COLLEGE of AMERICAN PATHOLOGISTS

Master

Immunology Checklist

CAP Accreditation Program

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Participants of the CAP accreditation programs may download the checklists by logging into cap.org and going to e-LAB Solutions Suite - Accreditation Checklists. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

CHECKLIST ACCREDITATION RESOURCES

CAP accredited laboratories have access to additional checklist accreditation tools and resources found on the CAP website (cap.org) by logging into e-LAB Solutions Suite - Accreditation Resources. Content found in Accreditation Resources includes:

- A library of past Focus on Compliance webinars and laboratory inspection preparation videos
- Answers to the most common checklist questions
- Customizable templates and forms (eg, competency assessment, personnel, validation/verification, quality management)
- Proficiency testing (PT) frequently asked questions, forms, and troubleshooting guides
- IQCP eligibility, frequently asked questions, forms, templates, and examples
- Laboratory director education and resources
- Quality management resources
- Inspector training and inspection tip sheets
- Self and post inspection toolbox

SUMMARY OF CHECKLIST EDITION CHANGES
Immunology Checklist
12/26/2024 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
   - Modifications that may require a change in policy, procedure, or process for continued compliance; or
   - A change to the Phase
3. Deleted/Moved/Merged**:**
   - Deleted
   - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
   - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for inspections and self-evaluations may not list all of these requirements.

Previously Cited Checklist Requirements

- The **inspector's version** of the checklist contains a listing of previously cited checklist requirements. Specific information on those citations, including the inspection date and inspector comments, is included following each related requirement within the checklist.
- Laboratories can access data on previously cited deficiencies by logging into e-LAB Solutions Suite on cap.org and going to Accreditation Reports - Inspection Summation Report.

NEW Checklist Requirements
None

REVISED Checklist Requirements

Requirement	Effective Date
IMM.33800	08/24/2023
IMM.33818	08/24/2023
IMM.40755	12/26/2024
IMM.41420	12/26/2024
IMM.41820	08/24/2023

DELETED/MOVED/MERGED Checklist Requirements
None

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect an immunology laboratory section or department.

Certain requirements are different for waived versus nonwaived tests. Refer to the checklist headings and explanatory text to determine applicability based on test complexity. The current list of tests waived under CLIA may be found at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/analyteswaived.cfm.

Policy/Procedure icon - The placement of this icon next to a checklist requirement indicates that a written policy or procedure is required to demonstrate compliance with the requirement. The icon is not intended to imply that a separate policy or procedure is required to address individual requirements. A single policy or procedure may cover multiple checklist requirements.

***Laboratories not subject to US regulations:** Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).*

QUALITY MANAGEMENT

CALIBRATION AND STANDARDS

Inspector Instructions:

READ	• Sampling of calibration and AMR policies and procedures • Sampling of calibration/calibration records • Sampling of AMR verification records • Sampling of patient reports and worksheets for verification of results outside of AMR
OBSERVE	• Sampling of calibration materials (labeling, storage, quality)
ASK	• What is your course of action if calibration is unacceptable? • When was the last time you performed a calibration procedure and how did you verify the calibration? • What is your course of action when results fall outside the AMR?
DISCOVER	• Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration, unacceptable calibration verification, and results outside the AMR

CALIBRATION AND VERIFICATION PROCESSES - WAIVED TESTS

IMM.33337 Calibration, Calibration/Verification - Waived Tests **Phase II**

For waived tests, testing personnel follow manufacturer instructions for calibration, calibration verification, and related functions.

Evidence of Compliance:

- ✓ Records for calibration/calibration verification/related functions as required by the manufacturer **AND**
- ✓ Records of recalibration or other appropriate corrective action when calibration verification is unacceptable

CALIBRATION AND VERIFICATION PROCESSES - NONWAIVED TESTS

The requirements in this checklist on CALIBRATION, CALIBRATION VERIFICATION, and ANALYTIC MEASUREMENT RANGE (AMR) VERIFICATION do not apply to waived tests.

This introduction discusses the processes of calibration, calibration verification, and AMR verification.

CALIBRATION: The process of adjusting an instrument or test system to establish a relationship between the measurement response and the concentration or amount of the analyte that is being measured by the test procedure.

CALIBRATION VERIFICATION: The process of confirming that the current calibration settings for each analyte remain valid for a test system.

Each laboratory must define limits for accepting or rejecting results of the calibration verification process. Calibration verification can be accomplished in several ways. If the manufacturer provides a calibration validation or verification process, it should be followed. Other techniques include (1) assay of the current calibration materials as unknown specimens, and (2) assay of matrix-appropriate materials with target values that are specific for the test system.

ANALYTICAL MEASUREMENT RANGE (AMR): The range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment that is not part of the usual assay process.

LINEARITY AND THE AMR
Linearity is a fundamental characteristic of many analytic measurement methods, whereby there is a straight-line relationship between ‘‘true’’ analyte concentrations and measured concentrations. In this context, linearity refers to the relationship between the predicted and observed measurement results and not to the relationship between instrument signal output and analyte concentration.

AMR VERIFICATION
Laboratories are required to verify that the appropriate relationship is maintained over the AMR. Laboratories may verify and use an AMR that is narrower than the range defined by the manufacturer. This may be appropriate when materials available for method validation and/or AMR verification are not available to verify the full range claimed by the manufacturer, or reporting values across the full range defined by the manufacturer is not clinically relevant. For many assays, results beyond the AMR can be reported through dilution or concentration studies (see IMM.33900 & IMM.33910). AMR verification is not required for calculated test results

(refer to the Definition of Terms in the All Common Checklist) as long as the individual results contributing to the calculation have AMR verification.

Minimum requirements for AMR verification can be met by using matrix appropriate materials, which include low, mid and high concentration or activity range of the AMR with recovery of results that fall within a defined range of the target value. Records of AMR verification must be available.

CLOSENESS OF SAMPLE CONCENTRATIONS OR ACTIVITIES TO THE UPPER AND LOWER LIMITS OF THE AMR
When verifying the AMR, it is required that materials used are near the upper and lower limits of the AMR. Factors to consider in verifying the AMR are the expected analytic imprecision near the limits, the clinical impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes. In such cases, reasonable procedures should be adopted based on available specimen materials. The closeness of sample concentrations or activities to the upper and lower limits of the AMR are defined at the laboratory director's discretion. The method manufacturer's instructions for verifying the AMR must be followed, when available. The laboratory director must define limits for accepting or rejecting verification tests of the AMR.

IMM.33374 Calibration Procedure **Phase II**

The laboratory calibrates each test system as defined and reviews calibration records for acceptability.

NOTE: Calibration of FDA-cleared/approved methods must be performed following the manufacturer's instructions, at minimum, including the number, type, and concentration of calibration materials, frequency of calibration, and criteria for acceptable performance. Calibration procedures are typically specified in the manufacturer's instructions but may also be established by the laboratory.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 1992(Feb 28):7165 [42CFR493.1217]
2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]
3) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Matrix Effects.* 4th ed. CLSI document EP14. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.
4) Miller WG. Quality control. In: Henry's Clinical Diagnostic and Management by Laboratory Methods, 21st Edition, ed McPherson RA, Pincus MR. Saunders Elsevier. 2007;99-111.

IMM.33448 Calibration and Calibration Verification Materials **Phase II**

High quality materials with test system and matrix-appropriate target values are used for calibration and calibration verification whenever possible.

NOTE: Calibration and calibration verification materials must have defined analyte target values and appropriate matrix characteristics for the clinical specimens and specific assay method. Many instrument systems require calibration materials with system-specific target values to produce accurate results for clinical specimens.

Suitable materials for calibration verification include, but are not limited to:
1. *Calibrators used to calibrate the analytical system*
2. *Materials provided by the manufacturer for the purpose of calibration verification*
3. *Previously tested unaltered patient/client specimens*
4. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
5. *Third party general purpose reference materials that are suitable for verification*

In general, routine control materials and proficiency testing materials are not suitable for calibration verification, except in situations where the material has been shown to be suitable (eg, specifically designated by the method manufacturer) or no other materials are available.

Evidence of Compliance:

✓ Records of calibration and calibration verification

REFERENCES

1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255]
2) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Matrix Effects.* 4th ed. CLSI document EP14. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

IMM.33670 Recalibration/Calibration Verification Criteria — Phase II

Criteria for the frequency and acceptability of recalibration or calibration verification are defined and followed.

NOTE: Laboratories must either recalibrate or perform calibration verification at least every six months and if any of the following occur:

1. *At changes of reagent lots unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results*
2. *If QC shows an unusual trend or shift or is outside of acceptable limits, and the system cannot be corrected to bring control values into the acceptable range*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

Single use devices, and other test devices that do not allow user calibration, do not require calibration verification.

Evidence of Compliance:

✓ Records of calibration verification at defined frequency

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707[42CFR493.1255(b)(3)]
2) Miller WG. Quality control. In: Henry's Clinical Diagnostic and Management by Laboratory Methods, 21st Edition, ed McPherson RA, Pincus MR. Saunders Elsevier. 2007:99-111.

IMM.33744 Recalibration — Phase II

The test system is recalibrated when calibration verification fails to meet the established criteria of the laboratory.

Evidence of Compliance:

✓ Records of recalibration, if calibration or calibration verification has failed

REFERENCES

1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255(a)(3)]

*****REVISED** 08/24/2023***

IMM.33800 AMR Verification Materials — Phase II

Verification of the analytical measurement range (AMR) is performed with matrix-appropriate materials which, at a minimum, include the low, mid and high range of the AMR, and appropriate acceptance criteria are defined.

NOTE: The matrix of the sample (ie, the environment in which the sample is suspended or dissolved) may influence the measurement of the analyte. In many cases, the method manufacturer will recommend suitable materials. Other suitable materials for AMR verification include the following:

1. *Linearity material of appropriate matrix, eg, CAP CVL Survey-based or other suitable linearity verification material*

2. *Previously tested patient/client specimens, that may be altered by admixture with other specimens, dilution, spiking in known amounts of an analyte, or other technique*
3. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
4. *Patient samples that have reference method assigned target values*
5. *Control materials, if they adequately span the AMR and have method specific target values.*

Factors to consider in verifying the AMR are the expected analytic imprecision near the limits, the clinical impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes. In such cases, reasonable procedures should be adopted based on available specimen materials. The closeness of sample concentrations and activities to the upper and lower limits of the AMR are defined at the laboratory director's discretion.

Evidence of Compliance:

✓ Records of AMR verification at least every six months

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707 [42CFR493.1255]
2) Shah VP, Midha KK, Dighe S, et al. Bioanalytical Method Validation - *Pharm Res.* 1992;9(4):588–92.
3) Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of bioanalytical chromatographic methods. *J Pharm Biomed Anal*. 1998;17(2):193–218.
4) Findlay JW, et al. Analytical Methods Validation - Bioavailability, Bioequivalence and Pharmacokinetic Studies. *Pharm Res*. 2000;17(12):1551-7.
5) Killeen AA, Long T, Souers R, Styler P, Ventura CB, Klee GG. Verifying Performance Characteristics of Quantitative Analytical Systems: Calibration Verification, Linearity, and Analytical Measurement Range. Arch Pathol Lab Med. 2014;138(9): 1773-81.

****REVISED** 08/24/2023***

IMM.33818 AMR Verification **Phase II**

Verification of the analytical measurement range (AMR) is performed at least every six months and following defined criteria. Records are retained.

NOTE: The AMR must be verified at least every six months after a method is initially placed in service and if any of the following occur:

1. *At changes of reagent lots unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results, and the range used to report patient/ client test data*
2. *If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

It is not necessary to independently verify the AMR if the calibration of an assay includes calibrators that span the full range of the AMR, with low, midpoint and high values represented (ie, three points) and if the system is calibrated at least every six months. A one-point or two-point calibration does not include all of the necessary points to verify the AMR.

AMR verification is not required for calculated test results as long as the individual results contributing to the calculation have AMR verification.

AMR verification is not required for methods that measure an analyte quantitatively or semi-quantitatively yet report a qualitative value based on concentration threshold. For such methods, refer to checklist requirement IMM.33905.

Evidence of Compliance:

✓ Records of AMR verification, as required, at least every six months

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):3707 [42CFR493.1255]

IMM.33900 Diluted or Concentrated Samples **Phase II**

If a result is greater than or less than the AMR, a numeric result is not reported unless the sample is processed by dilution, a mixing procedure or concentration so that the result falls within the AMR.

NOTE:

1. *A measured value that is outside the AMR may be unreliable and should not be reported in routine practice. Dilution, a mixing procedure* or concentration of a sample may be required to achieve a measured analyte activity or concentration that falls within the AMR. The result must be within the AMR before it is mathematically corrected by the concentration or dilution factor to obtain a reportable numeric result.*
2. *For each analyte, the composition of the diluent solution and the appropriate volumes of sample and diluent must be specified in the procedure manual. Specifying acceptable volumes is intended to ensure that the volumes pipetted are large enough to be accurate without introducing errors in the dilution ratio.*
3. *All dilutions, whether automatic or manual, should be performed in a way that ensures that the diluted specimen reacts similarly to the original specimen in the assay system. For some analytes, demonstrating that more than one dilution ratio similarly recovers the elevated concentration may be helpful.*
4. *This checklist requirement does not apply if the concentration or activity of the analyte that is outside the AMR is reported as "greater than" or "less than" the limits of the AMR.*

**This procedure is termed the "method of standard additions." In this procedure, a known quantity (such as a control) is mixed with the unknown, and the concentration of the mixture is measured. If equal volumes of the two samples are used, then the result is multiplied by two, the concentration of the known subtracted, and the concentration of the unknown is the difference.*

Evidence of Compliance:

✓ Patient reports or worksheets

IMM.33905 Cut-Off Values for Qualitative Tests **Phase II**

For qualitative tests that use a quantitative cut-off value to distinguish positive from negative results, the analytic performance around the cut-off value is verified or established initially, and reverified at least every six months thereafter.

NOTE: This requirement applies to tests that report qualitative results based on a quantitative measurement using a threshold (cut-off value) to discriminate between positive and negative results for clinical interpretation. It does not apply to methods where the laboratory is not able to access the actual numerical value from the instrument.

Appropriate materials for establishment and verification of the cut-off are identical to those recommended for calibration verification. The requirement can be satisfied by the process of calibration or calibration verification using calibrators or calibration verification materials with values near the cut-off. It may also be satisfied by the use of QC materials that are near the cut-off value if those materials are claimed by the method manufacturer to be suitable for verification of the method's calibration process.

Verification of the cut-off should also be performed at changes of lots of analytically critical reagents (unless the laboratory director has determined that such changes do not affect the cut-off); after replacement of major instrument components; after major service to the instrument; and when QC materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of accessing and correcting unacceptable control values fail to identify and correct the problem.

For FDA-cleared or approved tests, the clinical appropriateness of the cut-off value is evaluated as part of the clinical validation performed by the manufacturer. For laboratory-developed tests and modified FDA-cleared or approved tests refer to COM.40640 for validation of clinical claims.

Evidence of Compliance:

✓ Records of initial establishment and verification of cut-off value at defined frequency

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255].
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1253].

IMM.33910 Maximum Dilution **Phase II**

For analytes that may have results falling outside the limits of the AMR, the laboratory defines the maximum dilution that may be performed to obtain a reportable numeric result.

NOTE:

1. *For each analyte, the laboratory procedure defines the maximum dilution that falls within the AMR and that can be subsequently corrected by the dilution factor to obtain a reportable numeric result. Note that for some analytes, an acceptable dilution procedure may not exist because dilution would alter the analyte or the matrix causing erroneous results. Also note that, for some analytes, there may be no clinical relevance to reporting a numeric result greater than a stated value.*
2. *Analytes for which a dilution procedure is unable to bring the activity or concentration into the AMR should be reported as "greater than" the highest estimated values.*
3. *Establishment of allowable dilutions is performed when a method is first placed into service. The laboratory director is responsible for establishing the maximum allowable dilution of samples that will yield a credible laboratory result for clinical use.*

Evidence of Compliance:

✓ Patient results or worksheets

CONTROLS

Controls are samples that act as surrogates for patient specimens. They are processed like a patient sample to monitor the ongoing performance of the entire analytic process.

CONTROLS - WAIVED TESTS

Inspector Instructions:

READ	• Sampling of quality control policies and procedures • Sampling of QC records
ASK	• How do you determine when QC is unacceptable and when corrective actions are needed?

DISCOVER	• Review a sampling of QC data over the previous two-year period. Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory procedure or corrective action

IMM.33930 QC - Waived Tests **Phase II**

The laboratory follows manufacturer's instructions for quality control, reviews results, and records acceptability prior to reporting patient results.

NOTE: Quality control must be performed according to manufacturer instructions. To detect problems and evaluate trends, testing personnel or supervisory staff must review quality control data on days when controls are run prior to reporting patient results. The laboratory director or designee must review QC data at least monthly or more frequently if specified in the laboratory QC policy.

*With respect to internal controls, acceptable control results must be recorded at a minimum, once per day of patient testing for each device.**

**Acceptable internal control results need not be recorded, if (and only if) an unacceptable instrument control automatically locks the instrument and prevents release of patient results.*

Evidence of Compliance:

✓ Records showing confirmation of acceptable QC results

IMM.33940 QC Corrective Action - Waived Tests **Phase II**

The laboratory performs and records corrective action when control results exceed defined acceptability limits.

CONTROLS - NONWAIVED TESTS

Inspector Instructions:

READ	• Sampling of quality control policies and procedures • Sampling of QC records, including external and internal quality control processes
ASK	• How do you determine when quality control is unacceptable and when corrective actions are needed? • How does your laboratory verify or establish acceptable quality control ranges? • How does your laboratory perform QC for test procedures that report results as reactive, weakly reactive and nonreactive? • What is your course of action when you perform test procedures that do not have commercially available calibration or control materials?
DISCOVER	• Review a sampling of QC data over the previous two-year period. Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory policy for corrective action

- Use QC data to identify tests that utilize internal quality control processes to confirm that any individualized quality control plan (IQCP) is used as approved by the laboratory director

IMM.34120 Daily QC - Nonwaived Tests **Phase II**

The laboratory performs controls for quantitative and qualitative tests each day of testing, or more frequently if specified in manufacturer's instructions, laboratory procedure, or the CAP Checklist, and when changes occur that may impact patient results.

NOTE: The laboratory must define the number and type of quality control used and the frequency of testing in its quality control procedures. Control testing is not required on days when patient testing is not performed.

Controls must be run prior to resuming patient testing when changes occur that may impact patient results, including after a change of analytically critical reagents, major preventive maintenance, change of a critical instrument component, or with software changes, as appropriate.

Daily quality control must be run as follows:

1. *Quantitative tests - two controls at different concentrations at least daily*
2. *Qualitative tests - a negative control and a positive control (when applicable) at least daily*
3. *Tests producing a graded or titered result - a negative control and a control material with graded or titered reactivity, as applicable, at least daily (serially diluted positive controls are not required)*

Controls should verify assay performance at relevant decision points. The selection of these points may be based on clinical or analytical criteria.

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director defining the control process, including the frequency and use of external and internal controls. At a minimum, external control materials must be analyzed with new lots and shipments of reagents or more frequently if indicated in the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

Evidence of Compliance:

- ✓ Records of QC results including external and internal control processes **AND**
- ✓ Manufacturer product insert or manual

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register.* 1993(Jan 19):5232 [42CFR493.1256(d)(3)], [42CFR493.1256(d)(6)].
2) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Qualitative, Binary Output Examination Performance;* 3rd ed. CLSI document EP12. Clinical and Laboratory Standards Institute, Wayne, PA; 2023.
3) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
4) Department of Health and Human Services, Centers for Medicare and Medicaid Services. S & C: 16-20-CLIA: Policy Clarification on Acceptable Control Materials Used when Quality Control (QC) is Performed in Laboratories. April 8, 2016.

IMM.34140 Control Range Establishment or Verification **Phase II**

The laboratory establishes or verifies an acceptable control range for each lot of control material.

NOTE: For unassayed control materials, the laboratory must establish an acceptable control range by repetitive analysis in runs that include previously tested control material. For assayed control materials, the laboratory must verify control ranges supplied by the manufacturer.

Control values supplied by the manufacturer may be used without verification for qualitative (eg, positive or negative) testing.

Evidence of Compliance:

✓ Records for control range establishment or verification of each lot

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision of Quantitative Measurement Procedures. Approved Guideline.* 3rd ed. CLSI document EP05-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.
2) Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

IMM.34142 Calibrator Preparation — Phase II

If the laboratory prepares calibrators and controls in-house, these materials are prepared separately.

NOTE: In general, calibrators should not be used as QC materials. If calibrators are used as controls, then different preparations should be used for these two functions.

IMM.34145 Calibrators as Controls — Phase I

If a calibrator obtained from an outside supplier is used as a control, it is a different lot number from that used to calibrate the method.

NOTE: In general, calibrators should not be used as QC materials. However, the practice may be necessary for some methods when a separate control product is not available. In such cases, the calibrator used as a control must be from a different lot number than that used to calibrate the method.

Evidence of Compliance:

✓ QC/calibrator records

IMM.34170 Weakly Reactive Controls — Phase II

Reactive, weakly reactive and nonreactive controls are all used in test systems where results are reported in that fashion.

NOTE: Weakly reactive controls must be used when test results are reported in that fashion, unless such controls are not commercially available.

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director defining the control process, including the frequency and use of external and internal controls. At a minimum, external control materials must be analyzed with new lots and shipments of reagents or more frequently if indicated in the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

Evidence of Compliance:

✓ QC results

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. S & C: 16-20-CLIA: Policy Clarification on Acceptable Control Materials Used when Quality Control (QC) is Performed in Laboratories. April 8, 2016.

IMM.34250 QC Corrective Action — Phase II

The laboratory performs and records corrective action when results of controls exceed defined acceptability limits.

NOTE: The actions taken must be consistent with the laboratory's quality control program (GEN.30000). Patient/client test results obtained in an analytically unacceptable test run or since the last acceptable test run must be re-evaluated to determine if there is a significant clinical difference in patient/client results. Re-evaluation may or may not include re-testing patient samples, depending on the circumstances.

Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results. For example, evaluation could include comparison of patient means for the run in question to historical patient means, and/or review of selected patient results against previous results to see if there are consistent biases (all results higher or lower currently than previously) for the test(s) in question.

The corrective action for tests that have an IQCP approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on the problems identified (eg, trending for repeat failures, etc.).

Evidence of Compliance:

✓ Records of corrective action for unacceptable control results

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Oct 1):1046 [42CFR493.1282(b)(2)]

IMM.34270 QC Handling — Phase II

The laboratory tests control specimens in the same manner and by the same personnel as patient samples.

NOTE: Personnel who routinely perform patient testing must analyze QC specimens; however, this does not imply that each operator must perform QC daily. Personnel must participate in QC on a regular basis. To the extent possible, all steps of the testing process must be controlled.

Evidence of Compliance:

✓ Records reflecting that QC is performed by the same personnel performing patient testing

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(d)(8)]

IMM.34290 QC Confirmation of Acceptability — Phase II

Personnel review control results for acceptability before reporting patient/client results.

Evidence of Compliance:

✓ Records of control result approval

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7166 [42CFR493.1256(f)]

IMM.34315 QC Data — Phase II

Quality control data are organized and presented so they can be evaluated daily by the technical staff to detect problems, trends, etc.

NOTE: Results of controls must be recorded or plotted to readily detect a malfunction in the instrument or in the analytic system. These control records must be readily available to the person performing the test.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

IMM.34362 Monthly QC Review **Phase II**

The laboratory director or designee reviews and assesses quality control data at least monthly.

NOTE: The reviewer must record follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month may be reviewed when the tests are performed.

The review of quality control data for tests that have an IQCP approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on problems identified (eg, trending for repeat failures, etc.).

Evidence of Compliance:
- ✓ Records of QC review **AND**
- ✓ Records of corrective action taken when acceptability criteria are not met

IMM.34380 Numeric QC Data **Phase II**

For numeric QC data, quality control statistics (eg, SD and CV) are calculated monthly to define and monitor analytic imprecision.

NOTE: The laboratory must evaluate the imprecision statistics (eg, SD and CV, or other appropriate statistics) monthly to confirm that the test system is performing within acceptable limits. For whole blood methods, where stabilized whole blood or other suitable material is not available for QC, such statistics may be generated from previous patient/client samples using the SD of duplicate pairs or other patient data based statistical procedures.

This checklist requirement does not apply to external controls run only to verify new lots/ shipments of test materials. However, the laboratory should have defined acceptable limits for such controls (either from the manufacturer or developed by the laboratory).

Evidence of Compliance:
- ✓ QC records showing monthly monitoring for imprecision

REFERENCES

1) Rifai N, Horvath AR, Wittwer CT, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. St. Louis, MO: Elsevier; 2018.
2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7146 [42CFR493.1256(d)(10)(i)]

IMM.34450 Fluorescent/Enzyme Antibody Stain QC **Phase II**

Positive and negative controls are included with each patient run for all fluorescent or enzyme antibody stains.

NOTE: When examining tissue specimens, internal antigens, when present, may serve as positive controls (eg, IgA in tubular casts, IgG in protein droplets, and C3 in blood vessels). Non-reactive elements in the tissue specimen may serve as a negative tissue control. A negative reagent control in which the patient tissue is processed in an identical manner to the test specimen but with the primary antibody omitted must be performed for each patient tissue specimen. If internal controls are not present (eg, ANA IFA), external positive and negative controls must be included with each patient run.

Evidence of Compliance:
- ✓ Records of fluorescent/enzyme antibody stain QC at defined frequency

REFERENCES

1) Clinical and Laboratory Standards Institute. *Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline.* CLSI Document I/LA23-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2004.
2) Walker PD, *et al.* Practice guidelines for the renal biopsy. *Mod. Pathol.* 2004;17:1555-1563

IMM.34475 Alternative Control Procedures **Phase II**

If the laboratory performs test procedures for which control materials are not commercially available, the laboratory performs and records alternative control procedures to detect immediate errors and monitor test system performance over time.

NOTE: "Performance" includes elements of accuracy, precision, and clinical discriminating power. The following are examples of alternative procedures: split sample testing with another method or with another laboratory, the testing of previously tested patient specimens in duplicate, testing of patient specimens in duplicate, or other defined processes approved by the laboratory director.

Evidence of Compliance:
✓ Records of alternative control procedures

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(h)].

INSTRUMENTS AND EQUIPMENT

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

READ	• Sampling of incubator monitoring records • Sampling of radioimmunoassay policies and procedures • Sampling of calibration records • Sampling of background radioactivity records
ASK	• How does your laboratory verify concentration techniques? • What is your laboratory's course of action prior to using non-certified thermometers?

IMM.35070 Incubator QC **Phase II**

On days of use, the incubator is monitored for acceptable CO_2 concentration and humidity.

Evidence of Compliance:
✓ Incubator QC records

IMM.35275 Concentration Techniques **Phase I**

Concentration techniques for quantitative tests are verified.

NOTE: Techniques used to concentrate specimens for analysis must be verified at specified, periodic intervals (not to exceed one year or manufacturer's recommendations).

Evidence of Compliance:
- ✓ Records of concentration technique verification at defined frequency

RADIOIMMUNOASSAYS

Refer to the Laboratory General Checklist for requirements for use and storage of radioactive materials.

IMM.35965 Gamma Counter Calibration **Phase II**

Gamma counters and/or scintillation counters are calibrated, with the results recorded and compared to previous values each day of use.

Evidence of Compliance:
- ✓ Records of calibration

IMM.35975 Background Radioactivity **Phase II**

The background radioactivity is determined each day of use, including the background in each well of a multi-well counter, with defined upper limits of acceptability.

Evidence of Compliance:
- ✓ Records of background radioactivity determinations at defined frequency

IMM.35995 Counting Times **Phase II**

The laboratory defines counting times for quantitative procedures that are sufficiently long for statistical accuracy and precision.

ANALYTICAL BALANCES

COLORIMETERS, SPECTROPHOTOMETERS, AND FLUORIMETERS

The following requirements apply to stand-alone instruments; they are not applicable to instruments embedded in automated equipment for which the manufacturer's instructions must be followed.

Inspector Instructions:

READ	• Sampling of colorimeter/spectrophotometer policies and procedures • Sampling of manufacturer required system checks
ASK	• How does your laboratory verify calibration curves?

IMM.39500 Absorbance/Linearity **Phase II**

Absorbance and/or fluorescence linearity is checked and recorded at least annually or as often as specified by the manufacturer, with filters or standard solutions.

Evidence of Compliance:
- ✓ Records of absorbance and linearity checks at required frequency

IMM.39520 Spectrophotometer Checks **Phase II**

Spectrophotometer (including ELISA plate readers) wavelength calibration, absorbance and linearity are checked at least annually (or as often as specified by the manufacturer), with appropriate solutions, filters or emission line source lamps, and the results recorded.

NOTE: Some spectrophotometer designs, eg, diode array, have no moving parts that can alter wavelength accuracy and do not require routine verification. The manufacturer's instructions must be followed.

Evidence of Compliance:
- ✓ Records of spectrophotometer checks at required frequency

IMM.39530 Stray Light **Phase II**

Stray light is checked at least annually with extinction filters or appropriate solutions, if required by the instrument manufacturer.

Evidence of Compliance:
- ✓ Records of stray light checks, as applicable

IMM.39540 Calibration Curves **Phase II**

For procedures using calibration curves, all the curves are rerun at defined intervals and/or verified after servicing or recalibration of instruments.

NOTE: Calibration curves must be run following manufacturer's instructions, at minimum, and as defined in laboratory procedure.

Evidence of Compliance:
- ✓ Records of calibration curve rerun and/or verification at defined frequency

PROCEDURES AND TEST SYSTEMS

ANTI-NUCLEAR ANTIBODY TESTING

Inspector Instructions:

- Sampling of ANA result reports

IMM.39700 Anti-Nuclear Antibody Reporting **Phase I**

The method used for detecting anti-nuclear antibodies (ANA) is included on the report.

NOTE: Indirect immunofluorescence is traditionally used to detect antibodies with affinity for HEp-2 cells, and the pattern of ANA immunofluorescence is reported. Other methods (such as enzyme-linked immunoassay or multiplexed bead immunoassay) may not detect all of the same autoantibodies as the HEp-2 methodology, and these differences may be clinically significant. The ANA results report must include a brief description of the method used for ANA screening if the methodology is not explicit in the test name.

Evidence of Compliance:

✓ Records of ANA reports indicating method used

REFERENCES

1) Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis.* 2010;69:1420-1422.
2) American College of Rheumatology Position Statement: Methodology of Testing for Antinuclear Antibodies. American College of Rheumatology. August 2015.

TUMOR MARKER TESTING

Inspector Instructions:

- Sampling of tumor marker result reports
- Test reference guide or other communication to ordering providers

IMM.39800 Tumor Marker Result Reporting **Phase I**

The following information is available to clinicians for the reporting of tumor marker results:

- **Manufacturer and methodology of the tumor marker assay**
- **A statement indicating that patient results determined by assays using different manufacturers or methods may not be comparable.**

NOTE: As used in this checklist, a tumor marker is defined as any analyte that is serially measured over time primarily as an indicator of tumor burden.

Tumor marker results obtained can vary due to differences in assay methods and reagent specificity. If there is an assay change while monitoring a patient, the CAP recommends (but does not require) that the laboratory run parallel measurements with both assays.

The required information does not need to be reported with the test result if it is readily available elsewhere (eg, test reference guide).

Evidence of Compliance:

✓ Patient reports with required elements **OR**

✓ Test reference guide or other mechanism for providing ordering and interpretation information

REFERENCES

1) National Academy of Clinical Biochemistry. Sturgeon, CM, Diamandis, EP. (Eds.). *Laboratory Medicine Practice Guidelines. Use of tumor markers in clinical practice: quality requirements.* American Association for Clinical Chemistry; 2009.

BLOOD TYPE, GROUP, AND/OR ANTIBODY SCREENS

If the laboratory performs transfusion-related testing or any immunohematology tests other than blood group typing (ABO and Rh) antibody screens, or direct antiglobulin testing (DAT), the Transfusion Medicine Checklist must be used for inspection.

Inspector Instructions:

	• Sampling of blood type/group/antibody screen policies and procedures • Sampling of current package inserts • Sampling of QC records
	• Technologist performing testing (recording results at the time of testing)
	• What is your laboratory's course of action when ABO and Rh typing results are not in agreement with the patient's historical record? • How does your laboratory ensure that the direct antiglobulin test detects RBC-bound complement as well as IgG? • How do you confirm negative antiglobulin tests?
	• If there had been an instance when the ABO and Rh typing results were not in agreement with the patient's historical record, further evaluate the laboratory's responses, corrective actions and resolutions

IMM.40200 Package Inserts/Manufacturer's Instructions- Immunohematology Reagents Phase II

Current package inserts/manufacturer's instructions are available for immunohematology reagents.

NOTE: The laboratory must have a procedure that assures that:
- *The most current package inserts/manufacturer's instructions are in use.*
- *The relevant procedures are updated when changes to the instructions occur.*

Unless a manufacturer's package insert is being used as part of an approved procedure, laboratories are not required to retain discontinued package inserts; however, the laboratory must have a process to obtain expired package inserts from the manufacturer, if requested.

IMM.40250 Reagent Handling - Immunohematology Reagents Phase II

Immunohematology reagents are used according to manufacturer's instructions; or, if alternative procedures are used, validation records confirm that they perform as intended.

NOTE: Testing methods used for ABO, Rh and antibody screening that are different from the manufacturer's instructions are acceptable provided they are not prohibited by the manufacturer, have been demonstrated to be satisfactory, or, for laboratories subject to US regulations, have been approved by the Centers for Biologics Evaluation and Research (CBER).

Evidence of Compliance:
- ✓ Records of validation if instructions have been modified

REFERENCES
1) Food and Drug Administration. Guide to inspections of blood banks, Set 1994.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1271(a)(1)].

IMM.40300 Antisera/Reagent Red Cell QC **Phase II**

There are records of acceptable reactivity and specificity of typing sera and reagent red cells on each day of use, including a check against known positive and negative cells or antisera, or manufacturer's instructions for daily quality control are followed.

NOTE: Unless manufacturer instructions state otherwise, the following apply:

- *Typing reagents, including antisera (eg, anti-D, anti-K, anti-Fy(a)) and reagent red cells must be checked for reactivity and specificity on each day of use. Typing antisera must be checked with known positive and negative cells; reagent red cells must be checked with known positive and negative antisera.*
- *Each cell used for antibody screening must be checked each day of use for reactivity of at least one antigen using antisera of 1+ or greater avidity.*
- *Anti-IgG reactivity of antiglobulin reagents may be checked during antibody screening and crossmatching.*

This checklist requirement can be satisfied by testing one vial of each reagent lot each day of testing.

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):7171 [42CFR493.1271]

IMM.40440 Agglutination/Hemolysis Criteria **Phase II**

Criteria for agglutination and/or hemolysis are defined.

NOTE: Criteria must be defined to provide uniformity of interpretation of positive and negative agglutination and hemolysis results.

IMM.40580 Test Result Recording **Phase II**

Observations of all test results are recorded properly at the time the test is performed.

NOTE: Test results must be recorded at the time done in order to reduce the risk of transcription errors from delayed recording.

IMM.40720 Anti-D Controls **Phase II**

Appropriate control(s) are used for anti-D testing.

NOTE: If anti-D reagent contains a potentiating diluent, the appropriate control is the diluent alone. The selection of controls used must be consistent with the manufacturer's instructions.

Evidence of Compliance:

✓ Records of anti-D control results

*****REVISED** 12/26/2024***

IMM.40755 Historical Record Check **Phase II**

ABO/Rh results are compared with historical result records for each patient for at least the preceding 12 months.

NOTE: The purpose of this comparison is to detect sample/patient identification errors or other errors that might lead to the attribution of an incorrect blood type or antibody screen result to a patient. The historical record search can be performed manually by qualified laboratory personnel or with a validated computer system capable of performing historical checks. Acceptable ABO and Rh historical records for transfusion purposes are only those generated or entered by laboratory personnel into the health system's laboratory information system and performed by

an accredited laboratory/certified by the relevant government agency in its jurisdiction. If the laboratory performing the testing does not maintain records that would allow this check to be performed, the testing shall be reported with a disclaimer alerting the ordering physician that the check has not been performed and that verifications of the sample's identity and the test results are strongly recommended.

Evidence of Compliance:

- ✓ Records of historical checks **OR**
- ✓ Records of LIS historical check validations

IMM.40790 Typing Discrepancies - Investigation/Reconciliation **Phase II**

There are records of the investigation and reconciliation of all cases in which ABO and Rh typing results were not in accord with the patient's historical record.

NOTE: Available laboratory records for each patient must be routinely searched whenever testing is performed. Quality management records must include an investigation of all cases in which the ABO or Rh typing was not in accordance with the patient's laboratory historical record.

IMM.40795 Forward/Reverse Typing **Phase II**

For each patient, red blood cells are tested with anti-A, anti-B, and anti-D, and serum/plasma is tested using A1 and B reagent red cells.

NOTE: The ABO/Rh type of the patient's red blood cells must be determined by an appropriate test procedure. Tests on each sample must include forward and reverse grouping.

Evidence of Compliance:

- ✓ Logs or computer records with forward and reverse grouping

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1271(a)]

IMM.40800 Unexpected Antibody Screen **Phase II**

The antibody screen to detect unexpected red cell alloantibodies includes the following:

- **Incubation at 37°C**
- **Use of red cells that are not pooled**
- **Interpretation at the antiglobulin phase**

Evidence of Compliance:

- ✓ Logs or computer records indicating the reactions at the different phases of testing

IMM.40825 DAT Algorithm **Phase II**

When a direct antiglobulin test (DAT) is ordered by a patient's physician, the testing algorithm allows for detection of RBC-bound complement as well as IgG.

NOTE: The testing algorithm is intended to detect patients with complement-mediated hemolysis which may occur in paroxysmal cold hemoglobinuria, autoimmune hemolytic anemia, or drug-induced hemolytic anemia. Detection of complement is not required for the purpose of diagnosing hemolytic disease of the newborn.

The use of anti-IgG alone will fail to detect some cases of complement-mediated hemolysis because not all cases of complement-mediated hemolysis have detectable IgG coating the red blood cell. IMM.40860 and IMM.40980 also apply.

Evidence of Compliance:

✓ Records for DAT consistent with procedure

REFERENCES

1) Sokol RJ, *et al.* Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. *Brit Med J.* 1981;282:2023-2027
2) Packman CH, Leddy JP, Cryopathic hemolytic syndromes. In: Beutler E, *et al*, eds. William's Hematology, 5th ed. New York: McGraw-Hill, 1995:685-691
3) Vengelen-Tyler V, ed. American Association of Blood Banks Technical Manual, 13th ed. Bethesda, MD: AABB Press, 1999:259-262

IMM.40860 Antiglobulin Test Controls - Anti-IgG or Polyspecific Reagents Phase II

When performing an antiglobulin test with anti-IgG or polyspecific antiglobulin reagents, IgG-coated red blood cells are used as a control in all negative antiglobulin tests.

NOTE: IgG-coated red blood cells must be used to confirm all negative antiglobulin tests when the antiglobulin reagent used for testing has anti-IgG reactivity. Tests found negative by tube methodology must be verified by obtaining a positive test result after adding IgG-coated (control) red blood cells. If a licensed system is used that does not require verification of negative test results using IgG-coated cells, an appropriate quality control procedure must be followed, as recommended by the manufacturer.

Evidence of Compliance:

✓ Records of testing that include control results confirming negative antiglobulin tests

IMM.40980 Antiglobulin Test Controls - Anti-C3 Reagents Phase II

When performing an antiglobulin test with anti-C3 antiglobulin reagents, C3-coated red blood cells are used as a control in all negative antiglobulin tests.

NOTE: Complement-coated red blood cells must be used to confirm all negative antiglobulin tests when the antiglobulin reagent used for testing has anti-C3 reactivity. Tests found negative by tube methodology must be verified by obtaining a positive test result after adding C3-coated (control) red blood cells. If a licensed system is used that does not require verification of negative test results using C3-coated cells, an appropriate quality control procedure must be followed, as recommended by the manufacturer. If a polyspecific antiglobulin reagent is used, refer to checklist item IMM.40860.

Evidence of Compliance:

✓ Records of testing that include control results confirming negative antiglobulin tests

SYPHILIS SEROLOGY

Inspector Instructions:

READ	• Sampling of syphilis serology policies and procedures • Sampling of QC records • Needle delivery volume logs
ASK	• What is your laboratory's course of action prior to performing RPR, VDRL, TPPA, and/or USR patient testing using new antigen reagent lots?

IMM.41100 RPR Needles **Phase II**

If antigen is delivered by needles, the volume of delivery is checked under each of the following circumstances:

1. **Each time a new needle is used**
2. **When control patterns cannot be reproduced**
3. **When the antigen drop does not fall cleanly from the tip**

Evidence of Compliance:

✓ Records of needle verification

REFERENCES

1) Larsen SA, Pope V, Johnson RE, Kennedy EJ Jr, eds. *Manual of Tests for Syphilis.* Washington, DC. Amer Public Health Assn: 1998.

IMM.41400 New Reagent Lot/Shipment Confirmation of Acceptability - RPR, TPPA and VDRL **Phase II**

New reagent lots/shipments of antigen for RPR, TPPA, and VDRL tests are checked in parallel with the existing lot to confirm appropriate levels of reactivity.

NOTE: New reagent lots and shipments must be checked with samples (either patient specimens or controls) with known reactivity. For laboratories reporting only qualitative (positive/negative) results, a non-reactive sample along with a sample with low titer (for RPR and VDRL) or low reactivity (for TPPA) must be tested to verify detection of low-grade reactivity. Laboratories reporting RPR or VDRL titers or TPPA semi-quantitative reactivity must test at least one additional positive sample with known high titer or reactivity. Laboratories must have written criteria for acceptance of new lots (eg, acceptance of ±1 dilution of expected result).

Evidence of Compliance:

✓ Records of verification data of new lots/shipments

REFERENCES

1) Kennedy EJ, *et al*. Quality Control. In, SA Larsen *et al* (eds). A manual of tests for syphilis, 9th ed. Washington, DC: American Public Health Association, 1998; chap 4

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IMM.41420 Syphilis Antibody Screening **Phase II**

If the laboratory offers screening for syphilis, a complete screening algorithm is followed including appropriate confirmatory/secondary tests.

NOTE: Screening for infection by Treponemal pallidum can be performed by initial testing with either a nontreponemal (lipoidal antigen) antibody test (ie, traditional syphilis screening) or a treponemal antibody test (ie, reverse sequence syphilis screening). The reverse screening algorithm (with anti-treponemal antibody testing performed initially) may be preferred in cases of recent infection or in cases of late latent or tertiary syphilis when nontreponemal antibodies may not be detectable (even in the absence of adequate treatment).

Regardless of the method used, a positive (reactive) result in the primary screening assay must be reflexively tested by at least one secondary test method. In the traditional syphilis screening algorithm, a nontreponemal (lipoidal antigen) antibody screening assay must be reflexively tested by an anti-treponemal assay (such as EIA or TPPA).

In the reverse sequence screening algorithm, a treponemal antibody screening assay must be tested by a nontreponemal (lipoidal antigen) assay (such as RPR or VDRL). When discordant results are obtained (screening anti-treponemal antibody positive, nontreponemal (lipoidal antigen) negative), an additional anti-treponemal test (eg, TPPA or EIA) must be performed given the possibility of false positive results in anti-treponemal antibody screening assays.

Reflex testing in either algorithm may be performed on site or by a referral laboratory.

If the nontreponemal (lipoidal antigen) antibody test is performed to monitor treatment of patients with known syphilis infection (not as a screening tool), anti-treponemal antibody testing is not required. Because anti-treponemal antibodies persist after successful treatment, testing patients with previously diagnosed syphilis using a reverse algorithm approach is discouraged; therefore, laboratories should provide a clear option for providers to order nontreponemal (lipoidal antigen) titers directly for following serologic response to treatment.

This checklist requirement only applies to testing serum/plasma specimens. For testing CSF specimens, stand-alone anti-treponemal (eg, FTA-ABS or TPPA) and/or nontreponemal (lipoidal antigen) (eg, VDRL) tests may be used at the discretion of the laboratory director.

Evidence of Compliance:

✓ Records of confirmatory testing of positive screening antibody results with appropriate secondary assays

REFERENCES

1) Papp JR, Park IU, Fakile Y, Pereira L, Pillay A, Bolan GA, CDC laboratory recommendations for syphilis testing, United States, 2024. *MMQR Recomm Rep.*2024;73 (No. RR-1): 1-32.
2) Rhoads DD, et al. Prevalence of traditional and reverse-algorithm syphilis screening in laboratory practice. A survey of participants in the College of American Pathologists syphilis serology proficiency testing program. *Arch Pathol Lab Med.* 2017;141(1):93-97.

HIV PRIMARY DIAGNOSTIC TESTING

Inspector Instructions:

READ

- Sampling of HIV diagnostic testing policies and procedures
- Sampling of HIV result reports

IMM.41450 HIV Primary Diagnostic Testing - Supplemental and Confirmatory Testing Phase I

The laboratory follows public health recommendations or guidelines for HIV primary diagnostic testing, including primary screening and additional (supplemental and/or confirmatory) testing.

NOTE: If additional testing after a primary screening test is recommended by public health authorities, the laboratory:

- *Performs additional testing reflexively if the specimen is suitable and the test is performed in house, or*
- *Sends additional testing to a referral laboratory if the specimen is suitable, or*
- *Provides guidance to providers on submission of additional specimens, if needed for supplemental or confirmatory testing.*

The US Centers for Disease Control and Prevention (CDC) and Association of Public Health Laboratories (APHL) provide recommendations for HIV testing. Guidelines and recommended algorithms can be found on the CDC and APHL websites.

Evidence of Compliance:

✓ Patient reports with initial screening results and reflexive testing results and/or guidance

REFERENCES

1) Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. Available at http://stacks.cdc.gov/view/cdc/23447. Published June 27, 2014. Accessed 11/19/2019.
2) National Center for HIV/AIDS, Viral Hepatitis, and TB Prevention (US). Divisions of HIV/AIDS Prevention; Association of Public Health Laboratories. 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens, Available at: https://stacks.cdc.gov/view/cdc/50872. Published January 2018. Accessed 4/2/2023.

3) Association of Public Health Laboratories. Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm. January 2019. Available at APHL Publications. Accessed 11/19/2019.

WESTERN BLOT ASSAYS

Inspector Instructions:

- Sampling of western blot policies and procedures

IMM.41500 Molecular Weight Markers **Phase II**

Known molecular weight markers are included and reviewed with each Western blot assay of patient samples.

Evidence of Compliance:

✓ Records of QC for known markers

IMM.41600 Western Blot Separations **Phase II**

Western blot separations are satisfactory with sufficient resolution (low background, clear signal, absence of bubbles, etc.) to interpret band size easily.

IMM.41700 Acceptable Limits - Controls **Phase II**

Acceptable limits are set for controls of procedures where the Western blot bands are quantified.

NOTE: The criterion to designate a Western blot test as positive is based on the detection of a certain combination of positive bands. The laboratory should define a minimum intensity that allows a band to be considered positive.

Evidence of Compliance:

✓ Records of defined acceptable limits for control range of each lot

IMM.41800 Interpretation **Phase II**

Objective criteria are defined for interpretation of Western blot.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection.* 2nd ed. CLSI guideline M53. Clinical and Laboratory Standards Institute, Wayne, PA; 2023.
2) Clinical and Laboratory Standards Institute. *Western Blot Assay for Antibodies to Borrelia burgdorferi; Approved Guideline*. CLSI document M34-A. CLSI, Wayne, PA, 2000.
3) Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J Clin Microbol* 1995;33:419-22
4) Dressler F, Whelan JA, Reinhart BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993;167:392-400

DIRECT ANTIGEN TESTING

Inspector Instructions:

- Sampling of direct antigen testing policies and procedures
- Sampling of QC records

IMM.41810 Group A Streptococcus Direct Antigen Detection **Phase I**

If group A Streptococcus direct antigen testing is performed on pediatric patients, confirmatory testing is performed on negative samples.

NOTE: Cultures or other confirmatory tests must be performed on pediatric specimens that test negative when using antigen detection methods or if the manufacturer's guidelines include recommendations for culture follow-up. The laboratory policy must take into account the sensitivity of the assay in use, the age and clinical presentation of the patient, and other factors.

REFERENCES
1) Shulman S, Bisno A, Clegg H, et al. Clinical Practice Guideline for the Diagnosis and Management of Group A Streptococcal Pharyngitis: 2012 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2012;55(10). doi: 10.1093/cid/cis629.

*****REVISED** 08/24/2023***

IMM.41820 *Clostridioides (formerly Clostridium) difficile* **Phase II**

The laboratory defines criteria for the rejection of specimens for *C. difficile* and/or *C. difficile* toxin testing in stool.

NOTE: The laboratory, in collaboration with institutional stakeholders (eg, infection prevention and control, antimicrobial stewardship, infectious disease physicians), must develop criteria for rejection of inappropriate specimens submitted to the laboratory for C. difficile testing. For example, these criteria may include stool consistency (eg, test only unformed stool), repeat testing (eg, do not perform repeat testing during the same episode of diarrhea), and any exceptions. Reference or commercial laboratories may not have the ability to collaborate with stakeholders, but still need to define rejection criteria.

Evidence of Compliance:

✓ Records of specimen rejection such as rejection log or patient report

REFERENCES
1) Novak-Weekley SM, *et al. Clostridium difficile* testing in the Clinical Laboratory by Use of Multiple Testing Algorithms. *Journal of Clinical Microbiology* 2010; 48:889-893
2) Eastwood K, *et al*. Comparison of Nine Commercially Available *Clostridium difficile* Toxin Detection Assays, a Real-Time PCR Assay for *C. difficile tcdB* and a Glutamate Dehydrogenase Detection Assay to Cytotoxin Testing and Cytotoxigenic Culture Methods. *Journal of Clinical Microbiology* 2009; 47:3211-3217
3) Peterson LR and Robicsek A. Does my Patient have *Clostridium difficile* Infection? *Annals of Internal Medicine* 2009; 151:176-178

IMM.41830 CSF Back-Up Cultures **Phase II**

If bacterial antigen-detection methods are used, back-up cultures are performed on both positive and negative CSF specimens.

NOTE: Total dependence on a bacterial antigen test for the diagnosis of bacterial meningitis does NOT meet accreditation requirements. Meningitis may be caused by bacteria not detected by the antigen tests. In addition, it is important to recover the causative agent for susceptibility

testing. Thus, culture is essential for proper evaluation of bacterial meningitis, and must be performed on the patient specimen - if not performed on site by the laboratory, the inspector must seek evidence that a culture has been performed in a referral laboratory.

Evidence of Compliance:

- ✓ Records of back-up CSF cultures performed on-site **OR** records indicating that cultures are performed at another location **OR** records that order for CSF bacterial antigen was blocked by the computer due to no order for a culture

REFERENCES

1) Forward KR. Prospective evaluation of bacterial antigen detection in cerebral spinal fluid in the diagnosis of bacterial meningitis in a predominantly adult hospital. *Diagn Micro Infect Dis.* 1988;11:61-63
2) Maxson S, *et al.* Clinical usefulness of cerebrospinal fluid bacterial antigen studies. *J Pediat.* 1994; 125:235-238
3) Finlay FO, *et al.* Latex agglutination testing in bacterial meningitis. *Arch Dis Child.* 1995;73:160-161
4) Rathore MH, *et al.* Latex particle agglutination tests on the cerebrospinal fluid. A reappraisal. *J Florida Med Assoc.* 1995;82:21-23
5) Kiska DL, *et al.* Quality assurance study of bacterial antigen testing of cerebrospinal fluid. *J Clin Micro.* 1995;33:1141-1144

IMM.41840 Cryptococcal Antigen — Phase II

If cryptococcal antigen-detection methods are used on CSF, back-up cultures are performed on positive CSF specimens submitted for diagnosis.

NOTE: It is important to recover the causative organism for precise identification (C. neoformans vs. C. gattii) and potential susceptibility testing. Back-up cultures of follow-up specimens used for trending the antigen titer are not required. If culture is not performed on site by the laboratory, the laboratory must show evidence that it has been performed in a referral laboratory.

Evidence of Compliance:

- ✓ Records of back-up CSF cultures performed on-site **OR** records indicating that cultures are performed at another location

IMM.41850 Direct Antigen Test QC - Nonwaived Tests — Phase II

For nonwaived direct antigen tests performed on patient specimens, positive and negative controls are tested and recorded each day of testing, or more frequently if specified in the manufacturer's instructions, laboratory procedure or the CAP Checklist.

NOTE: This requirement pertains to nonwaived tests with a protein, enzyme, or toxin which acts as an antigen. Examples include, but are not limited to: Group A Streptococcus antigen, C. difficile toxin, fecal lactoferrin and immunochemical occult blood tests. For panels or batteries, controls must be employed for each antigen sought in patient specimens.

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director defining the control process, including the frequency and use of external and internal controls. At a minimum, external control materials must be analyzed with new lots and shipments of reagents or more frequently if indicated in the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

For each test system that requires an antigen extraction phase, as defined by the manufacturer, the system must be checked with an appropriate positive control that will detect problems in the extraction process. If an IQCP is implemented for the test, the laboratory's quality control plan must define how the extraction phase will be monitored, as applicable, based on the risk assessment performed by the laboratory and the manufacturer's instructions.

Evidence of Compliance:

- ✓ Records of QC results including external and internal control processes **AND**
- ✓ Manufacturer product insert or manual

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. S & C: 16-20-CLIA: Policy Clarification on Acceptable Control Materials Used when Quality Control (QC) is Performed in Laboratories. April 8, 2016.

MOLECULAR-BASED MICROBIOLOGY TESTING - WAIVED TESTS

The requirements in this section apply to molecular-based microbiology tests classified as waived. Microbiology testing performed by nonwaived molecular-based methods must be inspected with the Microbiology Checklist.

Inspector Instructions:

	• Sampling of QC statistics • Sampling of molecular microbiology specimen handling and processing policies and procedures • Sampling test reports (test methodology, clinical interpretation)
	• What is your course of action when monitored statistics increase above the expected positive rate?

IMM.41900 Quality Monitoring Statistics **Phase I**

The laboratory monitors for the presence of false positive results (eg, due to nucleic acid contamination) for all molecular microbiology tests.

NOTE: Examples include review of summary statistics (eg, monitoring percentage of positive results relative to current local and regional rates and increased positive Strep results above historical rate within a run or over multiple runs), performance of wipe (environmental) testing, and review and investigation of physician inquiries. Based on monitoring data, the laboratory may implement additional mitigation strategies to minimize the risk of contamination, such as process controls.

Evidence of Compliance:

✓ Records of data review, wipe testing, statistical data evaluation and corrective action if indicated

REFERENCES
1) Borst A, Box AT, Fluit AC. False-positive results and contamination in nucleic acid amplification assays: suggestions for a prevent and destroy strategy. *Eur J Clin Microbiol Infect Dis*. 2004; 23(4):289-99.
2) Cone RW, Hobson AC, Huang ML, Fairfax MR. Polymerase chain reaction decontamination: the wipe test. *Lancet*.1990; 336:686–687.
3) McCormack JM, Sherman ML, Maurer DH. Quality control for DNA contamination in laboratories using PCR- based class II HLA typing methods. *Hum Immunol*. 1997;54:82–88.
4) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments;* 1st ed. CLSI document MM19-A. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 2011.

IMM.41920 Specimen Handling **Phase II**

The laboratory uses appropriate processes to prevent specimen loss, alteration, or contamination during collection, transport, processing and storage.

NOTE: Specimen collection, processing and storage must follow manufacturer's instruction and limit the risk of preanalytical error. For example, there must be a procedure to ensure absence of cross-contamination of samples during processing/testing for respiratory specimens that may be sent for further testing.

It is also essential to follow the manufacturer's instructions for the handling of wastes (eg, used test cartridges) to prevent contamination.

IMM.41930 Safe Specimen Handling/Processing **Phase II**

The laboratory safely handles and processes specimens, including those suspected to contain highly infectious pathogens.

NOTE: Suggested topics to be considered in the development of policies and procedures include the need for tight sealing of containers, avoiding spills of hazardous materials, requirements for wearing gloves, the need for respirator protection, availability and use of vaccinations, and the hazards of sniffing plates.

For specimens suspected of containing highly infectious pathogens, laboratories must review national, federal, state (or provincial), and local guidelines for the handling of specimens from patients suspected to have high risk pathogens, such as Francisella tularensis, avian influenza, Ebola, MERS coronavirus, SARS coronavirus, SARS-CoV-2 coronavirus, or any infectious agent that has a high potential to cause disease in individuals and communities.

Evidence of Compliance:

✓ Records of universal precaution for all personnel handling suspected infectious pathogens

REFERENCES

1) Wooley DP, Byers KB, eds. Biological Safety, Principles and Practices, 5th ed. ASM Press. 2017.
2) Miller JM, Astles R, Baszler T, et al. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories, Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. MMWR. 2012; 61:1-102.
3) Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 6th ed. June 2020.

IMM.41940 Final Report **Phase I**

The final report includes a summary of the test method and information regarding clinical interpretation if appropriate.

NOTE: For tests that may be performed by either direct antigen or molecular-based methods (PCR), including the test method in the report is important for interpretation of the results. The report must include a brief description of the method if the methodology is not explicit in the test name.