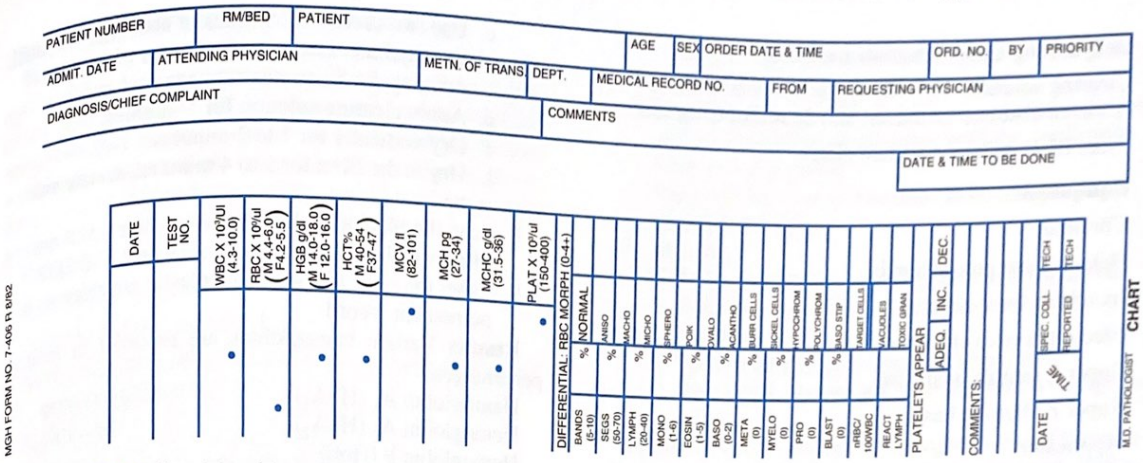


FIGURE 28–29 Hematology laboratory report sample form illustrating the complete blood count (CBC).

Procedure

1. Centrifuge the blood sample for 5 minutes at 2000 rpm.
2. Transfer the top layer of cells (approximately 0.5 mL) to a Wintrobe hematocrit tube. This layer will contain red cells.
3. Centrifuge the Wintrobe tube for 5 minutes at 2000 rpm.
4. Carefully remove the plasma without disturbing the cells.
5. Transfer the top layer of cells (approximately 0.03 mL) to a 20-mL plastic vial.
6. Prepare a 1% to 2% RBC suspension with normal saline in the plastic vial.
7. Add 3 drops of 22% bovine albumin to a 10-mL RBC suspension.
8. Add 6 drops of the suspension to the cytocentrifuge holders.
9. Centrifuge the specimen for 5 minutes at 1400 rpm.
10. Allow the slides to air-dry before staining with Wright-Giemsa stain.

Comments

1. This method of buffy coat preparation alleviates the tedious counting of a low number of WBCs on the peripheral blood smear and misrepresentation of a 100 white cell differential.
2. The amount of albumin added may vary depending on the presence of artifacts on the blood smear. If there is an excess of albumin, the cells will appear too dark.

➤ TESTS FOR HEMOGLOBINS

Hemoglobinometry

Principle Hemoglobin, the main component of the RBC, transports oxygen to and CO_2 from the body's tissues. Hemoglobin in circulating blood is a mixture of hemoglobin, oxyhemoglobin, carboxyhemoglobin, and minor amounts of other forms of this pigment. It is necessary to prepare a stable derivative involving all forms of hemoglobin in the blood in order to measure this compound accurately. The cyanmethemoglobin (HiCN) derivative can be conveniently and reproducibly prepared and is widely used for hemoglobin determination. All forms of circulating hemoglobin are readily converted to HiCN except for sulfhemoglobin, which is rarely present in significant amounts. Cyanmethemoglobin can be measured accurately by its absorbance in a colorimeter.

The basic principle of the cyanmethemoglobin (HiCN, hemoglobin-cyanide method) is that blood diluted in a solution of potassium ferricyanide yields oxidation to the ferric state (Fe^{3+}) to form methemoglobin (Hi-hemoglobin). This solution reacts with potassium cyanide to form stable cyanmethemoglobin, which is read on a spectrophotometer at 540 nm.

Procedure Automated hematology analyzers routinely perform hemoglobin determinations. The reader is referred to manufacturer instructions for specific methodology.

Interpretation

Normal Values (g/100 mL)

Man	14–18
Woman	12–16
Newborn	17–23
3-month-old	9–14
10-year-old	12–14.5

Hemoglobin Electrophoresis

Principle Electrophoresis is defined as the movement of charged particles in an electric field. The different normal and abnormal hemoglobins show different mobilities of migration patterns in an electric field at a fixed pH. The usual support medium is cellulose acetate at an alkaline pH of 8.5. The procedure that follows is from Helena Laboratories, and all reagents and apparatus are available through their organization.[24]

Reagents

Hemolysate reagent

Controls A_1FSC; normal A_1A_2 patient

Buffer: Supre-Heme buffer (one envelope is dissolved in distilled water and diluted to 980 mL tris-EDTA-boric acid buffer, pH 8.4)

Ponceau S stain

Destain: 5 mL of glacial acetic acid per 100 mL distilled water, a 5% solution

Dehydrating agent: Absolute methanol

Clearing solution: 150 mL of glacial acetic acid, 350 mL of absolute methanol, and 20 mL of Clear aid

Titan III-H cellulose acetate plates

Equipment

Cliniscan

Helena Titan power supply

Incubator-oven-dryer

Electrophoresis chamber

Super Z sample well plate

Super Z aligning base

Applicator

Zip-zone chamber wicks

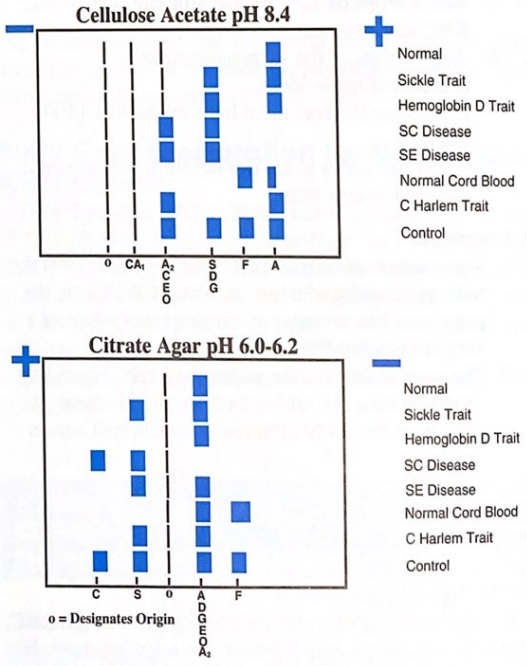
Procedure

1. Preparation of hemolysate: Spin EDTA blood for 20 minutes at 3000 rpm to pack the RBCs. Remove the plasma and buffy coat. Add 6 drops of hemolysate reagent to 1 drop of packed RBCs. Let stand for 1 minute; then vortex for 1 minute. Hemolysate may be frozen and then thawed to ensure complete hemolysis.
2. Preparation of electrophoretic chamber: Pour 100 mL of buffer into each outer compartment. Soak a wick in each compartment, and then drape it over the bridge, making sure it contacts the buffer. Cover the chamber.
3. Preparation of cellulose acetate plates: Number the plates on the bottom right of the glossy side. Wet the plates by slowly lowering the rack into a container of buffer. Allow them to soak for at least 5 minutes.
4. Preparation of sample well plates: Clean with distilled water and dry each well with a cotton swab. Prepare two rinse plates by filling the wells with distilled water. Prepare the patient samples by using a 5-lambda (λ) microdispenser to fill the wells on clean dry plates. Patient samples should be run in duplicate, and a normal A_1A_2 and A_1FSC control should be run on each plate. Cover with glass slide to prevent evaporation.
5. Loading the cellulose acetate plates:
   a. Prime the applicator by depressing several times into the same well plate and then depressing once on a blotter.
   b. Remove the cellulose acetate plate from the buffer; blot once firmly and place on the aligning base with the number at the bottom left.
   c. Load the applicator by depressing three times into the sample well plate; then transfer the applicator to the aligning base, and depress the bar firmly for 5 seconds.
   d. Place the plate, glossy side up, across the bridge in the electrophoresis chamber.
6. Electrophoresis at 350 volts for 25 minutes.
7. Staining:
   a. Apply Ponceau S for 5 minutes. Drain for 5 to 10 seconds.
   b. Four successive washes of 5% glacial acetic acid are used to destain. Leave in each for 2 minutes, draining for 5 seconds between each wash.
   c. Use two successive washes of absolute methanol to dehydrate. Leave for 2 minutes in each, draining for 5 seconds between each wash.
   d. Apply clearing solution for 5 minutes.
   e. Dry vertically for 1 to 2 minutes.
   f. Dry in the oven for 3 to 4 minutes, acetate side up.
8. Scan the plate with the Cliniscan using a 525-nm filter, slit size 5, and optics filter wheel V-2 O.D.
9. Label the plate and store in a plastic envelope as a permanent record.

Results Variant hemoglobins are reported in relative percentages.

Hemoglobin A_1 (HbA_1)	95%–97%
Hemoglobin A_2 (HbA_2)	2%–3%
Hemoglobin F (HbF)	
Birth	60.0%–90.0%
After 1 year	1.0%–2.0%

Comments At an alkaline pH, hemoglobins S and D have the same mobility, as do hemoglobins A_2, C, E, and O_{Arab}. These hemoglobins may be separated by electrophoresing on citrate agar at an acid pH (Fig. 28–30). Hemoglobin A_2 may also be quantitated by column. HbF separates from HbA in this system and migrates slightly closer to the origin. However, cellulose acetate electrophoresis is not recommended as an initial screening test during the neonatal period because large amounts of HbF form a heavy band overlapping the adjacent bands of HbA or HbS.[25] The procedure for separation and quantitation of HbF is based on acid or alkali resistance, or both (see Acid Elution Test for HbF).



➤ **FIGURE 28–30** Comparative hemoglobin electrophoresis. Hemoglobin electrophoresis on cellulose acetate and citrate agar, indicating patterns of mobility. The width of the band is not indicative of hemoglobin concentration.

Citrate Agar Hemoglobin Electrophoresis

Principle As mentioned earlier, electrophoresis is the movement of charged particles in an electric field. Using citrate agar at an acid pH facilitates the separation of hemoglobins that migrate together on other media (cellulose acetate) at a different pH (alkaline). The following procedure is that of Helena Laboratories, and all reagents and apparatus are available from their organization.

Reagents

Hemolysate reagent

Controls A_1FSC; normal A_1A_2

Buffer: Citrate buffer. Dissolve one package in distilled water and dilute to 1 L.

Stain:

10 mL of 5% glacial acetic acid

5 mL of toluidine in methanol

1 mL of sodium nitroferricyanide in water

1 mL of 3% hydrogen peroxide

Note: Prepare fresh on day of use

Titan IV citrate agar plates

Equipment

Helena Titan power supply

Electrophoresis chamber

Sample well plate

Aligning base

Applicator

Sponge wicks

Procedure

1. Preparation of hemolysate: Spin EDTA blood for 20 minutes at 3000 rpm to pack the red cells. Remove the plasma and buffy coat. Add 10 drops of hemolysate reagent to 1 drop of packed RBCs. Let stand for 1 minute, then vortex for 1 minute. Hemolysate may be frozen and then thawed to ensure complete hemolysis.
2. Preparation of electrophoresis chamber: Pour 100 mL of buffer into each outer compartment. Soak a sponge wick in each compartment, then place it so that the top of the sponge protrudes over the inner ridge of the compartment. Cover the chamber.
3. Preparation of sample well plates: Clean all wells with distilled water and dry with cotton swabs. Prepare the patient samples by using a 5-λ microdispenser to fill the wells. Patient hemolysates should be run in duplicate, plus an A_1FSC and an A_1A_2 control should be run on each plate. Cover the plate with a glass slide to prevent evaporation.
4. Loading of citrate agar plates:
   a. Prime the applicator by pressing several times into the sample well plate, and then dispensing once on a blotter.
   b. Place the Titan IV citrate agar plate on the aligning base.
   c. Load the applicator by pressing three times into the sample well plate; then transfer the applicator to the aligning base.
   d. Depress the applicator onto the gel surface using no pressure, and allow hemolysate to absorb for 1 minute.
6. Place the plate gel side down across the inner ridges of the electrophoresis chamber with the application point near the anode.
7. Electrophorese for 40 minutes, at 40 mA per plate and 50 V per plate.
8. Staining:
   a. Place the plate in a staining dish and puddle the stain over the surface. Let stand for 5 to 10 minutes.
   b. Rinse with distilled water for 10 minutes.
   c. Cover with another gel plate and seal with tape to store.

Results With this procedure, hemoglobin S and D can be separated. Hemoglobin D, instead of migrating with HbS as in an alkaline buffer, will migrate with HbA. This procedure also separates hemoglobins A_2 and E from HbC, as hemoglobins A_2 and E will migrate with HbA, leaving HbC by itself. The pattern from cathode to anode is hemoglobin C–hemoglobin S–hemoglobin A_1–hemoglobin A_2–hemoglobin D–hemoglobin E–hemoglobin F (see Fig. 28–30).

Hemoglobin A_2 by Column Chromatography

Principle This is an anion-exchange chromatography method. The anion exchange resin is a preparation of cellulose covalently coupled to small, positively charged molecules, which will attract negatively charged molecules. Hemoglobins have positive or negative charges, owing to properties of their component amino acids. Here buffer and pH favor net negatively charged hemoglobins, which are attracted and bound to the resin. Once bound, the hemoglobins can be selectively eluted and measured on a spectrophotometer. This procedure (the Sickle-Thal column method) is that of Helena Laboratories.

Reagents

Control: Quik column control

Sickle-Thal Quik Column

Hemoglobin A_2 developer

Hemoglobin S developer

Hemolysate reagent C

Note: All are available from various manufacturers.

Equipment

Column rack and collection tubes

Spectrophotometer

Procedure

1. Preparation of hemolysate: Add 50-µL EDTA blood plus 200-µL hemolysate reagent C to a small test tube. Vortex vigorously and allow to stand 5 minutes before use.
2. Preparation of columns:
   a. Allow to come to room temperature.
   b. Turn each column upside down twice, place it in the rack, remove top cap, and resuspend with a pipet.
   c. Remove the bottom cap and allow the buffer to drain out.
   d. After the resin repacks, remove any buffer

remaining at the top, being careful not to disturb the resin.
3. Slowly apply 100 μL of patient hemolysate to the column and allow it to absorb into the resin.
4. Put 100 μL of patient hemolysate in a large collection tube and quantity sufficient (Q.S.) to 15 mL with distilled water. Label this tube "total fraction."
5. Elution of HbA_2:
   a. Slowly apply 3 mL of HbA_2 developer and allow it to pass through the column into the small collection tube (approximately 30 minutes).
   b. Q.S. the tube to 3 mL with distilled water.
6. Elution of HbS (optional):
   a. Slowly add 10 mL of HbS developer to the column in aliquots of 3 mL, 3 mL, and 4 mL.
   b. Allow it to pass through the column into a large collection tube (approximately 1.5 to 2 hours).
   c. Q.S. the tube to 15 mL with distilled water.
7. Using the spectrophotometer at 415 nm, record the absorbance of the HbA_2 eluate, the HbS eluate, and the total fraction.

Results

$$\%\ HbA_2 = \frac{OD\ HbA_2\ eluate \times 100}{5 \times OD\ total\ fraction}$$

$$\%\ HbA_2 = \frac{OD\ HbS\ eluate \times 100}{OD\ total\ fraction}$$

where OD = optical density.

The HbS eluate is optional, as it can be picked up on alkaline electrophoresis. The normal range for HbA_2 is 1.5% to 4.0%. This can be used to separate HbA_2 and HbC or HbE. Elevated levels of HbA_2 may be useful in diagnosing beta (β)-thalassemia. Be careful not to underload or overload the column with the hemolysate. Overloading the column may cause incomplete separation of the HbA_2. Underloading the column may make visual collection of the HbA_2 fraction impossible.[26]

Solubility Test for Hemoglobin S

Principle Sickling hemoglobin, which is defined as any hemoglobin that causes erythrocytes to sickle under conditions of low oxygen tension, in a deoxygenated state will form a precipitate when exposed to a high-molarity phosphate buffer solution.[27] This precipitation is the result of tactoids forming from deoxygenated hemoglobin molecules, producing a turbid solution. This turbidity is qualitatively determined from the inability to visualize black type lines on a white background. This procedure utilizes packed RBCs.

Reagents and Equipment

12 × 75 mm disposable glass or plastic test tubes

Reading card with 14-point or 18-point black type in straight lines on a white background approximately 0.5 cm apart (see Fig. 11–11)

Centrifuge (1500–2000 g)

Stock solution:

Dibasic potassium phosphate, anhydrous (1.24 mol), K_2HPO_4, 216 g

Monobasic potassium phosphate, crystals (1.24 mol), KH_2PO_4, 169 g

Saponin, 10 g

Distilled water, qs to 1 L

Note: Reagent should be stored at 4°C for approximately 1 month and should be checked against a known positive and negative control. There are many commercial kits available that mimic this procedure (i.e., Sickle-quik, General Diagnostics), and each new reagent should be checked with known positive and negative controls.

Working Solution Sodium hydrosulfite (dithionite) $Na_2S_2O_4$ (5 mg/mL of stock reagent) is added on the day of testing.

Procedure

1. Pipet 2 mL of working solution into a labeled 12 × 75 mm test tube.
2. Allow the working solution to warm to room temperature.
3. Centrifuge whole blood (EDTA) at 1500 to 2000 g for 5 minutes to remove buffy coat and plasma.
4. Add 10 μL of packed erythrocytes.
5. Mix and wait 5 minutes.
6. Hold the tube approximately 2.5 cm in front of the white card with black lines.
7. Read for qualitative determination of turbidity.

Results A negative result (indicating no sickling hemoglobins) occurs when the black lines are visible through the test solution.

A positive result (indicating the presence of a sickling hemoglobin) is indicated by a very turbid solution in which the black lines cannot be seen through the test solution (see Fig. 11–11). Other sickling hemoglobins may be present; therefore, this test is not specific for hemoglobin S. Table 28–8 depicts hemoglobins that can be present from negative and positive results of this test. Both positive and negative controls must be run for each solubility test performed.

Comments

1. Erroneous results may be seen in normal blood transfused to an anemic patient whose native blood contains sickling hemoglobin, or in transfused blood that contains a sickling hemoglobin.
2. Cold reagent may cause inaccurate results; the same holds true when whole blood is used instead of packed cells, the latter because of excess gamma globulins, extreme leukocytosis, or hyperlipidemia.[25]

➤ Table 28–8
HEMOGLOBINS PRESENT POSITIVE/ NEGATIVE SOLUBILITY TESTS

Positive	Negative
HbC_{Harlem}	HbA
HbS	HbF
HbS_{Travis}	HbC
$HbC_{Ziguinchor}$	HbD
	HbG
	HbE
	Hb Lepore
	HbA_2

Acid Elution Test for Hemoglobin F

Principle The passage of erythrocytes from an Rh-positive fetus into the circulation of an Rh-negative mother results in the formation of specific Rh antibodies. In subsequent pregnancies, the Rh antibodies formed in the blood serum of the Rh-negative mother are readily transmissible through the placenta into the circulation of the fetus. The action of the antibodies on the Rh-positive cells of the fetus may result in a disease entity recognized as isohemolytic disease, or erythroblastosis.[28] The acid elution procedure will detect fetal red blood cells in maternal circulation based on the properties of adult hemoglobin and fetal hemoglobin. Adult hemoglobin (HbA) will dissolve out of solution, whereas fetal hemoglobin (HbF) will be resistant to the acid medium and stain pink. This procedure is performed on Rh-negative mothers who have been sensitized to the Rh factor as a consequence of giving birth to an Rh-positive fetus. Mothers are administered specific gamma globulin containing anti-Rh_o(D) to suppress the immune response. The amount of Rh immune globulin (RhIg) administered is calculated by assessing the magnitude of fetal/maternal hemorrhage.

Reagents

Red Cell Fixing Solution, Product No. 101–10 (Sure-Tech Diagnostics Inc.): Ethanol 80% v/v denatured

Citrate/Phosphate buffer, Product No. 101–20 (Sure-Tech Diagnostics): 0.2 mol/L

Hemoglobin Staining Solution, Product No. 101–30 (Sure-Tech Diagnostics, Inc): Erythrosin, 0.1%

Note: Reagents are stored at room temperature and are stable for the period indicated on the label. Do not pour used reagents back into original containers.

Specimen Maternal blood collected with EDTA or oxalate should be used. Samples should be stored at 2°C to 8°C until assayed. Blood-EDTA mixtures have been reported to be satisfactory for use up to 2 weeks when stored under refrigeration It is recommended that specimens be tested as soon as possible.

Quality Control A high-positive control may be prepared by adding 0.1 mL of cord blood to 0.9 mL adult blood. A low-positive control may be prepared by adding 0.05 mL of the same cord blood to 0.95 mL of the same adult blood. These spiked controls are then diluted with saline and assayed in the same manner as the patient sample. Normal adult blood may serve as the negative control.

Procedure

1. Mix the blood sample by gentle inversion.
2. Place 3 drops of 0.85% saline and 2 drops of blood into a glass test tube, and mix gently.
3. Place 1 drop of diluted blood on a glass slide near one end. Prepare a smear by drawing the edge of another slide through the drop and across the slide.
4. Air-dry the slide at room temperature.
5. Place the slide in a Coplin jar containing sufficient Red Cell Fixing Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the fixing solution, and allow the slide to remain in the solution at room temperature for 5 minutes.
6. Remove the slide from the fixing solution, rinse thoroughly with deionized water, and air-dry.
7. Place the dry slide in a Coplin jar containing sufficient Citrate/Phosphate buffer to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the buffer and allow the slide to remain in solution at room temperature for 10 minutes.
8. Remove the slide from the buffer solution, rinse briefly with deionized water, and blot excess water from the edges.
9. Place the wet slide in a Coplin jar containing sufficient Hemoglobin Staining Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the stain and allow the slide to remain in the stain at room temperature for 3 minutes.
10. Remove the slide from the Hemoglobin Staining Solution, rinse thoroughly with deionized water, and allow it to dry at room temperature.
11. Slides must be examined by using oil immersion. Fetal cells will stain a dark reddish-pink whereas adult cells will appear white to light pink with a slightly darker center (Fig. 28–31).

Calculations Results may be expressed as either % fetal cells or fetal/adult RBC ratio.

% Fetal Cells The percentage of erythrocytes containing fetal hemoglobin may be determined in several ways. The American Association of Blood Banks (AABB) recommends the following be used[29]:

1. Count the total number of adult red and fetal erythrocytes in as many fields as required to give a total count of at least 2000 cells.
2. Calculate % fetal cells in the total counted.

Example:

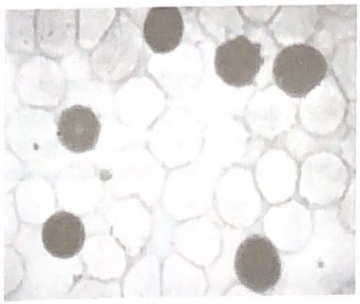
Total RBCs counted = 2150

Total fetal RBCs counted = 10

% Fetal cells = $10/2150 \times 100 = 0.46$

Fetal/Adult RBC Ratio

1. Count the total number of adult and fetal erythrocytes in as many fields as required to give a total count of at least 2000 cells.
2. Divide the total number of fetal cells by the total number of adult cells to obtain a ratio.



➤ **FIGURE 28–31** Kleihauer-Betke stain of blood from a newborn. Red-staining cells contain hemoglobin F; clear-staining cells contain hemoglobin A. (From Diggs, LW: Hematology. In Listen, Look and Learn. Health and Education Resources, Inc., Bethesda, MD, with permission.)

Example:

Total adult RBCs counted = 2150

Total fetal RBCs counted = 10

Fetal/adult RBC ratio = 20/2150 = 0.0046

RhIg Dosage The number of vials of RhIg necessary to protect against Rh immunization is based on the volume of fetomaternal hemorrhage and may be calculated using % fetal cells or fetal/adult RBC ratio.

Fetal Cells

Volume of fetomaternal hemorrhage (FMH) = % fetal cells × 50

Doses of RhIg required = FMH/30

Example:

If % fetal cells = 0.80; FMH = 0.80 × 50 = 40 mL

Doses of RhIg required = 40/30 = 1.3 doses (give 2 doses)

Note: when the number to the right of the decimal point is less than 5, round down and add one dose of RhIg. When the number to the right of the decimal point is 5 or greater, round up to the next number and add one dose of RhIg; for example, 2.9 doses (calculated) requires 4 doses.

Fetal/Adult RBC Ratio

Fetal/Adult RBC Ratio	Volume of FMH	No. of Vials
0.0–0.0045	up to 15 mL	1
0.0046–0.0090	15–30 mL	2
0.0091–0.0135	30–45 mL	3
0.0136–0.0180	45–60 mL	4
0.0181–0.0225	60–75 mL	5

Note: For each ratio interval of .0045, one additional vial of RhIg is indicated. If the dose calculation results in a fraction, administer the next number of whole vials of RhIg.

Staining for Heinz Bodies

Principle Heinz bodies are denatured hemoglobin precipitated in the RBC and attached to the RBC membrane. They are not visible with Wright's stain but show up with supravital staining (crystal violet) and phase microscopy (see Color Plate 83).

Reagents and Equipment

Crystal violet solution: 1.0 g of crystal violet dissolved in 50 mL of a 0.85% saline solution, which is shaken for 5 minutes and filtered before storage

Methyl violet solution: 0.5 g of methyl violet dissolved in 100 mL of a 0.85% saline solution, which is shaken for 5 minutes and filtered before storage

Glass slides and coverslip

Microscope

Procedure

1. Mix equal volumes of EDTA blood and stain in a small test tube. Either stain may be used.
2. Incubate for 20 minutes at room temperature.
3. Remix the blood-stain solution and transfer 1 drop to a slide.
4. Place a coverslip on the slide and examine for Heinz bodies under oil immersion.

Results Heinz bodies appear as irregular, refractile, purple inclusions, 1 to 3 μm in diameter, located on the periphery of the cell. They may even seem to be outside the cell. Reticulocytes are not stained by this technique.[30]

➤ TESTS FOR HEMOLYTIC ANEMIAS

Osmotic Fragility

Principle Whole blood is added to a series of saline dilutions. Exposure to hypotonic solution causes water to be drawn into the erythrocyte through osmosis. This eventually leads to swelling of the erythrocyte, leaking, and bursting of the cell. Once the cell bursts, hemoglobin is released and can be measured with a spectrophotometer. The presence or absence of hemolysis is an effective measure of erythrocyte susceptibility to hypotonic damage. This test is more than just an index of cell shape; it is also a measure of the surface-to-volume ratio. When an RBC's membrane surface decreases and its volume remains the same or increases, the cell becomes more turgid and less deformable. This is because the RBC membrane is flexible but not elastic. The result of this loss of surface-to-volume ratio is similar to what happens to a small plastic bag that is filled with more and more water.

Spherocytes, which have a decreased surface-to-volume ratio, demonstrate an increased osmotic fragility. This is because of their inability to swell in a hypotonic medium before leaking hemoglobin. Sickle cells, target cells, and other poikilocytes are relatively resistant to osmotic change and therefore demonstrate a decreased osmotic fragility.

Reagents and Equipment

Twenty-four 12 × 75 mm test tubes

Two 5-mL serologic pipets (TD), one 3-mL pipet

Parafilm squares

One heparinized normal control sample

One heparinized patient sample

Linear graph paper

1% NaCl solution:

Weigh 1.0 g NaCl crystals on an analytic balance.

Place crystals in a 100-mL volumetric flask and fill to the mark with distilled water.

Stir to completely dissolve NaCl.

Spectrophotometer

Note: Procedure can also be performed using Unopette Erythrocyte Fragility Test Kit by Becton Dickinson Vacutainer Systems. When using this kit, note that saline dilutions are prepackaged in individual reservoirs.

Procedure

1. Arrange two series of 12 tubes in the rack. Label both sets of tubes 1 through 12. The first series of tubes 1 through 12 is for the patient and the second series is for the control.
2. With a 5-mL pipet and 1% NaCl solution, and with the other 5-mL pipet, add distilled water into the series of patient tubes according to the following scheme: