D will not benefit from RhIG. However, it is important to distinguish passively acquired anti-D from actively produced anti-D. While the antibody pattern will be similar, actively made anti-D tends to have stronger reactions and higher titers than passively acquired anti-D. Review of the patient's medical history and timing of administered products can also be useful. Patients with only passively acquired anti-D are still candidates for RhIG prophylaxis.
- When the fetus/neonate is D-negative, there is both
 - No risk of HDFN to the fetus/neonate.
 - No risk of alloimmunization to D antigen that could affect future pregnancies of the mother.
- With that said, neonates that serologically type as D-negative should have further *RHD* genotyping to definitively confirm D-negative status since D variants may appear to be D-negative serologically but still be capable of inducing alloimmunization.

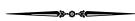


Some practices may find it easier just to give RhIG than pursue genotyping of the fetus/neonate.

Question 15: E. All of the above.

Explanation:

- Standard 5.30.2 “Rh Immune Globulin” of the 33rd edition of the *AABB Standards for Blood Banks and Transfusion Services* states that pregnant or recently pregnant women shall be considered for RhIG administration when all of the following apply:
 - The woman tests negative for D antigen (weak D testing being optional). This is because the patient would have the potential to make alloanti-D.
 - The woman is not known to be alloimmunized to the D antigen. This is because it is not too late for prophylactic intervention.
 - The Rh typing of the fetus/neonate is either unknown or positive for D or weak D. This because the mother could still be at risk for being sensitized by the fetus/neonate.
 - The standards also state that weak D testing is required if the fetus/neonate tests negative for D. This is because it is possible that serologic methods may not be sensitive enough to detect a weak D phenotype but where a fetus/neonate would still clinically at risk for inducing alloimmunization. Additional weak D testing (ie, genotyping) is needed to rule out this risk.



Question 16: D. The antibody results are likely due to previous RhIG administration. The patient should be interviewed and/or any available medical records should be checked for history of RhIG administration. In the meantime, the patient should be considered a candidate for RhIG.

Explanation:

- The goal of RhIG is to prevent alloimmunization to the D antigen. If a woman is already making anti-D, the purpose of administering RhIG has already been lost. Therefore, not all D-negative pregnant women are candidates for RhIG administration.



- The antibody panel and history are both consistent with the presence of passively acquired anti-D due to RhIG administration.
- The D antigen is highly immunogenic. Therefore, anti-D due to alloimmunization normally presents as a strongly positive antibody finding (3+ or 4+). Also, this is the mother's first pregnancy and she has an otherwise unremarkable medical history (no history to indicate prior alloimmunization).
- In this case, the antibody panel is showing a weaker presence of anti-D of 1+ reaction strength at a low titer of 1. This is suggestive that the anti-D is due to passively acquired anti-D (RhIG). The mother mostly likely received the standard first dose of RhIG at 28 weeks of gestation. Although reports vary, the half-life of RhIG has an approximate range of 2-4 weeks.
- Unless the mother is already known to be alloimmunized to the D antigen, RhIG should be given after any event that puts the mother at risk for FMH—even if she already received RhIG at 28 weeks, or if RhIG is still detectable in the antibody screen.
- Whenever there is any doubt, it is always safer to administer RhIG. The benefits far outweigh the perceived costs.
- Weak reactions of anti-D reagent to a patient's RBCs (weak+, 1+, 2+) are an indication that the patient's red cells may express weak D or partial D. This should not be confused with weak reaction of antibodies in a patient's serum/plasma sample to reagent D-positive red cells. However, it is true that a patient in whom weak-D vs partial-D typing is being considered, but not yet confirmed, should be managed as if she can make anti-D, and should be a candidate for RhIG prophylaxis pending genotyping results and until her RhD status is confirmed by genotyping (and as long as she is not already making anti-D).



Question 17: A. Within 72 hours of event. This patient would still benefit from RhIG.

Explanation:

- The ACOG practice guidelines recommend administration of RhIG within 72 hours of delivery or a potentially sensitizing event. However, RhIG has still been shown to be protective outside of this window.



- Given the risk of potential severe consequences of HDFN in future pregnancies with alloimmunization to D antigen, it is still worthwhile to provide RhIG prophylaxis to this mother at 4 days after the potentially sensitizing event, even if it is outside the recommended 72 hours.
-

Question 18: C. Further workup is needed for antibody identification. In the meantime, the patient should be considered a candidate for RhIG until workup and identification is complete.

Explanation:

- The patient's serum has reactivity against D-positive and C-positive cells. While there are D- and C-specific epitopes that may be recognized by anti-D and anti-C antibodies, respectively, the D and C antigens also share a common epitope, which is recognized by anti-G. Therefore, several different antibody combinations could present with the pattern seen above:
 - Anti-D and anti-C.
 - Anti-G.
 - Anti-G and anti-C.
 - Anti-G and anti-D.
 - Anti-G, anti-D, and anti-C.
- Discerning anti-G from anti-D and/or anti-C requires additional adsorption and elution steps, which is time and labor consuming. It is not unusual for a sample to be sent out to an immunohematology reference laboratory for this workup.
- The significance of anti-G is that, if it is misinterpreted as an anti-D by an inexperienced technologist, it may give the false impression to the clinical team that the patient already makes anti-D when the patient is actually not alloimmunized to D and would still benefit from RhIG prophylaxis.
- Anti-D, anti-C and anti-G are all associated with HDFN. One might wonder if there is any advantage to preventing alloimmunization to D in the presence of anti-G, since anti-G will react against D cells anyway. Since it is recognized that the presence of multiple antibodies can be associated with more severe HDFN, there is still an advantage in trying to prevent alloimmunization to D, even the mother already makes anti-G.



Question 19: B. First-time mother at first prenatal visit.

Explanation:

- The 2017 ACOG Practice guidelines recommend RhIG administration
 - At 28 weeks of gestation.
 - At delivery.
- RhIG should also be given when there is a risk factor for intrauterine hemorrhage (fetomaternal hemorrhage, FMH), which includes:
 - Abdominal trauma.
 - Placental previa.
 - Abruptio placentae.
 - Ectopic pregnancy.
 - Threatened termination.
 - Fetal death.
 - Amniocentesis.
 - Intrauterine manipulation.
 - Abortion.
- RhIG is also recommended for when there is a uterine evacuation of a molar pregnancy.
- RhIG is also indicated to suppress alloimmunization if a D-negative patient is transfused with D-positive cellular blood components (RBCs, platelets).
- By itself, the first prenatal visit is not reason enough to administer RhIG. In the absence of an at-risk event, RhIG is not indicated before 28 weeks of gestation because the observed rate of seroconversion before 28 weeks is very low.



Question 20: C. 50 µg RhIG; 5 mL of fetal whole blood.

Explanation:

- 50 µg of RhIG suppresses alloimmunization of 2.5 mL of fetal RBCs, or 5 mL of fetal whole blood.
- 300 µg of RhIG suppresses alloimmunization of 15 mL of fetal RBCs, or 30 mL of fetal whole blood.



- RhIG has a half-life of approximately 25 days. This means that approximately 10% of an RhIG dose given at 28 weeks is expected to be remaining in the mother at 40 weeks.
 - RhIG is supposed to be administered within 72 hours of birth.
 - In the United States, a single dose of 300 µg is given at 28 weeks. At delivery, the dose depends on the estimated volume of FMH.
-

Question 21: B. The mother received an inadequate dose of RhIG.

Explanation:

- Although RhIG can cause a positive anti-D pattern, it would not be expected to be at “4+” strength 1 year later, if at all. This would also apply to any antibody passively acquired anti-D from a transfused unit.
 - The RBC unit the patient received is almost certainly RhD negative and therefore not expected to be a cause of alloimmunization. Because RBC units are prelabeled with the donor’s RhD status (and the patient is group AB), there should be no real obstacle to the blood bank providing a D-negative unit, even in most emergency settings.
 - This history of the mother receiving a unit is an indication that there was significant bleeding, which may indicate a greater amount of FMH than typical.
-

Question 22: C. 10 mL.

Explanation:

- The rosette test is a semiquantitative screening test for FMH.
- It is performed by taking a blood sample from the D-negative mother, adding reagent anti-D, and then adding D-positive indicator red cells. If there are fetal D-positive cells present, the indicator red cells will cluster around with the aid of the anti-D reagent. This configuration of indicator red cells appears as a “rosette” around the fetal D-positive cell.
- A negative test (absence of visualized rosettes) indicates that any fetomaternal bleeding is less than 10 mL fetal red cells and that a



single 300- μ g vial of RhIG is sufficient for RhIG prophylaxis since one 300- μ g vial of RhIG should suppress alloimmunization by 15 mL fetal red cells (30 mL fetal whole blood).

Question 23: E. 6.

Explanation:

- The image shows the blood smear of the Kleihauer Betke test, also known as the KB acid elution test. This is a quantitative test used to estimate the amount of FMH and thereby to calculate the number of RhIG vials needed to prevent alloimmunization to the D antigen.
- The maternal blood smear is created on a slide that is then treated with acid. Fetal hemoglobin (HbF) in the fetal red cells is resistant to acid treatment but HbA in the mother's red cells is not. After the slide has been washed and stained, the fetal red cells (which have retained HbF) stain bright pink whereas the maternal red cells have a pale "ghost" appearance.
- FMH = maternal whole blood vol [mL] \times (# Fetal red cells / # total red cells).
 - In this case there are 4 fetal red cells out of 128 total = 3.125%
 - $FMH = 5000 \text{ mL} \times 3.125\% = 156.25 \text{ mL}$
- Remembering that one 300- μ g vial of RhIG counters 30 mL of fetal whole blood, an *initial approximation* of the number of vials needed = $FMH [\text{mL}] / 30 [\text{mL/vial}]$.
 - When the number behind the decimal point is < 5, one rounds down (eg, 2.4 vials \rightarrow 2 vials).
 - When the number behind the decimal point is ≥ 5 , one rounds up (eg, 2.5 vials \rightarrow 3 vials).
 - In this case $156.25 \text{ mL} / 30 \text{ mL} = 5.21$ vials, which rounds down to \rightarrow 5 vials.
- Because the KB test is subject to interpretation and sampling variability, there can be variation in the results even when repeated on the same sample. To guard against underestimating, one additional vial is added to the initial approximation to reach the final number of vials. In this case, one vial is added to the initial estimate of five vials: 5 vials + 1 vial = 6 vials.



Question 24: A. True.

Explanation:

- Because there is no reliable way to predict the level of FMH based on event or circumstance, the 2017 ACOG Practice guidelines recommend that all D-negative mothers who give birth to D-positive neonates undergo additional testing to screen and quantify (if needed) any FMH to ensure accurate administration of RhIG prophylaxis.
-

Question 25: B. First prenatal visit.

Explanation:

- ABO/Rh typing and antibody screen should be performed at the first prenatal visit.
-

Question 26: B. Plan to proceed with delivery at 37-38 weeks of gestation.

Explanation:

- For a fetus at risk for HDFN but with only mild anemia, recommendations are to continue monitoring and have a planned delivery by 37-38 weeks (instead of at 40 weeks) to decrease the time window of maternal antibody exposure.
 - Because the MCA-PSV is <1.5 MoM, there is no need for intrauterine transfusion at this time.
 - If MCA-PSV is >1.5 MoM and the fetus is at <35 weeks of gestation, the next step is cordocentesis to sample cord blood for fetal hematocrit and hemoglobin testing with the intention of planning for an intrauterine transfusion (IUT).
-

Question 27: B. Paternal (geno)typing.



Explanation:

- Once clinically significant antibodies are identified, the next step is to see if the fetus would be at risk for HDFN, ie, whether the fetal red cells would carry the target antigen. If the fetus is not expected to express the antigen, then there is no expected risk of HDFN.
- Because it is easier to type the father, it is recommended that the father is typed if there is certainty about the parentage. If the father is homozygous for the antigen, then the fetus has a 100% chance of inheriting the genotype and expressing the antigen. If the father is heterozygous, the fetus has a 50% chance of expression the antigen.
- Because there is no “d” antigen, determining homo- versus heterozygosity of the father in the presence of an RhD antigen requires genotyping.
- If fetal genotype cannot be inferred, either because parentage is uncertain or because the father is heterozygous for the antigen in question, fetal genotyping of fetal cell-free DNA in the maternal serum can be performed. This is noninvasive and therefore preferable to the older methods of amniocentesis or chorionic villus biopsy, which both have associated risks of bleeding and alloimmunization.
- Titers may typically be performed upfront for pregnant patients when a (clinically significant) antibody is identified. However, by themselves, titers are a moot point if the fetus/father is negative for the antigen.



Question 28: D. Neonates affected with HDFN will have lower levels of reticulocytes and amniotic bilirubin compared to neonates affected by anti-D.

Explanation:

- The antibody pattern seen in the panel for Question #27 is consistent with anti-K specificity.
- Most cases of alloimmunization to K are due to transfusion.
- The critical titer is defined as the titer at which the fetus is at risk for developing severe anemia and hydrops fetalis. Before a critical titer is reached, titers are monitored (typically every 2-4



weeks). Once a critical titer is reached, one should monitor the fetus for evidence of severe anemia that may require intervention.

- The 2018 ACOG Practice Bulletin uses a critical titer of 16 for alloanti-D, although individual practitioners may determine their own critical titers. Therefore, a critical titer for anti-D can be in the range of 8-32.
- Anti-K is notable for being associated with severe HDFN and early HDFN. Therefore, any titer of anti-K may be considered clinically significant (ie, eight or less) and the monitoring of titers of anti-K may be less useful because these do not correlate well with disease severity.
- Because K is expressed early on red cell precursors, HDFN with anti-K can present with significant anemia and reticulocytopenia. It may not always involve hemolysis or hyperbilirubinemia because the erythroid lineage cells may be destroyed before developing into red cells.
- A critical titer of 16-32 is used for other antibodies (non-K, non-D).



Question 29: D. Monitoring of peak systolic velocity (PSV) of middle cerebral artery (MCA).

Explanation:

- Once antibody titers approach the critical titer, the next step is to monitor the fetus for evidence of severe anemia.
- Doppler monitoring of peak systolic velocity (PSV) of middle cerebral artery (MCA) has now displaced 450 nm (ΔOD_{450}) spectral analysis of amniotic fluid bilirubin levels as the preferred means of evaluating for fetal anemia.
- Obtaining cord blood for fetal hematocrit and/or amniotic fluid for bilirubin levels are both invasive procedures.
- The Doppler MCA-PSV has the advantage of being noninvasive and sensitive for detecting moderate to severe anemia (fetal hematocrit <0.65). An MCA-PSV that is at least 1.5 times the multiples of the median (MoM) for gestational age is highly sensitive for detecting moderate to severe fetal anemia.
- Unconjugated hyperbilirubinemia is not expected during pregnancy since the mature maternal liver is capable of conjugating and clearing bilirubin. However, bilirubin levels may rise in the



neonate after it is separated from the protective effect of the maternal liver.

- Sampling cord blood for DAT testing is not recommended in the antenatal period. However, getting a cord blood sample for DAT and RBC typing shortly after delivery is recommended if monitoring for suspected HDFN in the postnatal period.
-

Question 30: A.

$$\frac{[\text{EFW(g)}] \times [0.14(\text{mL/g})] \times [\text{target Hct} - \text{pretransfusion Hct}]}{[\text{RBC unit Hct}]}$$

Explanation:

- Volume of RBC unit to transfuse = [Estimated fetal weight (g)] \times [0.14 (mL/g)] \times [target Hct – pretransfusion Hct] / [RBC unit Hct]
-

Question 31: C. IVIG with therapeutic plasma exchange (TPE) can delay development of severe anemia in the fetus.

Explanation:

- When there is a history of previous pregnancy affected by HDFN:
 - HDFN in subsequent pregnancies are predicted to be more severe and with earlier onset of symptoms.
 - Titers do not correlate well with severity of HDFN in subsequent pregnancies and therefore serial monitoring of titers becomes less useful.
- When managing HDFN at an early gestational age, IVIG with or without therapeutic plasma exchange are available interventions while waiting until such time when intrauterine transfusions are an option.
- Intraperitoneal transfusions have also been used as a means of delivering compatible red cells in early gestational stages when intravascular access is a challenge. Red cells appear to migrate into fetal circulation from the peritoneal space.

**Question 32: C.** Category III.**Explanation:**

- The 2023 ASFA guidelines determine red cell alloimmunization in pregnancies at <20 weeks of gestation to be a Category III indication. Category III indicates that the optimal role of TPE in this setting is not yet established. Therefore, the decision to use TPE should be made on an individual patient basis.
-

Question 33: E. If it is a challenge to get antigen negative units (eg, high-frequency antigen or multiple antigens to avoid) and the mother is used as the RBC donor, the maternal RBCs should be washed.

Explanation:

- Units selected and prepared for intrauterine transfusion should be:
 - Type O.
 - Negative for the antigen of concern.
 - Crossmatch compatible with the maternal serum. (There is no need to obtain fetal serum for crossmatching).
 - Irradiated, regardless of donor relationship or level of HLA match, to prevent transfusion-associated graft-vs-host disease (TA-GVHD).
 - CMV negative and/or CMV-reduced-risk (leukocyte-reduced).
 - Freshest possible (ideally within 5-7 days). However, it may not always be possible to have a unit <7 days old, especially depending on the feasibility of obtaining antigen-negative units. While units <7 days are ideal, “freshest possible” is the accepted principle.
 - Even if there is no family history of sickle disease involved, selection of HbS-negative RBCs avoids the sickling phenomenon that can occur at low oxygen levels.
 - If antigen-negative units are not readily identified and the mother is used as an RBC donor, the unit should be washed to remove the offending alloantibodies.



Question 34: C. Transfusion threshold <30%; target hematocrit >40%.

Explanation:

- While practices may vary, and thresholds may change with gestational age, in general a fetal hematocrit of <30% is referenced as a trigger threshold for initiating intrauterine transfusion and a fetal hematocrit of >40% is used as the posttransfusion target.
-

Question 35: C. The greatest concern for complications of hyperbilirubinemia is for the fetus/neonate in the period after delivery.

Explanation:

- Hyperbilirubinemia due to immune-mediated hemolysis is not a concern in utero because the functioning maternal liver is able to help conjugate and clear the released fetal bilirubin. However, after delivery, the neonatal liver is not able to adequately perform this function by itself, leading to the accumulation of bilirubin.
 - Untreated, the increase in bilirubin can lead to jaundice and, more dangerously, to chronic bilirubin encephalopathy (also known as kernicterus), which is a form of bilirubin-induced neurologic dysfunction (BIND) that may have severe and/or irreversible neurologic sequelae.
 - First-line management of neonatal hyperbilirubinemia involves phototherapy (notable decreases can occur within the first few hours of use). IVIG and exchange transfusions can be used in cases that require escalation of care.
-

Question 36: B. Symptoms are expected to be mild.

Explanation:

- This is a case of HDFN due to ABO incompatibility. This explains why the DAT result is positive but the eluate is negative against the three-cell screening panel where the reagent red cells are group O.



- Autoantibodies would be expected to have a positive eluate. Autoantibodies would also be expected to show up on previous antibody screens.
 - A positive DAT result due to an RhIG (anti-D) would be expected to be positive with the three-cell screening panel on the eluate.
 - HDFN due to ABO is typically mild and self-limiting.
-

Question 37: D. IVIG is associated with increased risk for necrotizing enterocolitis (NEC) and hemolysis.

Explanation:

- Phototherapy is the first line of treatment and converts bilirubin into the non-neurotoxic and more water-soluble lumirubin, which can be cleared through urine. Significant reductions in bilirubin can be seen within the first few hours of phototherapy.
 - IVIG is associated with increased risk for NEC.
 - Exchange transfusions can be considered when phototherapy and IVIG prove insufficient. A double-volume exchange is recommended to fully remove the unwanted red cells. Instead of using a whole-blood unit, *reconstituted whole blood* is used instead. This allows for the selection of both antigen-negative and antibody-negative components (eg, group O RBCs, group AB plasma).
 - As per the 2021 *Circular of Information for the Use of Human Blood and Blood Components*, pathogen-reduced platelet and plasma components are contraindicated for neonates undergoing phototherapy with devices that emit a peak energy wavelength <425 nm or with a lower bound of emission bandwidth <375 nm.
-

Question 38: B. 85% red cells; 50% total bilirubin.

Explanation:

- A double-volume exchange transfusion is expected to remove approximately 85% of circulating red cells and approximately 50% of total serum bilirubin (TSB).



- TSB levels are expected to increase after the exchange as re-equilibrium is reached between the extra- and intravascular spaces and may necessitate another exchange transfusion procedure.
-

Question 39: E, B, C, and D.**Explanation:**

- FNAIT is mediated by maternally produced alloantibodies that have specificity to paternally inherited antigens expressed on fetal/neonatal platelets. Maternal platelets will not be affected by alloantibodies and, therefore, the mother is physiologically not directly affected by FNAIT and should herself be clinically asymptomatic. (This contrasts with what we see with primary immune thrombocytopenia (ITP) where the culprit antibodies are autoantibodies).
 - Because the offending antibodies in FNAIT are alloantibodies, only the fetal/neonatal platelets will be affected.
 - FNAIT is often not diagnosed until after birth. The fetal/neonatal presentation is consistent with features of (immune-mediated) destruction of platelets and can include:
 - Thrombocytopenia (can range from mild to severe).
 - Petechiae and ecchymosis.
 - Gastrointestinal bleeding.
 - Intracranial hemorrhage (ICH) – this is the most concerning feature of FNAIT.
-

Question 40: D. 1 in 1000 (0.1%).**Explanation:**

- Although reports vary, the incidence of FNAIT is approximately 1 in 1000 (0.1%).
- The consequence of this low incidence is that health-care workers may be inexperienced in dealing with a case of FNAIT when it occurs.

**Question 41: A.** HPA-1a.**Explanation:**

- The classic scenario for FNAIT is a pregnant woman who is homozygous for HPA-1b (ie, negative for the HPA-1a antigen) and who makes alloantibodies against paternally inherited HPA-1a antigen that is expressed on fetal platelets.
- It should be recognized that anti-HPA-1a may not be the most likely causation of FNAIT in non-European populations. For example, in Asian populations, the causative antibody in FNAIT is more likely to be anti-HPA-4b.
- Rarely, non-HPA antibodies have been reported as a cause of FNAIT (eg, anti-A, anti-HLA).
- A 2004 study published a breakdown of 1162 FNAIT cases evaluated in the United States listed below:
 - 79% of FNAIT were caused by anti-HPA-1a.
 - 9% of FNAIT were caused by anti-HPA-5b.
 - 4% of FNAIT were caused by anti-HPA-1b.
 - 2% of FNAIT were caused by anti-HPA-3a.
 - 6% of FNAIT were caused by other and/or multiple antibodies.

**Question 42: C.** 2%.**Explanation:**

- 98% percent of the population expresses HPA-1a (may be homozygous or heterozygous). Therefore, 2% of the population is HPA-1a negative (homozygous for HPA-1b). This should be kept in mind when considering what % of platelet donors are expected to be HPA-1a negative.

**Question 43: A.** ICH is fatal in approximately 35% of cases.**Explanation:**

- ICH due to FNAIT affects approximately 1 in 5000 to 1 in 10,000 live births.



- Nonfatal cases of FNAIT-associated ICH are often (85%) associated with neurologic/developmental sequelae.
 - The majority of FNAIT-associated ICH cases occur in utero, with a reported majority occurring before 28 weeks of gestation. Ultrasound can be used to detect fetal ICH.
 - Preventing ICH is the primary goal of antenatal/perinatal interventions (see also answers #39 and #47).
-

Question 44: B. False.

Explanation:

- FNAIT can occur in a first pregnancy. This is in contrast to (non-ABO) HDFN, which generally requires a previous pregnancy to serve as an alloimmunizing (or sensitizing) event.
-

Question 45: D. A and B.

Explanation:

- The low frequency/incidence of FNAIT is such that not every mother needs to automatically be monitored for FNAIT during pregnancy.
- The likelihood of FNAIT—and the likelihood of severe FNAIT—is increased when a woman has a history of a pregnancy affected by FNAIT or suspected FNAIT.
- The Platelet Immunology Scientific Subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) issued a statement in 2018 where they specified the clinical criteria for suspecting FNAIT in an index pregnancy to include at least one of the following:
 - A nadir platelet count <100,000/ μ L at birth or within first 7 days of birth.
 - Fetal intracranial hemorrhage (ICH) in the absence of an alternate identifiable cause.
- If a female patient has a sister with history of pregnancy affected by FNAIT, this indicates that the sister was negative for a certain HPA antigen and the patient herself is at increased likelihood,



relative to the general population, for being negative for the same HPA antigen. Therefore, the patient has the potential to make the same alloantibody and have her own pregnancy affected by FNAIT.

- 2019 FNAIT guidelines issued by the International Collaboration for Transfusion Medicine Guidelines (ICTMG) (to be reviewed every 3 years) recommends typing HPA-1b/1b homozygous pregnant women for the *HLA-DRB*01:01* allele (of the HLA-DR52 antigen). There have been several studies that indicate that women who do not carry this *HLA-DRB3*01:01* allele (ie, the allele is absent) are at low risk for being alloimmunized to the HPA-1a antigen.



Question 46: D. If the father is not known and/or unavailable for HPA typing, fetal platelet genotyping is recommended.

Explanation:

- The recurrence rate of FNAIT in a subsequent pregnancy is approximately 72%. This is why a history of FNAIT in a previous pregnancy is clinically significant in obstetric/perinatal management.
- Evaluation for FNAIT includes:
 - Maternal HPA typing.
 - Maternal HPA antibody testing. (Note: Antibody titer does not have the same utility in FNAIT as it does with HDFN.)
 - Paternal HPA typing.
 - If the father is homozygous, the fetus should be positive for the HPA antigen of interest. However, if the father is heterozygous, the fetus has a 50% chance of a positive allele.
 - Fetal HPA testing is recommended if the father is heterozygous for the antigen of interest, or the father is unknown and/or unavailable for testing. Noninvasive testing using cell-free DNA (cfDNA) is preferred. Invasive options are available but have associated risks (amniocentesis – risk of bleeding; chorionic villus sampling – risk of alloimmunization).



Question 47: D. A platelet threshold of 30,000/ μ L is recommended as a transfusion threshold in nonbleeding neonates. For neonates



with life-threatening bleeding (ICH, gastrointestinal), a threshold of 100,000/ μ L should be used, followed by a threshold of 50,000/ μ L for at least 7 days.

Explanation:

- The ideal platelet unit to issue for an FNAIT-affected neonate is an irradiated, CMV-negative platelet unit that is negative for the HPA antigen that the mother makes an alloantibody against ("HPA-selected") because that will allow for the greatest post-transfusion increment increase.
- However, if an HPA-selected unit is not available, then proceeding with an HPA-unselected unit is still acceptable, even if it is expected to be less effective.
- Maternal platelets, as a directed donation, can be an option because they will be negative for the HPA antigen of interest, but collecting and processing these units comes with logistical challenges, and it would not be practical to wait for the availability of this unit in a clinically urgent/emergent setting.
- There is no current need or rationale for intrauterine administration of IVIG. IVIG administered directly to the mother is sufficiently effective. Also, intrauterine infusions/transfusions come with their own inherent risks.
- FNAIT can develop as early as 20 weeks of gestation. Therefore, if the mother has a history of FNAIT, early intervention at 12-16 weeks of gestation is recommended. Otherwise, if there is no history of FNAIT, interventions can be initiated at 20-24 weeks of gestation. Interventions may include IVIG and/or steroids.
- Neonatal platelet levels typically decrease after birth, reaching a nadir in the first week. It is recommended that neonatal platelet levels should be monitored until they reach normal levels (in the absence of any treatment).



Question 48: E. A and D.

Explanation:

- In FNAIT, the HPA alloantibodies target fetal/neonatal platelets, but maternal platelets are unaffected and therefore not correlative with fetal/neonatal levels.



- In ITP, maternal platelets decreased due to platelet autoantibodies that may also affect fetal/neonatal platelets. However, and perhaps counterintuitively, maternal platelet levels do not correlate with fetal/neonatal platelets levels and, therefore, are not used as a marker by which to predict fetal/neonatal risk for thrombocytopenia.
- ITP is a diagnosis of exclusion, therefore there are no laboratory findings that are considered diagnostic for ITP. By contrast, HPA typing and HPA antibody testing are utilized in making a diagnosis of FNAIT.
- In contrast to FNAIT, fetal/neonatal bleeding complications are rare in ITP, with risk of ITP-associated ICH being extremely rare. Also, if ICH occurs in ITP, it typically occurs neonatally. By comparison, FNAIT-associated ICH typically occurs in utero. Any neonatal bleeding complications seen with ITP-associated pregnancies tend to occur within the first 2 days.



Question 49: C. If the neonate presents with severe thrombocytopenia (platelets <50,000/ μ L), parental testing should be done to exclude NAIT.

Explanation:

- There are no recommendations to monitor fetal platelet levels during pregnancy, in part because there are not enough data to establish desirable fetal platelet levels.
- Interestingly, platelet antibody testing is not a good predictor of maternal or fetal/neonatal platelet levels. Therefore, platelet antibody testing is not recommended.
- Severe neonatal thrombocytopenia (platelets <50,000/ μ L) is seen in approximately 9%-15% of ITP-associated pregnancies. Therefore, when observed, it is important to consider and rule out NAIT as the etiology. The significance of distinguishing NAIT vs ITP as the etiology is that it affects neonatal management, given that HPA-selected platelet units are recommended for FNAIT but not for ITP-associated thrombocytopenia.
- There are no recommendations for platelet crossmatching as a means of selecting platelet units when platelet transfusions are required.



Question 50: C. Similar to FNAIT, IVIG or steroids administered to the mother are considered first-line management options.

Explanation:

- The primary goal of ITP management in pregnancy is to mitigate the risk of bleeding in the mother. In FNAIT, interventions are directed to preventing and managing bleeding complications of the fetus/neonate. Also of interest, management of thrombocytopenia in a pregnant woman with ITP has not been found to reliably prevent fetal/neonatal thrombocytopenia.
- In the absence of any bleeding or symptoms, a platelet level of 20-30,00/ μ L is currently considered safe for a pregnant woman with ITP.
- Other management options for ITP include combination therapies (eg, IVIG with steroids, high-dose methylprednisolone with azathioprine, etc), RhIG, splenectomy (ideally performed in the second trimester), rituximab (anti-CD20), thrombopoietin, and thrombopoietin receptor agonists. However, risks and benefits should be considered before moving to more aggressive options.
- Cranial ultrasounds to assess for ICH are recommended for neonates with a platelet count of <50,000/ μ L to assess for ICH.

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9

Massive Transfusion and Extracorporeal Membrane Oxygenation

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- Massive transfusion is most often defined as transfusion of 10 or more Red Blood Cell (RBC) units in a 24-hour period, although other definitions are also used (eg, 4 RBC units in 1 hour).
- Initial resuscitation of trauma patients is focused on early transfusion with plasma, platelets, and RBCs in a fixed ratio to approximate the transfusion of whole blood and prevent dilutional coagulopathy.
- Although much of the scientific evidence on massive transfusion protocols is from the study of trauma patients, massive transfusions also occur among other patient populations (eg, solid-organ transplantation, gastrointestinal bleeding, and cardiac/vascular surgery).
- Extracorporeal membrane oxygenation (ECMO) is a treatment in which blood is removed from the patient's venous circulation, circulated through a machine to remove carbon dioxide and replenish oxygen, and then returned to the patient.



QUESTIONS

Question 1: Based upon the 2015 PROPPR trial conducted in the United States, which statement(s) are true?

- A. Early administration of red cells, plasma, and platelets in a 1:1:1 ratio compared with a 2:1:1 ratio showed significant differences in mortality at 24 hours.
 - B. Early administration of red cells, plasma, and platelets in a 1:1:1 ratio compared with a 2:1:1 ratio showed a significant difference in mortality at 30 days.
 - C. Subgroup analysis revealed more patients in the 1:1:1 group achieved hemostasis and fewer experienced death due to exsanguination by 24 hours.
 - D. A and B are true.
 - E. A, B, and C are true.
-

Question 2: The best choice of treatment for hyperfibrinolytic bleeding is?

- A. Fresh frozen Plasma (FFP).
 - B. Tranexamic acid (TXA).
 - C. Fibrinogen concentrate.
 - D. Cryoprecipitate.
 - E. Plasminogen.
-

Question 3: The most common anticoagulant used in extracorporeal membrane oxygenation (ECMO) circuits is heparin, which causes low levels of which protein in the patient?

- A. Thrombin.
- B. Von Willebrand Factor (vWF).
- C. Antithrombin.
- D. Antiplasmin.
- E. Factor X.



Question 4: A 26-year-old male is hooked up to an ECMO circuit. A few small clots have been noticed in the circuit and additional heparin is added. An activated partial thromboplastin time (aPTT) test is sent out and comes back at 64 seconds (reference range 22.3-38.2 seconds). Additional heparin is added and another aPTT is sent and comes back at 63 seconds. A heparin anti-Xa level is then obtained and reported as 0.3 (goal range 0.3-0.7). Additional heparin is added to the circuit and the anti-Xa level comes back at 0.3 again. What is the most likely explanation?

- A. The samples are being mixed up and are likely from another patient.
 - B. The wrong concentration of heparin is being added.
 - C. The patient is resistant to heparin due to low antithrombin levels.
 - D. The coagulation assays being performed should be repeated with additional quality control (QC) measures as they are most likely being performed incorrectly.
 - E. Nothing is wrong.
-

Question 5: ECMO can lead to a prohemorrhagic phenotype by causing which of the following?

- A. Platelet dysfunction.
 - B. vWF proteolysis/degradation.
 - C. Coagulation factor consumption.
 - D. Antithrombin deficiency.
 - E. A, B, and C.
-

Question 6: In patients with a contraindication such as intracranial hemorrhage who require ECMO, anticoagulant-free ECMO can be tried. Which of the following make anticoagulant-free ECMO possible?

- A. Use of large amounts of antithrombin.
- B. Use of surface modifications such as endothelialization, biomimetic, and surface passivation.
- C. Use of citrate instead of heparin.



- D. Careful monitoring and supplementation of the circuit with FFP.
 - E. Use of direct thrombin inhibitors.
-

Question 7: Patients receiving ECMO treatments in which the circuitry is anticoagulated with unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) can develop heparin-induced thrombocytopenia (HIT). What are antibodies in HIT directed against?

- A. Platelets.
 - B. PF4.
 - C. PF6.
 - D. Thrombin.
 - E. Factor VIII.
-

Question 8: In the answer choices below, which ECMO type is matched correctly with what it is used to treat? (VA-ECMO = venoarterial-ECMO and VV-ECMO = venovenous-ECMO)

- A. VA-ECMO and respiratory failure.
 - B. VV-ECMO and refractory cardiogenic shock.
 - C. VA-ECMO and refractory cardiogenic shock.
 - D. VV-ECMO and respiratory failure.
 - E. C and D.
 - F. A and B.
-

Question 9: In the HECTIC study (involving hemostasis, coagulation, and thrombin in venoarterial and venovenous ECMO) which statements are true?

- A. Bleeding and thrombosis occurred earlier in VA-ECMO.
- B. Bleeding occurred earlier in VA-ECMO and thrombosis occurred earlier in VV-ECMO.
- C. Bleeding occurred earlier in VV-ECMO and thrombosis occurred earlier in VA-ECMO.
- D. Bleeding and thrombosis occurred earlier in VV-ECMO.
- E. None of the above.



Question 10: Which clotting factor has been shown to be activated very early in ECMO initiation because of the contact with the artificial surfaces?

- A. Factor VII.
 - B. Factor IX.
 - C. Factor X.
 - D. Factor XI.
 - E. Factor XII.
-

Question 11: In contrast to UFH, LMWH predominantly acts upon which coagulation factor?

- A. Thrombin.
 - B. Factor VIIa.
 - C. Factor IXa.
 - D. Factor Xa.
 - E. Factor Xla.
-

Question 12: ECMO circuits can be anticoagulated with direct thrombin inhibitors (DTIs) such as argatroban. Argatroban dosing and administration should be performed cautiously in which patients?

- A. Patients with renal impairment.
 - B. Patients with respiratory failure.
 - C. Patients with HIT.
 - D. Patients with a clotted ECMO circuit.
 - E. Patients with hepatic impairment.
-

Question 13: LMWH anticoagulation of an ECMO circuit can be monitored using which laboratory test?

- A. aPTT.
- B. PT.
- C. Anti-Xa.
- D. Activated clotting time (ACT).
- E. Thromboelastography (TEG).



Question 14: ACT testing can be affected by which of the following?

- A. Hemodilution.
- B. Hypothermia.
- C. Anemia.
- D. Thrombocytopenia.
- E. All of the above.



Question 15: Acquired von Willebrand syndrome is seen in ECMO patients and is caused by what source?

- A. Dilution due to transfusion of RBCs.
- B. Underproduction of proteins due to decreased blood flow to the liver.
- C. Shear stress causing functional deficiency.
- D. Increased clearance.
- E. Use of UFH as an anticoagulant.



Question 16: What can be used to reverse the effects of UFH anti-coagulation following decannulation and removal of ECMO?

- A. Plasma transfusion.
- B. Kcentra.
- C. Protamine.
- D. Cryoprecipitate.
- E. Antithrombin.



Question 17: What is the difference between three-factor prothrombin complex concentrate (PCC) and four-factor PCC?

- A. Thrombin.
- B. Factor VII.
- C. Factor IX.
- D. Factor X.
- E. Factor XI.



Question 18: In the massive transfusion study known as the BEST collaborative study, researchers found that the reason for massive transfusion was strongly associated with survival and that survival was inversely proportional to what?

- A. The number of units of blood components received.
 - B. The levels of Factor VIII.
 - C. The levels of protein C.
 - D. The levels of fibrinogen.
 - E. The levels of antithrombin.
-

Question 19: Which of the following pairs correctly matches the platelet receptors and their respective antigen?

- A. Glycoprotein (GP) IIb/IIIa:vWF.
 - B. GPIb/IX/V:fibrinogen.
 - C. GPIIb/IIIa:fibrinogen.
 - D. GPla/IIa:vWF.
 - E. GPIb/IX/V:collagen.
-

Question 20: Which of the following is found in platelet alpha granules?

- A. vWF.
 - B. Factor X.
 - C. Factor VII.
 - D. Serotonin.
 - E. Calcium.
-

Question 21: Reconstitution of a unit of RBCs in additive solution, a unit of plasma, and a unit of whole-blood-derived platelets results in a mixture with which of the following parameters?

- A. 35% hematocrit; 150,000/ μ L of platelets, and plasma with a concentration of 1.0 U/mL.
- B. 40% hematocrit; 175,000/ μ L of platelets, and plasma with a concentration of 0.81 U/mL.



- C. 29% hematocrit; 88,000/ μ L of platelets, and plasma with a concentration of 0.62 U/mL.
 - D. 32% hematocrit; 74,000/ μ L of platelets, and plasma with a concentration of 0.5 U/mL.
 - E. 25% hematocrit; 105,000/ μ L of platelets, and plasma with a concentration of 0.71 U/mL.
-

Question 22: What plasma type is the universal donor for massive transfusion protocols (MTPs)?

- A. Group O.
 - B. Group A.
 - C. Group B.
 - D. Group AB.
 - E. None of the above.
-

Question 23: Which MTP “pack” reflects a 1:1:1 ratio as indicated in the PROPPR trial?

- A. 1 unit of RBCs: 1 unit of FFP: 1 unit of apheresis platelets.
 - B. 4 units of RBCs: 4 units of FFP: 1 unit of apheresis platelets.
 - C. 1 unit of RBCs: 1 unit of FFP: 4 units of apheresis platelets.
 - D. 6 units of RBCs: 6 units of FFP: 1 unit of apheresis platelets.
 - E. 6 units of RBCs: 6 units of FFP: 3 units of apheresis platelets.
-

Question 24: Most MTPs are activated due to which of the following situations?

- A. Obstetric hemorrhage.
- B. Liver transplant.
- C. Trauma.
- D. Gastrointestinal bleeding.
- E. All of the above.



Question 25: Massive transfusion is traditionally defined as transfusion of how many units of RBCs in a 24-hour period?

- A. 4 units.
 - B. 6 units.
 - C. 8 units.
 - D. 10 units.
 - E. 12 units.
-

Question 26: The Assessment of Blood Consumption (ABC) score is frequently used to help determine the need for MTP activation in adult trauma patients with penetrating or blunt injuries. What four variables does the ABC score consist of? (FAST = Focused Assessment with Sonography for Trauma scan)

- A. Pulse >120 bpm, SBP <90 mmHg, +FAST, and penetrating torso injury.
 - B. Pulse >140 bpm, SBP <70 mmHg, +FAST, and penetrating torso injury.
 - C. Pulse >150 bpm, SBP <90 mmHg, +FAST, and blunt torso injury.
 - D. Pulse >150 bpm, SBP <75 mmHg, +FAST, and blunt torso injury.
 - E. Pulse >120 bpm, SBP <90 mmHg, +FAST, and blunt torso injury.
-

Question 27: For maximum effectiveness, damage control resuscitation (DCR) principles recommend what mechanism of delivery for RBCs, plasma, cryoprecipitate, and platelets?

- A. All four should be delivered by a rapid transfuser and through a blood warmer.
- B. All four should be delivered by a rapid transfuser without a blood warmer.
- C. The RBCs and plasma should be delivered by a rapid transfuser with a blood warmer and cryoprecipitate and platelets should not use a blood warmer.



- D. The RBCs, plasma, and cryoprecipitate should be delivered by a rapid transfuser with a blood warmer and the platelets should not use a blood warmer.
 - E. All of the products should be delivered cold to reduce blood loss and brain injury by inducing hypothermia.
-

Question 28: How frequently should MTP coolers be delivered once the MTP is activated?

- A. Every 10 minutes.
 - B. Every 15 minutes.
 - C. Every 30 minutes.
 - D. Every 45 minutes.
 - E. Every 60 minutes
-

Question 29: A hospital has both group O– and O+ RBCs available in a refrigerator in the trauma bay. A 59-year-old male presents with a gunshot wound to the abdomen with SBP of 50 mmHg and pulse of 165 bpm. The MTP is initiated. What should be given now?

- A. The group O– units.
 - B. The group O+ units.
 - C. Wait for the MTP cooler to arrive in 5 minutes.
 - D. Start crystalloid solutions such as Lactated Ringer's or normal saline solution.
 - E. Start 5% albumin.
-

Question 30: Which blood components should be placed in the temperature-controlled MTP cooler?

- A. RBCs, plasma, and cryoprecipitate.
- B. RBCs and plasma.
- C. RBCs, plasma, cryoprecipitate, and platelets.
- D. Cryoprecipitate and platelets.
- E. RBCs, plasma, and platelets.



Question 31: The trauma team calls you to ask what blood component to give to a patient with thromboelastography (TEG) tracing that shows an R-value of 16 minutes. You recommend which component(s)?

- A. RBCs.
 - B. Platelets.
 - C. Cryoprecipitate.
 - D. Plasma.
 - E. All of the above.
-

Question 32: Which of the following are performance indicators for massive transfusion?

- A. Time from calling MTP to infusion of first unit of RBCs.
 - B. Wastage rates for blood components products.
 - C. Adherence to a predetermined ratio or goal between 1 to 2 hours after initiation of the MTP.
 - D. Informing the transfusion service that MTP has been terminated within 1 hour of termination.
 - E. All of the above.
-

Question 33: Massive transfusion in the pediatric population has been defined as which of the following?

- A. RBC transfusion of 50% of total blood volume (TBV) in 3 hours.
 - B. RBC transfusion of 100% TBV in 24 hours.
 - C. RBC transfusion of >10% of TBV per minute.
 - D. Transfusion of 5 or more RBC units in 24 hours.
 - E. A, B, and C.
-

Question 34: Which of the following are a cause of trauma-induced coagulopathy?

- A. Release of tissue factor caused by tissue damage.
- B. Hypoperfusion caused increased thrombomodulin expression.



- C. Reduced plasminogen activator inhibitor (PAI-1).
 - D. Activated protein C (APC) inhibition of Factor Va and Factor VIIIa.
 - E. All of the above.
-

Question 35: Pediatric patients have large physiologic reserves in trauma and can maintain arterial pressure after loss of 20-49% of TBV. For this reason, signs and symptoms of hypovolemia are not a good indicator for MTP need. What is a good indicator in pediatric populations for hypovolemia and/or MTP need?

- A. Tachycardia.
 - B. Systolic hypotension.
 - C. Narrow pulse pressure.
 - D. Diastolic hypotension.
 - E. All of the above.
-

Question 36: Due to the amount of citrate anticoagulant in the blood components transfused in MTPs, what should be monitored to aid in coagulation?

- A. Magnesium.
 - B. Calcium.
 - C. Ionized calcium.
 - D. Iron.
 - E. Platelets.
-

Question 37: In the HALT-IT trial, involving patients with acute gastrointestinal bleeding, what effects did a high-dose 24-hour infusion of tranexamic acid have on death and thromboembolic events?

- A. High-dose tranexamic acid was associated with increased arterial thromboembolic events in patients with acute gastrointestinal bleeding.
- B. High-dose tranexamic acid was associated with increased venous thromboembolic events in patients with acute gastrointestinal bleeding.



- C. High-dose tranexamic acid was associated with significantly less bleeding in patients with acute gastrointestinal bleeding.
 - D. High-dose tranexamic acid was associated with increased arterial and venous thromboembolic events in patients with acute gastrointestinal bleeding.
 - E. All of the above.
-

Question 38: When looking at the EKG during MTP, ST segment prolongation can be seen in cases of what iatrogenic cause?

- A. Hypomagnesemia.
 - B. Hypercalcemia.
 - C. Hypermagnesemia.
 - D. Low hematocrit and low oxygen-carrier capacity.
 - E. Hypocalcemia.
-

Question 39: Which of the following changes in red cells during storage can impair resuscitation and oxygen delivery during MTPs?

- A. Decreased pH.
 - B. Increased potassium levels.
 - C. Decreased 2,3-diphosphoglycerate (2,3-DPG).
 - D. Improved deformability.
 - E. A, B, and C.
-

Question 40: A 26-year-old male received massive transfusion due to a gunshot wound to the left leg and abdomen. He received 15 units of group O+ RBCs before his type and screen came back and the MTP was switched to group-specific components. His blood group is B+. A new type and screen is performed 3 days after he received a total of 15 units of group O+ RBCs, 16 units of group B+ RBCs, 15 units of group AB plasma, 15 units of group B plasma, 1 unit of group A apheresis platelets, and 4 units of group B apheresis platelets. What would you expect to see on the forward and reverse typing?



- A. He is typing as mixed group O+ and B+ on forward typing. On reverse typing, the patient is typing as group B. The DAT result is positive.
 - B. He is typing as group B+ on forward typing and group B on reverse typing. The DAT result is positive.
 - C. He is typing as mixed group O+ and group B+ on forward typing. On reverse typing, the patient is typing as group B. The DAT result is negative.
 - D. He is typing as group B+ on forward typing and group B on reverse typing. The DAT result is negative.
 - E. He is typing as mixed group O+ and group B+ on forward typing and on reverse typing, the patient is typing as a mixed group A and B.
-

Question 41: A 19-year-old female presents to the trauma bay by ambulance following a terrible motor vehicle accident. Her blood pressure (BP) is 80/48 mmHg with a pulse of 150 bpm. A MTP is initiated. While waiting for the cooler to arrive from the blood bank, which components from the emergency room trauma refrigerator should be used?

- A. Infuse 2 units of group O– RBCs and 2 units of group AB plasma.
 - B. Infuse 2 units of group O+ RBCs and 2 units of group AB plasma.
 - C. Infuse 2 units of group O– RBCs, 2 units of group O+ RBCs, and 2 units of group AB plasma.
 - D. Do not infuse anything, wait 5-10 minutes for the cooler.
 - E. Infuse 2 units of group AB plasma and no RBCs because you do not know the patient's blood type.
-

Question 42: The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) study showed which of the following to be true?

- A. Patients who received plasma:RBCs in a 1:2 ratio had significantly better 6-hour survival, but survival at later time points did not differ.



- B. Patients who received plasma:RBCs in a 1:1 ratio had significantly better 6-hour survival, but survival at later time points did not differ.
 - C. Patients who received plasma:RBCs in a 1:2 ratio had significantly better 6-hour survival, as well as improved survival at later time points.
 - D. Patients who received plasma:RBCs in a 1:1 ratio had significantly better 6-hour survival, as well as improved survival at later time points.
 - E. Patients who received plasma:RBC in a 1:1 ratio had significantly better 6-hour survival, but decreased survival at later time points.
-

Question 43: According to AABB *Standards*, what type of whole blood can be used in trauma resuscitation?

- A. ABO identical.
 - B. ABO compatible.
 - C. Only group O-.
 - D. Only group AB-.
 - E. Only group AB+.
-

Question 44: A patient on ECMO is noted to have bleeding from mucus membranes as well as the urinary catheter site. All clotting factors come back within normal ranges. What test would you order?

- A. aPTT.
 - B. Anti-Xa.
 - C. PT.
 - D. vWF antigen and activity.
 - E. Antithrombin level.
-

Question 45: A 67-year-old male arrives by ambulance to the trauma bay. He was in a motor vehicle accident and hit his head on the dashboard and also has a broken pelvis and femur. Blood samples are sent to the laboratory and he is noted to have an elevated



PT and aPTT. It is suspected he is taking some sort of anticoagulation. What test(s) would you order next?

- A. Anti-Xa.
 - B. Factor VIII activity, Factor IX activity, Factor X activity, fibrinogen, and thrombin activity.
 - C. aPTT and PT mixing studies.
 - D. Lupus anticoagulant testing.
 - E. Platelet activity tests.
-

Question 46: The patient above is found to be taking warfarin for a history of recurrent deep venous thrombosis (DVT). What should be given to reverse the warfarin and help stop the patient's bleeding so that he can have surgery?

- A. Oral vitamin K.
 - B. IV vitamin K.
 - C. IV vitamin K and four-factor PCC.
 - D. Plasma.
 - E. Cryoprecipitate.
-

Question 47: Which of the following is in platelet-dense granules?

- A. vWF.
 - B. Factor VIII.
 - C. Fibrinogen.
 - D. Serotonin.
 - E. Platelet factor 4.
-

Question 48: A 45-year-old male patient presents to the trauma bay via ambulance from the site of a motor vehicle vs bicycle accident. He was the bicyclist and was thrown from the bicycle and hit his head, knee, and back. He says he is taking a Factor Xa inhibitor drug for a recent DVT and pulmonary embolism (PE) that he developed following a long plane trip from Taiwan. A CT scan shows that he has cranial bleeding. What is the best option to treat the bleeding?



- A. Infuse plasma.
 - B. Infuse four-factor PCC.
 - C. Perform emergent therapeutic plasmapheresis.
 - D. Give protamine.
 - E. Give Andexxa (recombinant inactivated coagulation Factor Xa).
-

Question 49: A MTP cooler is returned with unused components. Inside, you find 4 units of RBCs, 3 units of plasma, and 1 unit of apheresis platelets. All of the products are at 6 C. What should you do?

- A. Discard everything that was returned.
 - B. Discard the RBCs and platelets but restock the plasma on the correct shelf.
 - C. Return all products to the correct stock in the blood bank.
 - D. Return the RBCs and plasma to the blood bank stock and discard the platelets.
 - E. Discard the platelets and plasma but return the RBCs to the blood bank stock.
-

Question 50: A patient on the floor had an order for 1 unit of RBCs, but it was denied as the patient had a hemoglobin level of 10 g/dL and the resident said that the patient was not actively bleeding. Later in the day, the same order was placed again. The resident said the patient was not symptomatic or bleeding, but the team just wanted to transfuse the patient for discharge so that he could feel “like his best self” on vacation next week. This order is denied by the blood bank as it is outside transfusion guidelines. The blood bank then receives a MTP request for this patient. What should the blood bank do?

- A. Call to the floor to see if the patient is bleeding.
- B. Send the MTP cooler and ask questions later.
- C. Send the MTP cooler and have the blood bank director call the attending physician for this patient.
- D. Cancel the MTP, they have already tried to get blood twice before.
- E. Send one unit of RBCs.



ANSWERS

Question 1: C. Subgroup analysis revealed more patients in the 1:1:1 group achieved hemostasis and fewer experienced death due to exsanguination by 24 hours.

Explanation:

- The PROPPR trial showed that early administration of red cells, plasma and platelets in a 1:1:1 ratio compared with a 2:1:1 ratio *did not* show significant differences in mortality at 24 hours or at 30 days.
- The PROPPR study did show in subgroup analysis that more patients in the 1:1:1 group achieved hemostasis and fewer experienced death due to exsanguination by 24 hours.
- A more recent meta-analysis of 5 randomized controlled trials with approximately 1800 patients identified a significantly improved 24-hour mortality [odds ratio (OR) 0.69] and 30-day mortality (OR 0.78) for patients receiving high platelets:red cells ratio as compared with patients receiving a low ratio.



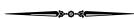
Question 2: B. Tranexamic acid.

Explanation:

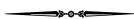
- Tranexamic acid (TXA) is a small molecule that is a lysine analog. It acts on fibrinolysis by blocking lysine binding sites which are important in plasminogen/plasmin binding and activity.
- TXA has been shown to be effective in hyperfibrinolytic bleeding.
- Fibrinogen concentrate and cryoprecipitate may be helpful in treating hypofibrinogenemia which will occur later.
- Plasminogen is not readily available by itself as recombinant drug and would make hyperfibrinolytic bleeding worse.
- FFP could help hyperfibrinolytic bleeding later as a replacement of used fibrinogen and clotting factors, but it would not be the best choice.

**Question 3: C. Antithrombin.****Explanation:**

- The most commonly used anticoagulant in ECMO is heparin. Heparin is also commonly used as an anticoagulant in hemodialysis. However, citrate is used as a common anticoagulant in apheresis and continuous renal replacement therapy.
- Heparin functions by binding to antithrombin and facilitating its inhibition of serine proteases involved in coagulation such as Factor Xa, Factor IXa, Factor XIa, or Factor XIIa.
- Antithrombin also acts as an inhibitor of thrombin but the heparin molecule acts in a template mechanism and binds to both the antithrombin and thrombin.
- Antithrombin levels are frequently low in ECMO circuits and recombinant antithrombin is frequently given to supplement the patient antithrombin levels to facilitate anticoagulation of the circuit.

**Question 4: C. The patient is resistant to heparin due to low anti-thrombin levels.****Explanation:**

- When additional amounts of heparin are added to a circuit or patient and the aPTT and/or heparin anti-Xa levels do not change, this suggests heparin resistance. Heparin resistance in ECMO circuits is quite common and due to low levels of anti-thrombin.

**Question 5: E. A, B, and C.****Explanation:**

- ECMO can lead to a prohemorrhagic phenotype due to consumption of coagulation factors, proteolysis/degradation of vWF, and platelet dysfunction.
- Antithrombin deficiency caused by heparin use in ECMO is associated with a clotting phenotype.



Question 6: B. Use of surface modifications such as endothelialization, biomimetic, and surface passivation.

Explanation:

- Antithrombin, citrate, and direct thrombin inhibitors are all anti-coagulants. *Supplementation of the circuit with FFP and more careful monitoring would not facilitate anticoagulant-free ECMO.
 - Mitigation of the bioincompatibility of the ECMO circuit itself has been shown to facilitate anticoagulant-free ECMO and reduce inflammatory effects and clotting.
-

Question 7: B. PF4.

Explanation:

- Antibodies in HIT are directed against heparin and PF4. The antibody directed against heparin-PF4 causes platelet activation.
 - HIT can develop in patients receiving LMWH or UFH. It is associated with thrombocytopenia and/or thrombosis.
 - When HIT is suspected, heparin should be stopped and another anticoagulant substituted.
-

Question 8: E. C and D.

Explanation:

- VA-ECMO is frequently used to treat refractory cardiogenic shock and VV-ECMO is used to treat respiratory failure.
 - In ECMO, a large cannula is placed in a large central vein to supply the ECMO circuit with patient blood.
 - A second cannula to return blood is either placed in the venous system (VV-ECMO) or arterial system (VA-ECMO). The return cannula contains oxygenated blood.
-

Question 9: A. Bleeding and thrombosis occurred earlier in VA-ECMO.

**Explanation:**

- In the HECTIC study, 25 bleeding events occurred in total (16 in VV-ECMO patients and 9 in VA-ECMO patients). Bleeding occurred earlier in VA-ECMO patients (0.30 vs VV-ECMO patients at 4.30 days, $p=0.001$).
 - Similarly, thrombosis occurred earlier in VA-ECMO patients as well. A total of 26 thrombotic events occurred (7 were in VA-ECMO and 19 were in VV-ECMO); $p<0.002$. The majority of thrombosis events in VV-ECMO were circuit clots.
-

Question 10: E. Factor XII.**Explanation:**

- Factor XII is activated to Factor XIIa via contact with the artificial surfaces in the ECMO circuit. This leads to activation of kallikrein and bradykinin, which in turn propagate coagulation and inflammation.
-

Question 11: D. Factor Xa.**Explanation:**

- LMWH is smaller than UFH and acts predominantly by binding to Factor Xa.
 - UFH acts with all the coagulation factors mentioned and uses a template mechanism to inhibit thrombin via antithrombin.
-

Question 12: E. Patients with hepatic impairment.**Explanation:**

- Argatroban is metabolized predominantly by the liver and has an expected half-life time of 45 minutes. In patients with liver impairment, the dose of argatroban may need to be greatly reduced.



- The DTI, bivalirudin is metabolized in both renal and hepatic pathways with a half-life time of 25 minutes.
-

Question 13: C. Anti-Xa.

Explanation:

- LMWH dosing and anticoagulation are measured and monitored using anti-Xa levels.
 - UFH heparin can be monitored using aPTT, anti-Xa, and the ACT.
-

Question 14: E. All of the above.

Explanation:

- ACT testing measures the time needed for a sample of whole blood to clot. A typical goal is 180-220 seconds.
 - ACT testing is affected by UFH, hemodilution, anemia, hypothermia, thrombocytopenia, platelet inhibitors, low anti-thrombin levels, low fibrinogen levels, and low clotting factor levels.
-

Question 15: C. Shear stress causing functional deficiency.

Explanation:

- Acquired deficiency seen in ECMO patients is caused by shear stress leading to a functional vWF deficiency. Acquired vWD is associated with bleeding and should be suspected in ECMO patients with bleeding.
-

Question 16: C. Protamine.

**Explanation:**

- UFH anticoagulation is reversed with protamine. The half-life of IV UFH is 45-90 minutes when at therapeutic range and protamine administration can be used to rapidly reverse the anticoagulation. The half-life of protamine is 7 minutes; thus, repeat administration may be needed to fully reverse UFH.
- Antithrombin would further anticoagulate the circuit. Transfusion with plasma or cryoprecipitate would not be appropriate and neither would use of Kcentra.
- Protamine can partially reverse LMWH.

**Question 17: B. Factor VII.****Explanation:**

- The four-factor PCC contains coagulation Factors II, VII, IX, and X as well as the endogenous inhibitor proteins S and C.
- The three-factor PCC contains Factors II, IX, X and only minimal amounts of Factor VII.
- Four-factor PCC is indicated for the urgent reversal of acquired coagulation factor deficiency induced by vitamin K antagonist therapy in adult patients with acute major bleeding.

**Question 18: A. The number of units of blood components received.****Explanation:**

- This study found that survival was inversely correlated with the number of blood products received (ie, those patients who received more products had worse survival).

**Question 19: C. GP IIb/IIIa:fibrinogen.****Explanation:**



- GPIa/IIa binds to collagen. GPIb/IX/V binds to vWF. GPIIa/IIIb binds to fibrinogen.
-

Question 20: A. vWF.

Explanation:

- Platelet alpha granules contain vWF, fibrinogen, Factor V, and thrombospondin. These proteins all aid in clot formation.
 - Platelet-dense granules contain serotonin, calcium, ADP, ATP, and mid-length chain polyphosphates.
-

Question 21: C. 29% hematocrit; 88,000/ μ L of platelets, and plasma with a concentration of 0.62 U/mL.

Explanation:

- Compared with whole blood collected in citrate, reconstituted blood has lower hematocrit, platelet, and coagulation factor levels.
-

Question 22: D. Group AB.

Explanation:

- The universal donor plasma is group AB as it does not contain any antibodies to A or B blood cells. However, only about 5% of the population is group AB and thus, not enough group AB plasma is available for MTP use.
 - A substitute that has been studied is using group A plasma.
-

Question 23: D. 6 units of RBCs: 6 units of FFP: 1 unit of apheresis platelets.

**Explanation:**

- One unit of apheresis platelets is equivalent to 5-6 units of whole-blood-derived platelets. Thus, answer D is considered a 1:1:1 ratio as described in the PROPPR trial.
 - The PROPPR trial compared a 1:1:1 ratio to a 1:1:2 ratio. The PROPPR trial found that in patients with severe trauma and major bleeding, early administration of plasma, platelets, and red blood cells in a 1:1:1 ratio compared with a 1:1:2 ratio did not result in significant differences in mortality at 24 hours or at 30 days.
 - The study also found that more patients in the 1:1:1 group achieved hemostasis and fewer experienced death due to exsanguination by 24 hours. Even though there was an increased use of plasma and platelets transfused in the 1:1:1 group, no other safety differences were identified between the two groups.
-

Question 24: E. All of the above.

Explanation:

- All of these categories are major causes of MTP activation and the predominant cause of MTP activation will vary from hospital to hospital depending upon their patient demographics and location. Typically, the largest category within this group for MTP activation is trauma.
- Massive transfusion protocols should be developed by a multidisciplinary committee that includes representatives from the transfusion service/blood bank, emergency department, anesthesia, and trauma service.
- The massive transfusion protocol should address:
 - Thresholds for initiating massive transfusion in trauma.
 - Resuscitation in the trauma bay (including MTP product availability, MTP product delivery, and MTP blood product transfusion).
 - Continuing MTP in the OR, angiography suite, and intensive care unit.
 - Transfusion service processes for delivery of blood products.
 - Transfusion targets.
 - The use of adjuncts for massive transfusion patients.
 - Termination of the MTP.
 - Performance improvement monitoring.



Question 25: D. 10 units.

Explanation:

- The traditional definition of massive transfusion is transfusion of ≥ 10 units of RBCs within a 24-hour period.
-

Question 26: A. Pulse >120 bpm, SBP <90 mmHg, +FAST, and penetrating torso injury.

Explanation:

- In the ABC score, each of these is assigned 1 point and a score of 2 points or more warrants MTP activation.
 - The ABC score overestimates the need for transfusion, with a positive predictive value of 50% to 55%, meaning that 45% to 50% of patients in whom MTP is activated will not need a massive transfusion.
 - However, the ABC score is excellent at identifying who will not need massive transfusion, with a negative predictive value of less than 5%, meaning it identifies more than 95% of all patients who will need a massive transfusion.
-

Question 27: C. The RBCs and plasma should be delivered by a rapid transfuser with a blood warmer and cryoprecipitate and platelets should not use a blood warmer.

Explanation:

- Warming of platelets will result in activation, which is not desirable.
 - Additionally, use of all cold products will induce hypothermia, which is associated with coagulopathy because the clotting factors have reduced activity at lower temperatures.
-

Question 28: B. Every 15 minutes.

**Explanation:**

- During MTP activation, coolers should be delivered every 15 minutes until the MTP is terminated.
 - The goal is to keep at least one MTP cooler ahead for the entire MTP activation.
-

Question 29: B. The group O+ units.

Explanation:

- The patient is a 59-year-old male and should receive the group O+ RBC units that are immediately available. The O- units should be preserved for females of childbearing potential so as to not introduce Rh immunization.
 - It would not be appropriate to wait for the MTP cooler as the patient has a very low SBP and elevated pulse and needs blood components immediately.
 - Also crystalloids should not be used especially when blood components are available.
 - Lastly, albumin would not be preferred when blood is available.
-

Question 30: B. RBCs and plasma.

Explanation:

- The platelets and cryoprecipitate should not be chilled in the cooler and should instead be outside the cooler.
 - For each 1 C decrease in temperature, coagulation factor activity decreases by 10%. At temperatures below 34 C, clotting times prolong, platelets pool in the spleen, and there is impaired platelet adherence and aggregation.
-

Question 31: D. Plasma.

**Explanation:**

- A prolonged R-value is indicative of hypocoagulation and the need for clotting factors that can be found in plasma. Plasma and/or cryoprecipitate and/or fibrinogen concentrate would be indicated for a k-time >4 minutes.
 - Cryoprecipitate/fibrinogen concentrate and/or plasma would be indicated for an α -angle $<60^\circ$.
 - Platelets would be helpful for a MA values <55 mm and tranexamic acid or another antifibrinolytic would be helpful for LY30 values $>7.5\%$.
-

Question 32: E. All of the above.**Explanation:**

- Performance indicators for massive transfusion include all of the following:
 - Wastage rates for blood products.
 - Time from calling MTP to infusion of first unit of RBCs.
 - Time from calling MTP to infusion of first unit of plasma.
 - Adherence to a predetermined ratio or goal between 1 to 2 hours after initiation of the MTP.
 - Informing the transfusion service that MTP has been terminated within 1 hour of termination.
-

Question 33: E. A, B, and C.**Explanation:**

- Massive transfusion in the pediatric population has been defined as RBC transfusion of 50% of total blood volume (TBV) in 3 hours, RBC transfusion of 100% TBV in 24 hours, or RBC transfusion of $>10\%$ of TBV per minute.
-

Question 34: E. All of the above.

**Explanation:**

- Tissue damage caused by trauma results in the release of tissue factor (TF) and subsequent activation of the coagulation cascade.
 - Hypoperfusion following blood loss causes increased expression of thrombomodulin which binds to thrombin and activated protein C (APC).
 - The APC inhibits Factor Va and Factor VIIIa.
 - Excess APC also depletes plasminogen activator inhibitor-1 (PAI-1), which results in less tissue plasminogen activator (tPA) inhibition and increased activation of plasminogen to plasmin and fibrinolysis.
-

Question 35: C. Narrow pulse pressure.

Explanation:

- In pediatric populations, a narrow pulse pressure is a more sensitive sign of hypovolemia than tachycardia or systolic hypotension.
 - Additionally, lactic acidosis secondary to hypoperfusion and decreased urine output are additional indicators of hypovolemia.
-

Question 36: C. Ionized calcium.

Explanation:

- The citrate in the blood components binds calcium in the patient's blood stream and leads to impaired coagulation.
 - It is recommended to give either 1-2 g of calcium chloride or 3-6 g of calcium gluconate per MTP round (6 units of RBCs).
 - The patient's ionized calcium (iCa) should be followed and a normal to mildly elevated iCa should be targeted.
-

Question 37: B. High-dose tranexamic acid was associated with increased venous thromboembolic events in patients with acute gastrointestinal bleeding.



Explanation:

- In the HALT-IT trial, death due to bleeding was equivalent in the tranexamic group and placebo group.
 - Arterial thromboembolic events (myocardial infarction or stroke) were similar in the tranexamic acid group and placebo group and venous thromboembolic events (deep vein thrombosis or pulmonary embolism) were higher in the tranexamic acid group than in the placebo group.
 - Thus, based upon this large randomized controlled study, tranexamic acid use is not recommended in patients with acute gastrointestinal bleeding.
-

Question 38: E. Hypocalcemia.

Explanation:

- The citrate in the blood component binds up free calcium in the patient's blood leading to low levels of iCa. These low-iCa levels can cause prolonged ST segments in an EKG.
 - Hypercalcemia causes shortening of the ST segment.
 - A myocardial infarction would cause ST elevation in multiple leads of an EKG.
-

Question 39: E. A, B, and C.

Explanation:

- Red cell preservation and storage result in changes over time that include decreased pH, increased potassium, decreased 2,3-diphosphoglycerate (2,3-DPG), and decreases in erythrocyte function and deformability, all of which may affect resuscitation and oxygen delivery.
-

Question 40: A. The patient is typing as mixed group O+ and B+ on forward typing. On reverse typing, he is typing as group B. The DAT result is positive.

**Explanation:**

- He received a large number of group O+ and B+ RBC unitss and would be expected to be mixed group O and B positive on a forward typing. On reverse typing, he would most likely look like a group B.
 - The DAT result would be positive due to the transfused anti-B in the A platelets and in the group O red cells binding to his own group B red cells and the transfused group B red cells.
-

Question 41: A. Infuse 2 units of group O– RBCs and 2 units of group AB plasma.

Explanation:

- The 19-year-old female is of childbearing potential and has unknown Rh status. The group O– RBCs should be used with the group AB plasma until the MTP cooler arrives.
 - The patient has low BP and elevated pulse and requires emergent resuscitative measures.
-

Question 42: B. Patients who received plasma:RBC in a 1:1 ratio had significantly better 6- hour survival, but survival at later time points did not differ.

Explanation:

- A later study, the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial, compared patient outcomes for 1:1:1 to 1:1:2 ratios of platelets:plasma:RBCs.
 - Notably, the primary outcomes that were measured (24-hour and 30-day survival) did not significantly differ between the two ratios.
 - The 1:1:1 group had fewer deaths from bleeding in 24 hours; thus, the 1:1:1 is frequently the preferred ratio for MTPs.
-

Question 43: B. ABO compatible.

**Explanation:**

- The AABB *Standards* permit ABO-compatible whole blood use. Thus, low-titer group O whole blood can be used.
-

Question 44: D. vWF antigen and activity.**Explanation:**

- The ECMO circuit destroys large-molecular-weight multimers of vWF due to the shear stress. Within a few hours of starting ECMO, large vWF multimers disappear. This leads to a decreased vWF activity in the setting of near normal vWF antigen levels. * Additional vWF can be infused from plasma, cryoprecipitate, or vWF concentrate.
-

Question 45: C. aPTT and PT mixing studies.**Explanation:**

- The mixing studies will tell if the patient has an inhibitor such as could be seen in acquired Factor VIII antibody or if he has been taking warfarin.
 - The aPTT and PT will correct in the setting of warfarin therapy, but not in Factor VIII antibody or anti-Xa inhibitor medications.
-

Question 46: C. IV vitamin K and four-factor PCC.**Explanation:**

- The patient should receive four-factor PCC now as he is bleeding due to his multiple injuries.
- He should also receive IV vitamin K so that he can start making functional clotting proteins.

**Question 47: D.** Serotonin.**Explanation:**

- Platelet alpha granules contain proteins such as vWF, platelet factor 4, thrombospondin, and fibrinogen.
 - Platelet-dense granules contain small molecules such as calcium, serotonin, polypyrophosphate chains, and ADP.
-

Question 48: E. Give Andexxa (recombinant inactivated coagulation Factor Xa).**Explanation:**

- The treatment to emergently reverse direct Factor Xa inhibitor drugs is to administer recombinant inactivated coagulation Factor Xa (Andexxa).
 - Plasma infusion would put the patient at risk for TRALI, TACO, as well as other transfusion reactions and require a large amount of plasma. Before the availability of Andexxa, 4-factor PCC was the treatment of choice.
 - Emergent plasmapheresis would not be a first or second choice given the site of his bleeding.
 - Protamine reverses heparin but not direct Factor Xa inhibitors.
-

Question 49: D. Return the RBCs and plasma to the blood bank stock and discard the platelets.**Explanation:**

- The RBCs and plasma were kept at the correct transport temperature; however, the platelets were not. The platelets were mistakenly placed in the cooler, which necessitates their discard.
- This is unfortunate as there are not enough blood products and someone took the time to donate that vital component only to have someone mistakenly place it in the wrong location.



- This error should be discussed with the team that was engaged in the MTP to make sure no additional products are wasted in the future.
-

Question 50: C. Send the MTP cooler and have the blood bank director call the attending physician for this patient.

Explanation:

- This situation is tough. Clinicians should not misuse MTP to get around blood transfusion guidelines that have been put in place for patient safety.
- That being said, the patient could be massively hemorrhaging and require MTP; at this point, it is unknown.
- The MTP cooler should be sent and the blood bank doctor should reach out to the team and/or attending physician of the patient to elucidate what is going on. Patient safety and wellbeing should always be prioritized.

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10

Noninfectious Complications of Transfusion

E. Alexander Dent, MD

Key Points from the *Technical Manual*

- The greatest risks associated with transfusion are noninfectious risks.
- Many transfusion reactions have signs or symptoms that may be present in more than one type of reaction. Early recognition of the reaction, prompt cessation of the transfusion, and further evaluation are key to the successful resolution of a reaction.
- Transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) are the leading causes of transfusion-related mortality reported to the Food and Drug Administration (FDA).
- Acute intravascular hemolytic transfusion reactions are often caused by sample or patient misidentification and are, therefore, preventable.
- Transfusion-associated graft-vs-host-disease (TA-GVHD) is fatal in >90% of cases and can be prevented by irradiation of blood components.
- Iron overload is a complication of chronic transfusion therapy; primary treatments include chelation and therapeutic phlebotomy.



QUESTIONS

Question 1: Which of the following factors is most likely to be associated with transfusion-associated hyperkalemia?

- A. Irradiation of Red Blood Cell (RBC) unit.
 - B. RBC units stored for longer than 30 days.
 - C. Volume of transfused RBCs.
 - D. Patient age between 1-5 years.
 - E. Patient kidney dysfunction.
-

Question 2: How does leukocyte reduction of blood components decrease the incidence of transfusion reactions?

- A. Poststorage leukocyte reduction more efficiently removes leukocytes that cause TRALI.
 - B. Leukocyte reduction decreases the total amount of protein in a unit, including allergic proteins.
 - C. Leukocyte reduction filters remove bacteria responsible for septic transfusion reactions.
 - D. Leukocyte reduction filters identify donors with sickle cell trait.
 - E. Prestorage leukocyte reduction decreases the content of inflammatory cytokines in a unit of blood.
-

Question 3: A patient with leukemia is receiving transfusions of both RBCs and platelets for anemia and thrombocytopenia. Which of the following is the most likely adverse outcome?

- A. Alloimmunization to red cell antigens.
 - B. Alloimmunization to HLA antigens.
 - C. Febrile nonhemolytic transfusion reaction (FNHTR).
 - D. Urticaria.
 - E. Transfusion-associated circulatory overload (TACO).
-

Question 4: Acute transfusion reactions occur:



- A. During transfusion of a blood component.
 - B. Within 4 hours of transfusion of a blood component.
 - C. Within 12 hours of transfusion of a blood component.
 - D. Within 24 hours of transfusion of a blood component.
 - E. Within 28 days of transfusion of a blood component.
-

Question 5: The most common presenting symptom of an acute hemolytic transfusion reaction is:

- A. Fever.
 - B. Rigors.
 - C. Flank pain.
 - D. Hypotension.
 - E. Red/dark urine.
-

Question 6: What is the most common cause of acute hemolytic transfusion reactions (AHTR)?

- A. Human error involving the interpretation of recipient ABO or Rh testing.
 - B. Human error involving the identification of specimen, patient, or blood unit.
 - C. Rh antigen differences between donor and recipient.
 - D. Kell antigen differences between donor and recipient.
 - E. Duffy antigen differences between donor and recipient.
-

Question 7: Transfusion-related sepsis (TRS) presents with _____ and is more severe with gram-_____ bacteria.

- A. Low-grade fever; negative.
- B. High-grade fever; negative.
- C. Low-grade fever; positive.
- D. High-grade fever; positive.
- E. No fever; both negative and positive equally.



Question 8: A patient receiving a unit of RBCs starts to complain of chills. The patient's temperature is measured at 102.1 F and the transfusion is stopped. The unit and all tubing associated with blood administration are returned to the blood bank for workup. What is the appropriate source of culture material related to this suspected reaction?

- A. Fluid in the blood administration kit tubing.
 - B. Fluid in the tubing segments that are still connected to the RBC unit.
 - C. Fluid in the RBC unit collected through the port connected to the blood administration kit.
 - D. Fluid in the RBC unit collected by cleaning and cutting open the bag.
 - E. Collect patient blood cultures if the patient has only itching.
-

Question 9: When is it permissible to resume transfusion of a unit implicated in a suspected transfusion reaction?

- A. Never.
 - B. If the patient has fever that resolves within 10 minutes.
 - C. If the patient has shortness of breath that resolves within 15 minutes.
 - D. If the patient has urticarial symptoms that resolve with antihistamines.
 - E. If the patient has itching that resolves without treatment.
-

Question 10: Which of the following is most likely to cause transfusion-related acute lung injury (TRALI)?

- A. Anti-HLA (human leukocyte antigens) in the recipient.
- B. Anti-HNA (human neutrophil antigens) from the donor.
- C. Anti-HPA (human platelet antigens) in the recipient.
- D. Anti-IgA from IgA-deficient blood donors.
- E. Cytokines elaborated by leukocytes that survive leukocyte reduction.



Question 11: What is the leading cause of transfusion-related mortality?

- A. Acute hemolytic transfusion reactions (AHTRs).
 - B. Delayed hemolytic transfusion reactions (DHTRs).
 - C. Transfusion-related acute lung injury (TRALI).
 - D. Transfusion-associated circulatory overload (TACO).
 - E. Transfusion-associated graft-vs-host disease (TA-GVHD).
-

Question 12: Antibodies to which of the antigens listed have been implicated most often in cases of posttransfusion purpura (PTP)?

- A. HPA-1a.
 - B. HPA-1b.
 - C. HPA-2a.
 - D. HLA-Class I antigens.
 - E. HLA Class II antigens.
-

Question 13: Which of the following transfusion donor/recipient pairs is most concerning for TA-GVHD?

- A. Donor is heterozygous for HLA alleles where the recipient is homozygous.
 - B. Donor is homozygous for HLA alleles where the recipient is heterozygous.
 - C. Donor is Rh Ce and the recipient is Rh CE.
 - D. Donor is Rh CE and the recipient is Rh Ce.
 - E. Donor is previously implicated in TA-GVHD.
-

Question 14: Patients taking ACE inhibitors have higher relative risk for hypotensive transfusion reactions due to the accumulation of vasoactive kinins. Which of the following factors is also known to increase concentrations of bradykinin?

- A. Recent prostatectomy.
- B. Use of cardiopulmonary bypass.
- C. Negatively charged bedside leukocyte reduction filters.



- D. Positively charged bedside leukocyte reduction filters.
 - E. All of the above.
-

Question 15: A 29-year-old female with a history of HbSS disease, iron overload, and silent cerebral infarcts undergoes red cell exchange. Ten days later, she is admitted from a sickle cell clinic with lower back and neck pain, as well as decreased hemoglobin level (from 7.1 g/dL post-exchange to 6.2 g/dL at admission). Additionally, her HbSS fraction was 7% post-exchange, and is 41% at admission. What is the most likely cause of her anemia?

- A. Delayed hemolytic transfusion reaction (DHTR) caused by anti-HLA.
 - B. DHTR caused by anti-Fy(a).
 - C. DHTR reaction with no identifiable antibody.
 - D. Delayed serologic transfusion reaction.
 - E. Vaso-occlusive pain crisis.
-

Question 16: Hyperhemolysis is a dreaded consequence of delayed hemolytic transfusion reactions. What is hyperhemolysis?

- A. A rate of hemolysis greater than 0.5 mL/kg/hour.
 - B. A rate of hemolysis greater than 1 mL/kg/hour.
 - C. Clinical or laboratory evidence of both hemolysis and macrophage activation.
 - D. Clinical or laboratory evidence of both hemolysis and reticulocytopenia.
 - E. Hemolysis of both donor and recipient red cells.
-

Question 17: Your facility performs blood compatibility testing for an offsite infusion center. What are the reporting requirements when a patient dies of a transfusion reaction or complication of blood transfusion?

- A. Report to your institution's Joint Transfusion Committee immediately via e-mail.
- B. Report to the blood supplier within 48 hours via phone.



- C. Report to the National Healthcare Safety Network within 48 hours via fax.
 - D. Report to the FDA within 7 days via written report.
 - E. Report to your institution's Chief Medical Officer as soon as possible via e-mail.
-

Question 18: Which of the following is true regarding iron overload?

- A. Therapeutic phlebotomy is the only therapy shown to prolong survival.
 - B. A unit of RBCs contains approximately 300-350 mg of iron.
 - C. Cumulative doses as few as 20 RBC units are associated with increased morbidity and mortality.
 - D. Iron chelation should be initiated once serum ferritin exceeds 1500 µg/L.
 - E. Iron-chelating agents bind iron indirectly, increasing its excretion in urine and feces.
-

Question 19: Which of the following patients does not have an indication for irradiated blood components?

- A. A leukemia patient receiving granulocyte transfusion.
 - B. A non-Hodgkin lymphoma patient treated with fludarabine therapy receiving RBCs.
 - C. A patient receiving intrauterine transfusion.
 - D. A patient receiving a component from a blood relative.
 - E. A CMV-negative patient receiving a component from a CMV-untested donor.
-

Question 20: What is the required minimum dose of irradiation to prevent TA-GVHD?

- A. 15 Gy delivered to the central portion of the container.
- B. 25 cGy delivered to any portion of the container.
- C. 25 Gy delivered to the central portion of the container.
- D. 1500 Gy delivered to any portion of the container.
- E. 2500 Gy delivered to the central portion of the container.



Question 21: In the context of blood transfusion for transplant recipients, which of the following is a minor incompatibility?

- A. Transfusions with mismatched HLA antigens that are cross-match-compatible.
 - B. Transfusions with mismatched HPA antigens that are cross-match-compatible.
 - C. Transfusions with mismatched red cell antigens that are cross-match-compatible.
 - D. Transfusions where the donor has preformed antibodies against the recipient.
 - E. Transfusions where the recipient has preformed antibodies against the donor.
-

Question 22: Intravascular hemolytic transfusion reactions are associated most commonly with which blood group system?

- A. Rh.
 - B. Kidd.
 - C. Kell.
 - D. Duffy.
 - E. ABO.
-

Question 23: A group A positive patient receives a liver transplant from a group O negative donor. During the transplant procedure, the recipient received multiple units of group A platelets and low-titer group O plasma. Two weeks later the patient is noted to have elevated lactate dehydrogenase and decreased hemoglobin. What is the most likely cause?

- A. Delayed hemolytic transfusion reaction.
- B. Passenger lymphocyte syndrome.
- C. Underlying disease.
- D. Delayed serologic transfusion reaction.
- E. Mechanical hemolysis.



Question 24: What is the utility of adding furosemide to the treatment of acute hemolytic transfusion reactions?

- A. To enhance renal cortical blood flow.
 - B. To monitor urine for signs of hemolysis.
 - C. To prevent concurrent development of TACO.
 - D. To reduce the cardiac preload.
 - E. To reduce serum potassium.
-

Question 25: Which of the following RBC donors is most likely to have a unit with nonimmune-mediated hemolysis during storage?

- A. 20-year-old male donor.
 - B. 20-year-old female donor.
 - C. 45-year-old male donor.
 - D. 45-year-old female donor.
 - E. Donor gender and age do not impact red cell storage hemolysis.
-

Question 26: Reported cases of TRALI have followed the transfusion of which component most frequently?

- A. Platelets.
 - B. Plasma.
 - C. RBCs.
 - D. Granulocytes.
 - E. Cryoprecipitate.
-

Question 27: Which of the following symptoms is the least common with TACO?

- A. Cough.
- B. Headache.
- C. Fever.
- D. Hypertension.
- E. Tachycardia.



Question 28: Which of the following premedications is effective in routine use to reduce instances of allergic transfusion reactions?

- A. Acetaminophen.
 - B. Eculizumab.
 - C. Diphenhydramine.
 - D. Ranitidine.
 - E. None of the above.
-

Question 29: What percentage of blood donations contain HLA or HNA antibodies?

- A. 1%.
 - B. 5%.
 - C. 10%.
 - D. 15%.
 - E. 20%.
-

Question 30: A patient awaiting liver transplant develops gastrointestinal bleeding that requires massive transfusion. Although he is now hemodynamically stable, an echocardiogram shows reduced cardiac function following transfusion. What is the most likely cause?

- A. Citrate toxicity.
 - B. Hyperkalemia.
 - C. Air embolism.
 - D. Hypothermia.
 - E. TACO.
-

Question 31: How is fever defined for transfusion reactions?

- A. ≥ 1 C rise in temperature to ≥ 38 C.
- B. ≥ 1 C rise in temperature to ≥ 39 C.
- C. ≥ 1.5 C rise in temperature to ≥ 38 C.
- D. ≥ 1.5 C rise in temperature to ≥ 39 C.
- E. ≥ 2 C rise in temperature to ≥ 38 C.



Question 32: A patient with leukemia is preparing to receive transfusion of autologous stem cells. While the transfusionist is connecting the unit to the infusion kit, the patient starts to complain of a bitter, metallic taste after the IV is flushed with saline. What is the most likely cause?

- A. Anxiety.
 - B. Vasovagal feedback.
 - C. Bacterial contamination of saline.
 - D. Volatile organic compounds in saline.
 - E. Volatile organic compounds in tubing.
-

Question 33: Does the gender or the parity of the blood donor affect mortality of patients receiving RBC transfusion?

- A. Yes, because female donors are more likely to have anti-HLA.
 - B. Yes, and there is a survival benefit among recipients of blood from female donors.
 - C. Yes, but the effect is dose dependent.
 - D. No, although units from female donors tend to have lower hemoglobin content.
 - E. No, although units from male donors tend to have lower fibrinogen content.
-

Question 34: In the United States, what entity was created to implement national surveillance of transfusion-associated adverse events aimed at improving patient safety, minimizing morbidity and mortality of transfusion recipients, and identifying emerging complications and pathogens associated with blood transfusion?

- A. The Food and Drug Administration Center for Biologics Evaluation and Research.
- B. The Centers for Disease Control and Prevention.
- C. The National Healthcare Safety Network Hemovigilance Module.
- D. The Association for the Advancement of Blood and Biotherapies.
- E. The Transfusion-Transmitted Infectious Monitoring System.



Question 35: The NHSN case definition of a febrile nonhemolytic transfusion reaction (FNHTR) requires that symptoms occur within how many hours of cessation of transfusion to be considered definitive?

- A. 4.
 - B. 8.
 - C. 12.
 - D. 24.
 - E. 48.
-

Question 36: A patient developed respiratory distress during transfusion. The transfusion is stopped, an investigation is initiated, and the patient is transferred to the intensive care unit (ICU), where the patient was intubated and provided vasopressor support. The patient's reaction is ultimately defined as transfusion-associated dyspnea (TAD). What is the severity of their reaction?

- A. Nonsevere.
 - B. Severe.
 - C. Very Severe.
 - D. Life-threatening.
 - E. Undefined.
-

Question 37: Transfusion-associated adverse reactions are classified according to the specific case definition, the severity of the reaction, and imputability criteria within the NHSN Hemovigilance Module Surveillance Protocol. Imputability may be definite, probable, or possible. If a patient's death is only possibly related to a transfused product, how should the severity of that reaction be classified?

- A. Nonsevere.
- B. Severe.
- C. Life-threatening.
- D. Death.
- E. Not determined.



Question 38: Resolving the cause of shortness of breath following transfusion can be challenging. Radiographic evidence is required for the definitive diagnosis of which of the following transfusion reactions according to the NHSN Hemovigilance Module Surveillance Protocol?

- A. Transfusion-associated circulatory overload (TACO).
 - B. Transfusion-related acute lung injury (TRALI).
 - C. Allergic reaction.
 - D. Transfusion-associated dyspnea (TAD).
 - E. None of the above.
-

Question 39: Transfusion-related immune modulation (TRIM) describes the tendency of transfused blood components to induce immune tolerance. Although some aspects of TRIM are poorly understood (and debated), it is clear that some blood components are more immune modulatory. Which of the following components would you expect to have the greatest immune modulatory effect?

- A. Whole blood.
 - B. RBCs, leukocyte reduced.
 - C. Platelets.
 - D. Plasma.
 - E. Cryoprecipitate.
-

Question 40: Can TRALI be diagnosed in a patient with pre-existing lung injury? [P/F ratio is the ratio of the PaO₂ (arterial oxygen partial pressure obtained from an arterial blood gas) to the FiO₂ (fraction of inspired oxygen expressed as a decimal).]

- A. Yes, as long as the patient had stable lung function in the 12 hours preceding transfusion.
- B. Yes, as long as the patient's P/F ratio was above 300 before transfusion.
- C. Yes, as long as the patient tests positive for anti-HLA.
- D. No, TRALI cannot be reliably distinguished from underlying lung injury.
- E. No, TRALI does not occur in patients with pre-existing lung injury.



Question 41: What is the most common sign or symptom of TA-GVHD?

- A. Fever.
 - B. Liver enzyme elevation.
 - C. Rash.
 - D. Pancytopenia.
 - E. Diarrhea.
-

Question 42: The definitive diagnosis of posttransfusion purpura (PTP) requires thrombocytopenia, defined as a decrease in platelets to less than ____% of pretransfusion count.

- A. 5.
 - B. 10.
 - C. 15.
 - D. 20.
 - E. 25.
-

Question 43: A 67-year-old female is admitted to the hospital and found to have a hemoglobin level of 4.5 g/dL. After receiving one unit of whole blood, she begins to complain of shortness of breath. The transfusion rate is slowed, and a total of 3 units of whole blood are transfused. Two hours later, she experiences cardiac arrest and requires intubation. Her B-type natriuretic peptide (BNP) is elevated and a chest x-ray shows increased pulmonary edema. Her vital signs are shown below:

Vital Sign	Pretransfusion	Posttransfusion
Temperature	36.8 °C	36.1 °C
Blood pressure	83/66 mmHg	180/144 mmHg
Pulse	99 bpm	100 bpm
Respiratory rate	18/minute	34/minute
O ₂ saturation	92%	91%



These findings are most consistent with which transfusion reaction?

- A. TACO.
 - B. TRALI.
 - C. TAD.
 - D. Allergic transfusion reaction.
 - E. Underlying disease / other.
-

Question 44: 69-year-old male with end-stage renal disease (ESRD) presents to the emergency department complaining of headache. A chemistry panel shows metabolic derangements and the patient receives urgent hemodialysis. A complete blood count (CBC) reveals a hemoglobin level of 6.6 g/dL, and the patient receives a unit of RBCs. Approximately 30 minutes into the transfusion, he begins to complain of anxiety and a racing heartbeat. His vital signs are shown below:

Vital Signs	Pretransfusion	Posttransfusion
Temperature	36.5 C	36.5 C
Blood pressure	167/109 mmHg	117/85 mmHg
Pulse	91 bpm	145 bpm
Respiratory rate	18/minute	20/minute
O ₂ saturation	100%	100%

The laboratory investigation is ongoing; however, the clinical team is eager to categorize the transfusion so the patient can receive additional product. These findings are most consistent with which transfusion reaction?

- A. TAD.
- B. TRALI.
- C. TACO.
- D. Hypotensive transfusion reaction.
- E. Underlying disease / other.



Question 45: 24-year-old female undergoing marrow transplantation begins to complain of itching at the end of her procedure. Her itching resolves with diphenhydramine, but an hour later the patient experiences rigors, shortness of breath, and develops a rash. The patient remains afebrile, an EKG shows normal sinus rhythm, and high-sensitivity troponin is not elevated. Her symptoms resolve following a saline bolus. A chest radiograph taken after symptom resolution shows pulmonary vascular redistribution. The blood bank is alerted the following day, and the patient has received multiple platelet products and IV fluids since her transplant. The treating clinicians say that the patient appears euvolemic, but are worried about a transfusion reaction.

The patient is group B positive and she has known alloantibodies against the K and Le^b antigens. Her marrow donor is male, group B positive, and negative for K and Le^b antigens. Although the patient has known HLA Class I antibodies, the donor lacks cognate Class I antigens. Which of the following is the most likely transfusion reaction?

- A. Hemolytic transfusion reaction.
 - B. TRALI.
 - C. TACO.
 - D. Allergic transfusion reaction.
 - E. Underlying disease / other.
-

Question 46: A 76-year-old male with history of atherosclerotic cardiovascular disease presents to the emergency department with double vision. He is hypotensive (65/45 mmHg) at presentation, is given a bolus of fluids, and re-evaluated. His repeat blood pressure is 144/65 mmHg and he is admitted to the intensive care unit with a hemoglobin concentration of 6.5 g/dL. In the ICU, he is transfused one unit of RBCs. Thirty minutes into the transfusion the blood pressure is noted to be 70/50 mmHg without other symptoms. The transfusion is stopped, a transfusion reaction investigation initiated, and the RBC unit is returned. You talk to the ICU and find out that his blood pressure has been re-checked in the opposite arm and is 175/67 mmHg. Which of the following is the most likely explanation for his fluctuating blood pressure?



- A. Hypotensive transfusion reaction.
 - B. Subclavian steal syndrome.
 - C. Peripheral artery disease of upper extremity.
 - D. Aortic stenosis.
 - E. Diabetic neuropathy.
-

Question 47: While giving informed consent for blood transfusion, a patient reports that they had a “bad reaction” to a unit of blood 13 years ago, but no record of this reaction can be located. The blood bank is preparing a unit of RBCs for this patient to be transfused nonemergently. What is the appropriate transfusion rate during the first 15 minutes of the transfusion?

- A. 1-2 mL/minute.
 - B. 3-4 mL/minute.
 - C. 5-6 mL/minute.
 - D. 7-8 mL/minute.
 - E. 9-10 mL/minute.
-

Question 48: A transfusion recipient is suspected of having an air embolism. How should this patient be positioned?

- A. Trendelenburg.
 - B. Reverse Trendelenburg.
 - C. Left lateral recumbent.
 - D. Right lateral recumbent.
 - E. Prone.
-

Question 49: The following statements about IgA deficiency are false, except:

- A. It is associated with AHTRs.
- B. Platelets for IgA-deficient patients should be washed before transfusion.
- C. Incidence rates for people of European ancestry are approximately 1 in 70,000 to 1 in 80,000.



- D. Anti-IgA will not be produced by most patients.
 - E. In most cases, IgA antibodies occur naturally.
-

Question 50: In the evaluation of a possible AHTR, which serologic test will be most useful?

- A. Rh type.
- B. Direct antiglobulin test (DAT).
- C. Antibody screen.
- D. Antibody identification panel.
- E. Crossmatch.

ANSWERS

Question 1: C. Volume of transfused RBCs.

Explanation:

- Transfusion-associated hyperkalemia (TAH) is a rare complication of RBC transfusion with a higher incidence and mortality rate in pediatric transfusion recipients. Pediatric patients aged one to five are most often reported as having TAH; however, the greatest risk for TAH occurs when the volume of transfused RBCs exceed 30% of a patient's estimated total blood volume.
- Comorbidities such as kidney dysfunction, liver dysfunction, and even respiratory distress may play a role in the development of TAH, and there is a high mortality rate in patients who develop TAH.
- There is evidence that the age of the RBC unit is *inversely* associated with development of TAH, probably because of the practice of many blood banks to provide younger units to younger patients.
- Irradiation may theoretically cause more potassium leakage from the cells but this is not observed clinically.



Question 2: E. Prestorage leukocyte reduction decreases the content of inflammatory cytokines in a unit of blood.

- Prestorage leukocyte reduction decreases the inflammatory cytokine content in a unit of blood and the incidence of nonhemolytic febrile transfusion reactions (NHFTR.)
- Poststorage leukocyte reduction is less effective and does not prevent the development of TRALI. Bacteria are smaller than the pores in a leukocyte reduction filter.
- Although sickle cell trait is a recognized cause of filter failure, that does not decrease the incidence of transfusion reactions.



Question 3: B. Alloimmunization to HLA antigens.

Explanation:

- HLA immunization is one of the most common consequences of transfusion as can be seen from the incidence of common transfusion reactions listed below.
- Patients being transfused with platelet components are exposed to HLA Class I antigens.
- Components containing red cells may have residual HLA Class I antigens, termed Bennett-Goodspeed (Bg) antigens. Bg^a corresponds to HLA-B7, Bg^b corresponds to HLA-B17, and Bg^c corresponds to HLA-A28. These antigens are expressed variably on red cells.

Transfusion Reaction	Incidence	Components
Alloimmunization to HLA antigens	1:10 (10%)	Platelets and white-cell-containing components
Alloimmunization to red cell antigens	1:100 (1%)	Red-cell-containing components
Urticaria	1:100-1:33 (1-3%)	Antibody to donor plasma protein



Transfusion Reaction	Incidence	Components
Circulatory overload	1:100 (1%)	Red-cell-containing components
Febrile nonhemolytic transfusion reaction	1:1000-1:100 (0.1-1%)	Lower with universal leukocyte reduction

Question 4: D. Within 24 hours of transfusion of a blood component.

Explanation:

- Acute or immediate transfusion reactions occur within 24 hours of blood product transfusion.
 - Acute transfusion reactions include hemolytic transfusion reactions, transfusion-related sepsis, TRALI, allergic reactions, TACO, air embolism, hypotensive reactions, FNHTRs, and others.
 - It is impossible to know the significance of acute transfusion reactions based on symptoms alone and all acute transfusion reactions require laboratory evaluation
-

Question 5: A. Fever.

Explanation:

- Fever is the most common presenting symptom of an immune-mediated acute hemolytic transfusion reaction (AHTR). Chills and/or rigors may accompany fever.
- All of the answer options may be presenting signs or symptoms of an AHTR but are less common than fever.
- Patients who are sedated, unconscious, or have altered mental status may have AHTRs that manifest only as red/dark urine and they may receive several units of incompatible blood before acute hemolysis is recognized.
- Symptom severity is related, among other things, to the amount of incompatible antigen transfused which is why if there are any signs of an acute transfusion reaction, the transfusion should be stopped immediately.



Question 6: B. Human error involving the identification of specimen, patient, or blood unit.

Explanation:

- Human error that results in the misidentification of specimen, patient, or blood unit is the most common cause of mistransfusion resulting in AHTR.
 - Interventions to reduce the likelihood of these errors is a must, but there is no foolproof method to eliminate these errors.
 - The use of barcode scanners, radiofrequency labels, and smart refrigeration systems have all been used to reduce the likelihood of such errors.
-

Question 7: B. High-grade fever; negative.

Explanation:

- Transfusion-related sepsis (TRS) usually presents during or shortly after transfusion with marked fever ($>38.5\text{ C}$ or 101 F) and is more severe with gram-negative bacteria.
 - Although gram-positive TRS may present with isolated fever, gram-negative TRS can manifest as shock, renal failure, or disseminated intravascular coagulation (DIC).
 - Historically, these reactions have been most commonly associated with transfusion of platelets.
-

Question 8: D. Fluid in the RBC unit collected by cleaning and cutting open the bag.

Explanation:

- When units are implicated in potential cases of transfusion-related sepsis, it is critical to increase culture sensitivity while decreasing the likelihood of introducing additional organisms. That is why the best source of culture material is the unit itself, not integral segments, and not the tubing of a blood administration kit.



- If the unit has an unused port, that port should be cleaned before collecting blood from the bag, but if no ports remain unused, the outside of the bag should be cleaned and cut open using sterile instruments.
 - The sensitivity of culture is directly related to the amount of blood inoculated into liquid media or onto solid plates.
-

Question 9: D. If the patient has urticarial symptoms that resolve with antihistamines.

Explanation:

- Urticaria, a mild manifestation of allergic reactions, is the only transfusion reaction where the administration of the component may be routinely resumed after prompt treatment, assuming that there are no other symptoms and the unit has not expired.
 - Such reactions are not required to be reported to or investigated by blood banks.
-

Question 10: B. Anti-HNA (human neutrophil antigens) from the donor.

Explanation:

- The neutrophil is the central culprit in TRALI, and most cases are caused by *donor* anti-HLA or anti-HNA in the blood unit.
- The most popular explanatory model for TRALI is the “two-hit” model. The first hit is a patient’s pre-existing inflammation that primes neutrophils and slows their transit through lung microvasculature where they eventually can move into the alveolar space. The second hit comes from the transfused product: HLA or HNA antibodies capable of activating neutrophils. These activated neutrophils elaborate cytokines that promote pulmonary edema leading to patients’ symptoms.
- This mechanism is sometimes simplified to “noncardiogenic pulmonary edema” to differentiate TRALI from TACO.



Question 11: D. Transfusion-associated circulatory overload (TACO).

Explanation:

- TACO has been the leading cause of transfusion-related mortality reported to the FDA since 2016. Even though it is underrecognized and underreported, it accounted for nearly one-third of fatalities reported from 2013 to 2017.
- Before 2016, TRALI was the leading cause of transfusion-related mortality. Plasma from multiparous females is more likely to have anti-HLA and anti-HNA, and efforts since 2006 to reduce plasma collection from such donors has led to a continued decrease in TRALI incidence.
- Acute and delayed hemolytic transfusion reactions may be fatal, but are less common.*Although TA-GVHD is almost uniformly fatal, its occurrence is rare.



Question 12: A. HPA-1a.

Explanation:

- Posttransfusion purpura (PTP) is an uncommon, but serious, transfusion reaction marked by the destruction of autologous and allogeneic platelets.
- PTP is caused by antibody formation against human platelet antigen (HPA-1a) in the majority of cases, but HPA-2b, and HLA antibodies have also been implicated.
- These antibodies most often form after transfusion of RBCs or whole blood, not platelets.



Question 13: B. Donor is homozygous for HLA alleles where the recipient is heterozygous.

Explanation:

- Although TA-GVHD is rare, it is almost always fatal. Donor T lymphocytes recognize the host's immune cells as foreign, so an



HLA homozygous donor with heterozygous recipient is the most concerning pair because the donor can recognize the recipient as foreign, but the recipient cannot recognize the donor as foreign.

- Transfusion recipients with impaired T-cell mediated immunity have a higher relative risk, but TA-GVHD has been reported in previously healthy blood recipients.
 - A patient's Rh haplotype does not confer risk, nor does a donor history of being implicated in TA-GVHD.
 - Irradiation and pathogen inactivation are considered equivalent to reduce the risk of TA-GVHD.
-

Question 14: E. All of the above.

Explanation:

- All of the listed factors are known to increase blood (or blood component) concentrations of bradykinins either through activation of the contact system or, in the case of prostatectomy, by the release of kallikreins from the prostate.
 - When hypotension is recognized in patients taking ACE inhibitors, stopping the transfusion is crucial and often sufficient to resolve symptoms. Some patients may require additional supportive care with IV fluids or more aggressive therapies such as vaso-pressors.
-

Question 15: C. Delayed hemolytic transfusion reaction with no identifiable antibody.

Explanation:

- This patient's decrease in hemoglobin and concurrent increase in hemoglobin S fraction indicates that she is experiencing a delayed hemolytic transfusion reaction. In up to one-third of DHTR cases in sickle-cell-disease patients, a specific antibody is not identified.
- Because of differences in blood donor and blood recipient antigen profiles, Rh (C, E) and Kell (K) antigens have accounted for the majority of identified antigens in DHTR.



- This is not a delayed serologic transfusion reaction because the patient has symptoms and signs of hemolysis.
-

Question 16: E. Hemolysis of both donor and recipient red cells.

Explanation:

- Hyperhemolysis is defined as a destruction of both donor and recipient red cells, which may result in hemoglobin levels lower than pretransfusions levels.
 - Proposed mechanisms of hyperhemolysis include bystander hemolysis caused by complement activation, erythropoiesis suppression, and macrophage-mediated RBC destruction.
-

Question 17: D. Report to the FDA within 7 days via written report.

Explanation:

- The FDA must be notified as soon as possible followed by a written report within 7 days.
- It is the responsibility of the facility performing the compatibility testing to report.
- The FDA can be contacted by e-mail (fatalities2@fda.hhs.gov), phone (240-402-9160), fax (301-827-0333), or by express mail at the following address:

US Food and Drug Administration
CBER Office of Compliance and Biologics Quality
Document Control Center
10903 New Hampshire Avenue
W071, G112
Silver Spring, MD 20993-0002

Question 18: C. Cumulative doses as few as 20 RBC units are associated with increased morbidity and mortality.



Explanation:

- Causes of iron overload can be primary (as in hereditary hemochromatosis) or secondary (as in chronic RBC transfusion) because each unit contains 200-250 mg of iron and the body does not have a physiologic mechanism to excrete excess iron.
 - Increased morbidity has been observed after 20 RBC unit transfusions. Iron chelation and therapeutic phlebotomy have been shown to prolong survival.
 - Iron chelators should be initiated once serum ferritin exceeds 1000 µg/L and work by directly binding iron, increasing its excretion in urine and feces.
-

Question 19: E. A CMV-negative patient receiving product from a CMV-untested donor.

Explanation:

- The following is a list of well-documented indications for irradiated components.
 - Intrauterine transfusions.
 - Prematurity, low birthweight (<1200 g), or erythroblastosis fetalis in newborns.
 - Congenital immunodeficiencies.
 - Hematologic malignancies or solid tumor (neuroblastoma, sarcoma, Hodgkin disease).
 - Peripheral blood stem cell/marrow transplantation.
 - Donations from family members or that are HLA-matched.
 - Fludarabine therapy.
 - Granulocyte components.
-

Question 20: C. 25 Gy delivered to the central portion of the container.

Explanation:

- AABB *Standards* require a minimum dose of 25 Gy (2500 cGy) delivered to the central portion of the container and a minimum



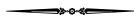
of 15 Gy (1500cGy) elsewhere. *Pathogen inactivation also prevents T-cell proliferation after transfusion and is an alternative to irradiation.



Question 21: D. Transfusions where the donor has preformed antibodies against the recipient.

Explanation:

- Minor incompatibility involves the transfusions where the blood donor has pre-existing antibodies against recipient antigens.
- Major incompatibility involves pre-existing antibodies in the recipient against donor antigens.



Question 22: E. ABO.

Explanation:

- Preformed, high-titer anti-A and/or anti-B are the most common antibodies implicated in acute intravascular HTRs.
- Anti-A and anti-B are naturally occurring antibodies that develop within a few months of birth. They are predominantly IgM antibodies, but they are reactive at 37 C.
- Anti-A and anti-B fix complement, having the ability to drive the complement cascade to completion and cause intravascular hemolysis.
- Antibodies directed against the Kidd, Kell, Rh, and Duffy blood group determinants are predominantly IgG. They have the ability to sensitize red cells but do not fix complement well, with the exception of Kidd antibodies. These IgG antibodies are more frequently implicated in delayed transfusion reactions with associated extravascular hemolysis.



Question 23: B. Passenger lymphocyte syndrome.

**Explanation:**

- This is the classic history of passenger lymphocyte syndrome (PLS) where lymphocytes in the transplanted organ elaborate antibodies that cause hemolysis of recipient red cells.
 - When a patient is suspected of having PLS, transfuse with donor-compatible RBCs and recipient ABO-group plasma/platelets.
 - Nothing reliably predicts which patients with minor ABO-mismatches will develop PLS. Organs with higher degrees of HLA-compatibility may be associated with higher risk of PLS because the recipient is less likely to clear donor immune cells.
-

Question 24: A. To enhance renal cortical blood flow.

Explanation:

- Maintaining renal blood flow is a critical goal of AHTR treatment. The urine flow rate goal is >1 mL/kg/hour and furosemide can enhance renal blood flow and urine output.
 - However, if acute tubular necrosis has already occurred, AHTR patients are at risk of developing pulmonary edema.
-

Question 25: C. 45-year-old male donor.

Explanation:

- Units from middle-aged male donors show a higher degree of hemolysis in stored RBC components. This is likely due to differences in hormone levels and the effects of those hormones on red cell function.
 - Units from younger and/or female donors have been associated with a reduced risk of red cell storage hemolysis.
-

Question 26: C. RBCs.

**Explanation:**

- Although TRALI may be caused by antibodies in plasma-containing components, it is important to remember that RBC units contain residual plasma and that many more RBC units are transfused compared to plasma units. Therefore, most cases of TRALI follow transfusion of RBCs, not plasma.
 - The evidence of TRALI risk based on component type is changing and some authors have reported that there is no longer a statistically significant difference in the risk for TRALI from different components without stratifying such data by gender.
 - In other words, there are no differences in the rate of TRALI from different components and the most commonly transfused component is the most likely to be implicated in TRALI.
-

Question 27: C. Fever.**Explanation:**

- Fever is the least common symptom of TACO but can occur. Fever does not exclude the diagnosis of TACO and some authors report inflammatory symptoms in up to one-third of TACO cases.
-

Question 28: E. None of the above.**Explanation:**

- There is no evidence to support routine premedication to prevent allergic transfusion reactions.
-

Question 29: C. 10%.**Explanation:**

- Approximately 10% of blood donations contain anti-HLA or anti-HNA. This is many more units than are implicated in TRALI, highlighting that the mere presence of these antibodies is not suf-



ficient to cause TRALI, but also that TRALI may be underrecognized and underreported.

Question 30: A. Citrate toxicity.

Explanation:

- This patient is likely showing symptoms of hypocalcemia caused by citrate anticoagulant used in the blood components he received during transfusion.
 - Numbness or tingling in digits or the perioral area is often reported as a common symptom, but the clinical status of the patient may exclude evaluation for this symptom.
-

Question 31: A. ≥ 1 C rise in temperature to ≥ 38 C.

Explanation:

- Fever is defined as a temperature greater than or equal to 38 C/100.4 F measured orally.
 - Fever alone may not satisfy the diagnosis of febrile reaction, because there should also be a change of at least 1 C/1.8 F from the pretransfusion temperature.
-

Question 32: D. Volatile organic compounds in saline.

Explanation:

- Patients may experience bad taste (or sometimes smell) because of volatile substances in saline.
- When these substances are injected into the bloodstream, they are metabolized, and eliminated by the respiratory system. The patients sense these metabolites in expired air as either a flavor, taste, or odor.



Question 33: D. No, although units from female donors tend to have lower hemoglobin content.

Explanation:

- Although it is recognized that plasma and platelet products from female or multiparous donors are associated with higher risk of transfusion reactions, there has been conflicting evidence about RBCs.
- This conflict probably arose because previous studies did not account for confounding factors, such as the lower hemoglobin counts and lower doses of hemoglobin in RBC units from female donors.



Question 34: C. The National Healthcare Safety Network Hemovigilance Module.

Explanation:

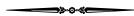
- This is the mission of the NHSN HV module.
- Although the NHSN HV module is a part of the CDC, the CDC was not created for this purpose.
- The FDA and the AABB have broader aims while the TTMS has a more narrow focus.



Question 35: A. 4.

Explanation:

- FNHTRs occur within 4 hours of cessation of transfusion and are accompanied by fever (greater than or equal to 38 C/100.4 F) measured orally and a change of at least 1 C/1.8 F from pretransfusion value or the presence of chills/rigors.



Question 36: D. Life-threatening.



Explanation:

- This patient required major intervention following their transfusion (eg, vasopressors, intubation, or transfer to intensive care) to prevent death; therefore, the reaction would be categorized as life-threatening.



Question 37: D. Death.

Explanation:

Category	NHSN Definition
Nonsevere	Medical intervention (eg, symptomatic treatment) is required but lack of such would not result in permanent damage or impairment of a bodily function.
Severe	Inpatient hospitalization or prolongation of hospitalization is directly attributable to the adverse reaction, persistent or significant disability or incapacity of the patient occurs as a result of the reaction, or a medical or surgical intervention is necessary to preclude permanent damage or impairment of a body function.
Life-threatening	Major intervention required following the transfusion (eg, vasopressors, intubation, transfer to intensive care) to prevent death.
Death	The recipient died as a result of the adverse transfusion reaction. Death should be used if death is possibly, probably, or definitely related to transfusion. If the patient died of a cause other than the transfusion, the severity of the reaction should be graded as appropriate given the clinical circumstances related to the reaction.
Not determined	The severity of the adverse reaction is unknown or not stated.



Question 38: B. Transfusion-related acute lung injury (TRALI).

**Explanation:**

- Although the 2019 consensus TRALI definition does not require radiographic evidence to make a diagnosis of TRALI, the CDC definition used in the NHSN Hemovigilance Module Surveillance Protocol requires radiographic evidence of bilateral infiltrates to make a definitive diagnosis.
 - TACO, TAD, and allergic reactions can be definitively diagnosed without radiographic evidence.
-

Question 39: A. Whole blood.

Explanation:

- Because of the content of white cells in whole blood, it is the product most effective in inducing TRIM. The established and proposed TRIM effects are as follows:
 - Decreased renal allograft rejection.
 - Improvement in autoimmune diseases.
 - Decreased repetitive spontaneous abortions.
 - Increased tumor recurrence.
 - Increased postoperative infection.
-

Question 40: A. Yes, as long as the patient had stable lung function in the 12 hours preceding transfusion.

Explanation:

- The 2019 consensus TRALI definition includes TRALI Type II, previously called possible TRALI, so that TRALI can be diagnosed in patients with existing lung injury or risk factors for acute respiratory distress syndrome (ARDS). TRALI Type II can be diagnosed in patients with P/F ratios between 200-300 if the patient's respiratory status was stable before transfusion.
- Although the P/F ratio may indicate changes in respiratory status, it should be interpreted in context of the patient's clinical picture because the P/F ratio can change without an actual change in the patient's respiratory status; for example, prone positioning.

**Question 41: C. Rash.****Explanation:**

- In a systematic review, rash was the most common symptom of TA-GVHD seen in 80.2% of patients.
- Other signs or symptoms include fever (68%), liver enzyme elevation (66%), pancytopenia (65%), diarrhea (43%), marrow aplasia (23%), and hepatomegaly (14%).

**Question 42: D. 20.****Explanation:**

- Thrombocytopenia in this context is defined as a decrease in platelets to less than 20% of pretransfusion count, but the patient must also demonstrate alloantibodies against HPA or other platelet-specific antigens.
- Patients who experience a 20 to 80% decrease in platelets may be diagnosed with probable or possible PTP, depending on the presence or absence of thrombocytopenia, and results of platelet antibody testing.

**Question 43: A. TACO.****Explanation:**

- This patient has new onset shortness of breath, radiographic evidence of increased pulmonary edema, and an elevated BNP, making the transfusion reaction most consistent with TACO.

**Question 44: E. Underlying disease/other.****Explanation:**

- This patient fails to meet any criteria for a definitive transfusion reaction without additional investigation. In stable patients,



efforts should be made to postpone transfusion until after the reaction investigation is complete; however, blood can be a life-saving therapy and should not be withheld if urgently needed.

- Although the patient experienced cardiac symptoms, the patient's pre- and posttransfusion blood pressure readings do not meet the criteria for a hypotensive transfusion reaction (drop in systolic BP of ≥ 30 mmHg and systolic BP ≤ 80 mmHg).
 - Further investigation may reveal laboratory or radiographic evidence that makes another reaction more likely.
-

Question 45: D. Allergic transfusion reaction.

Explanation:

- Although this product did not originate in the blood bank, we would categorize this reaction as an allergic transfusion reaction. The patient exhibited predominantly allergic symptoms with some respiratory component, which is likely bronchospasm.
 - TRALI should be considered, but is not favored, because the donor lacks Class I antigens for the patient's known HLA antibodies. Additionally, the donor is male and the imaging findings are not characteristic.
 - TACO should be considered given that the patient has received multiple infusions (including this product, multiple platelets, and IV fluids) but is not favored based on discussion with treating clinicians about her volume status.
 - The diagnosis of an allergic reaction/definitive requires two or more of the following occurring within 4 hours of the cessation of transfusion: conjunctival edema, edema of lips/tongue/uvula, erythema and edema of periorbital area, generalized flushing, hypotension, angioedema of the head and neck, maculopapular rash, itching, respiratory distress/bronchospasm, and urticaria. This patient had three of the conditions [pruritis (itching), bronchospasm, and urticaria (hives)]; therefore, her transfusion reaction is consistent with an allergic transfusion reaction.
-

Question 46: B. Subclavian steal syndrome.



Explanation:

- This is a case of subclavian steal syndrome (SSS) mimicking a hypotensive transfusion reaction and highlights the importance of investigating how vital signs were taken when performing a transfusion reaction investigation.
 - The pathophysiology of SSS involves the arm “stealing” blood from vertebrobasilar circulation, which can cause neurologic symptoms such as vision changes, dizziness, or nausea.
 - Although aortic stenosis and peripheral artery disease can also cause varied blood pressure readings between left and right arms, you would not expect double vision.
-

Question 47: A. 1-2 mL/minute.

Explanation:

- Life-threatening transfusion reactions most often occur within the first 15 minutes of transfusion. Nonemergency transfusions should start slowly, between 1-2 mL/minute, and the recipient should be closely monitored. Severe reactions can occur with transfusion volumes as small as 10 mL.
 - After 15 minutes, the patient should be reassessed and the transfusion rate can be increased as tolerated by the patient.
-

Question 48: C. Left lateral recumbent.

Explanation:

- Air embolism occurs in open transfusion systems or situations whenever oxygen has the opportunity to enter blood administration sets, such as when units of blood are being changed. Symptoms can range from cough to dyspnea to death.
- When air embolism is suspected, patients should be placed on their left side with the head down (left lateral recumbent) to displace any air bubble from the pulmonic valve.



Question 49: D. Anti-IgA will not be produced by most patients.

Explanation:

- IgA deficiency is the most common congenital immune deficiency, affecting approximately 1 in 700 to 1 in 800 individuals.
- From the perspective of a clinical immunology practice, a patient with a serum IgA level below 5 mg/dL is “IgA deficient.” However, these patients do not recognize IgA as a foreign molecule and, therefore, do not make anti-IgA.
- From the perspective of a transfusion medicine practice, only patients with IgA levels below 0.05 mg/dL are “IgA deficient.” These patients are at risk for developing class-specific IgA antibodies when exposed to IgA via pregnancy or transfusion.
- Up to 30% of severely IgA-deficient individuals have IgA class-specific (IgG or IgM) antibodies in their circulation.
- Previous transfusion or pregnancy is generally required to develop IgA antibodies.
- IgA-deficient transfusion recipients with anti-IgA are at risk for anaphylactic transfusion reactions.
- Anaphylactic reactions can occur suddenly and after transfusion of only a few milliliters of blood.
- The treatment of anaphylactic transfusion reactions includes discontinuation of the transfusion, IV fluid infusion, and epinephrine administration. The patient’s airway needs to be protected (eg, intubation). Patients may also require vasopressor support.
- The transfusion of blood components from IgA-deficient donors can prevent anaphylactic reactions in recipients who have experienced such reactions caused by IgA antibodies. These components can be obtained from larger blood centers. Washed or deglycerolized RBCs are also used for transfusion to prevent anaphylactic reactions.
- Although methods are available to wash platelets, they are cumbersome and washing may activate the platelets, making them less effective from the standpoint of hemostasis. Platelets from IgA-deficient donors, obtained by apheresis, are the product of choice for IgA-deficient donors at risk for anaphylaxis.
- Individuals with acquired IgA deficiency (eg, multiple myeloma) are not at risk for these reactions.



Question 50: B. Direct antiglobulin test (DAT).

Explanation:

- There is significant morbidity and mortality accompanying intravascular HTRs. Therefore, quick clinical intervention and laboratory evaluation are essential.
- The DAT is the most important serologic test to perform in the early evaluation of an AHTR. A newly reactive DAT or a DAT result that has increased in strength of reactivity in comparison with the pretransfusion sample strongly supports immune-mediated hemolysis.
- Other common serologic tests, including the antibody screen, the antibody identification panel, and Rh typing, are not likely to yield important information in the evaluation of an AHTR, as the vast majority of these reactions are caused by ABO antibodies.
- Repeat crossmatch testing is unlikely to be useful unless the root cause of the patient's reaction was a pretransfusion sample that actually belonged to another patient. In that case, crossmatching with a posttransfusion sample would be expected to confirm the incompatibility.
- The vast majority of intravascular HTRs are caused by clerical errors (ie, the intended recipient received the wrong donor unit). As such, a clerical check is an early critical step in the evaluation of these adverse reactions.
- Visual inspection of serum (for evidence of hemoglobinemia) and a centrifuged urine specimen (for evidence of hemoglobinuria) are rapid tests that are commonly used to determine if intravascular hemolysis has occurred.

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11

Infectious Complications of Blood Transfusion

Christina Dean, MD

Key Points from the *Technical Manual*

- Screening donors for infectious disease is accomplished by 1) questioning potential donors and excluding those who have an increased risk of infection, and 2) testing donated blood.
- Blood donated during the window period can transmit infections. The window period is a period of time after an individual is exposed and becomes infected when it is not yet possible to detect the presence of an infection. During the “window period” a recently infected individual can unknowingly transmit an infection through blood donation and the donor may still test negative because testing methods cannot detect this early infection.
- Maintaining a donor population that has a low incidence of infection continues to play a key role in preserving blood safety.
- Infections transmitted to humans by animal or insect vectors are being increasingly recognized as potential sources of transfusion-transmitted infection.
- Blood banks must have processes in place to ensure that donations with positive test results are not released for transfusion.



QUESTIONS

Question 1: What was the first infectious agent the Food and Drug Administration (FDA) required testing for in donated blood components?

- A. Hepatitis B virus (HBV).
 - B. Human immunodeficiency virus (HIV).
 - C. *Treponema pallidum*.
 - D. Cytomegalovirus (CMV).
 - E. Human T-cell lymphotropic virus-I/II (HTLV-I/II).
-

Question 2: Before the implementation of HBV screening in the 1970s, what percentage of patients receiving multiple transfusions developed posttransfusion hepatitis (PTH)?

- A. Less than 5%.
 - B. 15%.
 - C. 20%.
 - D. 25%.
 - E. More than 30%.
-

Question 3: All of the following measures may be used as part of a system to screen blood donors for infectious agents, except:

- A. Questioning only.
 - B. Collection of donor medical records.
 - C. Testing only.
 - D. Questioning and testing.
 - E. Donor education.
-

Question 4: FDA infectious disease testing requirements are outlined in which of the following documents?

- A. Title 21, Part 610.10 of the Code of Federal Regulations (CFR).
- B. Title 21, Part 610.40 of the CFR.



- C. Title 21, Part 610.41 of the CFR.
 - D. Title 21, Part 630.40 of the CFR.
 - E. Title 21, Part 640.10 of the CFR.
-

Question 5: The FDA requires the initiation of retrieval and quarantine of in-date components from previous donations within how many calendar days for a donor with a reactive HIV test result?

- A. 1.
 - B. 2.
 - C. 3.
 - D. 4.
 - E. 5.
-

Question 6: A look-back notification is required by law for which of the following infectious agents?

- A. HIV and HBV.
 - B. HIV and West Nile virus (WNV).
 - C. WNV and HTLV.
 - D. HIV and HCV.
 - E. HCV and HBV.
-

Question 7: To reduce the transmission of CMV, blood components can be leukocyte reduced. What is the estimated CMV transmission risk following transfusion with a leukocyte-reduced blood product?

- A. No residual transmission risk.
- B. 1-2%.
- C. 2-3%.
- D. 3-5%.
- E. 5-10%.



Question 8: With the introduction of nucleic acid testing (NAT), the window period (approximately) for detecting HIV in blood components has been reduced to how many days?

- A. 4.
 - B. 6.
 - C. 8.
 - D. 9.
 - E. 11.
-

Question 9: Which of the following is the correct estimated residual risk of transfusion-transmitted infection per donated unit following NAT implementation (approximately)?

- A. HBV: 1 in 800,000 to 1 in 1.2 million.
 - B. HCV: 1 in 1.1 million.
 - C. HIV: 1 in 1.5 million.
 - D. All of the above.
 - E. None of the above.
-

Question 10: The FDA requires donor screening with all of the following HBV tests except:

- A. HBsAg.
 - B. HBeAg.
 - C. HBV DNA.
 - D. Anti-HBc IgM.
 - E. Anti-HBc IgG.
-

Question 11: Which of the following is the correct window period (approximately) for the detection of HCV RNA and anti-HCV antibodies, respectively?

- A. 10 days; 15 days.
- B. 15 days; 25 days.
- C. 10 days; 2-3 months.



- D. 12 days; 1-1.5 months.
 - E. 7 days; 1.5-2 months.
-

Question 12: Which of the following is true concerning HTLV-I/II?

- A. 10% of HTLV infections in US blood donors are with HTLV-II.
 - B. Most HTLV infections are symptomatic.
 - C. The only FDA-approved screening tests for HTLV-I/II are IgG antibody assays.
 - D. Approximately 15% of HTLV-I infected individuals develop adult T-cell leukemia/lymphoma after a lag of 20-30 years.
 - E. HTLV-I/II is transmitted by acellular blood components.
-

Question 13: Which of the following is true regarding donor testing for syphilis?

- A. Most reactive tests represent a true infection with syphilis.
 - B. Most reactive tests are due to a biologic false-positive or persistent antibody detection in previously treated individuals.
 - C. Individuals with a history of treatment of syphilis must be deferred for 6 months after the completion of the treatment of syphilis.
 - D. A few cases of transfusion-transmitted syphilis are reported every year.
 - E. Several studies have shown that donor screening for syphilis provides value in detecting other bloodborne pathogens, such as HBV, HCV, and HIV.
-

Question 14: All the following are treponemal-specific assays, except:

- A. Automated reagin test.
- B. Enzyme immunoassays.
- C. Fluorescent treponemal antibody “absorbed” assays.
- D. Microhemagglutination assays.
- E. Particle agglutination assays.



Question 15: Transfusion with which of the following blood components is associated with the highest risk of sepsis and related fatality?

- A. Red Blood Cells (RBCs).
 - B. Thawed plasma.
 - C. Granulocytes.
 - D. Fresh Frozen Plasma (FFP).
 - E. Platelets.
-

Question 16: Per FDA guidance, all of the following are acceptable bacterial risk control strategies for 5-day apheresis platelets, except:

- A. Large-volume, delayed sampling (LVDS) ≥ 36 hours.
 - B. Pathogen reduction.
 - C. Primary culture ≥ 24 hours and secondary culture \geq day 3.
 - D. Rapid testing alone.
 - E. Primary culture ≥ 24 hours and secondary rapid testing.
-

Question 17: WNV is an RNA virus belonging to which family of viruses?

- A. *Flaviviridae*.
 - B. *Hepeviridae*.
 - C. *Hepadnaviridae*.
 - D. *Retroviridae*.
 - E. *Herpesviridae*.
-

Question 18: Which of the following statements is true concerning WNV?

- A. Approximately 80% of infections are symptomatic.
- B. Serologic testing for anti-WNV is required by both the FDA and AABB to screen blood donors.
- C. If a blood donor has a reactive WNV test, the donation deferral period is 120 days.



- D. If a blood donation is reactive for WNV, the blood collection center must retrieve and quarantine in-date products from previous collections dating back 60 days.
 - E. Minipool-NAT (MP-NAT) in pools of 6-16 donations is acceptable even during periods of high WNV activity in a geographic region because viral loads are typically high in infected individuals.
-

Question 19: Which of the following statements is true regarding donor deferral for individuals with initial reactive anti-HTLV-I/II test results?

- A. Individuals can undergo retesting with a new sample with at least two different, licensed anti-HTLV-I/II screening tests in 3 months and if they are reactive with both tests, they will be permanently deferred.
 - B. They can undergo retesting with a new sample with at least two different, licensed anti-HTLV-I/II screening tests in 6 months and if they are nonreactive with both tests, they can be reentered provided they meet all other donor eligibility criteria.
 - C. They can undergo retesting with a new sample with at least two different, licensed anti-HTLV-I/II screening tests in 3 months and if they are nonreactive with both tests, they can be reentered provided they meet all other donor eligibility criteria.
 - D. They can undergo retesting with a new sample with one licensed anti-HTLV-I/II screening test in 6 months and if they are nonreactive, they can be reentered provided they meet all other donor eligibility criteria.
 - E. There is no additional testing allowed. The individual is permanently deferred with one initial reactive anti-HTLV-I/II test.
-

Question 20: Zika virus is transmitted to humans through which mosquito species?

- A. *Anopheles gambiae*.
- B. *Anopheles earlei*.



- C. *Aedes aegypti*.
 - D. *Aedes polynesiensis*.
 - E. *Culex pipiens*.
-

Question 21: The FDA mandates screening for certain relevant transfusion-transmitted infections (RTTI). Which of the following is *not* considered a RRTI?

- A. Zika virus.
 - B. HIV.
 - C. Creutzfeldt-Jakob disease (CJD).
 - D. Variant Creutzfeldt-Jakob disease (vCJD).
 - E. *Plasmodium* species (malaria).
-

Question 22: Which of the following are possible routes of human-to-human *Trypanosoma cruzi* (*T. cruzi*) transmission?

- A. Blood transfusion.
 - B. Organ transplantation.
 - C. Tissue transplantation.
 - D. Congenital.
 - E. All of the above.
-

Question 23: Which of the following statements is true regarding *T. cruzi* and transfusion-transmitted *T. cruzi*?

- A. The FDA recommends one-time testing of each donor of blood and blood components for *T. cruzi*.
- B. 60% of infected individuals will later develop cardiac complications (cardiomegaly, arrhythmias, heart failure) or intestinal dysfunction (megacolon, megaesophagus).
- C. Approximately 2 million people unknowingly infected reside in the US.
- D. The FDA recommends asking the question “Have you ever had Chagas disease?” to potential blood donors.



- E. If a donor is reactive on an initial licensed screening test and negative on a licensed supplemental test, the donor is permanently deferred with no option to reenter the donor pool.
-

Question 24: In 2019 the FDA recommended year-round donor screening for *Babesia* in which states?

- A. All continental states and Washington, DC.
 - B. Connecticut, Maine, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Rhode Island, Wisconsin, and Washington, DC.
 - C. Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Vermont, Virginia, and Washington, DC.
 - D. Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Wisconsin, and Washington, DC.
 - E. Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Wisconsin, Michigan, Illinois, and Washington, DC.
-

Question 25: If a screening test is reactive for *Babesia* by NAT, the donor may be eligible to donate again, provided the new donation is NAT-negative and the donor has been deferred for how long?

- A. 3 months.
 - B. 6 months.
 - C. 12 months.
 - D. 18 months.
 - E. 24 months.
-

Question 26: Which *Babesia* species causes most infections in the Western United States?

- A. *B. microti*.



- B. *B. duncani*.
 - C. *B. felis*.
 - D. *B. lengau*.
 - E. *B. kiwiensis*.
-

Question 27: Which of the following statements is true concerning malaria and transfusion-transmitted malaria?

- A. There is no FDA-approved malaria test to screen US blood donations.
 - B. *P. malariae* causes the most severe disease and over 90% of deaths in sub-Saharan Africa.
 - C. Most US malaria cases each year are caused by non-US residents traveling from malaria-endemic countries.
 - D. Of the reported US transfusion-transmitted malaria cases, most have been from asymptomatic US residents returning from travel to a malaria-endemic country.
 - E. Plasma components are the most common source of transfusion-transmitted malaria in the US.
-

Question 28: Although rare, locally acquired malaria has caused small outbreaks in the US. These infections have been predominantly caused by which *Plasmodium* species?

- A. *P. falciparum*.
 - B. *P. malariae*.
 - C. *P. vivax*.
 - D. *P. ovale*.
 - E. *P. knowlesi*.
-

Question 29: Which of the following statements regarding malaria-related donor deferral is correct?

- A. Twelve-month deferral: Travel to a malaria-endemic area by residents of nonendemic countries.
- B. Three-year deferral: Prior residence in a malaria-endemic country.



- C. Two-year deferral: Travel to a malaria-endemic area by prior residents of a malaria-endemic country, if they have been a resident of a nonendemic country for less than 3 consecutive years.
 - D. Twelve-month deferral: Travel to a malaria-endemic area by prior residents of a malaria-endemic country, if they have been a resident of a nonendemic country for more than 3 consecutive years.
 - E. Permanent deferral: History of malaria.
-

Question 30: Familial CJD (fCJD) accounts for what percent (approximately) of CJD cases?

- A. 15%.
 - B. 25%.
 - C. 50%.
 - D. 75%.
 - E. 90%.
-

Question 31: Which of the following statements is true regarding vCJD as compared to CJD?

- A. vCJD is caused by the agent bovine spongiform encephalopathy from eating contaminated beef products.
 - B. vCJD typically affects individuals younger than 55 years old.
 - C. vCJD has a duration of illness lasting more than 6 months.
 - D. vCJD presents with psychiatric symptoms at illness onset and/or persistent painful sensory symptoms.
 - E. All of the above.
-

Question 32: Which of the following individuals is eligible to donate blood in the US, provided they meet all other eligibility requirements?

- A. An individual who has a blood relative diagnosed with fCJD.
- B. An individual who received cadaveric pituitary human growth hormone.



- C. An individual who received a human cadaveric dura mater transplant.
 - D. An individual who received a blood transfusion in the UK in 1990.
 - E. An individual who has been diagnosed with vCJD.
-

Question 33: Which of the following viruses lacks a lipid envelope making it resistant to pathogen inactivation technology?

- A. CMV.
 - B. Ebola virus.
 - C. HEV.
 - D. WNV.
 - E. HTLV-I/II.
-

Question 34: Pathogen inactivation technology with amotosalen/UV treatment can also inactivate white cells, which is associated with all of the following, except:

- A. Prevent transfusion-associated graft-vs-host disease.
 - B. Decrease formation and release of cytokines during storage.
 - C. Reduce febrile nonhemolytic transfusion reactions.
 - D. Reduce the rate of HLA antibody formation.
 - E. Reduce CMV transmission.
-

Question 35: Which of the following statements is correct concerning HIV-related donor deferrals?

- A. An individual who has taken oral medication to prevent HIV infection must be deferred for 6 months from the most recent dose.
- B. An individual who has taken medication by injection to prevent HIV infection must be deferred for 2 years from the most recent injection.
- C. An individual who has exchanged sex for money or drugs in the past 12 months must be deferred for 12 months from the most recent event.



- D. An individual who has engaged in nonprescription injection drug use in the past 6 months must be deferred for 6 months from the most recent event.
 - E. An individual who has had sex with a person who has ever had a positive test for HIV must be deferred for 12 months from the most recent sexual contact.
-

Question 36: Which of the following set of HBV test results would result in a permanent donor deferral (not eligible for reentry)?

- A. HBV NAT: positive; HBsAg: nonreactive; anti-HBc: repeatedly reactive.
 - B. HBV NAT: positive; HBsAg: repeatedly reactive; anti-HBc: nonreactive.
 - C. HBV NAT: positive; HBsAg: nonreactive; anti-HBc: nonreactive.
 - D. HBV NAT: positive; HBsAg: repeatedly reactive/not confirmed; anti-HBc: nonreactive.
 - E. HBV NAT: negative; HBsAg: nonreactive; anti-HBc: nonreactive.
-

Question 37: A donor of whole blood intended for transfusion has the following initial HBV screening test results: NAT - positive, HBsAg - negative, anti-HBc - negative. The donor may be eligible to donate again, provided they meet all of the following requalification criteria, except?

- A. It has been 6 weeks since the initial screening test.
 - B. Repeat HBsAg test is nonreactive.
 - C. An FDA-licensed HBV NAT with a sensitivity of ≤ 2 IU/mL at 95% detection rate is negative.
 - D. It has been 6 months since the initial screening test.
 - E. Repeat anti-HBc is nonreactive.
-

Question 38: A donor has the following initial HIV screening test results: HIV-1 NAT – reactive, anti-HIV-1/2 – negative, HIV-1 p24



EIA – negative. This donor may be eligible for reentry following a deferral period of how long?

- A. This individual is permanently deferred and not eligible for reentry.
 - B. 12 months.
 - C. 6 months.
 - D. 3 months.
 - E. 8 weeks.
-

Question 39: A donor had the following initial HCV screening test results: HCV NAT – reactive, anti-HCV – negative. After a 6-month deferral, the donor would like to be tested to reenter the donor pool. Which of the following test results would let this donor requalify for donation, provided they met all other eligibility criteria?

- A. Reactive individual HCV NAT (ID-NAT); nonreactive for anti-HCV by two different licensed screening tests.
 - B. Reactive HCV ID-NAT; reactive for anti-HCV by two different licensed screening tests.
 - C. Nonreactive HCV ID-NAT; nonreactive for anti-HCV by two different licensed screening tests.
 - D. Nonreactive HCV ID-NAT; nonreactive for anti-HCV by one licensed screening test.
 - E. Nonreactive HCV NAT; repeatedly reactive for anti-HCV by two different licensed screening tests.
-

Question 40: A 40-year-old doctor has returned from a medical mission in a country that is classified by the Centers for Disease Control and Prevention (CDC) as having widespread transmission of Ebola virus. While there, he treated several Ebola-infected patients. How long should he wait before donating blood (from departure date)?

- A. There is no deferral period if he is asymptomatic.
- B. 2 weeks.
- C. 4 weeks.
- D. 8 weeks.
- E. The donor should be indefinitely deferred.



Question 41: Which of the following statements is true regarding human parvovirus B19 and parvovirus B19 transmission in plasma-derived products?

- A. Manufacturers of plasma-derived products should ensure that parvovirus B19 DNA does not exceed 10^4 IU/mL in plasma pools via NAT testing.
 - B. Parvovirus B19 is a nonenveloped double-stranded DNA virus.
 - C. Heat and solvent/detergent treatments are highly effective at inactivating parvovirus B19 in plasma-derived products.
 - D. There are no reports of parvovirus B19 infections associated with administration of plasma-derived coagulation factors.
 - E. Parvovirus B19 is easily removed from plasma-derived products by filtration methods.
-

Question 42: All of the following patients should receive cellular blood components that have a reduced risk of transmitting CMV except:

- A. Fetuses (intrauterine infusion).
 - B. Seropositive pregnant women.
 - C. Seronegative HIV-positive patients.
 - D. Seronegative solid organ recipients from a seronegative donor.
 - E. Seronegative hematopoietic stem cell transplant recipients.
-

Question 43: Of the following, which infectious agent is the most likely to be transmitted through transfusion?

- A. *Treponema pallidum*.
 - B. Coagulase-negative staphylococci.
 - C. HBV.
 - D. HCV.
 - E. HIV.
-

Question 44: A blood donation is tested and receives repeat reactive results. With which screening test is this most likely?



- A. Anti-HBc.
 - B. HBsAg.
 - C. HTLV-I/II ELISA.
 - D. HIV-1/2 EIA.
 - E. HIV-1 p24 antigen.
-

Question 45: HBV vaccination in a nonimmunized donor 3 days before blood donation could lead to a positive result in a routine screening test. In which is this most likely?

- A. Anti-HBc.
 - B. Anti-HBsAg.
 - C. HBsAg.
 - D. Anti-HIV-1/2.
 - E. Anti-HCV.
-

Question 46: Which of the following protozoan parasites is not addressed categorically by *AABB Standards for Blood Banks and Transfusion Services*?

- A. *Trypanosoma cruzi*.
 - B. *Leishmania donovani*.
 - C. *Babesia MO-1*.
 - D. *Plasmodium ovale*.
 - E. *Plasmodium falciparum*.
-

Question 47: Choose the correct statement about syphilis:

- A. The presentation of syphilis transmitted through transfusion is acute, fulminant secondary syphilis.
- B. *T. pallidum* is easily transmitted by red cells because it is stable at 4 C.
- C. *T. pallidum* is easily transmitted by platelet products because it prefers an environment rich in oxygen.
- D. For a donor with positive nontreponemal and treponemal test results, a negative nontreponemal test result 12 months later would be the only requirement for donor reentry.



- E. A donor with a positive nontreponemal test result and no follow-up testing should be deferred for 6 months.
-

Question 48: Choose the correct statement about *T. cruzi*:

- A. *T. cruzi* is transmitted by infected mosquitoes to humans, usually in tropical areas.
 - B. Infection rarely results in chronic disease.
 - C. Acute infection usually includes symptoms such as esophageal and colonic dysfunction.
 - D. Most commonly, when transfusion-transmission of *T. cruzi* occurs, the blood component involved is platelets.
 - E. The incidence of transfusion-transmitted *T. cruzi* infection has been steadily increasing in the United States.
-

Question 49: Choose the correct statement about WNV:

- A. NAT is used to detect infection, but testing for IgM antibodies to WNV is an effective alternative.
 - B. The main reservoir for the virus is rodents.
 - C. Infected humans can transmit the virus to mosquitoes, completing the virus's life cycle.
 - D. Of those infected who develop West Nile fever, 1 out of 150 individuals will experience severe neurologic consequences.
 - E. Of those infected, 7% of individuals develop a chronic state.
-

Question 50: A 51-year-old woman has a sexual partner who has hemophilia A and routinely uses coagulation Factor VIII concentrate, sourced from human plasma. Choose the statement that reflects her status as a potential blood donor:

- A. Ineligible, indefinitely deferred.
- B. Ineligible, unless her partner switches to a recombinant form.
- C. Deferred for 12 months since last sexual intercourse.
- D. Deferred for 5 years since last sexual intercourse.
- E. Acceptable for blood donation.



ANSWERS

Question 1: C. *Treponema pallidum*.

Explanation:

- In the 1950s, the FDA mandated blood donor screening for the causative agent of syphilis, *T. pallidum*. The last reported case of transfusion-transmitted syphilis in the US was in 1966. The decline of cases is due to a combination of factors, including decreased incidence of syphilis cases and the inactivation of *T. pallidum* in cold-stored blood components.
- Continuing syphilis screening in blood products is a controversial topic, with some studies showing an added benefit and others showing no added benefit.
- As of 2023, syphilis testing is still required for all blood donors.



Question 2: E. More than 30%.

Explanation:

- Before the implementation of HBV screening in the 1970s, more than 30% of multitransfused patients developed PTH. HBV was discovered in the early 1970s and was shown to be the cause of 25% of cases of PTH.
- Following required testing for HBsAg and moving to a volunteer-only blood donation program, the rates of PTH declined rapidly.
- Unfortunately, it took nearly 2 decades to discover that HCV was the causative agent of the majority of PTH cases and implement testing for HCV.



Question 3: B. Collection of donor medical records.

Explanation:

- There are several approaches to screening donors for infectious diseases, including predonation questioning, postdonation test-



ing, a combination of both questioning and testing, as well as donor education.

Question 4: B. Title 21, Part 610.40 of the CFR.

Explanation:

- The FDA is the regulatory body that oversees the infectious disease testing requirements for all blood components intended for transfusion in the US. The FDA describes test requirements and defines transfusion-transmitted infections (TTI) in Title 21 CFR Parts 610.40 and 630.3, respectively.
 - In general, the FDA defines a TTI as a pathogen that is known to be fatal, to be life-threatening, or to cause severe impairment and that is potentially transmissible through the blood supply.
-

Question 5: C. 3.

Explanation:

- The FDA requires initiation of retrieval of in-date components within 3 calendar days for a donor with a reactive HIV, HCV, WNV, or *T. cruzi* test result and within 1 week of a reactive HBsAg, anti-HBc, or anti-HTLV screening test result.
-

Question 6: D. HIV and HCV.

Explanation:

- Title 21 CFR Parts 610.46 and 610.47 outline the requirements for the look-back process for donors reactive for HIV and HCV, respectively.
- A look-back consists of identifying blood and blood components from prior collections (up to 12 months) from a donor with reliably reactive (positive) HIV or HCV testing results.
- Subsequent actions include quarantine of in-date products, further donor testing, destruction or relabeling of potentially infec-



tious products, notification of recipients of identified blood products, or the recipient's physician of record.

- The recipient notification must occur within 12 weeks.
-

Question 7: C. 2-3%.

Explanation:

- Several options to minimize the risk of transfusion-transmitted CMV are available.
 - Patients can receive blood components from CMV-seronegative donors, however there is still an estimated 1-2% CMV transmission risk.
 - More commonly, blood components are leukocyte reduced to remove the white cells that carry CMV. Leukocyte-reduced blood components have an estimated 2-3% CMV transmission risk.
-

Question 8: D. 9.

Explanation:

- With the introduction of NAT, the window period for detecting HIV in blood components has been reduced to 9.1 days.
 - The infectious window period for HCV and HBV detection by NAT is 7.4 days and 26.5-18.5 days, respectively.
-

Question 9: D. All of the above.

Explanation:

- The estimated residual risk of transfusion-transmitted infection per donated unit following NAT implementation is 1 in 1.5 for HIV, 1 in 1.1 million for HCV, and 1 in 800,000 to 1.2 million for HBV. These estimated risks are based on window-period and incidence calculations.

**Question 10: B. HBeAg.****Explanation:**

- The FDA requires donor screening for HBsAg, HBV DNA, and total anti-HBc (IgM and IgG) to detect HBV infection at different stages of infection. HBV can be transmitted by blood from asymptomatic donors in the seronegative window (negative for HBsAg and anti-HBc).
 - HBV DNA can be detected 2 to 5 weeks after infection and approximately 40 days before detection of HBsAg.
-

Question 11: E. 7 days; 1.5-2 months.**Explanation:**

- The average window period for detection of HCV RNA is 7.4 days, while the detection of anti-HCV is 1.5 to 2 months. NAT for detection of HCV has been estimated to reduce the window period by 50 to 60 days compared to that for anti-HCV.
-

Question 12: C. The only FDA-approved screening tests for HTLV-I/II are IgG antibody assays.**Explanation:**

- HTLV-I/II are retroviruses that infect lymphocytes, resulting in life-long infections, although most individuals are asymptomatic. The viruses are spread through blood transfusion, breast feeding, and sexual contact. Because these viruses are cell-associated, viral transmission has not been demonstrated with acellular blood components, such as plasma.
- The FDA first recommended blood donor screening for HTLV-I in 1988 and HTLV-II in 1998 via serology. HTLV-I/II have specific disease associations, usually manifesting decades after infection.
- HTLV-I infections are associated with adult T-cell leukemia/lymphoma (2-5%), HTLV-associated myelopathy/tropical spastic paraparesis, and HTLV-associated uveitis.



- HTLV-II infections may be associated with hairy cell leukemia, though this connection has not been fully established. In the US, roughly 50% of HTLV infections are with HTLV-II.
-

Question 13: B. Most reactive tests are due to a biologic false-positive or persistent antibody detection in previously treated individuals.

Explanation:

- Most reactive tests do not represent a true infection with syphilis. Individuals with a history of syphilis or gonorrhea or treatment of syphilis or gonorrhea must be deferred for 3 months after the completion of treatment. No cases of transfusion-transmitted syphilis have been reported in the US in over 40 years. Several studies have shown that donor screening for syphilis provides no incremental value in detecting other bloodborne pathogens, such as HBV, HCV, or HIV.
-

Question 14: A. Automated reagin test.

Explanation:

- Nontreponemal assays, such as the rapid plasma reagin (RPR) test, the venereal disease research laboratory (VDRL) test, and the automated reagin test (ART), are nonspecific tests that detect reagin antibodies directed against the ubiquitous cardiolipin.
- Individuals with active or recently treated syphilis infections usually have reactive results with nontreponemal tests. Those who are uninfected or have completed successful treatment years earlier usually have nonreactive nontreponemal test results.
- Treponemal assays test for antibodies specific to treponemes and include enzyme immunoassays (EIA), fluorescent treponemal antibody “absorbed” assays (FTA-ABS), *Treponema pallidum* microhemagglutination assays (MHA-TPA) and *Treponema pallidum* particle agglutination assays (TP-PA).



- Treponemal assays are most useful in identifying recent and past infections, usually remaining reactive (positive) throughout an individual's life.
-

Question 15: E. Platelets.**Explanation:**

- Platelets are associated with a higher risk of sepsis and related fatality than any other transfusible blood component, most commonly occurring with transfusion of day 4 or 5 units. This is likely due to bacteria growth during platelet storage at room temperature.
 - Approximately 1/6000 apheresis units test positive for bacteria by culture. The rate of a septic transfusion reaction is approximately 1/100,000 per transfused apheresis platelet unit using passive surveillance, and a rate of 1/10,000 when active surveillance is used.
-

Question 16: D. Rapid testing alone.**Explanation:**

- In 2020, the FDA released new guidance with recommendations to control the risk of bacterial contamination of stored platelets via bacterial testing (culture-based or rapid detection) or pathogen-reduction methods. These recommendations include single-step and two-step strategies that are dependent on the type of platelet unit and storage duration. These strategies are summarized below:



Component	Strategy
Five-day Stored Platelet Units	
Apheresis platelets	<ul style="list-style-type: none"> -Large-volume delayed sampling (LVDS) ≥ 36 hours -Pathogen reduction -Primary culture ≥ 24 hours + secondary culture \geq day 3 -Primary culture ≥ 24 hours + secondary rapid testing
Prestorage pools of whole blood derived (WBD) platelets	<ul style="list-style-type: none"> -LVDS ≥ 36 hours -Primary culture ≥ 24 hours + secondary culture \geq day 3 -Primary culture ≥ 24 hours + secondary rapid testing
Single units of WBD platelets	<ul style="list-style-type: none"> -Rapid testing -Primary culture ≥ 24 hours -Primary culture ≥ 36 hours
Poststorage pools of WBD platelets	-Rapid testing

Seven-day Stored Platelet Units	
Apheresis platelets	<ul style="list-style-type: none"> -LVDS ≥ 48 hours -LVDS ≥ 36 hours + secondary rapid testing -LVDS ≥ 36 hours + secondary culture \geq day 4 -Primary culture ≥ 24 hours + secondary culture \geq day 4 -Primary culture ≥ 24 hours + secondary rapid testing
Prestorage pools of whole blood derived (WBD) platelets	N/A
Single units of WBD platelets	N/A
Poststorage pools of WBD platelets	N/A



Question 17: A. *Flaviviridae.*

Explanation:

- Other viruses in this family include yellow fever, dengue fever, Japanese encephalitis, and Zika virus.
-

Question 18: C. If a blood donor has a reactive WNV test result, the donation deferral period is 120 days.

Explanation:

- The donor should be notified and counseled. In addition, the blood collection facility should retrieve and quarantine all in-date products from previous collections dating back 120 days.
 - Eighty percent of WNV infections are asymptomatic, making donor history questions ineffective for screening.
 - The FDA approved NAT for WNV testing in donated blood components in 2005. Serologic testing is not appropriate because it is imperative to detect acute infections in patients with viremia.
 - Donation samples are tested by MP-NAT; however, the FDA and AABB recommend switching to individual donor NAT (ID-NAT) during periods of increased regional activity, as ID-NAT has higher sensitivity than MP-NAT.
 - Typically, viral loads are low during an acute infection.
-

Question 19: B. The individual can undergo retesting with a new sample with at least two different, licensed anti-HTLV-I/II screening tests in 6 months and if they are nonreactive with both tests, they can be reentered provided they meet all other donor eligibility criteria.

Explanation:

- Blood donors with an indefinite deferral because of a reactive anti-HTLV-I/II test may be eligible for reentry. Deferred donors are eligible for reentry if at the time of donation that prompted the deferral:



- An investigational or licensed supplemental test was negative or indeterminate.
 - A research-use-only supplemental HTLV algorithm was negative or indeterminate before a licensed supplemental test was available.
 - Not further tested for anti-HTLV-I/II before the licensed test was available.
 - After 6 months of deferral, a new sample can be collected and tested with two different, licensed anti-HTLV-I/II tests. If this individual is nonreactive with both tests, they can be reentered provided they meet all other donor eligibility criteria. If they are reactive by both tests, the donor will be permanently deferred. If the tests are discrepant, the donor will remain deferred, or a supplemental test may be performed.
-

Question 20: C. Aedes aegypti.**Explanation:**

- Zika virus is a flavivirus transmitted mainly by the *Aedes aegypti* mosquito, and to a lesser extent *Aedes albopictus* mosquitos. Zika virus can also be transmitted by blood transfusion, perinatally, intrauterine, and through sexual intercourse.
 - Other arborviruses carried by *Aedes* species mosquitos include dengue virus, yellow fever virus, and chikungunya virus.
 - *Culex* species mosquitos are the main insect vector for WNV, St. Louis encephalitis virus, and Japanese encephalitis virus.
 - *Anopheles* species mosquitos are the vector for *Plasmodia* species (malaria), as well as some other parasites.
-

Question 21: A. Zika virus.**Explanation:**

- In 2016, the FDA determined that Zika virus met the criteria for RTTI because it had sufficient incidence and/or prevalence to affect the potential donor population. However, in 2021 the FDA determined that Zika virus no longer met these criteria and removed the mandate for donor screening by blood establishments.



- There have been no reported cases of transfusion-transmitted Zika virus in the US and the last confirmed Zika-virus-positive blood donation in the US was in 2018.
 - As of 2023, the FDA considers the following infectious agents to be RTTIs: HIV1/2, HBV, HCV, HTLV-I/II, *T. pallidum*, WNV, *T. cruzi*, CJD, vCJD, *Babesia*, and *Plasmodium* species.
-

Question 22: E. All of the above.

Explanation:

- The parasite *T. cruzi* is the causative agent of Chagas disease, which is endemic in Mexico, Central America, and South America. It is transmitted to humans by a triatomine bug bite.
 - Human-to-human transmission can occur through blood transfusion, organ/tissue transplantation, congenitally, and orally via contaminated food products.
-

Question 23: A. The FDA recommends one-time testing of each donor of blood and blood components for *T. cruzi*.

Explanation:

- Acute *T. cruzi* infections are usually asymptomatic unless the individual is immunocompromised. The infection can persist throughout the life of the individual without symptoms, however approximately 30% will develop cardiac complications or intestinal dysfunction usually decades after the initial infection.
- Transfusion-transmission is more common in endemic areas, although these rates have decreased with the implementation of donor screening and decreased use of fresh whole blood.
- The presence of *T. cruzi* in US donors has increased due to immigration of infected individuals from endemic areas, with estimates of approximately 300,000 people unknowingly infected.
- In 2010, the FDA recommended one-time testing for donors and subsequent data showed zero cases of *T. cruzi* infection in 4.2 million donors over 4 years. In 2015, the FDA deemed *T. cruzi* a RTTI and thus one-time testing was made mandatory for all



donors. In 2010, the FDA recommended asking the question “Have you ever had Chagas disease?” to all potential donors and to indefinitely defer anyone who answers yes. However, in 2017 the FDA removed this recommendation, as the one-time testing is sufficient for identifying cases.

- The FDA outlines the eligibility criteria for donors to reenter the donor pool in a 2017 guidance. A donor who has a reactive result on an initial licensed *T. cruzi* screening test but is negative on a licensed supplemental test is eligible to undergo repeat testing with two different licensed screening tests after a 6-month deferral. If the donor is reactive on either or both tests, they are permanently deferred.



Question 24: D. Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Wisconsin and Washington, DC.

Explanation:

- Over 200 cases of transfusion-transmitted babesiosis have been reported with 99% coming from the states noted in choice D.
- The FDA requires that each donation be tested using a licensed NAT from these states. Alternatively, an FDA-approved pathogen-reduction device effective against *Babesia* can be used for plasma and platelet products.



Question 25: E. 24 months.

Explanation:

- A donor with a reactive NAT test for *Babesia* or who reports a diagnosis of babesiosis must be deferred from donating for 2 years. A reasonable attempt to notify the donor of their positive test result and deferral should occur within 8 weeks of determining that the donor is deferred.
- Additionally, all cellular components previously collected from that donor in the 12 months before the reactive test must be quarantined.

**Question 26: B. B. duncani.****Explanation:**

- In the Western US, most *Babesia* infections are due to the species *B. duncani*, likely carried by the tick vector *Ixodes pacificus*.
- In the Northeastern and Midwestern US, most infections are caused by the species *B. microti* transmitted by *I. scapularis* ticks.

**Question 27: A.** There is no FDA-approved malaria test to screen US blood donations.**Explanation:**

- Screening is conducted through the donor history questionnaire. There have only been 12 reported cases of transfusion-transmitted malaria in the US from 2000-2021; none of these cases were from US residents returning from travel to a malaria-endemic region.
- Of the transfusion-transmitted malaria cases, most have been from whole blood/RBCs (94%) or from platelets (6%). Of the five *Plasmodia* species, *P. falciparum* causes the most severe disease and over 90% of deaths in sub-Saharan Africa.
- Malaria infections can be asymptomatic, which is more likely for former residents of malaria-endemic countries when they return from travel to malaria-endemic countries. There are approximately 2000 reported malaria cases in the US each year with the majority (70%) of cases in US residents returning from travel.

**Question 28: C. P. vivax.****Explanation:**

- Although malaria was eradicated in the US in the 1950s, there have been 64 small outbreaks from local mosquito transmission of *Plasmodium* species. Most of these outbreaks were caused by *P. vivax*. In 2023 local transmission of *P. vivax* was reported in Florida, Texas, and Maryland, the first in 20 years.



Question 29: B. Three-year deferral: Prior residence in a malaria-endemic country.

Explanation:

- Below are the FDA recommendations concerning malaria-related donor deferrals:
- Three-month deferral: Travel to a malaria-endemic area by residents of nonendemic countries.
 - Platelet and/or plasma products may be collected from this group without a deferral period, if the components are pathogen reduced with a device effective against *P. falciparum*.
- Three-year deferral: Prior residence in a malaria-endemic country.
 - No pathogen reduction exception.
- Three-year deferral: Travel to a malaria-endemic area by prior residents of a malaria-endemic country, if they have been a resident of a nonendemic country for less than 3 consecutive years.
 - No pathogen reduction exception.
- Three-month deferral: Travel to a malaria-endemic area by prior residents of a malaria-endemic country, if they have been a resident of a nonendemic country for more than 3 consecutive years.
 - Platelet and/or plasma products may be collected from this group without a deferral period, if the components are pathogen reduced with a device effective against *P. falciparum*.
- Three-year deferral: History of malaria.
 - No pathogen reduction exception.



Question 30: A. 15%.

Explanation:

- Sporadic CJD (sCJD) is the most common form of CJD and accounts for 85-95% of CJD cases. The estimated incidence of sCJD is 1 per million worldwide.
- Familial CJD (fCJD) is inherited in an autosomal dominant pattern and accounts for 5-15% of CJD cases.
- Iatrogenic CJD (iCJD) accounts for less than 1% of CJD cases and has occurred in individuals who received dura mater transplants



from infected donors or contaminated injections of cadaveric pituitary human growth hormone.

Question 31: E. All of the above.

Explanation:

- Variant CJD is a unique form of CJD linked to the agent bovine spongiform encephalopathy, thought to be acquired from eating contaminated beef products. It was first recognized in the UK in 1985 and subsequently spread to Europe and other regions.
 - Of the approximately 230 vCJD cases identified, 4 were likely transfusion transmitted, all in the UK. As compared to CJD, vCJD usually affects younger individuals, presents with psychiatric symptoms, and has a longer course of illness (> 6 months).
-

Question 32: D. An individual who received a blood transfusion in the UK in 1990.

Explanation:

- The FDA blood guidance has been revised several times over the years concerning vCJD. In 2020, the FDA removed most of the geographic risks of possible exposure for most European countries, including time spent on military bases and receipt of blood transfusion. In 2022, the FDA updated this guidance to also include the UK, France, and Ireland.
- As a result of these changes, individuals previously deferred for some vCJD risks may be eligible for requalification. Below is a summary of recommendations in the 2022 FDA guidance:
 - Assess donors for a history of receiving a human cadaveric dura mater transplant via the donor history questionnaire.
 - Permanently defer donors if they:
 - Have ever been diagnosed with vCJD or CJD.
 - Have a blood relative that has been diagnosed with fCJD.
 - Have received a human cadaveric dura mater transplant.
 - Have received cadaveric pituitary human growth hormone.

**Question 33: C. HEV.****Explanation:**

- Unenveloped viruses, such as HEV, parvovirus B19, and hepatitis A virus (HAV) are resistant to current pathogen-inactivation technologies. As such, these viruses must be screened for in plasma derivative manufacturing.
-

Question 34: D. Reduce the rate of HLA antibody formation.

- Pathogen inactivation with amotosalen/UV light has been shown to inactivate white cells, which can prevent transfusion-associated graft-vs-host disease, reduce CMV transmission, decreases formation and release of cytokines during storage, and reduce febrile nonhemolytic transfusion reactions. However, they do not seem to influence HLA antibody formation.
-

Question 35: B. An individual who has taken medication by injection to prevent HIV infection must be deferred for 2 years from the most recent injection.**Explanation:**

- Donor deferral recommendations to reduce HIV transmission have evolved over the years. Individuals who have ever had a confirmed positive test result for HIV infection or who have ever taken antiretroviral therapy to treat HIV infection must be permanently deferred from blood donation. Both oral (short-acting) and injectable (long-acting) medications are available for pre-exposure prophylaxis (PrEP). These medications can reduce the viral load below the detectable level by current screening assays. As such, donors taking these medications are deferred from donating. An individual is deferred for 3 months following their last oral dose and 2 years from their last injectable dose.
- The deferral period for the other HIV risk factors is 3 months since the most recent event.



Question 36: B. HBV NAT: positive; HBsAg: repeatedly reactive; anti-HBc: nonreactive.

Explanation:

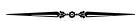
- Some individuals with an initial positive HBV NAT result may be eligible for reentry into the donor pool under certain circumstances.
- Below is a summary of donor and unit management for whole blood and blood components intended for transfusion when an initial HBV NAT is positive.

Permanent deferral, not eligible for reentry.

- HBV NAT: positive; HBsAg: repeatedly reactive/confirmed positive; anti-HBc: nonreactive.
- HBV NAT: positive; HBsAg: repeatedly reactive/confirmed positive; anti-HBc: repeatedly reactive.
- HBV NAT: positive; HBsAg: repeatedly reactive/not confirmed; anti-HBc: repeatedly reactive.

Indefinite deferral, may be eligible for reentry.

- HBV NAT: positive; HBsAg: nonreactive; anti-HBc: repeatedly reactive.
- HBV NAT: positive; HBsAg: nonreactive; anti-HBc: nonreactive.
- HBV NAT: positive; HBsAg: repeatedly reactive/not confirmed; anti-HBc: nonreactive.



Question 37: A. It has been 6 weeks since the initial screening test.

Explanation:

- The FDA outlines requalification criteria for donors of whole blood or blood components intended for transfusion that are indefinitely deferred based on initial HBV testing. There must be at least a 6-month deferral since the initial test results.
- The FDA recommends using an FDA-licensed HBV NAT that has a sensitivity of ≥ 2 IU/mL at 95% detection rate. If this test result is positive, the donor is permanently deferred from donation. The donor may be reentered if the NAT result is negative and repeat HBsAg and anti-HBc results are still nonreactive, provided the donor meets all other eligibility criteria.

**Question 38: E.** 8 weeks.**Explanation:**

- The FDA provides guidance on reentering donors with certain reactive HIV screening test results. Donors who are seronegative for HIV-1/2 antibodies with a reactive NAT test result may be eligible for reentry after an 8-week deferral and repeat testing using HIV-1 individual NAT and anti-HIV1/2 test.
 - If the repeat NAT test result is reactive, then the donor must be deferred permanently, regardless of anti-HIV-1/2 test results.
 - A donor who is non-reactive on both tests, may reenter the donor pool, provided all other eligibility criteria are met.
-

Question 39: C. Nonreactive HCV ID-NAT; nonreactive for anti-HCV by two different licensed screening tests.

- A donor with either of the following initial HCV screening test results may be retested for reentry:
 - HCV NAT – reactive; anti-HCV – negative.
 - HCV NAT – nonreactive or not performed; anti-HCV – repeatedly reactive.
 - The donor must be deferred for 6 months and undergo retesting with both HCV ID-NAT and two different licensed anti-HCV screening tests. If all three tests are nonreactive, the donor may be reentered, provided all other eligibility criteria are met.
 - The donor will be permanently deferred if the ID-NAT is reactive, regardless of anti-HCV results. If the ID-NAT is nonreactive and both anti-HCV tests are repeatedly reactive, the donor will be permanently deferred.
-

Question 40: D. 8 weeks.**Explanation:**

- Following the 2014 Ebola outbreak, the FDA released guidance for blood establishments regarding donor eligibility, donor deferral, and blood component management. Donors should be pro-



vided donor education material and assessed for a history of Ebola virus infection and travel/residence in a country considered to have widespread transmission of Ebola virus, as defined by the CDC.

- Donors with a history of Ebola virus infection are indefinitely deferred. A donor with a history of travel or residence in a country with widespread Ebola virus transmission is deferred for 8 weeks from departure date. Donors who had close contact with a person confirmed to have Ebola virus infection or a person under investigation with a pending diagnosis are also deferred for 8 weeks.
-

Question 41: A. Manufacturers of plasma-derived products should ensure that parvovirus B19 DNA does not exceed 10^4 IU/mL in plasma pools via NAT testing.

Explanation:

- Human parvovirus B19, a small, nonenveloped single-stranded DNA virus, is the causative agent of erythema infectiosum (fifth disease). Transient red cell aplasia can occur during an acute infection, which can be prolonged in immunocompromised individuals.
- Transfusion transmission of parvovirus B19 has been documented in cases where the viral load was very high ($>10^{12}$ IU/mL), including in plasma-derived coagulation factors and solvent/detergent-treated pooled plasma.
- Parvovirus B19 is highly resistant to inactivation methods, such as solvent/detergent, heat, and filtration due to its small size and lack of a viral envelope.
- Plasma-derived products are particularly at risk for having high levels of parvovirus B19 DNA because the manufacturing process requires pooling large numbers of plasma units. Low levels of parvovirus B19 DNA can be detected in almost all lots of plasma-derived products. Manufacturers of plasma-derived products must screen for high-titer parvovirus B19 via NAT, $>10^4$ IU/mL, and exclude these units from further manufacturing.

**Question 42: B.** Seropositive pregnant women.**Explanation:**

- CMV-seronegative populations at risk of morbidity from CMV transfusion transmission include pregnant women, fetuses (intrauterine transfusion), low-birthweight infants (<1250 g), patients with severe immunodeficiency (HIV/AIDS, SCID), hematopoietic stem cell transplant recipients/candidates, and solid-organ transplant recipients when the donor is also seronegative.
-

Question 43: B. Coagulase-negative staphylococci.**Explanation:**

- The estimated risk of bacterial contamination of blood components exceeds the risk of HIV, HBV, HCV, and HTLV contamination combined, and no cases of *T. pallidum* transmission have been reported for many decades.
- The most common bacterial flora isolated from platelets:
 - Coagulase-negative staphylococci.
 - Serratia marcescens*.
 - Streptococci.
- Factors contributing to bacterial contamination of platelets:
 - Storage: 22 C to 24 C for 5 days.
 - Because contamination usually results from skin cores during phlebotomy, pooled products (6 to 10 needlesticks) have a higher risk of contamination than apheresis platelet products (one needlestick).
- Yersinia enterocolitica* accounts for >50% of sepsis cases resulting from red cell transfusion.
- Factors contributing to *Y. enterocolitica* contamination of red cells:
 - Grows at 4 C (cryophilic or psychrophilic organism).
 - Calcium-free environment of anticoagulated blood.
 - Iron-rich environment of blood favors growth.
 - Potent endotoxin is formed during storage.
 - Chance of sepsis increases after 21 days of storage.
 - Donors harboring the organism are asymptomatic or have non-specific complaints. Donor selection procedures, history, and



physical examination may not rule out donors harboring *Y. enterocolitica*.

- AABB *Standards for Blood Banks and Transfusion Services* requires bacteria detection or pathogen inactivation in platelet components.



Question 44: A. Anti-HBc.

Explanation:

- Unfortunately, assays for anti-HBc are problematic when applied to donor screening. In these assays, a solid-phase reagent that consists of recombinant HBc antigen is incubated with donor serum. A labeled anti-HBc probe is then added and competes with any anti-HBc in the donor serum for antigen sites. The solid-phase reagent is washed, and the substrate for the indicator is added. The presence of anti-HBc in the donor serum results in a low signal, as it has bound to the HBc substrate with the result being the binding of less labeled anti-HBc. In the absence of anti-HBc in the donor's serum, more labeled anti-HBc binds, resulting in a higher signal. Competitive assays of this type are sensitive to operator error. In addition, because there is not a clear differentiation between positive and negative populations, numerous false-positive results are generated. In one report, anti-HBc testing resulted in more repeat-reactive donations (0.62%) than all of the remaining serologic tests for viral infection combined (0.47%).
- Anti-HBc can detect a small number of patients who are in the "window period" for HBsAg. During an acute infection with HBV, HBsAg declines over a period of weeks as anti-HBsAg titers rise. During this period, donors would still be infectious despite the absence of detectable HBsAg. Anti-HBc is present during this period and would identify these donors.
- Anti-HBc is the first antibody to appear after infection with HBV. The antibody appears shortly after the appearance of HBsAg and persists for the lifetime of the individual. Testing for anti-HBc was originally implemented as a surrogate marker for cases of non-A, non-B hepatitis, the majority of which were caused by HCV. In this setting, alanine aminotransferase and anti-HBc were effective in reducing the incidence of non-A, non-B hepatitis from 20



cases per 1000 recipients to 5 cases per 1000 recipients. Anti-HBc also served as a surrogate marker for HIV infection. With current testing for HCV and HIV, anti-HBc is not an effective surrogate marker and does not improve the safety of the blood supply concerning these viral agents.

- All of the remaining assays mentioned are EIA or ELISA tests. The screening test for HBsAg and HIV-1 p24 antigen is an EIA test, and the confirmatory test is a neutralization assay.



Question 45: C. HBsAg.

Explanation:

- Because of the extreme sensitivity of the HBsAg assay, the amount of HBsAg in a dose of HBV vaccine was found to be detectable for up to 5 days following immunization. As this represents “real” HBsAg, it would be neutralizable. It is not a false-positive test result in the usual sense.
- Although the vaccine is given to induce anti-HBsAg, this test is not a routine donor screening test. Also, in a donor who is receiving a first dose of HBV vaccine, a primary immune response (requiring 14 days before antibody is detectable) is what would be expected.
- HBV vaccine contains only HBsAg. Anti-HBc does not result from HBV immunization.
- Interestingly, HBV immunization has been listed as a cause for false-positive HIV-1/2 EIA results, but this is rare.



Question 46: B. *Leishmania donovani*.

Explanation:

- Deferral criteria are presented for *T. cruzi*, *Babesia*, and malaria, but AABB Standards does not state what should be done with regard to other protozoal infections such as *Toxoplasma gondii* or *Leishmania* species. As a result, decisions concerning the status of donors infected with these organisms are determined by the



medical director of the collecting facility, either on a case-by-case basis or through standard operating procedures.

- *T. gondii* circulates in the blood as an intracellular parasite. Transfusion-transmitted toxoplasmosis has been reported to have occurred only in the setting of granulocyte transfusions to immunosuppressed individuals.
 - *Leishmania* species circulate in the blood within mononuclear cells and granulocytes. Cases of transfusion transmission have occurred. In 1991 and 2003, 12-month deferrals were implemented by the US Department of Defense for personnel stationed in areas in Iraq where the parasite is endemic.
-

Question 47: A. The presentation of syphilis transmitted through transfusion is acute, fulminant secondary syphilis.

Explanation:

- This is characterized by spirochetemia with deposition of the organisms in all organs of the body. The characteristic finding is a mucocutaneous rash caused by deposition of the organisms within the skin.
- Poor viability of the organism at 4 C (red cells) and at high oxygen concentrations (platelets) is among the reasons that transfusion transmission is rare.
- FDA guidance in 2020 provided alternate algorithms for donor management depending on whether both nontreponemal and follow-up treponemal tests are used or only treponemal tests. In the former case, when a nontreponemal screening test is reactive, the donor is deferred indefinitely until a treponemal test is performed. If this follow-up treponemal test is also reactive, the donor is deferred indefinitely. Such a donor may be reentered if 1) the donor completed successful treatment for syphilis 3 months before the next donation, or 2) the donor was medically evaluated to determine infection was never present. Apart from satisfying these conditions, the donor remains deferred. When reentered, the next donation is tested like any other donation.



Question 48: D. Most commonly, when transfusion-transmission of *T. cruzi* occurs, the blood component involved is platelets.

Explanation:

- As mentioned in the answer #22, *T. cruzi* is a parasite transmitted by the reduviid (triatomine) bug. The bite itself does not transmit infection. When it takes a meal, the bug defecates. If this is rubbed into the bite or conjunctiva, infection occurs.
- Acute infection is characterized by fever, anorexia, lymphadenopathy, mild hepatosplenomegaly, and myocarditis. This syndrome lasts for 4 to 8 weeks. Treatment at this stage can cure infection. Infants, young children, and the immunocompromised can develop myocarditis or meningoencephalitis.
- Following the acute infection, chronic infection, which is incurable, occurs. Most infections become chronic but remain asymptomatic. Thirty percent of chronically infected individuals develop dementia, megacolon, megaesophagus, or cardiac failure. These result from destruction of neurons within the brain and myenteric plexus by the parasite.
- T. cruzi* has been transmitted by platelets and red cells. Platelet transfusion has been the most common route identified in North America. The parasite appears to have limited survival in refrigerated components.
- Most donors with positive results for *T. cruzi* in the United States are from areas of Latin America where it is endemic. In such areas, transmission rates of 10% to 20% have previously been reported. Since donor screening was implemented in the United States in 2007, however, reports of transfusion transmission have been exceedingly rare.



Question 49: D. Of those infected who develop West Nile fever, 1 out of 150 individuals will experience severe neurologic consequences.

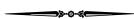
Explanation:

- WNV is transmitted by the bite of infected mosquitoes. The main reservoir for the virus is birds, with humans and other animals



representing dead-end hosts, incapable of completing the viral life cycle.

- WNV infection is a self-limited infection characterized by the development of permanent immunity.
- IgM antibodies to WNV appear early but persist for up to 512 days. As viremia is cleared within 104 days, the use of IgM testing would result in the deferral of donors and discard of products that were not viremic.
- Of those who are symptomatic, 1 out of 150 people with West Nile fever (WNF) develop meningitis, encephalitis, or a syndrome of flaccid paralysis mimicking acute inflammatory demyelinating polyneuropathy (Guillain-Barré syndrome). The mortality rate among those hospitalized with WNF is 4% to 14%.
- Individuals at either age extreme, the young or the elderly, are at greatest risk for WNF and neurologic complications.



Question 50: E. Acceptable for blood donation.

Explanation:

- As the result of current safety measures in the manufacture of coagulation factor concentrates, they are no longer considered to be a risk factor for HIV or hepatitis. Whether the factors are recombinant or derived from plasma, they are considered safe.
- Although individuals with hemophilia should still be deferred for their personal safety, their sexual partners have been eligible since the revision of the FDA guidance for reducing the risk of HIV transmission by blood components in December of 2015 (since superseded by guidance issued in 2020).

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12

Hematopoietic Cell Transplantation and Cellular Processing

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Key Points from the *Technical Manual*

- Hematopoietic stem cell transplantation and adoptive immunotherapy are recognized treatment strategies for many hematologic malignancies and for some nonmalignancies.
- Hematopoietic progenitor cells (HPCs) for transplantation can be collected either from marrow or peripheral blood after mobilization, or from an umbilical cord blood after delivery.
- HPC processing techniques can reduce product volume, plasma and red cell content, and (post-thaw) cryoprotectant. Their use is based on graft specifications and the recipient's clinical needs.
- Cryopreservation, thawing, infusion and infusion-related adverse-event monitoring require personnel and standard operating procedures that maximize patient safety and minimize toxicity.



QUESTIONS

Question 1: Select the answer choice that correctly pairs the type of transplant with the donor.

- A. Autologous transplant – related donor (eg, recipient's parent, sibling).
 - B. Allogeneic transplant – unrelated donor (eg, registry donor).
 - C. Xenogeneic transplant – the recipient's twin.
 - D. Syngeneic transplant – nonhuman donor.
 - E. Allogeneic transplant – recipient (ie, self-donation).
-

Question 2: Hematopoietic progenitor cells (HPCs) can be collected from which of the following sources?

- I. Marrow.
 - II. Peripheral blood.
 - III. Umbilical cord.
- A. I.
 - B. II.
 - C. III.
 - D. I and II.
 - E. All of the above.
-

Question 3: What is the marker that is most used to enumerate stem cells within HPC products?

- A. CD3.
 - B. CD19.
 - C. CD34.
 - D. CD56.
 - E. CD61.
-

Question 4: Which of the following engraftment terms is defined correctly?



- A. Neutrophil engraftment – a peripheral neutrophil count $>500 \times 10^6/L$ for three consecutive days.
 - B. Platelet engraftment – platelet transfusion independent for 4 days with platelet count $>20,000/\mu L$.
 - C. Full/complete T-cell engraftment – $>80\%$ donor T cells as determined by chimerism analysis.
 - D. Primary graft failure – failure of a graft that was previously functioning.
 - E. Secondary graft failure – failure to achieve engraftment within the first month after transplantation in the absence of disease relapse.
-

Question 5: The most common indication for autologous HPC transplantation in the United States is:

- A. Acute myelogenous leukemia.
 - B. Non-Hodgkin lymphoma.
 - C. Myelodysplastic syndrome.
 - D. Multiple myeloma.
 - E. Germ cell tumor.
-

Question 6: Beyond hematologic malignancies, HPC transplantation can be performed for other medical conditions. For which of the following types of disorders is HPC transplantation a common modality of treatment?

- I. Inherited metabolic disorders.
 - II. Infectious disease.
 - III. Autoimmune disease.
 - IV. Immunodeficiencies.
- A. I.
 - B. II.
 - C. I, III, IV.
 - D. III, IV.
 - E. All of the above.



Question 7: Which of the following statements is true concerning autologous HPC transplantation?

- A. As with blood donor collection, the Food and Drug Administration (FDA) requires questionnaires to screen for risk factors of relevant clinical diseases.
 - B. Infectious disease testing may preclude donation, as the HPC product cannot be cryopreserved with other patient products due to possible cross-contamination.
 - C. More immunosuppression is required for conditioning before transplantation compared to allogeneic HPC transplant.
 - D. The antitumor effect stems from the infusion of healthy HPCs and their impact on malignant cells.
 - E. It is most effective for malignancies with minimal marrow involvement.
-

Question 8: Title 21 of the Code of Federal Regulations (CFR) Part 1271 Subpart C outlines requirements relating to human cells, tissues, and cellular and tissue-based products. Which of the following HPC donor(s) would be subject to 21 CFR Part 1271?

- I. An allogeneic peripheral blood HPC donor.
 - II. An allogeneic marrow HPC donor.
 - III. An allogeneic umbilical cord HPC donor.
 - IV. An autologous peripheral blood HPC donor.
- A. I and II.
 - B. I, II, III.
 - C. I, II, III, IV.
 - D. I and III.
 - E. II, III, IV.
-

Question 9: For allogeneic HPC transplantation, the 21 CFR Part 1271 Subpart C requires infectious disease marker testing for which of the following diseases?

- A. *Treponema pallidum*.
- B. Zika.



- C. Malaria.
 - D. Variant Creutzfeldt-Jakob disease (vCJD).
 - E. Hepatitis E.
-

Question 10: In addition to HPC donation, 21 CFR Part 1271 Subpart C also regulates donation of other human cells and tissues. Which additional tests must donors of reproductive tissues undergo?

- A. *Chlamydia trachomatis* and vCJD.
 - B. *Chlamydia trachomatis* and *Neisseria gonorrhoea*.
 - C. *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and vCJD.
 - D. *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and herpes simplex virus (HSV).
 - E. *Chlamydia trachomatis*, *Neisseria gonorrhoea*, herpes simplex virus (HSV), and vCJD.
-

Question 11: Which of the following statements is true concerning allogeneic HPC transplantation?

- A. Immunosuppression is required only before transplantation, in the conditioning phase.
 - B. For umbilical cord HPC transplants, the newborn is screened and tested for relevant infectious diseases.
 - C. If an allogeneic donor tests positive for an infectious disease marker, there is no way to proceed to transplantation with the implicated donor.
 - D. Fewer allogeneic transplants are performed per year compared to autologous transplants.
 - E. If a standardized questionnaire reveals the donor to be at risk for transmission of Zika virus, the donor can still be eligible if RNA testing of plasma or serum is negative.
-

Question 12: Which of the following is an advantage of syngeneic HPC transplantation?

- A. There is no risk of relapse after HPC transplant procedures performed for genetic disorders.



- B. There is a good graft-vs-leukemia effect.
 - C. Minimal to no immunosuppression can be used after HPC transplantation.
 - D. There is no risk of communicable disease transmission.
 - E. HLA typing is not required because the recipient and donor are identical twins.
-

Question 13: For umbilical cord HPC transplantation, what do the National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR) recommend as the minimum HLA match (ie, number of matched alleles) between recipient and donor, and which HLA loci should be considered in the match?

- A. 3/6, HLA-A, -B, -C.
 - B. 4/6, HLA-A, -B, -DR.
 - C. 6/8, HLA-A, -B, -C, -DR.
 - D. 7/8, HLA-A, -B, -C, -DR.
 - E. 7/8, HLA-A, -B, -C, -DQ.
-

Question 14: A 4-year-old female is undergoing evaluation for acute myeloid leukemia (AML) with central nervous system involvement. In this case, umbilical cord donors were considered. None of the donors are HLA identical to the recipient. Which of the following donors would be considered the best match and have the most acceptable mismatches based on HLA phenotype?

	HLA-A	HLA-B	HLA-C	HLA-DR
Patient	01:01	08:01	07:01	03:01
	02:01	13:02	03:04	07:01
Father	01:01	08:01	07:01	03:01
	03:01	07:02	04:01	15:01



	HLA-A	HLA-B	HLA-C	HLA-DR
Mother	02:01	13:02	03:04	07:01
	11:01	18:01	01:02	01:02
Umbilical cord donor #1	23:01	44:03	07:01	10:01
	68:01	27:05	03:04	04:04
Umbilical cord donor #2	01:01	08:01	07:01	03:01
	68:01	27:05	03:04	04:04
Umbilical cord donor #3	02:01	13:02	06:02	07:01
	01:01	07:02	07:02	15:01
Umbilical cord donor #4	01:01	08:01	07:01	03:01
	11:01	18:01	16:02	07:01

- A. Umbilical cord donor #1.
- B. Umbilical cord donor #2.
- C. Umbilical cord donor #3.
- D. Umbilical cord donor #4.
- E. They are all equally acceptable as the patient's age and the immaturity of the umbilical cord T cells permits immunologic tolerance.



Question 15: Which of the following statements is true concerning haploidentical HPC transplantation?

- A. Compared to conventional matched-related and matched-unrelated donors, the HLA matching criteria for haploidentical HPC transplants are more stringent.
- B. It is important to test the donor for HLA antibodies to prevent graft failure.
- C. Donor procurement is often faster compared to conventional matched-related and matched-unrelated transplants.



- D. For most recipients, haploidentical donors are more difficult to find compared to conventional matched-related and matched-unrelated donors.
- E. Given that the donors are generally first- and second-degree relatives, there is minimal risk of graft-vs-host disease (GVHD).
-

Question 16: A 48-year-old female with AML needs HPC transplantation. The patient weighs 60 kg and is blood group A and cytomegalovirus (CMV) seronegative. Fortunately for the patient, she has a common HLA phenotype, and thus, multiple 12/12 HLA-matched donors are available in the donor registry. Which of the following HLA-matched donors would be the best selection for the recipient?

Choice	Age (years)	Gender	Weight (kg)	ABO Group	CMV Status
A.	48	Female	60	AB	+
B.	20	Male	75	A	-
C.	18	Female	50	O	-
D.	55	Male	60	A	-
E.	50	Male	75	A	+

Question 17: One of the hematologists at your hospital is inquiring about bringing KIR testing in-house to help evaluate HPC transplantation donors. What is KIR an abbreviation for?

- A. Killer-cell inhibitory receptor.
- B. Karyotype incompatibility range.
- C. Killer-cell immunoglobulin-like receptor.
- D. Killer-cell incompatibility report.
- E. Kinship incompatibility report.



Question 18: Which of the following recipient-donor pairs represents a minor ABO incompatibility without a major ABO incompatibility?

- A. Recipient group A; donor group A.
 - B. Recipient group O; donor group B.
 - C. Recipient group A; donor group B.
 - D. Recipient group B; donor group A.
 - E. Recipient group A; donor group O.
-

Question 19: Which of the following answer choices accurately describes the clinical risks that are associated with major ABO-incompatible HPC transplants, along with the intervention(s) that can mitigate these risks?

Choice	Clinical Risk				Intervention
	Hemolysis	Delayed Engraftment	Pure Red Cell Aplasia	Passenger Lymphocyte Syndrome	
A.	-	+	+	-	Plasma reduction of HPC product
B.	+	+	+	-	Red-cell reduction of HPC product
C.	+	+	+	+	Plasma reduction and red-cell reduction of HPC product
D.	+	-	-	+	Plasma reduction of HPC product
E.	-	-	-	-	None



Question 20: For transfusion support in the period between HPC product infusion and engraftment, what would be the first choice ABO group for red cell and platelet transfusion to a group B recipient matched with a group O donor?

Choice	Red Cells	Platelets
A.	Group O	Group AB
B.	Group B	Group O
C.	Group O	Group B
D.	Group AB	Group O
E.	Group B	Group AB



Question 21: Compared to HPCs collected by marrow harvest (HPC-M), which of the following is accurate concerning peripheral blood HPCs collected by apheresis (HPC-A)?

- A. Higher risk of chronic GVHD.
- B. Lower CD34+ cell dose.
- C. Slower engraftment.
- D. Less graft-vs-leukemia effect.
- E. Fewer are performed each year.



Question 22: Compared to HPC-A, which of the following is accurate concerning HPC-M?

- A. Requires mobilization before collection.
- B. Less invasive collection.
- C. Lower risk of relapse.
- D. Higher risk of graft failure.
- E. Contains more T cells.



Question 23: Which of the following is an advantage of HPCs collected from umbilical cord blood (HPC-CB)?

- A. High CD34+ cell dose.
 - B. Increased graft-vs-leukemia effect.
 - C. Readily available for transplantation.
 - D. Decreased risk of engraftment failure, especially with nonmyeloablative conditioning regimens.
 - E. Early immune reconstitution.
-

Question 24: Which of the following accurately describes harvesting and processing of HPC-M?

- A. Marrow harvest is considered a noninvasive procedure, as general anesthesia is not required.
 - B. The patient is laid supine to properly access the posterior iliac crests.
 - C. The procedure requires only one experienced physician to perform.
 - D. 18- to 20-gauge needles are used to aspirate the marrow, which is then added to a larger collection bag containing appropriate anticoagulants.
 - E. The HPC-M product should be filtered to remove contaminating debris, bone spicules, and other aggregates.
-

Question 25: When performing a marrow harvest, what is the maximum recommended volume that should be collected per the National Marrow Donor Program (NMDP) guidelines?

- A. 10 mL/kg.
 - B. 15 mL/kg.
 - C. 20 mL/kg.
 - D. 25 mL/kg.
 - E. 30 mL/kg.
-

Question 26: What is the minimum HPC-M cell dose that should be collected for proper engraftment?



- A. $2\text{-}3 \times 10^9 \text{ TNC/kg}$ of donor weight.
 - B. $1\text{-}2 \times 10^8 \text{ TNC/kg}$ of recipient weight.
 - C. $2.5 \times 10^6 \text{ TNC/kg}$ of recipient weight.
 - D. $1\text{-}2 \times 10^7 \text{ TNC/kg}$ of donor weight.
 - E. $2\text{-}3 \times 10^8 \text{ TNC/kg}$ of recipient weight.
-

Question 27: Which of the following are potential adverse events of a marrow harvest?

- I. Pain.
 - II. Nausea and dizziness.
 - III. Bruising and bleeding.
 - IV. Fat embolism.
 - V. Acute pancreatitis.
-
- A. I and II.
 - B. I and III.
 - C. I, II, and III.
 - D. I, II, III, and IV.
 - E. All of the above.
-

Question 28: Which of the following statements is accurate about HPC collection via apheresis (HPC-A)?

- A. HPC-A products are collected without mobilization of stem cells; however, mobilization can be performed for donors who may be deemed poor mobilizers.
- B. The minimum stem cell dosage to help ensure efficient HPC-A engraftment is $3 \times 10^6 \text{ CD34+cells/kg}$ recipient weight.
- C. For most healthy HPC-A donors, a temporary central venous catheter is used for vascular access.
- D. HPC-A collection can lead to a 30-50% reduction in platelet count.
- E. Large-volume leukapheresis (LVL) involves processing 2-3 total blood volumes (TBV) through the apheresis instrument.



Question 29: The collection efficiency (CE2) of an apheresis cell separator instrument can be calculated by the following equation:

$$\text{CE2 } (\% \times 10) = \frac{\text{Total CD34+ cell collected } (\times 10^8) \times 100}{\text{TBV processed (mL)} \times \text{Preprocedure peripheral [CD34+cell]} (\times 10^3/\text{mL})}$$

This equation can be reconfigured to predict the total CD34+ cell dose that will be collected.

$$\text{Predicted CD34+cells/kg} = \frac{\text{CE2 } (\% \times 10) \times \text{Preprocedure peripheral [CD34+cell]} (\text{per microliter}) \times \text{TBV possessed (mL)}}{\text{Recipient weight (kg)} \times 10,000}$$

What would be the predicted CD34+ cell yield for a donor provided the following data?

- Donor peripheral CD34+ cell count = 50/ μL
 - Recipient weight = 70 kg
 - Volume processed = 12 L
 - Volume of HPC-A product collected = 250 mL
 - CE2 of the apheresis cell separator instrument = 50%
- A. 0.6×10^6 CD34+cells/kg.
B. 1.5×10^6 CD34+cells/kg.
C. 3.4×10^6 CD34+cells/kg.
D. 4.3×10^6 CD34+cells/kg.
E. 5.7×10^6 CD34+cells/kg.
-

Question 30: Which of the following is an advantage of adding unfractionated heparin (UFH) to acid-citrate-dextrose solution A (ACD-A) as the anticoagulant for the HPC-A product compared to using ACD-A alone?

- A. Less bleeding risk.
B. Decreases the volume of anticoagulant needed.
C. Puts the patient at risk of heparin-induced thrombocytopenia (HIT).
D. Prevents development of citrate toxicity.
E. It is ideal for patients who have thrombocytopenia before initiating collection.



Question 31: Select the statement that is true concerning HPC-A mobilization:

- A. Chemotherapeutic agents are used to mobilize both autologous and healthy allogeneic donors.
 - B. Granulocyte colony stimulating factor (G-CSF) is given once, the day before HPC-A collection.
 - C. Acute leukemia is an independent risk factor of poor mobilization.
 - D. G-CSF is a C-X-C chemokine receptor type 4 (CXCR-4) antagonist, releasing stem cells from the marrow into peripheral circulation.
 - E. The stem cell yield using G-CSF in combination with plerixafor is equivalent to the yield obtained using G-CSF alone.
-

Question 32: Which of the following potential adverse events are associated with G-CSF mobilization?

- I. Bone pain.
 - II. Nausea.
 - III. Bruising and bleeding.
 - IV. Splenic rupture.
- A. I.
 - B. I and II.
 - C. I, II, and III.
 - D. I, III, and IV.
 - E. All of the above.
-

Question 33: Which of the following statements correctly describes HPCs collected from umbilical cord (HPC-CB)?

- A. HPC-CB are products to be collected only after the delivery of the placenta.
- B. More HPC-CB grafts are collected ex utero than in utero.
- C. Blood is aspirated directly from the umbilical vein using a syringe.



- D. The volume of HPC-CB collected correlates with the birth weight and placental weight.
 - E. Each double cord transplant unit should contain 3×10^7 TNC/kg before cryopreservation.
-

Question 34: After HPC collection in the apheresis unit or the operating room, the HPC product is transferred for processing in the cellular therapy laboratory. Which of the following assessments are part of HPC product evaluation/quality control?

- I. Cell enumeration.
 - II. Washing.
 - III. Flow cytometry.
 - IV. Sterility.
 - V. Potency/viability.
 - VI. Cryopreservation.
- A. I, IV, and V.
 - B. I, III, IV, and V.
 - C. I, II, III, and IV.
 - D. I, II, III, and VI.
 - E. All of the above.
-

Question 35: Calculate the CD34+ cell dose for an HPC-A product with the following specifications:

- Volume of product – 120 mL
 - TNC – $500 \times 10^3/\mu\text{L}$
 - Percent CD34+cells – 0.5%
 - Recipient weight – 60 kg
- A. 0.55×10^6 CD34+ cells/kg.
 - B. 2.0×10^6 CD34+ cells/kg.
 - C. 3.4×10^6 CD34+ cells/kg.
 - D. 5.0×10^6 CD34+ cells/kg.
 - E. 2.0×10^7 CD34+ cells/kg.



Question 36: Select the answer choice that correctly fills in the blanks of the following statement: _____ can deplete T cells based on cell size and density while _____ employs immunomagnetic beads covered in monoclonal antibody that can bind cells of interest.

- A. Cell expansion; cell selection.
 - B. Plasma reduction; elutriation.
 - C. Elutriation; red cell reduction.
 - D. Cell selection; cell expansion.
 - E. Elutriation; cell selection.
-

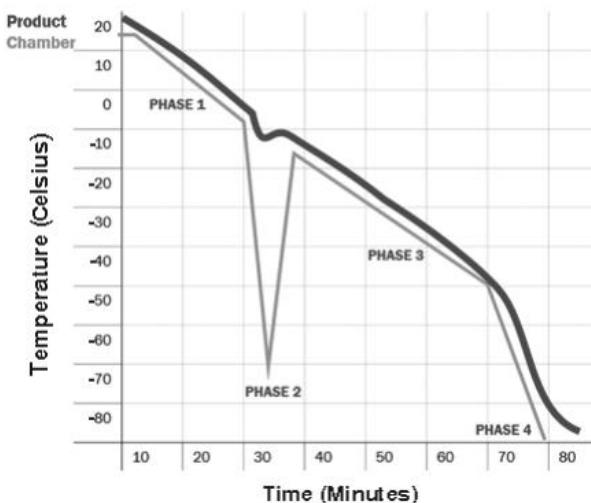
Question 37: Which of the following HPC products would most likely be given fresh (ie, no cryopreservation)?

- A. Autologous HPC-A for multiple myeloma.
 - B. HPC-CB.
 - C. Allogeneic HPC-A from a related donor that will be infused within 48 hours of collection.
 - D. Allogeneic HPC-A from an unrelated donor that will take 96 hours (about 4 days) to transport.
 - E. Autologous HPC-A for Hodgkin lymphoma.
-

Question 38: What is the most common cryoprotectant used for the cryopreservation of HPC products?

- A. Albumin.
 - B. Dimethyl sulfoxide (DMSO).
 - C. Hydroxyethyl starch (HES).
 - D. Dextran.
 - E. Saline.
-

Question 39: During cryopreservation of HPC products, the cooling curve in the accompanying figure is generated by the controlled-rate freezer. What is the rate of freezing for Phase 3?



- A. $-0.5\text{ C}/\text{minute}$.
- B. -1 to $-2\text{ C}/\text{minute}$.
- C. -5 to $-10\text{ C}/\text{minute}$.
- D. -10 to $-20\text{ C}/\text{minute}$.
- E. $-25\text{ C}/\text{minute}$.



Question 40: Referring to the cryogenic freezing curve in question #39, what transition is the HPC product undergoing during Phase 2?

- A. Sublimation.
- B. Deposition.
- C. Fusion.
- D. Condensation.
- E. Evaporation.



Question 41: For cryogenic storage, HPC products can be kept at what nitrogen phase and temperature?

- A. Liquid phase at -195 C .
- B. Liquid phase at -125 C to -150 C .
- C. Vapor phase at -195 C .
- D. Vapor phase at -80 C .
- E. Liquid phase at -80 C .



Question 42: To minimize the risk of DMSO toxicity, what is the maximum HPC product dose of 10% DMSO that can be administered to a patient?

- A. 0.5 mL/kg/hour.
- B. 1 g/kg/minute.
- C. 1 mL/kg/day.
- D. 2 g/kg/hour.
- E. 2 mL/kg/day.



Question 43: Which of the following reactions is considered an adverse event mostly attributed to DMSO toxicity?

- A. Allergic reaction.
- B. Circulatory overload.
- C. Febrile reaction.
- D. Nausea.
- E. Hemolytic reaction.



Question 44: Which of the following statements is true concerning the thawing of cryopreserved HPC products?

- A. HPC products are thawed in a 24 C waterbath.
- B. Thawing of the HPC product is performed in the cellular therapy processing laboratory and then transported to the patient's bedside.
- C. HPC products are transferred to the patient's bedside from the cellular therapy laboratory at room temperature.
- D. If the HPC plastic bag breaks, the product must be discarded to prevent contamination.
- E. For infusion of >1 HPC bag, thawing of each bag should occur separately after the infusion of the previous bag is complete.



Question 45: HPC collection of an unrelated registry donor occurs in Germany, but the recipient is in the United States. What is the term that would best describe the transit of the cells from the collection site to the site of infusion?



- A. Transporting.
 - B. Shipping.
 - C. Delivering.
 - D. Conveying.
 - E. Transferring.
-

Question 46: A patient with multiple myeloma underwent HPC collection, in which 10×10^6 CD34+cells/kg were collected, in a facility in New York. Half of the cells were infused during transplantation and the other half remained in cryogenic storage. Ten years later, the patient needs a second transplant, but now lives in Florida. The facility in Florida requests the cryopreserved cells from the facility in New York. Select the answer choice that correctly describes the transportation of the cells from the collection/storage facility to the receiving facility.

- A. The HPC product must be delivered and infused within 24-72 hours of shipping.
 - B. The HPC product must be accompanied and under the control of a trained courier.
 - C. The HPC product must be transported at a temperature of 2-8 C.
 - D. The HPC product may pass through x-ray machines if the product is being transported by air.
 - E. The temperature must be continuously monitored, and data stored in an electronic log.
-

Question 47: HPCs and other biologics are heavily regulated by the federal government. Which federal regulation grants the Department of Health and Human Services the authority to prevent the spread of communicable disease to and within the United States?

- A. Title 21 CFR Part 210.
- B. 21 CFR Part 600.
- C. 21 CFR Part 1271.
- D. Section 351 of the Public Health Service (PHS) Act.
- E. Section 361 of the PHS Act.



Question 48: Select the answer choice that accurately describes the intracellular and extracellular domains of the most common chimeric antigen receptor T cells (CAR-T cells).

Choice	Intracellular Domain	Extracellular Domain
A.	CD3+ cell activation domain	Single-chain immunoglobulin with portions of heavy and light chains.
B.	CD4+ cell activation domain	Double-chain immunoglobulin with portions of heavy and light chains.
C.	CD8+ cell activation domain	Single-chain immunoglobulin with portions of two heavy chains.
D.	CD3+ cell activation domain	Double-chain immunoglobulin with portions of the variable and constant regions.
E.	CD4+ cell activation domain	Single-chain immunoglobulin with portions of the variable and constant regions.



Question 49: Which CAR-T-cell-related adverse reaction is characterized by fever, hypoxia, hypotension, and multiorgan failure?

- A. CAR-T-cell-related encephalopathy syndrome (CRES).
- B. B-cell aplasia in anti-CD19 CAR-T cells.
- C. Tumor lysis syndrome.
- D. Cytokine release syndrome (CRS).
- E. Immune effector cell–associated neurotoxicity syndrome (ICANS).



Question 50: In addition to HPCs and CAR-T cells, what other cell types are investigated and processed in cellular therapy laboratories?

- A. Mesenchymal stromal cells.
- B. Dendritic cells.
- C. Natural killer cells.
- D. Induced pluripotent stem cells (iPS cells).
- E. All of the above.



ANSWERS

Question 1: B. Allogeneic transplant – unrelated donor (eg, registry donor).

Explanation:

- Hematopoietic progenitor cell (HPC) transplantation involves the transfer of pluripotent stem cells to a recipient with the goal of reconstituting hematopoietic function.
- Several types of HPC transplants can be carried out depending on the donor type:
 - Autologous transplant – involves collecting HPCs from the recipient and reinfusing the stem cell product into the recipient later. In this way, autologous transplants are akin to autologous red cell transfusions.
 - Allogeneic transplant – involves collecting HPCs from allogeneic donors. These donors can be either related to the recipient (eg, parent, sibling, etc) or unrelated. Unrelated donors usually come from marrow registries.
 - Syngeneic transplant – a type of related HPC transplant where the donor is an identical twin to the recipient.
 - Xenogeneic transplants – HPC transplant where the donor is a nonhuman animal (eg, pig, primate).



Question 2: E. All of the above.

Explanation:

- HPCs can be collected from multiple sources:
 - Marrow – HPCs are found among the mesenchymal elements that compose the marrow microenvironment. HPCs from bone marrow are obtained through marrow harvest, which is a rather invasive procedure.
 - Peripheral blood – HPCs can be collected from the peripheral blood via apheresis after mobilization, a process that releases HPCs from the marrow into peripheral circulation. In the US, peripheral collection is the most common source of HPCs.



- Umbilical cord – the blood from the umbilical cord is rich in HPCs and can be collected after birth without the need for invasive procedures associated with marrow harvest nor for mobilization before peripheral HPC collection.
-

Question 3: C. CD34.**Explanation:**

- CD34 is a marker found on HPCs and is a negatively charged, N-glycosylated, and O-glycosylated sialomucin. It is thought to be involved in the migration of HPCs through the marrow stroma. However, its exact function is still unclear and under investigation.
 - Different monoclonal CD34 antibodies are used to detect HPCs via flow cytometry to enumerate stem cells and calculate the transplant CD34+ cell dose.
 - CD3 and CD19 are markers of T cells and B cells respectively, whereas CD56 is found on natural killer (NK) cells.
 - CD61 is found on platelets and megakaryocytes.
-

Question 4: A. Neutrophil engraftment – a peripheral neutrophil count $>500 \times 10^6/\text{L}$ for three consecutive days.**Explanation:**

- Reconstitution of hematopoiesis through engraftment is the goal of HPC transplantation. Different cell lineages engraft at different rates, and it is good to be aware of what constitutes engraftment and engraftment failure.
- Some parameters such as neutrophil and platelet engraftment can be monitored by peripheral blood counts, while others like T-cell and myeloid-cell engraftment are monitored by chimerism testing.
- Neutrophil engraftment is often defined as maintaining an absolute neutrophil count (ANC) $>500 \times 10^6/\text{L}$ for three consecutive days.
- Platelet engraftment can be defined as maintaining a platelet count of $>20,000/\mu\text{L}$ for 7 days without platelet transfusion support.



- Complete CD3 engraftment is determined by chimerism analysis [eg, short tandem repeat (STR), polymerase chain reaction (PCR), next-generation sequencing NGS] of the T-cell compartment and is defined as 100% donor-derived DNA. However, some define complete CD3 engraftment as >90 or >95%, depending on the sensitivity of the assay used.
 - Primary graft failure can be defined as the inability or failure to achieve engraftment within the first month (or 28 days) after transplantation without disease relapse.
 - Secondary graft failure is the loss of engraftment, sometimes defined as ANC <500 × 10⁶/L, after the patient achieved engraftment.
 - Morbidity and mortality associated with primary graft failure are considered worse than those associated with secondary graft failure.
-

Question 5: D. Multiple myeloma.

Explanation:

- The number of autologous transplants performed for multiple myeloma is more than double that for any other indication for which autologous transplants are performed.
 - Autologous transplants are also performed for Hodgkin lymphoma, non-Hodgkin lymphoma, angioimmunoblastic T-cell lymphoma, and solid tumor malignancies such as germ-cell tumors, neuroblastoma, Wilms tumor, and Ewing sarcoma.
 - Acute myelogenous leukemia and myelodysplastic syndrome are the most common indications for allogeneic HPC transplant.
-

Question 6: C. I, III, and IV.

Explanation:

- HPC transplantation is an accepted treatment modality for a variety of medical conditions, including:
 - Inherited metabolic disorders: Gaucher disease, lysosomal storage disorders, leukodystrophies, mucopolysaccharidoses.
 - Congenital immunodeficiencies: chronic granulomatous diseases, DiGeorge syndrome, leukocyte adhesion deficiency, Wiskott-Aldrich syndrome, severe combined immunodeficiency.



- Autoimmune diseases: multiple sclerosis and systemic sclerosis.
 - Hemoglobinopathies: thalassemia and sickle cell anemia.
 - Marrow failure syndromes: severe aplastic anemia, Fanconi anemia, Diamond-Blackfan anemia, pure red cell aplasia.
 - HPC transplantation has also been used as a rescue therapy for transfusion-associated graft-vs-host disease.
 - HPC transplant procedures are generally not performed for infectious diseases. Rather, infectious diseases are often a complication following HPC transplantation.
-

Question 7: E. It is most effective for malignancies with minimal marrow involvement.

Explanation:

- Unlike blood donor collection and allogeneic HPC donation, the Food and Drug Administration (FDA) does not mandate the eligibility criteria for autologous HPC donation. Thus, a screening questionnaire is not required before collection. Likewise, testing for infectious disease markers (eg, HIV, HBV, HCV, HTLV) is not required.
- Although infectious disease marker testing is not required, it is recommended and often performed to prevent possible cross-contamination between stored cryopreserved HPC products from different individuals. If an autologous donor tests positive for one or more of the infectious disease markers, the HPC product should be stored in a manner that minimizes the risk of cross-contamination.
- One of the advantages of autologous transplantation over allogeneic is the need for less immunosuppression, which in turn is associated with less chemotherapy-related toxicity.
- In autologous transplantation settings, the infusion of HPCs serves to reconstitute the marrow and hematopoiesis. The antitumor effect comes from the chemotherapy and radiation used in the conditioning regimen for the transplant procedure.
- Autologous transplantation is most successful for patients whose disease has minimal marrow involvement. Significant marrow involvement by disease can adversely affect the mobilization of HPCs, thereby decreasing the yield. Additionally, there is the possibility of collecting and reinfusing the malignant cells.

**Question 8: D. I and III.****Explanation:**

- Regarding allogeneic HPC transplantation, Title 21 CFR Part 1271 Subpart C mandates that donors be subject to a physical examination, review of medical history, infectious disease marker testing, and a standardized screening questionnaire for relevant risk factors.
- Donors subject to 21 CFR Part 1271 include allogeneic peripheral blood HPC donors and allogeneic umbilical cord HPC donors.
- Allogeneic marrow HPC donors are subject to Sections 375 and 379 of the Public Health Service Act. Nonetheless, marrow donors are still evaluated similarly to allogeneic peripheral blood and umbilical cord donors, because accrediting organizations, such as AABB and the Foundation for the Accreditation of Cellular Therapy (FACT), require screening and testing of all allogeneic donors.
- Donor eligibility of autologous HPC donors is not subject to FDA regulations but is rather mandated by institutional policies.

**Question 9: A. *Treponema pallidum*.****Explanation:**

- Title 21 CFR Part 1271 requires that a specimen from all donors of human cells and tissues, including HPCs, be tested for the following:
 - Human immunodeficiency virus, type 1.
 - Human immunodeficiency virus, type 2.
 - Hepatitis B virus.
 - Hepatitis C virus.
 - *Treponema pallidum*.
- Additionally, because HPCs are leukocyte-rich products, donors must also be tested for:
 - Human T-lymphotropic virus, type I.
 - Human T-lymphotropic virus, type II.
 - Cytomegalovirus.
- All infectious disease markers must be tested by an FDA-approved assay.
- It is also customary to test for West Nile virus and *Trypanosoma cruzi*.



- In addition to testing donor specimens, other relevant communicable diseases (eg, Zika virus, variant Creutzfeldt-Jakob, malaria) that could be transmitted via HPC transplantation are screened for by a standardized questionnaire.
-

Question 10: B. *Chlamydia trachomatis* and *Neisseria gonorrhoea*.

Explanation:

- Donors of reproductive tissues must be tested for all the infectious disease markers that are required for HPC donors (see answer #9) in addition to *Chlamydia trachomatis* and *Neisseria gonorrhoea*. This is to reduce the transmission risk of relevant communicable diseases of the genitourinary tract.
 - Of note, anonymous semen donors are to be retested 6 months after donation to monitor for the possible development of relevant communicable diseases since donation.
 - Donors of dura mater should be tested for the same infectious disease markers as all donors of human cells and tissues (see answer #9). However, because dura mater is not leukocyte-rich, human T-lymphotropic virus, types I and II, and CMV testing are not required. Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoea* is also not required as dura mater does not represent reproductive tissue. However, dura mater donors do need to be tested for vCJD.
-

Question 11: D. Fewer allogeneic transplants are performed per year compared to autologous transplants.

Explanation:

- Allogeneic HPC transplantations constitute the minority of HPC transplants in the US at approximately 42%. The remaining 58% are autologous HPC transplants.
- Nonmyeloablative and myeloablative conditioning regimens are used before transplantation. However, immunosuppression is also required after transplantation to prevent and treat transplant-associated graft-vs-host disease.



- Although umbilical cord HPC products are subject to donor eligibility evaluation, it is the mother, not the newborn, who is screened and tested for relevant communicable infectious diseases.
- Allogeneic donors who test positive for infectious disease markers are deemed ineligible for donation. However, unlike blood donation, ineligible HPC donors are not indefinitely or permanently deferred. All parties – donor, recipient, and recipient's physician – are informed of the donor's ineligibility. If the benefit of receiving the transplant outweighs the risk of transmitting the infectious agent, the ineligible donor HPC product can still be used for transplant provided that the justification of "urgent medical need" is properly documented per FDA stipulations.
- The risk of Zika virus transmission is assessed by donor questionnaire rather than by direct testing. There are FDA-approved Zika virus assays, one of which tests for the presence of viral RNA in plasma and serum. However, it has been demonstrated that an HPC product can test positive for Zika virus even when no viral RNA is detected in the plasma/serum. Thus, a negative result of plasma or serum would not necessarily be indicative of decreased transmission risk.



Question 12: C. Minimal to no immunosuppression can be used following HPC transplantation.

Explanation:

- Advantages of syngeneic HPC transplantation include:
 - Because the recipient and donor are HLA identical, the risk of graft-vs-host disease (GVHD) after transplantation is minimal to none.
 - Given the minimal risk of GVHD, posttransplant immunosuppression can be minimized, and in some cases, no posttransplant immunosuppression is given.
 - In the setting of hematologic and solid-organ malignancies, donors of syngeneic HPC transplants are free of disease; therefore, there is no risk of residual disease contaminating the donor product as there is in the setting of autologous HPC transplant.
- Disadvantages of syngeneic HPC transplantation include:
 - It is thought that genetic differences between recipient and donor are important in graft-vs-leukemia effect. Because iden-



tical twins do not have many genetic differences, there is poor graft-vs-leukemia effect in syngeneic HPC transplantation. This decreased graft-vs-leukemia effect may help explain the increased risk of relapse seen in syngeneic HPC transplantation.

- Because the recipient and donor in syngeneic HPC transplantation are genetically identical, there is risk of relapse if the transplant were performed secondary to a genetic disorder, as the donor would be at risk for the same genetic disorder.
 - Given that a syngeneic transplant is a type of allogeneic transplant, there is still risk of transmission of communicable disease.
 - Despite identical twins being HLA identical, HLA typing of the recipient and donor are still required to confirm HLA phenotype. In fact, the National Marrow Donor Program (NMDP) requires recipients and donors to undergo initial HLA typing and confirmatory HLA typing before transplantation.
-

Question 13: B. 4/6, HLA-A, -B, -DR.

Explanation:

- Although HLA matching is important in umbilical cord HPC transplantation, the matching criteria are not as stringent as those for marrow and peripheral blood HPC transplantation (see Chapter 16, The HLA System, for questions concerning HLA matching in marrow and peripheral blood HPC transplantation). The increased permissiveness of HLA mismatch in umbilical cord HPC transplantation is largely attributed to the immature umbilical cord T cells.
- The NMDP and CIBMTR recommend high-resolution typing for the following loci: HLA-A, HLA-B, HLA-C, and HLA-DR.
- In terms of matching, a $\geq 4/6$ match is recommended. This matching scheme considers the following loci: HLA-A, HLA-B, and HLA-DR. Note: Matching for the A- and B- loci can be at the antigen level (ie, first-field) while matching for the DR locus is at the allele level (ie, second-field or higher). However, if high-resolution typing is taken into consideration for all loci, then $\geq 4/8$ matches are permissible.
- Studies have demonstrated similar outcomes comparing $\geq 4/6$ match umbilical cord transplants with HLA-matched unrelated transplants.



- Although HLA-C is not included in the matching scheme, studies have shown increased transplant-related mortality when HLA-C locus mismatches are present.
-

Question 14: D. Umbilical cord donor #4.

Explanation:

- Although criteria for HLA matching in umbilical cord HPC transplantation are less stringent, due to the immaturity of the umbilical cord T cells, it is still important to consider the degree of HLA match when evaluating potential donors.
 - Based on the answer #13, the donor should be at least a 4/6 HLA match at the HLA-A, -B, and -DR loci. As such, this rules out umbilical cord donor #1 who is a 0/6 match. This would also rule out donor #2 who is only a 3/6 HLA match with the patient.
 - This leaves donors #3 and #4, who are both 4/6 HLA matches with the patient. Looking at the mismatches, something to note is that donor #4 expresses *A*11:01* and *B*18:01*, which are not shared with the patient, but are shared with the mother. These alleles would be considered noninherited maternal antigens (NIMA).
 - It is theorized that during pregnancy, fetal exposure to maternal HLA in utero leads to tolerance to the maternal antigens not inherited by the fetus. Consequently, NIMA are considered acceptable mismatches.
 - Compared to non-NIMA-matched transplants, NIMA-matched transplants have improved engraftment and lower transplant-related mortality.
-

Question 15: C. Donor procurement is often faster compared to conventional matched-related and matched-unrelated transplants.

Explanation:

- Haploidentical HPC transplants have less stringent HLA-matching criteria compared to conventional matched-related and matched-unrelated transplants because only half of the HLA alleles (ie, haplotype) are matched (eg, 4/8, 5/10, 6/12).



- Most people will have a haploidentical donor in a parent, sibling, or second-degree relative; thus, finding a haploidentical donor is easier compared to finding a conventional matched-related or matched-unrelated donor. Given the availability of these donors, haploidentical HPC transplants have faster donor procurement.
- Because matching occurs for only half of the HLA alleles, this leaves the recipient at risk for GVHD, where donor T cells with mismatched HLA alleles can attack recipient cells. However, with the advent of posttransplantation cyclophosphamide and techniques such as T-cell depletion of HPC products, the risk of GVHD has been reduced, and recipient outcomes are much improved, comparable to conventional matched-related and matched-unrelated transplants.
- Given the HLA mismatch in haploidentical HPC transplantation, there is a possibility that the recipient could possess HLA antibodies against the potential donor. This risk is increased when the recipient has been transfused or pregnant. Mothers receiving HPCs from an offspring are particularly at risk of having donor-directed HLA antibodies given the exposure to mismatched paternal antigens during pregnancy.
- The presence of donor-specific antibodies (DSAs) in the setting of HPC transplantation is a risk factor for primary graft failure. If DSAs to a certain donor are present, it is recommended to select another donor. If no other donor is available, steps to reduce antibody burden (eg, immunosuppression, plasma exchange, IVIG) should be taken.



Question 16: B.

Choice	Age (years)	Gender	Weight (kg)	ABO Group	CMV Status
B.	20	Male	75	A	-

Explanation:

- Although HLA matching is paramount when selecting an HPC donor, other factors are also taken into consideration, particularly when multiple donors with similar degrees of HLA match to the recipient are available.



- These considerations include:
 - ABO-blood-group matching – It is preferred to select a donor who is ABO identical to the recipient to prevent complications of major and/or minor ABO incompatibility (see Questions #18 - #20).
 - CMV status – An attempt should be made to match recipient and donor CMV status. If a recipient is CMV seronegative, then it is preferable to select a CMV-seronegative donor to decrease the risk of CMV infection and/or reactivation.
 - Gender – male donors are preferred over female donors, potentially due to recognition of Y chromosome-derived minor histocompatibility antigens by female donor T cells.
 - Age – recipient survival has been shown to be greater with younger donors.
 - Weight/size – larger donors compared to recipient size are preferable. Larger donors typically yield satisfactory HPC counts for transplant.
 - Clinical health – Donors should be evaluated for underlying diseases and other comorbidities to ensure successful HPC collection.
- Taking these factors into account, healthy, larger, young, and ABO-identical males with matching CMV status to the recipient would be considered ideal donors after controlling for HLA compatibility.

**Question 17: C. Killer cell immunoglobulin-like receptor.****Explanation:**

- KIRs are found on natural killer (NK) cells, which are part of the innate immune system.
- KIRs are part of an immunoglobulin superfamily that was formally called killer cell inhibitory receptors.
- KIR genes, located on chromosome 19, are highly polymorphic and are inherited in a haplotype. These genes encode surface proteins that have two to three immunoglobulin domains, a transmembrane domain, and a cytoplasmic tail.
- KIR proteins can engage certain HLA-Class I molecules and have either inhibitory or activating effects.



- NK-cell alloreactivity can occur when the donor expresses inhibitory KIR that do not engage recipient HLA Class I molecules. Failure of donor NK cells to detect self-ligands is theorized to lead to graft-vs-leukemia effect.
 - Given the potential of KIR to contribute to graft-vs-leukemia effect, some guidelines include selection of donors based on NK-cell alloreactivity. There are various proposed KIR-matching schemes, each with pros and cons; thus, there is no consensus on which matching scheme should be used.
-

Question 18: E. Recipient group A; donor group O.

Explanation:

- Approximately 40-50% of HPC transplants cross the ABO barrier, meaning that half of recipient-donor pairs are ABO mismatched. Unlike transfusion and solid-organ transplantation, HPC recipient-donor pairs can be ABO incompatible. Once engrafted, the recipient takes on the donor ABO phenotype.
- Before HPC transplantation, a two-way crossmatch is performed between the recipient and donor. A two-way crossmatch includes the major crossmatch and the minor crossmatch.
- The major crossmatch is what is performed before red cell transfusion. Recipient plasma is incubated with donor red cells looking for the presence of donor-directed antibodies.
- The minor crossmatch is used to detect donor-derived antibodies directed toward the recipient's red cells by mixing donor plasma with recipient red cells.
- When assessing recipient-donor pairs, there are four categories of ABO compatibility:
 1. ABO compatibility – major and minor crossmatches are negative.
 2. ABO major incompatibility – major crossmatch is positive and minor crossmatch is negative, indicating the presence of recipient-derived antibodies toward the donor red cells.
 3. ABO minor incompatibility – minor crossmatch is positive and major crossmatch is negative, indicating the presence of donor-derived antibodies toward the recipient red cells.
 4. Bidirectional ABO incompatibility – major and minor crossmatches are positive, indicating the presence of recipient-



derived antibodies directed toward the donor red cells and the presence of donor-derived antibodies directed toward the recipient cells.

- Major and minor incompatibilities can also be due to antibodies targeting minor blood group antigens. This is particularly an issue when transplanting for hemoglobinopathies (eg, sickle cell disease) in which the recipients may have multiple alloantibodies.



Question 19: B.

Choice	Hemolysis	Delayed Engraftment	Pure Red Cell Aplasia	Passenger Lymphocyte Syndrome	Intervention
B.	+	+	+	-	Red cell reduction of HPC product

Explanation:

- ABO incompatibility poses increased clinical risk depending on the category of incompatibility. Fortunately, there are HPC processing methods that can mitigate these risks (see table below for summary).

ABO Incompatibility Category	Clinical Risk					Mitigation Strategy/ Intervention
	Hemolysis	Delayed Engraftment	Pure Red Cell Aplasia	Passenger Lymphocyte Syndrome		
Compatible	-	-	-	-	-	-
Major incompatibility	+	+	+	-	Red cell reduction of HPC product, plasma exchange	



Minor incompatibility	+	-	-	+	Plasma reduction of HPC product, monitoring for hemolysis, red cell exchange
Bidirectional incompatibility	+	+	+	+	Plasma reduction and red cell reduction of HPC product along with other strategies outlined above

- In the case of major ABO incompatibility, the recipient's ABH antibodies can hemolyze red cells in the donor HPC product at the time of infusion. Subsequently, these antibodies can lead to delayed red cell engraftment, and in severe cases pure red cell aplasia (PRCA), where the antibodies attack erythrocytes and lead to failure of erythropoiesis. To help mitigate hemolysis and delayed engraftment, the HPC product can be red cell reduced/depleted to decrease the number of red cells infused with the product. In patients with high ABH titer, plasma exchange may be considered.
- For minor ABO incompatibility, the donor ABH antibodies can hemolyze recipient red cells. Moreover, donor lymphocytes in the HPC product can continue making donor ABH antibodies days to months after transplantation. This is termed passenger lymphocyte syndrome and it can lead to chronic hemolysis, ranging from subclinical to severe. To help mitigate hemolysis, the HPC product can be plasma reduced to decrease the ABH antibody load of the HPC product. It may also be helpful to monitor for hemolysis after transplantation (direct antiglobulin test, complete blood count, bilirubin, haptoglobin, lactose dehydrogenase, reticulocyte count). Hemolysis abates after red cell engraftment. In severe cases, red cell exchange with group O red cells has been performed to decrease antibody target on native recipient cells.

**Question 20:**

Choice	Red Cells	Platelets
C.	Group O	Group B

Explanation:

- Following HPC infusion, transfusion support is critical to maintain viable hematologic cell counts while awaiting engraftment. This can be particularly challenging in the setting of ABO mismatched transplants, as blood components must be compatible with the recipient and the donor. See table below for summary of first choice ABO group for Red Blood Cells (RBCs) and platelet/plasma transfusion.

Major ABO Incompatibility			
Recipient ABO	Donor ABO	First Choice ABO RBCs	First Choice ABO Platelets/Plasma
Group O	Group A	Group O	Group A
Group O	Group B	Group O	Group B
Group O	Group AB	Group O	Group AB
Group A	Group AB	Group A	Group AB
Group B	Group AB	Group B	Group AB

Minor ABO Incompatibility			
Recipient ABO	Donor ABO	First Choice ABO RBCs	First Choice ABO Platelets/Plasma
Group A	Group O	Group O	Group A
Group B	Group O	Group O	Group B
Group AB	Group O	Group O	Group AB
Group AB	Group A	Group A	Group AB
Group AB	Group B	Group B	Group AB



- For bidirectional ABO incompatibility (group A to B or group B to A), first-choice RBCs are always group O and first-choice platelets/plasma is always group AB.
 - Once engraftment is complete, donor-type RBCs and platelet/plasma can be provided.
-

Question 21: A. Higher risk of chronic GVHD.

Explanation:

- By far, most HPC transplantsations are performed using HPC-A as the graft source. In fact, approximately 70% of allogeneic transplants and 99% of autologous transplants are performed using HPC-A.
- Increased use of nonmyeloablative conditioning regimens has contributed to preference of HPC-A grafts given the high CD34+ cell dosage and higher graft-vs-leukemia effect.
- The following table compares HPC-A and HPC-M grafts.

Aspect/Outcome	HPC-A	HPC-M
Engraftment	Faster	Slower
Chronic GVHD*	Higher risk	Lower risk
Engraftment failure	Lower risk	Higher risk
Disease relapse	Lower risk	Higher risk
Graft-vs-leukemia effect	More activity	Less activity
Immune reconstitution	Faster	Slower
Graft failure	Lower risk	Higher risk
TNC and CD34+cell dose	Higher	Lower
T-cell content	Higher	Lower
Mobilization before collection	Required	Not required
Collection procedure	Less invasive (vascular access)	More invasive (marrow harvest)

*Rates of acute GVHD are similar for HPC-A and HPC-M.



Question 22: D. Higher risk of graft failure.

Explanation:

- See table in answer #21.
-

Question 23: C. Readily available for transplantation.

Explanation:

- In the US, far fewer HPC-CB transplant procedures are performed compared to HPC-A and HPC-M transplants.
 - Advantages of HPC-CB grafts include:
 - Units are readily available because they are cryopreserved. There is no need for a donor to undergo apheresis collection or marrow harvest.
 - Fewer restrictions regarding HLA matching.
 - Decreased risk of GVHD due to presence of immature T cells. However, risk is also dependent on the degree of HLA mismatch between recipient and donor.
 - Disadvantages of HPC-CB grafts include:
 - Low cell dosage—on average, HPC-CB grafts contain one log fewer total nucleated cells, including CD34+ stem cells. Consequently, for adult transplantation, 2 HPC-CB units are required to achieve an adequate dosage. Although 2 units are infused, only one engrafts; however, the second unit can contribute to cellular immunity early in the posttransplant period.
 - Increased risk of engraftment failure, in part due to immature T cells.
 - Delayed immune reconstitution.
 - As the entire HPC-CB unit is infused, the ability to perform stem-cell boosts and donor-lymphocyte infusions from the same donor is compromised.
-

Question 24: E. The HPC-M product should be filtered to remove contaminating debris, bone spicules, and other aggregates.



Explanation:

- Marrow harvest is considered an invasive procedure, which is performed using sterile techniques and under general anesthesia. Because this is an operative procedure, the patient must be informed of the potential adverse events (see answer #27) during the consenting process.
- The procedure is performed with the patient in the prone position to facilitate easy access to the posterior iliac crests, the most common location accessed for HPC-M harvest.
- In terms of operators, there must be at least two practitioners, one of whom is a senior physician with qualified experience.
- The operators use 11- to 14-gauge needles to aspirate marrow from the iliac crest.
- Small volumes are aspirated at a time to prevent peripheral blood contamination of the marrow. The needle can be rotated between aspirations to access more marrow. The syringe's contents are then emptied into a larger collection bag filled with anticoagulant to prevent clotting of the HPC-M product. The process is repeated at different sites along the iliac crest until the target TNC value is reached. Efforts should be made to minimize the number of skin puncture sites throughout the procedure.
- The collection bag should be filtered to prevent debris, aggregates, and bone spicules from contaminating the HPC-M product.



Question 25: C. 20 mL/kg.

Explanation:

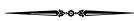
- During marrow harvest, peripheral blood is aspirated along with the marrow. Consequently, donors often experience a decrease in hemoglobin. In the past, a substantial percentage of patients often received blood transfusion. Many donors would donate autologous units before the harvest in case they required a transfusion after the procedure. As of late, the collection of autologous units for marrow harvest has come into question.
- To prevent significant blood loss, the NMDP recommends that a maximum of 20 mL/kg be collected from the donor.



Question 26: E. $2-3 \times 10^8$ TNC/kg of recipient weight.

Explanation:

- Though HPCs come from the donor, the cell dosage is based on recipient weight. Thus, a low recipient-to-donor weight ratio may be advantageous. Thus, a larger donor may not have to donate as much marrow relative to their size to provide an adequate TNC dose for a smaller donor. Conversely, in cases of high recipient-to-donor weight ratios, a small donor may have to donate a lot of marrow relative to their size if they have a larger recipient. This may put the donor at increased risk for significant blood loss.
- The recommended HPC-M cell dose to facilitate proper engraftment is $2-3 \times 10^8$ TNC/kg of recipient weight.
- To estimate that an adequate cell dose is being collected during marrow harvest, a cell count is performed on an aliquot from the collection bag halfway through the procedure, (marrow harvest lasts about 60 minutes). The resulting TNC can provide an estimate of how much more marrow should be collected to reach the goal TNC. Stem cell counts (CD34+) are not performed intra-operatively given that the prolonged time required to run flow cytometry would necessitate exposing the donor to additional anesthesia and associated risks.



Question 27: E. All of the above.

Explanation:

- Adverse events of marrow harvest range from minor to severe or life-threatening.
- Fortunately, most common adverse events are minor with approximately 50% of HPC-M donors experiencing local discomfort, which resolves within 3 weeks. Bruising is also quite common and expected given the procedure itself.
- Uncommon complications occurring in 1-10% of HPC-M donors include fever, postoperative nausea, urinary retention, persistent pain for greater than 3 weeks, spinal headaches, and hematomas that can cause pressure neuropathies.
- Rare and potentially life-threatening complications occur in less than 1% of HPC-M donors. These include septicemia, pulmonary



embolism, fat embolism, acute pancreatitis, mechanical ileus, ilium fracture, arrhythmia, and cardiopulmonary arrest.

- As referenced in answer #25, bleeding is another risk of marrow harvest. Historically, some studies have quoted upward of 80% of donors receiving autologous blood transfusions. Of note, if allogeneic red cells or platelets are to be transfused, they should be irradiated to prevent contaminating the HPC-M graft with white cells from the donated blood units. Consent for use of blood components should be obtained before the procedure if the probability of transfusion is considerable.



Question 28: D. HPC-A collection can lead to a 30-50% reduction in platelet count.

Explanation:

- HPC-A collection can be performed on an outpatient basis and is less invasive than marrow harvest.
- Vascular access in healthy donors can usually be obtained using peripheral veins via two 16- to 17-gauge needles. However, approximately 10% of healthy donors will require a temporary central venous catheter, which is most often placed in the internal jugular vein or sometimes in the subclavian. The risks associated with line placement are bleeding, thrombosis, infection (especially with femoral access), and pneumothorax.
- Before collection, all allogeneic and autologous HPC-A donors undergo mobilization (see answers #31 and #32 for more information about mobilization).
- In standard HPC-A collection, 2-3 TBVs are processed, which equates to about 10-12 L. Large-volume leukapheresis (LVL) entails the processing of 3-6 TBVs, or 15-30 L. LVL is usually reserved for select cases, such as in children and poor mobilizers undergoing autologous collection, in which large volumes need to be processed to collect sufficient CD34+ cells.
- A dose of 2×10^6 CD34+cells/kg of recipient weight is usually cited as the minimum required to obtain efficient engraftment of HPC-A product. However, a cell dose of 5×10^6 CD34+cells/kg is often preferred.
- HPC-A collection is associated with the same adverse events as those seen with therapeutic plasma exchange (see Chapter 14,



Hemapheresis) such as fatigue, hypotension, allergic reaction, syncope, and citrate toxicity. In specific, HPC-A collection can also lead to a 30-50% reduction in platelet count; thus, it is recommended that a CBC be obtained before collection to evaluate for severe thrombocytopenia.



Question 29: D. 4.3×10^6 CD34+cells/kg.

Explanation:

- It has been shown that the CD34+ cell dose collected is proportionally related to the donor's peripheral CD34+ cell count and the collection efficiency (CE2) of the apheresis cell separator instrument.
- This is just a matter of plugging in the numbers and calculating the expected dose using the equation:

$$\text{Predicted CD34+cells/kg} = \frac{\text{CE2 } (\% \times 10) \times \text{Preprocedure peripheral [CD34+cell] (per microliter)} \times \text{TBV possessed (mL)}}{\text{Recipient weight (kg)} \times 10,000}$$
$$\text{Predicted CD34+cells/kg} = \frac{0.5 \times 10 \times 50 \times 12,000}{70 \times 10,000} = 4.3$$

- Thus, this donor would be expected to collect 4.3×10^6 CD34+ cells/kg, which would be higher than the minimum 2.0×10^6 CD34+ cells/kg required for engraftment. However, if the transplant team had a goal of 5×10^6 CD34+ cells/kg or higher, then this patient would have to undergo additional days of collection.
- CE2 assumes that the CD34+ cell concentration is constant throughout the procedure and tends to underestimate the actual yield.
- There is another collection efficiency, CE1, that can be calculated as: (pre-CD34 and post-CD34)/2. This CE1 considers the average CD34+ cell concentration during the procedure. Thus, CE1 approximates the CD34+ cell yield better than CE2. However, this requires measurement of a CD34+ cell concentration before and after collection, which is associated with additional costs, time, and resources. Given the need for a postprocedural count and the additional cost, CE1 is not generally used to predict CD34+ cell yields.



Question 30: B. Decreases the volume of anticoagulant needed.

Explanation:

- In HPC-A collection, the circuit can be anticoagulated with ACD-A or ACD-A plus UFH.
- The following table outlines the advantages and disadvantages of the two anticoagulation strategies.

Anticoagulant(s)	Advantage	Disadvantage
ACD-A	Lower risk of bleeding	Higher risk of citrate toxicity
ACD-A + UFH	Lower risk of citrate toxicity Less total volume of anticoagulant needed (reduced risk of volume overload)	Higher risk of bleeding (especially if donor is thrombocytopenic) Potential risk of heparin-induced thrombocytopenia



Question 31: C. Acute leukemia is an independent risk factor of poor mobilization.

Explanation:

- Before HPC-A collection, allogeneic and autologous donors are mobilized to increase the peripheral CD34+ cell count. This is accomplished through pharmacologic agents that serve to release CD34+ cells from the marrow into the circulation.
- There are three main mobilization agents that are commonly employed:
 - Chemotherapeutic agents – These function by transient marrow suppression with substantial increase in CD34+ cell counts appearing with recovery 9-11 days (about one and a half weeks) after chemotherapy. The CD34+ cell count can be further amplified by the addition of G-CSF. Chemotherapeutic agents are used in autologous stem cell collections in patients with hematologic malignancies. They are not used in healthy donors.
 - Hemopoietic growth factors (G-CSF) – These function by expanding the number of granulocytes within the marrow.



Additionally, they stimulate the release of proteases that disrupt adhesion between HPCs and marrow stroma. G-CSF is usually given at a dose of 10 µg/kg daily for 4-5 days before collection. The aim is to increase peripheral cell count for an optimal CD34+ cell dose. A peripheral CD34+ cell concentration of $\geq 10/\mu\text{L}$ is considered an acceptable threshold to initiate collection.

- Plerixafor – This is a CXCR4 receptor antagonist that prevents the receptor-ligand interaction between the HPC CXCR4 and stromal derived factor 1 (SDF-1) thereby facilitating the release of HPCs into the peripheral circulation. Plerixafor is generally dosed at 240 µg/kg approximately 11 hours before collection. Studies have shown that mobilization with G-CSF plus plerixafor is better compared to G-CSF alone.
 - Whereas healthy allogeneic donors usually mobilize successfully, some autologous donors are poor mobilizers necessitating many days of collection to achieve even a minimum CD34+ cell dose. Risk factors of poor mobilization include increasing age, underlying disease (eg, acute leukemia, indolent lymphoproliferative disorders, non-Hodgkin lymphoma), previous radiation of the marrow, and previous chemotherapy with agents that are toxic to HPCs.
-

Question 32: E. All of the above.

Explanation:

- Mobilization agents each come with their own set of potential adverse events:
 - G-CSF:
 - Common adverse events: bone and musculoskeletal pain (40-95%), fatigue (30-70%), headache (12-70%), insomnia, flu-like symptoms, bruising, and bleeding gums/nosebleed due to thrombocytopenia.
 - Less common: sweating, anorexia, fever, chills, and nausea.
 - Severe: marked thrombocytopenia with severe bleeding, splenic rupture, and acute lung injury.
 - Plerixafor:
 - Gastrointestinal symptoms – nausea, vomiting, bloating, flatulence, and diarrhea.
 - Injection site reactions.



- Paresthesia – perioral and facial.
 - Headache, lightheadedness, and dizziness.
 - Chemotherapeutic agents: Immunosuppression, infection, and other agent-specific adverse effects.
-

Question 33: D. The volume of HPC-CB collected correlates with the birth weight and placental weight.

Explanation:

- HPC-CB can be collected in utero or ex utero (before or after placental delivery, respectively) with in utero collections being more common. As such, collection can be performed before or after placental delivery.
 - Collection is carried out by cannulating the umbilical cord vein and letting the blood drain into collection container by gravity. A closed collection system will reduce the risk of bacterial contamination.
 - HPC-CB volumes range from approximately 50 to 200 mL. Increased HPC-CB volumes and TNC counts are associated with certain factors such as cesarean section, prolonged labor, early cord clamping, birth weight, placental weight, female gender, first-born children, and induction.
 - For HPC-CB, 3×10^7 TNC/kg before cryopreservation is generally considered an adequate dose to facilitate engraftment. However, given the small volumes of the units, achieving this dose requires double cord transplantation with the use of HPC-CB units from two donors. Some institutions stipulate that each unit of a double cord transplant contains at least 1.5×10^7 TNC/kg. For single-unit HPC-CB transplants, some centers have set a minimum dose of 2.5×10^7 TNC/kg.
-

Question 34: B, I, III, IV, and V.

Explanation:

- All HPC products, no matter the source, must be evaluated to ensure they are suitable for transplantation. Quality metrics that are assessed include:



- Cell enumeration – the TNC needs to be determined to calculate the CD34+ cell dose (see answer #35 for calculation of CD34+ cell dose) and ensure that the dose is sufficient for engraftment. If a hematology analyzer is used, then the TNC equates to the white cell count but may be overestimated if there are a lot of nucleated red cells or other immature myeloid cells.
- Flow cytometry – the percent CD34+ cells in the HPC product also needs to be determined to calculate the CD34+ cell dose. Flow cytometry employs fluorescently conjugated anti-CD34 to label the CD34+ cells.
- Sterility – small aliquots of the HPC product are assessed for the presence of microbial organisms, which could pose a risk to the recipient if infused. Both aerobic and anaerobic cultures are sent for assessment. Sources of HPC product contamination can be intrinsic, from the cell collection source (ie, donor) or extrinsic, from processing secondary to reagents, material, environment, and/or personnel.
- Viability – the CD34+ cells must be assessed for potency/viability to ensure they are capable of proliferating and differentiating once infused. Commonly, viability is assessed by flow cytometry using 7-aminoactinomycin D (7-ADD), which is a viability dye that will penetrate dead cells but not live cells with intact membranes. The colony-forming unit (CFU) measure is considered the gold standard for the evaluation of potency and is used to detect cells' ability to proliferate and form colonies. CFU is more laborious and time consuming, so it is not performed as much as flow cytometry.
- Washing and cryopreservation can be part of cellular processing, but they are not necessarily performed on all HPC products. Washing and cryopreservation would be steps that occur after the quality metrics are assessed.



Question 35: D. 5.0×10^6 CD34+ cells/kg.

Explanation:

- To calculate the CD34+ cell dose, you can use the following equation.



CD34+ cells ($\times 10^6/\text{kg}$) =

$$\frac{\text{HPC product volume (mL)} \times \text{TNC} (\times 10^3/\mu\text{L}) \times \text{percent CD34+ cells}}{\text{Recipient weight (kg)}}$$

- Remember to multiply by 1000 $\mu\text{L}/\text{mL}$ (WBC is per μL and HPC volume is in mL).

CD34+ cells ($\times 10^6/\text{kg}$) =

$$\frac{120 \text{ (mL)} \times \text{TNC} \times 10^3/\mu\text{L} \times 0.005 \times 1000 \mu\text{L}/\text{mL}}{60 \text{ (kg)}} = 5.0 \times 10^6/\text{kg}$$

Question 36: E. Elutriation; cell selection.

Explanation:

- HPC products are often modified or manipulated before storage and/or infusion. Modifications include:
 - Red cell reduction – performed when there is a major ABO incompatibility between recipient and donor to reduce risk of hemolysis during infusion and to reduce the risk of delayed red cell engraftment and pure red cell aplasia posttransplant. Usually performed when ABO titers are greater than 16; however, some labs may universally perform red cell reduction on all components in which there is a major ABO incompatibility. Red cells are reduced to 20-30 mL/component for adults and 0.2-0.4 mL/kg for pediatric patients.
 - Plasma reduction – performed when there is a minor ABO incompatibility between recipient and donor to reduce the risk of hemolysis during infusion and to reduce the risk of passenger lymphocyte syndrome after transplantation. Plasma reduction is often carried out by washing the product. Both red cell and plasma reduction can lower CD34+ cell yield and increase the risk of contamination. See answer #19 for more information about red cell and plasma reduction.
 - Elutriation – process that separates cells based on size and density. It is a process that works well to separate different cell populations and was historically used for T-cell depletion of



HPC products, which would help decrease the risk of acute GVHD. Centrifugation would separate cells based on density, and a counterflow applied in the opposite direction of sedimentation would separate cells based on size.

- Cell selection – can be positive or negative selection. In positive selection, monoclonal antibodies targeting cell surface markers (eg, CD34+ cells) are conjugated to immunomagnetic beads and used to pull out cells of interest. CD34+ cell selection is a passive method for T-cell depletion in which a CD34+ cell recovery of 50-100% and a cell purity of 90-99% is desired. In negative selection, the undesired cells are targeted (eg, CD3+ cells, CD19+ cells). This permits retention of other immune cells (eg, NK cells) in the final product that may have immunologic benefits.
- Cell expansion – ex-vivo cell expansion to increase the dose of desired cells. Increased CD34+ cell dose has been shown to expedite engraftment and improve outcomes. This method employs a variety of cytokines, media, cultures, and proprietary reagents to promote cellular proliferation. It is not commonly used in the clinical setting, but different methods are currently being investigated.



Question 37: C. Allogeneic HPC-A from a related donor that will be infused within 48 hours of collection.

Explanation:

- Most allogeneic HPA-A and HPC-M products are infused fresh within 48 hours. In the time between collection and infusion, HPC-A products should be stored at 2-8 C while HPC-M can be stored at room temperature. If there is a delay, the products should be cryopreserved within 72 hours (3 days) to maintain the integrity of the cells.
- HPC-CB products are cryopreserved shortly after collection and stored in cryobanks.
- Autologous HPC-A products, no matter the indication, are cryopreserved while the patient undergoes chemotherapy before transplantation.

**Question 38: B.** Dimethyl sulfoxide (DMSO).**Explanation:**

- Often used at a concentration of 10%, DMSO penetrates the cell membrane and prevents the formation of intracellular ice crystals, guarding against dehydration, which can both decrease viability/potency and subsequently increase the time to engraftment.
 - Hydroxyethyl starch (HES) can also be used for cryoprotection. Unlike DMSO, HES is extracellular and is thought to coat the cell, thereby impeding the efflux of water and preventing dehydration.
 - Sometimes HES is used in combination with DMSO to decrease the concentration of the former and decrease the risk of DMSO toxicity (see answer #43).
 - Dextran is another cryoprotectant that is commonly used with other cryoprotectant(s). The combination of HES and dextran is often used to cryopreserve HPC-CB products.
 - Plasma, saline, and albumin are additives that are used in the cryopreservation process in addition to the cryoprotectants.
-

Question 39: B. –1 to –2 C/minute.**Explanation:**

- Cryopreservation serves to preserve cellular structure and function during short- or long-term storage. Use of a controlled-rate freezer permits the standardization of gradual cooling to subzero temperatures.
- The figure provided in the question is an example of a normal controlled-rate cooling curve, or a cryogenic freezing curve. The typical cooling process is broken up into four phases:
 1. Phase 1 – the initial cooling phase where HPC product temperature is brought down gradually from room temperature to approximately –3 to –8 C, which is the point at which rapid cooling starts. Most freezers are programmed to cool at a rate of –1 to –2/ minute, although sometimes higher rates can be observed. During this time the product temperature (top line) should run parallel to the freezer chamber temperature (bottom line).



2. Phase 2 – the rapid cooling phase (superfreezing) in which formation of ice nucleation and transition from liquid to solid phase occurs. During this phase, the freezer chamber temperature is lowered to -70 C at an approximate rate of $-25\text{ C}/\text{minute}$. At this point there will be a gap of $>50\text{ C}$ between the HPC product temperature and the freezer chamber temperature, as the product temperature decreases by only a few degrees Celsius. This minimal change in product temperature is due to the latent heat of fusion as ice crystals form and the product transitions from liquid to solid. The rapid cooling rate is designed to offset the latent heat of fusion. Once the chamber reaches -70 C , the freezer chamber temperature increases at an approximate rate of $15\text{ C}/\text{minute}$ until a temperature of -14 C is reached.
 3. Phase 3 – the freezer chamber returns to gradual cooling at an approximate rate of -1 to $-2\text{ C}/\text{minute}$, like Phase 1. Also, like Phase 1, the HPC product temperature (top line) should run parallel to the freezer chamber temperature (bottom line). This phase continues until approximately -45 C .
 4. Phase 4 – the final phase when the freezer chamber temperature decreases at an approximate rate of -10 C until a temperature of -90 C , at which point the HPC product is safely able to be transferred to liquid nitrogen freezers for storage.
- HPC products can also be cryopreserved using noncontrolled rate freezers. In this method, HPC products are placed directly into -80 C freezer after the addition of cryoprotectants and additives. The product can be insulated in styrofoam or absorptive padding, which can help control the rate of cooling, approximately -1 to $-2\text{ C}/\text{minute}$. Outcomes (eg, potency/viability, recovery, engraftment) have been shown to be similar between controlled-rate and noncontrolled-rate frozen HPC products.
-

Question 40: C. Fusion.**Explanation:**

- Fusion is the transition from the liquid phase to the solid phase. See Phase 2 in answer #39.
- Sublimation is the transition from solid phase to gaseous phase.



- Deposition is the direct transition from gaseous phase to solid phase without going through liquid phase.
 - Condensation is the transition from the gaseous phase to the liquid phase.
 - Evaporation is the transition from the liquid phase to the gaseous phase.
-

Question 41: A. Liquid phase at –195 C.

Explanation:

- After cryopreservation, facilities will store HPC products (eg, HPC-CB and autologous HPC-A) in liquid nitrogen freezers.
 - Products can be stored either in the liquid nitrogen phase or in the vapor nitrogen phase.
 - Storage temperature for the liquid phase is –195 C.
 - Storage temperature for the vapor phase is –150 C to –125 C. HPC products that test positive for infectious disease markers or that have positive sterility results are stored in the vapor phase to prevent possible cross-contamination of other HPC products.
 - Cell viability after storage has been shown to be comparable for the liquid phase and solid phase up to storage times of 15 to 23.5 years.
-

Question 42: C. 1 mL/kg/day.

Explanation:

- The maximum amount of HPC product stored in 10% DMSO that can be given is 1 mL/kg/day or 1 g/kg/day. This limit is placed to prevent DMSO-related toxicity (see answer #43 for manifestations of DMSO toxicity).
-

Question 43: D. Nausea.



Explanation:

- Adverse events associated with administration of HPC products can be related to the cellular components of the product or the additives, primarily DMSO.
- Adverse events related to the cellular components manifest much like other transfusion reactions: febrile nonhemolytic reactions, allergic reactions, circulatory overload, and hemolysis.
- Adverse events related to DMSO occur either during or shortly after infusion and include:
 - Gastrointestinal symptoms – nausea, vomiting, diarrhea, abdominal cramps, halitosis, garlic/metallic taste.
 - Cardiac symptoms – hypotension, hypertension, tachycardia, bradycardia, chest tightness, left cardiac insufficiency, asystole.
 - Respiratory symptoms – shortness of breath/dyspnea, cough, edema, hypoxia.
 - Neurologic symptoms – sedation, seizure, amnesia, transient palsy (cranial nerves III,V), encephalopathy.
 - Urogenital symptoms – dysuria, pelvic pain, renal/urinary disorders.
 - Other: Facial flushing, fever, chills, dizziness, weakness, hyponatremia.



Question 44: E. For infusion of >1 HPC bag, thawing of each bag should occur separately after the infusion of the previous bag is complete.

Explanation:

- HPC products are transported to the patient's bedside from the cellular processing laboratory in liquid nitrogen and thawed at the patient's bedside to minimize the time that DMSO is in liquid state to curtail possible DMSO toxicity.
- As with blood transfusion, before infusion of HPC products, proper patient identification must be carried out according to standards and institutional policies.
- The HPC product bag is placed in a secondary bag and submerged in a 37 C waterbath. The product is gently massaged to help break up ice crystals. The secondary bag permits collection of any spillage from possible breakage in the primary bag.



- If a product bag does break, the defect should be clamped with a hemostat to prevent further leakage. The product and cells recovered from the secondary bag can be aseptically transferred into a transfer bag and still infused into the patient. In these scenarios, a sample must be sent for culture to monitor for contamination and the risk/benefit ratio must be weighed. In some cases, the patient may be empirically treated with antibiotics.
 - It is good practice to thaw and infuse products sequentially after the infusion of the previous product. This practice limits the time of DMSO in the liquid state and permits monitoring for adverse events before thawing and administrating the next product unit.
-

Question 45: B. Shipping.

Explanation:

- To ensure the quality and integrity of HPC products, the transporting and shipping of HPC products is governed by a variety of regulatory agencies and accreditation organizations: FDA, AABB, Foundation for the Accreditation of Cellular Therapy (FACT), Department of Transportation, International Civil Aviation Organization, and the International Air Transport Association.
- These agencies and organizations provide requirements and standards addressing the packaging, labeling, temperature monitoring, and accompanying documentation of HPC products during shipment and transportation.
- Shipping refers to the transit in which the personnel in the facility that collected, stored, distributed, or received the product lose or hand over control of that product. Shipping often occurs when the collection center is not the same center as the infusion center. Such is the case in this scenario.
- Transporting describes the process where the trained personnel of a facility retain control or oversight of a product as it is transferred within or between facilities. For example, transporting occurs when the cellular processing laboratory personnel transfers the product from cryogenic storage to the patient's bedside.



Question 46: E. The temperature must be continuously monitored, and data stored in an electronic log.

Explanation:

- Both allogeneic and autologous HPC products are shipped between the transfer facility (ie, collection facility) and the receiving facility (ie, where the patient is located). However, the way they are shipped can differ.
 - Allogeneic HPC products (including HPC-A and HPC-M) are usually shipped fresh and accompanied by a trained courier, who always maintains control of the product. The product should be delivered between 24 and 72 hours and kept between 2 and 8 C to preserve HPC viability.
 - Autologous HPC products are usually shipped cryopreserved in a liquid nitrogen shipper, which should be validated to maintain temperatures <–150 C for up to 2 weeks. The shipper's temperature should be continuously monitored throughout transit and recorded in a data log.
 - HPC-CB products are also shipped cryopreserved like autologous HPC products.
 - All products, whether allogeneic or autologous, should be accompanied by the appropriate documentation, including chain of custody. Additionally, HPC products should be inspected manually and not be subjected to x-rays or gamma irradiation.
-

Question 47: E. Section 361 of the PHS Act.

Explanation:

- Title 21 of the Code of Federal Regulations contains a codification of FDA regulations. This includes several regulations that are applicable to cellular therapy, including HPCs.
 - 21 CFR 210 – *Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs* – This code addresses the minimum requirements for the facilities and methods used to manufacture, process, and store a drug to uphold safety and quality standards.
 - 21 CFR 600 – *Biological Products: General* – This code addresses both licensed biological products regulated by the



Center for Biologics Evaluation and Research (CBER) and products regulated by the Center for Drug Evaluation and Research (CDER). These regulatory bodies process biologics license applications (BLAs), fatality reports, deviation reports, and other relevant communications. Cellular therapy products are subject to this code.

- 21 CFR 1271 – *Human Cells, Tissues, and Cellular and Tissue-Based Products* – This code addresses cellular therapy products, including HPCs, and covers general provisions; procedures for registration and licensing; donor eligibility; current good tissue practice regulations; and requirements, inspection, and enforcement of establishments or institutions collecting, processing, and/or receiving cellular therapy products.
- The Public Health Service (PHS) Act is a federal law that grants the federal government, particularly the United States Public Health Service, the authority to prevent spread of infectious diseases into and within the United States. The act includes regulations that are applicable to cellular therapy products including HPCs.
 - Section 351 – This section defines and regulates biological products. Per this section biologic products can include vaccines, blood, blood components, blood derivatives, viruses, toxins, antitoxins, or analogous products designed to prevent or treat/cure human disease. Of note, human hormones are not considered biological products.
 - Section 361 – This section is called, “Control Communicable Diseases,” and grants the federal government, in specific the Surgeon General in conjunction with the Secretary’s approval, authority to create and enforce regulations designed to prevent the spread of infectious diseases into the United States and between states and territories.



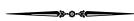
Question 48:

Choice	Intracellular Domain	Extracellular Domain
A.	CD3+ cell activation domain	Single chain immunoglobulin with portions of heavy and light chains.



Explanation:

- CAR-T cells are engineered ex vivo to recognize targets on malignant cells.
- The most common CAR-T cells consist of an extracellular single-chain variable fragment (scFv) composed of a portion of heavy chain linked to a portion of light chain. The extracellular antibody domain is coupled to an intracellular CD3 activation domain that can contain costimulatory domains to propagate signal activation.
- When the antibody domain recognizes its target, the T cell becomes activated and attacks the target cell bearing the cognate antigen.
- Collection and processing of CAR-T cells used in the clinical setting involves the following:
 - Collection of T cells via leukocytapheresis. Most protocols involve autologous collection, but some do employ allogeneic donors.
 - Shipping T cells to manufacturer.
 - Reprogramming/engineering of CAR-T cells.
 - CAR-T cell expansion.
 - Cryopreservation.
 - Shipping T cells to treating facility.
 - CAR-T cells infused into the patient after conditioning.
- The most common CAR-T cells used in the clinical setting are engineered to target hematologic malignancies, particularly B-cell malignancies, such as diffuse large B-cell lymphoma and B-acute lymphoblastic leukemia, where CD19 is the target. However, CAR-T cells can also be used to target solid tumors.



Question 49: D. Cytokine release syndrome (CRS).

Explanation:

- As CAR-T cells mediate cellular destruction; the targeted cells release cytokines and other inflammatory mediators. This release can cause a cytokine storm and the development of what has been termed cytokine release syndrome (CRS). Common symptoms of CRS include fever, hypotension, tachycardia, hypoxia, and chills. More serious symptoms are atrial fibrillation, ventricular tachycardia, cardiac arrest, renal insufficiency, capillary leak syndrome, and hemophagocytic lymphohistiocytosis. Symptom



onset is approximately 2 days after CAR-T cell infusion, with median duration of approximately 7 days. For mild cases, supportive care is sufficient while more severe cases can be treated with tocilizumab (anti-IL-6 receptor antagonist) and/or corticosteroids.

- Neurologic adverse events associated with CAR-T-cell therapy are termed CAR-T-cell-related encephalopathy syndrome (CRES) or immune effector cell-associated neurotoxicity syndrome [ICANS]. Common symptoms of CRES/ICANS include encephalopathy, tremor, dizziness, delirium, confusion, and agitation. More serious symptoms are seizures, leukoencephalopathy, cerebral edema, aphasia, and obtundation. Symptom onset is approximately 4 days after CAR-T-cell infusion, with median duration of approximately 17 days. For mild cases, supportive care is advised while more severe cases are treated with high dose steroids. If there is concurrent CRS, tocilizumab can be added to the treatment regimen.
- B-cell aplasia can occur in cases when CAR-T cells target B-cell markers (eg, CD19), because the CAR-T cells not only target malignant B cells but will also engage healthy B cells. B-cell aplasia can lead to marked hypogammaglobulinemia and put the patient at risk for severe infections.
- Like CRS, tumor lysis syndrome (TLS) is a consequence of cellular destruction, which can result in the release of nucleic acids, potassium, and phosphate. One of the main sequelae of TLS is acute kidney injury due to uric acid (secondary to nucleic acid breakdown) and calcium phosphate (secondary to hyperphosphatemia) deposition in the renal tubules. Hyperkalemia can cause cardiac arrhythmias. As nucleic acids are broken down, uric acid can be deposited in renal tubules. Other symptoms of TLS include nausea, vomiting, anorexia, lethargy, tetany (secondary to hypocalcemia), and seizures.



Question 50: E. All of the above.

Explanation:

- Mesenchymal stromal cells are an attractive candidate for cellular therapy as they can easily be isolated from many different tissue types, have a high expansion capacity, possess multilineage differentiation potential, provide stromal support of HPCs, and have immunomodulatory capabilities.



- NK cells are innate immune cells and do not require prior exposure to initiate effector functions. They are capable of cytotoxic function even in the absence of target cell major histocompatibility complex (MHC) presentation. Because many malignant cells downregulate their MHC, NK cells are an attractive cancer immunotherapy candidate.
- Dendritic cells are antigen-presenting cells that survey and take up a variety of antigens from their surrounding environment. As they mature, they become proficient in presenting these antigens to lymphocytes and are even able to modulate downstream responses. In doing so, dendritic cells can both activate the immune system and induce tolerance, two characteristics that are being exploited in cellular therapy.
- Through overexpression of certain genes (eg, Sox2, Oct4, cMyc, Klf4), somatic cells can be reprogrammed to revert to pluripotent stem cells, which could then be programmed to differentiate to the cell of interest. The generation of such induced pluripotent stem cells (iPSC) have vast potential clinical applications and avoids the ethical quandary surrounding the use of human embryonic stem cells.

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13

Neonatal and Pediatric Transfusion

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- A full-term newborn has a blood volume of approximately 85 mL/kg; a premature newborn has a blood volume of approximately 100 mL/kg.
- Iatrogenic losses from repeated phlebotomy for laboratory samples can contribute to the need for Red Blood Cell (RBC) transfusion.
- The therapy of choice to reduce the risk of stroke recurrence in patients with sickle cell disease is chronic RBC transfusion of indefinite duration to maintain hemoglobin S levels below 30%.
- Patients with sickle cell disease have the highest rates of alloimmunization to red cell minor antigens of any patient group. The most commonly formed antibodies are to RH, KEL, FY, and JK system antigens.
- If aliquots are made with a sterile connection device, they are considered to have been prepared in a “closed system,” and the original unit’s expiration date can be used for the aliquot.
- To decrease the risk of transfusion-transmitted cytomegalovirus (CMV) infection in susceptible patients such as low-birthweight neonates and immunocompromised children, should receive blood components that have either been leukocyte reduced or are from CMV-negative donors.



QUESTIONS

Question 1: In infants and young children, transfusion with 10-15 mL/kg of red cells is expected to raise the hemoglobin level by what amount?

- A. 0.5 g/dL.
 - B. 1 g/dL.
 - C. 2-3 g/dL.
 - D. 3-4 g/dL.
 - E. 5 g/dL.
-

Question 2: Transfusion with what amount of platelets would be expected to raise the platelet count of an infant or small child by 50,000-100,000/ μ L?

- A. 25 mL/kg.
 - B. 15-20 mL/kg.
 - C. 10-15 mL/kg.
 - D. 5-10 mL/kg.
 - E. 2-5 mL/kg.
-

Question 3: Transfusion with what amount of Fresh Frozen Plasma (FFP) or thawed plasma is expected to raise the factor activity of an infant or small child by 15-20%?

- A. 2-5 mL/kg.
 - B. 5-10 mL/kg.
 - C. 10-15 mL/kg.
 - D. 15-20 mL/kg.
 - E. 25 mL/kg.
-

Question 4: What amount of cryoprecipitate is expected to raise the fibrinogen level of an infant or small child 60-100 mg/dL?

- A. 1 unit/kg.



- B. 1-2 units/2.5 kg.
 - C. 1-2 units/5 kg.
 - D. 1-2 units/10 kg.
 - E. 1-2 units/15 kg.
-

Question 5: Transfusion of neonates or small children undergoing extracorporeal membrane oxygenation (ECMO), cardiopulmonary bypass, or exchange transfusion with fresh Red Blood Cell (RBC) units less than 5-14 days old are preferred for what primary reason?

- A. Older components carry less oxygen.
 - B. Older components have lower 2,3-diphosphoglycerate (2,3-DPG) levels.
 - C. Older components have more free hemoglobin.
 - D. Older components have higher levels of extracellular potassium.
 - E. Older components have less deformability of the red cells.
-

Question 6: According to AABB *Standards*, leukocyte-reduced RBC components must contain less than what amount of leukocytes/unit?

- A. $<2.0 \times 10^6$ leukocytes/unit and retain 85% of the original red cells.
 - B. $<5.0 \times 10^6$ leukocytes/unit and retain 75% of the original red cells.
 - C. $<3.0 \times 10^6$ leukocytes/unit and retain 80% of the original red cells.
 - D. $<2.0 \times 10^6$ leukocytes/unit and retain 75% of the original red cells.
 - E. $<5.0 \times 10^6$ leukocytes/unit and retain 85% of the original red cells.
-

Question 7: RBC units can be washed to remove plasma components such as cytokines, potassium, or immunoglobulins. What can RBCs be washed and resuspended in?



- A. Lactated Ringer's solution.
 - B. Normal saline.
 - C. Sterile water.
 - D. One-half normal saline.
 - E. None of the above.
-

Question 8: Prophylactic platelet transfusions are indicated in older children who have platelet counts less than what volume?

- A. <10,000/ μ L.
 - B. <25,000/ μ L.
 - C. <50,000/ μ L.
 - D. <75,000/ μ L.
 - E. <100,000/ μ L.
-

Question 9: A 9-month-old infant with platelet count of 26,000/ μ L requires platelet transfusion for bleeding. The infant is group A. The only platelets in the blood bank are group O. What should be done?

- A. Release the desired amount of the group O platelets for transfusion.
 - B. Order a group A platelet from the blood supplier and wait for its arrival; keep the team updated.
 - C. Volume-reduce the group O platelets and then release for transfusion.
 - D. Call the attending physician and ask if the platelets are really needed and offer plasma instead.
 - E. None of the above.
-

Question 10: A 10-kg infant requires platelet transfusion and 100 mL of platelet is ordered for transfusion. The hospital transfusion service prepares a 100-mL aliquot for the infant in a syringe using a sterile connection device. How long does the service have to transfuse the aliquot?

- A. 1 hour.
- B. 2 hours.



- C. 3 hours.
 - D. 4 hours.
 - E. 6 hours.
-

Question 11: A child has an international normalized ratio (INR) of 1.3 and an order is placed for plasma. You are covering the transfusion service and are called regarding the order. The child is not bleeding and is set to go to interventional radiology for a line placement. What should you do?

- A. Approve the plasma.
 - B. Offer four-factor prothrombin complex concentrate (PCC) instead.
 - C. Call the ordering provider and explain that plasma has an INR of ~1.5 and would not correct the child's INR of 1.3. Then explain that an INR of 1.3 is not correlated with any increased rate of bleeding and it is safe to proceed.
 - D. Call the ordering provider and explain that plasma has an INR of ~1.5 and would not correct the child's INR of 1.3. Then explain that the INR should be corrected with vitamin K and the procedure should be delayed.
 - E. Call the ordering provider and explain that plasma has an INR of ~1.5 and would not correct the child's INR of 1.3. Then explain that cryoprecipitate instead of plasma could be used to correct the INR.
-

Question 12: Which statement is true?

- A. Plasma is indicated for routine use as a volume expander.
- B. Plasma can be used as a source of protein for nutritional support.
- C. Plasma can be associated with risk of viral transmission.
- D. Plasma should not be used as a replacement fluid in plasma exchange for thrombotic thrombocytopenic purpura (TTP).
- E. Plasma is not associated with a risk of transfusion-related acute lung injury (TRALI).



Question 13: Solvent/detergent-treated plasma has lower levels of certain proteins. Which of the following proteins are lower in solvent/detergent-treated plasma?

- A. Protein S, protein C, and IgA.
- B. Protein S, IgA, and α_2 -antiplasmin.
- C. Protein C, IgA, and α_2 -antiplasmin.
- D. Protein S, IgG, and α_2 -antiplasmin.
- E. Protein S, protein C, and IgG.



Question 14: Which statement is true regarding granulocyte transfusion?

- A. Granulocytes do not need to be crossmatched.
- B. Granulocytes must be transfused within 24 hours of collection.
- C. Granulocytes should not be irradiated.
- D. Granulocytes should be stored at 1-6 C.
- E. Granulocytes should be agitated during storage.



Question 15: Neonatal alloimmune thrombocytopenia (NAIT) is commonly caused by maternal antibodies against what antigen?

- A. HPA-1a.
- B. HPA-1b.
- C. HPA-5b.
- D. HPA-15b.
- E. HPA-3a.



Question 16: A 3.5-kg male infant is born at full term to a G1P1 mother. The infant is found to have bleeding from his umbilical stump and has petechia on his body. His platelet count is 9000/ μ L and he is diagnosed with neonatal alloimmune thrombocytopenia (NAIT). The team orders 55 mL of platelets. What should the transfusion lab do?

- A. Send a 55-mL aliquot of ABO-compatible platelets from the shelf.



- B. Collect the mother's platelets, wash them, and then give them to the infant.
 - C. Order HPA-1a-negative platelets from the blood supplier and wait for them to arrive.
 - D. Perform platelet antibody testing using the mother's plasma and then test all of the platelets on the shelf for compatibility.
 - E. Collect the father's platelets, wash them, and then give them to the infant.
-

Question 17: Which statement is *false*?

- A. Thrombin generation and clot lysis are reduced in neonates compared to older children and adults.
 - B. At birth, levels of Factors II, VII, IX, and X are ~50% of adult values.
 - C. Newborn levels of von Willebrand factor (vWF) are decreased.
 - D. Preterm neonates have decreased platelet activation, responsiveness, and granule secretion.
 - E. Newborns have plasminogen ~50% of adult levels.
-

Question 18: Nonimmune hemolysis can be caused by all of the following except?

- A. Artificial heart valve.
 - B. Mechanical pumps.
 - C. ECMO.
 - D. Use of small-gauge needle for RBC transfusion.
 - E. Concurrent transfusion of RBCs with normal saline IV fluid.
-

Question 19: Febrile nonhemolytic transfusion reactions occur less commonly in which group?

- A. Adults.
- B. Young children.
- C. Older children.
- D. Infants.
- E. The rate is equivalent in all groups.



Question 20: Pediatric patients who have never been transfused or never had a transfusion reaction should/should not receive premedication with acetaminophen and/or diphenhydramine.

- A. Should.
 - B. Should not.
 - C. Not sure, they both sound good.
 - D. It depends on whether there is a shortage of acetaminophen or diphenhydramine.
 - E. It depends on what component is being transfused.
-

Question 21: Which of the following groups below are at increased risk of hypocalcemia related to transfusion?

- A. Premature infants.
 - B. Children with liver disease.
 - C. Term infants.
 - D. A and B.
 - E. A, B, and C.
-

Question 22: A 7-year-old girl with sickle cell disease presents with a pain crisis. Her hemoglobin level upon admission is 6.5 g/dL. She receives 1 unit of crossmatch-compatible, ABO- identical RBCs that are matched for C/c, D, E/e, and Kell antigens. Her hemoglobin level following transfusion is 8.2 g/dL. Three days later, another unit of RBCs is requested and a type and screen test is performed. A new antibody is found to Jk^a. The DAT result is positive for IgG and complement. Her hemoglobin level is tested to check for evidence of hemolysis because the transfused unit was found to be Jk^a positive. Her hemoglobin level is found to be 5.5 g/dL. What is the most likely explanation?

- A. She has hemolyzed the unit of RBCs that were Jk^a positive.
- B. She is having another sickle crisis.
- C. She hemolyzed the recently transfused unit and also has hyperhemolysis.



- D. She is bleeding somewhere and should have a full body scan to determine the source.
 - E. The laboratory result is likely wrong and was from a different patient.
-

Question 23: Which group below is *not* at increased risk for significant cytomegalovirus (CMV) infection?

- A. Premature infants during infancy.
 - B. Recipients of intrauterine transfusions.
 - C. Organ transplant recipients.
 - D. Patients with congenital immunodeficiency diseases.
 - E. Patients with IgA deficiency.
-

Question 24: Which of the following are ways to reduce transfusion-transmitted CMV?

- A. Use prestorage, leukocyte-reduced blood components for transfusion.
 - B. Irradiate before transfusion.
 - C. Use only components from CMV-negative donors.
 - D. A, B, and C.
 - E. A and C.
-

Question 25: Which of the following patients should *not* receive irradiated blood components?

- A. 4-month-old male with severe combined immunodeficiency disease (SCID).
- B. 16-year-old male with acute lymphoblastic leukemia (ALL) who received an allogeneic hematopoietic stem cell transplant (allo-HSCT).
- C. 3-year-old female receiving transfusion of blood components from her parents.
- D. 17-year-old female with aplastic anemia who received antithymocyte globulin (ATG).
- E. 3-year-old male with respiratory syncytial virus (RSV) and asthma



Question 26: A 4-year-old female who received an allo-HSCT for acute myelogenous leukemia (AML) requires transfusion with platelets and RBCs. Which components below can she safely receive?

- I. Irradiated ABO-compatible RBCs.
 - II. Irradiated ABO-compatible RBCs from a CMV-negative donor.
 - III. Irradiated ABO-compatible platelets.
 - IV. Irradiated ABO-compatible platelets from a CMV-negative donor.
 - V. Irradiated ABO-compatible leukocyte-reduced (LR) RBCs.
 - VI. Irradiated ABO-compatible LR platelets.
 - VII. Irradiated ABO-compatible platelets that have been treated with an FDA-approved pathogen reduction system.
-
- A. All of the above.
 - B. II and IV only.
 - C. II, IV, and VII.
 - D. V and VI.
 - E. II, IV, V, VI, and VII.



Question 27: Residual leukocytes in blood components can be associated with all of the following except?

- A. Febrile nonhemolytic transfusion reactions.
- B. CMV infection.
- C. HLA alloimmunization.
- D. Allergic transfusion reactions.
- E. Immune modulation.



Question 28: A blood collection center tested 100 LR-RBC components and found eight that had large numbers of remaining leukocytes. Some had 5-10 times the upper limit for acceptable number of leukocytes remaining to be classified as leukocyte reduced. The donors of these units were further tested and tracked. Units from two of the donors had failed leukocyte reduction before and aggregated the filters. Another unit from one of the donors was discarded



as it, too, had clogged the filter and resembled currant jelly. What is most likely the problem?

- A. The donors have sickle cell trait.
 - B. The donors have thalassemia.
 - C. The donors have sickle cell disease.
 - D. There is a bad batch of filters causing the problem.
 - E. The donors have babesiosis.
-

Question 29: Platelets collected in platelet additive solution (PAS) contain how much less plasma compared to normal apheresis platelets?

- A. 20% less plasma.
 - B. 25% less plasma.
 - C. 33% less plasma.
 - D. 42% less plasma.
 - E. 50% less plasma.
-

Question 30: Which statement below about the ABC PICU study and the ARIPI trial is *false*?

- A. The ABC PICU study involved pediatric intensive care patients.
 - B. The ARIPI trial studied premature infants.
 - C. Both studies found that the age of blood is associated with increased mortality in children and neonates.
 - D. Both studies found that the age of blood is not associated with increased mortality in children and neonates.
 - E. In these studies, there was no control for the additive or anticoagulant solution used.
-

Question 31: Which of the following conditions has been associated with development of necrotizing enterocolitis in neonates?

- A. Transfusion of RBCs.
- B. Transfusion of platelets.
- C. Transfusion of plasma.



- D. Anemia.
 - E. All of the above.
-

Question 32: Some physicians prefer to transfuse neonates with RBCs collected in CPDA-1, CP2D, or CPD instead of using RBCs that have additive solution (AS) added. Why?

- A. The units would be more concentrated.
 - B. The units do not contain mannitol.
 - C. The units have little or no adenine.
 - D. There is less dextrose.
 - E. All of the above.
-

Question 33: A 10-year-old girl with AML is undergoing an allo-HSCT. She is blood group O-positive and the donor is blood group A-positive. What RBCs, platelets, and plasma should she receive before complete engraftment?

- A. Group A RBCs, group AB plasma, and group A platelets.
 - B. Group O RBCs, group O plasma, and group O platelets.
 - C. Group O RBCs, group A plasma, and group A platelets.
 - D. Group A RBCs, group AB plasma, and group O platelets.
 - E. Group O RBCs, group O plasma, and group A platelets.
-

Question 34: A 14-year-old boy with ALL is undergoing an allo-HSCT. He is group B-positive and the donor is group A-positive. What RBCs, platelets, and plasma should he receive before complete engraftment?

- A. Group A RBCs, group AB plasma, and group A platelets.
- B. Group O RBCs, group O plasma, and group O platelets.
- C. Group B RBCs, group AB plasma, and group AB platelets.
- D. Group O RBCs, group AB plasma, and group AB platelets.
- E. Group O RBCs, group AB plasma, and group O platelets.



Question 35: A 7-year-old girl with hypoplastic left heart is scheduled for a heart transplant. She is group O-positive and the donor heart is group A-positive. Which RBCs, platelets, and plasma should be prepared?

- A. Group A RBCs, group AB plasma, and group A platelets.
 - B. Group O RBCs, group O plasma, and group O platelets.
 - C. Group O RBCs, group O plasma, and group A platelets.
 - D. Group A RBCs, group AB plasma, and group O platelets.
 - E. Group O RBCs, group A plasma, and group A platelets.
-

Question 36: According to AABB Standard 5.17.1.3, how long should records of selection of compatible units be kept when initial antibody testing for a neonate shows clinically significant antibodies?

- A. 1 year.
 - B. 3 years.
 - C. 7 years.
 - D. 10 years.
 - E. 18 years.
-

Question 37: According to AABB Standard 5.14.9(3), how long should records of difficulty in blood typing, clinically significant antibodies, significant adverse events to transfusion, and special transfusion requirements should be kept?

- A. 1 year.
 - B. 3 years.
 - C. 7 years.
 - D. 10 years.
 - E. Indefinitely.
-

Question 38: According to AABB Standard 5.1.8.1.3.1, if granulocytes are kept in an open storage area and the ambient temperature recorded every 4 hours what is the minimum amount of time this record should be kept?



- A. 1 year.
 - B. 3 years.
 - C. 7 years.
 - D. 10 years.
 - E. Indefinitely.
-

Question 39: The recently completed Transfusion of Prematures (TOP) trial found which of the following to be *false*?

- A. There were no differences between the liberal and restrictive arms in terms of neurodevelopment (ND).
 - B. The lower hemoglobin threshold arm had higher rates of retinopathy of prematurity stage ≥ 3 .
 - C. There were no differences in terms of death or disability at 22–26 months of corrected age.
 - D. There were no differences between the two arms in terms of grade 3 or 4 intraventricular hemorrhage, cystic periventricular leukomalacia, or ventriculomegaly.
 - E. There were no differences between the two arms in terms of development of necrotizing enterocolitis.
-

Question 40: The recently completed effects of liberal vs restrictive transfusion thresholds on survival and neurocognitive outcomes in extremely low birthweight infants (ETTNO study) found which of the following to be *false*?

- A. There were no differences between the liberal and restrictive arms in terms of neurodevelopment (ND).
- B. There were no differences in terms of death or disability at 24 months of corrected age.
- C. There were no differences between the liberal and restrictive arms in terms of cerebral palsy or cognitive impairment.
- D. There were no differences between the liberal and restrictive arms in terms of severe visual or hearing impairment.
- E. The rate of necrotizing enterocolitis was higher in the liberal transfusion group.



Question 41: Which of the following is the blood volume of a full-term infant?

- A. 50 mL/kg.
 - B. 60 mL/kg.
 - C. 75 mL/kg.
 - D. 85 mL/kg.
 - E. 100 mL/kg.
-

Question 42: A full-term infant is found to have a bilirubin level of 28 mg/dL and an exchange transfusion is ordered. The infant weighs 3.25 kg and a two-volume exchange transfusion is ordered. What volume of RBCs resuspended in ABO-compatible plasma should be prepared?

- A. 510 mL.
 - B. 553 mL.
 - C. 455 mL.
 - D. 420 mL.
 - E. 650 mL.
-

Question 43: Several studies have shown that in sickle cell disease, the risk of recurrent stroke is decreased to less than 10% if hemoglobin levels are maintained at what levels and hemoglobin S is maintained at what percentage?

- A. 6-7 g/dL and <30%.
 - B. 9-10 g/dL and <50%.
 - C. 8-9 g/dL and <10%.
 - D. 8-9 g/dL and <30%.
 - E. 9-10 g/dL and <10%.
-

Question 44: In sickle cell disease patients, most red cell antibodies are directed against which antigens?

- A. Rh and Ss.
- B. Rh and U.



- C. Rh, Kell, Duffy, and Kidd.
 - D. Rh, Ss, and Lewis.
 - E. Kell and Lewis.
-

Question 45: Which of the following sentences regarding coagulation parameters in preterm neonates and full-term neonates are true?

- A. The prothrombin time (PT), activated partial thromboplastin-time (aPTT), and thrombin time are longer in preterm neonates compared to term neonates.
 - B. The PT, aPTT, and thrombin time are shorter in preterm neonates compared to term neonates.
 - C. The PT and aPTT are longer and the thrombin time is shorter in preterm neonates compared to term neonates.
 - D. The PT and aPTT are shorter and the thrombin time is longer in preterm neonates compared to term neonates.
 - E. The PT and thrombin time are longer and the aPTT is shorter in preterm neonates compared to term neonates.
-

Question 46: Compared to older children or adults, the clotting time and clot formation time in ROTEM/TEG for neonates are which?

- A. The same.
 - B. Shorter.
 - C. Longer.
 - D. The clotting time is longer and the clot formation time is shorter.
 - E. The clotting time is shorter and the clot formation time is longer.
-

Question 47: Which statement below is true?

- A. Newborn infants have increased erythropoietin (EPO) production in response to hypoxia in contrast to older children.
- B. Newborns have decreased EPO production in response to hypoxia in contrast to older children.



- C. Premature neonates produce more EPO compared to term infants in response to hypoxia.
 - D. Premature neonates produce less EPO compared to term infants in response to hypoxia.
 - E. B and D.
-

Question 48: Neonates are very sensitive to hypothermia and blood warmers are frequently used for large-volume transfusions or exchanges. Hypothermia in neonates can worsen or exaggerate which of the following?

- A. Hypoglycemia.
 - B. Increased metabolic rate.
 - C. Metabolic acidosis.
 - D. Apneic events.
 - E. All of the above.
-

Question 49: Which of the following is a problem with storing platelets in a syringe for a neonate?

- A. The platelets get activated.
 - B. The pH increases.
 - C. The pH decreases.
 - D. The amount of microparticles drastically increases.
 - E. The platelets aggregate.
-

Question 50: An RBC unit with an expiration date in 28 days is used in a closed system with a sterile connection device to make an aliquot of 38 mL for a neonate. What is the new expiration date for the aliquot of red cells?

- A. 4 hours.
- B. 24 hours.
- C. 7 days.
- D. 28 days.
- E. 6 hours.



ANSWERS

Question 1: C. 2-3 g/dL.

Explanation:

- A transfusion of 10-15 mL/kg of red cells should raise the hemoglobin level of an infant or small child 2-3 g/dL.
-

Question 2: D. 5-10 mL/kg.

Explanation:

- A transfusion of 5-10 mL/kg of platelets should raise the platelet count of an infant or small child by 50,000-100,000/ μ L.
-

Question 3: C. 10-15 mL/kg.

Explanation:

- A transfusion of 10-15 mL/kg of FFP/thawed plasma should raise the factor level by 15-20% in an infant or small child.
-

Question 4: D. 1-2 units/10 kg.

Explanation:

- Transfusion with 1-2 units/10 kg of cryoprecipitate is expected to raise the fibrinogen level of an infant or small child by 60-100 mg/dL.
-

Question 5: D. Older components have higher levels of extracellular potassium.

**Explanation:**

- The main reason fresher components are preferred for neonates and children undergoing massive transfusion as occurs with ECMO, exchange transfusion, or cardiopulmonary bypass is the large amount of extracellular potassium that is in older RBC components.
 - All of the other options are also true but are not the primary reason for preference of fresher RBC components in young patients.
-

Question 6: E. $<5.0 \times 10^6$ leukocytes/unit and retain 85% of the original RBCs.

Explanation:

- The preferred time to perform leukocyte reduction is following collection and before storage. If the leukocytes are removed before storage, the amount of cytokines that accumulate during storage is reduced.
 - Thus, prestorage leukocyte reduction is associated with a lower rate of febrile transfusion reactions compared to poststorage leukocyte reduction.
-

Question 7: B. Normal saline.

Explanation:

- RBCs can be washed and resuspended in normal saline. Usually, the RBCs are resuspended in a final volume of ~220 mL for a final hematocrit of 70-80%.
 - When the RBCs are washed in an open system, they can be stored for 24 hours at 1-6 C.
-

Question 8: A. $<10,000/\mu\text{L}$.

**Explanation:**

- Prophylactic platelet transfusion is indicated in older children without bleeding who have platelet counts <10,000/ μ L.
 - It is frequently recommended to maintain the platelet count >50,000/ μ L in older children who are actively bleeding or undergoing major surgery.
 - Lastly, in older children with central nervous system (CNS) bleeding or planned CNS surgery, it is recommended to maintain the platelet count >75,000-100,000/ μ L.
-

Question 9: C. Volume-reduce the group O platelet unit and then release for transfusion.

Explanation:

- The best option is to quickly volume-reduce or perform saline replacement on the group O platelet unit and release it. This will minimize resultant hemolysis of the infant's group A red cells by the anti-A in the plasma of the platelet unit.
 - If the infant required a platelet transfusion and the transfusion medicine doctor and attending doctor both thought there was not enough time to volume-reduce, releasing the group O platelets and monitoring for hemolysis could be an option.
 - Because the infant is bleeding and has a low platelet count, ordering group A platelets and waiting is also not the best option. Depending upon the blood supplier location and availability of platelet products, this could take several hours.
 - Offering plasma instead of platelets is also not the best choice.
-

Question 10: D. 4 hours.

Explanation:

- The platelet aliquot is stable and acceptable for transfusion for up to 4 hours.



- Notably, blood manufacturers cannot create aliquots because there are no FDA-approved systems for the purpose of aliquot creation.
-

Question 11: C. Call the ordering provider and explain that plasma has an INR of ~1.5 and would not correct the child's INR of 1.3. Then explain that an INR of 1.3 is not correlated with any increased rate of bleeding and it is safe to proceed.

Explanation:

- The provider is safe to proceed and should not be offered four-factor PCC or cryoprecipitate.
 - If the child had been on warfarin, administration of vitamin K could help to correct the INR.
-

Question 12: C. Plasma can be associated with risk of viral transmission.

Explanation:

- The other statements are all false.
 - Plasma should not be used for nutritional support or as a volume expander as safer options are available for both of these indications that are not associated with risk of TRALI or infectious disease transmission.
 - Plasma is the replacement fluid of choice during plasma exchange for TTP.
-

Question 13: B. Protein S, IgA, and α_2 -antiplasmin.

Explanation:

- Solvent/detergent-treated plasma has lower levels of protein S, IgA, and α_2 -antiplasmin. Thus, it should not be used in certain settings such as treatment of congenital protein S deficiency.



Question 14: B. Granulocyte products must be transfused within 24 hours of collection.

Explanation:

- Granulocytes outdate 24 hours after collection. They should be stored at room temperature without agitation.
 - Due to the large volume of red cells in granulocytes, crossmatching is required.
 - Irradiation should also be performed to prevent transfusion-associated graft-vs-host disease (TA-GVHD).
-

Question 15: A. HPA-1a.

Explanation:

- NAIT is most commonly attributable to antibodies against HPA-1a particularly in people of European ancestry.
 - The most common cause of NAIT in individuals of Asian ancestry is HPA-4b, and in people of African ancestry, HPA-2 and HPA-5 are commonly indicated as the cause.
 - Incompatibility to HPA-1 occurs in ~2% of pregnancies and antibodies result in 5-10% of cases. Overall, the incidence of NAIT is estimated at 1-10/10,000 live births.
-

Question 16: A. Send a 55-mL aliquot of ABO-compatible platelets from the shelf.

Explanation:

- The correct thing to do is to select an ABO-compatible platelet unit from the shelf, aliquot 55 mL, and send it to the NICU for the patient.
- The mother's platelets would be negative for the antigen; however, the collection, washing, and preparation would take a long time and not all centers can collect the mother's platelets.



- You could assume that the cause of the NAIT is HPA-1a and order negative platelets, but it typically takes several days to get HPA-1a negative platelets.
 - Collection of the father's platelets would not be the best option as off-the-shelf platelets likely also have the antigen and are readily available and have been tested for infectious disease.
 - The mother's plasma should be tested for the identity of the antibody; however, this is not needed to dispense platelets. The platelets off the shelf will likely get bound by the circulating antibodies until no more antibodies exist and then the platelet count will start rising.
-

Question 17: C. Newborn levels of vWF are decreased.

Explanation:

- All of the statements are true except C. Newborns actually have higher levels of vWF.
 - Thrombin generation and clot lysis are reduced in neonates compared to older children and adults, and at birth, levels of Factors II, VII, IX, and X are ~50% of adult values.
 - At birth, plasminogen levels are also ~50% of adult levels.
 - Lastly, preterm neonates have decreased platelet activation, responsiveness, and granule secretion.
-

Question 18: E. Concurrent transfusion with normal saline IV fluid.

Explanation:

- Use of fluids other than saline will result in hemolysis.
- Red cells can be physically damaged by high infusion pressure, overheating, freezing, or microbe contamination.
- Red cells can be mechanically damaged by ECMO, artificial heart valves, mechanical pumps, dialysis, and/or apheresis procedures.
- Lastly, transfusion through smaller-gauge needles can result in red cell damage and hemolysis.

**Question 19: D. Infants.****Explanation:**

- Febrile nonhemolytic transfusion reactions (FNHTRs) occur infrequently in infants.
 - Children are actually believed to have more FNHTRs than adults.
 - FNHTRs are attributed to cytokines in the blood component as well as antibodies present in the recipient's plasma that are directed against transfused leukocytes.
-

Question 20: B. Should not.**Explanation:**

- Many studies have investigated premedication in children and adults.
 - Premedication in pediatric or adults with no history of transfusion or transfusion reactions is not effective at reducing allergic or FNHTR transfusion reactions.
 - Moreover, premedication can delay recognition of a transfusion reaction or delay diagnosis of sepsis.
-

Question 21: D. A and B.**Explanation:**

- Children with liver disease are more likely to develop hypocalcemia because citrate in the blood components is metabolized largely via the liver.
 - Additionally, preterm infants have a blunted parathyroid response to hypocalcemia compared to term infants.
-

Question 22: C. She hemolyzed the recently transfused unit and also has hyperhemolysis.

**Explanation:**

- Hyperhemolysis is a condition that can be more frequently seen in sickle cell patients, where following a delayed hemolytic transfusion reaction as seen here with the Jk^a antibody, the patient also has bystander hemolysis of their own cells. The bystander hemolysis is called hyperhemolysis.
 - Avoiding further transfusion unless absolutely necessary for life-saving measures is a treatment for hyperhemolysis.
 - The C5 antibody, eculizumab, has also been used to treat hyperhemolysis.
-

Question 23: E. Patients with IgA deficiency.

Explanation:

- Patients with IgA deficiency are not at increased risk for significant CMV infection. All of the other groups are at increased risk.
 - Additionally, patients receiving an allogeneic hematopoietic stem cell transplant, cancer patients receiving intense chemotherapy such as for acute leukemias, and fetuses of CMV-negative females are also at risk for CMV infection.
-

Question 24: E. A and C.

Explanation:

- Two ways to reduce transfusion-transmitted CMV is to use prestorage leukocyte-reduced blood components and/or to use components from donors who test CMV negative.
 - Irradiation of blood components does not affect CMV transmission.
-

Question 25: E. 3-year-old male with RSV and asthma.

**Explanation:**

- All of the patients in answer choices A, B, C, and D should receive irradiated components. The 3-year-old with asthma and RSV has no indication for irradiated blood components.
-

Question 26: E. II, IV, V, VI, and VII.**Explanation:**

- The patient can safely receive irradiated ABO-compatible components that have either been leukocyte reduced (LR) or collected from CMV-negative donors.
 - She can also receive platelets that are irradiated and not leukocyte reduced if they have been treated with an FDA-approved pathogen reduction system.
-

Question 27: D. Allergic transfusion reactions.**Explanation:**

- Residual leukocytes in blood components are associated with all of the responses except allergic transfusion reactions.
-

Question 28: A. The donors have sickle cell trait.**Explanation:**

- Sickle trait can cause the LR filters to clog and form aggregates.
- RBC components from sickle cell trait donors that have successfully passed through LR filters have been found to have abnormally elevated levels of leukocytes.
- Thalassemia and babesiosis would not cause this scenario and it is highly unlikely that 7% of donors would have sickle cell disease.



Question 29: C. 33% less plasma.

Explanation:

- Platelets collected with PAS are associated with decreased febrile and allergic transfusion reactions.



Question 30: C. Both studies found that the age of blood is associated with increased mortality in children and neonates.

Explanation:

- All of the statements are true except for choice C. These studies found that there was no association between the age of blood and mortality in the studied population.
- However, there are a few caveats with the studies that have precluded wide acceptance of using any age of blood.
 - The studies did not include many populations of children that could be very sensitive to the age of blood such as children undergoing cardiovascular surgery, oncology patients, and sickle cell disease patients.
 - The ARIPI trial did not designate thresholds for transfusion of RBCs.
 - Another issue with the ARIPI trial was that most RBCs used were not older than 21 days. Thus, by convention, the components were relatively young because “old” components between 35-42 days were not brought into comparison for the study.



Question 31: D. Anemia.

Explanation:

- Anemia has been found to be associated with development of necrotizing enterocolitis in neonates, not transfusion.



Question 32: E. All of the above.



Explanation:

- The adenine and mannitol that are present in additive solution (AS) have been shown at high concentrations to be associated with nephrotoxicity in lab animals.
 - Additionally, mannitol has diuretic effects and could affect the neonate's intracerebral pressure.
 - AS has additional amounts of dextrose and neonates have been shown to have difficulty regulating dextrose.
 - The units without AS would be more concentrated as well and thus have less volume for transfusion which is helpful in avoiding TACO.
 - RBC units without AS are difficult to find and typically must be requested from the blood supplier.
 - There has not been a clinical trial comparing transfusion of AS-containing RBC units to non-AS units. Thus, most usage is based upon physician preference and ability to obtain it.
-

Question 33: C. Group O RBCs, group A plasma, and group A platelets.

Explanation:

- She should not receive group O platelets or group O plasma because it will contain anti-A, which could cause hemolysis.
-

Question 34: D. Group O RBCs, group AB plasma, and group AB platelets.

Explanation:

- He should not receive group A or B plasma because it will contain anti-A or anti-B, which could cause hemolysis of his group B red cells or of his newly made group A red cells.
-

Question 35: E. Group O RBCs, group A plasma, and group A platelets.

**Explanation:**

- She could also receive group AB plasma and /or group AB platelets, but these are much harder to come by.
 - She should not receive group O plasma or group O platelets because they will contain anti-A, which could bind to group A antigens on the donor heart.
-

Question 36: D. 10 years.**Explanation:**

- Records of the intrauterine transfusion policy and records of cellular component irradiation should also be retained for a minimum of 10 years.
-

Question 37: E. Indefinitely.**Explanation:**

- These records should be kept indefinitely.
-

Question 38: D. 10 years.**Explanation:**

- This record should be retained for a minimum of 10 years.
-

Question 39: B. The lower hemoglobin threshold arm had higher rates of retinopathy of prematurity stage ≥ 3 .**Explanation:**

- The Transfusion of Prematures (TOP) trial was a multicenter, randomized, controlled trial conducted in 41 neonatal intensive



care units (NICUs). This study enrolled 1824 infants who were <1000 g (mean birthweight, 756 g; mean gestational age, 25.9 weeks). The investigators sought to evaluate whether liberal vs restrictive transfusion in extremely low-birthweight infants improves a composite of death or neurodevelopmental impairment (cognitive delay, cerebral palsy, or hearing or vision loss) at 22 to 26 months of age, corrected for prematurity.

- There were no differences between the liberal and restrictive arms in terms of neurodevelopment (ND).
- There were no differences in terms of death or disability at 22-26 months of corrected age.
- There were no differences between the two arms in terms of grade 3 or 4 intraventricular hemorrhage, cystic periventricular leukomalacia, or ventriculomegaly.
- There were no differences between the two arms in terms of development of necrotizing enterocolitis.
- There was essentially no difference in the rate of retinopathy of prematurity stage ≥ 3 (however there were slightly more cases in the higher transfusion threshold group).
- It is notable that the actual differences in pretransfusion mean hemoglobin values between the two groups was only 1.9 g/dL.



Question 40: E. The rate of necrotizing enterocolitis was higher in the liberal transfusion group.

Explanation:

- The ETTNO trial was a randomized clinical trial conducted in 36 level III/IV NICU units in Europe. The investigators studied 1013 infants with birthweights of 400 g to 999 g at less than 72 hours after birth. They sought to evaluate whether liberal vs restrictive transfusion in extremely low-birthweight infants improves neurodevelopmental outcome at 24 months of corrected age.
- There were no differences between the liberal and restrictive arms in terms of neurodevelopment (ND).
- There were also no differences in terms of death or disability at 24 months of corrected age.
- There were no differences between the liberal and restrictive arms in terms of cerebral palsy or cognitive impairment.



- There were no differences between the liberal and restrictive arms in terms of severe visual or hearing impairment, and there were essentially no differences between the two arms in terms of necrotizing enterocolitis.



Question 41: D. 85 mL/kg.

Explanation:

- The blood volume of a full-term infant is 85 mL/kg. The blood volume of a preterm infant is 100 mL/kg. The blood volume of an adult is estimated at 70 mL/kg.



Question 42: B. 553 mL.

Explanation:

- The infant is full term and a blood volume of 85 mL/kg would be used.

$$85 \text{ mL/kg} \times 3.25 \text{ kg} = 276.25 \text{ mL} \times 2 = 553 \text{ mL.}$$



Question 43: D. 8-9 g/dL and <30%.

Explanation:

- Transfusion has been shown to be more effective than hydroxyurea and phlebotomy or observation in children with sickle cell disease and silent cerebral infarcts.
- Red cell exchange is more effective than transfusion in reducing iron overload.



Question 44: C. Rh, Kell, Duffy, and Kidd.

**Explanation:**

- In patients with sickle cell disease, most red cell antibodies that are developed are typically against the Rh, Kell, Duffy, and Kidd systems. Thus, most hospitals/blood banks perform extended RBC phenotype for each sickle cell patient before beginning transfusion therapy and provide antigen-matched RBCs.
 - The standard of care is to provide RBCs matched for Rh and Kell; however, some facilities provide more extended matched RBCs.
-

Question 45: A. The PT, aPTT, and thrombin time are longer in preterm neonates as compared to term neonates.

Explanation:

- The aPTT is longer in neonates compared to older children or adults and the PT and thrombin time are the same or longer in neonates as compared to older children or adults.
-

Question 46: B. Shorter.

Explanation:

- The clotting time and clot formation time is the same in preterm neonates and full-term neonates.
 - However, both of these are shorter in neonates compared to older children or adults. Adult values for these parameters are reached around 3 months of age.
-

Question 47: E. B and D.

Explanation:

- Newborns have decreased EPO production in response to hypoxia in contrast to older children.
- Premature neonates produce less EPO compared to term infants in response to hypoxia. This is likely to prevent polycythemia in



the intrauterine environment which is hypoxic. In preterm infants, EPO production is liver based and also contributes to the low levels of EPO.

- Typically, EPO production does not occur in the kidney until around the time of full-term delivery.



Question 48: E. All of the above.

Explanation:

- Hypothermia in a neonate can worsen or exaggerate hypoglycemia, increased metabolic rate, metabolic acidosis, and apneic events.



Question 49: C. The pH decreases.

Explanation:

- The pH of platelets in a syringe system has been shown to decrease rapidly. Thus, components for neonates should be prepared as close to as possible to when they will be dispensed and transfused for neonates, particularly with regards to platelets in syringes.



Question 50: D. 28 days.

Explanation:

- Because a sterile connection device was used and a closed system was kept, the expiration date of the aliquot is the same as the original unit.



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14

Hemapheresis

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Key Points from the *Technical Manual*

- Therapeutic apheresis treats diseases by removal or extra-corporeal manipulation of pathologic plasma constituents, white cells, platelets, or red cells.
- The apheresis process can be accomplished by accomplished by the use of centrifugation, filtration, selective adsorption, or photopheresis.
- Medical evaluation of the therapeutic apheresis patient should include the disease indication; type of procedure; frequency, number, and duration of treatments; therapeutic goal; patient tolerance of the procedure; vascular access; replacement fluids; and medications.
- The American Society for Apheresis publishes updated guidelines and recommendations approximately every 3 years for the use of apheresis in clinical practice.
- Adverse effects of apheresis can include hypocalcemia, hypotension, urticaria, and nausea. Other consequences of apheresis may include coagulopathy, hypogammaglobulinemia, and removal of certain drugs and biologics.



QUESTIONS

Question 1: During the process of apheresis, using centrifugation to separate blood components, which component is located closest to the axis of rotation?

- A. Plasma.
 - B. Platelets.
 - C. Lymphocytes.
 - D. Red cells.
 - E. Granulocytes.
-

Question 2: In continuous-flow centrifuge apheresis, how do the elements of blood separate?

- A. Size.
 - B. Granularity.
 - C. Specific gravity.
 - D. Density.
 - E. Viscosity.
-

Question 3: The American Society for Apheresis (ASFA) classifies diseases/disorders treated by apheresis to assist in determining the appropriateness of treating a patient with this modality. One of the categories is defined as “Disorders for which apheresis is accepted as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment.” Which one of the following is defined as above?

- A. Category I.
- B. Category II.
- C. Category III.
- D. Category IV.
- E. None of the above.



Question 4: Which of the following statements is true for continuous-flow centrifugation separators?

- A. Blood is processed continuously, with individual layers being removed and retained or returned to the patient/donor.
 - B. They have a higher rate of citrate toxicity than intermittent-flow centrifugation separators.
 - C. Procedures performed on continuous-flow centrifugation separators are shorter than those using intermittent-flow centrifugation separators when processing the same amount of blood.
 - D. A and C are both true.
 - E. A, B, and C are all true.
-

Question 5: In therapeutic plasma exchange (TPE), what percent of substance in the plasma is removed in a 1.0-volume plasma exchange?

- A. 33%.
 - B. 50%.
 - C. 66%.
 - D. 72%.
 - E. 90%.
-

Question 6: Which blood component is removed during a leukapheresis procedure?

- A. Plasma.
 - B. Platelet-rich plasma.
 - C. Red cells.
 - D. Buffy coat.
 - E. Cholesterol and triglycerides.
-

Question 7: Which type of processing is used during rheopheresis?

- A. Continuous centrifugation.
- B. Intermittent centrifugation.
- C. Fractionation.



- D. Gravity separation.
 - E. Filtration.
-

Question 8: Which of the following statements concerning leukocytapheresis (therapeutic leukapheresis) is true?

- A. It may be used instead of chemotherapy to treat all patients with chronic myelogenous leukemia (CML).
 - B. It can be used to prevent tumor lysis syndrome in patients with acute myelogenous leukemia (AML).
 - C. It is associated with improved long-term survival in patients with AML.
 - D. It is most often performed in the setting of acute lymphoblastic leukemia (ALL).
 - E. It is frequently used in patients with chronic lymphocytic leukemia (CLL) to lower the white cell count instead of chemotherapy.
-

Question 9: Which of the following statements about red cell exchange is true?

- A. It is routinely used to treat uncomplicated malaria.
 - B. It is routinely used to treat uncomplicated babesiosis.
 - C. It has more long-term complications than surgery when used to treat priapism in sickle cell disease.
 - D. Red cell exchange is superior to transfusion in sickle cell patients with a history of stroke.
 - E. Transfusion is superior to red cell exchange in the treatment of acute chest syndrome in sickle cell disease.
-

Question 10: Which of the following statements regarding the use of red cell exchange for sickle cell disease complications is true?

- A. The fraction of cells remaining (FCR) should be 50%.
- B. Postprocedure hemoglobin S should be 10% or less.
- C. The red cells used for replacement should be screened for the presence of hemoglobin S.



- D. The final hematocrit target at the end of the procedure should be 42%.
 - E. The red cells should be phenotypically matched for all known red cell antigens.
-

Question 11: Fresh Frozen Plasma (FFP) is used as a replacement fluid in TPE procedures for which condition?

- A. Thrombotic thrombocytopenic purpura (TTP).
 - B. Myasthenia gravis.
 - C. Chronic inflammatory demyelinating polyneuropathy.
 - D. Lambert-Eaton myasthenic syndrome.
 - E. All of the above.
-

Question 12: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, TPE is what level of indication for acute inflammatory demyelinating polyradiculoneuropathy [Guillain-Barré syndrome (GBS)]?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. TPE is never used to treat this disorder.
-

Question 13: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, TPE is what level of indication for transplant-associated thrombotic microangiopathy (TA-TMA)?

- A. Category I.
- B. Category II.
- C. Category III.
- D. Category IV.
- E. TPE is never used to treat this disorder.



Question 14: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, TPE is what level of indication for N-methyl D-aspartate receptor (NMDAR) encephalitis?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. TPE is never used to treat this disorder.
-

Question 15: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, TPE is what level of indication for acute attack or relapse of neuromyelitis optica spectrum disorder (NMOS)?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. TPE is never used to treat this disorder.
-

Question 16: Which statements regarding extracorporeal photopheresis (ECP) are *false*?

- A. ECP should not be performed in patients with aphakia.
 - B. ECP patients should wear sunglasses and sunscreen for 24 hours after each procedure.
 - C. There is a risk of venous thromboembolism in patients receiving ECP.
 - D. ECP can be used to treat graft-vs-host disease (GVHD) in patients after hematopoietic stem cell transplantation (HSCT).
 - E. ECP can effectively be performed on patients with low white cell counts and anemia.
-

Question 17: Which of the following is an advantage of immunoadsorption over TPE?



- A. Immunoadsorption does not require a replacement fluid such as 5% albumin or FFP.
 - B. Immunoadsorption is associated with increased risk of adverse events.
 - C. Immunoadsorption is associated with increased risk of transfusion transmitted diseases.
 - D. Immunoadsorption is associated with increased bleeding risk.
 - E. Immunoadsorption is associated with more allergic reactions.
-

Question 18: According to the ASFA Guidelines on the Use of Therapeutic Apheresis in Clinical Practice, TPE is what level of indication for diffuse alveolar hemorrhage in anti-glomerular basement membrane (anti-GBM) disease?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. TPE is never used to treat this disorder.
-

Question 19: According to the ASFA Guidelines on the Use of Therapeutic Apheresis in Clinical Practice, TPE is what level of indication for severe cold agglutinin disease (CAD) and severe warm autoimmune hemolytic anemia (WAIHA)?

- A. Category II for both.
 - B. Category II for WAIHA and Category III for CAD.
 - C. Category III for WAIHA and Category II for CAD.
 - D. Category III for both.
 - E. Category IV for both.
-

Question 20: According to the ASFA Guidelines on the Use of Therapeutic Apheresis in Clinical Practice, red cell exchange is what level of indication for severe babesiosis infection?

- A. Category I.
- B. Category II.



- C. Category III.
 - D. Category IV.
 - E. Red cell exchange is never used to treat this disorder.
-

Question 21: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, ECP is what level of indication for erythrodermic mycosis fungoides/Sézary syndrome?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. ECP is never used to treat this disorder.
-

Question 22: According to the ASFA *Guidelines on the Use of Therapeutic Apheresis in Clinical Practice*, ECP is what level of indication for acute GVHD following allogeneic HSCT?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. ECP is never used to treat this disorder.
-

Question 23: Which type of psoralen is used in ECP?

- A. 4-MOP.
 - B. 6-MOP.
 - C. 8-MOP.
 - D. 10-MOP.
 - E. 12-MOP.
-

Question 24: If the protein to be removed is IgG instead of IgM, what is the impact on TPE?



- A. Saline is indicated as the replacement fluid.
 - B. Increased fraction removed.
 - C. Red cells should be used for priming the instrument.
 - D. Increased fraction remaining.
 - E. Plasma is indicated as the replacement fluid.
-

Question 25: Which of the following statements about the pathophysiology of TTP is true?

- A. A normal distribution of von Willebrand factor (vWF) multimers is present in the plasma during active disease.
 - B. Activity of ADAMTS13, a metalloproteinase that cleaves multimers of vWF, is deficient in many cases of TTP.
 - C. The thrombi in the microvasculature consist of platelets, fibrin, and other activated coagulation factors.
 - D. Idiopathic TTP does not recur.
 - E. An autoantibody that decreases ADAMTS13 activity is present in all cases of congenital TTP (Upshaw-Schulman syndrome).
-

Question 26: A 72-year-old woman with Waldenström macroglobulinemia (WM) is seen in the clinic for chronic nose bleeds, headaches, and blurry vision. You are called for advice regarding TPE for hyperviscosity. Which of the following is correct?

- A. Waldenström macroglobulinemia symptoms are caused by elevated levels of IgG.
 - B. Patients become symptomatic even with minor elevations in viscosity.
 - C. IgG tends to cause hyperviscosity symptoms at the lowest concentration (relative to IgA and IgM).
 - D. IgM tends to cause hyperviscosity symptoms at the lowest concentration (relative to IgG and IgA).
 - E. Measurement of plasma viscosity is useful in guiding therapy.
-

Question 27: TPE is recommended as first-line therapy in which of the following diseases?



- A. Complement-mediated thrombotic microangiopathy due to mutations in complement genes.
 - B. Coagulation-mediated thrombotic microangiopathy due to mutation in THBD, DGKE, and/or PLG.
 - C. Complement-mediated thrombotic microangiopathy due to autoantibody to Factor H.
 - D. Infection-associated thrombotic microangiopathy.
 - E. Transplant-associated thrombotic microangiopathy.
-

Question 28: Anticoagulation with heparin is required for which procedure below?

- A. Low-density lipoprotein (LDL) apheresis.
 - B. TPE.
 - C. Red cell exchange.
 - D. Hematopoietic stem cell collection.
 - E. Therapeutic leukocyte reduction.
-

Question 29: A 29-year-old woman with primary biliary cirrhosis is suffering from pruritus that is unresponsive to medical management. Her gastroenterologist contacts you to ask about the role of TPE in this patient. What ASFA category indication is this?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. It is not listed in the ASFA guidelines.
-

Question 30: Hyperviscosity syndrome may occur in patients with markedly elevated plasma protein levels. Which of the following statements is true of hyperviscosity syndrome and its treatment?

- A. It is commonly seen with polyclonal increases in immunoglobulins.
- B. The decision to institute TPE should be based on serum viscosity levels.



- C. It more commonly occurs with multiple myeloma than with Waldenström macroglobulinemia.
 - D. Concurrent therapy with chemotherapeutic agents is not indicated when initiating TPE for hyperviscosity.
 - E. Waldenström macroglobulinemia resistant to chemotherapy has been reported to be effectively treated with TPE for extended periods.
-

Question 31: A 22-year-old female was in a car accident and received 6 units of group O-positive Red Blood Cells (RBCs). The following day, the resident taking care of her calls you to ask about anti-D prophylaxis. The patient is group B negative and she hoping to have children when she is older. You estimate that approximately 50% of her circulating red cells are currently Rh positive. What is the thought process for performing a red cell exchange for this patient?

- A. Remove all Rh-positive cells, eliminating the need for anti-D therapy.
 - B. Achieve an FCR that is unlikely to cause alloimmunization.
 - C. Achieve an FCR that can be treated with anti-D therapy.
 - D. Remove Rh-positive cells, replacing with phenotype matched cells to minimize alloimmunization.
 - E. Red cell exchange is not indicated. Anti-D prophylaxis is an ASFA Category IV indication for red cell exchange and it would expose the patient to additional allogeneic RBC units.
-

Question 32: Anti-basement membrane antibody syndrome is characterized by pulmonary and renal hemorrhage. Which of the following statements is true of this disorder and its treatment?

- A. TPE should be instituted only when the patient is dialysis dependent.
- B. In a patient with no hope of renal recovery, TPE is indicated if pulmonary hemorrhage occurs.
- C. TPE for longer than 6 months after onset is common.
- D. The disorder usually presents with pulmonary hemorrhage only.
- E. TPE should be implemented only after the failure of cytotoxic and immunosuppressive therapy.



Question 33: A 59-year-old female receives maintenance ECP for chronic GVHD. The tunneled catheter doesn't draw or return when the nurse tries to use it for the procedure. Which medication below may be infused into the line to restore it?

- A. Heparin sulfate.
 - B. 4% trisodium citrate.
 - C. Aminocaproic acid.
 - D. Tissue plasminogen activator (tPA).
 - E. Tranexamic acid.
-

Question 34: A healthy 19-year-old male is donating plasma by apheresis. He is near the end of the procedure and alerts the staff that he feels nauseous, that his fingers and hands are tingling, and that he has numbness and tingling around his mouth. What is the most likely cause?

- A. Hypotension caused by a vasovagal reaction.
 - B. Hypotension caused by hypovolemia as a result of a large extracorporeal volume.
 - C. Allergic reaction to ethylene oxide used to sterilize the disposable kit.
 - D. The donor has anxiety.
 - E. Presence of a low ionized calcium due to anticoagulation.
-

Question 35: Which condition in cardiac transplantation is a Category III indication for TPE?

- A. Desensitization before transplant.
 - B. Cellular rejection.
 - C. Treatment of antibody-mediated rejection.
 - D. Determination of donor-specific antibodies.
 - E. No established role.
-

Question 36: Which of the following is a risk factor for developing citrate toxicity during an apheresis procedure?



- A. Hypoventilation.
 - B. Acid-citrate-dextrose formula B (ACD-B) as the anticoagulant compared to ACD-A.
 - C. Slow rate of infusion.
 - D. Use of a continuous-flow centrifugation instrument.
 - E. Use of FFP as a replacement fluid.
-

Question 37: A donor is donating platelets and develops perioral paresthesia due to the citrate anticoagulant. What treatment would be effective?

- A. Increase the whole-blood-to-citrate ratio.
 - B. Ignore the symptom as it will likely go away
 - C. Discontinue the procedure and restart the procedure without any anticoagulant.
 - D. Give the donor or patient intravenous (IV) magnesium.
 - E. Increase the rate of reinfusion.
-

Question 38: A 45-year-old man with myasthenia gravis is undergoing a TPE procedure. Serum albumin (5%) is used as the replacement fluid with 3 units of plasma at the end of the procedure. With 5 minutes remaining in the procedure, the patient experiences hypotension, shortness of breath, and flushing. Which of the following is most likely responsible for this reaction?

- A. Ethylene oxide used to sterilize the disposable kit.
 - B. Angiotensin-converting enzyme (ACE) inhibitor taken the morning of the procedure.
 - C. Sodium caprylate used as a preservative in the albumin.
 - D. Albumin.
 - E. Plasma.
-

Question 39: A 16-year-old girl with hepatic failure has an ABO-incompatible living familial liver donor. The transplant team orders TPE for desensitization. What ASFA Category indication is this?

- A. Category I.



- B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. It is not listed in the ASFA guidelines.
-

Question 40: A female patient has a tunneled central line for repeat TPE for myasthenia gravis. The nurse comes to you and says she is having frequent access alarms and difficulty aspirating from the line. What do you suspect and what would you do?

- A. Suspect the line is not in the right place and order an x-ray.
 - B. Suspect a fibrin sheath and order tPA use.
 - C. Suspect an infection and order cultures from the line.
 - D. Suspect the line is bad and schedule the patient to have it replaced.
 - E. Tell the nurse to keep trying.
-

Question 41: What percentage of a patient's total blood volume is the maximum safe extracorporeal volume (ECV) for an apheresis procedure?

- A. 5%.
 - B. 7.5%.
 - C. 15%.
 - D. 20%.
 - E. 25%.
-

Question 42: What is the total plasma volume (TPV) of a 37-year-old male weighing 75 kg assuming a blood volume of 70 mL/kg and a hematocrit of 40%?

- A. 5250 mL.
- B. 4250 mL.
- C. 3250 mL.
- D. 3150 mL.
- E. 2150 mL.



Question 43: What is the red cell volume (RCV) of a 42-year-old male weighing 60 kg assuming a blood volume of 70 mL/kg and hematocrit of 29%?

- A. 2982 mL.
 - B. 1218 mL.
 - C. 1764 mL.
 - D. 1818 mL.
 - E. 4200 mL.
-

Question 44: A female donor requires ECP for GVHD. The donor weighs 40 kg. Assume a blood volume of 60 mL/kg. What is the maximum ECV that she could tolerate?

- A. 200 mL.
 - B. 240 mL.
 - C. 360 mL.
 - D. 480 mL.
 - E. 960 mL.
-

Question 45: If the tubing of the ECP machine in question #44 requires 450 mL, what should you plan to do?

- A. Nothing, because the ECV that she will tolerate is more than 450 mL.
 - B. She cannot undergo the procedure because the ECV that she could tolerate is less than 450 mL.
 - C. Prime the machine with saline.
 - D. Prime the machine with red cells.
 - E. Call the manufacturer of the ECP machine.
-

Question 46: A 45-year-old male with CIDP is admitted to the hospital and you are asked to perform plasmapheresis for him emergently with 5% albumin. He weighs 83 kg and has a hematocrit of 42%. You decide to do a 1.5 TPV exchange. What volume of plasma do you plan to remove, assuming a blood volume calculation of 70 mL/kg?



- A. 5055 mL.
 - B. 5810 mL.
 - C. 3370 mL.
 - D. 2440 mL.
 - E. 3660 mL.
-

Question 47: In question #46, how many bottles of 5% albumin should you order, assuming each bottle is 500 mL?

- A. 5.
 - B. 7.
 - C. 11.
 - D. 8.
 - E. 12.
-

Question 48: You are asked to start performing red cell exchanges as prophylaxis for a sickle cell patient with a history of stroke. What are the current ASFA guideline recommendations?

- A. Category I.
 - B. Category II.
 - C. Category III.
 - D. Category IV.
 - E. There is no current recommendation.
-

Question 49: Multiple sclerosis is a Category II indication for TPE with acute attack or relapse. How many procedures should be performed over what time?

- A. One TPE weekly for 3 months.
- B. TPE daily for 14 days.
- C. TPE daily for 1 week.
- D. Five to seven TPEs over 2 weeks.
- E. One TPE every 2 weeks for 6 months.



Question 50: You are asked to perform red cell exchange for a 15-year-old female with acute chest syndrome. Her weight is 55 kg and her hematocrit is 27%. Assuming her blood volume is 60 mL/kg, what is her red cell volume?

- A. 3300 mL.
- B. 2409 mL.
- C. 1205 mL.
- D. 891 mL.
- E. 500 mL.

ANSWERS

Question 1: A. Plasma.

Explanation:

- Centrifugal force separates the various components of blood according to their specific gravity (density). The densest components collect the farthest from the axis of rotation, while the least dense layer collects the closest.
- The order of the layers of separation from the axis of rotation outward is plasma, platelets, lymphocytes, granulocytes, and red cells.
- Red cells and granulocytes have an overlap in densities, resulting in poor separation. This is why granulocyte products contain large numbers of red cells. Greater separation can be achieved by the addition of hydroxyethyl starch. This aggregates the red cells, resulting in their having a greater density than the granulocytes.



Question 2: D. Density.

Explanation:

- In continuous-flow centrifuge apheresis, the components of blood separate by density.



- By modifying the pump and centrifuge speed, one can optimize separation to better facilitate selective removal of the pathogenic component.
-

Question 3: B. Category II.**Explanation:**

- Most diseases treated by apheresis are “orphan diseases” that have a limited incidence. As a result, large, randomized, placebo, controlled trials are uncommon. This means that the available literature consists most often of cases, case series, and trials with historical controls.
- Because of the quality of the literature, the ASFA Clinical Application Committee reviews the medical literature on all diseases reported to be treated by apheresis every 3 years. The committee categorizes the disorders into categories on the basis of the evidence in the literature.
 - Category I: Disorders for which apheresis is accepted as first-line therapy, either as a primary stand-alone treatment or in conjunction with other modes of treatment.
 - Category II: Disorders for which apheresis is accepted as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment.
 - Category III: Optimum role of apheresis therapy is not established. Decision making should be individualized.
 - Category IV: Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful.
- Third-party payers will usually reimburse for Category I and II disorders and will almost never reimburse for Category IV diseases. Category III disorders may or may not be reimbursed and may require communication between the apheresis physician and the appropriate physician for the payer. This is not a trivial concern, given the cost of providing these procedures and the potential adverse consequences to patients and their families caused by repeated, expensive therapeutic interventions.
- Not all diseases have been categorized. Whether to treat a patient with an uncategorized disease is a decision to be made after reviewing the pertinent medical literature, discussing



options with the requesting physician, and discussing risks and benefits with the patient and the patient's family.

Question 4: D. A and C are both true.

Explanation:

- In intermittent-flow centrifugation separators, blood is pumped into the separation chamber. When it is filled, the chamber is spun to separate the various components into layers. The component of interest is removed, the centrifuge is stopped, and the remaining contents are returned to the patient/donor.
 - In intermittent-flow centrifugation separators, processing occurs in batches, with a large bolus of anticoagulated blood being returned to the patient/donor as opposed to a continuous infusion.
 - Citrate toxicity occurs more frequently with intermittent instruments. In addition, the extracorporeal blood volume tends to be larger and a higher incidence of hypotensive reactions is seen.
 - Because the separation chambers must fill and empty and there is not a continuous flow of blood from the patient, intermittent-flow instruments tend to take longer than continuous-flow instruments to process the same amount of blood.
- In continuous-flow centrifugation separators, blood is pumped into a spinning separation chamber continuously, with the layers being removed and retained or returned to the patient/donor. As blood leaves the separation chamber, it is continuously replaced with fresh whole blood.
 - Continuous-flow centrifugation separators can be used in single-needle procedures. In these, blood fills a reservoir that then feeds the separation chamber. The blood from the reservoir flows into the separation chamber, replacing blood being returned to the patient/donor. The machine does have to refill the reservoir, but the separation chamber does not empty completely as in an intermittent instrument.
 - Because of the presence of the reservoir and the resulting larger extracorporeal volume, when using a single-needle kit, there is the possibility of a greater frequency of hypotensive reactions.



- When a two-needle kit is used with a continuous-flow instrument, the extracorporeal volumes are usually smaller than those seen in intermittent-flow instruments.
 - Most instruments currently used are continuous-flow centrifuge separators.
-

Question 5: C. 66%.**Explanation:**

- The removal of substances located within the intravascular compartment is defined by the equation

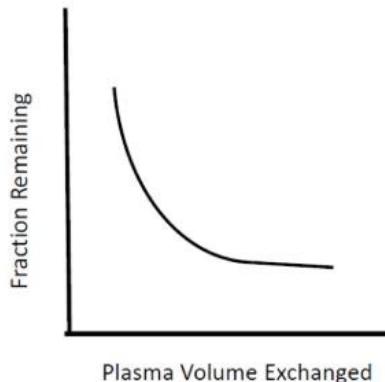
$$Y/Y_0 = e^{-x}$$

where Y = final concentration of the substance

Y_0 = the initial concentration of the substance

x = the number of times a patient's plasma volume is exchanged

- This equation shows that the relationship is an exponential, rather than a linear, one (see accompanying figure).



- As plasma is removed, it is replaced with a replacement fluid such as albumin, which acts to dilute the amount of substance of interest that is left. In other words, with each plasma volume processed, a fixed percentage of the substance of interest remaining



is removed. This is why most TPE procedures process only 1 to 1.5 plasma volumes.

- TPE is nonselective as everything in the plasma is removed including both undesired (eg, LDL cholesterol, autoantibodies) and desirable (eg, HDL cholesterol, coagulation factors, normal antibodies, some drugs) solutes.
-

Question 6: D. Buffy coat.

Explanation:

- During a leukapheresis procedure, the buffy coat is removed. The buffy coat contains white cells. Leukapheresis would be performed for patients with leukemia and high white cell counts.
 - The buffy coat is also removed during an extracorporeal photopheresis (ECP) procedure; however, this buffy coat is reinfused to the patient after treatment with a psoralen compound and UVA light.
-

Question 7: E. Filtration.

Explanation:

- Rheopheresis, which selectively decreases the concentration of high-molecular-weight molecules, is performed via a special filtration method. Selective adsorption for hypercholesterolemia also uses filtration. Because filtration separates the cellular components to return to the patient as a first step, these devices cannot be used for cytapheresis procedures.
- Centrifugation, either continuous or discontinuous, can be used for most therapeutic procedures.
- Fractionation is performed industrially, during production of plasma protein concentrates, not therapeutic apheresis.
- Gravity separation is sometimes used by cellular therapy laboratories to reduce the red cell content of stem cell products that have a major ABO incompatibility with the recipient.



Question 8: B. It can be used to prevent tumor lysis syndrome in patients with acute myelogenous leukemia (AML).

Explanation:

- Leukocytapheresis may be indicated when patients become symptomatic from high white cell counts. These symptoms usually result from hyperviscosity of blood caused by the elevated white cell counts or leukostasis with the leukemic blasts “clogging” the microvasculature. This takes the form of either neurologic symptoms (eg, transient ischemic attack, or stroke) or poor oxygenation associated with chest pain (eg, pulmonary leukostasis). In addition, removal of large numbers of white cells before the start of chemotherapy can prevent disseminated intravascular coagulation (DIC) and renal damage from uric acid and phosphate that can result from the death of these cells when chemotherapy is begun (ie, tumor lysis syndrome).
- Prophylactic leukocytapheresis (ie, leukocytapheresis in the absence of symptoms) is indicated for counts $>100,000/\mu\text{L}$ in AML. In studies of patients with AML, a white cell count $>100,000/\mu\text{L}$ at presentation has been found to be associated with a greater frequency of CNS infarction and hemorrhage as well as pulmonary complications when compared with those with counts $<50,000/\mu\text{L}$. The use of leukocytapheresis in those with white cell counts $>100,000/\mu\text{L}$ is associated with improved 2- to 3-week mortality. Long-term survival is *not* improved by leukocytapheresis.
- In ALL, leukocytapheresis may be indicated at white cell counts $\geq400,000/\mu\text{L}$. At these counts, there is a greater frequency of complications, primarily CNS infarction and hemorrhage.
- Leukocytapheresis is usually not indicated in CLL or chronic phase CML. The exception is in the setting of CML in pregnant women. Leukocytapheresis has been used to avoid chemotherapy in women with chronic phase CML until the neonate is delivered.



Question 9: D. Red cell exchange is superior to transfusion in sickle cell patients with a history of stroke.



Explanation:

- Red cell exchange can be performed using either manual or automated methods. The automated method allows for better control of how much of the patient's own red cells are left at the end of the procedure and, as a result, it is more effective.
- Red cell exchange has been used to treat both babesiosis and malaria. In the case of *Babesia* infection, it is used to treat an erythrocyte parasitemia >10% in symptomatic disease or in an asplenic individual. Asplenic patients are at great risk for rapid deterioration when infected with *Babesia*. It is not routinely used in uncomplicated cases of babesiosis. In malaria, it is most commonly used to treat infections with *Plasmodium falciparum*, as they are most frequently associated with symptomatic hyperparasitemia. It is indicated when the erythrocyte parasitemia is >10% or if the patient has severe malaria manifested by altered mental status, nonvolume overload pulmonary edema, or renal complications.
- Red cell exchange is also used to treat threatened end-organ damage in sickle cell disease. In this setting, hemoglobin-S-containing red cells are removed and replaced with normal red cells, thereby improving blood flow and tissue oxygenation. It allows for the operator to determine the fraction of hemoglobin-S-containing cells to be left at the end of the procedure while maintaining a selected hematocrit. It has the benefit over simple transfusion in that it does not result in an increase in hematocrit with an accompanying increase in blood viscosity and also helps to avoid iron overload. Red cell exchange is indicated in sickle cell disease for the treatment of acute chest syndrome, priapism, cerebrovascular accident, retinal artery vaso-occlusion, hepatic failure, and septic shock.
- Pharmacologic intervention, such as instillation of alpha agonists, is used as first-line therapy for priapism. Surgery may be required for unresponsive cases. Red cell exchange may be used in an attempt to avoid surgical intervention, which is associated with a high frequency of erectile dysfunction.



Question 10: C. The red cells used for replacement should be screened for the presence of hemoglobin S.



Explanation:

- The goal of red cell exchange is to reduce the amount of hemoglobin-S-containing red cells below 30%; below 10% is not necessary.
- The fraction of cells remaining (FCR) is the desired number of the patient's red cells remaining after the completion of the procedure. A procedure treating 1 red cell volume (RCV) will remove 70% of the patient's red cells, resulting in an FCR of 30%. If a patient with sickle cell anemia has not been transfused recently, the amount of hemoglobin S remaining would reach the 30% goal after the exchange of 1 RCV. If the patient has been transfused, the percentage of red cells containing hemoglobin S will be less than 100%, and as a result a smaller RCV will need to be exchanged. Hemoglobin electrophoresis can be used to determine the starting hemoglobin S level and to determine the goal FCR.
- Because the goal is to reduce the hemoglobin S level below 30%, it is necessary to use hemoglobin-S-negative red cells in performing the procedure. The replacement red cells should be screened for hemoglobin S.
- It is undesirable to increase the final hematocrit above 30% or above the hematocrit typically seen in sickle cell disease patients. Their endothelium is abnormally "sticky" and a "normal" hematocrit can result in increased viscosity and the potential for triggering sickling by producing hypoxia caused by poor perfusion.
- It would be impossible to match for all red cell antigens. It has been suggested that RBC units be matched for C, E, and Kell as well as ABO and D. Additionally, the products should be leukocyte reduced and, in order to maximize the time of survival, as fresh as possible.



Question 11: A. TTP.

Explanation:

- FFP can transmit viral infections and is associated with a number of transfusion reactions, including allergic reactions (both simple and anaphylactic), febrile reactions, posttransfusion purpura, and transfusion-related acute lung injury (TRALI). As a result, the use



of FFP as a standard replacement fluid is limited to only a few diseases.

- Most procedures use a combination of albumin and/or saline as replacement fluids because of the greater safety of these products. Those disorders in which FFP is indicated are ones in which FFP replaces a factor missing in the patient's plasma. FFP is primarily used in treating TTP and hemolytic uremic syndrome (HUS).
- FFP may also be used to replace coagulation factors lost during the nonselective removal of plasma components in patients at risk for bleeding, such as those with recent biopsies or planned surgical procedures. In this setting, partial FFP replacement can be given at the end of the procedure. The timing of this is important. If the FFP is given at the beginning of the procedure, then the coagulation factors will be removed as part of the procedure itself. Some facilities monitor fibrinogen levels in patients, providing FFP as part of the replacement if the levels fall below 150 mg/dL.



Question 12: A. Category I.

Explanation:

- The pathogenesis of GBS is autoimmune antibody-mediated damage to peripheral nerve myelin. TPE can improve motor recovery, decrease time on the ventilator, and decrease time to attainment of other clinical milestones.
- Several studies have shown that TPE has beneficial effect in severely and mildly affected individuals; with a significantly increased proportion of patients able to walk after 4 weeks.
- Typically, 1-1.5 plasma volumes are exchanged using 5% albumin for 5-6 times over 10-14 days, some patients may need additional treatments.



Question 13: C. Category III.



Explanation:

- The use of TPE in post-HSCT-associated thrombotic microangiopathy (TA-TMA) is based on extrapolation of its effectiveness for idiopathic TTP.
 - However, numerous studies have confirmed that plasma ADAMTS13 levels are not severely deficient nor are ADAMTS13 inhibitors detectable in patients with TA-TMA.
 - Typically, 1-1.5 plasma volumes are exchanged using FFP daily as is done for TTP treatment.
-

Question 14: A. Category I.

Explanation:

- NMDAR encephalitis is an acute inflammatory brain disorder characterized by IgG antibodies targeting subunits of the NMDAR. NMDAR encephalitis typically affects children and young adults, with a female predominance. Up to 58% of affected young female patients have an ovarian teratoma.
 - Typically, 1-1.5 plasma volumes are exchanged using 5% albumin for 5-12 treatments with TPE over 1-3 weeks.
-

Question 15: B. Category II.

Explanation:

- Neuromyelitis optica (NMO or Devic's disease) is an inflammatory CNS syndrome, a distinct disease entity from multiple sclerosis, that is associated with serum aquaporin-4 immunoglobulin G antibodies (AQP4-IgG). Antibodies to AQP4, the principal water channel on astrocyte foot processes at the blood brain barrier, are pathogenic in NMOSD.
- Acute NMOS attacks are typically managed with high dose IV methylprednisolone. Acute attacks or NMOS relapse can be treated with 1-1.5 plasma volume TPE exchange with 5% albumin every day or every other day.
- TPE for maintenance of NMOS is a Category III indication.



Question 16: E. ECP can effectively be performed on patients with low white cell counts and anemia.

Explanation:

- ECP should not be performed in patients with aphakia (absence of lens) due to increased risk for retinal damage. Additionally, patients should be instructed to wear eye and skin protection for 24 hours after ECP treatment due to increased photosensitivity from the infused psoralen.
- A 2018 MedWatch Safety Alert issued by the US Food and Drug Administration (FDA) warned of a risk of venous thromboembolism in patients receiving ECP.
- Lastly, patients with very low white cell counts should not have ECP as collection of a buffy coat to treat with psoralen is necessary for the effectiveness of ECP. Different centers use various white cell cut-offs.



Question 17: A. Immunoabsorption does not require a replacement fluid such as 5% albumin or FFP.

Explanation:

- Immunoabsorption removes immunoglobulins by binding them to select ligands on the backing matrix surface (membranes or beads) of the adsorber column. A major advantage of IA is that no substitution of albumin or plasma is necessary.
- Immunoabsorption devices can be categorized as those with non-regenerative or regenerative columns. Non-regenerative columns are single and limited to the treatment of one patient's plasma volume and have their main indication in acute situations of autoantibody-mediated diseases. Regenerative adsorber systems consist of column pairs, which are sequentially regenerated during a treatment session, and may be reusable. They can treat up to three plasma volumes in a single session.
- Most immunoabsorption columns are broad immunoglobulin adsorbers that use various ligands to bind all major immunoglobulin subclasses.

**Question 18: A.** Category I.**Explanation:**

- Anti-glomerular basement membrane (anti-GBM) disease is a small-vessel vasculitis which affects the glomerular capillaries, pulmonary capillaries, or both along with anti-GBM autoantibody deposition. The disease most commonly presents with rapidly progressive glomerulonephritis (RPGN) and hematuria. Kidney biopsy typically will show crescent formation within the glomeruli. Pulmonary hemorrhage is commonly present and may range from cough associated with a mild anemia reflective of blood loss within the alveoli to massive hemoptysis requiring invasive respiratory support. It is a Category I indication for disease with DAH or dialysis-independent disease. TPE in anti-GBM disease that is dialysis dependent with no diffuse alveolar hemorrhage is a Category III indication.
- TPE usually consists of 1-1.5 plasma volumes with exchanges performed daily or every other day for 10-20 days. The exchange fluid is FFP when diffuse alveolar hemorrhage is present and 5% albumin for other cases.

**Question 19: C.** Category III for WAIHA and Category II for CAD.**Explanation:**

- Autoimmune hemolytic anemia (AIHA) represents a group of disorders in which autoantibodies mediate intravascular hemolysis either by the terminal complement complex (C5b-C9) or by extravascular destruction in the spleen via the macrophage-phagocytic system.
- AIHA can be classified into two major types, warm autoimmune hemolytic anemia (WAIHA) and cold agglutinin disease (CAD)/cold autoimmune hemolytic anemia (CAIHA). WAIHA is typically caused by IgG that bind to RBCs at 37 C. Typically, the DAT result is positive for IgG and sometimes for C3d. CAD is caused by IgM antibodies that react at much colder temperatures and are sometimes directed against the RBC antigens I/i. CAD is frequently seen in patients following viral or bacterial illnesses or in



patients with lymphoproliferative disorders. The DAT result in CAD is typically positive only for C3d.

- The cold-reactive IgM autoantibody produced after *Mycoplasma pneumoniae* infection typically has anti-I specificity, whereas the autoantibody associated with Epstein-Barr virus infection (infectious mononucleosis) demonstrates anti-i specificity. TPE is performed using 5% albumin and a 1-1.5 plasma volume is exchanged daily or every other day. In CAD, if the IgM antibody agglutinates red cells at room temperature, the room temperature and machine temperature may need to be increased to 37°C to prevent red cell agglutination in the tubing and centrifuge.



Question 20: C. Category III.

Explanation:

- Red cell exchange is a Category III indication for patients with babesiosis and heavy parasitemia (typically >10%) or those who have significant comorbidities such as significant hemolysis, DIC, pulmonary, renal, or hepatic compromise.



Question 21: A. Category I.

Explanation:

- Mycosis fungoides (MF) and the leukemic variant, Sézary syndrome (SS), account for 55% and 5% of cutaneous T cell lymphoma (CTCL) cases, respectively. ECP is not recommended for non-erythrodermic disease as it is thought to require blood involvement to be effective. Non-erythrodermic MF is a Category III indication for ECP treatment.
- ECP involves leukapheresis, treatment with 8-methoxysoralen (8-MOP) and UVA light, and subsequent reinfusion of the treated cells. Treatment induces apoptosis of malignant cells, which are phagocytosed by antigen-presenting cells. In addition, ECP stimulates monocyte differentiation to myeloid dendritic cells with a Th1 phenotype. The Th1 cells then attack the malignant clone.
- ECP treatments consists of one cycle comprised of two daily ECP procedures every 2 or 4 weeks.

**Question 22: B. Category II.****Explanation:**

- Acute GVHD occurs before day 100 following HSCT and is characterized by inflammatory tissue injury and necrosis with skin and gastrointestinal (GI) tract inflammation and denudation, cholangiohepatic liver injury, and cholestatic jaundice.
- In patients with acute GVHD, one cycle is performed weekly comprised of two daily ECP procedures until disease improvement and then tapered to every-other-week before discontinuation.
- In patients with chronic GVHD, one cycle of ECP is performed weekly for 4 weeks and then one cycle every 2 weeks for at least 8 weeks. Acute GVHD and chronic GVHD are both Category II indications for ECP.

**Question 23: C. 8-MOP.****Explanation:**

- 8-methoxysoralen is added to the leukocytes. The mixture is then treated with UVA light and returned to the patient.

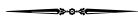
**Question 24: D. Increased fraction remaining.****Explanation:**

- IgM antibodies are restricted to the intravascular space; however, IgG antibodies are distributed both intra- and extravascularly. With an expanded volume of distribution, IgG will have an increased fraction remaining after TPE. This apparent decrease in efficiency is due to recruitment of extravascular IgG into the vascular space during the procedure. This redistribution of IgG antibodies causes an apparent decrease in the fraction removed.
- Exclusive use of saline as the replacement fluid in TPE is contraindicated because this would create a deficit of colloidal pressure. Plasma is the replacement fluid in TPE if the diagnosis is



TTP, atypical hemolytic uremic syndrome (aHUS), or if there is a concern for coagulopathy.

- A red cell prime is indicated when the extracorporeal volume is greater than 10% to 15% of the patient's blood volume.



Question 25: B. Activity of ADAMTS13, a metalloproteinase that cleaves multimers of vWF, is deficient in many cases of TTP.

Explanation:

- The pathophysiology of TTP includes:
 - Abnormal vWF multimer patterns with unusually large forms (ie, >10,000,000 kD) are detected in the plasma of patients with chronic relapsing TTP during symptom-free intervals and these unusually large vWF multimers are considered potential important mediators of the platelet aggregation that underlies the clinical manifestations of this disease.
 - Unusually large vWF multimers are produced by human endothelial cells (in Weibel-Palade bodies) and platelets (in α -granules) and these multimers are most effective in aggregating platelets under high shear stress situations and in vitro in response to ristocetin.
 - Episodes of disease are regularly associated with decreased levels of the unusually large forms of vWF.
 - The abnormal release of unusually large vWF multimers and/or their inability to undergo appropriate processing into smaller forms leads to abnormal platelet aggregation and endothelial adhesion in the microcirculation, with resulting thrombosis and microangiopathic hemolysis.
 - ADAMTS13 (A Disintegrin And Metalloprotease with Thrombospondin type I motifs 13), a metalloproteinase that cleaves multimers of vWF, is deficient in many cases of TTP. A genetic deficiency in enzyme production is the cause of the congenital Upshaw-Schulman syndrome.
 - Idiopathic TTP is frequently caused by an autoantibody that decreases ADAMTS13 activity by inhibiting the enzyme and/or increases its clearance.
 - Platelet aggregates in TTP consist of platelets and vWF. They do not contain activated coagulation factors.



- In contrast to the thromboses of DIC, the intravascular thrombi formed during episodes of TTP stain strongly for vWF antigen, but only weakly for fibrinogen.



Question 26: D. IgM tends to cause hyperviscosity symptoms at the lowest concentration (relative to IgG and IgA).

Explanation:

- Waldenström macroglobulinemia (WM) is also called lymphoplasmacytic lymphoma. In WM, individuals make large amounts of monoclonal IgM (called macroglobulin). The increased IgM leads to symptoms of hyperviscosity such as headaches, blurry vision, and increased bleeding.
- IgM tends to cause symptomatic hyperviscosity when at the lowest concentration (relative to IgG and IgA). IgM can become symptomatic when levels increase beyond 3 g/dL, whereas IgG tends to cause symptoms beyond 4 g/dL and IgA tends to cause symptoms beyond 6 g/dL. Because minor elevations in viscosity typically do not cause symptoms, measurement of viscosity is not useful for guiding apheresis treatment.



Question 27: C. Complement mediated thrombotic microangiopathy due to autoantibody to Factor H.

Explanation:

- TPE is recommended as first-line therapy for thrombotic microangiopathy due to autoantibodies to Factor H. It is a Category III indication for complement-mediated thrombotic microangiopathy due to mutations in complement genes; coagulation-mediated thrombotic microangiopathy due to mutation in THBD, DGKE, and/or PLG; infection-associated thrombotic microangiopathy; and transplant-associated thrombotic microangiopathy.



Question 28: A. LDL apheresis.



Explanation:

- Heparin is the only acceptable anticoagulant in the two FDA-approved LDL apheresis instruments. In the heparin-induced extracorporeal LDL precipitation method, acidified heparin is used to precipitate LDL from plasma. Although the other procedures mentioned may use full or partial heparin anticoagulation, it is not required. Alternatively, citrate may be used.



Question 29: C. Category III.

Explanation:

- Pruritus due to hepatobiliary diseases is a Category III indication, meaning that the optimum role for apheresis is not established and decision-making should be individualized. This is a Grade 1C recommendation, meaning a strong recommendation based on low-quality or very low-quality evidence. Patients may experience some relief after the second TPE, which lasts for a variable period. Some patients respond to an acute treatment course, whereas other patients require long-term, chronic TPE.



Question 30: E. Waldenström macroglobulinemia resistant to chemotherapy has been reported to be effectively treated with TPE for extended periods.

Explanation:

- Hyperviscosity syndrome results from the presence of large concentrations of paraprotein that increases blood viscosity by causing sludging of the red cells. It results in occlusion of the microcirculation with organ ischemia. Symptoms usually occur when the viscosity increases to 4 to 6 Ostwald units (normal blood viscosity is 1.5 to 1.8 Ostwald units), but patients may be asymptomatic at very high protein concentrations and viscosity levels (8 to 10 Ostwald units) or symptomatic at low levels (3 to 4 Ostwald units).



- Hyperviscosity syndrome is characterized by mental status changes, a bleeding diathesis, retinopathy, and hypervolemia with congestive heart failure. It occurs in less than 5% of multiple myeloma patients but in as many as 70% of patients with Waldenström macroglobulinemia. It is only rarely seen with polyclonal increases in immunoglobulin.
- The treatment of hyperviscosity syndrome is directed at decreasing paraprotein production with chemotherapy and improving blood flow by acutely decreasing paraprotein levels. This second goal can be achieved with TPE.
- In hyperviscosity syndrome caused by an IgM monoclonal protein, one to two TPEs may be sufficient to remove enough protein to improve viscosity, because IgM is located almost entirely within the intravascular space. With IgG or IgA paraproteins, the immunoglobulin is also present within the extravascular space, and more procedures may be necessary to decrease paraprotein levels.
- The primary goal of therapy should be relief of symptoms, with subsequent frequency tailored to the needs of the patient. In instances of Waldenström macroglobulinemia resistant to therapy, repeated TPE alone has been used to control hyperviscosity for extended periods.



Question 31: E. Red cell exchange is not indicated. Anti-D prophylaxis is an ASFA Category IV indication for red cell exchange and it would expose the patient to additional allogeneic RBC units.

Explanation:

- In the ASFA guidelines, prevention of RhD alloimmunization after red cell exposure is a Category IV indication with a Grade 2C recommendation. Red cell exchange could achieve a decreased percentage of Rh-positive cells remaining after the procedure, which can be safely treated with Rh Immune Globulin.
- Because apheresis instruments operate by percentage removal, no extent of procedure could eliminate the indication for Rh Immune Globulin. Unfortunately, by undergoing a red cell exchange, the patient will be exposed to additional blood donors and would be at risk for further alloimmunization. These argu-



ments are on the other side of the scale when considering which decision is best for a particular patient.

- Phenotypically similar cells are used for frequently transfused patients, such as those with sickle cell disease. This is typically not the case for recovering trauma patients.
-

Question 32: B. In a patient with no hope of renal recovery, TPE is indicated if pulmonary hemorrhage occurs.

Explanation:

- Anti-basement membrane antibody disease is a rare disorder characterized by the combination of pulmonary hemorrhage and renal hemorrhage (nephritic syndrome). When both occur together, it is referred to as Goodpasture syndrome or anti-basement antibody syndrome. It more commonly affects men and is most frequent between 18 and 35 years of age.
 - It is caused by a transient autoantibody directed against the chains of type IV collagen found in glomerular and pulmonary basement membranes. The autoantibody appears to be triggered by damage to the respiratory system. Anti-basement membrane antibody syndrome frequently presents with pulmonary symptoms of cough, hemoptysis, and dyspnea with laboratory evidence of renal failure and renal inflammation. The mortality rate may be as high as 50%.
 - Treatment is focused on suppressing antibody production and inflammation with immunosuppressive and cytotoxic agents as well as removing the antibody with TPE. TPE is usually performed daily with concurrent immunosuppression. Because the antibody is transient, treatment longer than 6 months is uncommon. TPE should be initiated early in the course of therapy for this disorder, as recovery of renal function is unlikely once scarring and atrophy of glomeruli and tubules occur.
 - Severe renal disease (defined by oliguria, dialysis dependency, or a serum creatinine >6.8 mg/dL) and diffuse alveolar hemorrhage are Category III indications for TPE.
-

Question 33: D. Tissue plasminogen activator (tPA).



Explanation:

- The patient has a thrombosed central line. Infusion of a fibrinolytic agent may salvage the line. Urokinase and tissue plasminogen activator (uPA and tPA) activate the fibrinolytic system to dissolve the thrombosis.
 - Heparin and citrate are two anticoagulants that are often infused into lines to prevent thrombosis; however, once blood is thrombosed, these anticoagulants are not helpful. Aminocaproic acid and tranexamic acid are both fibrinolytic inhibitors. They inhibit the breakdown of formed clots and would, therefore, be counterproductive for a thrombosed line.
-

Question 34: E. Presence of a low ionized calcium due to anticoagulation.

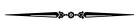
Explanation:

- Citrate is used as the primary anticoagulant in both donor and therapeutic apheresis procedures because it effectively prevents coagulation and is short acting and easily reversible, unlike heparin. Citrate chelates calcium ions, producing a soluble complex, making them unavailable for biologic reactions (eg, coagulation).
- Within the apheresis instrument, plasma citrate concentrations reach 15 to 24 mmol/L, lowering the calcium ion concentration below 0.2 to 0.3 mmol/L, the level necessary for coagulation. This level of anticoagulation requires the infusion of approximately 500 mL of acid-citrate-dextrose formula A (ACD-A) solution.
- It would be expected that the infusion of this volume of solution into the donor or patient would result in a calcium ion concentration of 0.2 mmol/L, a level incompatible with life. This does not occur, however. Upon return of the blood from the apheresis instrument to the donor or patient, the citrate is diluted throughout the total extracellular fluid. In addition, the liver, kidneys, and muscles rapidly metabolize citrate, releasing the bound calcium.
- Finally, the body also responds to the decrease in ionized calcium by increasing parathyroid hormone levels with a mobilization of calcium from skeletal stores as well as increased absorption by the kidneys. In TPE procedures using either FFP or



cryoprecipitate-reduced plasma as replacement fluids, an additional source of citrate is infused in the form of the anticoagulant present in these solutions. Again, the effects of this citrate load are minimized as described above.

- Despite compensatory mechanisms, citrate infusion can result in a decrease in ionized calcium levels to a point where symptoms develop in the patient. These result from a decrease in ionized calcium to levels where the excitability of nerve membranes increases until spontaneous depolarization can occur.
- The signs and symptoms of citrate toxicity include circumoral paresthesias, acral paresthesias, shivering, light-headedness, twitching, and tremors. In addition, some patients experience nausea and vomiting. As the ionized calcium levels fall further, these symptoms may progress to carpopedal spasm, tetany, and seizure.
- It is important to elicit the presence of the early symptoms of citrate toxicity from the patient so that interventions can occur before the more severe symptoms. In addition to the symptoms described above, prolongation of the QT interval on electrocardiograms as well as depressed myocardial contractility have been seen, and fatal arrhythmias have been reported.



Question 35: C. Treatment of antibody-mediated rejection.

Explanation:

- Antibody-mediated rejection in cardiac transplant patients is a Category III indication for TPE treatment. Cellular rejection and rejection prophylaxis in cardiac transplant patients are a Category II indications for TPE.
- Cellular rejection, recurrent rejection, and rejection prophylaxis in cardiac transplant patients are Category II indications for ECP. If a patient experiences recurrent episodes of rejection, ECP appears to attenuate the severity of the rejection and permits decreased immunosuppression.
- TPE can remove circulating antibodies, whereas ECP temporarily removes just the buffy coat for treatment. Determination of donor-specific antibodies is based on the HLA typing of the donor organ and determining the antibody profile of the recipient.

**Question 36: E.** Use of FFP as a replacement fluid.**Explanation:**

- Factors that have been found to influence the rate of citrate reactions in donor and therapeutic apheresis include the following:
 - Alkalosis caused by hyperventilation (decreases ionized calcium).
 - Type of anticoagulant solution used, with ACD-A having more reactions than ACD-B (half the concentration of citrate compared to ACD-A solution).
 - Rapid rate of infusion of the anticoagulant solution.
 - Amount of citrate infused, including the infusion of replacement fluids, such as FFP, which contain citrate.
 - Low serum albumin (less albumin-bound calcium to buffer the decrease in calcium).
 - Intermittent-flow apheresis procedures tend to have a greater frequency of citrate reactions because there is a higher rate of citrate infusion when the separation chamber is emptied, as compared with continuous apheresis procedures.

**Question 37: A.** Increase the whole-blood-to-citrate ratio.**Explanation:**

- Hypocalcemia caused by citrate toxicity is characterized by peri-oral and acral paresthesias, shivering, lightheadedness, twitching, tremors, nausea, and hypotension. If not treated, these can progress to carpopedal spasm, tetany, and seizure activity.
- The treatment of citrate reactions includes slowing the reinfusion rate to allow for dilution of citrate and metabolism of the calcium-citrate complex, increasing the blood-to-citrate ratio to decrease the amount of citrate infused; giving oral calcium in the form of calcium antacids; and giving intravenous calcium gluconate or calcium chloride. Calcium gluconate or chloride infusions are usually not necessary in donor procedures but may be required with therapeutic procedures and hematopoietic progenitor cell collections. This is especially true when plasma products (anticoagulated with citrate) are used as replacement fluids or when lengthy procedures are instituted.



- Citrate can cause hypomagnesemia because it will bind magnesium, a divalent cation. Hypomagnesemia is less common than hypocalcemia. It can present as hypocalcemic symptoms that fail to respond to calcium infusion and should be considered in this clinical situation.
- Studies have demonstrated limitations in the use of oral calcium supplementation. While increasing ionized calcium levels and reducing severity of paresthesias, it did not prevent symptom development. Administration of IV solutions before the procedure or at the time of symptoms has been found in some studies not to be effective.
- Finally, studies have suggested that continuous infusions result in fewer reactions and higher ionized calcium levels. In most cases, anticoagulation is required to perform an apheresis procedure. Attempts to perform procedures without citrate or heparin anticoagulation will result in thrombosis of the extracorporeal circuit.



Question 38: E. Plasma.

Explanation:

- The reaction was most likely caused by plasma that was used for replacement during the end of the procedure. The blood bank should be notified of the allergic reaction to the plasma. Allergic, anaphylactoid, and anaphylactic reactions can occur in both donors and patients undergoing apheresis procedures.
- Causes of allergic reactions include the following:
 - Antibodies generated toward ethylene oxide used to sterilize disposable kits. This occurs after repeated procedures or donations where ethylene oxide in the kit binds to plasma proteins, inducing an immune response. Some studies of platelet donors have reported an incidence of 1%.
 - Antibodies have been found against sodium caprylate used as a preservative in albumin. In addition, aggregates in albumin preparations can trigger allergic-like reactions. Finally, the processing of the albumin may modify the molecule such that it appears foreign and is capable of eliciting an immune response.



- Hydroxyethyl starch, used as a colloidal replacement fluid or to enhance separation of red cells from white cells, can activate complement and produce allergic-like phenomena.
- In IgA-deficient individuals, antibodies to IgA can cause allergic reactions, ranging from hives to anaphylaxis. These reactions are most commonly seen in procedures using plasma products as replacement fluids, but IgA may be present in normal serum albumin as well.
- ACE inhibitors inactivate kinase I and II. These enzymes normally inactivate bradykinin. During TPE, prekallikrein-activating factor present in the albumin preparation can result in the production of bradykinin. In selective removal systems, such as LDL apheresis, the negatively charged columns can also cause bradykinin generation. Bradykinin is normally rapidly inactivated upon reinfusion of the blood. Individuals taking ACE inhibitors cannot do this, however, and may experience flushing, hypotension, bradycardia, and dyspnea. Patients or donors should discontinue ACE inhibitors a minimum of 24 to 48 hours before apheresis procedures. Longer lengths of time may be required for ACE inhibitors with longer half-lives.

**Question 39: A. Category I.****Explanation:**

- In ABO-incompatible living donor liver transplantation, TPE is extensively used as part of a desensitization protocol to lower antibody titer(s) below a critical threshold before the transplant procedure. Additionally, basiliximab, IVIG, and other agents are occasionally used before and/or after transplantation.
- TPE has been used in the setting of liver transplant allograft antibody-mediated rejection to decrease levels of HLA donor-specific antibodies and anti-A or anti-B isoagglutinins. Retrospective studies have indicated that TPE with enhanced immunosuppression may be effective in reversing humoral rejection of the liver allograft.



Question 40: B. Suspect a fibrin sheath and order tPA use.

Explanation:

- Difficulty aspirating from a central line and frequent access alarms can indicate a fibrin sheath is present. Fibrin sheaths can partially or completely occlude the line during efforts to aspirate from a catheter. The fibrin sheath can be on the inside of the catheter or can deposit on the coating of the catheter tip.



Question 41: C. 15%.

Explanation:

- Most people can safely tolerate an ECV of up to 15% of their total blood volume. When the ECV exceeds 15%, priming of the apheresis circuit with plasma, albumin, or red cells may be needed.



Question 42: D. 3150 mL.

Explanation:

- Calculations are as follows:

$$\begin{aligned}\text{Total blood volume (TBV): } & 75 \text{ kg} \times 70 \text{ mL/kg} = 5250 \text{ mL} \\ \text{Total plasma volume (TPV): } & 5250 \text{ mL} \times (1 - 0.40) = 3150 \text{ mL}\end{aligned}$$



Question 43: B. 1218 mL.

Explanation:

- Calculations are as follows:

$$\begin{aligned}\text{Total blood volume (TBV): } & 60 \text{ kg} \times 70 \text{ mL/kg} = 4200 \text{ mL} \\ \text{Red cell volume (RCV): } & 4200 \times 0.29 = 1218 \text{ mL}\end{aligned}$$

**Question 44: C. 360 mL****Explanation:**

- The maximum ECV is 15%. The patient's total blood volume (TBV) = $60 \times 40 = 2400$ mL; $2400 \times 0.15 = 360$ mL
-

Question 45: D. Prime the machine with red cells.**Explanation:**

- The patient can tolerate a maximum ECV of 360 mL (15% TBV) and the ECP machine tubing and bowl requires 450 mL. Therefore, the machine and tubing should be primed with red cells.
-

Question 46: A. 5055 mL.**Explanation:**

- Calculations are as follows:

$$\text{Total blood volume (TBV)} = 83 \text{ kg} \times 70 \text{ mL/kg} = 5810 \text{ mL}$$

$$\text{Total plasma volume (TPV)} = 5810 \times (1 - 0.42) = 3370 \text{ mL}$$

$$\text{For a 1.5 PV exchange} = 3370 \times 1.5 = 5055 \text{ mL}$$

Question 47: C. 11 bottles.**Explanation:**

- The calculation is as follows:

$$5055 \text{ mL}/500 \text{ mL} = 10.11 \text{ and round up to 11}$$

Question 48: A. Category I.

**Explanation:**

- Sickle cell disease stroke prophylaxis is a Category I recommendation for red cell exchange. Pregnancy and recurrent vaso-occlusive pain crises in non-acute sickle cell disease are Category II indications for red cell exchange. Preoperative management in non-acute sickle cell disease is a Category III indication for red cell exchange. Acute stroke is a Category I indication for red cell exchange and severe acute chest syndrome is a Category II indication for red cell exchange.
-

Question 49: D. Five to seven TPEs over 2 weeks

Explanation:

- The current recommendation is five to seven procedures over 10-14 days.
-

Question 50: D. 891 mL.

Explanation:

- Calculations are as follows:

$$\begin{aligned} \text{TBV} &= 55 \text{ kg} \times 60 \text{ mL/kg} = 3300 \text{ mL} \\ \text{RCV} &= 3300 \text{ mL} \times 0.27 = 891 \text{ mL} \end{aligned}$$

- The apheresis machine will calculate the volume of red cells needed to perform the red cell exchange. It will be more than 3 units because there will be a constant mixing of the new non-sickle red cells with the sickle red cells. The red cells should be matched for C/c, E/e, and Kell at a minimum and also be negative for any antigens that the patient has ever had antibodies against.



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15

Coagulation

Amy E. Schmidt, MD, PhD

Key Points from the *Technical Manual*

- Hemostasis is a delicate balance between procoagulant and anticoagulant factors. Disturbances in hemostasis can be congenital or acquired.
- Congenital hemostatic disorders include von Willebrand disease (types 1, 2, and 3), inherited platelet disorders (eg, Bernard-Soulier syndrome, Glanzmann thrombasthenia, gray platelet syndrome, Chediak-Higashi syndrome, Hermansky-Pudlak syndrome), hemophilia A, hemophilia B, and several rare deficiencies in clotting factors.
- Acquired hemostatic disorders can result from acquired von Willebrand syndrome, acquired platelet disorders, liver disease, disseminated intravascular coagulation (DIC), acquired coagulation inhibitors, and hyperfibrinolysis disorders.
- Thrombophilias can be congenital or acquired.
- Coagulation is a normal response to injury and involves the interactions of platelets, plasma proteins (coagulation factors), and endothelium at the site of injury.
- Anticoagulants include heparin, warfarin, vitamin K antagonists, direct thrombin inhibitors, and direct Factor Xa inhibitors.



QUESTIONS

Question 1: A 40-year-old female with no history of bleeding, presents with repeated epistaxis. She does have a history of Janus kinase 2 (JAK2) and BCR-ABL-negative essential thrombocythemia. She also has an enlarged spleen. Her blood type is group AB-positive. Her coagulation test results are given below. What is the most likely cause of her nose bleeds?

Test	Patient Results	Reference Range
Prothrombin time	11.9 seconds	10.2-14.2 seconds
International normalized ratio	1.1	
Activated partial thromboplastin time	35.8 seconds	26.8-41.2 seconds
Fibrinogen	252 mg/dL	223-372 mg/dL
Factor VIII	59%	55-145%
Von Willebrand antigen	59%	55-200%
Von Willebrand activity	52%	55-200%
Platelets	1,075,000/ μ L	150,000-400,000/ μ L

- A. She has type 1 von Willebrand disease.
- B. She has type 2A von Willebrand disease.
- C. She has type 2N von Willebrand disease.
- D. She has acquired von Willebrand syndrome.
- E. She has type 3 von Willebrand disease.



Question 2: A 64-year-old male with heart failure, coronary artery disease (CAD), and amyloidosis is found to have a prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). He has no bleeding history. What is the cause of the prolonged PT and aPTT?



Test	Patient Results	Reference Range
Prothrombin time	16.5 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	43.4 seconds	26.8-41.2 seconds
aPTT 1:1 mix	31.4 seconds	
Factor X	37%	75-147%
Factor VIII	120%	55-145%
Factor IX	107%	60-140%

- A. The patient has hemophilia A.
 - B. The patient has acquired Factor VIII antibody.
 - C. The patient has decreased Factor X due to amyloid binding.
 - D. The patient has congenital Factor X deficiency.
 - E. The patient has hemophilia B.
-

Question 3: A 13-year-old visually impaired female who recently moved from Puerto Rico is seen for nausea and weight loss of 10 lb over 2 weeks. She had a gastrointestinal virus recently and after that had ongoing nausea and weight loss. Emesis sometimes occurs after eating too much. She has abdominal pain several times per day for a few minutes. No correlation to meals or activity. She also has a life-long history of easy bruising, mostly on her arms and legs. No hematomas. The bruising has been worse than usual for the past few months, and some have been larger than usual. She also has menorrhagia with heavy cramps. Her periods range from 3 days to 2 weeks, and most recently lasted 1 week. She misses 1-2 days per month of school due to cramps or migraines. On physical exam she is noted to have nystagmus, oculocutaneous albinism, and bruising to her arms and legs. Her test results are as follows:



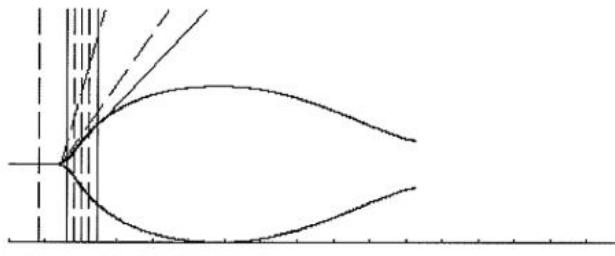
Test	Patient Results	Reference Range
White cells	$10.3 \times 10^9/\text{L}$	$3.8-10.4 \times 10^9/\text{L}$
Hemoglobin	13.1 g/dL	11.9-14.8 g/dL
Hematocrit	40%	35-43%
Red cells	$4.4 \times 10^{12}/\text{L}$	$3.8-5.0 \times 10^{12}/\text{L}$
Platelets	283,000/ μL	153,000-361,000/ μL

What would you expect to see on a peripheral smear or electron microscopy?

- A. Absence of dense granules in platelets.
- B. Elliptocyte red cells.
- C. Giant platelets.
- D. Hypersegmented neutrophils.
- E. Nucleated red cells.



Question 4: A 42-year-old male fell from a ladder and was taken by ambulance to the emergency room. His viscoelastic testing (on a TEG machine) results are shown below. Which statement is the best treatment?



Sample data:	Units:	Normal values:
R: 8.0	min	(4 — 10)
K: 4.2	min	<high> (1 — 3)
Angle: 44.4	deg	<low> (53 — 73)
MA: 40.2	mm	<low> (50 — 72)
LY30: 25.6	%	<high> (0 — 8)
TMA: 25.5	min	
Cl: -6.3		<low> (-3 — 3)



- A. The patient is hypercoagulable and would benefit from anticoagulation with heparin.
 - B. The patient is hypocoagulable and would benefit from plasma transfusion.
 - C. The patient is hypocoagulable and would benefit from platelets/fibrinogen and tranexamic acid.
 - D. The patient has hyperfibrinolysis and needs only tranexamic acid.
 - E. The patient is receiving heparin and a cuvette with heparinase was not used.
-

Question 5: A 56-year-old female with hepatitis C and no bleeding history presents to the emergency department with bruising, nose bleeding, and a swollen knee. She fell recently and is noted to have a very large hematoma on her thigh that measures 6 × 7 inches and is purple and red. Testing is ordered and results are shown below. What do you suspect and what should you order next?

Test	Patient Results	Reference Range
Prothrombin time	11.4 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	101.3 seconds	26.8-41.2 seconds
Fibrinogen	292 mg/dL	223-372 mg/dL
Factor VIII	<5%	55-145%
Factor IX	106%	60-140%
Von Willebrand antigen	145%	55-200%
Von Willebrand activity	96%	55-200%
Platelets	290,000/ μ L	150,000-400,000/ μ L

- A. Suspect hemophilia A and order an aPTT mixing study.
- B. Suspect acquired Factor VIII inhibitor and order an aPTT mixing study and inhibitor titer.



- C. Suspect warfarin use and order an aPTT mixing study and levels of Factor VII.
 - D. Suspect vWF type 2N and order a multimer study.
 - E. Suspect lupus anticoagulant and order an aPTT mixing study and dilute Russell viper venom time (dRVVT) testing.
-

Question 6: A 12-year-old female presents to the pediatrician with heavy periods. Her periods last 5 days and she saturates two tampons per hour on most days. She is also noted to have had excessive bleeding when she had a tooth pulled as well as occasionally when she brushes her teeth. Several of her female relatives who live in Israel also have similar bleeding. Her testing results are below. What is the most likely cause of the bleeding?

Test	Patient Results	Reference Range
Prothrombin time	11.6 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	48.1 seconds	26.8-41.2 seconds
aPTT 1:1 mix, immediate	27.5 seconds	
aPTT mix, 1 hour	28.7 seconds	
Factor IX	102%	60-140%
Factor XI	3%	65-130%
Factor VIII	105%	55-145%
von Willebrand antigen	126%	55-200%
von Willebrand activity	134%	55-200%

- A. Hemophilia C.
- B. Hemophilia A.
- C. Hemophilia B.
- D. von Willebrand disease, type 1.
- E. von Willebrand disease, type 2A.



Question 7: A 30-year-old female with history of bleeding and menorrhagia requires a gynecologic procedure and lysis of adhesions. Her preoperative test results are shown below. The obstetric resident calls and asks what these results mean and what the patient should receive before the surgery. What do you recommend?

Test	Patient Results	Reference Range
Prothrombin time	11.9 seconds	10.2-14.2 seconds
Activated partial thrombo-plastin time	36.5 seconds	26.8-41.2 seconds
Fibrinogen	526 mg/dL	223-372 mg/dL
Factor VIII	41%	55-145%
von Willebrand antigen	24%	55-200%
von Willebrand activity	14%	55-200%
Platelets	288,000/ μ L	150,000-400,000/ μ L
Blood type	Group A-positive	

- A. The patient has lupus anticoagulant and no blood components are needed, but anticoagulation may be helpful.
- B. The patient has hemophilia A and requires Factor VIII replacement before surgery.
- C. There is nothing wrong with the patient and no components are needed.
- D. The patient has type 1 vWD and should receive DDAVP if she is a responder or vWF replacement before surgery.
- E. The patient has hemophilia B and requires Factor IX replacement before surgery.



Question 8: A 57-year-old male who is in the intensive care unit (ICU) has a prolonged aPTT. His test results are shown below. What is the most likely cause?



Test	Patient Results	Reference Range
Prothrombin time	17.3 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	111.4 seconds	26.8-41.2 seconds
aPTT hezyme	39.2 seconds	

- A. The patient has liver failure and is not making any clotting factors.
 - B. The patient is taking warfarin.
 - C. The patient is receiving heparin.
 - D. This is a lab error.
 - E. The patient is taking a direct Factor Xa inhibitor.
-

Question 9: A 56-year-old male had a deep vein thrombosis in his left lower leg diagnosed in the emergency room. Before initiation of anticoagulation, samples were drawn and test results are shown below. What is the most likely cause of his clot?

Test	Patient Results	Reference Range
Prothrombin time	12.1 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	49.6 seconds	26.8-41.2 seconds
aPTT mix, immediate	48.9 seconds	
aPTT mix, 1 hour	48.7 seconds	
Hexagonal phase	Positive	
Dilute Russell viper venom time	42.3 seconds	<45 seconds
Fibrinogen	234 mg/dL	223-372 mg/dL
Factor VIII	107%	55-145%
Antithrombin	102%	74-13%
Protein S activity	124%	60-150%
Protein C	113%	65-135%



- A. Protein S deficiency.
 - B. Elevated Factor VIII levels.
 - C. Antithrombin deficiency.
 - D. Lupus anticoagulant.
 - E. Protein C deficiency.
-

Question 10: What does a prolonged R value on a TEG tracing indicate?

- A. Fibrinolysis.
 - B. Hypercoagulability.
 - C. Low clotting factor function.
 - D. Decreased platelet function.
 - E. Increased platelet function.
-

Question 11: A TEG tracing shows normal R and K values as well as a normal α angle. However, the maximum amplitude is decreased. What blood component(s) might be indicated for transfusion?

- A. Platelets or cryoprecipitate.
 - B. Prothrombin complex concentrate.
 - C. Red Blood Cells.
 - D. Plasma.
 - E. Recombinant Factor VIII.
-

Question 12: What is the most likely explanation for the following TEG results?





- A. The patient is very sick.
 - B. The patient is receiving heparin and a heparinase cuvette was not used.
 - C. The patient is taking warfarin and a heparinase cuvette was used.
 - D. The patient is not receiving heparin and a heparinase cuvette was used.
 - E. The patient is taking dabigatran and a cuvette containing tissue factor was used.
-

Question 13: What is the difference between TEG and ROTEM testing?

- A. They are performed the same way; the only difference is the company that makes the machine.
 - B. In TEG the cup rotates through an angle of 4-45° and in ROTEM the pin rotates.
 - C. In TEG the pin rotates through an angle of 4-45° and in ROTEM the cup rotates.
 - D. In ROTEM the cup rotates through an angle of 4-45° and in TEG the pin and cup do not move.
 - E. In TEG, the cup and pin both move and in ROTEM, neither moves.
-

Question 14: What is the function of kaolin in TEG and ROTEM?

- A. It inactivates heparin.
- B. It activates the platelets directly allowing better measurement of maximal amplitude.
- C. It activates the contact pathway of coagulation and generates a standardized, faster waveform tracing.
- D. It eliminates the effects of aspirin and clopidogrel on platelet function.
- E. It eliminates the effects of warfarin on clotting.



Question 15: When TEG is used in the setting of cardiopulmonary bypass, the testing cuvettes used often have tissue factor and heparinase. Which TEG measurement can no longer be used in this case and why?

- A. MA because tissue factor makes it artificially increased.
 - B. α angle because tissue factor makes it artificially increased.
 - C. LY30 because tissue factor prevents fibrinolysis from occurring.
 - D. R value because tissue factor makes it artificially too short.
 - E. K time because tissue factor makes it artificially increased.
-

Question 16: The aPTT is normal in patients with severe deficiency of which of the following clotting factors?

- A. Factor V.
 - B. Factor VIII.
 - C. Factor IX.
 - D. Factor XI.
 - E. Factor VII.
-

Question 17: Which of the following plasma coagulation factors is found in cryoprecipitate?

- A. Factor II.
 - B. Factor VIII.
 - C. Factor IX.
 - D. Factor XIII.
 - E. Both B and D.
-

Question 18: Which of the following is *not* localized or synthesized in a platelet?

- A. Arachidonic acid.
- B. Serotonin.
- C. Prostacyclin.
- D. Thromboxane A₂.
- E. Calcium.



Question 19: A 47-year-old man needs to have a knee replacement surgery. He was diagnosed with a bleeding disorder as a child but does not remember his diagnosis. He does remember not being allowed to play outdoors with the other kids. He has received several blood components and infusions over the years, associated with trauma and medical procedures. A battery of laboratory tests demonstrates prolonged PT, prolonged aPTT, normal thrombin time (TT), and prolonged dilute Russell viper venom time (dRVVT). These results are consistent with a congenital deficiency of which coagulation factor?

- A. Factor I.
 - B. Factor VII.
 - C. Factor IX.
 - D. Factor X.
 - E. Factor XIII.
-

Question 20: Which of the following statements concerning Factor XII (Hageman factor) is true?

- A. Its activation triggers the extrinsic pathway of coagulation.
 - B. Deficiency of Factor XII is associated with a mild bleeding disorder.
 - C. Factor XII deficiency has a prevalence of about 1 in 100,000.
 - D. Deficiency of Factor XII causes a marked prolongation of the PT.
 - E. It is an important determinant of in-vitro hemostasis but is not a determinant of physiologic hemostasis.
-

Question 21: Which of the following statements regarding the thrombin time and reptilase time is true?

- A. Reptilase time (RT) is prolonged in the presence of heparin.
- B. Both are prolonged in the presence of an abnormal fibrinogen.
- C. Both will be prolonged by vitamin K deficiency.
- D. Thrombin time (TT) is prolonged by the addition of protamine.
- E. They both measure the conversion of prothrombin to thrombin.



Question 22: A male neonate is noted to have extensive bruising soon after birth as well as excessive bleeding following blood collection for his bilirubin level. Neither parent has had any issues with bleeding or bruising. Initial coagulation testing reveals a normal PT, TT, and complete blood count (CBC); however, he has a very prolonged aPTT. What tests should be ordered next?

- A. Factor levels (I, II, XIII).
 - B. Factor levels (II, V, VII, X).
 - C. Factor levels (VIII, IX, XI).
 - D. Platelet function analyzer-100 (PFA-100).
 - E. TEG.
-

Question 23: Testing on the patient in Question 22 confirms a diagnosis of hemophilia A. If the family history is probed deeper, which family member is *most* likely to have a bleeding history?

- A. Father.
 - B. Paternal grandfather.
 - C. Maternal grandfather.
 - D. Paternal grandmother.
 - E. Maternal grandmother.
-

Question 24: The differentiation of type 2B von Willebrand disease (vWD) from all other subtypes depends on which of the following laboratory tests?

- A. Bleeding time.
 - B. Low-strength ristocetin platelet aggregation.
 - C. Full-strength ristocetin platelet aggregation.
 - D. Multimeric assay of Factor VIII.
 - E. Ristocetin cofactor assay.
-

Question 25: Which of the following is the best screening test for the presence of lupus anticoagulant (LA)?

- A. PT.



- B. Fibrinogen.
 - C. Dilute aPTT.
 - D. Factor X activity.
 - E. Antithrombin.
-

Question 26: An elevated level of D-dimer is specific for which of the following?

- A. Primary fibrinolysis.
 - B. Disseminated intravascular coagulation (DIC).
 - C. A blood clot.
 - D. All of the above.
 - E. None of the above.
-

Question 27: Which of the following is the *best* test to monitor the treatment of thrombotic thrombocytopenic purpura (TTP)?

- A. ADAMTS13 activity.
 - B. Fibrinogen level.
 - C. Platelet count.
 - D. D-dimer.
 - E. Hemoglobin.
-

Question 28: Which of the following results is most suggestive of primary fibrinolysis?

- A. Elevated levels of plasminogen and fibrin degradation products (FDPs).
- B. Low levels of all clotting factors and positive monochloroacetic acid (MCA) lysis.
- C. Thrombocytopenia, low levels of all clotting factors, short euglobulin lysis time, and elevated FDPs.
- D. Short euglobulin lysis time and decreased fibrinogen.
- E. None of the above.



Question 29: A 10-year-old boy presents to the emergency room with new onset of seizures and severe headache. A head computerized tomography (CT) scan shows a thrombus in the cerebral sinus. His mother tells the physician in the emergency department that she had a pulmonary embolism when she was 19 years old and a deep vein thrombosis when she was 25 years old. She is on daily anticoagulant therapy now. Which of the following is the most likely diagnosis?

- A. Protein S deficiency.
 - B. Bernard-Soulier syndrome.
 - C. Glanzmann thrombasthenia.
 - D. Factor X deficiency.
 - E. Alpha₂-antiplasmin deficiency.
-

Question 30: What skin condition can be associated with protein C deficiency?

- A. Livedo reticularis.
 - B. Cryoglobulinemia.
 - C. Pyoderma gangrenosum.
 - D. Warfarin-induced skin necrosis.
 - E. Panniculitis.
-

Question 31: Vitamin K deficiency affects the function of which of the following proteins?

- A. Antithrombin.
 - B. Factor VIII.
 - C. Protein S.
 - D. Factor XII.
 - E. Factor V.
-

Question 32: Which of the following can cause elevation of FDPs?

- A. Recent thrombosis.
- B. Renal and liver disease.



- C. Recent surgery.
 - D. Primary fibrinolysis.
 - E. All of the above.
-

Question 33: Which of the following has been associated with an abnormal activated protein C resistance (APCR) test?

- A. A mutation in the Factor V gene.
 - B. Decreased thrombin ability to bind thrombomodulin.
 - C. A mutation in the Factor VIII gene.
 - D. Defective release of thrombomodulin.
 - E. A genetic defect in protein S.
-

Question 34: A 10-year-old boy with a history of nose bleeds presents to the hospital with a nose bleed that will not stop. Some laboratory testing is performed with results below.

Test	Patient Results	Reference Range
Prothrombin time	12.2 seconds	10.2-14.2 seconds
Activated partial thromboplastin time	31.7 seconds	26.8-41.2 seconds
Fibrinogen	304 mg/dL	223-372 mg/dL
von Willebrand antigen	120%	55-200%
von Willebrand activity	101%	55-200%
Platelets	245,000/ μ L	150,000-400,000/ μ L

Platelet aggregometry is then performed and shows a normal response to ristocetin, but no response to other agonists. What is the diagnosis?

- A. Bernard-Soulier syndrome.
- B. Scott syndrome.
- C. Quebec platelet syndrome.



- D. Storage pool disorder.
 - E. Glanzmann thrombasthenia.
-

Question 35: Which of the following statements is true regarding the international normalized ratio (INR)?

- A. It is useful for comparing the PT and aPTT results with those of different laboratories.
 - B. It is useful for monitoring patients receiving heparin therapy.
 - C. It is recommended for monitoring dabigatran during the first few days of therapy.
 - D. It is calculated as [patient PT ÷ control PT]^{ISI}.
 - E. It is most sensitive to Factor II levels.
-

Question 36: Which of the following factors has the longest half-life?

- A. Factor VII.
 - B. Factor XIII.
 - C. Factor VII.
 - D. Factor XII.
 - E. Factor X.
-

Question 37: Which of the following are true regarding heparin-induced thrombocytopenia (HIT)?

- A. Typically occurs 3 to 14 days after heparin administration.
 - B. Can be associated with heparin flushes.
 - C. Associated with thrombosis.
 - D. Largely remains a clinical rather than a laboratory diagnosis.
 - E. All of the above.
-

Question 38 A 10-year-old boy presents with thrombocytopenia, eczema, and immunodeficiency. Which of the following is the most likely diagnosis?



- A. Wiskott-Aldrich syndrome.
 - B. Hermansky-Pudlak syndrome.
 - C. Quebec platelet syndrome.
 - D. Scott syndrome.
 - E. Chediak-Higashi syndrome.
-

Question 39: Which of the following is true regarding low-molecular-weight heparin (LMWH)?

- A. It exerts its major effect against Factor X.
 - B. It is monitored by the anti-Xa assay.
 - C. It is not associated with risk of HIT.
 - D. It can be reversed with plasma transfusion.
 - E. A and B.
-

Question 40: Which of the following statements concerning DIC is true?

- A. Protein C and protein S activity are decreased.
 - B. Decreased levels of antithrombin are associated with increased mortality.
 - C. Factor VIII levels are variable and can be high or low.
 - D. All of the above.
 - E. A and B.
-

Question 41: The aPTT is not sensitive to which of the following coagulation factors?

- A. Factor XII.
- B. Factor V.
- C. Factor VII.
- D. Factor IX.
- E. Factor X.



Question 42: A 42-year-old man with hemophilia A requires a knee replacement. The morning of surgery he is given an appropriate dose of recombinant Factor VIII to obtain a factor level of 100%; however, 2 hours after infusion his Factor VIII level is only 36%. Surgery is delayed while another dose of Factor VIII is given, but again the postinfusion factor level is less than expected. Which type of vWD could this be?

- A. Type 1.
 - B. Type 2A.
 - C. Type 2B.
 - D. Type 2N.
 - E. Type 2M.
-

Question 43: A 71-year-old female presents with a history of cough, fever, and right chest pain. After the appropriate workup, a diagnosis of deep vein thrombosis with pulmonary embolism is made. Symptoms resolve with bed rest and heparin anticoagulation. Long-term anticoagulation with warfarin is instituted and heparin is discontinued. Six months later, the patient is brought to the emergency department and is unresponsive. A CT scan shows an intracerebral hemorrhage. Her INR is 9. What is the most effective immediate treatment?

- A. Intramuscular (IM) vitamin K.
 - B. Prothrombin complex concentrate (PCC).
 - C. Fresh Frozen Plasma (FFP).
 - D. Intravenous (IV) vitamin K.
 - E. Cryoprecipitate.
-

Question 44: Which of the following factors has the shortest half-life?

- A. Factor II.
- B. Factor V.
- C. Factor VII.
- D. Factor IX.
- E. Factor X.



Question 45: Which of the following factors is *not* screened for by the PT test?

- A. Factor II.
 - B. Factor V.
 - C. Factor VII.
 - D. Factor IX.
 - E. Factor X.
-

Question 46: A 17-year-old male with severe hemophilia is scheduled for surgery. He weighs 65 kg, has a Factor VIII activity of <1%, and his hematocrit is 45%. How many units of Factor VIII would be required to raise the patient's Factor VIII level to 50%? Assume 70 mL/kg for blood volume.

- A. 625.
 - B. 1000.
 - C. 1250.
 - D. 2500.
 - E. 4550.
-

Question 47: A 40-year-old woman presents for surgical removal of a benign-appearing ovarian cyst. The surgery goes well; however, she is urgently returned to the operating room on postoperative day 1 for increased surgical drain output. The patient appeared to be bleeding from several sites in her abdomen, which required administration of multiple blood components. Laboratory testing showed: normal PT, normal aPTT, normal TT, normal bleeding time, and there was lysis when the patient's clot was placed in 5 M urea. What is the most likely diagnosis?

- A. Factor VII deficiency.
- B. Factor XII deficiency.
- C. Factor XIII deficiency.
- D. Plasminogen deficiency.
- E. Tissue plasminogen activator deficiency.



Question 48: How long is the half-life of Factor VIII in an average healthy person?

- A. 6 hours.
 - B. 12 hours.
 - C. 24 hours.
 - D. 36 hours.
 - E. 48 hours.
-

Question 49: What is the pattern of inheritance for hemophilia A?

- A. Autosomal recessive.
 - B. X-linked dominant.
 - C. Autosomal dominant.
 - D. X-linked recessive.
 - E. Autosomal codominant.
-

Question 50: A 30-year-old woman presents with an acute right-sided hemiparesis. Imaging confirms a thrombus in the left anterior cerebral artery, without hematoma formation. She is immediately given thrombolytic therapy. She has a negative family history for thrombosis. The patient is not obese, doesn't smoke, and doesn't have hypertension. She had been treated with ravulizumab-cwvz for approximately 1 year before losing her insurance. She does not remember why she received ravulizumab-cwvz but remembers she had "anemia and yellow eyes" before she was started on it. What is the most likely diagnosis?

- A. Antiphospholipid disease.
- B. Factor V Leiden.
- C. MTHFR mutation.
- D. Paroxysmal nocturnal hemoglobinuria.
- E. Prothrombin gene mutation.

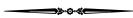


ANSWERS

Question 1: D. She has acquired von Willebrand syndrome.

Explanation:

- Acquired von Willebrand syndrome is frequently associated with essential thrombocythemia, autoimmune disorders, cardiovascular disorders, and monoclonal gammopathies. These diseases either inhibit the vWF or enhance clearance of the vWF. Acquired von Willebrand syndrome occurs in people with no history of bleeding.
- vWF levels are associated with blood type, with group O patients having lower vWF levels as compared to group A, group B, or group AB. Frequently, group O patients can be diagnosed as type 1 vWD.
- The patient's lab results seem to indicate a type 1 vWD; however, group AB patients would be expected to have much higher vWF activity and antigen. The donor is also not vWD type 2A. Type 2A is a qualitative defect where there is a mismatch between the vWF activity and vWF antigen. Usually, the ratio is <0.6. However, type 2A and type 1 would both be expected to be associated with a bleeding history. Type 2N is a defect in binding to Factor VIII and is associated with normal levels of vWF activity and antigen but very low levels of Factor VIII. It frequently can look like hemophilia A.



Question 2: C. The patient has decreased Factor X due to amyloid binding.

Explanation:

- The patient has no history of bleeding, which would exclude congenital Factor X deficiency. This is also known as Stuart-Power factor deficiency and is rare. Factor X deficiency is inherited in an autosomal recessive pattern.
- If the patient had acquired Factor VIII inhibitor, the aPTT would be prolonged and the PT would be normal as well as the Factor



VIII would be low and Factor X would be normal. Additionally, the aPTT would not correct in Factor VIII inhibitors.

- Hemophilia A is due to a congenital Factor VIII deficiency and would have a prolonged aPTT and low Factor VIII level.
- This patient has acquired Factor X deficiency due to amyloid binding. Amyloid fibrils are known to bind to circulating Factor X causing decreased levels and bleeding. Other clotting factors (ie, Factors II, VII, and IX) can be bound by amyloid fibrils; however, Factor X is more efficiently bound and cleared.



Question 3: A. Absence of dense granules in platelets.

Explanation:

- The patient has Hermansky Pudlak syndrome (HPS). This disease is rare in individuals from various populations. However, the incidence is 1:1800 in northwestern Puerto Rico.
- HPS is associated with oculocutaneous albinism, horizontal nystagmus, easy bruising, prolonged bleeding after dental procedures/surgery/childbirth, menorrhagia, granulomatous colitis, and pulmonary fibrosis.
- The platelets have a storage pool deficiency of dense granules. Platelet-dense granules store adenosine diphosphate (ADP), serotonin, calcium, adenosine triphosphate (ATP), and phosphate.
- Elliptocytes are seen in hereditary elliptocytosis and would not be associated with easy bruising, menorrhagia, or the other findings in the patient.
- Giant platelets can be seen in Bernard-Soulier syndrome, May-Hegglin anomaly, and gray platelet syndrome but these conditions would not present in this patient.
- Lastly, hypersegmented neutrophils are seen in B12 deficiency and folate deficiency.



Question 4: C. The patient is hypocoagulable and would benefit from platelets/fibrinogen and tranexamic acid.



Explanation:

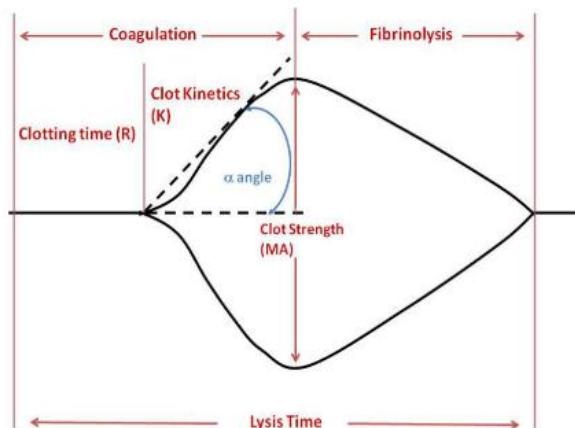
- The patient has a decreased maximum amplitude (MA), decreased alpha angle, decreased K, negative coagulation index (CI), and elevated LY30. See accompanying figure and table.
 - The MA reflects clot strength and can indicate a need for platelets or fibrinogen when low.
 - K is the coagulation time and is a measure of the speed of clot formation and strengthening. A low K can indicate a need for fibrinogen.
 - The α angle is the angle between baseline and a line defined by the points R and K and is a measure of the speed of clot formation. A low α angle can indicate a need for fibrinogen.
 - R is the reaction time and measures the time from zero to the beginning of clot formation. A low R can indicate hypercoagulability and a possible need for anticoagulation. A prolonged R can indicate a need for plasma infusion.
 - LY30 is the % clot lysis at 30 minutes. In this case, the patient has hyperfibrinolysis.
 - The CI, or coagulation index, takes into effect all values in TEG and gives an overall picture of the patient's coagulation status, which in this case is hypocoagulable.
- The patient would benefit from tranexamic acid as well as platelets/fibrinogen.

Measurement	TEG	ROTEM
Clotting time (time from start to 2 mm amplitude)	R (reaction time) 2-8 minutes*	CT (clotting time) 100-240 seconds*
Clot kinetics (time from 2 mm to 20 mm amplitude)	K (kinetics) 1-3 minutes*	CFT (clot formation time) 30-110 seconds*
Clot strengthening	α angle 55-78 degrees*	α angle 70-83 degrees*
Maximum strength of clot	MA (maximum amplitude) 51-69 mm*	MCF (maximum clot firmness) 50-72 mm*



Measurement	TEG	ROTEM
Clot lysis at a specific time	LY30, LY60 (clot lysis) LY30: 0-8%*	CLI30, CLI60 (clot lysis index) CLI30: 94-100%*

*Normal ranges given are for assays using kaolin as an activator.



Schematic representation of thromboelastograph. The clot time or reaction time (R value) reflects the time to initiate fibrin formation. The kinetics of clot formation (K value) is the time at which the amplitude reflecting clot strength reaches 20 mm. The R and K values are measurements of coagulation. The α angle is the angle between midline and a line tangential to the developing TEG trace. The α angle represents the clot kinetics of clot build-up and fibrin crosslinking. The maximum amplitude (MA) is the maximum width of the TEG trace. It is a measurement of the ultimate clot strength and reflects platelet number and function.



Question 5: B. Suspect an acquired Factor VIII inhibitor and order a PTT mixing study and inhibitor titer.



Explanation:

- The patient has no bleeding history and has presented with bleeding, bruising, and hematomas. Because hemophilia A is congenital and X-linked recessive, it is unlikely she has hemophilia A. Her Factor IX levels are also normal and Factor IX is a vitamin-K-dependent protein that is affected by warfarin. Thus, it is unlikely she is taking warfarin. vWF type 2N has normal vWF levels and low Factor VIII levels due to defective vWF binding of Factor VIII. This would also be congenital and the patient has no bleeding history.
- New bleeding in an older patient with prolonged aPTT and low Factor VIII levels is likely to be an acquired inhibitor. The most common acquired inhibitor is to Factor VIII. A mixing study would possibly correct immediately and after 30 minutes to 1 hour of incubation would be prolonged.
- A titer of the Factor VIII antibody would be helpful in deciding treatment. A low-titer antibody could possibly be overcome with Factor VIII replacement and a high-titer antibody would require FEIBA and possibly recombinant Factor VIIa treatment.



Question 6: A. Hemophilia C.

Explanation:

- The patient has a prolonged aPTT that corrects in a 1:1 mix immediately and stays corrected for 1 hour. This indicates that it is most likely a factor deficiency and not an antibody or inhibitor.
- Hemophilia A and B are both X-linked recessive inheritance patterns and unlikely to occur in a female. Moreover, both would likely present with bleeding earlier in life and present with significant joint bleeding and large hematomas.
- The patient has normal levels of Factor VIII, vWF antigen, and vWF activity which excludes vWD type 1.
- Hemophilia C is caused by a deficiency of Factor XI and is inherited autosomally recessively. Factor XI deficiency affects males and females equally. Hemophilia C occurs at a frequency of ~1:100,000 in the general population and is more common in people of Ashkenazi Jewish ancestry, where it affects ~8% of the population.



Question 7: D. The patient has type 1 vWD and should receive DDAVP if she is a responder or vWF replacement before surgery.

Explanation:

- The patient has low vWF antigen and activity as well as low Factor VIII levels. She also has a bleeding history. Type I vWD patients can have a trial of DDAVP to see if they respond and release enough vWF. DDAVP would not be appropriate for treating patients with qualitative defects in vWF such as type 2A and type 2B vWD. If the patient is not a responder, treatment with vWF replacement can be started.
- There is no indication that she has a lupus anticoagulant.
- As described previously, hemophilia A is X-linked recessive and uncommon in females.



Question 8: C. The patient is receiving heparin.

Explanation:

- The patient is most likely receiving heparin as indicated by a prolonged aPTT and slightly prolonged PT. Moreover, hezyme treatment results in full correction of the aPTT. Helyzyme is an enzyme that degrades heparin in samples. Given that the aPTT corrects with hezyme, the patient is unlikely to have a prolonged aPTT due to warfarin use or liver failure.



Question 9: D. Lupus anticoagulant.

Explanation:

- The patient has a lupus anticoagulant as evidenced by his prolonged aPTT that does not correct with mixing immediately or with incubation. To test for lupus anticoagulants, excess lipids are added, and the tests are repeated. With additional lipids, the antibodies bind to the excess lipids and coagulation can normalize.
- If the aPTT-based lupus anticoagulant testing is negative, the dRVVT assay can be performed. If this is prolonged, excess phos-



pholipids are added and it is repeated. A sensitive screen for lupus anticoagulants is a dRVVT or dilute aPTT where the phospholipids are diluted, making the antibody more likely to be detected.

- Antithrombin, protein S, and protein C are all anticoagulant proteins that can be associated with hypercoagulable states if they are deficient.
- Lupus anticoagulants are present in ~30% of lupus patients. Thus, most people with lupus anticoagulants do not have lupus.



Question 10: C. Low clotting factor function.

Explanation:

- The R value reflects clotting proteins and a prolonged R value indicates hypocoagulability and possible need for plasma transfusion. Decreased platelet number or function would likely be reflected by a low MA. Hypercoagulability could be reflected by a shortened R value, elevated MA, or a shortened K time. Fibrinolysis is reflected in the LY30.



Question 11: A. Platelets or cryoprecipitate.

Explanation:

- A decreased MA is most indicative that transfusion with platelets or cryoprecipitate may be needed. A prolonged R value would indicate a possible need for plasma or in a rare case, prothrombin complex concentrate. There is no value on the TEG that would indicate that RBCs are needed.



Question 12: B. The patient is receiving heparin and a heparinase cuvette was not used.



Explanation:

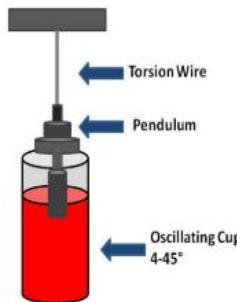
- A heparinase cuvette would have no effect on blood from a patient who was taking warfarin. Similarly, a heparinase cuvette would have little to no effect on blood from a patient who was not receiving heparin.



Question 13: B. In TEG the cup rotates through an angle of 4-45° and in ROTEM the pin rotates.

Explanation:

- In TEG, the cup rotates through an angle of 4-45 degrees with each rotation cycle taking 10 seconds. Then, as the blood clots and fibrin strands develop, the cup's movement is impeded; this is transmitted to the pin and a tracing is generated. See accompanying figure.



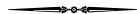
In TEG, the pin is suspended from a torsion wire and is inserted into the cuvette or cup which rotates through an angle of 4-45°. As fibrin strands form in the cuvette/cup, the impeded movement is transmitted via the pin and torsion wire to a tracing.

- In ROTEM, the pin transmits a signal via an optical detector system and not a torsion wire as in TEG. The pin rotates through an angle and as the blood clots and fibrin strands form, the pin's movement is impeded and as in TEG, a tracing is generated.
- Some of the differences in the measurements reported by TEG and ROTEM can be explained by the differences in cups and pins used in each system. Cups and pins in ROTEM are composed of a plastic with an increased surface charge as compared to those



used in TEG. Hence, there is greater contact pathway activation in ROTEM. There are also differences in composition and concentration of components in the proprietary activators used in each system.

- Both the TEG and ROTEM tests have added modifications that allow for better interpretation of clotting under various circumstances.



Question 14: C. It activates the contact pathway of coagulation and generates a standardized, faster waveform tracing.

Explanation:

- Most commonly, TEG and ROTEM are used on citrated whole blood. To initiate coagulation, these samples need to have calcium added and often kaolin is also used as an activator of blood coagulation. Kaolin use allows the tests to be standardized and decreases the time it takes to get a result.
- Use of citrated samples allows the samples to be stored for 30 minutes to 4 hours, during which time they can be transported to a central lab if needed. However, before a citrated sample can be analyzed, it must sit or rest for at least 30 minutes.
- A native whole blood TEG or ROTEM test usually takes 30-60 minutes. The results of the thromboelastography tests are usually made available to clinicians in real-time on monitors in the emergency department and operating rooms.



Question 15: D. R value because tissue factor makes it artificially too short.

Explanation:

- During cardiac surgery in which patients are on cardiopulmonary bypass (CPB), platelets are activated and degranulated via contact/mechanical activation (ie, by passing through the tubing and CPB circuit components) as well as via direct thrombin activation generated as a result of CPB-induced contact/tissue factor activation. Thus, tissue-factor activation of the TEG can be used after



CPB and allows the MA to be measured in approximately 10 minutes; however, because the tissue factor shortens the R value, it makes interpretation of the R value problematic.

Question 16: E. Factor VII.

Explanation:

- The aPTT is a measure of the function of the intrinsic pathway (contact factors and Factors XI, IX, and VIII) and common pathways (Factors X, V, thrombin, and fibrinogen) of coagulation. To perform the test, calcium is added to a mixture of citrated plasma and thromboplastin and kaolin or another negatively charged substance. The endpoint is the time to clot formation, measured in seconds.
 - Patients with vWD may have a prolonged aPTT as a result of the decrease in Factor VIII that accompanies vWD abnormalities. Although the prolongation of the aPTT may be minimal or absent in mild cases, in type 3 vWD and type 2N vWD, the Factor VIII is markedly reduced, and the aPTT is prolonged.
 - Factor VII and tissue factor are part of the extrinsic pathway and along with Factors X and V, thrombin, and fibrinogen are measured in the PT. A deficiency of Factor VII would result in a prolonged PT and normal aPTT.
-

Question 17: E. Both B and D.

Explanation:

- Cryoprecipitate is the insoluble fraction of plasma that precipitates when FFP is thawed at 1 to 6 C. Cryoprecipitate contains concentrated forms of Factor VIII, vWF, fibrinogen, and Factor XIII.
- The most common indication for cryoprecipitate use is hypofibrinogenemia caused by consumption (eg, DIC) or loss (eg, massive bleeding). Other uses of cryoprecipitate include treatment of hereditary dysfibrinogenemia and in bleeding associated with uremia.



- Notably, a functional defect in the vWF-platelet interaction is thought to contribute to platelet dysfunction in uremia. Administration of cryoprecipitate has been shown to shorten the bleeding time in some patients with uremia. Results are highly variable, but when effective, a response in bleeding time is seen within 4 to 12 hours, with some responses as early as within 1 hour. In uremic patients, cryoprecipitate is usually given only to those who are undergoing surgical procedures or experiencing difficult-to-control bleeding.
- Cryoprecipitate was originally developed for the treatment of hemophilia and vWD. However, it is no longer used for this purpose because recombinant and pathogen-reduced factor concentrates are available for management of these bleeding disorders. Cryoprecipitate, FFP, and Factor XIII concentrate are acceptable options for Factor XIII replacement. If Factor XIII concentrate is not available and volume is not an issue for the patient, then FFP is the preferred product. This is because cryoprecipitate only moderately concentrates Factor XIII.

Question 18: C. Prostacyclin.**Explanation:**

- Prostacyclin, a platelet-aggregation inhibitor, is produced in the vascular endothelium. Arachidonic acid is cleaved from the platelet membrane by phospholipases. Cyclo-oxygenase is located in the dense tubular system of the platelet. It metabolizes arachidonic acid to an intermediate substance, which thromboxane synthetase converts to thromboxane A₂. Calcium and serotonin are both located in dense granules.

Question 19: D. Factor X.**Explanation:**

- Prolongation of the PT and aPTT suggests a congenital deficiency within the common pathway (Factors X, V, II, and I). Factor II is commonly referred to as thrombin and Factor I is fibrinogen.



- A prolonged dilute Russell viper venom time measures direct activation of the common pathway at Factor X.
 - The normal thrombin time rules out deficiency of Factor I. Deficiencies of Factors X, V, and II remain in the differential diagnosis; however, of these, only Factor X is listed as a choice.
-

Question 20: E. It is an important determinant of in-vitro hemostasis but is not a determinant of physiologic hemostasis.

Explanation:

- Factor XII is a component of the intrinsic system. Factor XII (Hageman factor), prekallikrein (Fletcher factor), and Factor XI are protein zymogens that are known as the “contact system” because of the autoactivation of Factor XII when it comes in contact with negatively charged surfaces (eg, glass tubes). Although important for in-vitro measurement of coagulation, Factor XII is not physiologically necessary for coagulation, as evidenced by the finding that individuals with Factor XII deficiency do not exhibit a bleeding disorder.
 - In-vitro, homozygous deficiency of Factor XII will cause a marked prolongation of the aPTT (~200 seconds). Heterozygous deficiency prolongs the aPTT to a less significant degree.
 - Factor XII does participate in other physiologic functions, including fibrinolysis, complement activation, inflammation, and chemotaxis. Its importance in these functions remains largely undetermined.
 - The prevalence of homozygous Factor XII deficiency is about 1:1,000,000. Heterozygous Factor XII deficiency is more common and is often detected as an otherwise unexplained prolongation of the aPTT.
-

Question 21: B. Both are prolonged in the presence of an abnormal fibrinogen.



Explanation:

- The TT measures the conversion of fibrinogen to fibrin. In the thrombin time test, bovine or human thrombin is added to the patient's citrated plasma sample, then the time to clot formation is measured in seconds.
- The TT is prolonged when the sample contains heparin or direct thrombin inhibitors, the patient has hypofibrinogenemia or hyperfibrinogenemia, the patient has dysfibrinogenemia, or the patient sample contains elevated FDPs.
- Reptilase is an enzyme derived from snake venom. Like thrombin, it splits fibrinogen to fibrin and generates fibrinopeptide A. Unlike thrombin, it does not split fibrinopeptide B from fibrin.
- Notably, reptilase is not inhibited by heparin; therefore, a RT will help distinguish elevation in TT due to heparin from other causes of elevated TT. The RT is otherwise prolonged by all of the same causes of TT prolongation
- Because the TT and RT measure conversion of fibrinogen to fibrin after addition of exogenous thrombin or reptilase, it is not affected by deficiency in vitamin K or treatment with warfarin or direct Factor Xa inhibitors. Protamine neutralizes the effect of heparin and may be added to specimens suspected of containing heparin as a means of demonstrating that a TT prolongation is attributable to heparin. Protamine will not prolong the TT.



Question 22: C. Factor levels (VIII, IX, XI).

Explanation:

- A prolonged aPTT in the setting of a normal PT indicates that Factors VIII, IX, XI, and XII may be low. A mixing study could be performed as well as a repeat of the aPTT with hepczyme to ensure that the cause is not heparin.
- The patient has a normal number of platelets and platelet disorders are quite rare.
- A TEG would not help to elucidate the cause of the infant's bruising and bleeding.



Question 23: C. Maternal grandfather.

Explanation:

- The infant has hemophilia A, which is inherited in an x-linked recessive pattern; therefore, the mother was a carrier and the maternal grandfather was potentially affected.



Question 24: B. Low-strength ristocetin platelet aggregation.

Explanation:

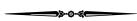
- vWD is categorized into three major types. Types 1 and 3 are characterized by a quantitative defect in vWF and type 2 is characterized by a qualitative difference in vWF.
- Type 1 accounts for ~75% of cases and is a partial quantitative deficiency of vWF. Bleeding symptoms are usually mild to moderate; some cases may be asymptomatic until a hemostatic challenge.
- Type 2 accounts for ~20% of cases and has functionally deficient vWF protein.
 - Type 2A decreases the intermediate and high-molecular-weight vWF multimers that are necessary for hemostasis.
 - In type 2B, vWF has an increased ability to bind to the platelet receptor glycoprotein 1b (GP1b). High-molecular-weight multimers readily bind to platelets and are lost from circulation. Type 2B may also cause clearance of platelets from circulation because of agglutination. The use of desmopressin (DDAVP) may increase platelet clearance and produce thrombocytopenia. Therefore, DDAVP is contraindicated for management. This is a distinct and separate type compared to platelet-type vWD.
 - Type 2M is rare and is characterized by reduced binding to GPIb. Type 2M can be associated with significant bleeding.
 - Type 2N is also rare and is characterized by vWF not binding to Factor VIII, which leads to rapid clearance of Factor VIII. This type of vWD can look similar to hemophilia A.
 - Platelet-type (or pseudo vWD) is characterized by abnormal GPIb receptor binding on the platelet and has normal vWF. It appears similar to type 2B.



- Type 3 is rare and characterized by the absence of vWF. Because vWF binds and protects Factor VIII from clearance, these patients also have very low Factor VIII levels, <10%. The patients also have severe bleeding that is both mucosal as well as soft tissue/joint bleeding.
- Testing for vWD can be divided into tests that answer the following questions:
 1. How much vWF antigen is present?
 2. How much vWF activity is present?
 3. Is the quality of the vWF interaction with platelets normal?
 4. Is the multimeric composition of the vWF normal?
 5. Is Factor VIII activity normal?
 - Plasma vWF antigen (vWF:Ag) is a quantitative measure of the amount of vWF antigen in the circulation. vWF:Ag is reduced when vWF production is decreased. Examples would be vWD types 1 and 3.
 - Plasma vWF activity (ristocetin cofactor activity, or vWF:RCO) is a measure of the ability of vWF to interact with platelet GPIb. vWD types characterized by a qualitative difference in vWF will have a low vWF:RCO relative to vWF:Ag. Decreased vWF:RCO would be seen in vWD types 1, 2A, 2B, and 3.
 - RIPA: Ristocetin binds to platelet GPIb and to vWF. Platelets with bound vWF will aggregate in the presence of ristocetin. Ristocetin-induced platelet aggregation is a measure of the affinity with which vWF binds to GPIb. Ristocetin is added to aliquots of patient platelet-rich plasma at various concentrations. The vWF of type IIB vWD readily binds GPIb; therefore, ristocetin will induce aggregation at lower concentration than is seen with normal individuals or other forms of vWD. An excellent way to remember this is the mnemonic "type 2B Binds Better."
 - Multimer analysis: vWF multimer distribution is a gel electrophoresis test used to qualitatively assess the quantity and molecular weight distribution of the patient's vWF multimers. It provides a visual estimate of the amount of vWF present and whether it is compositionally normal.
 - Factor VIII activity is a coagulation factor assay. Factor VIII activity is reduced when the amount of vWF is reduced, or when there is a defect in the vWF binding site for Factor VIII.
 - PFA-100 assessment of platelet aggregation in plug formation may also be included in the evaluation of vWD.

**Question 25: C. Dilute aPTT.****Explanation:**

- Phospholipid antibodies are directed at phospholipids and phospholipid-binding proteins such as cardiolipin and β_2 -glycoprotein. LA is the term applied to phospholipid antibodies that interfere with the function of coagulation and anticoagulation factors. In the body, the interaction of LA with their target phospholipid and antigen is prothrombotic and can manifest as a clot. In the laboratory, a LA actually appears to inhibit coagulation and prolongs the clotting time in various clotting-based assays.
- Notably, the aPTT is prolonged only in approximately 50% of patients with LA. When LA is strongly suspected, coagulation testing should be performed by methods that are more sensitive to phospholipid levels. The dilute PT, dilute aPTT, and DRVVT are low-phospholipid-concentration clotting tests that are recommended for the initial detection of LA.
- In the presence of LA, the phospholipid in these tests is not sufficient to support coagulation. If there is prolongation of a phospholipid-dependent test, the next step is to determine if a 1:1 mixing test corrects the prolongation. If the prolongation does not correct, then the presence of LA is suggested, and must be confirmed by demonstrating that addition of excess phospholipid shortens the prolonged coagulation test.
- It is important to note that the presence of other inhibitors of coagulation must be excluded.

**Question 26: E. None of the above.****Explanation:**

- The presence of D-dimer indicates that the sequence of coagulation, crosslinking of fibrin, and fibrinolysis has occurred. The D-dimer assay detects a specific FDP and is an indicator of fibrinolysis of *any cause*. It is not an indicator of fibrinogenolysis because D-dimer depends on the crosslinking of fibrin monomers (not fibrinogen), followed by plasmin lysis.
- D-dimers are elevated in DIC but they are not specific to DIC. D-dimers are elevated with deep vein thrombosis, pulmonary



emboli, sepsis, pregnancy (especially when preeclampsia is present), following surgery, or with large hematomas.

Question 27: C. Platelet count.

Explanation:

- The platelet count is the most consistently abnormal test in TTP, and platelets play a clear role in the pathologic process, resulting in renal and neurologic dysfunction. Although some facilities may use lactate dehydrogenase (LDH) levels and creatinine levels to monitor a patient with TTP, the platelet count is the best indicator of response to therapy.
 - Although TTP is always associated with a decreased platelet count, TTP is not a primary platelet disorder. TTP platelet activation and microthrombi development are secondary to another underlying defect. The presence of abnormal, unusually large von Willebrand factor (ULvWF) multimers and endothelial injury are the two most commonly implicated mechanisms of platelet activation in TTP.
 - *ADAMTS13 is a metalloproteinase necessary for the cleavage of ULvWF multimers. Deficiency of ADAMTS13 because of either congenital mutations or development of autoantibodies is one mechanism by which ULvWF may accumulate in the circulation. ADAMTS13 levels are not always decreased in TTP, and although decreased levels of ADAMTS13 (<5% of normal) are highly specific for TTP, severe decreases have also been reported with sepsis and DIC.*ADAMTS13 levels may remain persistently low even in the presence of excellent clinical responses to therapy; therefore, levels are not used to monitor response to therapy. The PT, aPTT, and fibrinogen levels are typically within normal range in patients with TTP. Also, at most hospitals, ADAMTS13 is a send-out test and not readily available to monitor treatment response.
-

Question 28: D. Short euglobulin lysis time and decreased fibrinogen.



Explanation:

- Primary fibrinolysis may be caused by an increase in plasminogen activators (tissue-type or urokinase-type plasminogen activators), a decrease in plasmin inhibitors (α_2 -antiplasmin, thrombin-activatable fibrinolysis inhibitor), or decreased inhibition of plasminogen activators (plasminogen activator inhibitors 1 and 2).
- In addition to congenital abnormalities of the fibrinolytic system, primary fibrinolysis occurs in association with many common clinical settings, including cardiopulmonary bypass, severe liver disease, hepatic resection or transplantation, genitourinary tract surgery (associated with urokinase-type plasminogen activator release), oral surgery, trauma, and malignancy.
- If direct lysis of fibrinogen (as well as fibrin) occurs, it is called fibrinogenolysis, which is always pathologic.
- The euglobulin fraction refers to fibrinogen, plasminogen, tissue plasminogen activator, and other proteins that precipitate when citrated plasma is diluted at low pH and low ionic strength. The precipitated euglobulins are resuspended in buffer and then clotted by the addition of thrombin. The time to lysis of that clot is a measure of fibrinolytic activity and is reported as the euglobulin lysis time. A shortened euglobulin lysis time combined with a disproportionate decrease in fibrinogen (Factor I) is suggestive of primary fibrinolysis.
- An important distinction between primary hyperfibrinolysis and secondary hyperfibrinolysis as seen with DIC is that with primary fibrinolysis there is a disproportionate consumption of fibrinogen and plasminogen relative to consumption of coagulation factors. Consumption of coagulation factors and platelets in the presence of a short euglobulin time is supportive of DIC. FDPs are elevated with both.
- Plasminogen and antiplasmin levels may be useful for confirming the presence of fibrinolysis, but their levels decrease rather than increase because of consumption.
- Notably, in DIC, fibrinogen is decreased in proportion to other clotting factors.
- The MCA lysis test is a screening test for Factor XIII deficiency. In the absence of Factor XIII, MCA will solubilize the patient's clotted plasma. Factor levels of 1% to 2% are sufficient to prevent solubilization.

**Question 29: A. Protein S deficiency.****Explanation:**

- Deficiency of protein S, antithrombin, and/or protein C results in a thrombotic phenotype, because they are natural anticoagulants. Protein C along with its cofactor, protein S, cleaves Factors Va and VIIIa, which are critical for the prothrombinase and tenase complexes, respectively. Without protein C or protein S, critical negative feedback is taken away from the procoagulant cascade, which results in thrombosis. Antithrombin acts to bind and inhibit thrombin and Factors VIIa, Xa, IXa, and Xia.
- The other options are associated with a bleeding phenotype.

**Question 30: D. Warfarin-induced skin necrosis.****Explanation:**

- Warfarin-induced skin necrosis typically occurs within the first week after a patient with protein C deficiency is given a vitamin-K-antagonist anticoagulant. This condition is characterized by thrombosis of skin and subcutaneous tissues over relatively fat-rich tissues. This is a rare situation where a thrombotic disease may be effectively treated with plasma. Either plasma or protein C concentrate may restore the natural anticoagulant.

**Question 31: C. Protein S.****Explanation:**

- Coagulation Factors II (prothrombin), VII, IX, and X, as well as anticoagulation proteins C, S, and Z, are vitamin-K dependent. Vitamin K is a necessary cofactor for γ -carboxylation of glutamic acid residues on vitamin-K-dependent coagulation proteins. Carboxylation of glutamic acid residues leads to binding of Ca^{2+} and allows these proteins to bind phospholipids on cell membranes. In the absence of carboxylation, the complexes necessary for



coagulation proteins to effectively interact are not formed, rendering the proteins functionally inactive.

- Warfarin inhibits vitamin K reductase (vitamin K 2,3-epoxide reductase) and blocks the regeneration of the active form of vitamin K.



Question 32: E. All of the above.

Explanation:

- FDPs form whenever plasmin cleaves soluble fibrin monomers formed as a result of clotting. FDPs form as a result of plasmin's action on fibrinogen. D-dimer is a specific type of fibrin degradation product formed when plasmin acts on crosslinked fibrin; it is an indication that both coagulation and fibrinolysis have occurred. Any disorder associated with increased coagulation or increased generation of plasminogen activator may result in the formation of FDPs.
- FDPs cause feedback inhibition of thrombin and reptilase and may prolong the thrombin time (TT) and reptilase time (RT). Therefore, they have clot inhibitory properties. This is not usually clinically significant except in a situation such as DIC, where degradation products are sometimes grossly elevated and may contribute to the already abnormal hemostatic state.



Question 33: A. A mutation in the Factor V gene.

- Addition of APC prolongs the aPTT in normal patients. Absence of prolongation indicates resistance to the action of protein C. Recent reports show that abnormal resistance test results may occur in 40% of patients with otherwise unexplained thrombosis. In addition, 95% of those with abnormal resistance test results have been found to have Factor V Leiden.
- Protein C is activated by thrombin complexed with thrombomodulin. Activated protein C inactivates Factor Va by sequentially cleaving Factor Va in three locations. The first proteolytic cleavage is at the Arg506 residue and is necessary for cleavage at the other two sites. The Factor V Leiden gene has a guanine-to-



adenine point mutation at nucleotide 1691 that results in a glutamine in place of an arginine at position 506 of the protein.

- Factor V Leiden is not susceptible to APC cleavage at position 506; therefore, inactivation of Factor Va is impaired. Factor V Leiden heterozygotes and homozygotes both have thromophilia; however, the risk of thrombotic events is greater for homozygotes. APC in combination with deactivated Factor V and protein S plays a role in the deactivation of Factor VIIIa, but this is also dependent on the prior cleavage of Factor Va at residue 506. Thrombin and thrombomodulin are necessary to generate APC, but the APCR test assesses defects downstream of APC generation.



Question 34: E. Glanzmann thrombasthenia.

Explanation:

- Glanzmann thrombasthenia is a congenital disorder of platelet function. In this disorder, there are mutations in the platelet's fibrinogen receptor (GPIIb/IIIa) that result in a defect of platelet aggregation. The typical pattern in aggregometry studies is aggregation with ristocetin only (failure to aggregate with all other agonists). This is because ristocetin causes aggregation via the glycoprotein Ib receptor, which is normal in patients with Glanzmann thrombasthenia.
- Bernard-Soulier syndrome is another congenital disorder of platelet function that is characterized by mutations in the GPIb receptor. This results in a defect of platelet adhesion. The typical pattern in aggregometry studies is aggregation with all agonists, except for ristocetin (exactly the opposite pattern of Glanzmann thrombasthenia).
- Storage pool diseases typically lack a secondary wave, which is indicative of an issue with secretion.
- Scott syndrome is characterized by an impaired ability to expose negatively charged phospholipids in response to stimulation. The platelet aggregation studies in these patients are normal.
- Quebec platelet syndrome is characterized by increased degradation of alpha granule content. This is thought to be due to the accumulation of urokinase plasminogen activator within the



alpha granules. The pattern of platelet aggregation described is abnormal aggregation with collagen and ADP.



Question 35: D. It is calculated as $[\text{patient PT} \div \text{control PT}]^{\text{ISI}}$.

Explanation:

- The INR prevents confusion arising from the use of different PT assays in different laboratories by correcting for the sensitivity of the thromboplastin used in each assay compared to an international standard. Therefore, an INR from one laboratory can be directly compared to an INR from another without knowing a control value for the PT and without concern for the type of assay either laboratory uses.
- For most clinical situations, the recommended range for the INR is between 2.0 and 3.0. Patients with mechanical cardiac valves are an exception and should have an INR between 2.5 and 3.5. The INR is most sensitive to the inhibition of Factor VII because it has the shortest half-life (4-6 hours) of any of the vitamin-K-dependent factors.
- The INR should not be routinely used to follow patients with liver disease or vitamin K deficiency and is not accurate in the early phases of warfarin therapy. In these cases, the PT should be used. The INR is not relevant to the aPTT assay.



Question 36: B. Factor XIII.

Explanation:

- The half-life of Factor VII is 4 to 6 hours (coagulation factor with the shortest half-life) and the half-life of Factor XIII is 144 hours (coagulation factor with the longest half-life).



Question 37: E. All of the above.



Explanation:

- HIT occurs in about 2% to 5% of patients receiving unfractionated heparin (UFH) for more than 4 days. It usually appears 3 to 14 days after the start of heparin administration and may occur with minute doses, including heparin flushes. Onset of HIT may be very rapid in those with previous exposure to heparin. The risk of developing HIT is greatest with use of UFH, with surgical rather than medical patients, and in females. More cases have been associated with bovine (85%) than porcine (15%) heparin.
- The diagnosis of HIT should be considered when there is an otherwise unexplained decrease in platelet count of 50%, even if still above normal range, within 3 to 14 days after heparin therapy. Thrombocytopenia without thrombosis is the most frequent presentation of HIT. The thrombocytopenia is usually not severe, with median platelet counts remaining in the 60,000/ μ L range. There is rarely a need to consider platelet transfusion, and because of the risk of thrombosis, prophylactic platelet transfusion is relatively contraindicated.
- In contrast, posttransfusion purpura (PTP) and immune thrombocytopenic purpura (ITP), which are often included in the differential diagnosis, are associated with platelet counts <20,000/ μ L. HIT is associated with a high risk of thrombosis. Thrombotic events may precede the development of thrombocytopenia; therefore, HIT should be considered as a cause of otherwise unexplained thrombosis. The risk of thrombosis in patients managed by heparin discontinuation alone is in the range of 20% to 50%. Venous thrombosis is by far more common than arterial thrombosis, but arterial thrombosis is seen frequently enough to have garnered HIT the acronym *white clot syndrome*.
- HIT is an immune-mediated disorder caused by antibodies directed at an epitope of platelet factor 4 (PF4) that is available only when heparin is complexed with PF4. The PF4-heparin complexes attach to platelets and induce activation. Diagnostic assays for HIT either directly detect heparin-dependent antibodies or look for functional evidence of the antibodies.
- Enzyme-linked immunosorbent assays use PF4 complexed with heparin (or similar anionic molecules) affixed to microtiter wells as a target for detecting antibodies in patient serum. This is a highly sensitive but not very specific assay, as many patients without HIT may have demonstrable antibodies. A negative result is



strong evidence against the diagnosis of HIT, but a positive result may be misleading.

- Functional assays include serotonin release and heparin-induced platelet aggregation. These assays evaluate whether heparin at therapeutic levels enables the patient's plasma to activate platelets. The serotonin release assay, which is highly sensitive and specific, is the "gold standard" for laboratory diagnosis of HIT. However, this assay is not always easily available. Platelet aggregation assays are specific but not sensitive.
- The available HIT tests are not considered diagnostic; rather, the diagnosis of HIT remains largely clinical. Once a clinical diagnosis of HIT has been made, nonheparin, rapidly acting anticoagulation therapy should be promptly initiated. Discontinuation of heparin therapy is not sufficient for protecting the patient from thrombotic complications, as the highest risk for thrombosis appears to be during the first few days after stopping heparin. In the presence of HIT, warfarin should not be started without other rapidly acting anticoagulants because of an increased risk of warfarin-induced skin necrosis.



Question 38: A. Wiskott-Aldrich syndrome.

Explanation:

- Wiskott-Aldrich syndrome is X-linked and often associated with the triad of thrombocytopenia, eczema, and immunodeficiency.
- Hermansky-Pudlak syndrome is autosomal recessive and characterized by lack of dense granules and oculocutaneous albinism. Additional features, such as pulmonary fibrosis and granulomatous colitis, are dependent upon the particular mutation inherited.
- Chediak-Higashi syndrome is autosomal recessive and also characterized by a lack of dense granules and oculocutaneous albinism. However, in contrast to Hermansky-Pudlak syndrome, Chediak-Higashi syndrome is associated with neutropenia/immunodeficiency and neurologic complications.
- Neither Scott syndrome nor Quebec platelet syndrome have been associated with additional clinical problems outside of platelet dysfunction at this point. Both are very rare.

**Question 39: E. A and B.****Explanation:**

- Because of its short size, LMWH is largely unable to simultaneously bind antithrombin and thrombin to exert a direct effect on thrombin. This is largely because heparin works with thrombin and antithrombin in a template mechanism and LMWH is not long enough. LMWH exerts its major action against Factor Xa. The aPTT is not usually prolonged by LMWH; response to therapy is monitored by measuring anti-Xa activity.
- Advantages of LMWH include:
 - Response is correlated to body weight; therefore, fixed dosing is possible.
 - Ongoing monitoring is not required for most patients.
 - Pediatric, obese, and renal failure patients may require more frequent monitoring.
 - May be administered in the outpatient setting.
 - Is less likely to induce heparin-associated antibodies.
- Notably, patients with established HIT can respond to LMWH administration in the same manner as heparin. It has no role in the management of HIT. LMWH is at least equally effective as UFH for deep vein thrombosis prophylaxis, and anticoagulation before warfarin therapy in patients with mechanical valve replacement. LMWH is also indicated for the prevention of deep vein thrombosis and pulmonary emboli in patients undergoing hip replacement. LMWH is reversed by protamine sulfate, but not as efficiently as heparin is reversed.

**Question 40: D. All of the above.****Explanation:**

- The critical step in the pathogenesis of acute DIC is the formation of thrombin at sites of endothelial injury by the action of the tissue factor-activated Factor VII (TF-FVIIa) system. Massive generation of thrombin triggers systemic coagulation, which overwhelms anticoagulation and fibrinolytic pathways. In addition to consumption, impaired synthesis/regulation of antithrombin, protein C, and protein S contributes to the low levels of these



proteins seen in DIC. Low antithrombin levels in DIC are associated with increased mortality.

- Although both coagulation and fibrinolysis are activated in DIC, activation of coagulation is disproportional to activation of fibrinolysis. End-organ damage in DIC is attributable to widespread fibrin deposition and tissue ischemia. The platelet count is a sensitive test in DIC, particularly if it dramatically decreases over a short time. About 95% of patients with DIC have thrombocytopenia.
- However, low or decreasing platelet counts may be associated with a variety of disorders, including bleeding. A normal platelet count is strong evidence against this diagnosis. Fibrinogen is an acute-phase reactant; therefore, fibrinogen levels alone are not a very sensitive test for DIC. Fibrinogen levels are decreased in less than 50% of patients with DIC and are lowest in patients with severe acute uncompensated DIC. No single test or combination of tests is specific or sensitive enough to give a definitive diagnosis of DIC. Routinely available tests that are of value in the diagnosis are:
 - FDPs and D-dimers should both be elevated in DIC.
 - Soluble fibrin monomers are increased.
 - PT and aPTT are elevated as a result of consumption of coagulation factors.
 - Fibrinogen may be decreased.
 - TT and RT are prolonged.
 - Platelets are decreased.
 - Antithrombin levels are decreased.
 - Factor V and Factor VIII levels may be decreased in acute DIC. Factor VIII levels are variable because, like fibrinogen, Factor VIII is an acute-phase reactant.



Question 41: C. Factor VII.

Explanation:

- The aPTT is a screening test for all coagulation factors except Factors VII and XIII. The aPTT is less sensitive to the proteins of the common pathway than is the PT. The PT is a screening test for Factors I, II, V, VII, and X (extrinsic and common pathway).

**Question 42: D. Type 2N****Explanation:**

- In vWD type 2N, the vWF molecule has a defective coagulation Factor VIII binding site. As a result, there is rapid Factor VIII clearance from circulation. Although type 2N is very rare, it is common for it to be misdiagnosed as the far more common diagnosis, hemophilia A. Failure to achieve expected postinfusion Factor VIII levels, in the absence of an inhibitor, should make one consider a diagnosis of vWD type 2N.
-

Question 43: B. Prothrombin complex concentrate (PCC).**Explanation:**

- The choice of method for warfarin reversal is dependent on the urgency of the situation and the thrombotic risk consequent to interrupting anticoagulation. When there is major life-threatening bleeding such as intracranial hemorrhage, the goal is to effect rapid and complete reversal of warfarin. PCCs, in combination with intravenous vitamin K, will reverse warfarin within 10 to 15 minutes. If PCCs are not available, FFP may be used.
- In the absence of bleeding or with only minor bleeding, a decision must be made about whether to simply discontinue warfarin or to discontinue warfarin and administer vitamin K. Vitamin K administration is usually not necessary when the INR is <5, may be necessary when the INR is >5 but <9, and is frequently necessary when the INR is >9. When making the decision to withhold or administer vitamin K, careful attention must be given to the balance of bleeding and thrombotic risks.
- Vitamin K may be administered via IV, IM, and oral routes. IM administration is not recommended because of the risk of IM hemorrhage, variable absorption, and the possibility of a residual effect on warfarin reinstatement. IV vitamin K will have an effect within 4 to 6 hours and is not adequate as a sole source of reversing warfarin with severe bleeding. Recommended dosing is 5 to 10 mg for life-threatening bleeding. Doses of 1 mg are appropriate for marked elevations in INR (>9) in the absence of bleeding. Oral vitamin K takes effect within 24 hours. Doses are generally 1



to 2 mg orally, unless the INR is markedly elevated, in which case doses may be in the 2- to 5-mg range. In all cases of warfarin intoxication, warfarin should be discontinued until it is safe to resume.

- Cryoprecipitate does not have a role in warfarin reversal.
-

Question 44: C. Factor VII.

Explanation:

- The half-life of Factor VII is 4 to 6 hours (coagulation factor with the shortest half-life). The half-life of Factor XIII is 144 hours (coagulation factor with the longest half-life).
-

Question 45: D. Factor IX.

Explanation:

- The PT is a screening test for Factors I, II, V, VII, and X (extrinsic and common pathway).
-

Question 46: C. 1250.

Explanation:

- The initial dosage to achieve the desired plasma level of Factor VIII (% activity) is calculated as follows:
 - Blood volume (mL) = $70 \text{ mL/kg} \times 65 \text{ kg} = 4550 \text{ mL}$
 - Plasma volume (mL) = blood volume (mL) $\times (1 - \text{Hct})$; $4550 \times (1 - 0.45) = 2503 \text{ mL}$
 - Factor VIII requirement = plasma volume \times (desired Factor VIII level % – pretransfusion Factor VIII level %); $2503 \times (0.5 - 0.0) = 1250 \text{ units}$
-

Question 47: C. Factor XIII deficiency.



Explanation:

- The normal PT rules out the diagnosis of Factor VII deficiency. The normal aPTT rules out the diagnosis of Factor XII deficiency. None of the test results presented directly assess tissue plasminogen activator or plasminogen deficiency.
- However, the clot lysis assay is a screen for Factor XIII deficiency. Factor XIII is not assessed in the common clot-based assays. The role of Factor XIII is to crosslink insoluble fibrin monomers. This action makes a clot resistant to lysis in 5 M urea. Therefore, the fact that the patient's clot did dissolve in the urea suggests that Factor XIII is extremely low. This assay is poorly sensitive, even 5% of Factor XIII is sufficient for a normal result (persistence of the clot).
- Despite challenges assessing Factor XIII, it is clinically significant. Patients with low Factor XIII are at high risk for intracranial bleeding as well as delayed surgical/traumatic bleeding.



Question 48: B. 12 hours.

Explanation:

- Half-life of Factor VIII is 8 to 12 hours.



Question 49: D. X-linked recessive.

Explanation:

- Both hemophilia A and B are inherited X-linked recessive.



Question 50: D. Paroxysmal nocturnal hemoglobinuria.

Explanation:

- Paroxysmal nocturnal hemoglobinuria is one of the few diseases that is known to cause both arterial and venous thrombosis. Anti-phospholipid disease, Factor V Leiden, and prothrombin gene



mutation are known risk factors for venous thrombosis. Of course, if there is a patent foramen ovale, a venous thrombosis may embolize to the arterial circulation. MTHFR mutation is a risk factor for a risk factor, so is generally not tested in current thrombosis evaluations.

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16

The HLA System

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Key Points from the *Technical Manual*

- The HLA complex in humans is a critical component of the immune system and plays a significant role in the ability to distinguish self from nonself.
- HLA genes are located within several highly polymorphic loci on the short arm of chromosome 6.
- HLA genes are normally inherited as haplotypes—a linked set of genes from the mother and father. Together, the maternal and paternal haplotypes are referred to as the genotype.
- Class I proteins (HLA-A, -B, and -C) are expressed ubiquitously. Class II proteins (HLA-DR, -DQ, and -DP) have restricted tissue distribution.
- Current HLA typing is most often performed by molecular methods and has rapidly improved in the last few years, especially with the use of next-generation sequencing (NGS).
- Solid-phase assays have become the “gold standard” for detecting and identifying HLA antibodies.



QUESTIONS

Question 1: Select the answer choice that fills in the blanks appropriately. β_2 -microglobulin associates with HLA Class _____ and is encoded by a gene on chromosome _____.

- A. I; 15q.
 - B. II; 15p.
 - C. I; 6p.
 - D. II; 6p.
 - E. I; 9q.
-

Question 2: Which statement accurately describes the variable regions of HLA Class II proteins (ie, where does the majority of polymorphism that confers antigen specificity reside)?

- A. The variable regions reside in the $\alpha 1$ and $\alpha 2$ domains of the alpha heavy chain.
 - B. The variable regions reside in the $\alpha 1$ and $\alpha 3$ domains of the alpha heavy chain.
 - C. The variable regions reside in the $\alpha 1$ and $\beta 1$ domains of the α and β chains.
 - D. The variable regions reside in the $\alpha 2$ and $\beta 2$ domain of the α and β chains.
 - E. The variable regions reside in the $\alpha 1$ and $\beta 2$ domain of the α and β chains.
-

Question 3: Which of the following cells would not be expected to express HLA Class I antigens?

- A. Platelets.
- B. B lymphocytes.
- C. Monocytes.
- D. Neurons.
- E. Endothelial cells.



Question 4: In *HLA-A*31:01:02:51Q*, identify the digits in the field that differentiate alleles based on presence of synonymous (“silent”) nucleotide substitutions.

- A. 31.
 - B. 01.
 - C. 02.
 - D. 51.
 - E. 31:01.
-

Question 5: What is the difference between *HLA-A*02:02:01:01* and *HLA-A*02:02:01:03*?

- A. There is a genetic variation in one of the introns that results in a different protein.
 - B. There is a genetic variation in one of the introns that results in the same protein.
 - C. There is a genetic variation in one of the exons that results in the same protein.
 - D. There is a genetic variation in one of the exons that results in a different protein.
 - E. There is a genetic variation in one of the introns that results in an allele of a different serologic group.
-

Question 6: A patient with end-stage renal disease secondary to hypertension is undergoing evaluation for renal transplantation. The results of HLA typing are as follows:

<i>HLA-A</i>	<i>HLA-B</i>	<i>HLA-C</i>	<i>HLA-DRB1</i>	<i>HLA-DQB1</i>
02	08	07	01	05
XX	07	10	15	06

What does the XX designation for the second allele of the A locus most likely signify?

- A. Homozygosity for *A*02*.



- B. A null allele.
 - C. The allele is not further defined and could be any number of possible alleles.
 - D. A newly discovered allele.
 - E. Allelic drop out.
-

Question 7: Which of the following is true about “public” HLA antigens?

- A. Public epitopes are generally present on a very limited number of different HLA specificities.
 - B. The common determinants between alleles are generally in a hypervariable region.
 - C. The most common public antigens are Bw4 and Bw8.
 - D. Developing an antibody to a public antigen generally has limited clinical implications.
 - E. Antibody development to a public antigen can resemble the presence of many discrete HLA antibodies.
-

Question 8: Which answer choice accurately describes the biologic functions of HLA Class I proteins?

Choice	Type of Peptide Presented	Location of Protein Degradation into Peptide	Type of T-Cell Interaction
A.	Endogenous	Large multifunctional protease (LMP)	CD4
B.	Exogenous	Endosome	CD4
C.	Endogenous	Endosome	CD8
D.	Endogenous	LMP	CD8
E.	Exogenous	LMP	CD4



Question 9: Which answer choice accurately describes the biologic functions of HLA Class II proteins?

Choice	Type of Peptide Presented	Location of Peptide Loading	Length of Peptide Presented	Type of T-Cell Interaction
A.	Exogenous	Endosome	12-25 amino acids	CD4
B.	Exogenous	Endoplasmic reticulum	8-9 amino acids	CD4
C.	Endogenous	Endosome	12-25 amino acids	CD8
D.	Endogenous	Endoplasmic reticulum	8-9 amino acids	CD8
E.	Exogenous	Endosome	8-9 amino acids	CD4



Question 10: Which of the following is a nonclassical HLA gene?

- A. A.
- B. DQ.
- C. G.
- D. C4A.
- E. TNF.



Question 11: What is the probability that any two full siblings will share at least one HLA haplotype?

- A. 0%.
- B. 25%.
- C. 50%.
- D. 75%.
- E. 100%



Question 12: A 50-year-old male with acute myelocytic leukemia (AML) needs a hematopoietic stem cell transplant. His hematologist inquires if he has any siblings who may be eligible to donate. He replies that he has five siblings, including two brothers and three sisters. What are the chances that the patient will have at least one HLA-identical sibling among his five brothers and sisters?

- A. 25%.
 - B. 33%.
 - C. 50%.
 - D. 65%.
 - E. 76%.
-

Question 13: The HLA types for a family are provided in the table below.

Family Member	Haplotype	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Father	a	24	51	15	8	4
	b	30	7	7	4	7
Mother	c	2	60	10	13	7
	d	68	53	4	12	5
Child #1	a	24	51	15	8	4
	c	2	60	10	13	7
Child #2	a/b	24	51	15	4	7
	d	68	53	4	12	5
Child #3	b	30	7	7	4	7
	c	2	60	10	13	7
Child #4	b	30	7	7	4	7
	d	68	53	4	12	5



Which of the following describes the a/b haplotype in child #2?

- A. A paternal crossover between the *HLA-C* and *HLA-DR* loci.
 - B. A maternal crossover between the *HLA-C* and *HLA-DR* loci.
 - C. A paternal crossover between the *HLA-B* and *HLA-DR* loci.
 - D. A maternal crossover between the *HLA-B* and *HLA-DR* loci.
 - E. A crossover between the paternal *HLA-C* and the maternal *HLA-DR* loci.
-

Question 14: The table below supplies the HLA types of a family.

Family Member	HLA-A	HLA-B	HLA-DRB1
Father	33	44	4
	2	58	11
Mother	68	72	15
	34	7	13
Sibling #1	2	44	15
	68	7	4
Sibling #2	34	58	13
	33	72	11
Sibling #3	2	72	4
	34	44	13
Sibling #4	68	58	15
	33	7	11

What are the haplotypes of sibling #3 (assuming no crossover events during gametogenesis)?

- A. A2,B72,DR13/A34,B44,DR4.
- B. A34,B44,DR13/A2,B7,DR4.
- C. A34,B72,DR13/A33,B58,DR11.
- D. A33,B58,DR11/A68,B7,DR15.
- E. A34,B72,DR13/A2,B44,DR4.



Question 15: The table below supplies the HLA types of a family.

Family Member	HLA-A	HLA-B	HLA-DRB1
Father	3	44	11
	32	35	17
Mother	2	42	4
	30	38	18
Sibling #1	2	35	18
	3	42	17
Sibling #2	X	44	4
	30	38	11
Sibling #3	2	44	18
	32	42	11

What is the HLA-A antigen (X) of sibling #2 (assuming no crossover event during gametogenesis)?

- A. A2.
- B. A3.
- C. A30.
- D. A32.
- E. Cannot be determined.



Question 16: In the United States, the frequency of DR17 and DQ2 in those of European ancestry is 12.9% and 23%, respectively. If HLA alleles were randomly inherited, then the frequency of expressing both would be $2 \times 0.129 \times 0.23 = 0.059$. However, the DR17 and DQ2 haplotype has a frequency of approximately 13%. What term describes this discrepancy observed between the expected and the actual allele frequencies?



- A. Hardy-Weinberg equilibrium.
 - B. Linkage disequilibrium.
 - C. Lyonization.
 - D. Haplotype inheritance.
 - E. Linkage equilibrium.
-

Question 17: The table below supplies the HLA types of four siblings.

Sibling	HLA-A	HLA-B	HLA-DRB1
#1	24	8	17
	1	18	11
#2	2	13	7
	3	7	15
#3	3	8	7
	1	13	17
#4	2	18	11
	24	7	15

Which of the following represent the parental haplotypes (assuming no crossover event during gametogenesis)?

- I. A24,B13,DR7/A2,B7,DR15
 - II. A2,B7,DR15/A1,B8,DR17
 - III. A2,B18,DR11/A3,B13,DR7
 - IV. A3,B13,DR7/A24,B18,DR11
- A. I and II.
 - B. I and III.
 - C. II and III.
 - D. II and IV.
 - E. III and IV.



Question 18: Which of the following statements is true regarding serologic-based methods for HLA typing?

- A. Require only a small number of cells for nucleic acid extraction.
- B. Could detect the difference between an $A^*01:01$ and $A^*01:02$.
- C. Have high sensitivity and can reliably detect antigens with low expression.
- D. Offer high-resolution typing.
- E. Viable lymphocytes are needed for antigen detection.



Question 19: Which of the following answer choices correctly pairs the HLA typing method with the expected resolution and result for DR4 antigen/allele?

Choice	Typing Method	Resolution (low, intermediate, high)	DR4 Result
A.	Next-generation sequencing (NGS)	Low	<i>DR4</i>
B.	Complement-dependent cytotoxicity (CDC)	High	<i>DR4</i>
C.	Sequence-specific primer (SSP)	Low	<i>DRB1*04:01</i>
D.	NGS	High	<i>DRB1*04:01:01G</i>
E.	CDC	Low	<i>DRB1*04:01</i>



Question 20: When comparing a cell-based complement-dependent cytotoxicity (CDC) crossmatch to a flow cytometric crossmatch (FXM), which of the following is true?

- A. The CDC requires viable lymphocytes but the FXM does not.
- B. Like the CDC, the FXM also requires assessment of cell lysis.
- C. The FXM is more sensitive than the CDC.



- D. T-cell and B-cell crossmatches can simultaneously be assessed using the CDC or FXM.
 - E. Anti-human globulin is required to increase sensitivity of the FXM but not the CDC.
-

Question 21: If a physical crossmatch between a recipient and a prospective donor were performed and resulted as both T-cell and B-cell crossmatch positive, which of the following statements could be true?

- I. Presence of Class I antibodies only.
 - II. Presence of Class II antibodies only.
 - III. Presence of Class I and II antibodies.
 - IV. The class of HLA antibodies cannot be distinguished.
-
- A. I.
 - B. II.
 - C. I and III.
 - D. II and III.
 - E. IV.
-

Question 22: Regarding the solid-phase assay used in testing for HLA antibody detection, which of the following statements is true?

- A. Microparticle beads can be coated either with recombinant HLA antigens or with Class I or II phenotypes from cultured lymphocytes.
- B. Solid-phase assays detect Class I and II HLA antibodies using specialized lymphocytes.
- C. Solid-phase assays can detect the absence or presence of HLA antibodies, but are not ideal for detecting the specificities of HLA antibodies.
- D. Low-level antibodies detected by solid-phase assays but not CDC are always considered clinically significant.
- E. Solid-phase assays are less sensitive than complement-dependent cytotoxicity assays.



Question 23: Regarding HLA compatibility between renal transplant recipients and prospective donors, which of the following statements is true?

- A. Recipient-donor pairs should be HLA matched at the *HLA-A*, *-B*, *-C*, *-DR*, and *-DQ* loci.
 - B. A positive T-cell crossmatch is generally considered a contraindication to transplantation.
 - C. A positive physical crossmatch is predictive of long-term allograft survival.
 - D. A negative physical crossmatch is predictive of delayed graft function.
 - E. As with hematopoietic stem cell transplantation, recipient-donor pairs need not be ABO compatible.
-

Question 24: To tabulate a calculated panel-reactive antibody (cPRA), what input data are required?

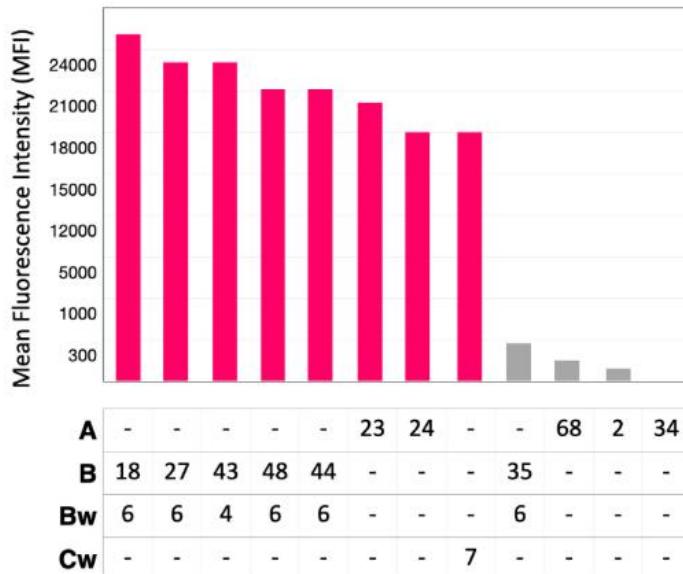
- A. All antibodies tested and nonreactive by any method.
 - B. Antigens to which the recipient has the corresponding HLA antibodies by solid-phase testing.
 - C. All donor antibodies tested and reactive by solid-phase testing.
 - D. Recipient and donor HLA type.
 - E. Donor HLA type alone.
-

Question 25: Which cPRA value would correspond to the widest pool of potential histocompatible donors?

- A. 99%.
- B. 50%.
- C. 80%.
- D. 20%.
- E. 99.9%.



Question 26: A blood-group-O, renal-transplant candidate has the following antibody profile on Class I single-antigen bead analysis:



No Class II antibodies were detected. Which of the donors would be considered compatible?

Choice	Blood Group	Class I HLA Phenotype
A.	O	A11, A24, B18, B35, C1, C7
B.	B	A1, A2, B13, B53, C1, C4
C.	O	A3, A34, B57, B75, C2, C14
D.	AB	A11, A24, B18, B35, C1, C7
E.	O	A1, A2, B48, B60, C10, C12



Question 27: A 4-year-old patient with GATA2 mutation is being evaluated for stem cell transplantation. The blood group and HLA type of the patient and his family members are provided in the following table:

	Blood Group	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Patient	0	03:01	35:01	04:01	03:01(17)	02:01:01G
		23:01	44:02	05:01	04:05	03:02(8)
Sister #1	AB	03:01	35:01	04:01	03:01(17)	02:01:01G
		23:01	44:02	05:01	04:05	03:02(8)
Brother #1	0	03:01	35:01	04:01	03:01(17)	02:01:01G
		23:01	44:02	05:01	04:05	03:02(8)
Sister #2	0	32:01	44:02	05:01	11:04	03:01(7)
		30:04	44:03	07:01	07:01	02:01:01G
Brother #2	A	03:01	35:01	04:01	11:04	03:01(7)
		23:01	44:02	05:01	04:05	03:02(8)
Cousin	0	23:01	44:02	05:01	04:05	03:02(8)
		01:01	08:01	07:02	03:01(17)	02:01

Which family member should be selected as the most appropriate donor?

- A. Sister #1.
- B. Brother #1.
- C. Sister #2.
- D. Brother #2.
- E. Cousin.



Question 28: Referring to the family typing provided in Question #27, what do the numbers in parentheses in *DRB1*03:01(17)*, *DQB1*03:01(7)*, and *DQB1*03:02(8)* indicate?



- A. The public epitope that the allele contains.
 - B. The crossreactive epitope group (CREG) to which the allele pertains.
 - C. The serologic equivalent of the allele.
 - D. The split of the allele.
 - E. The parent antigen of the allele.
-

Question 29: Referring to the family typing provided in Question #27, the patient, the sisters, and brother #1 all type as a *DQB1*02:01:01G*. What does the "G" indicate?

- A. The G indicates that the HLA nomenclature provided is generic.
 - B. The G indicates that the typing provided is reflective of the gene sequence.
 - C. The G follows alleles with identical amino acid sequences spanning the domains that compose the peptide-binding groove.
 - D. The G follows alleles with identical nucleotide sequences spanning the exons encoding the domains that compose the peptide-binding groove.
 - E. The G follows alleles with identical nucleotide and amino acid sequences of the membranous domains of the encoded HLA protein.
-

Question 30: The National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR) donor selection guidelines recommend at least a 7/8 or 8/8 HLA match between recipient and donor. To meet this recommendation, which HLA loci would have to be typed by molecular methods?

- A. *A, B, DRB1, DQB1*.
- B. *A, C, DRB1, DPB1*.
- C. *A, B, C, DQB1*.
- D. *A, DRB1, DQB1, DPB1*.
- E. *A, B, C, DRB1*.



Question 31: Which of the following would be an advantage of selecting HLA-matched platelets?

- A. HLA-matched platelets widen the donor pool compared to HLA antigen-negative platelets.
 - B. HLA-matched platelets require recipient HLA antibody testing.
 - C. HLA-matched platelets account for the presence of HPA incompatibility between recipient and donor.
 - D. HLA-matched platelets limit further HLA sensitization to mismatched HLA antigens between the recipient and donor.
 - E. HLA-matched platelets necessitate retyping donor and recipient after the recipient has been transfused multiple times.
-

Question 32: Which of the following would be a disadvantage of selecting HLA antigen-negative platelets compared to HLA-matched platelets?

- A. HLA antigen-negative platelets widen the donor pool compared to HLA-matched platelets.
 - B. HLA antigen-negative platelets do not account for the presence of HPA incompatibility between recipient and donor.
 - C. HLA antigen-negative platelets require continued donor HLA antibody testing to monitor for development of new HLA antibodies.
 - D. HLA antigen-negative platelets do not require recipient HLA typing.
 - E. HLA antigen-negative platelets do not require donor HLA typing.
-

Question 33: When administering HLA-matched platelets, what product modification should be performed?

- A. Irradiation.
- B. Washing.
- C. Volume reduction.
- D. Leukocyte reduction.
- E. Deglycerolization.



Question 34: A 55-year-old male with AML needs a hematopoietic stem cell transplantation (HSCT). His only sibling passed away 2 years ago in a motor vehicle accident. Thus, a search was performed in the donor registry for prospective donors. The table below provides the HLA types of the registry donors from the search.

	Age	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Patient	55	29:02	27:02	02:02	07:01	02:01:01G
		32:01	44:03	16:01	11:01	03:01
Registry donor #1	20	29:02	27:02	02:02	07:01	02:01:01G
		32:01	44:03	03:03	11:01	03:01
Registry donor #2	44	29:02	27:02	02:02	07:01	02:01:01G
		32:01	27:05	16:01	12:01	03:01
Registry donor #3	34	03:01	08:01	02:02	01:01	05:01
		32:01	44:03	16:01	11:03	03:01
Registry donor #4	60	01:01	27:05	02:02	15:01	06:02
		32:01	44:02	16:04	13:03	03:01
Registry donor #5	50	29:02	27:02	02:02	07:01	02:01:01G
		32:01	44:03	03:03	11:01	03:01

Which registry donor should be selected as the most appropriate donor?

- A. Registry donor #1.
- B. Registry donor #2.
- C. Registry donor #3.
- D. Registry donor #4.
- E. Registry donor #5.



Question 35: The patient in question #34 underwent HSCT. Two years later, he experienced a relapse. A new search in the registry was performed, but this time, HLA-DPB1 typing was added. The accompanying table provides the HLA types of the registry donors from the search. Which registry donor should be selected as the most appropriate donor?

- A. Registry donor #1.
 - B. Registry donor #2.
 - C. Registry donor #3.
 - D. Registry donor #4.
 - E. Registry donor #5.
-

Question 36: If a transplant center wanted to extend HLA matching to search for possible 12/12 HLA matches between recipient-donor pairs, which two loci would have to be typed in addition to the loci needed to satisfy the NMDP's recommended 7/8 or 8/8 HLA match?

- A. *DQA1, DQB1.*
 - B. *DPA1, DPB1.*
 - C. *DQA1, DPA1.*
 - D. *DQB1, DPB1.*
 - E. *DRB3/4/5, DQB1.*
-

Question 37: High-resolution HLA typing for hematopoietic stem cell transplantation requires HLA typing to at least which field?

- A. First field (eg, "01" in *A*01:02:03:04*).
- B. Second field (eg, "02" in *A*01:02:03:04*).
- C. Third field (eg, "03" in *A*01:02:03:04*).
- D. Fourth field (eg, "04" in *A*01:02:03:04*).
- E. There are no requirements for high-resolution typing if a molecular method is used for HLA typing.

Table for Question #35

	Age	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1	DPB1 Permissiveness
Patient	55	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	16:01	11:01	03:01	03:01
Registry donor #1	20	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	03:03	11:01	03:01	06:01
Registry donor #2	20	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	16:01	11:01	03:01	04:02
Registry donor #3	30	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	16:01	11:01	03:01	04:02
Registry donor #4	20	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	16:01	11:01	03:01	06:01
Registry donor #5	18	29:02	27:02	02:02	07:01	02:01:01G	04:01
		32:01	44:03	03:03	11:01	03:01	06:01



Question 38: A 54-year-old blood-group-AB male patient with a recent diagnosis of AML has persistent thrombocytopenia (3000/ μ L) despite daily platelet transfusions. Besides his current chemotherapy regimen, other nonimmune causes of platelet transfusion refractoriness have been ruled out. At 15 minutes after platelet transfusion, his corrected count increment (CCI) is 3000. Which of the following is most likely contributing to this patient's apparent refractoriness to platelet transfusions?

- A. Anti-GPIIa/IIIb.
 - B. Anti-HLA-DRB1.
 - C. Anti-HLA-A.
 - D. Naturally occurring anti-B.
 - E. Anti-HLA-C.
-

Question 39: The clinical team for the patient from question #38 calls the transfusion medicine service to obtain HLA-compatible platelets—either HLA-matched- or HLA antigen-negative units. To obtain HLA antigen-negative units, what laboratory testing needs to be ordered?

- I. Recipient HLA typing.
 - II. Donor HLA typing.
 - III. Recipient HLA antibody testing.
 - IV. Donor HLA antibody testing.
 - V. Platelet crossmatching.
- A. I and II.
 - B. I and III.
 - C. II and III.
 - D. II and IV.
 - E. V alone.
-

Question 40: In the workup of the patient in question #38, HLA antibody testing and HPA antibody testing are ordered. Single-antigen bead testing revealed the presence of multiple Class I antibodies, corresponding to a cPRA of 99.99%. HPA antibody testing was negative. Considering these results, which of the following platelet products should be selected for the patient?



- A. ABO-identical platelets.
 - B. HLA-matched platelets.
 - C. HLA antigen-negative platelets.
 - D. Crossmatched platelets.
 - E. HPA-matched platelets.
-

Question 41: The patient from question #38 is HLA typed in the hopes of finding HLA-matched platelet donors. Fortunately for the patient, he appears to be homozygous for a common HLA Class I haplotype, and there are many potential donors. The table below provides the Class I typing of the patient and one of the prospective donors.

ABO Group	HLA-A	HLA-B	HLA-C
Patient	AB	2,XX	8,XX
Potential donor	O	2,XX	8,XX

The patient is given multiple units from this donor. However, the patient's platelet count does not appear to respond. Corrected count increments have continued to be low following transfusion, ranging from 2000-4000. To investigate this discrepancy, a platelet cross-match is performed between the recipient and the donor. The cross-match is positive even when repeated by a different person with a serum sample drawn on a separate day. Which of the following reasons could explain the positive platelet crossmatch between this recipient and HLA-matched platelet donor?

- I. Technical laboratory error.
 - II. Intravenous immunoglobulin (IVIG).
 - III. HPA antibodies.
 - IV. ABO antibodies.
 - V. Allele-specific HLA antibody.
- A. I.
 - B. I and II.
 - C. III and IV.
 - D. II and III.
 - E. V.



Question 42: A 45-year-old immunocompetent female underwent cardiovascular surgery, during which she received multiple blood components. Fourteen days after surgery, the patient developed pancytopenia and was suspected to have TA-GVHD. Of the following HLA-typing results of implicated donors, which one is most likely to have the highest risk of causing TA-GVHD in this recipient?

Recipient	HLA-A*02:01, *03:01	HLA-B*44:03, *45:01
A. Donor 1	HLA-A*02:01, *03:01	HLA-B*44:03, *45:01
B. Donor 2	HLA-A*02:02, *03:01	HLA-B*44:03, *45:01
C. Donor 3	HLA-A*02:01, *03:01	HLA-B*44:03, *57:01
D. Donor 4	HLA-A*02:01, *03:01	HLA-B*44:03, *44:03
E. Donor 5	HLA-A*02:01, *24:01	HLA-B*44:03, *45:01



Question 43: Besides TA-GVHD, HLA compatibility can also play a role in the pathogenesis of which of the following transfusion reactions?

- A. Allergic transfusion reactions.
- B. Febrile nonhemolytic transfusion reactions.
- C. Transfusion-associated cardiovascular overload.
- D. Transfusion-associated dyspnea.
- E. Hemolytic transfusion reactions.



Question 44: Select the answer choice the most appropriately fills in the blanks. Transfusion-related acute lung injury (TRALI) is most attributed to inflammation resulting from the interaction between _____ antibodies and _____.

- A. Recipient HLA; recipient HLA.
- B. Donor neutrophil; recipient neutrophil antigen.
- C. Donor HLA; recipient HLA.
- D. Recipient neutrophil; recipient neutrophil antigens.
- E. Recipient HLA; donor HLA.



Question 45: A 35-year-old female received a renal transplant for end-stage renal disease secondary to lupus nephritis. Now, a year later, the patient presents with rising creatinine. A biopsy reveals histologic findings consistent with antibody-mediated changes including glomerulitis, peritubular capillaritis, and positive C4d staining. Single-antigen bead testing reveals multiple donor-specific antibodies, along with the corresponding mean fluorescent intensity (MFI) values. Which of the antibodies would be predicted to have the best response to reduction by therapeutic plasma exchange?

- A. Anti-HLA-A2 at 10,000 MFI.
 - B. Anti-HLA-B8 at 20,000 MFI.
 - C. Anti-HLA-DR17 at 20,000 MFI.
 - D. Anti-HLA-DR52 at 15,000 MFI.
 - E. Anti-HLA-DQ2 at 10,000 MFI.
-

Question 46: Which of the following statements is true concerning HLA disease susceptibility?

- A. HLA disease associations refer only to increased susceptibility to individual diseases.
 - B. All associations between HLA genes and individual diseases are due to the linkage disequilibrium that exists between the HLA gene and the disease-causing gene.
 - C. When an HLA allele is associated with a particular disease, most individuals with that particular allele have the disease.
 - D. If a disease is associated with a certain HLA antigen, all alleles within the antigen group will be associated with the disease.
 - E. Relative risk can describe the strength of the association between a given disease and a certain HLA antigen/allele.
-

Question 47: Which of the following diseases is associated with a gene within the major histocompatibility complex, representing a monogenic disease association?

- A. Sickle cell anemia.
- B. Cystic fibrosis.
- C. Celiac disease.



- D. Hereditary hemochromatosis.
 - E. Ankylosing spondylitis.
-

Question 48: Which of the following diseases is associated with the *DQB1*06:02* allele?

- A. Ankylosing spondylitis.
 - B. Celiac disease.
 - C. Behçet's disease.
 - D. Narcolepsy.
 - E. Psoriasis.
-

Question 49: Which of the following alleles is associated with a hypersensitivity reaction to abacavir, an antiretroviral used in the treatment of HIV infection?

- A. *B*15:02*.
 - B. *B*51:01*.
 - C. *B*57:01*.
 - D. *B*58:01*.
 - E. *B*59:01*.
-

Question 50: A new disease has emerged and it is thought to be associated with HLA-*DQB1*04:01*. A disease association study is carried out. Of the 200 individuals with the disease, 40% express *DQB1*04:01*. Of the 500 individuals without disease (controls), the prevalence of *DQB1*04:01* is 10%. What is the approximate relative risk that an individual who expresses *DQB1*04:01* will develop this new disease?

- A. 1.8.
- B. 2.3.
- C. 2.9.
- D. 4.0.
- E. 6.0.

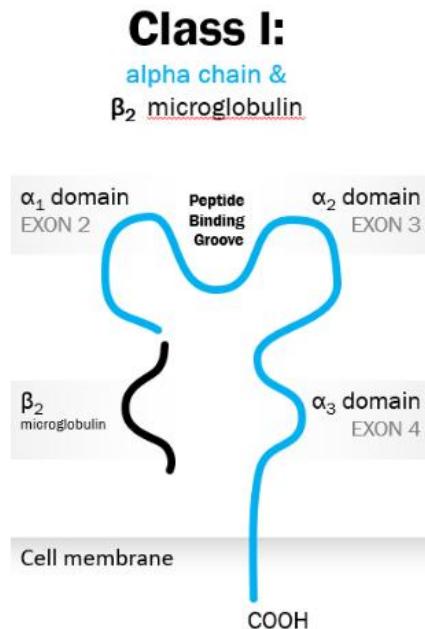


ANSWERS

Question 1: A. I; 15q.

Explanation:

- β_2 -microglobulin noncovalently associates with glycoprotein alpha heavy chain of HLA Class I molecules. It does not cross the membrane but rather interacts with the α_3 domain of the heavy chain, which does cross the cell membrane (see figure below).



- β_2 -microglobulin is encoded by a gene on chromosome 15q.
- Chromosome 6p is home to the human majority histocompatibility complex, which is where the HLA loci reside. Class I loci include *HLA-A*, *-B*, and *-C*; and the Class II loci include *HLA-DRB1*, *-DRB3*, *-DRB4*, *-DRB5*, *-DQA1*, *-DQB1*, *-DPA1*, and *-DPB1*.
- The ABO gene resides at 9q34.2 and encodes for the glycosyltransferases responsible for addition of the respective terminal sugar moieties. The gene consists of 7 exons (18 kb) with the protein's open reading frame mostly in exons 6 and 7. A and B



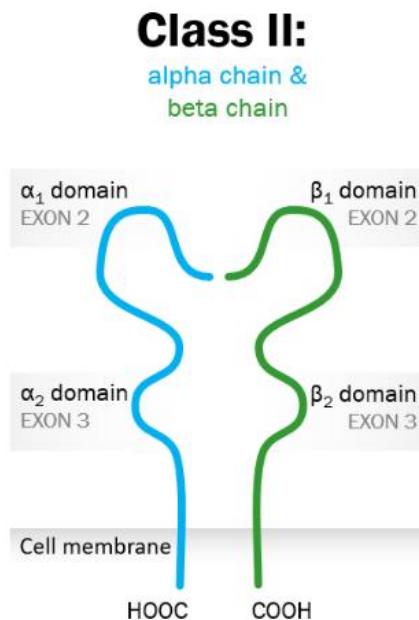
alleles are autosomal codominant; whereas, the O phenotype is an autosomal recessive trait, resulting from the inheritance of two ABO genes, encoding nonfunctional glycosyltransferases.



Question 2: C. The variable regions reside in the α_1 and β_1 domains of the α and β chains.

Explanation:

- HLA Class II proteins consist of an α and β chain, that are structurally similar. Both chains contain two domains: α_1 and α_2 on the α chain, and β_1 and β_2 chain on the β chain (see figure below).



- The variable regions reside in the α_1 and β_1 domains of the α and β chains, respectively. These domains are encoded by exon 2 of the genes.
- The variable regions for Class I reside in the α_1 and α_2 domains of the alpha heavy chain. These domains are respectively encoded by exon 2 and 3 of the gene.



- The variable regions of HLA Class I and II molecules are important. Not only do they confer antigen specificity, differentiating the proteins from each other, but the variability also permits the HLA protein to bind and present a wide variety of peptides. The ability to present different peptides provides an immunologic advantage to guard against a wide variety of pathogens.
 - Given that the variable regions confer specificity thereby differentiating HLA proteins, they can stimulate alloimmunization. In the setting of transfusion and transplantation, foreign HLA proteins can lead to the production of anti-HLA.
-

Question 3: D. Neurons.

Explanation:

- The table below summarizes HLA Class I and II expressions on different cell types.

Tissue	MHC Class I	MHC Class II
Lymphoid tissue		
T cells	+++	-
B cells	+++	+++
Macrophages	+++	++
Other antigen-presenting cells	+++	+++
Epithelial cells of the thymus	+	+++
Other nucleated cells		
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Brain	+	-
Nonnucleated cells		
Red cells	-	-



- Class I HLA antigens are present on most nucleated cells in the body as well as on platelets.
- Although generally only nucleated cells express Class I HLA proteins, some mature red cells express Class I HLA proteins termed Bg antigens (Bennet-Goodspeed antigens). These antigens are thought to be vestigial remnants of HLA antigens. Some HLA types are better expressed than others (eg, HLA-B7,-B57,-B58, -A60,-A69).
- Exceptions to Class I expression: some nucleated cell types do not express Class I antigens. These cells include germinal cells, trophoblasts, neurons, and corneal epithelial cells.
- Class II HLA antigens are usually restricted to antigen-presenting cells (eg, B lymphocytes, monocytes, dendritic cells, and macrophages). In quiescent states, T cells generally express only Class I, but once activated, they can be induced to express Class II antigens.



Question 4: C. 02.

Explanation:

- HLA nomenclature has evolved over the years to accommodate the growing list of identified HLA alleles. This number has exponentially increased in recent years largely due to increased use of next-generation sequencing for HLA typing.
- In short, an allele name consists of following components:
 - Locus – the HLA gene (eg, *A*, *B*, *C*, *DR*, *DQ*, *DP*). For Class II antigens the locus will also include the protein chain (α or β).
 - Asterisk (*) – denotes that the allele typing was obtained by molecular methods. If this is not present, then the allele could have been typed by serology.
 - First field – corresponds to the serologic equivalent/serologic specificity.
 - Colon (:) – each field is separated by a colon.
 - Second field – corresponds to the HLA protein. In other words, this field designates a unique sequence that differentiates the protein from others particularly at the variable regions (exons 2 and 3 for Class I and exons 2 for Class II). The number is usually assigned based on the order of sequence identification.
 - Third field – corresponds to nucleotide sequence differences that encode the same amino acid codon. Thus, even though the genetic sequence is different, the amino acid sequence will



be the same (ie, the protein will be identical). Therefore, they are termed synonymous or silent mutations.

- Fourth field – corresponds to nucleotide sequence differences in the introns, or the 3' or 5' untranslated regions of the gene. As these variations are in noncoding regions, they are generally not expressed.
- Nomenclature can include expression modifiers at the end:
 - N = null (gene product not expressed).
 - L = low expression.
 - S = secreted (ie, not on cell surface).
 - Q = questionable expression level.
 - C = cytoplasmic expression (ie, not on cell surface).
- The following table explains the different components of HLA nomenclature, using the allele in question #4 as an example.

Species	Locus	Antigen Equivalent	HLA Protein	Silent Mutation	Intronic Mutation	Expression Modifier
HLA	A	* 31 : 01	: 02	: 51		Q

First field Sec-
ond
field Third
field Fourth
field

Question 5: B. There is a genetic variation in one of the introns that results in the same protein.

Explanation:

- See answer #4.
-

Question 6: A. Homozygosity for A*02.

Explanation:

- The typing provided represents low resolution with reporting of only the first field. This patient types as *HLA*02, XX*. In this case, XX most likely represents homozygosity for the A2 antigen. If



higher-resolution typing were used, two different alleles of A2 may have been detected (eg, *02:01 and *02:06).

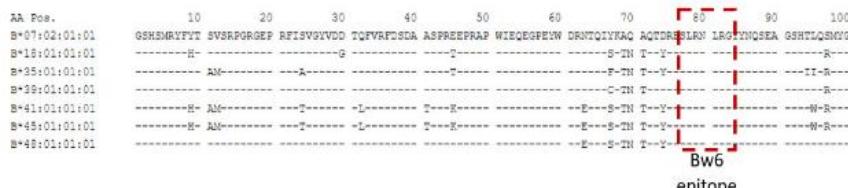
- Historically, XX was used to designate that a second allele was not detected, which could have meant homozygosity or that the method employed may have missed the second allele. This could be due to not having the appropriate antisera in serologic testing or having limited primers to detect the various HLA antigens in molecular testing. However, with improved molecular methods, XX is taken to mean homozygosity at that locus.
- It is important to note that if typing was reported as *HLA*02:XX* with the XX in the second field, then this is a code signifying that the allele is not further defined. Hence, all that is certain is the serologic equivalent or antigen family *02. The allele could be any one of a substantial number of possibilities (eg, *A*02:01/A*02:02/A*02:03/A*02:04/A*02:05/A*02:06/A*02:07*, etc).
- A null allele would be designated with the expression modifier 'N' (see answer #4).
- Allelic dropout could be why a second allele may not be detected and reported out as XX, but this is not what XX signifies.
- New alleles would be noted with an explanatory comment detailing detection of a suspected new allele along with the differences/similarities to other known alleles.



Question 7: E. Antibody development to a public antigen can resemble the presence of many discrete HLA antibodies.

Explanation:

- Public antigens are common amino acid determinants, or epitopes, and are shared among many HLA specificities (see figure below). These epitopes represent more conserved regions of the HLA protein.



Note: top row is the reference allele with its amino acid sequence. All dashes represent the amino acid in the reference sequence (ie, identical to reference sequence at that position).



- The two most common public antigens are Bw4 and Bw6. The accompanying figure shows several B alleles that all contain the Bw6 public epitope. The amino acid sequences between amino acid positions 77 and 83 (in the dashed box) are identical among the different HLA alleles.
- Exposure to just a single specificity could alloimmunize an individual to a public epitope. In this case, antibody testing would reveal reactivity among all the reagent targets that bear the public epitope. This reactivity will make it appear that they have antibodies to multiple alleles.
- Given that an antibody to a public antigen could give a person broad alloreactivity to all specificities bearing the epitope, this can have major clinical implications when trying to find a compatible donor in the setting of transplantation and platelet transfusion.



Question 8: D.

Choice	Type of Peptide Presented	Location of Protein Degradation into Peptide	Type of T-Cell Interaction
D.	Endogenous	LMP	CD8

Explanation:

- The main biologic function of the HLA molecule is to present peptides to T cells. Although this function applies to both Class I and II, there are differences in the types of peptides presented, the way peptides are processed, and the type of T cells with which they interact. The following table compares the biologic function of Class I and II HLA proteins.

Function	HLA Class I	HLA Class II
Synthesis of HLA protein – location	Endoplasmic reticulum (ER)	ER
Type of proteins loaded in peptide binding groove	Endogenous <ul style="list-style-type: none">• Self-proteins• Viral proteins• Cancer proteins	Exogenous <ul style="list-style-type: none">• Self-proteins• Bacterial proteins• Parasitic proteins

cont'd



Function	HLA Class I	HLA Class II
Proteins degradation into peptides – location	<ul style="list-style-type: none"> Large multifunctional protease (LMP) in cytosol Peptides transported to ER by TAP molecule 	Endosome
Peptide loading – location	ER	Endosome - here the HLA-DM molecule removes the invariant chain, which was inserted in the binding groove in the ER, and inserts the peptide
Length of peptide in the peptide binding groove	8-9 amino acids	12-25 amino acids
Type of T cell the peptide is presented to	CD8 – activation elicits cytotoxic cell properties	CD4 – activation promotes cytokine secretion, which is important in the development of antibodies



Question 9: A.

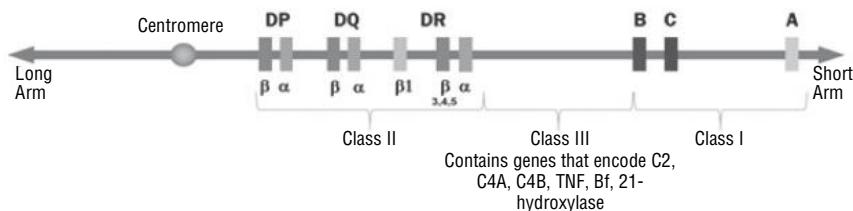
Choice	Type of Peptide Presented	Location of Peptide Loading	Length of Peptide Presented	Type of T-Cell Interaction
A.	Exogenous	Endosome	12-25 amino acids	CD4

Explanation:

- See the table in answer #8.

**Question 10: C. G.****Explanation:**

- HLA genes are on chromosome 6p21.3 and distributed into two main regions, Class I and Class II. Between Class I and II genes are Class III genes, although this region contains no HLA genes (see figure below for schematic).



Note: Figure is not drawn to scale.

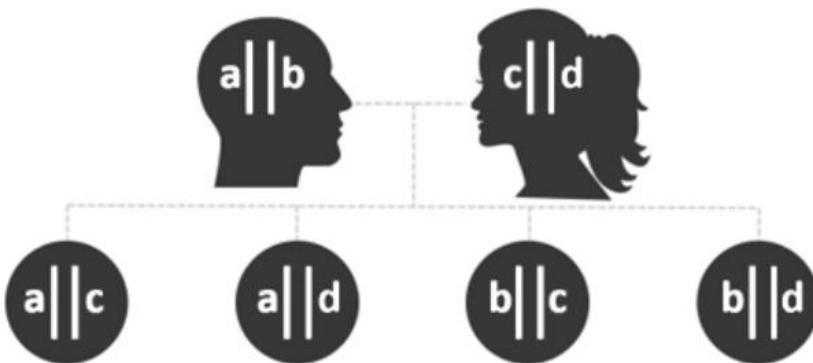
- Class I genes include *HLA-A*, *HLA-B*, and *HLA-C*. These are the classical genes.
- There are nonclassical genes, or Class Ib, which include *HLA-E*, *HLA-F*, *HLA-G*, *MICA*, and *MICB* among others. These genes are similar in structure to classical genes; however, they are not as polymorphic, are expressed in lower levels, and have limited tissue distribution. Of note, some nonclassical genes are actually pseudogenes (eg, *HLA-H*), as they encode no proteins or non-functional proteins.
- Class II genes encode both the α and β subunits of the Class II proteins. The α genes are generally less polymorphic than the β genes. The Class II genes include *DRA1*, *DRB1*, *DRB3*, *DRB4*, *DRB5*, *DQA1*, *DQB1*, *DPA1*, and *DPB1*. Like the Class I region, the Class II region also includes some pseudogenes.
- Class III includes genes encoding for proteins that are involved in immunologic responses such as cytokines [ie, tumor necrosis factor (TNF)], complement (bf, C2, C4A, C4B), and steroid enzymes (21-hydroxylase).

**Question 11: D. 75%.**



Explanation:

- HLA genes are inherited en bloc in a haplotype with one maternal and one paternal haplotype. A haplotype will include all Class I and II genes. Maternal and paternal haplotypes are codominantly expressed on the cell surface, so the maternal and paternal antigens will be present.
- Haplotype inheritance is depicted in the figure below. It is customary to designate the paternal haplotypes as 'a' and 'b' and the maternal haplotypes as 'c' and 'd.' As illustrated, there are four different haplotype combinations that the offspring can inherit.



- The chance that any two siblings will be HLA identical is $1/4$ (25%). At the same time, there is also a 25% chance that two siblings will not share either haplotype or a 50% chance that they will share only one haplotype. Therefore, there is a 75% ($25\% + 50\%$) chance of any two siblings sharing at least one haplotype.



Question 12: E. 76%.

Explanation:

- Even though the probability that two siblings will be HLA identical is 25%, the probability increases when comparing an individual with all their siblings. If an individual has 'n' number of siblings, the chance that at least one of the siblings will be HLA identical can be determined by the following equation: $1 - (0.75)^n$, where 'n' is the number of siblings. Application of this



equation to this question in which the patient has five siblings, results in $1 - (0.75)^5 = 0.76$ (76%).

Question 13: C. A paternal crossover between the HLA-B and HLA-DR loci.

Explanation:

- A crossover haplotype occurs during meiosis or gametogenesis when segments of HLA genetic material are exchanged between sister chromatids. As such, the crossover occurs either between the paternal haplotypes or the maternal haplotypes, not between a paternal and maternal haplotype (eliminating choice E).
 - The rate or frequency of crossover events is proportionately related, at least in part, to the distance between genes. The farther apart two genes are from each other, the more likely a crossover event will occur between them. For example, the distance between the *DQ* and *DP* loci is relatively far, thus the rate of crossover between these two loci is rather common. Alternatively, the closer two genes are to each other, the less likely a crossover event will occur. The *B* and *C* loci are very close together, so the rate of crossover between these two loci is rare.
 - In the family study provided, the a/b haplotype occurred in the father (remember paternal haplotypes are designated 'a' and 'b.' In this haplotype, the Class I antigens appear to come from haplotype 'a'; whereas the Class II antigens appear to come from haplotype 'b'. Thus, it may be tempting to say the crossover occurred between the *C* and *DR* loci. However, on chromosome 6p, the *DR* locus is centromeric to the *B* locus. Hence, the crossover would be between the *B* and *DR* loci (see figure in answer #10).
-

Question 14: E. A34,B72,DR13/A2,B44,DR4.

Explanation:

- Recall that HLA is inherited in haplotypes, one paternal and one maternal. This means when looking at each sibling's HLA type, one allele at each locus comes from the father while the other allele comes from the mother.



- When HLA types are resulted, the two antigens/alleles that are detected are reported but they are not generally reported by haplotype. In other words, in the example provided, each row of an individual HLA type does not necessarily correspond to the two haplotypes. However, when the HLA types of a family are provided, the HLA haplotypes can be deduced by determining how the maternal and paternal antigens/alleles segregate as they are passed to the offspring.
- In this case, the HLA-A antigens of sibling #3 are A2 and A34, so the corresponding haplotypes must include these two antigens. This eliminates choices C and D.
- Sibling #3 can be compared to one of the parents to see what antigens are shared. The patient and the father share A2, B44, and DR4, indicating that they were inherited together as a haplotype. This can be verified by determining if the same haplotype can be found in another case. In this case, sibling #1 also has the A2,B44,DR4 haplotype.
- The remaining antigens in the HLA type of sibling #3 – A34,B72,DR13 – should correspond to a maternal haplotype. This haplotype can be found in the mother's HLA type and that of sibling #2, helping to verify this second phenotype.
- Thus, the two haplotypes are A2,B44,DR4 and A34,B72,DR13.
- Choice C shows the haplotypes of sibling #2 and choice D shows the haplotypes of sibling #4.
- The haplotypes in choices A and B actually represent crossover haplotypes between father and mother haplotypes, which is not seen. Crossover haplotypes are the result of exchange of genetic material between sister chromatids.



Question 15: D. A32.

Explanation:

- The aim here is to determine the missing antigen, so identification of the complete haplotype can reveal the other haplotype, thereby identifying X.
- A potential approach would be to start by examining the HLA type of sibling #2. The known HLA-A antigen is A30, which must be part of the maternal haplotype since the mother's HLA type includes A30, and the father's does not. Next, the HLA-B and -C



antigens that sibling #2 shares with the mother can be determined. It can be seen that the haplotype inherited from the mother is A30, B38, DR4. By default, the remaining antigens must be of paternal origin, making sibling #2's paternal haplotype X, B44, DR11.

- A paternal A antigen is needed to continue, and the only two viable options are either A3 or A32. The next step is to determine which of these two choices is inherited with B44 and DR11. Siblings #1 and #2 have the A3 antigen but neither of their haplotypes would carry the B44 nor the DR11. However, sibling #3 expresses the A32 along with the B44 and DR11. Therefore, the paternal haplotype would be A32, B44, DR11, making X = A32.
-

Question 16: B. Linkage disequilibrium.

Explanation:

- Linkage disequilibrium refers to the phenomenon whereby the expected and observed frequencies between genes are discordant. In other words, the frequency of two genes being inherited together is higher than expected by random distribution alone.
- The closer two genes are to each other, the tighter the linkage disequilibrium will be. That is, the more often they will be inherited together.
- Certain HLA alleles are inherited together in haplotypes quite often in a given population. Combinations of alleles tend to vary between different ethnic populations.
- Although haplotype inheritance does explain why genes are inherited together, it is not the term that describes the discrepancy between predicted and actual allele frequencies.
- Linkage equilibrium refers to distribution of genes that are randomly inherited together.
- The Hardy-Weinberg principle is an equation used to describe the frequency of allele distribution in a population. For this principle to apply, certain assumptions must be met including random mating, no migration, and no evolutionary selection, among others. If these assumptions are met, then it is assumed that allele frequencies in a population will remain constant.
- Lyonization refers to the inactivation of one of the two X chromosomes in females during embryogenesis.

**Question 17: D. II and IV.****Explanation:**

- This case is a little more difficult to determine haplotypes because the parental HLA types are not provided. However, the haplotypes can be deduced by using the HLA types of the siblings.
- It is helpful to start with two siblings that share the same antigen at a locus and then examine the rest of the type to see what other antigens they share. In this way a haplotype can be built. For example, siblings #2 and #3 both share A3; and looking at the B locus, it appears that they share the B13. Moving on to the DR locus, siblings #2 and #3, also share DR7. Thus, A3, B13, and DR7 represent a haplotype.
- The other antigens present in siblings #2 and #3 should represent the other haplotype. So, for sibling #2 the second haplotype would be A2, B7, DR15; and in sibling #3, the second haplotype would be A1, B8, DR17.
- With identification of three of the four parental haplotypes, it may be possible to match the identified haplotypes in other siblings. If you can identify them in another sibling, then the other antigens should represent the last haplotype. For instance, the A1, B8, DR17 haplotype is also present in sibling #1, which would leave A24,B18,DR11 as the fourth haplotype. And you can verify that the A24,B18,DR11 haplotype is also present in another sibling, sibling #4 in this case.
- Another approach would be to note that sibling #1 expresses HLA-A24 and A1, and sibling #3 expresses HLA-A3 and A1. So, HLA-A1 is present in one of the parents without A3 or A24, but rather the fourth alternative (A2). Therefore, one parental haplotype combination would include A1,A2 and the other would contain the remaining A3,A24. Answer choice D would be the only one that fits the bill.

**Question 18: E. Viable lymphocytes are needed for antigen detection.****Explanation:**

- There are several methods that provide HLA typing. Complement-dependent cytotoxicity (CDC) is an older serologic method sup-



planted by molecular-based methods, which employ polymerase chain reaction (PCR).

- In short, the CDC required antisera containing antibodies of known specificity. These antisera would be incubated with lymphocytes from the individual being typed. Rabbit complement would then be added to induce cell lysis. For example, an antiserum containing A1 antibodies would be incubated with a recipient's cells. If cell lysis was observed, then the interpretation would be that cells likely expressed the corresponding HLA-A1 antigen.
- This test requires viable lymphocytes for testing, because the main outcome of interest is cell lysis. Nucleic acid is not required for serologic typing.
- Additionally, serologic typing has low sensitivity; in fact, it does not reliably detect low expressing antigens, such as C locus antigens.
- In terms of resolution, it provides only serologic-level typing, which is low resolution. It could distinguish between an A1 allele and an A2 allele, but it could not usually distinguish one allele from another within the same antigen family.



Question 19: D.

Choice	Typing Method	Resolution (low, intermediate, high)	DR4 Result
D.	NGS	High	<i>DRB1*04:01:01G</i>

Explanation:

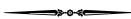
- The accompanying table provides a summary of the resolution, example of a DR4 result, and clinical application for the different methods of HLA typing.



Method	Resolution (low, intermediate, high)	Example of DR4 Result	Clinical Application
CDC	Low	DR4	Platelet-refractoriness
Sequence-specific primers (SSP)	Low-to-intermediate [†]	DRB1*04:01/03/ 07/09 [‡]	Solid-organ transplantation
Sequence-specific oligonucleotide probe (SSOP)	Low-to-intermediate [†]	DRB1*04:01/03/ 07/09 [‡]	Solid-organ transplantation
NGS	High	DRB1*04:01:01	Hematopoietic stem cell transplantation

[†]If more primers for SSP and more probes for SSOP are used, then high-resolution typing can be obtained for some alleles; however, most of the time there are still ambiguities.

[‡]The low-to-intermediate typing results in a string of alleles, which cannot be further distinguished due to limitations of the assay. In this example, SSP and SSOP narrowed down the typing to four alleles: DRB1*04:01, DRB1*04:03, DRB1*04:07, and DRB1*04:09.



Question 20: C. The FXM is more sensitive than the CDC.

Explanation:

- Before solid-organ transplant procedures, a prospective physical crossmatch is performed using recipient serum and donor lymphocytes to detect preformed, donor-specific antibodies to the potential donor. Such preformed antibodies could mediate hyperacute or acute rejection.
- As with HLA typing, described in answer #18, the complement-dependent cytotoxicity assay can be used to perform the cross-match. In short, recipient serum is incubated with donor lympho-



cytes followed by the addition of rabbit complement. If cell lysis ensues, then this is considered a positive crossmatch, which is generally a contraindication to transplantation. To bind complement, a certain titer of antibody must be present. Thus, the sensitivity of the CDC is low in the face of low-level antibodies. The addition of antihuman globulin, the so-called enhanced CDC, can increase the sensitivity of the assay.

- The flow cytometric crossmatch also uses viable cells. However, after serum incubation, fluorescently labeled antihuman globulin is added. The secondary antibody is directly targeting the HLA antibody on the cell. Therefore, the sensitivity of the FXM is higher than that of the CDC, which is reliant not only on the binding of complement but also on its ability to subsequently induce cell lysis.
- By adding anti-CD3 and anti-CD19 /anti-CD20 to the FXM, T cells and B cells can be assessed simultaneously, allowing for the individual analysis of T- and B-cell crossmatches.



Question 21: C. I and III.

Explanation:

- Resting T cells express only Class I HLA antigens whereas B cells express both Class I and II HLA antigens. Therefore, differential reactivity can indicate the class of HLA antibodies present in a given serum.
- The following table summarizes the Class of HLA antibodies present based on T- and B-cell crossmatch results.

T-Cell Crossmatch Result	B-Cell Crossmatch Result	HLA Antibodies
-	-	None
-	+	Class II
+	+	Class I only OR Class I and II

Note: T-cell positive, B-cell negative crossmatch reactivity pattern is not generally encountered because B cells actually express more Class I HLA antigens than T cells.



Question 22: A. Microparticle beads can either be coated in recombinant HLA antigens or with Class I or II phenotypes from cultured lymphocytes.

Explanation:

- Solid-phase assays employ a solid-phase medium, usually in the form of microparticle beads, in lieu of lymphocytes.
- The microparticle beads can either be single-antigen beads, which are coated with recombinant HLA antigens, or phenotypic beads, which are coated in Class I or II phenotypes.
- After incubation of serum with solid-phase medium, a secondary fluorescently labeled antihuman globulin is employed. Detection methods include flow cytometry, flow microarray, and less commonly ELISA.
- Solid-phase assays are considered more sensitive than cell-based assays and can even detect very low levels of HLA antibodies. However, although the clinical significance of high-level antibodies is well described, low-level antibodies may not always result in untoward clinical outcomes, especially when these antibodies are detected only by solid-phase assays.
- Single-antigen beads can provide the specificity of antibodies, in other words able to differentiate antibodies targeting different antigens, and in some cases, antibodies directed toward certain alleles within the same antigen family, so-called allele-specific antibodies.



Question 23: B. A positive T-cell crossmatch is generally considered a contraindication to transplantation.

Explanation:

- Unlike recipient-donor pairs in hematopoietic stem cell transplantation, pairs in renal transplantation need not be well matched at the HLA loci. Similar to the setting of transfusion, recipient-donor pairs in solid-organ transplant should be serologically compatible. The recipient should not possess HLA antibodies corresponding to donor HLA antigens.



- A positive crossmatch is predictive of acute rejection and delayed graft function, whereas a negative crossmatch is predictive of long-term graft survival.
- A positive T-cell crossmatch is generally a contraindication to transplantation, as is a positive B-cell crossmatch, if it is determined to be caused by HLA antibodies directed towards the donor.
- Regarding ABO compatibility, recipient-donor pairs should be ABO compatible to prevent antibody-mediated rejection. In some institutions, the ABO barrier is crossed after desensitizing the patient by reducing anti-ABO titers through various protocols, which include various combinations of plasma exchange and administration of IVIG, rituximab, and other immunomodulatory agents.



Question 24: **B.** Antigens to which the recipient has the corresponding HLA antibodies by solid-phase testing

Explanation:

- Single-antigen bead testing allows for the detection of antibody specificity (ie, the identity of the HLA protein target). As these antibodies are associated with poor outcomes, the corresponding antigens that they target can be considered “unacceptable” antigens. In other words, donors expressing these antigens would be considered unacceptable donors.
- By determining the prevalence of the “unacceptable” antigen(s) in the donor population, the calculated panel-reactive antibody (cPRA) can be determined. In the US, this is accomplished with an online calculator supplied by the United Network of Organ Sharing (UNOS). After inputting all the “unacceptable” antigens, the calculator will provide a percentage, 0-100%.
- The resulting cPRA value correlates with the percentage of potential donors who would be considered unacceptable for transplantation.



Question 25: **D.** 20%.



Explanation:

- Given that the cPRA correlates with the percentage of prospective donors who would be unacceptable for transplantation, then the acceptable donor pool would correlate to the remaining donors. Hence, the lower the cPRA, the wider the pool of compatible donors. Conversely, the higher the cPRA, the smaller the pool of compatible donors.
- To calculate the percentage of histocompatible or “acceptable” donors, simply subtract the cPRA from 100.



Question 26: C.

Choice	Blood Group	Class I HLA Phenotype
C.	O	A3, A34, B57, B75, C2, C14

Explanation:

- To answer this question, the reader must perform a virtual crossmatch. This is done by comparing the recipient antibody profile to the prospective donor HLA type. If the recipient possesses no HLA antibodies to donor antigens, then this is a negative virtual crossmatch and the donor is considered “acceptable.” However, if the recipient does possess HLA antibodies to the prospective donor, this is a positive virtual crossmatch, and depending on antibody strength, the donor may be considered “unacceptable” for transplantation.
- The histogram provided in the question is from single-antigen bead analysis, where the positive threshold for considering a bead reactive or positive is 1000 MFI, which is provided on the y-axis. The x-axis displays the bead and the antigen it bears.
- All bars above 1000 MFI would be considered positive or reactive specificities and the corresponding bead on the x-axis provides the “unacceptable” antigen.
- Answer choices A, C, and E would be the only acceptable ones from an ABO- compatibility perspective (the recipient is group O and can receive only group O donor organs).
- The donor in choice ‘A’ expresses multiple antigens (A24, B18, C7) to which the recipient has antibodies; and the donor in



choice 'E' also expresses an antigen (B48) to which the recipient has antibodies.

- The donor in choice 'C' is ABO compatible and expresses no HLA antigens to which the recipient has antigens, making the donor in choice 'C' the most appropriate selection.
-

Question 27: B. Brother #1.

Explanation:

- High-resolution HLA typing at the *A*, *B*, *C*, *DRB1*, and *DQB1* loci for the patient and five family members is provided in the question. To select the most appropriate donor in this scenario, we are looking for the best X/10 HLA match among the family members.
 - Sister #1 and Brother #1 are both 10/10 HLA matches to the patient. Therefore, both would be good from an HLA perspective. However, the ABO group should also be considered. Although ABO-incompatible stem-cell transplants are often performed, with the recipient usually assuming donor ABO group with engraftment, guidelines recommend selecting donors who are ABO identical. ABO-identical transplants are associated with fewer complications than ABO-incompatible transplants.
 - The cousin represents a haploidentical HLA match and Sister #2 does not appear to share either haplotype with the patient.
 - Brother #2 is matched for one *DRB1* allele and one *DQB1* allele, making him an 8/10 HLA match, which is less ideal than the 10/10 HLA matches. Examining how the haplotypes are distributed among the family members, Brother #2 appears to have a crossover haplotype. Parental HLA typing would help determine between which haplotypes the crossover occurred.
-

Question 28: C. The serologic equivalent of the allele.

Explanation:

- The number in parentheses refers to the serologic equivalent of the allele. Thus, the serologic equivalent of *DRB1*03:01* is DR17 and the serologic equivalent of *DQB1*03:01* is DQ7. The sero-



logic equivalents represent the antigen names assigned by serologic testing before molecular techniques became commonplace. With the advent of molecular methods, the genetic sequences and structures of the antigens became known, and new names were assigned based on the new data acquired. However, serologic-equivalent names are still very much used in the HLA community.

- Answer #7 describes public epitopes, which are common amino acid determinants shared among many HLA proteins (eg, Bw4 and Bw6).
- Crossreactive epitope groups (CREGs) are similar to public epitopes in that they refer to common determinants shared among several HLA proteins. However, the common determinants in CREGs are limited to fewer HLA proteins than a public epitope. One example includes the A2 CREG, which is composed of A2, A23, A24, A68, A69, B57, and B58.
- Parent and split antigens are related to one another. Parent antigens were usually discovered first and characterized by certain antisera. Over time, it was observed that some antisera, with more discriminating specificity, would differentiate a subset(s) of the parent antigen, essentially dividing the parent into two or more new split antigens. In the case of DQ3, it was discovered that it consisted of at least three split antigens, or daughter antigens: DQ7, DQ8, and DQ9. All three of these splits share common determinants, which likely account for the initial characterization of the parent DQ3 antigen.



Question 29: D. The G follows alleles with identical nucleotide sequences spanning the exons encoding the domains that compose the peptide-binding groove.

Explanation:

- For Class I, this would include exons 2 and 3 of the alpha domain; and for Class II, this would include exons 2 of the alpha and beta chains. G codes are written to the third field (eg, *DQB1*02:01:01G*).
- The P code designates alleles with identical amino acid sequences spanning the domains that compose the peptide binding groove. P codes are written to the second field (eg, *DQB1*02:01P*).



- Given the peptide-binding groove region is where the majority of the hypervariability between HLA alleles is located, identical sequences between alleles would indicate a high degree of similarity between them.
- Differences between alleles of the same G or P code would likely be limited to the membranous regions of the protein and thus, not immunologically accessible.
- From a clinical standpoint in hematopoietic stem cell transplantation, alleles within the same G or P code are considered matched. For example, *DQB1*02:01* and *DQB1*02:02* are both part of the *DQB1*02:01:01G* code. As such, if the recipient typed as a *DQB1*02:01,XX* and the donor typed as *DQB1*02:02, XX* they would still be considered matched at this locus.



Question 30: E. A, B, C, DRB1.

Explanation:

- The National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR) donor selection guidelines recommend at least a 7/8 or 8/8 HLA match between recipient and donor.
- The eight alleles that this guideline includes are the two alleles at the following four loci: *A, B, C, and DRB1*. Mismatches at all these loci have been associated with increased risk of GVHD, relapse, and transplant-related mortality.
- Mismatches at the other loci should be minimized when possible.



Question 31: D. HLA-matched platelets limit further HLA sensitization to mismatched HLA antigens between the recipient and donor.

Explanation:

- Advantages of HLA-matched platelets include the utility in highly sensitized patients (ie, high HLA antibody burden) and the prevention of further sensitization to potentially mismatched HLA antigens between recipient and donor. If HLA-matched platelets



are supplied, recipient HLA antibody testing is not necessarily needed. Donor HLA antibody testing is not needed for the provision of HLA-matched or HLA antigen-negative units.

- Disadvantages of HLA-matched platelets include restricting the donor pool to HLA-matched donors and requiring donor and recipient HLA typing (which is expensive and time consuming). Also, supplying HLA-matched platelets will not improve response to platelet transfusion if refractoriness is due to HPA antibodies.
- Once a donor and recipient are HLA typed, retyping is not necessary, as HLA phenotype should not change unless an individual undergoes an HLA-mismatched hematopoietic stem cell transplantation, in which the recipient's hematopoietic cells will convert to donor HLA phenotype following engraftment.



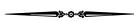
Question 32: B. HLA antigen-negative platelets do not account for the presence of HPA incompatibility between recipient and donor.

Explanation:

- HLA antigen-negative platelets do not express the HLA antigen(s) to which the recipient has HLA antibodies.
- Advantages of HLA antigen-negative platelets include widening of donor pool when compared to HLA-matched platelets and obviating the need for recipient HLA typing.
- As with HLA-matched platelets, HLA antigen-negative platelets will not improve the response to platelet transfusion if refractoriness is due to HPA antibodies. However, unlike HLA-matched platelets, provision of HLA antigen-negative platelets does increase the risk of further sensitization to mismatched HLA antigens between recipients and donors. Hence, repeated recipient HLA antibody testing should be considered to monitor the development of additional HLA antibodies to donor(s) mismatched antigens. Furthermore, although recipient HLA typing is not needed, donor HLA typing is still required in order to supply HLA antigen-negative units.

**Question 33: A. Irradiation.****Explanation:**

- Irradiation is performed on cellular blood components to prevent transfusion-associated graft-vs-host disease (TA-GVHD), which is almost universally fatal. Attack of recipient marrow by proliferative donor T cells leads to pancytopenia, resulting in death by infection or bleeding. Irradiation of the donor blood components can prevent the proliferation of T cells, thereby reducing the risk of TA-GVHD.
- Those who are severely immunocompromised are at risk for TA-GVHD because their immune system may not be able to mount a proper defense against the proliferating donor T cells. Others at risk are those receiving blood components from donors with similar HLA phenotypes. This is because cells from donors with similar HLA types may be able to evade the recipient immune system because donor HLA types could appear as "self" to the recipient. See answer #42 for additional details.
- Given the risk for TA-GVHD, the AABB recommends irradiating blood component that are selected based on HLA compatibility and blood components from family members, who are likely to share similar HLA types to the recipient.
- Of note, the process involved in pathogen reduction also targets cellular DNA to prevent cellular proliferation. Thus, pathogen-reduced platelets are low risk for TA-GVHD and do not need irradiation.
- The remainder of the modifications would not prevent TA-GVHD.

**Question 34: A. Registry donor #1.****Explanation:**

- Per the guidelines from the National Marrow Donor Program (NMDP)/Center for International Blood and Marrow Transplant Research (CIBMTR), if no donor is a complete HLA match, then the donor with a single allele/antigen mismatch should be selected if available.



- None of the donors in the question are a 10/10 HLA match. However, registry donor #1 and registry donor #5 are both 9/10 HLA matches. Both are mismatched at the C locus: the recipient is a *C*16:01* and the donors are both *C*03:03*. Now, the question becomes, how to elect the better match between the two 9/10 HLA matched donors.
 - The NMDP/CIBMTR guidelines also recommend the selection of younger donors. In this case, this would be registry donor #1.
 - See answers #35 and #36 for additional recommendations from the NMDP/CIBMTR guidelines.
-

Question 35: D. Registry donor #4.**Explanation:**

- This time the match appears to have resulted in donors who were a better HLA match than during the search for his first transplant.
- None of the donors are 12/12 HLA matches. However, registry donors #2, #3, and #4 are 11/12 HLA matches, each mismatched for one DPB1 antigen/allele (registry donors #1 and #5 are 10/12 HLA matches, each mismatched for one C and one DPB1 antigen/allele).
- Per the guidelines from the National Marrow Donor Program (NMDP)/Center for International Blood and Marrow Transplant Research (CIBMTR), donors with a permissive DPB1 mismatch should be selected. In this case, registry donor #4 with the permissive mismatch should be selected.
- HLA-DPB1 mismatches can be classified as permissive and non-permissive, based on T-cell-epitope groups, with some DPB1 antigen/alleles bearing T-cell-epitope groups that are more reactive, or immunogenic, with T cells. These T-cell-epitope groups can be classified as low, intermediate, and high-predicted immunogenicity. Even when the recipient and donor are mismatched at the DPB1 locus, the mismatch may be tolerated if both recipient and donor are classified with the same predicted immunogenicity (ie, permissive). When the immunogenicity between the mismatched DPB1 antigen(s)/allele(s) is not the same, then the mismatch may pose increased risk (ie, nonpermissive).
- See answers #34 and #36 for additional recommendations from the NMDP/CIBMTR guidelines.

**Question 36: D. DQB1, DPB1.****Explanation:**

- Although NMDP/CIBMTR recommend a minimum of 7/8 or 8/8 HLA match between recipient and donor, some institutions attempt to find donors who are 10/10 or even 12/12 HLA matches.
- To find donors who are 10/10 HLA matches, typing of the *DQB1* locus is required in addition to typing of the *A*, *B*, *C*, and *DRB1* loci.
- To find 12/12 HLA matches, typing would need to include *DQB1* and *DPB1* loci in addition to the *A*, *B*, *C*, and *DRB1* loci.
- Matching at the *DRB3/4/5*, *DQA1*, and *DPA1* is not generally performed, because few data are available on the clinical benefit that matching at these loci would provide.

**Question 37: B. Second field (eg, "02" in A*01:02:03:04).****Explanation:**

- High-resolution typing is required for hematopoietic stem cell transplantation (HSCT). High-resolution typing is also termed as “allele-level” typing or two-field typing. Thus, HLA typing must extend at least to the second field.
- Two-field typing distinguishes HLA proteins, based on the differences in the amino acid sequence in the peptide binding groove or antigen recognition domain.
- Although third-field and fourth-field typing are considered high resolution, current guidelines do not require HLA typing to include these fields in the matching of recipient-donor pairs for HSCT.
- High-resolution typing can also be designated with P or G groups (see answer #29), which are codes that designate a group of alleles that are identical across their peptide binding grooves/antigen recognition domains.

**Question 38: C. Anti-HLA-A.****Explanation:**

- Approximately 60-80% of platelet transfusion refractory cases are due to nonimmune causes, which include splenomegaly, infection, bleeding, disseminated intravascular coagulopathy, chemotherapy, and other drugs (eg, vancomycin) among others.
- The minority of cases are due to immune-mediated etiologies, the chief of which is anti-HLA. Platelets express only HLA Class I antigens, specifically HLA-A and -B antigens. HLA-C antigens have low expression on platelets. Consequently, only antibodies to HLA-A and -B antigens are thought to be clinically relevant in the setting of platelet transfusion refractoriness.
- Although antibodies directed to human platelet antigens (HPA), such as platelet glycoproteins, can mediate platelet transfusion refractoriness, these antibodies are found only in a small percentage of platelet refractoriness cases, around 2-8%. Even when HPA antibodies are present, there is usually concurrent HLA antibody present.
- Platelets express ABH antigens, and so, anti-ABH can also mediate posttransfusion platelet clearance in the setting of ABO-mismatch platelet transfusions. For this reason, it is recommended to perform the corrected count increment after the transfusion of ABO-identical platelets. In this question, the patient is blood group AB and would not be expected to have anti-ABH that could mediate enhanced platelet clearance.

**Question 39: C. II and III.****Explanation:**

- The management of immune-mediated platelet transfusion refractoriness involves the selection of specialized platelets such as HLA-matched platelets, HLA antigen-negative units, or cross-matched platelet units.
- HLA-matched platelets are platelets from donors who express the same HLA antigens/alleles as the recipients. In order to provide HLA-matched platelets, the HLA phenotypes of the recipient and



donor are needed. Thus, HLA typing for the recipient and donor would be required.

- HLA antigen-negative platelets refers to the selection of platelets from donors who do not express HLA antigens/alleles corresponding to recipient antibodies. In this way, HLA antigen-negative platelets follow the same principles of donor selection in red cell transfusion and solid-organ transplantation. This method compares the recipient HLA antibody profile to the donor HLA phenotype; thus, recipient HLA antibody testing and donor HLA typing are both required.
- Crossmatched platelets are selected based on reactivity between recipient serum and donor platelets. The most common assay used is the solid-phase red cell adherence (SPRCA) assay. In short, donor platelet fragments line microwells (a different donor per microwell) and are incubated with recipient serum. Next, an indicator red cell coated in antihuman globulin is used for antibody detection. This test will be positive in the presence of anti-HLA and anti-HPA. False-positive reactivity can also occur in the presence of intravenous immunoglobulin.
- HPA-matched or HPA-antigen-negative platelets may also be procured, but the testing required is not widely available, and in many cases, crossmatched platelets will suffice.
- Donor HLA antibody testing is not required in the management of patients with platelet transfusion refractoriness.



Question 40: B. HLA-matched platelets.

Explanation:

- Although ABO-identical platelets would be appropriate for the calculation of the corrected count increment (CCI) in the initial evaluation of a patient with suspected platelet transfusion refractoriness, this patient has already been shown to have a low CCI of 3000. Depending on the institution, a CCI of less than 5000 or 7500 assessed 10 minutes to 1 hour after platelet transfusion is generally considered corroborative of platelet transfusion refractoriness. Now that this patient has been shown to have a high HLA antibody burden, ABO-identical platelets are no longer the most appropriate product to select.



- When an individual is highly sensitized, such as this patient, crossmatched platelets will likely be of low yield. Recall that the higher the cPRA, the more difficult it will be to find a donor who is histocompatible with a given recipient. In somebody with a cPRA of 99.99%, crossmatching random platelet donors will likely result in positive crossmatches with most donors. In this case, more than 100 random donors may need to be cross-matched in order to find one compatible platelet donor.
- Although HLA antigen-negative units are an option for sensitized patients, an individual with a cPRA of 99.99% will likely be reactive with most antigens excluding self-antigens. These patients will likely be compatible only with donors who are closely HLA matched. In these cases, HLA typing of the recipient can be performed and those results can be matched against results from a pool of HLA-typed platelet donors. HLA-matched platelets will also prevent increased HLA sensitization against potential mismatches that occur when HLA antigen-negative units are selected.
- Given that HPA antibody testing was negative, HPA-matched platelets are not warranted.



Question 41: E. V alone.

Explanation:

- All of the provided choices are known causes of positive platelet crossmatches.
- Although technical error in the laboratory testing is a possibility, the crossmatch was repeated by a different medical laboratory scientist using a different serum sample from the same patient, and the crossmatch was still positive. Thus, laboratory error is unlikely.
- HPA antibodies can also cause positive platelet crossmatches, but as we know from this patient's history given in Question #40, the patient's HPA antibody testing was negative.
- IVIG can cause false-positive platelet crossmatches; however, nothing in the patient's history indicates that the patient received IVIG. IVIG was not provided as a stand-alone choice either, it was either paired with technical error or HPA, which are not likely in this case.



- Because platelets express ABH antigens, ABH antibodies can also cause positive platelet crossmatches when the recipient and donor are ABO mismatched. This patient is group AB, so he should not have ABH antibodies that could cause a reactive platelet crossmatch.
- An allele-specific antibody is an antibody against an allele within an antigen group. In this case, only low-resolution HLA typing results are provided. However, it could be possible that the recipient expresses a rarer A2 allele like an *A*02:06*. If this were the case, the patient could make an antibody to the more common *A*02:01*. Thus, although the recipient and patient appear HLA matched at low-resolution typing, they may actually be mismatched at high-resolution typing.

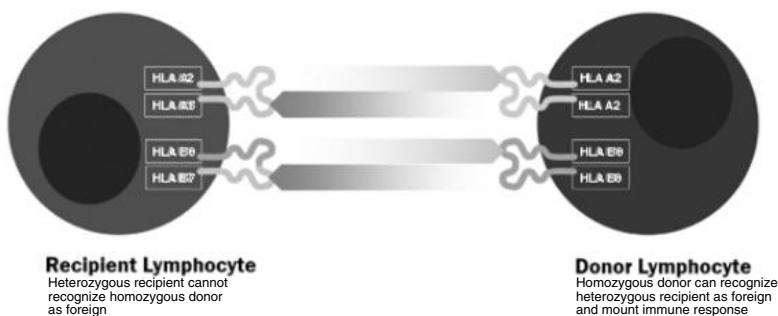


Question 42: D.

Choice	HLA-A*02:01, *03:01	HLA-B*44:03, *45:01
Donor 4	<i>HLA-A*02:01, *03:01</i>	<i>HLA-B*44:03, *44:03</i>

Explanation:

- As stated in answer #33, risk of TA-GVHD increases when the HLA types between donor and recipient are similar. This risk is greatest when the donor is homozygous at a locus where the recipient is heterozygous (see figure below). In this scenario, the recipient would not recognize the donor as foreign; however, the donor can recognize the recipient as foreign and mount an immune response, especially when the recipient is sufficiently





immunocompromised. Of the answer choices, only D represents a scenario where the donor is homozygous ($B^*44:03, B^*44:03$) at a locus where the recipient is heterozygous ($B^*44:03, B^*45:01$).

Question 43: B. Febrile nonhemolytic transfusion reactions.

Explanation:

- HLA antibodies have been implicated in the development of febrile nonhemolytic transfusion reactions (FNHTR). Recipient HLA antibody engagement of corresponding HLA antigens on donor cells (eg, leukocytes, platelets) can lead to the release of cytokines, such as interleukin-1, which can in turn elicit fever. HLA antibodies in donor plasma products could also engage HLA antigens on recipient cells and lead to a similar release of cytokines.
 - Other antibodies, such as those directed against HPA and granulocytes have also been implicated in the pathogenesis of FNHTR, as have free or extracellular cytokines in donor products, particularly those that are not leukocyte reduced.
 - HLA compatibility is not thought to play a key role in the development of the remaining transfusion reactions listed in other answer choices.
-

Question 44: C. Donor HLA; recipient HLA.

Explanation:

- Transfusion-related acute lung injury (TRALI) describes the acute noncardiogenic pulmonary edema that occurs after transfusion of plasma-based blood components (eg, FFP, platelets).
- The pathogenesis of TRALI most commonly involves HLA antibodies in donor components engaging recipient HLA on white cells, particularly granulocytes in the pulmonary vasculature. However, donor neutrophil antibodies directed against recipient neutrophil antigens have also been reported. Even rarer is so-called reverse TRALI, in which recipient HLA antibodies engage HLA on donor leukocytes.



- The interaction between antibody and leukocyte can lead to complement engagement and subsequent capillary damage and leakage with resulting pulmonary edema.
-

Question 45: A. Anti-HLA-A2 at 10000 MFI.

Explanation:

- Therapeutic plasma exchange (TPE) is a treatment modality in the management of antibody-mediated rejection after renal transplantation. The rationale for TPE is the physical removal of the donor-specific antibodies (DSAs) in the transplant recipient's plasma. Treatment regimens generally consist of at least five TPE procedures performed daily or every other day. Antibody-mediated rejection in the setting of renal transplantation is classified as a Category I indication for TPE, which indicates first-line treatment, based on recommendations by the American Society for Apheresis (ASFA).
 - Although TPE is effective at reducing HLA antibody levels, not all antibodies respond to TPE in the same way. It appears that the class of HLA antibody and the amount of HLA antibody may influence the degree of reduction. The units of mean fluorescence intensity (MFI) provided by single-antigen bead assays are considered a semiquantitative assessment of HLA antibody. However, there are instances when MFI can be misleading, either underestimating or overestimating the amount of HLA antibody. Nonetheless, antibodies with lower MFI values generally respond better to TPE. Additionally, studies have shown that Class I antibodies generally experience greater reduction compared to Class II antibodies. Thus, a Class I antibody with lower MFI value would generally be expected to respond better to TPE, which describes answer choice A.
-

Question 46: E. Relative risk can describe the strength of the association between a given disease and a certain HLA antigen/allele.



Explanation:

- Certain HLA alleles are associated with certain (especially autoimmune) diseases. Most of these associations link certain HLA phenotypes with increased susceptibility to the disease, but some of the associations appear to confer protection from the disease.
- In the past, it was thought that these associations were simply due to the linkage disequilibrium between the HLA allele and the actual disease-causing gene. However, more recent research has shown that certain HLA alleles may be involved in the pathogenesis of certain diseases. Proposed mechanisms include improper presentation of self-peptides or presentation of crossreactive non-self peptides.
- Although certain HLA alleles are associated with a risk of disease, not every individual with the allele will have disease. For example, even though upwards of 90% of patients with ankylosing spondylitis express HLA-B27, only 20% of individuals expressing HLA-B27 actually have the disease. Thus, HLA typing can help support a suspected diagnosis, but is not diagnostic on its own.
- Not all alleles within an antigen group will be associated with a particular disease. For instance, *HLA-B*27:02* and *B*27:03* are associated with increased risk for ankylosing spondylitis, however, *HLA-B*27:06* and *B*27:09* are not associated with disease and may even be protective.
- Relative risk is often used to describe the strength of the association between a given disease and a certain HLA antigen/allele. See answer #50 for more details on relative risk.



Question 47: D. Hereditary hemochromatosis.

Explanation:

- Monogenic diseases or disorders are caused by a variation in a single gene. These genetic polymorphisms are sufficient to cause disease. Examples include sickle cell anemia, which is caused by a point mutation in the hemoglobin beta gene, and cystic fibrosis, which is due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein.
- Monogenic diseases are also found in the MHC region, prominently located in the Class III region of the MHC complex. Exam-



ples of these MHC monogenic disease associations are provided in the table below.

Disease	Gene
Sialidosis	<i>NEU1</i>
Hereditary hemochromatosis	<i>HFE</i>
Congenital adrenal hyperplasia	<i>CYP21A2</i>
Ehler-Danlos syndrome	<i>TNXB</i>
C2 deficiency	<i>C2</i>
C4 deficiency	<i>C4</i>

- Narcolepsy and celiac disease are examples of polygenic diseases, which are genetic disorders that are caused by the combined action of more than one gene. See answer #48 for examples of polygenic diseases associated with HLA genes.
-

Question 48: D. Narcolepsy.

The following table provides examples of diseases associated with particular HLA antigens/alleles.

HLA Antigen/Alelle	Disease
A29	Birdshot chorioretinopathy
B27	Ankylosing spondylitis
B35	Subacute thyroiditis
B51	Behçet's disease
C6	Psoriasis
DR4	Rheumatoid arthritis
DR8	Juvenile rheumatoid arthritis
DR15	Multiple sclerosis
DR17	Grave disease
<i>DQA1*05/DQB1*02</i> heterodimer	Celiac disease
DQB1*06:02	Narcolepsy
DQ8	Type 1 diabetes



Question 49: C. B*57:01.

Explanation:

- In addition to disease susceptibility, associations between some HLA alleles and increased risk to drug hypersensitivity reactions have been established. The following table provides examples of HLA alleles associated with Stevens-Johnson Syndrome/toxic-epidermal-necrolysis-type reactions when exposed to certain drugs.

HLA Allele	Drug
B*15:02	Carbamazepine
B*57:01	Abacavir
B*58:01	Allopurinol



Question 50: C. 2.9.

Explanation:

- In relation to HLA-disease association, relative risk represents a ratio of the probability that disease will occur in an individual expressing a certain HLA allele vs the probability of disease occurring in an individual not expressing said allele.
- To calculate the relative risk, it is helpful to create a 2×2 contingency table.

		Disease	
		Yes	No
Allele Expression	Yes	A	B
	No	C	D

- Relative risk can be calculated using the equation:

$$\text{Relative Risk} = \frac{A/(A+B)}{C/(C+D)}$$



- A relative risk greater than one represents increased risk due to presence of the allele. Relative risk is often used in prospective studies.
- Thus, for the scenario posed in this question, the 2×2 table would be:

<i>DQB1*04 Expression</i>	Disease	
	Yes	No
Yes	80	50
No	120	450

- Relative risk would be $\frac{80/(80 + 50)}{120/(120 + 450)} = \frac{80/130}{120/570} = 2.9$
- Answer choice E represents the odds ratio, which is the ratio of the odds that disease occurs in an individual expressing a given allele vs the odds of disease occurring in an individual who does not express the allele of interest. Odds ratio is often used in retrospective studies and can be calculated using the equation:

$$\text{Odds ratio} = \frac{a/b}{c/d}$$

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17

Tissue Banking and Organ Transplantation

Reuben P. Jacob, MD

Key Points from the *Technical Manual*

- Human tissue allografts and solid organs may be obtained from living or deceased donors, who must meet stringent donor screening criteria and other donor testing requirements similar to those applied to blood donors.
- Human tissue allografts are used for various surgical applications to treat acquired disease, trauma, and other defects.
- Tissue banks are engaged in screening and testing of donors and recovery, labeling, processing, storage, and distribution of human tissue for transplantation. Such facilities are regulated by the Food and Drug Administration (FDA).
- Hospital-based tissue services are not subject to FDA regulatory oversight if their activities are limited to receiving, storing, and dispensing tissue for use within their own facilities.
- Transfusion support for solid-organ transplantation requires preparation for possible massive transfusion and special considerations for support of ABO-mismatched transplants.

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QUESTIONS

Question 1: Which of the following relevant communicable diseases or disease agents (RCDADs) are specifically listed in Title 21 Code of Federal Regulations (CFR) 1271 for all human cells and tissues?

- A. Human transmissible spongiform encephalopathy (TSE), including Creutzfeldt-Jakob disease (CJD).
 - B. Human T-lymphotropic virus, type I and II (HTLV-I/II).
 - C. *Chlamydia trachomatis*.
 - D. West Nile virus.
 - E. All of the above.
-

Question 2: A donor would be deemed ineligible by the Food and Drug Administration (FDA) after the required donor evaluation process is completed and the donor determined to have which of the following?

- A. Risk factors for, and/or clinical evidence of, infection due to RCDADs, identified through donor screening.
 - B. Risk factors for communicable-disease risks related to xenotransplantation, identified through donor screening.
 - C. One or more positive/reactive donor screening test results.
 - D. Donor plasma is sufficiently diluted to affect the validity of the RCDAD donor screening test results.
 - E. All of the above.
-

Question 3: Donor evaluation is based on both screening and testing methods for all the following infectious disease entities except:

- A. Human immunodeficiency virus, types 1 and 2 (HIV-1/2).
- B. Hepatitis B.
- C. HTLV-I/II.
- D. *Neisseria gonorrhoea*.
- E. Human TSE, including CJD.



Question 4: According to American Association of Tissue Banks (AATB) standards, which of the following required infectious disease testing is different depending on whether the donor is living or deceased?

- A. HIV-1/2.
 - B. Hepatitis B.
 - C. Hepatitis C.
 - D. *Treponema pallidum* (syphilis).
 - E. West Nile virus (WNV).
-

Question 5: A donor is considered ineligible if donor testing for relevant communicable disease agents is positive or reactive, except for which of the following?

- A. *Treponema pallidum* (syphilis).
 - B. HIV-1/2.
 - C. Hepatitis B.
 - D. Hepatitis C.
 - E. HTLV-I/II.
-

Question 6: Human cells, tissues, and cellular and tissue-based products (HCT/Ps) from an ineligible donor may be used for transplantation in all the following circumstances, except:

- A. Allogeneic use in a first-degree or second-degree blood relative.
 - B. HCT/P consists of reproductive cells or tissue from a directed reproductive donor.
 - C. There is a documented urgent medical need.
 - D. Veterinary use.
 - E. Nonclinical purposes.
-

Question 7: When can an abbreviated donor screening procedure be performed for repeat donors?

- A. Complete donor screening procedure performed on a living donor within the previous 9 months.



- B. Record of physical exam performed in the last 90 days.
 - C. Complete donor screening procedure performed on a living donor within the previous 12 months.
 - D. Donor history questionnaire completed within the previous 12 months.
 - E. None of the above.
-

Question 8: Donor testing is required to be performed on all the following donor samples, except:

- A. Donor 1 month of age or younger.
 - B. Donor greater than 1 month of age.
 - C. Autologous blood was used for the donor.
 - D. Adult donor who received a transfusion of 1 L of Red Blood Cells (RBCs).
 - E. Adult donor who received 2 L of crystalloid solution.
-

Question 9: When must a donor specimen be collected for testing?

- A. At time of cell or tissue recovery.
 - B. Within 7 days before or after cell or tissue recovery.
 - C. Up to 30 days before collection for hematopoietic progenitor cells (HPCs).
 - D. Up to 30 days before collection for oocytes.
 - E. All of the above.
-

Question 10: Once screening and testing of the allogeneic donor is completed and eligibility is determined, the product must always be accompanied by all the following, except:

- A. A unique identifier (alphanumeric) that links the donor to the product and donor records.
- B. A statement indicating that the donor is “eligible” or “ineligible.”
- C. A summary record/report showing donor testing results and interpretations.



- D. A summary record/report showing the name and address of the establishment that made the donor evaluation determination.
 - E. Statement of urgent medical need.
-

Question 11: Which of the following diseases have been unexpectedly transmitted from donor to recipient?

- A. Renal cancer.
 - B. Hemochromatosis.
 - C. Cryptococcus.
 - D. Strongyloides.
 - E. All of the above.
-

Question 12: According to FDA guidance, which of the following deferrals for donation of HCT/Ps is correct?

- A. Men who have sex with men (MSM) within the past 3 months.
 - B. Men who have sex with men (MSM) within the past 12 months.
 - C. Persons with hemophilia or other related clotting disorders who have received human-derived clotting factor concentrates in the preceding 5 years.
 - D. Persons treated for or had syphilis within the preceding 3 months.
 - E. Reproductive HCT/P donors who have been treated for or had *Chlamydia trachomatis* or *Neisseria gonorrhoea* infection in the preceding 6 months.
-

Question 13: Tissue banks must ensure which of the following conditions are met for laboratories that perform donor infectious disease testing for them?

- A. The laboratory is registered with the FDA as a tissue establishment and lists “testing” as a function.
- B. The laboratory uses the appropriate FDA-licensed, -approved, or -cleared donor screening tests.
- C. The laboratory is certified in accordance with the Clinical Laboratory Improvement Amendments of 1988 and 42 CFR part



- 493 or has met equivalent requirements as determined by the Centers for Medicare and Medicaid Services.
- D. The laboratory retains records of donor infectious disease testing for 10 years.
 - E. All of the above.
-

Question 14: AATB standards require which of the following of semen donors?

- A. Younger than 40 years of age.
 - B. Tested for HTLV-I/II and for CMV.
 - C. A physical examination must be performed.
 - D. Tested for *Neisseria gonorrhoea* and *Chlamydia trachomatis*.
 - E. All of the above.
-

Question 15: The FDA defines relevant medical records with regards to Title 21 CFR 1271 as which of the following?

- A. Current donor medical history interview.
 - B. Physical examination of a living donor.
 - C. Coroner and autopsy reports.
 - D. Police records.
 - E. All of the above.
-

Question 16: In order for an HCT/P to be regulated solely under Section 361 of the Public Health Service (PHS) Act and Title 21 CFR Part 1271, it must meet all of the following criteria except:

- A. Minimally manipulated.
- B. Manipulated such that the biological or relevant functional characteristics of the cells or tissues are altered.
- C. Intended for homologous use only.
- D. Not combined with a device or drug, except for sterilizing, preserving, or storage agents that do not raise clinical safety concerns.
- E. Not have a systemic effect or be dependent on the metabolic activity of living cells for its primary function; unless the HCT/P



is for: 1) autologous use; 2) allogeneic use in a first-degree or second-degree blood relative; or 3) reproductive use.

Question 17: Which of the following is considered minimal manipulation with regards to HCT/Ps?

- A. For structural tissue, processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement.
 - B. For cells or nonstructural tissues, processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement.
 - C. For structural tissue, processing of the HCT/P does not alter the relevant biological characteristics of cells or tissues.
 - D. Based on the effect of manufacturing on the original relevant characteristics of the HCT/P as the HCT/P exists in the recipient, and not based on the intended use of the HCT/P in the donor.
 - E. None of the above.
-

Question 18: Which of the following are considered HCT/Ps as defined by Title 21 CFR 1271?

- A. Semen.
 - B. Pancreas.
 - C. Genetically modified pig heart.
 - D. Minimally manipulated marrow for homologous use.
 - E. Collagen.
-

Question 19: All of the following are considered minimally manipulated, except:

- A. Bone shaped into screws and pins.
- B. Grinding bone to form bone chips and particles.
- C. Exposing bone to acid at elevated temperatures to demineralize bone and dissolve collagen to form a gel.
- D. Processing skin by mechanical meshing and cryopreservation.



- E. Processing amniotic membrane to preserve it and package it in sheets for use as an eye cover during intraocular repair.
-

Question 20: Which of the following is a homologous use of an HCT/P?

- A. Marrow-derived autologous stem cells used for improvement of heart function in patients with refractory angina and chronic heart failure.
 - B. Autologous marrow suspended in autologous plasma used for the treatment of osteoarthritis.
 - C. Stromal vascular fraction cells derived from adipose tissue used to treat tendonitis.
 - D. HPCs from cord blood used through intravenous infusion to treat cerebral palsy.
 - E. Pericardium used as a wound covering for dura mater defects.
-

Question 21: All of the following are exempted from the requirements in Title 21 CFR Part 1271, according to the Same Surgical Procedure Exception, except?

- A. Removal and implantation of the HCT/Ps into the same individual from whom they were removed.
 - B. Coronary artery bypass surgery involving autologous vein grafting or artery.
 - C. Autologous tissue subjected only to processes including rinsing, cleansing, sizing, and/or shaping.
 - D. Craniotomy or craniectomy with subsequent implantation of the bone flap.
 - E. Adipose tissue recovered by liposuction and processed by enzymatic digestion to isolate cellular components, commonly referred to as stromal vascular fraction.
-

Question 22: Xenografts are regulated as which of the following:

- A. Biological product.
- B. Drug.



- C. Medical device.
 - D. All of the above.
 - E. None of the above.
-

Question 23: Which of the following are considered to be xeno-grafts but not xenotransplantation products?

- A. Bovine heart valves.
 - B. Genetically engineered pig heart.
 - C. Baboon marrow.
 - D. Encapsulated bovine adrenal chromaffin cells.
 - E. None of the above.
-

Question 24: Which of the following is *not* considered an example of processing of an HCT/P?

- A. Testing for microorganisms.
 - B. Sterilization/steps to inactivate or remove adventitious agents.
 - C. Culturing.
 - D. Preservation for storage.
 - E. Recovery.
-

Question 25: Which of the following are considered steps in manufacturing of HCT/Ps?

- A. Recovery.
 - B. Storage.
 - C. Labeling
 - D. Distribution.
 - E. All of the above.
-

Question 26: Transplant programs must develop and comply with a written protocol for blood type determination and reporting that includes all the following, *except*:



- A. Candidate blood samples must be drawn on two separate occasions.
 - B. Candidate blood samples must have different collection times.
 - C. Candidate blood samples must be submitted as separate samples.
 - D. Verification of correct data entry into the national match system by a qualified health professional.
 - E. The transplant program must include a process to address conflicting or indeterminate primary blood type results in their written protocol.
-

Question 27: Which of the following must be true to be eligible for an intended ABO-incompatible heart?

- A. Be registered as status 2.
 - B. Have reported isohemagglutinin titers less than or equal to 1:16 for blood group A or B antigens to the Organ Procurement and Transplantation Network (OPTN) within the last 30 days.
 - C. Have reported isohemagglutinin titers less than or equal to 1:32 for blood group A or B antigens to the OPTN within the last 30 days.
 - D. Is registered before 20 years of age.
 - E. The candidate must have received treatments to reduce isohemagglutinin titers to 1:16 or less within 30 days of when blood sample was collected.
-

Question 28: Cold ischemic time is defined as which of the following?

- A. Time from subjecting cardiac tissue to cold rinse (or transport) solution at recovery to beginning of disinfection.
- B. Time interval from asystole to subjecting valvular tissue to perfusion solution.
- C. Time interval from asystole to subjecting tissue to disinfection solution.
- D. Time interval from asystole to subjecting vascular tissue to transport solution and wet ice temperatures at recovery.
- E. None of the above.



Question 29: Lyophilization is one method of tissue preservation and is best described as which of the following?

- A. Tissue dehydrated for storage by conversion of the water content of frozen tissue to a gaseous state under a vacuum that extracts moisture.
 - B. The removal of water from tissue using heat.
 - C. Freezing with the addition of, or in a solution containing, an additive that minimizes osmotic imbalances to limit cell damage caused by cell shrinkage and intracellular ice crystallization.
 - D. Drying process at high temperature and high pressure allowing conversion of liquid to gas without crossing any phase boundary.
 - E. None of the above.
-

Question 30: Which of the following tissue types does *not* require continuous temperature monitoring?

- A. Refrigerated skin tissue.
 - B. Frozen, cryopreserved cardiac tissue.
 - C. Lyophilized, dehydrated, desiccated musculoskeletal tissue.
 - D. HPCs stored in liquid nitrogen.
 - E. Tissue stored at room temperature within the temperature range specified in package insert by manufacturer.
-

Question 31: Tissue banks may support which of the following services?

- A. Obtaining authorization and/or informed consent.
 - B. Recovery/collection of tissue.
 - C. Processing of tissue.
 - D. Distribution and dispensing of tissue.
 - E. All of the above.
-

Question 32: Receivers of validated shipping containers are required to verify which of the following?



- A. Visual inspection for damage to container.
 - B. Received within the specified time frame indicated on the outside of the container.
 - C. Continuous measurement of the ambient and internal temperatures throughout the duration of shipment.
 - D. Opened within the specified time frame indicated on the outside of the container.
 - E. All of the above.
-

Question 33: The Joint Commission requires documentation of which of the following to be placed into the recipient's medical record?

- A. Donor tissue type.
 - B. Time of death for deceased donors.
 - C. Site of organ procurement.
 - D. Time of organ procurement.
 - E. All of the above.
-

Question 34: According to The Joint Commission standards, accredited health-care facilities must do which of the following?

- A. Confirm annually that tissue suppliers are registered with the FDA as tissue establishments and that they maintain a state license when required.
 - B. Ensure qualification information for each supplier is reviewed and approved every 10 years by the hospital tissue service.
 - C. A documented internal review or audit to ensure compliance must be performed every 10 years.
 - D. Tissue dispensing services records must be maintained for a minimum of 5 years after expiration of the tissue or, in the case of tissue with no expiration date, 5 years after dispensing.
 - E. None of the above.
-

Question 35: Which of the following organs have the highest risk of passenger lymphocyte syndrome (PLS) after transplantation?



- A. Intestine.
 - B. Liver.
 - C. Kidney.
 - D. Heart-Lung.
 - E. Pancreas.
-

Question 36: Which of the following is the most transplanted solid organ in the United States?

- A. Liver.
 - B. Kidney.
 - C. Heart.
 - D. Lung.
 - E. Intestine.
-

Question 37: Which of the following tissue allografts require HLA and/or ABO compatibility testing?

- A. Kidney.
 - B. Bone.
 - C. Skin.
 - D. Cornea
 - E. Dura mater.
-

Question 38: Which of the following solid organs have the highest survival rates?

- A. Heart.
 - B. Lung.
 - C. Intestine.
 - D. Liver.
 - E. Kidney.
-

Question 39: The following are all standard indications for irradiation of cellular blood products, except:



- A. Liver transplant.
 - B. Marrow transplant.
 - C. Congenital immunodeficiency syndromes.
 - D. Directed donations from first-degree relatives.
 - E. Granulocyte transfusions.
-

Question 40: Which of the following have been used to expand solid-organ shortages?

- A. Kidney paired donation.
 - B. Plasma exchange.
 - C. ABO-incompatible kidney transplants.
 - D. Xenografts.
 - E. All of the above.
-

Question 41: Which of the following makes a potential HPC donor *ineligible* under FDA requirements?

- A. Previous work in prostitution, ending 9 years ago.
 - B. Having a roommate with asymptomatic HCV infection who moved out a year and a half ago.
 - C. Receipt of smallpox vaccination 31 days ago, after which the scab fell off spontaneously.
 - D. Used IV drugs 10 years ago.
 - E. Receipt of a porcine artificial heart valve.
-

Question 42: Which of the following potential HPC donors is *eligible* under FDA requirements?

- A. Treated successfully for syphilis more than 1 year ago.
- B. Spouse had Parkinson disease and received a transplant of live monkey brain cells 7 years ago.
- C. Uncle was diagnosed with familial CJD 15 years ago.
- D. Had an undiagnosed illness around 2 months ago that a friend who is a physician suspects was West Nile virus.
- E. None of the above.



Question 43: Choose the correct statement about storage conditions and shelf life:

- A. Lyophilized fascia can be stored at room temperature for 10 years.
 - B. Bone can be stored at 1 to 10 C for 5 days.
 - C. Skin can be stored at 1 to 10 C for 3 weeks.
 - D. Frozen bone can be stored at -20 C for 12 months.
 - E. Frozen bone can be stored at -40 C for 10 years.
-

Question 44: Choose the correct statement about bone grafting:

- A. When selecting frozen bone grafts, matching the ABO and HLA type of the recipient with that of the donor is an important consideration.
 - B. Alloimmunization to RhD does not occur with the use of fresh bone grafts.
 - C. A greater inflammatory response and more rapid revascularization is seen with cortical bone vs cancellous bone.
 - D. Cortical bone grafts require more protection from mechanical stress than cancellous bone grafts.
 - E. Using frozen or lyophilized bone will produce faster results in graft revascularization and remodeling than autologous bone.
-

Question 45: Which of the statements about frozen or lyophilized bone grafts is true?

- A. The mechanical properties of the bone are unaffected by freezing and thawing.
- B. Without the need for thawing, the advantage of lyophilized bone over frozen bone is time savings because it can be implanted directly from the package.
- C. Calcium in the donor graft is necessary for successful incorporation of the graft and bone formation.
- D. Osteoblasts and osteoclasts in the donor graft are necessary for it to successfully incorporate into the defect.
- E. All of the above.



Question 46: Which of the statements about processing, storage, and distribution of tissue grafts is true?

- A. A hospital's logs will show a record of receipt of tissue products for transplanted products only.
 - B. When grafts are produced at one time from multiple donors using one set of instruments and supplies, it is referred to as a "lot."
 - C. Pooling of tissues from multiple donors during processing is acceptable if all donors involved meet all eligibility criteria.
 - D. Ethylene oxide or gamma irradiation can be used to sterilize tissues, such as tendons and fascia.
 - E. Inspection of tissue products by hospital staff is required only at the time of receipt.
-

Question 47: Which statement about transfusion support in HCT/P transplantation is true?

- A. All CMV-seronegative recipients of CMV-seropositive organ transplants receive blood components that are CMV-reduced-risk (CMV seronegative or leukocyte reduced) when receiving transfusion.
 - B. To avoid HLA alloimmunization in transplant recipients, blood components transfused both before and after transplantation should be leukocyte reduced.
 - C. For a recipient of an ABO-incompatible kidney, plasma products used should match the ABO group of the recipient.
 - D. Because of the significant risk of transfusion-associated graft-vs-host disease (TA-GVHD), irradiated blood components are routinely used for organ transplant recipients.
 - E. In TA-GVHD, donor lymphocytes attack host cells, including marrow, similar to GVHD in stem cell transplantation.
-

Question 48: Which statement about the role of the ABO blood group in organ transplantation is true?

- A. ABO incompatibility has little effect in heart transplantation in adults.



- B. In ABO-incompatible kidney transplantation, there are currently no effective options for recipients with high antibody titers to avoid hyperacute rejection.
 - C. The formation of ABO antibodies occurs after transplantation in response to the antigens present on the transplanted organ.
 - D. Transplant rejection due to ABO incompatibility generally begins to appear 1 to 2 weeks after transplantation.
 - E. ABO-incompatible heart transplants have been successful in infants when plasma exchange is used.
-

Question 49: A label on a tissue product will contain what type of information?

- A. Disinfection or sterilization procedure.
 - B. Name and address of the tissue bank(s) responsible for determining donor eligibility, processing, and distribution.
 - C. Potential residues of processing agents/solutions.
 - D. Quantity of tissue.
 - E. All of the above.
-

Question 50: Which of the following organizations issues requirements related to tissue handling that do *not* address activities at hospitals?

- A. Eye Bank Association of America (EBAA).
- B. American Association of Tissue Banks (AATB).
- C. The Joint Commission.
- D. AABB.
- E. FDA.

ANSWERS

Question 1: A. Human transmissible spongiform encephalopathy, including Creutzfeldt-Jakob disease.



Explanation:

- Title 21 CFR 1271 specifically lists HIV-1/2, hepatitis B virus, hepatitis C virus, human TSE (including CJD), and *Treponema pallidum* as relevant communicable diseases or disease agents for all human cells and tissues.
- In addition to the above listed diseases, viable leukocyte-rich cells and tissues, such as hematopoietic stem/progenitor cells (HPCs) and semen, HTLV-I/II are listed, while for reproductive cells or tissues, *Chlamydia trachomatis* and *Neisseria gonorrhoea* are listed.
- Although not specifically listed, other diseases may be considered relevant if any of the following apply:
 - There may be a risk of transmission.
 - The disease could be fatal or life-threatening.
 - Screening measures or licensed, approved, or cleared tests have been developed. West Nile virus is an example of a disease that is still determined to be relevant even though it is not specifically listed.
- FDA has determined which infectious diseases are the highest and most relevant risks, and it refers to them as RCDADs. The FDA identifies RCDADs in two ways:
 - Listing them specifically in the CFR.
 - Publishing a guidance document to communicate any changes or additions to RCDADs.



Question 2: E. All of the above.

Explanation:

- The regulations under Title 21 CFR part 1271 set out requirements for determining donor eligibility, including donor screening and testing, for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps).
- The FDA requires an assessment of screening and testing of donors of any allogeneic product defined as an HCT/P by the FDA.
- The required extent of the donor eligibility process may vary between autologous and allogeneic donors. For autologous



donors, there may be differences in state requirements, as well as between accrediting bodies and individual institutions.

- All donor evaluation determination processes for both living and cadaveric donors must be addressed in standard operating procedures (SOPs) documented in paper or electronic records.
 - When the donor screening and testing and donor evaluation process are completed as defined by the FDA, with no evidence or identified risk of RCDADs, xenotransplant risk, or reactive/positive RCDAD donor test results, as described above, the donor is then deemed “eligible.”
-

Question 3: E. Human TSE, including CJD.

Explanation:

- Donor screening is accomplished by reviewing the donor’s relevant medical records for risk factors for, or clinical evidence of, RCDADs, including communicable-disease risk factors associated with xenotransplantation, when relevant.
- Screening and testing methods for donor evaluation exist for the majority of infectious disease entities (RCDADs and non-RCDADs). However, for a handful of disease entities, FDA-licensed, -approved, or -cleared donor screening tests do not exist. These include TSE (including CJD), vaccinia infection, and sepsis, which have only screening options such as knowledge of the donor’s medical, family/travel/immunization history, or clinical evidence concerning/relevant for the disease. See table below.

Infectious Disease Entity	Donor Evaluation Type	
	Screening	Testing
<i>Relevant Communicable Disease Agents and Diseases (RCDADs)</i>		
All HCT/Ps		
HIV-1/2	X	X
HBV	X	X
HCV	X	X

(continued)



Infectious Disease Entity	Donor Evaluation Type	
	Screening	Testing
TSE including CJD	X	
<i>Treponema pallidum</i> (syphilis)	X	X
Viable, leukocyte-rich HCT/Ps only		
HTLV-I/II	X	X
CMV		X
Reproductive HCT/Ps only		
<i>Chlamydia trachomatis</i>	X	X
<i>Neisseria gonorrhoea</i>	X	X
<i>Non-RCDADs</i>		
West Nile virus	X	X
Sepsis	X	
Vaccinia	X	

Adapted from Areman E, Loper K, eds. Cellular therapy: Principles, methods, and regulations. 2nd ed. Bethesda, MD; AABB Press, 2016.

- Although CMV is not a relevant communicable disease agent or disease, establishments are required to test donors of viable, leukocyte-rich cells or tissues for CMV. A donor who tests positive or reactive for CMV (total antibody) is not necessarily ineligible to donate HCT/Ps. Screening as part of donor evaluation for CMV is not commonplace and is not usually present on donor history questionnaires.



Question 4: E. West Nile virus (WNV).

Explanation:

- Per AATB standards WNV testing is required for living donors but not for deceased donors.



- Establishments located within the United States are required to test all living donors, except autologous donors, when recovery, collection, or acquisition occurs from June 1st through October 31st every year. However, tissue establishments located outside the US are required to perform testing for WNV year round.
-

Question 5: A. *Treponema pallidum* (syphilis).

Explanation:

- An exception for donor testing and eligibility exists for syphilis. A donor who tests positive or reactive on a nontreponemal screening test and negative or nonreactive on a specific treponemal confirmatory test may be determined to be eligible. However, if the donor tests positive or reactive on either a specific treponemal confirmatory test for syphilis or on a treponemal screening test, then the donor is ineligible.
 - Nontreponemal assays, such as the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test, detect nonspecific antibodies to the cardiolipin antigen present in host tissues as well as in treponemes. These assays are useful in monitoring the progression of disease and response to therapy but are nonspecific. Treponemal assays, such as the fluorescent treponemal antibody with absorption test (FTA-ABS), use specific treponemal antigens and detect specific antibodies to these antigens. Unlike nontreponemal assays, results remain positive or reactive throughout an individual's life, even after successful treatment for syphilis.
-

Question 6: D. Veterinary use.

Explanation:

- American Association of Tissue Banks (AATB) standards specifically state that human tissue for transplantation shall not be offered or distributed for veterinary use except if such use is granted in a document of gift/authorization or in a record of informed consent. This is regardless of eligibility of the donor.



- There are limited uses of HCT/P from ineligible donors that are not prohibited including allogeneic use in a first-degree or second-degree blood relative, when HCT/P consists of reproductive cells or tissue from a directed reproductive donor, or there is a documented urgent medical need. In these instances, one must prominently label the HCT/P with a biohazard legend and with the statement "WARNING: Advise patient of communicable disease risks," and, in the case of reactive test results, "WARNING: Reactive test results for (name of disease agent or disease)." The establishment that manufactured the HCT/P from an ineligible donor is required to document that the physician using the HCT/P was notified of the results of testing and screening. HCT/Ps from ineligible donors may be used for nonclinical purposes as long as they are labeled with "For Nonclinical Use Only" and the biohazard legend.
- Directed reproductive donor is defined as a donor of reproductive cells or tissue (including semen, oocytes, and embryos to which the donor contributed the spermatozoa or oocyte) to a specific recipient, and who knows and is known by the recipient before donation.
- The FDA does not prohibit use of a product from an ineligible donor but does require documentation that the transplant center physician has been notified of the results of the donor screening and testing and documentation of informed consent of the donor and recipient.



Question 7: E. None of the above.

Explanation:

- A complete donor screening process for the living donor (including donor history questionnaire, physical examination, and review of any new medical records) must have been completed within 6 months prior to use to perform an abbreviated donor screening procedure. This abbreviated procedure must determine and document any changes in the donor's medical history since the previous donation that would make the donor ineligible, such as risk factors for, and clinical evidence of, RCDADs as well as communicable disease risks associated with xenotransplantation.



Question 8: A. Donor 1 month of age or younger.

Explanation:

- If a donor is 1 month of age or younger, a specimen from the birth mother is used instead of the donor. If this specimen is unavailable, the donor is ineligible. The specimen for testing from the birth mother must be collected within 7 days of donation by the infant, unless the donation consists of hematopoietic progenitor cells (HPCs) or marrow. The donor's only risk is what the donor was exposed to through the birth mother. Another concern is the ability to obtain enough sample volume from a newborn. It may also not be feasible to get enough sample to perform test validations in this patient population.
- Transfusion or infusion might dilute plasma, making test results potentially unreliable. A specimen taken before the transfusion or infusion and up to 7 days before recovery of cells or tissue is appropriate. If a pretransfusion/infusion specimen is not available, an appropriate algorithm to determine whether plasma dilution is or is not sufficient to affect test results can be applied. Example maximum volumes of transfused/infused products that can be given at certain points before sample collection are provided in guidance documents relating to donor eligibility.
- Plasma dilution must be suspected in adult donors if the donor has received greater than 2 L of blood or colloids within 48 hours immediately preceding the collection of a pre-mortem specimen for testing; or within 48 hours immediately preceding death, if the specimen for testing is collected post-mortem, whichever occurred earlier.
- In the case of crystalloids if the donor received more than 2 L of crystalloids within either 1 hour immediately preceding the collection of a pre-mortem specimen for testing; or within 1 hour immediately preceding death, if the specimen for testing is collected post-mortem, whichever occurred earlier.



Question 9: E. All of the above.

**Explanation:**

- In general, donor specimens for testing must be collected at the same time as when cells or tissue are recovered from the donor, or within 7 days before or after the recovery of cells and tissue.
- An exception is made for HPCs (peripheral blood or marrow) and oocytes, in which the donor specimen may be collected up to 30 days before donation. The rationale behind this is that the recipient may begin myeloablative chemotherapy more than 7 days before the transplant, hence donor eligibility for the allogeneic donor may need to be determined before this time. In the case of donation of oocytes that do not undergo a period of cryopreservation before implantation, an oocyte donor might need to be qualified before the 7 days before donation due to the time necessary for receiving hormonal stimulation.

**Question 10: E. Statement of urgent medical need.****Explanation:**

- Urgent medical need is defined as when there is no comparable HCT/P available and the recipient is likely to suffer death or serious morbidity without the HCT/P. A statement of urgent medical need is required only when donor screening and/or testing was not performed according to the FDA-defined requirements before release of the product or when donor testing and/or screening of the donor indicate that the donor may be at increased risk for transmission of a communicable disease agent to the recipient.

**Question 11: E. All of the above.****Explanation:**

- Despite clinical screening and testing of donors for transmissible disease, unexpected transmission of disease from the donor to recipient remains an inherent risk of organ transplantation.
- Expected donor-derived disease (DDD) (eg, CMV) is frequent and thus posttransplant management is often needed. On the other



hand, unexpected DDD transmissions occur in less than 1% of recipients. Infectious disease agents are most involved, but malignancies and, interestingly in rare instances, metabolic or allergic diseases may also be transmitted. The metabolic and allergic types that have been reported are most commonly peanut allergy, followed by amyloidosis, hemochromatosis, and ornithine-transcarbamylase deficiency.

- Although rare, DDD can be associated with significant morbidity and mortality. The Disease Transmission Advisory Committee (DTAC) was created by the Organ Procurement and Transplantation Network (OPTN) to review and classify reports of potential disease transmission and use this information to inform national policy and improve patient safety. Reports of potential donor disease transmission events are required by OPTN policy.
-

Question 12: C. Persons with hemophilia or other related clotting disorders who have received human-derived clotting factor concentrates in the preceding 5 years.

Explanation:

- Donor deferrals are based on FDA guidance that determine eligibility. Overall, there are many similarities between blood donor and HCT/P donor eligibility criteria. These include:
 - 12-month deferral for potential donors who:
 - have been in juvenile detention, lockup, jail, or prison for 72 hours or more.
 - had sexual contact or lived with someone with hepatitis.
 - 120-day deferral for potential donors who:
 - have had a medical diagnosis or suspicion of WNV infection should defer for 120 days following diagnosis or onset of illness, whichever is later.
 - have tested positive or reactive for WNV infection using an FDA-licensed or investigational WNV NAT donor screening test in the preceding 120 days.
 - 8-week deferral for potential donors who had contact with someone who was vaccinated for smallpox.
 - Permanent deferral for potential donors who:
 - have been diagnosed with or are suspected of having variant CJD (vCJD) or other forms of CJD.



- have blood relatives with CJD.
 - recipients of dura mater graft.
- Differences between blood donor and HCT/P donor eligibility criteria, particular to HCT/P donors include:
 - 5-year deferral for potential donors who:
 - are MSM.
 - have a history of intravenous (IV) drug use.
 - have hemophilia or other related clotting disorders who have received human-derived clotting factor concentrates.
 - have engaged in sex in exchange for money or drugs.
 - 12-month deferral for potential donors who:
 - have received allogeneic transfusion/come into contact with someone else's blood.
 - have undergone tattooing, ear piercing, or body piercing in which sterile procedures were not used.
 - have been treated for or had syphilis.
 - are reproductive HCT/P donors and have been treated for or had *Chlamydia trachomatis* or *Neisseria gonorrhoea* infection.
 - Children born to mothers with or at risk for HIV infection:
 - if 18 months of age or younger.
 - if breast-fed within the preceding 12 months.
 - Permanent deferral for potential donors who:
 - have had a diagnosis of clinical, symptomatic viral hepatitis after their 11th birthday, unless evidence from the time of illness documents that the hepatitis was identified as being caused by hepatitis A virus, Epstein-Barr virus (EBV), or cytomegalovirus (CMV).
 - are deceased and have a documented medical diagnosis of sepsis or have documented clinical evidence consistent with a diagnosis of sepsis that is not explained by other clinical conditions at the time of death.
 - have been diagnosed with dementia or any degenerative or demyelinating disease of the central nervous system or other neurologic disease of unknown etiology.
 - are xenotransplantation product recipients or intimate contacts of a xenotransplantation product recipient.
 - have had travel/residence restrictions that place them at increased risk of exposure to vCJD.
- The FDA guidance document from 2020 modified the deferral period from 12 months to 3 months for blood donation for MSM,



based on the FDA's current thinking on deferral recommendations for individuals with increased risk for transmitting HIV infection. In addition to the deferrals noted above for MSM, the FDA determined that the recommended deferrals for commercial sex work and IV drug use be changed from indefinite deferrals to 3-month deferrals. For similar reasons, the 12-month deferral for a recent tattoo or piercing and receipt of allogeneic blood products/contact with someone else's blood was reduced to 3 months.

- Finally, previous deferral recommendations for blood donors related to geographic risk for vCJD (eg, time spent in the United Kingdom from 1980 to 1996) were removed by the final FDA guidance dated May 2022. However, vCJD travel-related restrictions still apply to HCT/P donors.



Question 13: E. All of the above.

Explanation:

- AATB standards require that the tissue bank ensure all of the choices listed as well as ensure that the laboratory follows manufacturers' instructions for these tests and establishes a policy to collect and preserve serum, plasma, or hematopoietic tissue samples from donors for an appropriate duration after the recovery, collection, or acquisition date.



Question 14: E. All of the above.

Explanation:

- A semen donor can be a directed donor or an anonymous donor and is intended for use in artificial insemination and assisted reproductive technology procedures, such as in-vitro fertilization.
- According to AATB standards, semen donors should be younger than 40 years of age to minimize the risk of genetic anomalies except with the written agreement of the user physician. A physical examination must be performed on all anonymous and directed semen and oocyte donors. A repeat physical examination is also performed on anonymous semen donors at least every 6 months while the donor is actively collecting samples.



- As a leukocyte-rich cell, semen testing for HTLV-I/II and CMV is required.
 - As reproductive cells, testing for *Neisseria gonorrhoea* and *Chlamydia trachomatis* is required.
 - Anonymous semen donors require a new specimen to be collected and repeat testing at least 6 months after the donation. The donated semen must be quarantined until the retesting is complete and the donor is determined to be eligible.
-

Question 15: E. All of the above.

Explanation:

- Screening a potential cell or tissue donor requires the review of relevant medical records for the purpose of evaluating for risk factors for, and clinical evidence of, RCDADs. Relevant medical records include a current donor medical history interview, a current report of the physical assessment of a cadaveric donor, or the physical examination of a living donor.
 - If available, laboratory test results, medical records, coroner and autopsy reports, and records or other information including police reports also meet this definition.
-

Question 16: B. Manipulated such that biological or relevant functional characteristics of the cells or tissues are altered.

Explanation:

- HCT/Ps are defined in Title 21 CFR 1271 as articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient.
- The federal Food, Drug, and Cosmetic Act and the PHS Act provide the legal framework for FDA regulation of biological products, including HCT/Ps.
- Section 361 of the PHS Act and Title 21 CFR Part 1271, focus on preventing the introduction, transmission, or spread of communicable disease to the HCT/P recipient through donor eligibility and current good tissue practice (cGTP) requirements.



- Other HCT/Ps, considered as higher-risk products, are regulated as biological products, drugs, or medical devices, and evidence of their safety and effectiveness is required before they are marketed (see table below).

Criteria Determining the HCT/P Regulatory Path	
PHS Act Section 361— Title 21 CFR Part 1271	PHS Act Section 351
<p>Regulated solely under Section 361 of the PHS Act and Title 21 CFR Part 1271, HCT/Ps must meet all four criteria:</p> <ol style="list-style-type: none">1. Minimally manipulated.2. Intended for homologous use only.3. Not combined with a device or drug, except for sterilizing, preserving, or storage agents that do not raise clinical safety concerns.4. Not have a systemic effect or be dependent on the metabolic activity of living cells for its primary function, unless the HCT/P is for:<ul style="list-style-type: none">• Autologous use.• Allogeneic use in a first-degree or second-degree blood relative.• Reproductive use.	<p>Sections 351 and 361 of the PHS Act and premarket approval authorities apply if any of the following are applicable:</p> <ol style="list-style-type: none">1. Manipulated such that biological or relevant functional characteristics of the cells or tissues are altered.2. Genetically modified.3. Ex vivo expanded.4. Nonhomologous use.5. Combined with a drug, device, or biologic that may raise clinical safety concerns.6. Active systemically or dependent on the metabolic activity of the living cells for their primary function, unless minimally manipulated for:<ul style="list-style-type: none">• Autologous use.• Used in a first-degree or second-degree blood relative.• Reproductive use.

Adapted from Areman E, Loper K, eds. Cellular therapy: Principles, methods, and regulations. 2nd ed. Bethesda, MD; AABB Press, 2016.



Question 17: A. For structural tissue, processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement.

Explanation:

- HCT/Ps that function as a barrier or conduit, or connect, cover, or cushion are generally considered structural tissues.



- HCT/Ps that serve metabolic or other biochemical roles such as hematopoietic, immune, and endocrine functions, are generally considered cells/nonstructural tissues.
- Title 21 CFR Part 1271 provides two definitions of minimal manipulation, one that applies to structural tissue and one that applies to cells or nonstructural tissues. For structural tissue, minimal manipulation means that the processing of the HCT/P does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement. For cells or nonstructural tissues, minimal manipulation means that the processing of the HCT/P does not alter the relevant biological characteristics of cells or tissues.
- Original relevant characteristics of structural tissues generally include the properties of that tissue in the donor that contribute to the tissue's function(s). Similarly, relevant biological characteristics of cells or nonstructural tissues generally include the properties of the cells or nonstructural tissues in the donor that contribute to the cells or tissue's function(s). Processing that alters the original characteristics of the HCT/P, raises increased safety and effectiveness concerns for the HCT/P because there would be less basis on which to predict the product's function after transplantation. Thus, the determination of whether an HCT/P is minimally manipulated is based on the effect of manufacturing on the original relevant characteristics of the HCT/P as the HCT/P exists in the donor, and not based on the intended use of the HCT/P in the recipient.
- HCT/Ps are considered either structural tissues or cells/nonstructural tissue, based on the characteristics of the tissue in the donor. This distinction, which was first described in the 1997 proposed approach for the regulation of cell and tissue-based products, is reflected in the definitions of minimal manipulation for structural tissue and cells/nonstructural tissue. Structural HCT/Ps generally raise different safety and efficacy concerns than do cells or non-structural tissues.

**Question 18: A. Semen.**



Explanation:

- HCT/Ps are considered human cells or tissue intended for implantation, transplantation, infusion, or transfer into a human recipient.
- Examples of HCT/Ps include, but are not limited to, bone, skin, dura mater, cardiac valves, cornea, HPCs from peripheral and cord blood, and semen or other reproductive tissue.
- Vascularized organs, as opposed to those listed above are not considered HCT/Ps. Vascularized organs and minimally manipulated marrow for homologous use are regulated by the Health Resources and Services Administration (HRSA), a division of the US Department of Health and Human Services. HRSA issues contracts to provide national tissue and organ transplantation services. NMDP is issued a contract to manage marrow and cord blood transplantation and UNOS operates OPTN for solid-organ transplantation.
- Secreted or extracted human products, such as milk, collagen, and cell factors are not considered HCT/Ps, except for semen.
- Xenotransplantation products are also not included in the definition.



Question 19: C. Exposing bone to acid at elevated temperatures to demineralize bone and dissolve collagen to form a gel.

Explanation:

- HCT/Ps in this scenario are generally considered more than minimally manipulated because the processing (demineralization with acid) alters the bone's original relevant characteristics relating to its utility to support the body and protect internal structures (now in gel form to be used for adhesive purposes). Other examples include cell culture and ex-vivo expansion gene modification. Using hematopoietic stem/progenitor cells (HPCs) to produce terminally differentiated cells by culturing the cells under specific conditions would be another example. These HPCs would generally be considered more than minimally manipulated because the processing alters the cells' relevant biological characteristics of multipotency and capacity for self-renewal.



- Original relevant characteristics of bone relating to its utility to support the body and protect internal structures include strength, and resistance to compression. Milling, grinding, and other methods for shaping and sizing bone may generally be considered minimal manipulation when they do not alter bone's original relevant characteristics relating to its utility to support the body and protect internal structures.
- The original relevant characteristics of skin relating to its utility to serve as a protective covering generally include its large surface area, keratinized, water-resistant epithelial layer (epidermis), and dense, strong, and flexible connective tissue layer (dermis). The HCT/P generally is considered minimally manipulated because the processing does not alter the original relevant characteristics of the skin relating to its utility as a protective covering.
- Original relevant characteristics of amniotic membrane as related to its utility to serve as a barrier generally include its physical integrity, tensile strength, and elasticity. Thus, the original relevant characteristics of its use as a barrier are not being altered in the example of covering the eye during surgery; amniotic membrane serves as a barrier for movement of nutrients between external and in-utero environment and protecting the fetus in utero.



Question 20: E. Pericardium used as a wound covering for dura mater defects.

Explanation:

- Homologous use is defined as an HCT/P-based product that is used for a normal function that is analogous to that of the cells or tissues being repaired, replaced, reconstructed, or supplemented. These may be recipient cells or tissues that are identical to the donor and perform the same basic functions in the recipient as the donor, or not identical as long as they also perform similar functions. The HCT/P product may perform the same basic function even when it is not used in the same anatomic location where it existed in the donor.
- In the above example, the use of pericardium as a wound covering is considered homologous use because the pericardium is



intended to serve as a covering in the recipient, which is one of the basic functions it performs in the donor.

- The determination of homologous use is not always straightforward, however when treatment involves an unproven clinical use that involves a new function, it is likely to be a nonhomologous use. It is important to also note that the distinction between homologous and nonhomologous use also applies to autologous use of HCT/Ps.
-

Question 21: E. Adipose tissue recovered by liposuction and processed by enzymatic digestion to isolate cellular components, commonly referred to as stromal vascular fraction.

Explanation:

- In addition to the exceptions to Title 21 CFR Part 1271 that include using HCT/Ps for nonclinical scientific or educational purposes, there also exists the “same surgical procedure exception.” To fulfill this requirement, establishments must perform all the following:
 - Remove and implant HCT/Ps into the same individual from whom they were removed (autologous use).
 - Use HCT/Ps within the same surgical procedure.
 - Maintain HCT/Ps in their original form.
- Although the same surgical procedure usually means that it occurs on the same day and consists of one procedure, removal and implantation may be a few days apart as is the case for auto cranial flaps (ACF). A facility that removes autologous tissue that is intended to be shipped to another facility for implantation is not exempt except in certain cases such as ACF and parathyroid tissue.
- Examples of processing steps that will allow HCT/Ps to remain in their original form are rinsing, cleansing, sizing, and shaping. This contrasts with centrifugation or filtration, for cell isolation/expansion/activation, or enzymatic digestion.
- Establishments are not required to comply with the requirements of Title 21 CFR Part 1271 if they are the same establishment that removes HCT/Ps from an individual and implants said HCT/Ps into the same individual during the same surgical procedure.

**Question 22: C. Medical device.****Explanation:**

- Xenografts are regulated as medical devices.
 - This contrasts with xenotransplantation products, which can be considered a biological product, drug, or medical device. There is a subtle difference between the two; xenotransplantation is any procedure that involves the transplantation, implantation, or infusion into a human recipient of either:
 - Live cells, tissues, or organs from a nonhuman animal source.
 - Human body fluids, cells, tissues, or organs that have had ex-vivo contact with live nonhuman animal cells, tissues, or organs.
 - Xenografts are highly processed and consist of nonliving cells, tissues, or organs from nonhuman animals, such as bovine bone products, porcine insulin, and porcine heart valves, and are not considered xenotransplantation products.
-

Question 23: A. Bovine heart valves.**Explanation:**

- See answer #22 for further explanation. Answer choices B-D all consist of nonhuman living cells.
 - Porcine and bovine valve tissues are chemically treated with low concentrations of glutaraldehyde to reduce their antigenicity and to stabilize the tissue against proteolytic degradation following implantation. Both types of valve tissues are also treated with various other chemical agents to minimize their propensity to calcify over time.
-

Question 24: E. Recovery.**Explanation:**

- Processing is defined as any activity performed on an HCT/P, other than recovery, donor screening, donor testing, storage,



labeling, packaging, or distribution, such as testing for microorganisms, preparation, sterilization, steps to inactivate or remove adventitious agents, preservation for storage, and removal from storage. It also includes cutting, grinding, shaping, culturing, enzymatic digestion, and decellularization.

Question 25: E. All of the above.

Explanation:

- According to Title 21 CFR Part 1271, manufacturing includes processes such as recovery, processing, storage, labeling, packaging, or distribution of any human cell or tissue, and the screening and testing of HCT/Ps, but is not limited to these.
- An establishment that manufactures an HCT/P that is regulated solely under Section 361 of the PHS Act and 21 Title CFR Part 1271 is required to do all of the following:
 - Register with the FDA.
 - Submit a list of each HCT/P manufactured.
 - Comply with all applicable requirements contained in Title 21 CFR Part 1271.
 - Registration must be done within 5 days of starting operations and annually in December, except in cases such as changes of ownership or location or if there are changes to the establishment's name or contact information. In such cases, an amendment to the registration is required to be submitted within 30 days.



Question 26: D. Verification of correct data entry into the national match system by a qualified health professional.

Explanation:

- The Organ Procurement and Transplantation Network (OPTN) states that the candidate can only appear on a match run only after the transplant program completes verification and reporting of blood type by two different qualified health-care professionals rather than just a single person. All the other answer choices are part of the requirements.



Question 27: B. Have reported isoantibodies titers less than or equal to 1:16 for blood group A or B antigens to the OPTN within the last 30 days.

Explanation:

- Eligibility for intended ABO-incompatible heart includes all of the following:
 - Pediatric status 1A and 1B. Status 1A are patients who must remain in the hospital as in-patients and require high doses of intravenous drugs, require a ventricular assist device (VAD), are dependent on a ventilator, or have a life expectancy of a week or less without a transplant. Pediatric status 1B patients are generally not required to stay in the hospital as in-patients. They may require a VAD or low doses of intravenous medications. The other way to meet pediatric status 1B is to be less than 1 year old at initial registration with a diagnosis of hypertrophic or restrictive cardiomyopathy. Pediatric status 2 includes all other candidates for heart transplantation.
 - A candidate who is registered on the waiting list before turning 18 years of age remains eligible for pediatric status.
 - Transplant program reports updated isoantibodies titer information every 30 days.
 - The candidate meets one of the following:
 - Less than 1 year old at the time of the match run.
 - At least 1 year old at the time of the match run, and has titers less than or equal to 1:16, and has not received treatments that may have reduced isoantibodies titers to 1:16 or less within 30 days of when this blood sample was collected.
- Isoantibodies titers must be reported for recipients of an intended incompatible blood type heart, who were registered prior to 2 years old.



Question 28: A. Time from subjecting cardiac tissue to cold rinse (or transport) solution at recovery to beginning of disinfection.



Explanation:

- As defined by AATB Standards, perfusion time is the time interval from asystole to subjecting valvular tissue to perfusion solution. Total ischemic time is the time interval from asystole to subjecting tissue to disinfection solution. This is the sum of warm and cold ischemic time. Warm ischemic time is the time interval from asystole to subjecting vascular tissue to transport solution and wet ice temperatures at recovery.
-

Question 29: A. Tissue dehydrated for storage by conversion of the water content of frozen tissue to a gaseous state under a vacuum that extracts moisture.

Explanation:

- Dehydration is the removal of water from tissue. Desiccation is the process of extreme drying, which can be accomplished with a desiccant that is hygroscopic (attracts and holds water). Dehydration/desiccation methods include chemical (alcohol), critical/supercritical drying, air drying, or drying in a desiccator.
- Lyophilization or cryodesiccation, is a low-temperature dehydration process that involves freezing the product, lowering the pressure, and removing the ice by sublimation. This contrasts with dehydration by most conventional methods that evaporate water using heat.
- Cryopreservation is a method of freezing with an additive that prevents cell damage. Glycerol or dimethylsulfoxide (DMSO) are two examples of commonly used cryoprotectants.
- Although similar to lyophilization, supercritical drying is the removal of solvent from a sample without passing through the liquid-gas boundary, by immersion in a supercritical fluid and depressurization. The conversion from liquid to gas does not cross any phase boundary (distinction between gas and liquid ceases to apply), instead passing through the super critical region, where it is above the critical point. The critical point (or critical state) is the endpoint of a phase equilibrium in thermodynamics.



Question 30: C. Lyophilized, dehydrated, desiccated musculoskeletal tissue.

Explanation:

- Lyophilized, dehydrated, desiccated tissue is stored at ambient temperature (defined as the temperature of the immediate environment) and does not require temperature monitoring. For tissue stored at room temperature, continuous temperature monitoring is not required, unless a temperature range is specified by the manufacturer in their package insert.
- According to The Joint Commission, hospitals must maintain daily records to demonstrate that tissues requiring a controlled environment are stored at the required temperatures. The types of storage conditions include refrigerated, frozen/cryopreserved, and liquid-nitrogen storage. Hospitals must have a means to monitor the temperatures of refrigerators, freezers, and nitrogen tanks. Continuous temperature recording is not required but may be available with some continuous temperature monitoring systems. Storage equipment used to store tissues at a controlled temperature is required to have functional alarms and an emergency backup plan in cases where emergency transfer of tissue is needed.



Question 31: E. All of the above.

Explanation:

- Tissue banks provide the following services involving both living or deceased donors: obtaining authorization and/or informed consent, assessing donor eligibility, recovery, collection, acquisition, processing, storage, labeling, distribution, and dispensing of tissue.
- Tissue dispensing services and distribution intermediaries on the other hand perform more limited services. The former is responsible for the receipt, maintenance, and delivery of tissue to the end user; and the latter acquire and store tissue for further distribution only.



Question 32: A. Visual inspection for damage to container.

**Explanation:**

- As long as the shipping container is validated, the receiver needs to verify only that there is no damage to the container and that it was received *and* opened within the specified time frame posted on the outside of the shipping container. Validation protocols for shipping containers are well established.
 - Other means of verification such as review of data loggers that allow for continuous temperature monitoring are not required. Once the shipping container is returned to the shipping facility, recorded data, such as temperature tracings, can be downloaded and forwarded to the receiving facility. This is especially useful for quality investigations in cases of process deviations.
-

Question 33: A. Donor tissue type.

Explanation:

- The Joint Commission requires that documentation of the donor tissue type as well as its unique identifier be placed into the recipient's medical record. The unique donor identifier allows for traceability of the tissue from the donor to final disposition of each tissue. This allows one to determine the other choices if an investigation is needed in the event of recalls, look-backs, deviations, or adverse outcomes.
-

Question 34: A. Confirm annually that tissue suppliers are registered with the FDA as tissue establishments and that they maintain a state license when required.

Explanation:

- TJC standards require annual confirmation of tissue suppliers' FDA registration.
- Accrediting bodies generally require annual or biannual tissue supplier qualification and internal review/audits. Tissue dispensing services records must be maintained for a minimum of 10 years. As a general rule, records in tissue banking are required to be maintained for at least 10 years.

**Question 35: D. Heart-Lung.****Explanation:**

- PLS occurs when the recipient is immunocompromised and is incapable of mounting an effective immunologic reaction against lymphocytes from the immunocompetent graft. The risk of PLS is due to viable memory B cells from graft organs producing antibodies and is directly related to the amount of lymphoid tissue present in the organ allograft. The risk varies by transplanted organ and the following rates have been observed: 70% risk for heart-lung, 29% risk for liver, 9% risk for kidney. Along with anti-A (most reported and seen in minor ABO incompatibility), anti-D, anti-K, and anti-Fy^a have been reported.
-

Question 36: B. Kidney.**Explanation:**

- UNOS' online database, UNetSM, contains OPTN data regarding every organ donation and transplant event occurring in the US. In 2021, kidneys were the most transplanted solid organ (24,670), followed by liver (9236), heart (3817), and lung (2524).
-

Question 37: A. Kidney.**Explanation:**

- Although some tissue allografts may provoke an immune response resulting in an inflammatory response, these do not usually result in failure of the allograft, likely due to a lack of abundant residual cellular material in processed grafts. Therefore, it is not necessary to match most allografts to recipient's HLA type or ABO group in contrast to solid organs such as kidneys. HLA antibodies have been associated with corneal transplant rejection; however, it is still not common practice to perform HLA matching for this type of transplant.

**Question 38: E. Kidney.****Explanation:**

- Since the first successful transplant procedure, involving a living kidney donor in 1954, significant progress has been made in survival rates.
- Although survival rates differ by organ type, the majority of transplant recipients survive at least 1 year following the transplantation. Kidney recipients tend to have a higher 1-year survival rate than heart-lung or intestine recipients. Patient survival rates decrease over time, but the rate of decrease generally slows after the first year.
- OPTN data for graft survival rates for transplants performed between 2008 and 2015 show that primary kidney transplant had a survival rate of 94.7%.
- For some organs, graft failure, or loss of the transplant, would immediately jeopardize the patient's life without a repeat transplant. For organs such as heart, liver, and lung, graft survival is very similar to patient survival. For the loss of other organs, such as the kidney or pancreas, a person may survive a graft failure with other medical treatment.

**Question 39: A. Liver transplant.****Explanation:**

- Solid-organ transplantation is not considered a standard indication for irradiation by most authorities. If GVHD occurs, it is due to lymphocytes from the transplanted organ and not from transfusion-associated graft-vs-host disease (TA-GVHD). All the other options are considered high risk for TA-GVHD and thus irradiation is considered by most to be a requirement.

**Question 40: E. All of the above.**



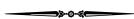
Explanation:

- According to UNOS data, there are over 100,000 people in need of a solid-organ transplant, however there are only around 20,000 organ donors available as of 2022. As a result, multiple efforts have been undertaken to expand access to organs.
- Kidney paired donation (KPD) is a living donation transplant option for candidates who have a living donor who is medically able but cannot donate a kidney to their intended candidate because they are incompatible with each other. In KPD, living donor kidneys are swapped so each recipient receives a compatible transplant. This allows two transplant candidates to receive organs and two donors to give organs even though the original recipient/donor pairs were incompatible. All donors and patients in the group of donor-patient pairs enter into a single agreement to donate and receive the kidneys, respectively, according to biological compatibility within the group.
- A living donor chain is another option for matching up altruistic donors with compatible recipients. A kidney chain begins with one altruistic donor who decides to donate a kidney to a stranger. The altruistic donor gives to a person waiting for a transplant, and that recipient's willing – but incompatible – donor gives to another person who is waiting for a kidney, and so on. Each living donor in this system gives to a stranger, and the chain of donors is kept going as long as possible.
- Plasma exchange allows for the reduction of isoantibodies titers in ABO-incompatible (ABOⁱ) heart-lung transplants to acceptable levels. It is also sometimes used in desensitization protocols in conducting ABOⁱ and/or positive crossmatch organ transplants, along with intravenous immunoglobulin and/or immunosuppressive medication.
- ABOⁱ kidney transplantation also allows for blood group B or O transplantation with a kidney from a blood group non-A1 (A2) donor. Antigenic expression of the A carbohydrate antigen N-acetylgalactosamine is reduced on the surfaces of group A2 donor kidneys, making these kidneys less antigenic to recipients. Given the longer waiting times of blood group B and O candidates, the allocation of A2 kidneys to select blood-group-B and -O candidates has been studied, demonstrating significantly reduced wait times with no significant increase in graft loss or death. The OPTN kidney allocation system implemented in 2014 permits allocation of group A2 and A2B kidneys to blood-group-



B candidates meeting center-specific criteria including prespecified, low anti-A titers.

- Although the potential benefits are considerable, the use of xenotransplantation raises concerns regarding the potential infection of recipients with both recognized and unrecognized infectious agents and the possible subsequent transmission to their close contacts and into the general human population. Of public health concern is the potential for cross-species infection by retroviruses, which may be latent and lead to disease years after infection. Moreover, new infectious agents may not be readily identifiable with current techniques. The FDA regulates most xenotransplantation products as biological products. The Center for Biologics Evaluation and Research (CBER) regulates biological products, including cellular therapies, under the authority of Section 351 of the PHS Act.



Question 41: C. Receipt of smallpox vaccination 31 days ago, after which the scab fell off spontaneously.

Explanation:

- See answers #12 (deferral criteria) and #22 (xenografts and xenotransplantation products).
- Previous deferral recommendations for blood donors related to geographic risk for vCJD (eg, time spent in the United Kingdom from 1980 to 1996) were removed by final FDA guidance in May 2022. As mentioned in answer #12, similar travel-related restrictions still apply to HCT/P donors.



Question 42: A. Treated successfully for syphilis more than 1 year ago.

Explanation:

- See explanation for questions 12 (deferral criteria) and 22 (xenografts and xenotransplantation products).
- Notwithstanding the exceptions for vCJD described in the previous explanation, having a blood relative with CJD is still a permanent deferral, as specified in answer #12 and in the 2022 FDA guidance.

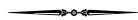


Question 43: B. Bone can be stored at 1-10 C for 5 days.

Explanation:

- See the table below for the shelf life of tissues under various storage conditions. For some conditions, a maximum shelf life has not been defined.

Tissue	Storage Condition	Shelf Life
Bone	-40 C	5 years
	-20 C	6 months
	1-10 C	5 days
	Lyophilized, room temperature	5 years
	Liquid nitrogen	
Tendon	-40 C	5 years
Fascia	-40 C	5 years
	Lyophilized, room temperature	5 years
Cartilage	-40 C	5 years
	1-10 C	5 days
	Liquid nitrogen	
Skin	1-10 C	14 days
	-40 C	
	Lyophilized, room temperature	
Cornea	2-6 C	14 days
HPCs	Liquid nitrogen (immersed or vapor phase)	
Semen	Liquid nitrogen (immersed or vapor phase)	
Heart valve	Liquid nitrogen (immersed or vapor phase)	
Dura mater	Lyophilized, room temperature	



Question 44: D. Cortical bone grafts require more protection from mechanical stress than cancellous bone grafts.



Explanation:

- HLA antigens and ABO antigens do not affect incorporation of the bone, and matching is not necessary.
- Immunization to the D, Fy^a, and Jk^b antigens has been reported with the implantation of fresh as well as frozen unprocessed bone. This is caused by red cells present in the bone. Lyophilized bone has not been reported to result in alloimmunization. Fresh or frozen unprocessed bone implanted in an Rh-negative female of childbearing potential should be Rh negative.
- Because of its greater surface area, cancellous bone induces a greater inflammatory response and more rapid vascularization. This means that there is a greater osteoblast response.
- With cortical bone, there is slower vascularization and a predominant osteoclastic response, resulting in an early phase of bone weakening compared with cancellous bone. The result is that while it is being incorporated, greater protection from mechanical stress is needed with cortical bone compared to cancellous bone.
- Autologous bone, usually harvested from the iliac crest, has more rapid revascularization and remodeling. Unfortunately, the disadvantages of this material include lack of adequate bone volume required to fill large defects and increased morbidity associated with the harvesting, such as greater time for surgical recovery, increased blood loss, more complex surgery, etc. As a result, allogeneic bone has become the preferred alternative in many cases.



Question 45: A. The mechanical properties of the bone are unaffected by freezing and thawing.

Explanation:

- Freezing bone does not alter its mechanical properties.
- Because lyophilized bone is very brittle and requires rehydration before use, preparation involves soaking the bone in a sterile solution for 2 to 4 hours before implantation. Frozen bone is thawed by soaking in a sterile solution, but much less time is required.
- Calcium is not essential for bone graft incorporation. Demineralized bone matrix is cortical bone that has been ground and had



the calcium removed with hydrochloric acid. This putty of matrix proteins can be used to fill defects and is preferred by periodontists and oral surgeons to augment bone growth around teeth or fill defects in facial bones. It is also used by spinal surgeons.

- Frozen or lyophilized bone does not contain viable cells, which are not involved in the incorporation process.
-

Question 46: D. Ethylene oxide or gamma irradiation can be used to sterilize tissues, such as tendons and fascia.

Explanation:

- The Joint Commission requires hospitals to keep records showing receipt of tissue, regardless of whether the tissue is transplanted or not.
 - A “lot” is defined as the tissue from one donor produced at one time using one set of instruments and supplies.
 - Pooling tissues from multiple donors during processing is prohibited by AATB standards, as it can lead to cross-contamination of tissue.
 - Either ethylene oxide or gamma irradiation may be used to sterilize tissues. Those sterilized by the former may contain toxic metabolites and have higher failure rates, whereas the latter has no adverse effect on the tissue, up to 25 kGy.
 - Packaging should be inspected not only at receipt, but also at the time of use. If the integrity of the packaging is compromised or labeling is not correct, the tissue should not be used, it should be quarantined, and the problem should be reported to the manufacturer. This requirement is shared by AABB, AATB, and Joint Commission standards.
-

Question 47: B. To avoid HLA alloimmunization in transplant recipients, blood components transfused both before and after transplantation should be leukocyte reduced.



Explanation:

- CMV infection in immunocompromised patients can cause severe infection, and attempts should be made to avoid infection in at-risk recipients such as CMV-seronegative recipients of CMV-seronegative organs. In a CMV-seronegative recipient receiving a CMV-seropositive organ, CMV infection will result from the organ, and some may not provide CMV-safe blood components in this setting.
- HLA alloimmunization can result in the development of antibodies that could lead to hyperacute rejection if formed before transplantation (organ never functions, as a result of immediate thrombosis of vasculature caused by complement activation and endothelial injury) or antibody-mediated rejection if formed after (organ functions initially but after some time—days to weeks to months—demonstrates a decline in function as antibody titers rise and damage the graft). Leukocyte-reduced blood components should be given to patients to help prevent alloimmunization.
- In ABO-incompatible solid-organ transplantation, there is usually major ABO incompatibility between the donor and the recipient (eg, a group A kidney for a group O recipient). The goal in these transplantations is to reduce the antibody titers of antibodies directed toward the donor. Providing recipient-type plasma products and platelets would infuse donor antibodies. These patients should be given plasma products that are compatible with both the recipient and the donor.
- TA-GVHD is an invariably fatal disease similar to GVHD seen in stem cell transplantation. It results from the infusion of viable lymphocytes into a recipient who has compromised cell-mediated immunity such that he or she cannot reject and destroy the cells. It is characterized by diarrhea, hepatitis, and jaundice. In addition, it is characterized by marrow hypoplasia as the infused lymphocytes also attack the marrow, something that does not occur in GVHD in stem cell transplantation, where the marrow is donor derived. Four cases of TA-GVHD have been reported in transplant recipients. Many more cases of transplant-associated GVHD, where the lymphocytes are derived from the organ, have been reported. Irradiated blood components are not routinely provided for transplant recipients because of the extreme rarity of TA-GVHD. (See also answer #39.)



Question 48: E. ABO-incompatible heart transplants have been successful in infants when plasma exchange is used.

Explanation:

- ABO antigens are expressed on the vascular endothelium of organs. Antibodies to ABO antigens are naturally occurring, present in people without previous blood exposure. The result is that ABO compatibility is required between donor and recipient or else the preformed antibodies will result in endothelial damage and organ ischemia as soon as blood flow is restored. This is called hyperacute rejection.
- In heart transplantation, ABO compatibility is almost universal except in the context of pediatric transplantation. To resolve difficulties in recipient and donor chest size, ABO-incompatible transplantations have been performed in infants. Plasma exchange before, during, and after transplantation has been used to decrease ABO antibody titers to prevent hyperacute rejection. ABO-incompatible heart transplantation is an ASFA Category I indication for plasma exchange in desensitization.
- ABO-incompatible renal transplants have been performed by two methods. In the first, a kidney from a living donor with a weak subgroup (usually group A2) is transplanted in a group O or group B individual. Enhanced immunosuppression and, possibly, splenectomy are performed. The second method involves determining the titer of donor-specific antibody. In individuals with low titers, enhanced immunosuppression and intravenous immunoglobulin are used to suppress antibody levels further. In patients with higher titers, plasma exchange is added to the enhanced immunosuppression to reduce antibody levels to the point that hyperacute rejection will not occur. ABO-incompatible renal transplantation involving a living donor is a Category I indication for plasma exchange conditioning according to ASFA.



Question 49: E. All of the above.

Explanation:

- All of the items listed are essential elements of the package label.



- Items on the label include the following:
 - Descriptive name of the tissue.
 - Name and address of the tissue bank(s) responsible for determining donor eligibility, processing, and distribution.
 - Expiration date.
 - Quantity of tissue.
 - Storage conditions.
 - Disinfection or sterilization procedure.
 - Preservative.
 - Potential residues of processing agents/solutions.
 - Reference to the package insert.
-

Question 50: A. Eye Bank Association of America (EBAA).

Explanation:

- EBAA standards cover all aspects of tissue banking except those associated with the institutions that dispense the tissue (hospitals).
- AATB's *Standards for Tissue Banking* provides a comprehensive foundation for guiding tissue banking activities, including the organizational requirements of a tissue bank, records management, procedure manuals, quality assurance, consent, donor screening, tissue recovery and collection, donor and tissue testing, processing and preservation, labeling, storage, release, distribution, and dispensing (hospitals).
- Both New York State and the State of Florida license and inspect tissue banks that provide tissue to facilities within their state. They inspect such facilities even if they are not located within their respective states. California, Georgia, and Maryland license facilities that procure and process tissue that may be used within their respective states, but they do not have inspection programs for these facilities.
- AABB's *Standards for Blood Banks and Transfusion Services* applies standards for blood components to tissues, including standards on sterility, receipt and inspection, and traceability. AABB's *Standards for Cellular Therapy Services* covers not only the dispensing (hospital) but also the procurement and processing aspects of HPCs and other cellular-based therapies.



- The Joint Commission's standards focus on the dispensing institution and lack requirements concerning procurement. Standards require designated oversight and responsibility for tissues at an institution and identification of the positions responsible for compliance, accreditation, and regulatory requirements.
- The College of American Pathologists also focuses solely on dispensing institutions.
- The FDA regulates donor eligibility with regard to infectious disease as well as all other aspects of tissue manufacturing. The agency does not have requirements for hospitals who obtain tissue from outside suppliers. Also, a hospital that acquires tissue for use within the same facility does not need to register with the FDA. If, however, the hospital distributes tissue to another unaffiliated facility, it must register with the FDA.

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18

Laboratory Management

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Key Points from the *Technical Manual*

- The fields of transfusion medicine and biotherapies are highly regulated, involving multiple government agencies and accrediting organizations.
- The Centers for Medicare and Medicaid Services (CMS) regulates all US medical laboratories under the Clinical Laboratory Improvement Amendments (CLIA). CLIA regulations establish requirements for certification, including the use of adequate facilities, qualified personnel commensurate with the complexity of testing, and ongoing successful performance in proficiency testing by CMS-approved vendors.
- Space allocations, building utilities, ventilation, sanitation, trash, and hazardous substance disposal must support the organization's operations.
- The policies and procedures governing a facility's personnel should address adequate staffing levels; staff selection, orientation, training, and competency assessment; and any specific regulatory or accreditation requirements.

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QUESTIONS

Question 1: Delegating responsibility is a component of which principle of management?

- A. Planning.
 - B. Organizing.
 - C. Directing.
 - D. Controlling.
 - E. Prioritization.
-

Question 2: Select the answer choice that correctly pairs the management type with a corresponding responsibility?

- A. Operations management – staffing and recruitment.
 - B. Human resource management – departmental budgets.
 - C. Financial management – advertising.
 - D. Marketing management – test benchmarking.
 - E. None of the above.
-

Question 3: What does the acronym in SWOT analysis stand for?

- A. Strengths, Weaknesses, Opportunities, Threats.
 - B. Strategic planning, Workflow, Operations, Time management.
 - C. Six Sigma, Workload, Objective, Trial run.
 - D. Safety, Weaknesses, Outcome, Testing.
 - E. Standards, Workflow, Organization, Turnaround time.
-

Question 4: A laboratory is in the process of deciding whether to add ABO genotyping by molecular sequencing to its test menu. In developing a strategic business plan a market assessment is performed. Which of the following key strategies of market assessment consists, in part, of sales support, customer service, and communication?

- A. Production strategies.



- B. Service strategies.
 - C. Distribution strategies.
 - D. Marketing and sales strategies.
 - E. Pricing strategies.
-

Question 5: Which of the following is an example of a laboratory performance indicator?

- A. Employee satisfaction.
 - B. Patient satisfaction.
 - C. Test turnaround time.
 - D. Hospital revenue.
 - E. Hospital admissions.
-

Question 6: Which of the following items is an example of a fixed cost?

- A. Reagents.
 - B. Office supplies.
 - C. Processing labor.
 - D. Collection supplies (eg, tubes, needles).
 - E. Laboratory information system.
-

Question 7: Technical labor would be an example of which type of cost?

- A. Direct costs.
 - B. Indirect costs.
 - C. Fixed costs.
 - D. Variable costs.
 - E. Incremental costs.
-

Question 8: The cost of an item under ideal or normal circumstances is referred to as what type of cost?



- A. Average cost.
 - B. Marginal cost.
 - C. Actual cost.
 - D. Standard cost.
 - E. Step-fixed cost.
-

Question 9: On average, your blood bank performs 100 ABO typings per day by a solid-phase method. If the fixed cost of running ABO testing is \$400/day and the variable cost is \$3/test, what would be the cost to perform one ABO test (ie, unit cost) on an average day?

- A. \$2/test.
 - B. \$5/test.
 - C. \$7/test.
 - D. \$8/test.
 - E. \$10/test.
-

Question 10: Your blood bank is thinking about switching from solid phase testing to gel method for ABO typing. Per the vendor, the purchase of the instrument will cost \$40,000 but the variable cost per test will remain \$3/test. Assuming a charge of \$8/test, how many tests will have to be performed in order to reach the breakeven point after purchasing the new instrument?

- A. 2,000 tests.
 - B. 4,000 tests.
 - C. 6,000 tests.
 - D. 8,000 tests.
 - E. 10,000 tests.
-

Question 11: The manufacturer of the gel-based ABO typing method offers three options for acquiring the instrument: purchase, leasing, and reagent rental. Which of the following statements is true in regard to these options?

- A. Leasing can be considered a tax-deductible overhead expense.



- B. Reagent rental agreements allow for the option to buy at the end of the term.
 - C. Leasing requires funds from the capital budget.
 - D. Purchasing has the highest reagent cost/test.
 - E. Reagent rentals are more cost-effective for low-volume tests.
-

Question 12: Which of the following is an indicator of high employee retention?

- A. Low turnover rate with a high number of new hires/total full-time equivalents (FTE).
 - B. High agency staff.
 - C. Negative employee feedback.
 - D. High number of vacancies.
 - E. Decreased overtime hours/pay period.
-

Question 13: Which of the following is a potential consequence of staffing shortages?

- A. Decreased turnaround time.
 - B. Decreased overtime.
 - C. Increase in the number of tests offered.
 - D. Increased overtime.
 - E. Decreased errors.
-

Question 14: A hospital laboratory has decided to start a blood bank, which will perform serologic testing (eg, serotyping, indirect antibody screens, direct antiglobulin test) in addition to storing and releasing blood products. The laboratory has been approved to conduct serologic testing but has yet to be inspected to ensure compliance with Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). What type of CLIA certificate does the blood bank currently have?

- A. Certificate for provider-performed microscopy procedures.
- B. Certificate of compliance.
- C. Certificate of registration.



- D. Certificate of accreditation.
 - E. Certificate of waiver.
-

Question 15: What is the primary purpose of laboratory accreditation?

- A. To increase laboratory revenue.
 - B. To promote laboratory safety and quality.
 - C. To streamline laboratory operations.
 - D. To expand laboratory services.
 - E. To reduce laboratory costs.
-

Question 16: After an inspection by the College of American Pathologists (CAP), your laboratory is issued a Phase II deficiency for failing to confirm ABO group and Rh type for a patient. Phase II deficiencies require which of the following actions?

- A. Written response of the corrective action taken to address the deficiency within 45 days of inspection.
 - B. Written response demonstrating compliance with the cited checklist item within 30 days of inspection.
 - C. Supporting documentation demonstrating compliance with the cited checklist item.
 - D. A and C.
 - E. B and C.
-

Question 17: Laboratory testing services provided for outpatients aged 65 years or older would be reimbursed by which federal insurance program?

- A. Medicare part A.
- B. Medicare part B.
- C. Medicaid.
- D. Medicare Part D.
- E. All the above.



Question 18: Which of the following Medicare codes is required for reimbursement of inpatient hospital procedures?

- A. ICD-10-PCS (Procedure Coding System).
 - B. Health Care Procedural Coding System (HCPCS) Level I.
 - C. HCPCS Level II.
 - D. Current Procedural Terminology (CPT).
 - E. None of the above.
-

Question 19: Which national organization develops, maintains, and copyrights the CPT codes?

- A. American Medical Association (AMA).
 - B. Centers for Medicare and Medicaid Services (CMS).
 - C. Centers for Disease Control (CDC).
 - D. American Dental Association (ADA).
 - E. All the above.
-

Question 20: What is the purpose of a laboratory information management system (LIMS)?

- A. To manage laboratory finances and budgets.
 - B. To track employee attendance and productivity.
 - C. To automate laboratory processes and workflows.
 - D. To monitor laboratory safety and compliance.
 - E. To maintain inventory of laboratory supplies and equipment.
-

Question 21: Which of the following appropriately defines a Lean manufacturing system?

- A. A system that strives to decrease wastage while improving efficiency.
- B. Quality improvement methodology that strives to reduce the variability in production thereby reducing the number of defects or errors.
- C. A process approach that integrates multiple facets of quality and product improvement.



- D. A set of 12 essential components for laboratory quality and competence.
 - E. The comparison of one's own performance against that of peers or a best practice standard (AKA, a "gold standard").
-

Question 22: Which of the following process improvement strategies is considered a Lean method?

- A. DMAIC process.
 - B. 5S pillars.
 - C. PDCA cycle.
 - D. B and C.
 - E. A, B, and C.
-

Question 23: A test for a new blood analyte has a coefficient of variation of 0.6% and a test bias of 0.15%. The laboratory determines that the preset allowable test error is 2%. What would the approximate sigma value for this test be?

- A. 2.
 - B. 3.
 - C. 4.
 - D. 5.
 - E. 6.
-

Question 24: Which of the following sigma values would represent a process that has the least variability?

- A. 2-sigma.
- B. 3-sigma.
- C. 4-sigma.
- D. 6-sigma.
- E. 7-sigma.



Question 25: Which of the following statements is true regarding the relationship between an assay's sigma value and the quality control (QC) that should be run for the assay?

- A. The higher the sigma, the more frequently QC should be run.
 - B. The higher the sigma, the more control measurements should be run.
 - C. The lower the sigma, the more frequently QC should be run.
 - D. The lower the sigma, the fewer control measurements should be run.
 - E. There is no relationship between the sigma value and QC.
-

Question 26: Which of the following is a commonly used financial benchmarking ratio for a hospital's transfusion service and blood bank?

- A. Billable ABO grouping/total FTE.
 - B. Billable indirect antibody screens/testing FTE.
 - C. Billable direct antiglobulin tests/inpatient day.
 - D. Blood expense/total hospital expenses.
 - E. Billable ABO grouping/outpatient visit.
-

Question 27: Your blood bank wants to hire a new medical laboratory scientist for the evening shift. What elements should be included in the posted job description?

- A. Job title.
 - B. Minimum qualifications.
 - C. Job duties and responsibilities.
 - D. Work environment.
 - E. All the above.
-

Question 28: Which of the following questions would be illegal to ask during a job interview in some states?

- A. Are you at least 18 years of age?
- B. Have you ever been arrested?



- C. Can you work on the weekends?
 - D. Are you authorized to work in the US?
 - E. Are there any duties in the job description that you could not perform?
-

Question 29: A veteran with limited mobility due to a combat injury applies for a job in the blood bank located at a Veterans Affairs Hospital. Which of the following employment regulations prohibits the discrimination of an applicant based on mental or physical disability?

- A. Age Discrimination in Employment Act of 1967.
 - B. American with Disabilities Act of 1990.
 - C. Civil Rights Act of 1964.
 - D. Vocational Rehabilitation Act of 1973.
 - E. Equal Pay Act of 1963.
-

Question 30: Where in a budget are employee salaries allocated?

- A. Supply portion of the operational budget.
 - B. Capital budget.
 - C. Expense portion of the operational budget.
 - D. Cash budget.
 - E. Revenue budget.
-

Question 31: Which of the following describes the comparison between a laboratory's actual performance, including volume and expenses, and the laboratory's budget?

- A. Budget variance analysis.
- B. Net present value (NPV).
- C. Return on investment.
- D. Payback period.
- E. Depreciation analysis.



Question 32: You are interviewing candidates for testing personnel in the blood bank to perform high-complexity testing. Which of the following education summaries meets the educational requirements for the position?

- A. Bachelor's degree in medical technology.
 - B. Bachelor's degree in chemical, biological, physical, or clinical laboratory science.
 - C. 60 semester hours in an accredited university or college consisting of 24 semester hours of science courses.
 - D. A and B.
 - E. All the above.
-

Question 33: An applicant with which of the following educational qualifications would meet the requirements of a technical supervisor in an immunohematology laboratory?

- A. Medical doctor (MD) or Doctor of Osteopathic Medicine (DO) who has 6 months of training or experience with high-complexity testing.
 - B. Doctorate degree in chemical, biological, physical, or clinical laboratory science who is certified by a Department of Health and Human Services (DHHS)-approved certification board.
 - C. MD or DO who is board certified in anatomic and/or clinical pathology.
 - D. Master's degree in chemical, biological, physical, or clinical laboratory science with 2 years of training or experience with high-complexity testing in an immunohematology laboratory.
 - E. Doctorate degree in chemical, biological, physical, or clinical laboratory science who is certified by a Department of Health and Human Services (DHHS)-approved certification board and who has 1 year of training or experience with high-complexity testing in an immunohematology laboratory.
-

Question 34: In addition to the directorship, which other roles in the blood bank could the laboratory director fill?

- A. Technical supervisor.



- B. General supervisor.
 - C. Clinical consultant.
 - D. Testing personnel.
 - E. All the above.
-

Question 35: How many laboratories can a laboratory director direct?

- A. Only one.
 - B. Two.
 - C. Three.
 - D. Five.
 - E. Ten.
-

Question 36: Which of the following is the responsibility of a general supervisor?

- A. Provide consultation to laboratory clients.
 - B. Oversee the overall operation and administration of the laboratory.
 - C. Supervise personnel performing routine testing and help resolve issues.
 - D. Select the testing methods to meet the needs of the patient population.
 - E. Process the specimens and perform the tests.
-

Question 37: Your blood bank is moving into a brand-new space, and you are working with the developer to help design the laboratory. Which of the following are standard requirements when designing a laboratory?

- A. 150-200 square feet (excluding walls, hallways, closets, etc) per FTE.
- B. Laboratory counter width/depth: 4 feet.
- C. Eyewash units should be within 200 feet of the workspace.
- D. Counter-to-counter clearance: 2.5 feet.
- E. One exit for each room larger than 100 square feet.



Question 38: Safety of laboratory employees is paramount considering the exposure to chemical hazards and potentially infectious patient samples. Which governmental agency makes recommendations and provides resources (information, research, education, and training) on health and workplace safety but has no authority to enforce its recommendations?

- A. Occupational Safety and Health Administration (OSHA).
 - B. National Institute of Occupational Safety and Health (NIOSH).
 - C. Environmental Protection Agency (EPA).
 - D. US Department of Health and Human Services (DHHS).
 - E. State Departments of Health.
-

Question 39: Which of the following states requires state licensure for laboratory personnel?

- I. New York.
 - II. Colorado.
 - III. Florida.
 - IX. California.
 - V. Ohio.
-
- A. I and II.
 - B. I, III, IV
 - C. II, IV, V.
 - D. II, III, IV, V.
 - E. All the above.
-

Question 40: The hospital is running out of pink top collection tubes due to a national shortage. What other tube could be used in the interim for blood bank testing (eg, indirect antibody tests, direct antiglobulin tests)?

- A. Red top.
- B. Light blue top.
- C. Lavender top.
- D. Gray top.
- E. Green top.



Question 41: As part of a safety orientation a new employee is shown where safety data sheets (SDSs) are located. What is the purpose of laboratory SDS?

- A. To track the use of laboratory supplies.
 - B. To document laboratory incidents and accidents.
 - C. To provide information about the hazards of laboratory chemicals.
 - D. To ensure that laboratory equipment is properly calibrated.
 - E. To monitor the performance of laboratory personnel.
-

Question 42: OSHA has developed industry-specific guidelines for abatement to assist employees and employers in minimizing injuries. Which of the following is a recommended guideline for proper ergonomics in a laboratory setting?

- A. Use a chair without armrests for better mobility.
 - B. Place the computer monitor below eye level.
 - C. Use a stool without an adjustable height.
 - D. Maintain an upright posture and avoid slouching.
 - E. Use a microscope without proper adjustment.
-

Question 43: An employee in your lab consistently creates a disruptive work environment for others. Despite multiple documented meetings with the employee to discuss expectations and consequences of continued actions, the problem behaviors have continued. The decision has been made to terminate this person's employment. Which of the following is an important consideration when firing an employee?

- A. Compliance with laws regulations, and company policies.
- B. Maintaining confidentiality.
- C. Safeguarding laboratory resources.
- D. Communication with staff.
- E. All of the above.



Question 44: Blood banks rely on skilled and experienced technologists to ensure that donated blood is properly tested, processed, and distributed to patients in need. High turnover rates can lead to disruptions in the blood supply chain, decreased productivity, and increased training costs. Which of the following is least likely to improve retention?

- A. Competitive compensation and benefits.
 - B. Short-term retention bonuses.
 - C. Professional development opportunities.
 - D. Employee recognition practice.
 - E. Flexible scheduling.
-

Question 45: Burn-out is a type of work-related stress which may manifest as physical or emotional exhaustion. Often described in health-care settings, burn-out may result in decreased employee performance, low workplace morale, and increased turnover. Strategies to prevent workplace burn-out include:

- A. Distribute repetitive or mundane tasks to lower-performing individuals.
 - B. Increase workday flexibility by making lunch and other breaks optional.
 - C. Express gratitude regularly.
 - D. Demonstrate a commitment to productivity by increasing your own hours.
 - E. Reward those who do not take vacation.
-

Question 46: Continuing education for laboratory personnel is a responsibility of:

- A. Operations management.
- B. Human resource management.
- C. Financial management.
- D. Marketing management.
- E. Executive management.



Question 47: A medical laboratory scientist has a concern about practices in her facility that appear to negatively impact the quality and safety of blood components. What is true about reporting such a concern?

- A. She must report it to her own organization's executive management.
 - B. She must first report it to her direct supervisor and, if not resolved, may then elevate the report to higher levels of management.
 - C. The organization must provide the option for her to report such concerns either to executive management or to AABB.
 - D. She has a responsibility to gather sufficient evidence of harm before reporting a concern.
 - E. If standard operating procedures are being followed, there is no basis for reporting the concern.
-

Question 48: When an equipment failure occurs in a blood bank or transfusion service, which of the following would *not* normally be a part of the investigation procedure that follows the failure?

- A. Removal of the equipment from service.
 - B. Review of the organization's process for qualifying the manufacturer.
 - C. Assessment of all products provided since the equipment was last known to be operating within manufacturer specifications.
 - D. Determination of effect on patient and donor safety and on donor eligibility.
 - E. Reporting the failure to the manufacturer.
-

Question 49: In the supplier qualification process, what information about a potential supplier is *least* useful?

- A. International Organization for Standardization (ISO) certification/compliance.
- B. Whether the supplier has a quality manual.
- C. Cost of products and parts.
- D. Data from IRS Form 941.
- E. Data from IRS Form 990.



Question 50: Which of the following is true concerning root causes of a problem or nonconformance in the laboratory?

- A. Root causes are specific underlying causes.
- B. Root causes are those that can reasonably be identified.
- C. Root causes are those management has control to fix.
- D. Root causes are those for which effective recommendations for preventing recurrences can be generated.
- E. All of the above.

ANSWERS

Question 1: C. Directing.

Explanation:

- Directing is a key principle of management because it is required to manage people and their actions/tasks. Good directing entails the following elements:
 - Communication – whether verbal or written, communication is essential to convey messages, understand assignments, set expectations, and give and receive feedback.
 - Delegation – the assignment or distribution of responsibilities to other team members.
 - Motivation – whether derived internally or externally, inspiration is a key determinant of accomplishing goals.
 - Management of change – introducing change in a manner that is least disruptive and most likely to be accepted by team members, who can often be resistant to changes that alter the status quo.
 - Coaching – teaching others through training and continuing education in order to ensure competence and increase productivity.
- Planning is another essential component of management that entails the development of aims and deciding the steps and components necessary to accomplish them. Fundamentals of planning include strategic planning, selecting a planning team, environmental analysis, SWOT analysis, vision statement, mis-



sion statement, goals, strategies, prioritization, accountability, and measuring success metrics.

- Organizing is a process that structures employees, tasks, and inputs such as materials with the objective of accomplishing a planned goal. Key elements of organization include time management, policies, procedures, workflow, and staffing.
 - Controlling is the manner whereby leadership and/or management assess that all is following a set plan. Components of controlling include setting performance standards, evaluating team member performance, problem solving when issues arise, and decision making, which is often a result of problem solving.
-

Question 2: E. None of the above.

Explanation:

- There are different types or categories of management, each with their own components and responsibilities.
- The table below highlights four types of management with examples of corresponding list of responsibilities.

Operations Management	Human Resource Management
<ul style="list-style-type: none"> Policies and procedures Productivity assessment Quality assurance Benchmarking Regulation compliance Strategic planning Continuing education 	<ul style="list-style-type: none"> Staffing Recruitment Job descriptions Competency assessments Performance evaluations Personnel records Employee discipline Dismissals
Financial Management	Marketing Management
<ul style="list-style-type: none"> Departmental budgets Test cost analysis Maintenance of fee schedule Billing and coding Compliance with financial regulations 	<ul style="list-style-type: none"> Marketing Advertising Customer service and client education

**Question 3: A.** Strengths, Weaknesses, Opportunities, Threats.**Explanation:**

- SWOT analysis is a method of assessment to evaluate the strengths and weakness of a laboratory along with opportunities for advancement and potential threats.
 - Leveraging the strengths of the laboratory will facilitate capitalizing on opportunities that may arise.
 - Identifying the weaknesses of a laboratory in the context of potential threats that could hinder staff, operations, and patient care can aid in the prevention of errors.
-

Question 4: D. Marketing and sales strategies.**Explanation:**

- A market assessment is a process that can be implemented to develop a strategic business plan. Market assessment is a useful tool to employ when deciding whether to offer a new service or a new product.
- When a strategic business plan is created, the following key components of the plan that should be considered:
 - I. Production (ie, product planning) – includes research and development and evaluating the inputs for services and product production times.
 - II. Service (ie, distribution) – determines the method of delivery of products and/or services.
 - III. Marketing and sales (ie, promotion) – involves sales support, customer service, communication as well as other elements of marketing such as market research, marketing, and advertising such as promotions and publications.
 - IV. Pricing – can be influenced by public opinion/preferences and laboratory regulations but must be profitable and justifiable.
- These strategies should be considered in context of environmental variables including legal variables, competitive variables, economic variables, and societal variables.

**Question 5: C.** Test turnaround time.**Explanation:**

- Laboratory performance indicators are measures used to evaluate the quality and efficiency of laboratory operations. Test turnaround time is a common laboratory performance indicator. Other commonly followed transfusion medicine performance metrics include number of adverse reactions, component discard rates, and crossmatch to transfusion ratios.
-

Question 6: E. Laboratory information system.**Explanation:**

- Laboratory cost and price analysis requires consideration of a number of materials, service, and overhead. The following table contains definitions and examples of common terms.

Term	Definition	Examples
Direct costs	Costs associated with performing tests and obtaining results from a lab specimen	- Reagents - Technical labor - Equipment depreciation
Indirect costs	Costs associated with attaining specimens for testing and with billing for the tests	- Courier service - Billing department - Office supplies - Administrative costs
Fixed costs	Costs that remain constant regardless of volume (as volumes increase, fixed costs decrease)	- Rent - Lease - Laboratory information system - Instruments - Overhead



Term	Definition	Examples
Variable costs	Costs that change proportionately to changes in test volume (as volumes increase, variable costs increase)	- Sample collection supplies. - Processing labor - Printer - Telephone/fax lines - Maintenance
Total costs	All costs associated with performing a test: Direct and indirect costs as well as fixed and variable costs	N/A
Incremental costs (ie, marginal costs)	Costs associated with performing an additional test after accounting for direct costs	N/A

**Question 7: A. Direct costs.****Explanation:**

See answer #6.

**Question 8: D. Standard cost.****Explanation:**

- Standard cost is the cost of an item under ideal or normal circumstances.
- Actual cost is the current or real-time cost of an item. This can either be higher or lower than the standard cost depending on market pricing.
- Average cost is the total or full cost divided by the unit of service. For example, if the full cost for running a test was \$10,000 and the number of tests performed was 5000, then the average cost would be \$2/test.



- Marginal cost is also known as incremental cost and is generally considered the increase in total cost relative to the increase in volume. In the laboratory, marginal cost is also often described as the cost of performing one more test or unit of service.
- Step-fixed costs are the fixed costs that remain constant until a certain level of activity is reached, at which point fixed costs increase. For example, one ABO typing instrument may be able to perform up to 500 samples in a day. Thus, the fixed cost of the instrument will remain constant up until the test volume reaches 500 samples. If the laboratory needed to accommodate 700 samples per day, a second instrument would need to be acquired and so fixed cost would increase in a step-wise fashion.

**Question 9: C. \$7/test.****Explanation:**

- Unit cost = Total cost/number of tests.
- Total cost = fixed cost + variable cost.
- Thus, unit cost = (fixed cost + variable cost)/number of tests.
- For this scenario, unit cost = $[\$400 + (\$3/\text{test} \times 100 \text{ tests})]/100 \text{ tests per day} = (\$400 + \$300)/100 \text{ tests} = \$700/100 = \$7/\text{test}$.
- Given that fixed cost is constant and the variable cost per test remains the same, the unit cost will decrease as the number of tests increases. For example, if the daily ABO typing volume went up to 250, then the unit cost would $\$4.60/\text{test}$ $[\$400 + (\$3/\text{test} \times 250 \text{ tests})/250 \text{ tests per day} = (\$400 + \$750)/250 \text{ tests} = \$1150/250 = \$4.60/\text{test}]$.

**Question 10: D. 8000 tests.****Explanation:**

- The breakeven point is considered the number of tests needed to be performed at which point total revenue is equal to total costs or, put in other words, where the net income equals zero.
- Breakeven point can be written out mathematically as: $0 = \text{revenue (R)} - \text{total cost}$. However, remember that total cost = fixed



cost (FC) – variable cost (VC). Thus, the breakeven point can be calculated by the following equation: $0 = R - FC - VC$.

- Considering that revenue and VC depend on the number of tests performed, the breakeven point equation can be written as: $0 = (R \times Z) - FC - (VC \times Z)$, where Z is the number of tests.
 - This equation can be solved for Z to determine the number of tests at the breakeven point: $Z = FC/(R - VC)$.
 - For this scenario, $Z = \$40,000/(\$8 - \$3) = 8000$ tests.
 - Hence, after the purchase of the new instrument, the blood bank would break even after performing 8000 ABO typings. If the test volume remained 100/day, it would take 80 days, or approximately 2.5 months, to reach the breakeven point.
-

Question 11: A. Leasing can be considered a tax-deductible overhead expense.

Explanation:

- When it comes to acquiring expensive laboratory equipment, there are often three options to consider: purchasing, leasing, and reagent rental. Each option comes with advantages and disadvantages.
- When equipment is purchased, the manufacturer may offer a discount. Interest rates on loans for equipment are generally low as well. Reagent costs are usually lower for laboratories that opted to purchase equipment. However, purchasing equipment requires capital budget funds, so it may not always be feasible if funds are unavailable. If assets are liquidated to purchase equipment, then the interest lost on the assets is foregone. Purchasing also locks you into the technology, which can be a detriment if the technology in the field is quickly evolving.
- Funds for leasing equipment generally come from the operating budget so no capital funds are needed, and the equipment can be purchased when needed or desired (ie, no need to wait for capital budget cycle request). Service and maintenance can be added to the leasing agreement, and some leases can be counted as tax-deductible overhead expenses. Also, many lease agreements come with an option to buy at the end of the term. Unlike purchasing, leasing permits the ability to change or upgrade instruments to keep up with evolving technology. However, leasing



generally comes with higher interest rates and higher costs of reagents compared to purchasing. Consequently, at the end of the leasing term, you may incur greater costs compared to purchase.

- The cost for reagent rental is usually factored by cost per reportable test (CPR). CPR can generally be negotiated lower with high test volumes. Therefore, costs are more favorable for high-volume tests. However, in general reagent costs are generally highest for reagent rental. Additionally, calibration and controls are not figured into the costs. At the end of the rental term, there is no option to buy as with leasing. Nevertheless, like leasing, reagent rental avoids use of capital funds; maintenance and consumables are figured into the CPR; and the upgrading of equipment is included.



Question 12: E. Decreased overtime hours/pay period.

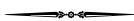
Explanation:

- Employee requirement and retention are instrumental in the overall success, longevity, and productivity of a laboratory.
- Recruitment strategies include national advertising, competitive salaries, sign-on bonuses, interview expenses, relocation expenses, flexible work hours, and tuition reimbursement.
- Several retention metrics (see table below) can be monitored that can be reflective of employee job satisfaction. If favorable, these metrics can help in recruitment.

Metric	Indicative of Low Retention	Indicative of High Retention
Vacancies	High number of vacancies	Low number of vacancies
Turnover rate	Increased number of hires/total FTE	Decreased number of hires/total FTE
Staff feedback	Negative employee feedback/ comments	Positive employee feedback/ comments
Overtime	Increased overtime hours/pay period	Decreased overtime hours/pay period
Agency staff	Increased hours paid/pay period	Decreased hours paid/pay period

**Question 13: D.** Increased overtime.**Explanation:**

- Monitoring staff retention is important, as staffing shortages can have detrimental consequences for the laboratory and patient care.
- Negative potential consequences of staffing shortages include increase in number of laboratory errors, increase in the turn-around times (ie, delays in providing test results), increase in overtime (which can lead to burnout and fatigue), increase in staff turnover, and increase in use of agency staff.
- Additionally staffing shortages can lead to a decrease in the number and variety of tests the laboratory can offer its customers, which in turn can lead to a decrease or loss of customers who are dissatisfied with the laboratory's services.

**Question 14: C.** Certificate of registration.**Explanation:**

- Depending on the complexity of testing that a laboratory provides, different CLIA certificates are required.
- Laboratories that perform only waived tests require a certificate of waiver. Waived tests are defined by CLIA as tests that are simple to perform and have minimal risk if an incorrect result is obtained. Examples of waived tests include rapid strep test, spun hematocrit, fecal occult tests, and tests that can be performed at home.
- Laboratories in which a practitioner (eg, physician, physician assistant, nurse practitioner, dentist, etc) performs only microscopy-related procedures require a certificate for provider-performed microscopy procedures (PPMP). PPMP is considered a moderately complex test and includes wet mounts, microscopic urinalysis, and cervical or vaginal preparations. This certificate grants the laboratory the authority to perform waived tests as well.
- A certificate of registration grants a laboratory temporary authority to perform moderate and/or high-complexity testing until the laboratory is inspected and determined to be CLIA-compliant.



Other than PPMP, examples of moderately complex tests include urine culture, Gram stain, and automated tests that do not require manual manipulation during analytic phase. Examples of high-complexity tests include ABO typing and serologic testing, manual cell counts, flow cytometry, hemoglobin electrophoresis, and cytopathologic evaluations.

- Once a laboratory has been inspected and is found to be CLIA-compliant, a certificate of compliance can be issued.
 - A laboratory that is accredited by an accrediting organization with CMS deemed status can receive a certificate of accreditation.
-

Question 15: B. To promote laboratory safety and quality.

Explanation:

- The primary purpose of laboratory accreditation is to promote laboratory safety and quality. Accreditation involves a rigorous evaluation of laboratory performance and adherence to quality standards. Accredited laboratories are recognized as having met high standards for laboratory safety and quality, and are often preferred by patients, health-care providers, and regulatory agencies.
-

Question 16: E. B and C.

Explanation:

- During CAP inspections, the inspector assesses laboratory compliance with requirements listed in the CAP Checklist. Each checklist item is designated as Phase I or II depending on the severity of consequences that could result from noncompliance.
- Phase I deficiencies require only a written response detailing the corrective action carried out to comply with the corresponding checklist item.
- Phase II deficiencies require both a written response as well as accompanying documentation showing compliance with the



cited checklist item. The written response is expected to detail the reasoning behind submitting the supporting documentation.

- Responses to both Phase I and II deficiencies are to be submitted to CAP within 30 days (about 4 and a half weeks) of the inspection.
 - Even when a laboratory is found to be compliant with a checklist item (ie, no deficiency is issued), an inspector may make recommendations on process improvements. No written responses are required for recommendations.
-

Question 17: B. Medicare part B.

Explanation:

- A basic understanding of how reimbursement works is important when managing a clinical laboratory. Although many private insurers are available, many patients rely on federally funded programs.
- Medicare Part A (hospital insurance) covers services for inpatient hospitalizations as well as services in skilled nursing facilities, hospice facilities, and services provided for end-stage renal disease services. For hospital stays, Medicare payments are reimbursed as part of diagnosis-related group (DRG), which are assigned based on criteria such as diagnosis and treatment types. A DRG reimburses the hospital a set amount based on the DRG regardless of how much the patient's care costs.
- Medicare Part B (medical insurance) covers outpatient services including laboratory testing as well as physician services. Of note, hospitals that provide testing for outpatients, in addition to inpatient testing, will submit a UB-92 form under Part A reimbursement, however, the payment is based on fee schedules from Medicare Part B.
- Medicare Part C, also known as Medicare Advantage Plan, offers Part A and B benefits through contracts between private companies and Medicare. Examples of Part C plans include preferred provider organizations (PPOs) and health maintenance organizations (HMOs). Part C plans also often provide additional coverage for prescriptions, dental, vision, hearing, and wellness programs.



- Medicaid (Title XIX) is jointly funded by federal and state governments to provide health coverage for low-income individuals who meet certain criteria as well as those in need.
 - Medicare Part D offers drug coverage plans.
-

Question 18: A. ICD-10-PCS (Procedure Coding System).

Explanation:

- Medicare has different code sets that provide guidance to providers, insurers, medical coders, and billing staff when submitting claims for diagnoses, services, procedures, supplies, drugs, and medical equipment.
 - ICD-10-CM codes are diagnosis codes that describe the reason the patient is seeking and/or receiving health-care services. These codes are used for both inpatient and outpatient claims. They are used to determine certain benefits and coverage but not the amount paid for services rendered.
 - ICD-10-PCS codes are for inpatient procedure claims and are submitted in conjunction with ICD-10-CM.
 - Level I HCPCS codes are CPT codes that are used for professional services and medical procedures rendered in the outpatient and ambulatory settings. CPT codes are used in conjunction with ICD-10-CM codes but not with ICD-10-PCS codes. CPT codes provided on claims do help determine payment.
 - Level II HPCS codes supply additional information concerning the drugs, supplies, and equipment provided during the patient's care. Physicians, suppliers, hospital-based outpatient department, and other outpatient facilities can submit level II HPCS codes to report and receive payment for services and supplies provided to the patient during their care. As with level I HPCS codes, level II HPCS codes are used in conjunction with ICD-10-CM codes but not with ICD-10-PCS codes. Level II HPCS codes are maintained by CMS with exception of the Current Dental Terminology (CDT) codes, which are developed, maintained, and copyrighted by the American Dental Association (ADA).
-

Question 19: A. American Medical Association (AMA).



Explanation:

- CPT codes are developed, maintained, and copyrighted by the AMA.
 - ICD-10-CM codes are developed and maintained by the CDC.
 - ICD-10-PCS codes are developed and maintained by CMS.
 - Level II HPCS codes are maintained by CMS with exception of the Current Dental Terminology (CDT) codes, which are developed, maintained, and copyrighted by the ADA.
-

Question 20: C. To automate laboratory processes and workflows

Explanation:

- A laboratory information management system (LIMS) is a software system that is designed to automate and manage laboratory processes and workflows. A LIMS typically includes features for managing samples, tracking test results, generating reports, and communicating with other health-care systems.
-

Question 21: A. A system that strives to decrease wastage while improving efficiency.

Explanation:

- The Lean system was originally developed in and applied to production in the automobile industry. Its focus was to make the production line as lean or streamlined as possible with a focus on reducing wastage while improving efficiency. Anatomic and clinical pathology laboratories began to adopt Lean practices to reduce wastage in their process, thereby improving services.
- Six Sigma is another method from the manufacturing world, particularly from Motorola, that has been adopted by laboratories. The focus of Six Sigma is to reduce or eliminate process variation thereby minimizing the number of defects or errors in the final product. Sigma is an expression of statistical deviation from the optimal or preset performance metric. It usually reflects the number of defects or errors per million opportunities. The perfor-



mance goal of six-sigma is 3.4 defects per million opportunities, which is a performance yield of 99.99966%.

- Total quality management describes an approach that integrates multiple facets of quality improvement. Its application aids in product and process improvement. Lean and Six Sigma would be considered elements or methods that could be integrated as part of a laboratory's total quality management.
- The Clinical and Laboratory Standards Institute (CLSI) developed a set of 12 essential components for laboratory quality and competence, which incorporates quality requirements from the International Organization for Standardization (ISO), particularly from ISO 15189. The 12 essentials include: Organization, personnel, equipment, purchasing and inventory, process control, information management, documents and records, occurrence management, assessment, process improvement, customer service, and facilities and safety.
- Benchmarking is a comparison of one's own performance against that of peers or a best practice standard (AKA, a "gold standard"). It is an assessment tool that permits evaluation of current practices and processes while at the same time allowing for avenue to quality and process improvement. Benchmarking can be external (comparison with an outside institution or laboratory) or internal (comparison with another department or laboratory within the same institution or system).



Question 22: D. B and C.

Explanation:

- The 5S pillars is a Lean improvement process whose aim is to decrease waste and increase productivity through workplace organization. It is often the first Lean method implemented by an organization. The 5S's are Sort, Set in order, Shine, Standardize, and Sustain:
 - Sort – organization by ridding the work areas of the unnecessary items that are not required for production operations.
 - Set in order – organization through creation of effective and efficient storage of essential items that can be easily found, used, and stowed away.



- Shine – thorough cleaning of work areas to facilitate identification of potential malfunctions in processes and equipment. This requires daily maintenance and cleaning.
- Standardization – method standardization to maintain Sort, Set in Order, and Shine. Standardization can be achieved by using tools such as task charts, signs, scoreboards, and checklists to assign responsibilities; integrating these pillars into normal work duties; and checking on the maintenance of the pillars. Standardization also involves preventing the reaccumulation of unnecessary items, the breakdown of procedures, and the dirtying of equipment.
- Sustain – change in behaviors to maintain the 5S pillars. Without this step, the workplace and process can revert to disorderliness. Tools such as performance reviews, team check-ins, newsletters, and posters can help sustain the 5S pillars.
- The PDCA cycle, or the PDSA cycle as it is sometimes referred to as, is another Lean improvement process that has its foundation in the scientific method and is also known as the Deming Cycle (named after W. Edwards Deming who introduced the method). PDCA stands for Plan, Do, Check, and Act:
 - Plan – determine the goals or aims and the methods by to achieve them.
 - Do – implement the necessary changes or modifications through education and training.
 - Check (Study in PDSA) – evaluate the results and effects that the implemented changes have on performance.
 - Act – take action to standardize the implemented changes if results are desirable or begin the cycle anew if the results did not achieve the predetermined goals.
- DMAIC is a quality improvement strategy that is data-driven and considered to be an important component of the Six Sigma method. DMAIC stands for Define, Measure, Analyze, Improve, Control:
 - Define – describe the problem, the areas for improvement, the improvement strategy, and the goals of the process.
 - Measure – evaluate the performance of the process through such tools as process maps, charts, and capability analysis.
 - Analyze – examine the process to ascertain the causes of poor performance, defects, and/or variation using tools such as root cause analysis and failure mode and effects analysis.



- Improve – ameliorate or enhance the process by targeting and/or eliminating the causes of problems identified in the analyze phase.
 - Control – manage and maintain the newly improved process through various methods including Lean methods such as the 5S pillars.
-

Question 23: B. 3.**Explanation:**

- Sigma can be calculated with the following data inputs:
 - Total allowable error (TE_a) – a preset limit of a test; any result obtained that is beyond this limit would be considered an error.
 - Coefficient of variation (CV) – the standard deviation (SD) of a test that is expressed a percentage of the mean ($CV = SD/\text{mean} \times 100$). The higher the CV, the more variability is present.
 - Bias – the difference between a laboratory's result and a reference value.
- The formula to calculate sigma is:

$$\text{Sigma} = \frac{TE_a - \text{Bias}}{CV}$$

- Thus, in this scenario, $\sigma = (2\% - 0.15\%)/0.6\% = 3.08$
-

Question 24: E. 7-sigma.**Explanation:**

- In general, the more controlled a process the less variability there is and the more likely the process is to meet the preset performance goals. Regarding laboratory testing, the lower the coefficient of variation, the higher the sigma value.
- Less variability correlates with a higher sigma. For example, a 3-sigma process has more variability than a 6- or 7-sigma process.



- In health care, most applications of the Six-Sigma system operate between 2- and 4-sigma. Proficiency testing for most laboratory analytes requires an accuracy rate of 80%-100%, which translates to a sigma of approximately between 3 and 4. However, in the US airline industry, they aim to maintain at least 7-sigma for the number of fatalities/1,000,000 flights.
-

Question 25: C. The lower the sigma, the more frequently QC should be run.

Explanation:

- The purpose of QC is to ensure that an assay is performing as expected and producing accurate results. It permits the interrogation of the process and intervention if QC fails.
 - Processes with a high sigma value would be expected to produce only a few errors or defects per million opportunities. Thus, it would stand to reason that QC failure would be an uncommon occurrence.
 - Indeed, the higher the sigma value, the less frequently QC needs to be performed and less often control measurements need to be taken. In fact, if a test method were to have a 6-sigma, then approximately 500 patient samples or more could be run between QC activities. Moreover, there would be less need to monitor for QC variation.
 - On the other hand, tests with lower sigma value require more frequent QC and more control measurements to ensure that erroneous results are not obtained. This would correlate with an increase in the need to monitor QC outliers. If a test method had an extremely low sigma value, then the amount and frequency of QC efforts may make the test impractical to operate.
-

Question 26: D. Blood expense/total hospital expenses.

Explanation:

- Numerous types of ratios can be evaluated for benchmarking purposes.



- Commonly used financial benchmarking ratios include expense of blood/total expenses, total testing expense/FTE, total expense/inpatient day, consumable expense/billable test, and maintenance expenses/total expenses.
 - Commonly used productivity benchmarking ratios include the number of billable tests/total FTE, billable tests/per inpatient day, billable tests/testing FTE, billable tests/outpatient visit, and billable tests/inpatient discharge.
 - Other commonly employed benchmarking ratios include worked hours/paid hours, in-hours billable test/total billable tests, testing FTE/total FTE, inpatient billable tests/total billable tests, and outpatient billable tests/total billable tests.
-

Question 27: E. All the above.

Explanation:

- A criterion-based job description generally contains the following components:
 - Job title – the title should correlate with the position offered and be consistent within the institution and similar with respect to similar jobs at the local and national. Job titles will often be followed by a brief job summary.
 - Minimum qualifications – requirements can include regulatory licensure, educational degrees, accrediting certifications, on-the-job training, and work experience. Minimum qualifications can be supplemented with desired skills, abilities, and qualifications (eg, multilingualism, presentation skills, effective communication, etc).
 - Job duties and responsibilities – job-related tasks should be provided detailing general, technical, and administrative duties expected of a prospective employee. If the position entails teaching or research responsibilities, these should be included as well.
 - Workplace environment – the location where most of the tasks and work performed will take place should be included (eg, processing center, blood bank, core laboratory, etc).
 - Job relationships within the organizational structure of the workplace are often provided and describe to whom the applicant is to report and who is to report to the applicant.



- Physical demands of the job such as standing, walking, and lifting heavy objects may also be specified.
-

Question 28: B. Have you ever been arrested?

Explanation:

- When conducting an interview, the interviewer should prepare for the interview by carefully reviewing the applicant's resume. The applicant should be welcomed and made to feel comfortable. The interview process and expected follow-up should be explained.
 - Different lines of questioning can be informative such as direct questions, open-ended questions, and hypothetical questions. Leading questions are not usually as informative because they generate expected answers.
 - In the United States, it is not only unacceptable, but illegal for an employer to discriminate against a job applicant because of race, color, religion, sex (including gender identity, sexual orientation, and pregnancy), age, national origin, or disability. Questions about these topics may be considered evidence of intent to discriminate.
 - Although there is no federal law barring the inquiry of arrest and conviction records, some states and local governments do have laws or ordinances preventing such inquiries from being posed on applications or during interviews or preliminary screenings.
 - Interviewers should also be cautious to avoid questions that could be discriminatory in nature. See answer #29 for more on this topic.
-

Question 29: D. Vocational Rehabilitation Act of 1973.

Explanation:

- It is important to be familiar with equal opportunity employment regulations when interviewing candidate employees to avoid violation of federal and local regulations. There should be policies in place to avoid potential discrimination against current and prospective employees.



- The Equal Pay Act of 1963 prohibits gender-based discrimination in regard to compensation.
 - The Civil Rights Act of 1964 established the Equal Employment Opportunity Commission (EEOC) and proscribes discrimination on the basis of race, color, gender, religion, or national origin.
 - The Age Discrimination in Employment Act of 1967 forbids age-based discrimination in employment.
 - The Vocational Rehabilitation Act of 1973 prevents employment discrimination due to mental or physical disability. This act applies to programs receiving federal funds and federal contractors.
 - The Americans with Disabilities Act of 1990 prohibits discrimination based on disability in employment, public services, telecommunications, and public accommodations.
-

Question 30: C. Expense portion of the operational budget.

Explanation:

- Most laboratories operate using two main types of budgets – the operating budget and capital budget.
- The operating budget is dependent on revenue and expenses as determined by volumes.
 - The statistical portion of the operating budget (ie, statistical budget or volume budget) is the projected activity/productivity of the laboratory.
 - The revenue section of the operating budget (ie, revenue budget) determines the generated charges based on projected volumes.
 - Most of the expense portion of the operating budget (ie, expense budget) covers labor including employee salaries, benefits, overtime, and temporary staffing.
 - The operating budget also covers supplies (ie, supply budget) which include reagents and consumables; long-term financial agreements such as leases, maintenance contracts, and rental agreements; and miscellaneous expenses including utility bills, reference laboratory testing, and general laboratory and office supplies.



- Given the number of components and the degree of financial planning required, the operating budget preparations usually start at least 6 months before the next fiscal year.
 - The capital budget constitutes requests for significant investments for the laboratory or organizations. In the laboratory the main type of capital requests are the replacement of equipment, purchase of new equipment, and renovations or new construction. Because capital budget requests represent large investments, purchase requests are usually accompanied by operation and financial justifications such as risk-benefit analyses and financial analyses.
 - There is a third type of budget that some organizations developed known as the cash budget. The cash budget trends the flow of cash in and out of the organization and helps the laboratory or organization with managing cash reserves, disbursements, borrowing, and investments. As such, the cash budget is important to ensure the laboratory or organization remains in business.
-

Question 31: A. Budget variance analysis.**Explanation:**

- Budget variance analysis involves analyzing the differences between a laboratory's or organization's actual performance to the projected budget. This process permits corrective actions to be implemented and decisions to be made in order to improve operations and better approximate the budget if performance is not up to par.
- Net present value, return on investment, and payback period are all standard financial analyses that can be performed to help justify capital expenditures.
- Depreciation analysis involves calculating the decrease in an asset's value over the course of its useful life when it is predicted to last for >1 year. For example, if a piece of capital equipment cost \$5000 and has a projected lifespan of 5 years, then the depreciation expense would be \$1000/year. There are various calculation methods that can be performed to determine depreciation. Although depreciation is an important concept to bear in mind, it is not usually a consideration when investing in capital acquisition as it is not a cash flow item.



Question 32: E. All the above.

Explanation:

- Testing personnel are responsible for specimen processing, test performance, and test result reporting. They should follow laboratory policies and procedures, run quality controls, and maintain appropriate records. Additionally, they should be able to recognize and identify problems that could adversely affect test results and document the corrective actions taken to address these problems.
- When considering candidates for various positions in the laboratory, it is important to be familiar with the required qualifications as outlined by federal regulations.
- Testing personnel performing high-complexity testing, as is performed in most blood banks, are required to have up-to-date state licensure if required by the state in which the laboratory is located and one of the following:
 - MD, DO, doctorate of podiatric medicine and board licensure from the state in which the laboratory is located.
 - Doctoral, master's, or bachelor's degree in chemical, biological, physical, clinical laboratory science, or medical technology from an accredited college or university.
 - Associate degree in medical laboratory technology or laboratory science from an accredited institution.
 - Equivalent of an associate degree in medical laboratory technology or laboratory science, which would include:
 - 60 semester hours from an accredited institution that includes either a medical laboratory technology course (24 semester hours) or science courses (24 semester hours) consisting of: 1) chemistry (6 semester hours), and 2) biology (6 semester hours), and a combination of chemistry, biology, and/or laboratory medicine (12 semester hours).
 - Laboratory training consisting of either an accredited clinical laboratory training program or 3 months of laboratory training in the specialty areas in which the laboratory performs high-complexity testing.



Question 33: C. MD or DO who is board certified in anatomic and/or clinical pathology.

**Explanation:**

- A technical supervisor is responsible for overseeing the scientific and technical aspects of the laboratory. Some of the responsibilities of a technical supervisor include selecting the appropriate testing methods for the patient population, verifying test characteristics and procedures, selecting and enrolling into a proficiency testing program, creating a QC program, resolving technical problems that arise with testing, developing the required training for testing personnel, and evaluating competencies of the training personnel.
- A technical supervisor of a laboratory performing high-complexity immunohematology testing, as is performed in most blood banks, is required to have:
 - Up-to-date state licensure if required by the state in which the laboratory is located.
 - One of the following:
 - MD or DO with board licensure from the state in which the laboratory is located and is certified in anatomic pathology and clinical pathology.
 - MD, DO, doctorate of podiatric medicine with board licensure from the state in which the laboratory is located and at least 1 year of training and/or experience in a laboratory performing high-complexity immunohematology testing.
- The qualifications outlined above for the technical supervisor for an immunohematology laboratory performing high-complexity testing are more restrictive than the qualifications for the position in other laboratories. In chemistry, microbiology, and hematology laboratories, individuals with doctoral, master's, and bachelor's degrees as well as varying years training and/or experience in high-complexity testing may be qualified as technical supervisors.

**Question 34: E. All the above.****Explanation:**

- A laboratory director oversees the overall operation and administration of the laboratory. The responsibilities are many but include approving all policies and procedures, ensuring that the



workspace is safe and adequate for the testing performed, making certain that comprehensive quality assurance and quality control programs are in place, confirming that the laboratory is enrolled in an appropriate proficiency testing program, guaranteeing that consultative services are available to the laboratory personnel, verifying that the laboratory personnel are performing tests as outlined in the procedures, corroborating that validation and verification of processes and testing methods are adequate and complete, and assigning duties to individuals commensurate with their qualifications, level of training, and experience.

- A laboratory director of a laboratory performing high-complexity testing, as is performed in most blood banks, is required to have:
 - Up-to-date state licensure if required by the state in which the laboratory is located.
 - One of the following:
 - MD or DO with board licensure from the state in which the laboratory is located and is certified in anatomic pathology and clinical pathology.
 - MD, DO, doctorate of podiatric medicine with board licensure from the state in which the laboratory is located and at least 2 years of training and/or experience in a laboratory performing high-complexity immunohematology testing.
 - Doctoral degree in chemical, biological, physical, or clinical laboratory science and be certified by a DHHS-approved certification board.
- In addition to directorship, a laboratory director's qualifications also qualify him or her to serve as the technical supervisor, clinical consultant, general supervisor, and testing personnel as long as the other qualifications of these positions are met.



Question 35: D. Five.

Explanation:

- Per the Code of Federal Regulations, Title 42 CFR Part 493, a laboratory director can direct up to five laboratories.
- Accordingly, Clinical Laboratory Improvement Amendments (CLIA) permits a director to hold up to five CLIA licenses, one for each laboratory he or she directs.



Question 36: C. Supervise personnel performing routine testing and help resolve issues.

Explanation:

- General supervisors oversee the day-to-day operations of the laboratory and activities of the laboratory personnel. They should be available and accessible to testing personnel for questions and help in resolving problems. Additionally, general supervisors monitor specimen adequacy, test analyses, and test result reporting.
- The laboratory director and technical supervisor can delegate responsibilities to the general supervisor as well. Delegated responsibilities may include orienting new personnel, performing annual personnel evaluations, and ensuring that remedial and/or corrective actions are taken after detected deviations.
- A general supervisor of a laboratory performing high-complexity testing, as is performed in most blood banks, is required to have:
 - Up-to-date state licensure if required by the state in which the laboratory is located.
 - One of the following:
 - Meet the qualifications of a laboratory director or technical supervisor (see answer #31 and #30, respectively).
 - MD or DO with board licensure from the state in which the laboratory is located and is certified in anatomic pathology and clinical pathology.
 - MD, DO, doctorate of podiatric medicine, doctoral degree, master's degree, or bachelor's degree (nonmedical degrees should be in a chemical, biological, physical, or clinical laboratory science) and at least 1 year of training and/or experience in a laboratory performing high-complexity testing.
 - Meet the qualifications of testing personnel (see answer #29) and at least 2 years of training and/or experience in a laboratory performing high-complexity testing.
- The clinical consultants provide consultation to laboratory clients on test selection, test interpretation, as well as quality and specifications of testing. They also help ensure that result reports contain sufficient information for proper interpretation. An individual can qualify as a clinical consultant if they meet the qualifications of a laboratory director or have an MD, DO, or doctorate of podi-



atric medicine with board licensure from the state in which the laboratory is located.

Question 37: A. 150-200 square feet (excluding walls, hallways, closets, etc) per FTE.

Explanation:

- During planning and designing for a laboratory, there are many considerations to take into account including standard space and dimension requirements.
- Adequate spacing is important to ensure mobility and safety. In general, requirements state 150-200 net square ft (excluding walls, hallways, closets, etc)/FTE or 27-40 net square feet/hospital bed.
- There should be two exits for rooms larger than 100 square feet (about the area of an apartment bedroom).
- Corridors used for personnel should be 3 feet 8 inches wide, while those used for patients should be 8 feet wide.
- Eyewash units are required to be within 100 feet of workspaces.
- Laboratory counter width/depth should be 2 feet 6 inches; counter-to-wall clearance should be 4 feet; and counter-to-counter clearance should be 7 feet.
- Desk space should be 3 square feet with a height of 30 inches.
- Other considerations include:
 - Base cabinets, which would be under the laboratory counters, provide more storage space compared to suspended cabinets.
 - Biologic safety cabinets and fume hoods are required to be away from doorways and high-traffic areas to prevent or minimize air current drafts.
 - Modular furniture provides flexibility for possible future changes.
 - HVAC should maintain room temperature (~68-76 F) and control humidity (~20-60%) and air flow to avoid extremes that could negatively impact equipment, reagents, personnel, and/or patients.



Question 38: B. National Institute of Occupational Safety and Health (NIOSH).

Explanation:

- NIOSH is an agency that is part of DHHS. Although it offers recommendations on safety hazards and supplies occupational safety resources (such as information, research, education, and training), it has no means by which to enforce these recommendations.
- OSHA, on the other hand, is part of the US Department of Labor, and does have the authority to develop and enforce workplace safety and health standards meant to protect workers. OSHA has recommendations concerning chemical safety, blood-borne pathogens, ergonomics, personal protective equipment, as well as other hazardous situations. Laboratories must comply with OSHA standards.
- The EPA is the agency in charge of protecting the environment and enforces requirements addressing the handling and disposal of hazardous materials used in the laboratory.
- DHHS is the main governmental agency tasked with protecting and enhancing the health of American citizens. It oversees the following governmental agencies:
 - The US Food and Drug Administration (FDA).
 - Centers for Medicare and Medicaid Services (CMS).
 - NIOSH.
 - National Institutes of Health (NIH).
 - Centers for Disease Control and Prevention (CDC).
 - Office of the Inspector General (OIG).
- State Departments of Health are run by individual states and each state department regulates laboratories to varying degrees. Some states inspect and license laboratories and even have CLIA deemed status while others do not. Personnel state licensure is required by some, but not all, states.



Question 39: B. I, III, IV.



Explanation:

- The following states require state licensure of laboratory personnel: California, Florida, Georgia, Hawaii, Louisiana, Montana, Nevada, New York, North Dakota, Rhode Island, Tennessee, West Virginia.
 - Puerto Rico, which is a US territory, also requires licensure of laboratory personnel.
-

Question 40: C. Lavender top.

Explanation:

- Pink top tubes are the preferred collection tube for blood bank testing. They contain spray-dried K₂EDTA as anticoagulant. Pink top tubes can be centrifuged, separating the plasma and red cells. The plasma can be used to perform tests such as indirect antibody screens, antibody identification panels, and crossmatches. The red cells can be used to perform direct antiglobulin tests and red cell phenotyping.
- Lavender top tubes also contain K₂EDTA as anticoagulant but are used for general hematology testing. The main difference between pink top tubes and lavender top tubes is the size—ie, pink top tubes are ~10 mL while lavender top tubes are ~5 mL. Higher volume is preferred for blood bank testing as more plasma is required for serologic testing, especially if multiple panels and techniques (ie, elutions, autoadsorptions, alloadsorptions, etc) are required for antibody identification.
- Red top tubes contain either no anticoagulant or a clot activator and are meant to obtain serum after the red cells clot. They are used for serology tests and certain chemistry tests. As such, red top tubes could be used in the blood bank if only serologic tests needed to be performed. However, any testing involving red cells could not be performed after clotting.
- Light blue top tubes contain sodium citrate as anticoagulant and are used to perform coagulation tests.
- Gray top tubes contain sodium fluoride as anticoagulant are used to perform glucose testing.
- Green top tubes contain sodium heparin or lithium heparin as anticoagulant and are used to perform chemistry tests.



Question 41: C. To provide information about the hazards of laboratory chemicals.

Explanation:

- The purpose of a laboratory Safety Data Sheet (SDS), formerly known as Material Safety Data Sheets (MSDS), is to provide information about the hazards of laboratory chemicals. Each chemical sheet includes properties of the chemical; the physical, health, and environmental health hazards; protective measures; and safety precautions for handling, storing, and transporting the chemical. SDSs are required by law for all hazardous chemicals in the laboratory, and are provided by the chemical manufacturer, distributor, or importer. They must be readily accessible to employees for all hazardous chemicals in their workplace.
-

Question 42: D. Maintain an upright posture and avoid slouching.

Explanation:

- The laboratory director has a responsibility to create and maintain a safe working environment for laboratory staff, and ergonomics is an important aspect of this. Proper ergonomic design can reduce the risk of workplace injuries, such as repetitive strain injuries and musculoskeletal disorders, and improve overall worker comfort and productivity. A director should work to implement ergonomic best practices and make necessary changes to equipment, workstations, and processes as needed to optimize ergonomics in the laboratory.
- Maintaining an upright posture and avoiding slouching can help reduce strain on the muscles and joints of the back, neck, and shoulders. Laboratory workers should be trained to maintain good posture and take frequent breaks to stretch and move around throughout the day.
- Armrests help support the arms and reduce shoulder and neck strain during tasks that require fine motor control, such as pipetting or microscopy. Therefore, chairs with armrests are often recommended for laboratory settings.
- Placing a computer monitor below eye level can cause neck and shoulder strain. Monitors should be at or slightly above eye level



to promote proper posture and reduce strain on the neck and shoulders.

- Adjustable stools are recommended for proper ergonomics in a laboratory setting to promote proper posture. Using a stool that is too high or too low can cause strain on the lower back, legs, and feet.
 - Proper adjustment of the microscope height, angle, and focus is important for proper ergonomics and can help reduce the risk of eyestrain, neck pain, and back pain.
-

Question 43: E. All of the above.

Explanation:

- Although many aspects of laboratory management, such as hiring and firing, may be delegated to other individuals, the laboratory director is ultimately responsible for the overall operation and administration of the laboratory, including personnel.
- Firing an employee is a serious matter that should be handled with care and consideration. Within a laboratory setting, there are several important considerations that should be taken into account when terminating an employee.
- It is important to ensure that the termination process complies with legal and ethical standards, including any relevant laws, regulations, and company policies. This can include providing the employee with proper notice, following the company's disciplinary procedures, and avoiding discrimination or retaliation.
- The privacy and confidentiality of the employee should be respected throughout the termination process. This includes keeping the reasons for termination confidential and not discussing them with others unless necessary.
- The laboratory director should ensure that laboratory resources, including equipment, samples, and data, are safeguarded before and during the termination process. This can include disabling access to laboratory resources and securing any confidential or sensitive information. This may be summarized in a standardized separation checklist.
- The laboratory director should communicate the decision to terminate an employee with other staff members in a professional and respectful manner. This can include explaining the reasons



for termination without divulging confidential information and reassuring other employees that the decision was made in the best interest of the laboratory and its goals.

- Firing an employee can have an impact on other staff members in the laboratory. It is important to provide support and resources to employees who may be affected by the termination, including counseling or other forms of support as needed.
-

Question 44: B. Short-term retention bonuses.

Explanation:

- Although bonuses can be effective in the short term, they may not have a lasting impact on employee retention. Once the bonus is paid out, employees may still be dissatisfied with their job and choose to leave. Similarly, isolated salary increases without addressing the underlying areas of employee dissatisfaction and turnover are unlikely to be successful for retention purposes.
 - Conducting employee engagement surveys can help identify areas where the organization can improve and address employee concerns.
 - Providing a competitive salary and comprehensive benefits package can improve employee retention by demonstrating the organization's commitment to its employees.
 - Providing opportunities for employees to continue learning and growing in their field can increase job satisfaction and motivation.
 - Acknowledging and rewarding employees for their hard work and accomplishments can improve morale and increase job satisfaction.
 - Offering flexible scheduling options can help employees balance work and personal responsibilities and improve job satisfaction.
 - By implementing retention strategies, blood banks can reduce turnover and maintain a highly skilled and dedicated workforce.
-

Question 45: C. Express gratitude regularly.



Explanation:

- Lack of clear expectations, work overload, feeling unappreciated, dysfunctional workplace dynamics, and work-life imbalance can all contribute to employee burnout. Strategies to aid burned-out employees include:
 - Evenly distribute difficult or repetitive work.
 - Keep the workload manageable with reasonable deadlines.
 - Regularly acknowledge employee efforts and express gratitude.
 - Provide opportunities for professional development and additional training.
 - Limit reliance on consistent overtime via staffing or workload changes.
 - Encourage full use of breaks, lunches, and vacations.
 - Set a good example as the leader.
-

Question 46: A. Operations management.

Explanation:

- See answer #2.
-

Question 47: C. The organization must provide the option for her to report such concerns either to executive management or to AABB.

Explanation:

- Staff should be trained to identify and report nonconformances, which include errors and accidents in the laboratory, as well as adverse reactions in donors and recipients. Often, these may be easily communicated through regular supervisory channels, but in other cases there may appear to be more systemic issues in the facility, for example, that an employee feels less comfortable pointing out.
- *AABB Standards for Blood Banks and Transfusion Services (Standards)* requires institutional members to have a process for personnel to anonymously communicate concerns about quality or



safety, with the option to communicate concerns *either* to their own executive management *or* to AABB, *or both* (Standard 1.6). Contact information for AABB must be readily available to all personnel.



Question 48: B. Review of the organization's process for qualifying the manufacturer.

Explanation:

- AABB *Standards* lists elements that should be included in an investigation and follow-up after an equipment malfunction, failure, or adverse event (Standard 3.5.2):
 - Assessment of blood, blood components, tissue, derivatives, and services provided since the equipment was last known to be functioning per manufacturer's written instructions, or facility-defined specifications.
 - Assessment of the effect on donor eligibility and donor and patient safety.
 - Steps to ensure that the equipment is removed from service.
 - Investigation of the malfunction, failure, or adverse event, and a determination if other equipment is similarly affected.
 - Steps for requalification of the equipment.
 - Reporting the nature of the malfunction, failure, or adverse event to the manufacturer, when indicated.
- The process of supplier qualification for manufacturers who supply equipment to the facility is completed before equipment is purchased and installed. Although the possibility that a flaw in the process of selecting a supplier/manufacturer eventually made some contribution to the problem may not be completely excluded, the above steps are likely to have much greater relevance in addressing the problem.



Question 49: D. Data from IRS Form 941.



Explanation:

- IRS Form 941 (also known as the employer's quarterly federal tax return) is routinely used by employers to report taxes withheld from employee pay and to pay the employer's portion of Social Security/Medicare tax. The information on it is not useful for the purpose of supplier qualification.
- In contrast, IRS Form 990, submitted annually, provides justification for maintaining tax-exempt status, based on qualitative data as well as financial details. Because it explains the organization's mission and activities and is publicly available, it is useful in the process of supplier qualification.
- ISO certification, a quality manual, and estimated costs would all be useful.
- See the accompanying table for a list of appropriate factors to consider in supplier qualification.

Factor	Examples
Licensure, certification, or accreditation	FDA, ISO, EU
Supplier-relevant quality documents	Quality manual, complaint-handling methodology
Results of audits or inspections	Previous FDA inspections, supplier qualification audit
Supply or product requirements	Ability to meet functional requirements
Cost of materials and services	Product cost, maintenance fees, parts costs
Delivery arrangements	Standing orders, turnaround time for stat
Financial security and market position	How long the organization has been in business, IRS 990
Support after sale	Training, validation guidance, contract/agreement review meetings

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FDA = Food and Drug Administration; EU = European Union; IRS 990 = Internal Revenue Service Form 990; ISO = International Organization for Standardization.



Question 50: E. All of the above.

Explanation:

- Root cause analysis, which is used as a quality improvement strategy, can involve a variety of techniques to uncover the base cause or causes of a problem. Although there is a lack of agreement among scholars on the precise definition of *root cause*, all the choices listed in this question have consensus.
- Some of the tools used in root cause analysis are brainstorming, the fishbone diagram, failure mode effects analysis (FMEA), and the five whys.
- The analysis is followed by selection of appropriate corrective action for the nonconformance.

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Bonus Test

QUESTIONS

Question 1: All of the following are rare conditions or diseases except:

- A. Anti-GBM disease.
 - B. Hermansky-Pudlak syndrome.
 - C. Bernard-Soulier syndrome.
 - D. Chediak-Higashi syndrome.
 - E. Thalassemia.
-

Question 2: Generally, the following comparisons of TRALI and TACO are true, except:

- A. TACO and TRALI may be found in the same patient at the same time.
 - B. Diuretics may be effective in TACO but produce minimal response in TRALI.
 - C. Acute dyspnea is associated with TRALI, but no dyspnea is associated with TACO.
 - D. Hypotension may present in TRALI, and the opposite in TACO.
 - E. Chest x-rays may be useful in both TRALI and TACO.
-

Question 3: Which is not a risk factor for a clotting disorder?

- A. Prothrombin G20210A mutation.



- B. Factor V deficiency.
 - C. Protein C deficiency.
 - D. Protein S deficiency.
 - E. Antithrombin deficiency.
-

Question 4: In heparin-induced thrombocytopenia (HIT), the antibody is directed against what antigen?

- A. Heparin and platelet factor 2.
 - B. Heparin and platelet factor 4.
 - C. Heparin and platelet factor 5.
 - D. Platelet factor 4 only.
 - E. Platelet factor 5 only.
-

Question 5: With regard to argatroban and fondaparinux, which of the following is true?

- A. They are direct thrombin inhibitors.
 - B. They are indirect Factor Xa inhibitors.
 - C. They are reversed by recombinant Factor VIIa (rFVIIa).
 - D. They are reversed by PCCs.
 - E. Argatroban is a direct thrombin inhibitor; fondaparinux is an indirect Factor Xa inhibitor.
-

Question 6: You notice on a patient's medical record that an apheresis consult has been requested for NMDAR encephalitis. What does NMDAR stand for?

- A. Nonmyeloid D-axion receptor.
- B. N-methyl-D-aspartate receptor.
- C. Neuro-mediated dendritic α -region.
- D. Neuromuscular dystrophy after relapse.
- E. Nonmalignant-dysplasia autoimmune reaction.



Question 7: A patient has an activated protein C resistance (APCR) ratio of 1.5. What is the interpretation?

- A. This is normal and the patient is not at increased risk for thrombosis.
 - B. This is abnormal and the patient is at increased risk for thrombosis.
 - C. This is abnormal and the patient is at increased risk of bleeding.
 - D. This is normal and the APCR cannot be used to determine if a patient is at risk for thrombosis.
 - E. None of the above.
-

Question 8: A 5-year-old girl with blond silvery hair is being evaluated for recurrent infections and easy bruising and gum bleeding. Her skin burns easily in the sunlight and she wears sunglasses everywhere due to photosensitivity. She also has nystagmus. On platelet aggregation studies, a storage pool deficiency is seen. On peripheral blood smear, white cells contain large granules. What is your diagnosis?

- A. Hermansky-Pudlak syndrome.
 - B. Griscelli syndrome.
 - C. Chediak-Higashi syndrome.
 - D. Vici syndrome.
 - E. Familial hemophagocytic lymphohistiocytosis.
-

Question 9: A 45-year-old man is brought to the emergency department with acute chest pain and S-T segment elevations on electrocardiogram. An emergency cardiac catheterization reveals a single high-grade obstruction of the left anterior descending artery. An attempt at angioplasty is made but is unsuccessful. Abciximab was administered during the attempted angioplasty. The patient is now taken for emergent coronary bypass surgery. Regarding the administration of abciximab, which of the following statements is true?



- A. Abciximab is a coagulation factor inhibitor; therefore, Fresh Frozen Plasma should be transfused to reverse the anticoagulation.
 - B. The patient should receive 2 units of platelets before surgery.
 - C. The patient should undergo dialysis to remove free abciximab from the plasma.
 - D. Platelets should be made available for surgery and transfused if there is evidence of excessive bleeding.
 - E. The patient will likely need Cryoprecipitated AHF transfusion during surgery.
-

Question 10: Several months ago, a 71-year-old female was treated for a deep vein thrombosis and pulmonary embolism and discharged on rivaroxaban. Today, she was transferred by ambulance from a motor vehicle collision where she sustained severe life-threatening injuries and requires emergent surgery. Laboratory work from the emergency department shows:

$$\text{PT} = 16.7 \text{ seconds (normal, 10.2-14.2 seconds)}$$
$$\text{aPTT} = 47.4 \text{ seconds (normal, 26.8-41.2 seconds)}$$

What should be given to reverse the rivaroxaban before surgery?

- A. Plasma.
 - B. Cryoprecipitated AHF.
 - C. Prothrombin complex concentrate.
 - D. Andexanet alfa.
 - E. Idarucizumab.
-

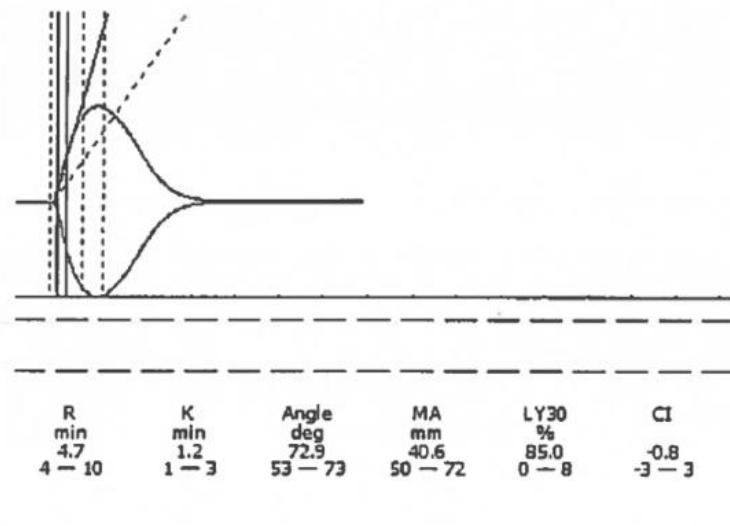
Question 11: A 26-year-old male with hemophilia A is taking HEM-LIBRA (emicizumab-kxwh). The patient presents to the emergency room after a car accident with head trauma and is found to have intracranial bleeding as well as spleen laceration and a broken femur. Which of the following tests will not be affected by the HEM-LIBRA (emicizumab-kxwh)?

- A. aPTT.
- B. Clotting-based Factor VIII activity.



- C. Chromogenic Factor VIII assay with human Factor IXa and Factor X.
 - D. Chromogenic Factor VIII assay with bovine Factor IXa and Factor X.
 - E. All of the above would be affected.
-

Question 12: A 23-year-old male is in the emergency room with a gunshot wound to the abdomen. Blood is drawn for laboratory testing, including type and screen. The following thromboelastography (TEG) tracing is generated.



What is the best description of the TEG?

- A. Hypercoagulable with hyperfibrinolysis.
 - B. Hypocoagulable with hyperfibrinolysis.
 - C. Hyperfibrinolysis only.
 - D. Hypocoagulable only.
 - E. Hypercoagulable only.
-

Question 13: Based on the TEG tracing in question #12, what should be given to the patient?



- A. Tranexamic acid.
 - B. Plasma.
 - C. Heparin.
 - D. Prothrombin complex concentrate.
 - E. Vitamin K.
-

Question 14: A 34-year-old male was shot in the abdomen and received 3 units of Red Blood Cells (RBCs), 2 units of plasma, and a unit of platelets before surgery. Following surgery, he was in the intensive care unit (ICU) and received another RBC unit. Ninety minutes into the transfusion, the patient coded and died. How long do you have to transmit a written report to the Center for Biologics Evaluation and Research (CBER)?

- A. 1 day.
 - B. 3 days.
 - C. 7 days.
 - D. 14 days.
 - E. 30 days.
-

Question 15: Allogeneic skin grafts have been involved in the transmission of which disease agent?

- A. Harmful species of bacteria.
- B. Toxic fungi.
- C. Cytomegalovirus.
- D. Human immunodeficiency virus (HIV).
- E. All of the above.

ANSWERS

Question 1: E. Thalassemia.



Explanation:

- Anti-GBM (glomerular basement membrane) disease is a rare (1 in 1,000,000) autoimmune disorder that occurs when the immune system mistakenly attacks and destroys healthy body tissue. It used to be known as Goodpasture syndrome.
- Hermansky-Pudlak syndrome is a rare (1-9 in 1,000,000) autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding diathesis, and other organ involvement specific to one of 11 types.
- Bernard-Soulier syndrome is a bleeding disorder associated with large platelets, thrombocytopenia, easy bruising, and bleeding. It affects approximately 1 in 1,000,000 people. In Bernard-Soulier syndrome, platelets are defective in binding to vWF.
- Chediak-Higashi syndrome is a condition that affects many parts of the body, particularly the immune system. A rare (1 in 1,000,000) autosomal recessive disorder, the syndrome is characterized by recurrent bacterial infections.
- Worldwide, the incidence of thalassemia is 4.4 in 10,000. The term represents a group of congenital disorders affecting hemoglobin production, particularly prevalent in the Mediterranean, Middle East, Africa, central Asia, the Indian subcontinent, and the Far East.



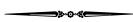
Question 2: C. Acute dyspnea is associated with TRALI, but no dyspnea is associated with TACO.

Explanation:

- In fact, acute dyspnea is a similar feature in these two adverse reactions to transfusion, although it is important to note that patients with either transfusion-related acute lung injury (TRALI) or transfusion-associated circulatory overload (TACO) may lack some typical features of these conditions.
- Furthermore, patients with TRALI may have some features suggestive of TACO (or vice versa). In addition, TRALI and TACO can on occasion present concurrently.

**Question 3: B.** Factor V deficiency.**Explanation:**

- Prothrombin G20210A mutation arises from a single missense mutation [G→A] at position 20210 of the prothrombin gene. The mutation affects the terminal 3' nucleotide of the 3' untranslated region of the prothrombin mRNA and leads to elevated plasma levels of prothrombin. Patients who are heterozygous for the prothrombin G20210A mutation have prothrombin levels that are increased by ~30%, and homozygous patients have prothrombin levels increased by ~70%.
- Increased prothrombin levels are associated with increased thrombin generation and correlate with an increased risk of thrombosis. Similarly, decreased levels of antithrombin, protein S, or protein C are associated with increased clotting risk, as each of these functions as an anticoagulant.
- Factor V deficiency would result in a tendency to bleed and not clot. Factor V Leiden mutation is associated with a thrombotic predisposition.

**Question 4: B.** Heparin and platelet factor 4.**Explanation:**

- HIT is an immune-mediated disorder in which heparin binds to platelet factor 4 (PF4) and leads to the formation of IgG PF4/heparin antibodies and the formation of IgG heparin/PF4 immune complexes. The antibodies can bind to the Fc receptor on platelets, leading to platelet activation.
- Overall, HIT is a thrombogenic state. In HIT, the platelet count usually declines within 5 to 14 days of the start of heparin but may decrease more rapidly in individuals who have been previously exposed to heparin and who have developed PF4/heparin IgG antibodies. The HIT antibodies are frequently undetectable after 100 days. After cessation of heparin, the platelet counts usually rise within 2 to 3 days and usually normalize within 10 to 14 days.



Question 5: E. Argatroban is a direct thrombin inhibitor; fondaparinux is an indirect Factor Xa inhibitor.

Explanation:

- Until recently, anticoagulation therapy has been based on unfractionated heparin (UFH), low-molecular-weight heparin (LMWH), and vitamin K antagonists. A high risk of adverse consequences and difficulty of use have driven a search for alternative agents. Alternatives currently available (or in the process of clinical evaluation) include drugs targeted to the inhibition of thrombin, activated Factor X, and the tissue factor (TF)-FVIIa complex.
- Argatroban and bivalirudin are direct thrombin inhibitors that are administered intravenously and monitored using the activated partial thromboplastin time (aPTT). For argatroban, a target of 1.5 to 3 times the aPTT steady-state baseline is recommended. The Food and Drug Administration (FDA) has approved their use for HIT and percutaneous coronary interventions.
- Reversal of direct thrombin inhibitors:
 - Direct thrombin inhibitors have short half-lives and are administered as continuous infusions. Reversal is dependent primarily on stopping the infusion.
 - If rapid reversal is required, activated prothrombin complex concentrates (PCCs) have been reported to be more effective than rFVIIa.
- Fondaparinux is an indirect Factor Xa inhibitor that is FDA approved for the prevention and treatment of venous thromboembolism. It is a pentasaccharide that binds to antithrombin (AT), inducing a conformational change that increases the natural Factor-Xa-inhibitory effect of AT 300-fold. Upon AT binding to Factor Xa, fondaparinux is released and is available to act upon other free AT molecules. Bound Factor-Xa/antithrombin complexes are cleared from the circulation.
- Fondaparinux is not reversed by administration of Fresh Frozen Plasma, PCCs, or protamine.



Question 6: B. N-methyl-D-aspartate receptor.



Explanation:

- NMDAR encephalitis is an acute inflammatory brain disorder characterized by IgG antibodies targeting subunits of the NMDAR. NMDAR encephalitis typically affects children and young adults, with a female predominance. Up to 58% of affected young female patients have an ovarian teratoma.
 - NMDAR is a Category I indication for therapeutic plasma exchange (TPE). Typically, 1 to 1.5 plasma volumes are exchanged using 5% albumin for 5 to 12 treatments with TPE over 1 to 3 weeks.
 - The other choices are fictitious.
-

Question 7: B. This is abnormal and the patient is at increased risk for thrombosis.

Explanation:

- The original test for APC resistance (APCR) involved measuring the aPTT of a plasma sample with and without the addition of exogenous APC. In a plasma sample without APCR, addition of APC inactivates Factor Va and Factor VIIIa and thus prolongs the clotting time of the aPTT. In contrast, in a sample with the Factor V Leiden mutation or another mutation, the prolongation in the clotting time is less.
 - A ratio is derived from: $[aPTT + APC]/[aPTT - APC]$.
 - A limitation of this test is that it requires that the patient have a normal aPTT. Individuals without the Factor V Leiden mutation generally have a ratio of >2.0 , and individuals who are heterozygous for the Factor V Leiden mutation have a ratio <2.0 .
-

Question 8: C. Chediak-Higashi syndrome.

Explanation:

- In Chediak-Higashi syndrome, white cells contain abnormally large granules. These abnormal granules affect the ability of the white cells to fight infection. Children are susceptible to frequent



bacterial, viral, and fungal infections, particularly of the skin and respiratory tract. Platelets are normal in number, but they do not function properly, causing easy bruising or prolonged bleeding.

- Griscelli syndrome is a rare inherited disorder characterized by partial albinism and abnormalities of platelets and white cells. The symptoms are similar to those seen in Chediak-Higashi syndrome. In Griscelli syndrome, the white blood cells do not have the giant granules like those seen in Chediak-Higashi syndrome.
- Hermansky-Pudlak syndrome is a rare inherited disorder characterized by reduced skin, hair, and/or eye pigmentation, abnormal platelets, and the excessive storage of ceroid in various parts of the body. The symptoms of Hermansky-Pudlak syndrome include reduced color in the skin, hair, and eyes, impaired vision, and excessive bleeding. Fatty deposits of ceroid in the lungs, intestines, heart, and/or kidneys may cause impaired function in many organs of the body.
- Familial hemophagocytic lymphohistiocytosis (FHL) is characterized by highly active macrophages and T lymphocytes and enlarged liver and spleen. Neurologic symptoms also may present in the form of pressure in the brain, irritability, stiff neck, low muscle tone, spastic muscles, convulsions, cranial nerve palsies, loss of muscle control, hemi/quadriplegia, blindness, and coma. Median survival for children is less than 2 months, usually due to infection. This disease is autosomal recessive and caused by a mutation in one of the FHL1-FHL5 genes.
- Vici syndrome is characterized by a lack of pigment, immunodeficiency, lack of development of the corpus callosum, cataracts of both eyes, and cleft lip and palate. Cognitive impairment, seizures, and severe respiratory infections have also been observed with this syndrome.



Question 9: D. Platelets should be made available for surgery and transfused if there is evidence of excessive bleeding.

Explanation:

- Platelet-mediated coronary thrombosis is the primary pathophysiological mechanism of acute coronary syndromes and acute ischemic complications of percutaneous interventions. Inhibition of



platelet aggregation is an essential therapeutic intervention in the management of acute coronary syndromes.

- Abciximab (ReoPro, Centocor BV) is a GPIIb/IIIa receptor antagonist. Two other GPIIb/IIIa antagonists are available for use: tirofiban (Aggrastat, Medicure Pharma) and eptifibatide (Integrelin, Schering-Plough).
 - Abciximab is a monoclonal antibody with a strong affinity for the GPIIb/IIIa receptor.
 - Although the serum half-life is only 15 to 30 minutes, it takes approximately 48 hours to return to 50% of baseline platelet function.
- Eptifibatide and tirofiban are drugs that have weaker affinity for the GPIIb/IIIa receptor.
 - Their half-lives are 2.5 and 2 to 2.5 hours, respectively.
 - After discontinuation of infusion, platelet aggregation returns to baseline within 4 to 8 hours.
 - Patients who require emergency cardiac surgery following attempted angioplasty are likely to have received a GPIIb/IIIa inhibitor and are at increased risk of bleeding during surgery.
 - Whenever possible, the drug should be discontinued at least 12 hours before surgery. The risk of bleeding with abciximab is directly related to the dose of heparin used for coronary artery bypass graft (CABG). At low doses of heparin, the incidence of major hemorrhage with abciximab is comparable to that of patients who did not receive abciximab. Because there is little free abciximab in the plasma, platelet infusion replenishes the number of GPIIb/IIIa receptors available for interacting with fibrinogen. When surgery cannot be delayed, platelet transfusion should be administered to those patients who demonstrate unexpected bleeding. Evidence to support the routine administration of platelets to all patients on abciximab is lacking.
 - Tirofiban and eptifibatide do circulate free in the plasma. Because they undergo significant renal elimination, platelet function returns to baseline within 4 to 8 hours of discontinuation. The drugs should be discontinued a minimum of 4 hours before surgery. If immediate reversal is necessary, the presence of free drug makes platelet transfusion much less effective.



- Clopidogrel
 - Clopidogrel is an irreversible inhibitor of adenosine diphosphate (ADP)-induced platelet aggregation and the subsequent ADP-mediated activation of the GPIIb/IIIa complex.
 - Clopidogrel is used in combination with aspirin in the management of acute coronary syndromes. Patients taking clopidogrel are at higher risk of excessive bleeding during CABG.
 - The half-life is 8 hours.
 - Whenever possible, the drug should be discontinued at least 5 to 7 days before surgery.
 - In case of an urgent invasive procedure or surgery, it is desirable to wait at least 6 hours (if possible) after a dose of clopidogrel, to avoid the inhibitory effect of the drug on the platelets transfused to correct the hemostasis.
 - Platelets may be administered for reversal of clopidogrel. The efficacy of this therapy, or the number of doses required, has not been formally studied.
- Aspirin
 - Aspirin irreversibly inhibits platelet cyclooxygenase-reducing synthesis of thromboxane A₂, a potent platelet aggregator and vasoconstrictor.
 - Doses of 75 mg/day achieve complete blockage of platelet cyclooxygenase.
 - As new platelets enter the circulation, platelet response to aggregating agents is restored.
 - Platelets inhibited with aspirin are capable of participating in adhesion and aggregation with platelets not exposed to aspirin.
 - Aspirin inhibition of platelets does not usually necessitate platelet transfusion.



Question 10: D. Andexanet alfa.

Explanation:

- The patient is taking a direct Factor Xa inhibitor, rivaroxaban, as evidenced by her medical record as well as prolonged prothrombin time (PT) and prolonged aPTT. She should receive andexanet alfa, which is FDA approved to reverse rivaroxaban and apixaban.



- Andexanet alfa is a recombinant-modified Factor Xa protein that binds the circulating direct Factor Xa inhibitor. Andexanet alfa acts as a decoy and sequesters rivaroxaban or apixaban, inhibiting them from binding to the patient's own Factor Xa. The increase in available Factor Xa reduces anticoagulant action, which can be measured by anti-Factor-Xa activity, thrombin generation, or unbound Factor-Xa-inhibitor plasma concentration from baseline. Idarucizumab can be used for reversal of dabigatran.
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Question 11: D. Chromogenic Factor VIII assay with bovine Factor IXa and Factor X.

Explanation:

- Emicizumab is an engineered IgG4 bispecific antibody that binds both Factor IXa and Factor X. This interaction colocalizes the components of the intrinsic tenase complex and improves coagulation. It is effective in hemophilia A patients with and without Factor VIII inhibitors. It affects a variety of coagulation-based assays and shortens the aPTT assay. The PT is minimally affected and the thrombin time and Clauss fibrinogen assay are unaffected by emicizumab. The chromogenic assays for anti-Xa, protein C, and antithrombin are not affected by emicizumab.
 - Emicizumab also interferes with Factor VIII chromogenic assays that use human Factor IXa and human Factor X; however, it does not affect chromogenic Factor VIII assays where bovine Factor IXa and bovine Factor X are used. Several studies have indicated that the chromogenic Factor VIII assay can be used with human Factor IXa and bovine Factor X with minimal interference by emicizumab.
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Question 12: C. Hyperfibrinolysis only.

Explanation:

- The patient has an elevated LY30 (clot lysis at 30 minutes after maximum clot strength) of 85% and a decreased maximum



amplitude (MA). The overall coagulation index (CI) is –0.8. The patient has hyperfibrinolysis and normal CI despite the low MA.

- The patient would benefit from tranexamic acid to treat the fibrinolysis, and platelets and/or cryoprecipitate to address the reduced MA.
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Question 13: A. Tranexamic acid.

Explanation:

- See answer #12.
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Question 14: C. 7 days.

Explanation:

- More information can be found in the FDA Guidance for Industry: Notifying FDA of Fatalities Related to Blood Collection or Transfusion (September 2003).
- The FDA is composed of several centers that regulate different areas. CBER is responsible for the regulation of biological products such as blood and vaccines. Other centers include the Center for Drug Evaluation and Research (CDER), Center for Devices and Radiological Health (CDRH), and the Center for Food Safety and Applied Nutrition (CFSAN).
- 21 CFR 606.170(b) states: “When a complication of blood collection or transfusion is confirmed to be fatal, the Director, Office of Compliance and Biologics Quality, CBER, must be notified by telephone, facsimile, express mail, or electronically transmitted mail as soon as possible. A written report of the investigation must be submitted to the Director, Office of Compliance and Biologics Quality, CBER, by mail, facsimile, or electronically transmitted mail [for mailing address, see 21 CFR 600.2(a)], within 7 days after the fatality by the collecting facility in the event of a donor reaction, or by the facility that performed the compatibility tests in the event of a transfusion reaction.”



- In the event that the report submitted at 7 days is not complete, the FDA requires that the report be amended by submitting the missing material as it becomes available.
- Materials to be included in the written report include: 1) discharge summary and/or death certificate; 2) autopsy report (if performed); and 3) conclusions and follow-up actions if appropriate.
- Additional information that should also be included is outlined in the FDA guidance. For blood donors, this includes the following:
 - The deceased's donor record file that includes the donation just before the fatal incident and information on all donations during the past 2 years.
 - Lot numbers and expiration dates of collection sets or harnesses; if replacement fluid(s) was given during the collection, indication should be given for which fluid(s) and the unit or lot number(s) and any other relevant information, such as manufacturer's notices, contamination warnings, or replacement fluid recalls.
 - Performance log for the device and any other relevant performance logs, maintenance records, manufacturer's notices, or recalls on significant machine part(s) on the device/system during the past 2 years.
 - If the donor was hospitalized due to the reaction, any relevant documents should be provided (eg, reports of laboratory tests) that may help determine the cause of the fatality.
- Similar notification is required if a patient's death *may* be caused by transfusion, a therapeutic apheresis procedure, or a therapeutic phlebotomy. The requested information varies according to which treatment is associated with the death.



Question 15: E. All of the above.

Explanation:

- All of the infectious diseases have been transmitted by allogeneic skin grafts.
- Skin banks perform microbial cultures before grafts are released for transplantation in order to minimize disease transmission.
- Standards of the American Association of Tissue Banks require that skin be discarded if pathogenic fungi or bacteria are present.



This includes coagulase-positive *Staphylococci*, group A beta-hemolytic *Streptococci*, *Enterococci*, Gram-negative organisms, *Clostridia* species, yeast, and fungi.

Note: For references related to the answers in this bonus test, see the respective chapters containing related content.

