COLLEGE of AMERICAN PATHOLOGISTS

Master

Chemistry and Toxicology Checklist

CAP Accreditation Program

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Chemistry and Toxicology Checklist

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ON-LINE CHECKLIST DOWNLOAD OPTIONS

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
Chemistry and Toxicology 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

Requirement	Effective Date
CHM.12925	12/26/2024
CHM.15225	12/26/2024
CHM.18610	08/24/2023
CHM.18620	08/24/2023
CHM.18640	08/24/2023
CHM.31150	12/26/2024

REVISED Checklist Requirements

Requirement	Effective Date
CHM.13550	08/24/2023
CHM.13600	08/24/2023
CHM.18600	08/24/2023
CHM.18700	08/24/2023
CHM.18825	08/24/2023
CHM.18850	08/24/2023
CHM.29100	08/24/2023
CHM.30150	08/24/2023
CHM.30700	08/24/2023
CHM.30800	08/24/2023
CHM.31950	12/26/2024
CHM.32300	12/26/2024
CHM.32800	12/26/2024
CHM.33900	12/26/2024

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
CHM.18800	08/23/2023
CHM.31100	12/25/2024
CHM.31200	12/25/2024
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CHM.31500	12/25/2024
CHM.31550	12/25/2024
CHM.31600	12/25/2024
CHM.31650	12/25/2024
CHM.31700	12/25/2024
CHM.32100	12/25/2024

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a chemistry 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).*

CHEMISTRY & TOXICOLOGY GENERAL ISSUES

PROFICIENCY TESTING

Inspector Instructions:

- Records of hemoglobin A1C accuracy-based proficiency testing results evaluation, as applicable

***NEW** 12/26/2024*

CHM.12925 Hemoglobin A1C Testing **Phase I**

For laboratories that use accuracy-based proficiency testing (PT) for hemoglobin A1C, the laboratory evaluates its results based on acceptable performance criteria of +/- 6% from the target value, with appropriate corrective action taken for each unacceptable result.

NOTE: The CAP recommends use of accuracy-based PT products, when possible, to evaluate the accuracy of hemoglobin A1C results. Due to limitations in product stability, this may not be available for laboratories outside of the US.

The Centers for Medicare and Medicaid Services (CMS) have established acceptable performance criteria for hemoglobin A1C as a regulated analyte at +/- 8% from the target value. The CAP and all CAP-accepted PT providers must use the +/- 8% criteria in the formal grading of the PT for reporting non-waived results to the CMS. For laboratories participating in the CAP's accuracy-based PT program for hemoglobin A1C, the CAP will also evaluate their results against the target value using +/- 6% performance criteria. This is provided in the participant evaluation

and participant summary report. Laboratories must review their performance against the +/- 6% criteria and perform corrective action for each unacceptable result.

Evidence of Compliance:

✓ Records of accuracy-based PT evaluation using the +/- 6% performance criteria

REFERENCES

1) Sacks DB, Arnold M, Bakris GL, et al. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. *Clin Chem*. 2023; 69(8):808-68.

QUALITY MANAGEMENT

CALIBRATION AND STANDARDS

Inspector Instructions:

	• Sampling of calibration and AMR policies and procedures • Sampling of calibration/calibration verification records • Sampling of AMR verification records • Sampling of patient reports and worksheets for verification of results outside of AMR • Current DEA license (for US laboratories that handle pure controlled substance(s))
	• Sampling of calibration materials (quality)
	• 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? • What is your course of action when you receive calibration materials for non-FDA cleared/ approved assays? • How does your laboratory verify concentration techniques?
	• Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration, and unacceptable calibration verification

CALIBRATION AND VERIFICATION PROCESSES – WAIVED TESTS

CHM.12950 Calibration, Calibration/Verification - Waived Tests **Phase II**

For waived tests, testing personnel follow manufacturer's 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 remaining 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 must 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 method.

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. For most assays, this relationship is linear within the AMR.

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 CHM.13710 & CHM.13720). 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.

CHM.13000 Calibration Procedure — Phase II

The laboratory calibrates each test system as defined and reviews the 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

CHM.13100 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 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) ISO 17511:2020 In vitro diagnostic medical devices, Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples. International Organization for Standardization. 2020.

CHM.13125 Calibration Materials - Non-FDA Cleared/Approved Assays — Phase II

The quality of all calibration materials used for non-FDA cleared or approved assays is evaluated and recorded.

NOTE: Commercial standards used to prepare calibrators require certificates of quality from the manufacturer, or a quality check as part of the initial assay validation process. The laboratory must ensure the accuracy of a new lot of calibrators by checking the new lot against the current lot.

CHM.13175 Pure Controlled Substances **Phase II**

If the laboratory uses chemicals (for standards, controls, etc.) covered by the Controlled Substances Act, it maintains appropriate licenses.

NOTE: The intent is to be compliant with national, federal, state (or provincial), and local laws and regulations.

For US laboratories, a DEA license, and in some states, a state license is required for controlled substances. A DEA license is not required for certain commercial solutions of controlled substances.

CHM.13400 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 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.

CHM.13500 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 and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1255(a)(3)]

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

CHM.13550 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

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) Shah VP, Midha KK, G, 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***

CHM.13600 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, eg, drugs of abuse, refer to checklist requirement CHM.13750.

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): [42CFR493.1255]

CHM.13710 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

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):[42CFR493.1282(b)(1)(ii)]

CHM.13720 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, eg, free drugs or free hormones. 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 reports or worksheets

CHM.13730 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

CHM.13750 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 assessing 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 the 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].

CHM.13810 Neonatal Bilirubin Testing **Phase II**

Neonatal bilirubin results in the range of 5 to 25 mg/dL are accurate and suitable for use with standardized clinical practice interpretive guidelines, with accuracy verified at least annually.

NOTE: Each laboratory must assess the accuracy of its instrument/test system over the range of bilirubin values appropriate for the clinical guidelines (5-25 mg/dL). In many cases, acceptable performance can be verified using proficiency testing materials with assigned reference values.

In other cases, the laboratory can meet the objective by using patient samples to perform correlation studies against 1) a reference method; OR 2) an alternate method that consistently demonstrates good performance in a proficiency testing program (based on the method mean value as compared to the reference value). In all cases, such comparisons should include at least one or two samples annually in the target clinical range of 5-25 mg/dL.

The reference method for total bilirubin is described in Doumas et al, Candidate reference method for determination of total bilirubin in serum: development and validation. Clin Chem, 1985.

Evidence of Compliance:

✓ Written assessment of adequacy for the agreement with target values in the range of the clinical guidelines for clinical purposes, at least annually, by the laboratory director or designee

REFERENCES

1) Lo SF, Doumas BT, Ashwood ER. Bilirubin proficiency testing using specimens containing unconjugated bilirubin and human serum: results of a College of American Pathologists study. *Arch Pathol Lab Med* 2004;128:1219-1223
2) American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* 2004;114:297-316
3) Doumas BT, Kwok-Cheung PP, Perry BW, et al. Candidate reference method for determination of total bilirubin in serum: development and validation. *Clin Chem* 1985; 31:1779-1789.
4) Lo SF, Doumas BT. The status of bilirubin measurements in U.S. Laboratories: Why is accuracy elusive? *Semin Perinatol* 2011; 35:141-147.
5) Barrington KJ, Sankaran K. Canadian Paediatric Society, Fetus and Newborn Committee. Guidelines for detection, management, and prevention of hyperbilirubinemia in term and late preterm newborn infants. http://www.cps.ca/documents/position/hyperbilirubinemia-newborn. Accessed August 18, 2014.

CONTROLS

Controls are used to ensure that a test system is performing correctly. Traditionally, controls are samples that act as surrogates for patient/client specimens, periodically processed like a patient/client sample to monitor the ongoing performance of the entire analytic process. Under certain circumstances, other types of controls (electronic, procedural, built-in) may be used. (Details are in the checklist requirements in this section, below.)

CONTROLS – WAIVED TESTS

Inspector Instructions:

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

CHM.13840 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's 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 the reporting of 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

CHM.13860 QC Corrective Action - Waived Tests **Phase II**

The laboratory performs and records corrective action when control results exceed defined acceptance 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? • What is your course of action when monthly precision data changes significantly from the previous month's data? • 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 procedure 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

CHM.13900 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.* 2003(Jan 24):5232 [42CFR493.1256(d)(3)], [42CFR493.1256(d)(6)].
2) Steindel SJ, Tetrault G. Quality control practices for calcium, cholesterol, digoxin, and hemoglobin. A College of American Pathologists Q-Probes study in 505 hospital laboratories. *Arch Pathol Lab Med.* 1998;122:401-408
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) Ye JJ, *et al*. Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol.* 2000;113:240-248
5) 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.
6) Clinical and Laboratory Standards Institute. *Laboratory Quality Control Based on Risk Management; Approved Guideline.* CLSI document EP23-A. Clinical and Laboratory Standards Institute, Wayne, PA, 2011.
7) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Brochure #11. CLIA Individualized Quality Control Plan Introduction. July 2013. http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIAbrochure11.pdf
8) Centers for Medicare and Medicaid Services (CMS), Individual Quality Control Plan (IQCP) for Clinical Laboratory Improvement Amendments (CLIA) laboratory nonwaived testing. http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/IQCP-announcement-letter-for-CLIA-CoC-and-PPM-labs.pdf (Accessed June 2014).
9) 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.

CHM.13950 Fluorescent Antibody Stain QC — Phase II

Positive and negative controls are included with each patient run for all fluorescent antibody stains (eg, ANA IFA).

Evidence of Compliance:

- ✓ Records of fluorescent 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.

CHM.14000 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.

CHM.14125 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.

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):3708 [42CFR493.1256(d)(9)]

CHM.14150 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, this 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

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):3708 [42CFR493.1256(d)(9)]

CHM.14200 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)].

CHM.14300 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.

CHM.14500 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) 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)]
3) Ross JW, Lawson NS. Analytic goals, concentrations relationships, and the state of the art for clinical laboratory precision. *Arch Pathol Lab Med.* 1995;119:495-513
4) 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.
5) Brooks ZC, *et al*. Critical systematic error supports used of varied QC rules in routine chemistry. *Clin Chem.* 2000;46:A70

CHM.14600 QC Corrective Action — Phase II

The laboratory performs and records corrective action when control results 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)]
2) 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):[42CFR493.1282(b)(1)(i)]

CHM.14800 QC Handling — Phase II

The laboratory tests control specimens in the same manner and by the same personnel as patient/client 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 run 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)]
2) *ibid*, 2003(Jan 24):3708[42CFR493.1256(d)(7-8)

CHM.14900 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)]
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):3708 [42CFR493.1256(d)(6)]

CHM.14916 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

RESULTS REPORTING

Inspector Instructions:

	• Sampling of reporting policies and procedures • Sampling of patient reports (reference interval and cut offs included)
	• How is information on the equations used for calculating eGFR and LDL results communicated to clinicians?

*****NEW** 12/26/2024***

CHM.15225 eGFR and LDL Cholesterol Calculated Test Results **Phase I**

Clinicians have access to information regarding the equation used to calculate results for estimated glomerular filtration rates (eGFR) and low-density lipoprotein (LDL) cholesterol.

NOTE: Calculated results may differ based on which equation is used. This may limit clinical assessment of results and/or comparability of calculated results across laboratories, particularly when the source equation is not readily available to providers.

The information can be made available to clinicians using different approaches, such as on the patient report, test reference guide, or inclusion of the equation name in the test name.

Evidence of Compliance:

✓ Patient reports with information on the calculation used **OR**
✓ Test reference guide or other mechanism for providing calculation information

REFERENCES

1) Inker LA, Eneanya ND, Coresh J, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. *N Engl J Med.* 2021; 385(19):1737-49.
2) Sampson M, Ling C, Sun Qian, et al. A New Equation for Calculation of Low-Density Cholesterol in Patient With Normolipidemia and/or Hypertriglyceridemia. *JAMA Cardiol.* 2020; 5(5):540-48.

CHM.15250 Toxicology Results **Phase II**

The laboratory follows written procedures for the reporting of toxicology results.

NOTE: In addition to the requirements found in the Laboratory General Checklist, the following information must be included in toxicology reports:

1. *If appropriate, substances or classes of substances analyzed as part of the toxicology test*
2. *Specimen type*
3. *Report status for positive results (ie, unconfirmed, confirmed or pending confirmation)*
4. *For immunoassays, the assay cut-off concentration for each drug or drug class**
5. *If the report includes unconfirmed screening results, a statement that such results are to be used only for medical (ie, treatment) purposes. Unconfirmed screening results must not be used for non-medical purposes (eg, employment testing)*

**The cut-off concentrations may either be included in the report or in a separate chart/ memorandum available to clinicians.*

Laboratories are encouraged to identify the detected drugs as parent compounds, metabolites, or impurities of drugs in the report or in a separate chart/memorandum available to clinicians.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Toxicology and Drug Testing in the Medical Laboratory.* 3rd ed. CLSI guideline C52. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.

METHODS, INSTRUMENT SYSTEMS, 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:

- If problems are identified during the review of the methods, instrument systems, and equipment or when asking questions, further evaluate the laboratory's responses, corrective actions and resolutions
- Select a representative assay and follow the entire process from specimen receipt to final result reporting

RADIOIMMUNOASSAYS

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

Inspector Instructions:

- Sampling of radioimmunoassay policies and procedures
- Sampling of calibration records
- Sampling of background radioactivity records

CHM.15900 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

CHM.16000 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

CHM.16200 Counting Times **Phase II**

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

REFERENCES
1) Klee G, Post G. Effect of counting errors on immunoassay precision. *Clin Chem.* 1989;35:1362-1366

CHROMATOGRAPHY AND MASS SPECTROMETRY

THIN LAYER CHROMATOGRAPHY (TLC)

Inspector Instructions:

- Sampling of TLC policies and procedures
- Sampling of control, standards/calibrator records

CHM.16300 Standard/Calibration Materials **Phase II**

Appropriate standards, calibrators, or controls (as applicable) are included with each TLC plate.

NOTE: Appropriate standards must include compounds that test the chromatographic range of the TLC plate, and that test all phases of the staining/development system. This may consist of a standard, previously tested positive patient sample, or dot that contains appropriate compounds.

Evidence of Compliance:

✓ Records showing use of appropriate standards/calibrators with each plate

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Toxicology and Drug Testing in the Medical Laboratory.* 3rd ed. CLSI guideline C52. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.

CHM.16400 Daily QC - TLC **Phase II**

Negative and appropriate positive controls are extracted and run through the entire procedure.

NOTE: Positive and negative controls must be extracted and carried through the entire procedure with each plate or card.

Appropriate positive controls must include drugs/compounds that test the extraction, chromatographic range of the TLC plate, and the staining/development system.

Evidence of Compliance:

✓ QC records at defined frequency

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.* 2003(Jan 24): [42CFR493.1256(d)(4)].
2) Clinical and Laboratory Standards Institute (CLSI). *Toxicology and Drug Testing in the Medical Laboratory.* 3rd ed. CLSI guideline C52. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.

CHM.16500 Solvent Mixtures **Phase II**

Solvent mixtures are prepared fresh as needed.

NOTE: If a mixture of solvents is used, certain components will evaporate with time faster than others. This leads to poor extraction or reproducibility of migration rates. If a commercial kit is used, the manufacturer's instructions should be followed.

GAS CHROMATOGRAPHY (GC) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Inspector Instructions:

READ	• Sampling of GC/HPLC policies and procedures • Sampling of control, calibration/standards records • Sampling of column verification records • Sampling of records of sample order • Records of signal intensity monitoring
ASK	• How does your laboratory evaluate the effectiveness of hydrolysis? • How does your laboratory evaluate potential carryover? • How have you determined the limit of detection and the AMR?

CHM.16550 Calibration and Calibration Verification — Phase II

Appropriate calibration or calibration verification is performed on each day of patient testing or following the manufacturer's instructions.

NOTE: For qualitative assays, an appropriate calibrator should be run at normal and abnormal levels. For quantitative assays, a multipoint calibration may be required if the measurement has a non-linear response. For some assays, a level near the assay's limit of detection (LOD) or at critical decision point(s) is needed. For measurement systems that have a linear response verified by periodic multipoint calibration verification and AMR verification protocols, a calibration procedure that uses a single calibrator at an appropriate concentration is acceptable. Analyses based on a single point calibration must be controlled by appropriate quality control samples. In addition, inclusion of a negative control (reagent blank) is good laboratory practice.

Evidence of Compliance:

✓ Records of calibration/calibration verification

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) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

CHM.16650 Daily QC - GC and HPLC — Phase II

Appropriate controls are extracted and run through the entire procedure on each day of patient testing.

NOTE: Controls used in chromatographic procedures must evaluate as much of the complete testing process as is technically feasible. The control process includes any pre-treatment, pre-purification or extraction steps, unless non-pretreated control material is appropriate. For qualitative assays, the negative and positive controls should be at concentrations that meaningfully confirm performance below and above the decision threshold for the analyte. For quantitative assays, appropriate controls must include at least one normal sample, and at least one sample reflecting a disease range. For some assays, an additional control concentration may be useful to confirm performance near the assay's LOD, LOQ** or cut-off, if appropriate, or at a concentration consistent with highly abnormal levels that test the AMR.*

**LOD - limit of detection*

***LOQ - limit of quantitation*

If a hydrolysis step is required in the assay, the laboratory includes a control (when available) with each batch to evaluate the effectiveness of hydrolysis.

Evidence of Compliance:

✓ QC records at defined frequency

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.* 2003(Jan 24):5232 [42CFR493.1256(d)(3)(ii)].
2) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

CHM.16750 Sample Run Order — Phase II

A record of sample run order is maintained for review.

NOTE: The run list must include blanks, standards, controls, and patients included in each run and be stored with the results of each batch run.

CHM.16770 Chromatographic Characteristics/Column Performance — Phase II

Chromatographic characteristics and column performance are reviewed and approved for each run before results are released.

NOTE: Checks should record testing variables such as flow rate of carrier gas and amount of sample injected and indications of error, including split peaks, doublets, and tailing.

Evidence of Compliance:

✓ Records of review and approval

CHM.16800 Carryover Detection — Phase II

The laboratory has a process to detect and evaluate potential carryover.

NOTE: No matter what type of injection is used, the process must address criteria for the evaluation of potential carryover from a preceding elevated (high concentration) sample to the following sample in each analytical batch analysis.

Evidence of Compliance:

✓ Records of reassessment of samples with potential carryover

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Preliminary Evaluation of Quantitative Medical Laboratory Measurement Procedures.* 4th ed. CLSI guideline EP10. Clinical and Laboratory Standards Institute, Wayne, PA; 2024.
2) Society of Forensic Toxicologists/American Academy of Forensic Sciences. *Forensic Toxicology Laboratory Guidelines.* 2002; 8.2.8:13
3) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

CHM.16850 Column Verification — Phase II

New columns are verified for performance before use.

Evidence of Compliance:

✓ Records of column verification

CHM.16950 Column/Detector Monitoring — Phase II

The performance of the column and detector is monitored each day of use.

NOTE: Unextracted standards and extracted calibrators or controls typically containing the target compound(s), may be analyzed each day to monitor critical aspects of column performance. Appropriate criteria for evaluating such parameters as retention time, relative retention time, separation of closely eluting compounds of interest, chromatography quality, and detector response should be established and monitored.

Evidence of Compliance:
- ✓ Records for column and detector monitoring

CHM.17050 Gas Leakage - GC **Phase I**

Gas lines and connections are checked for leaks every time tubing or a connection has been manipulated.

Evidence of Compliance:
- ✓ Records of gas line checks

CHM.17100 Reagent Grade **Phase II**

Reagents, solvents and gases are of appropriate grade.

CHM.17150 Limit of Detection/AMR **Phase II**

The limit of detection (sensitivity) and the AMR for quantitative methods have been determined for each procedure.

Evidence of Compliance:
- ✓ Records of limit of detection and AMR determination

REFERENCES

1) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

MASS SPECTROMETRY (MS)

Inspector Instructions:

READ	• Sampling of MS policies and procedures • Sampling of calibration and tuning records • Identification criteria compliance
ASK	• How does your laboratory identify possible ion-suppression or enhancement?

CHM.18400 Instrument Calibration **Phase II**

The laboratory calibrates the mass spectrometer and reviews calibration records for acceptability.

REFERENCES

1) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.
2) Clinical and Laboratory Standards Institute (CLSI). *Quantitative Measurement of Peptides and Proteins by Mass Spectrometry.* CLSI guideline C64. 1st ed. Clinical and Laboratory Standards Institute, Wayne, PA; 2021.
3) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods.* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

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

CHM.18600 Mass Spectrometer Tuning **Phase II**

The mass spectrometers are tuned as defined based on the particular platform in use, assay performance requirements, and specimen types tested.

NOTE: Instruments must be tuned at least as frequently as recommended by the manufacturer. Acceptable tolerance limits for tune parameters must be defined, and tuning records retained.

Evidence of Compliance:

✓ Records of tuning

***NEW** 08/24/2023*

CHM.18610 Extracted Calibrators **Phase II**

Appropriate extracted calibrator(s) are analyzed, or appropriate calibration verification is conducted, with each batch of samples.

NOTE: At least one extracted calibrator at the commonly accepted cut off or reporting threshold for single-point calibration, or multiple calibrators above and below that value for multipoint calibration, must be analyzed with each run. Appropriate calibration verification, including historical calibration, may be acceptable based on assay performance criteria and validation.

***NEW** 08/24/2023*

CHM.18620 Analytical Performance Monitoring of MS Assays **Phase II**

The laboratory monitors analytical performance of mass spectrometric assays using defined performance criteria and quality metrics and performs corrective action when acceptance criteria are not met.

NOTE: The performance criteria and quality metrics used must be based on the assay design, instrumentation and associated configuration(s), calibration strategy, specimen type, and reporting strategy.

The monitoring of assay performance includes the review and recording of the quality metrics of each run and at defined intervals. Examples of performance criteria and quality metrics include:

- *System suitability testing*
- *Adequacy and stability of internal standards response within and across runs*
- *For quantitative assays, performance criteria for the defined analytical measurement range*
- *Thresholds for re-injection of specimens or re-extraction*
- *Monitoring of assay performance when multiple instruments and/or instrument components are used.*
- *Performance checks after significant maintenance (eg, cleaning or replacing the ion source, changing voltages or gas flow parameters, and cleaning or replacing other hardware, such as, quadrupole rails/rods) for impact on sensitivity, accuracy, and precision where needed.*

Evidence of Compliance:

✓ Records of monitoring **AND**

✓ Records of corrective actions taken

REFERENCES

1) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

2) Clinical and Laboratory Standards Institute (CLSI). *Quantitative Measurement of Peptides and Proteins by Mass Spectrometry.* CLSI guideline C64. 1st ed. Clinical and Laboratory Standards Institute, Wayne, PA; 2021.
3) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods;* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

*****NEW** 08/24/2023***

CHM.18640 Validation, Monitoring, and Annual Verification of MS Data Analysis Tools Phase II

The laboratory validates data analysis tools used for compound identification and quantification when first installed and after any modifications, as applicable, and verifies performance at least annually.

NOTE: Data analysis tools may be used for various processes, such as integration of targeted and untargeted peaks, evaluating acceptability of calibration and control performance, stability of baseline, calculation of ion mass ratios, discrimination of positive and negative results, and assessing risk of carryover. Data analysis tools (eg, software or code-based rules, algorithms, machine learning) used for automated data analysis must be verified using defined acceptability criteria. Version control of custom data analysis tools is required. Reassessment of lower limit of quantification (LLOQ) and other decision points may be used to ensure that a shift has not occurred due to instrument performance or another factor impacting assay performance.

Customized data analysis tools, and modifications to that software, should be appropriately documented and records should allow for tracking to identify persons that have added or modified that software. The purpose of the computer program, the way it functions, and its interaction with other programs must be clearly stated. The level of detail should be adequate to support troubleshooting, system modifications, or additional programming.

Evidence of Compliance:

- ✓ Records of validation and revalidation after modifications **AND**
- ✓ Records of monitoring for changes to software update tools and other change impacting performance

REFERENCES

1) Vincente FB, Lin DC, Haymond S. Automation of chromatographic peak review and order to result data transfer in a clinical mass spectrometry laboratory. *Clin Chim Acta.* 2019;498(11):84-9.

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

CHM.18700 Identification Criteria - Mass Spectrometry Phase II

The identification criteria for analytes detected by mass spectrometry (eg, GC/MS, LC-MS/MS) are defined.

NOTE: For single-stage mass spectrometry, one acceptable criterion for compound identification using ion ratios is that the unknown result must have ion ratios within a prescribed acceptance or tolerance limit of calibrator ratios. This limit should be supported by either literature references or through experimental means. Such ion ratio tolerance limits may differ based on the technique applied (eg, GC/MS versus LC/MS) as well as the analyte(s) being determined (eg, compounds with mainly ions of low abundance); thus, a defined limit to cover all methods and analytes cannot be given.

In tandem mass spectrometry using multiple reaction monitoring (MRM), there is at least one transition monitored for the internal standard and another for the analyte.

Evidence of Compliance:

- ✓ QC and test records

REFERENCES

1) Clinical and Laboratory Standards Institute. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.
2) Official Journal of the European Communities. Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (17.8.2002)
3) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods;* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

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

CHM.18825 Matrix Effect Assessment of Mass Spectrometry Assays - Validation **Phase II**

There is a record of assessment of matrix effects in development and validation of mass spectrometry assays.

NOTE: Matrix effects can affect analyte ionization and performance in both directions: suppression or, less frequently seen, enhancement. Evaluation of matrix effects must be performed during assay development and validation.

Examples of evaluation protocols may include:

1. *Post Column Infusion - Constant infusion of analyte followed by injection of blank matrix specimen extracts to measure ionization response*
2. *Mobile Phase/Post Extractions Spiking - Compare response of analyte spiked into mobile phase to that of analyte spiked into blank matrix specimen extracts*
3. *Internal standard monitoring - Evaluate trend in internal standard abundance and signal to noise ratios during an analytical run that includes blank and spiked matrix specimen extracts.*

The minimum number of different matrix sources may vary based on the matrix, analytical targets, and assay design. Associated data should be used to evaluate the impact of matrix effect on results and determine appropriate acceptance criteria for each reportable analyte during routine testing of patient samples.

REFERENCES

1) Annesley, TM. Ion Suppression in Mass Spectrometry. *Clin. Chem.* 49, pp. 1041-1044 (2003)
2) Krull, I, and Swartz, M. Quantitation in Method Validation. *LC-GC*, 16, pp. 1084-1090 (1998)

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

CHM.18850 Matrix Effect Assessment of Mass Spectrometry Assays - Routine Monitoring **Phase II**

The laboratory evaluates mass spectrometry assays for possible ion-suppression or enhancement in patient samples during routine testing.

NOTE: Ion suppression (or less frequently, ion enhancement) is a recognized analytical anomaly in mass spectrometry assays. Such suppression can lead to false negative results or poor quantitative analyses (especially near assay limit of quantitation). While difficult to predict and observe from specimen to specimen, certain precautions should be used to try to detect ion suppression or enhancement.

Routine monitoring of the signal intensity of internal standard(s) is an effective way to recognize signal suppression/enhancement in a single patient sample, due to unexpected interfering components of the matrix. Internal standards to be used are those that cover the areas of the elution profile where matrix effects are most pronounced, and that the suitability of these internal standards has been determined (ie, with acceptance limits) during assay development and validation. Internal standard abundance acceptance criteria may be based on signal to noise ratio or may be compared to internal standard abundance in QC samples. As an example, for isotopically-labeled internal standards, if there is poor recovery of the internal standard, a signal to noise ratio greater than 3:1 should still suffice for acceptance of the specimen in question. If recovery of the isotopically-labeled internal standard is considered poor, then an alternate analysis should be considered, eg, the method of standard addition. For analogue-type internal standards, internal standard recovery may be used as a guide for identification of ion suppression/enhancement, although another option, such as the method of standard addition, would be a reasonable alternative. It should be noted that even isotopically-labeled internal standards do not always readily identify ion suppression or enhancement.

Evidence of Compliance:

✓ Records of monitoring of internal standards **OR** records of alternative methods used

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods;* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.

INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY (ICP/MS)

Inspector Instructions:

READ	• Sampling of ICP/MS policies and procedures • Sampling of calibration records
ASK	• How does your laboratory verify assay performance each day of use?

CHM.20400 Tuning Solution **Phase II**

An appropriate tuning solution or autotune is used to verify assay performance each day of use.

NOTE: Tuning solutions may contain a single element or multiple elements. Use of such solutions and/or autotuning verifies general system performance, control for potential interferences and mass resolution, optimization of ion lens voltages and check signal stability. Failure to use a tuning solution or autotune may affect ICP/MS sensitivity and selectivity.

Evidence of Compliance:

✓ Records of instrument tuning at defined frequency

CHM.20500 Peak Width **Phase I**

The peak width is optimized.

NOTE: ICP/MS peak width must be optimized. In quadrupole ICP/MS experiments, there is generally mass unit resolution. If a mass spectral peak is too broad, a false positive finding may occur, since it may overlap with another analyte. If a mass spectral peak is too narrow, sensitivity is sacrificed. Most manufacturers of ICP/MS instrumentation designate an acceptable peak width range. The peak width range is generally determined using a tuning solution. Some software packages automatically check and alter peak width range. Peak width optimization is generally verified daily. There may be times when it may be desirable to go outside the manufacturer's specified peak width range. For example, brass is an alloy of copper and zinc. In ICP/MS, copper peaks surround that of zinc. Therefore, the copper peaks may interfere with the ability to detect zinc. Hence, by narrowing the zinc peak width, the possible interference due to copper may be mitigated or eliminated. With high resolution ICP/MS, it may be acceptable to have designated acceptable peak width range levels for different analytes.

Evidence of Compliance:

✓ Records of verification of peak width optimization

CHM.20600 Common Interferences Minimized **Phase II**

When appropriate, oxides and doubly-charged species are minimized.

NOTE: Oxides and doubly-charged species are common interferences in ICP/MS. Oxides of various elements may have overlapping signals with elements of the same mass, thus leading to false-positive findings. Special techniques such as high resolution ICP/MS, dynamic-reaction cell and collision-reaction cell processes may eliminate the concern for oxide interference. Elements with a second ionization potential greater than or equal to 15.8 eV (the ionization potential of argon) may be doubly-charged. Such doubly-charged species may suggest the presence of an element that is not truly present. For example, gadolinium has an isotope at m/e154. It has a doubly-charged species at m/e 77, which is also the same mass as an isotope of selenium.

CHM.20700 Dual Detector Mode **Phase II**

If the dual detector mode is applied, the calibration is verified.

NOTE: In ICP/MS, calibration can be performed in two modes – pulse counting for lower concentrations and analog for higher concentrations. If a range is necessary that overlaps with both modes, then the laboratory should employ a cross-calibration. This is generally accomplished by use of a tuning solution whereby a full calibration is performed in both modes followed by software adjustment for a smooth transition. If a concentration range is needed that only encompasses one mode or the other, then a cross-calibration is unnecessary as long as the appropriate mode is employed.

Evidence of Compliance:

- ✓ Records of calibration verification and cross-calibration, if needed

CHM.20800 Reaction/Collision Cell **Phase I**

If a reaction/collision cell is utilized, the reaction/collision gases are optimized.

NOTE: Optimization of reaction/collision gases will allow for maximization of sensitivity and minimization of background counts. Such optimization is generally accomplished through use of a separate tuning solution and is controlled by a separate part of most software packages than that used for autotuning.

Evidence of Compliance:

- ✓ Records of optimization of reaction/collision gases

CHM.20900 Calibration Curve **Phase II**

An adequate and appropriate calibration curve is established for quantitative testing.

CHM.21000 Instrument Drift **Phase II**

Performance criteria are defined to detect drift in ICP/MS equipment.

NOTE: Procedures for ICP/MS equipment must include criteria for performance and procedures to detect drift, which can occur rapidly. One way in which instrument drift can be detected is by evaluating control materials at defined intervals during a run.

CHM.21100 Isotope/Standard Criteria **Phase II**

Appropriate criteria are defined for selection of both the isotope(s) and the associated internal standard(s) related to each quantified element.

NOTE: When isotopes and internal standards are measured by ICP/MS, interferences (isobaric and polyatomic species) and relative abundances must be considered and described in written procedures and/or assay validation materials.

CHM.21200 Contamination **Phase I**

Laboratory processes minimize and detect contamination of results obtained by ICP/MS.

NOTE: Potential sources of contamination include specimen collection, reagent handling, carryover between samples, and engineering controls within the analytical environment.

CHM.21300 Gas/Reagent Purity **Phase I**

The purity of each gas and reagent used with ICP/MS is defined and appropriate for the intended use.

NOTE: Purity of gasses and reagents (including water) used with ICP/MS should be defined and validated to identify and minimize interferences and sources of contamination.

CHM.21400 Controls/Calibrators/Blanks **Phase I**

Controls, calibrators and blanks are matrix-matched to the sample type.

NOTE: The matrices of controls, calibrators and blanks may affect the ions generated and should be considered in the design and validation of each ICP/MS assay. If matrices are not an issue, the laboratory should have a record that matrix-matching is not necessary.

IMAGING MASS SPECTROMETRY

Imaging Mass Spectrometry (IMS) is an emerging technology used to provide molecular information on tissue section specimens through visualization of the spatial distribution of proteins, lipids and other molecules by their molecular masses. It is used in conjunction with other pathology findings to make a tissue diagnosis or provide other information on the tissue specimen.

IMS combines the following methods to evaluate the tissues:
- *Whole slide imaging*
- *Matrix-assisted laser desorption ionization mass spectrometry (MALDI MS)*
- *Individual molecular mapping of a tissue*
- *Data analysis process*

This checklist section is not applicable to use of MS imaging for education or research-only use.

Inspector Instructions:

READ

- Sampling of imaging Mass Spectrometry policies and procedures
- Sampling of calibration and maintenance records
- Sampling of quality control records

CHM.21405 Instrument Calibration **Phase II**

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

NOTE: The manufacturer provides detailed instructions for this process along with minimum specifications for each instrument. This basic calibration should be performed each time the instrument is cleaned or serviced with a record of performance retained by the laboratory.

Evidence of Compliance:
- ✓ Records of service and calibration at defined frequency

CHM.21410 Mass Spectrometer Tuning — Phase II

The mass spectrometers are tuned each day of patient/client testing, or according to manufacturer's recommendations, and tuning records are retained.

NOTE: Acceptable tolerance limits for tune parameters must be defined, and tuning records retained. A specific suitable standard sample covering the m/z range of the patient samples must be used.

Evidence of Compliance:
- ✓ Records of tuning parameters

CHM.21415 Mass Spectrometry Calibration — Phase II

A calibration consisting of at least five standard compounds is included on each target used for patient testing within the m/z range acquired from patient samples. Calibration is performed with each insertion of the target plate, and these records are retained.

NOTE: Tolerance limits for calibration parameters must be defined in accordance with instrument specifications.

Evidence of Compliance:
- ✓ Records of calibration

CHM.21420 MS Performance Evaluation for Patient Samples — Phase II

Exogeneous standards are placed on like tissue (with respect to the type of patient tissue to be analyzed) on each day of patient testing to measure signal-to-noise and overall performance specifications for one or more analytes.

NOTE: For MS/MS (tandem MS) assays, identification criteria for tandem mass spectrometry (MS/MS) are validated and recorded. For MS tests using multiple reaction monitoring (MRM) there is at least one transition monitored for the internal standard and another for the analyte.

Evidence of Compliance:
- ✓ Records of performance values and test records

REFERENCES

1) Kaletas BK, van der Wiel IM, Stauber J, et al. Sample preparation issues for tissue imaging by imaging MS. *Proteomics.* 2009; 9(10):2622-33.

CHM.21425 Mass Spectrometer Control Tissue — Phase II

Appropriate control tissues are tested on each day of patient testing representing the diagnostic state being considered.

NOTE: Control tissue must be subjected to the same testing conditions throughout the testing procedure as patient specimens.

In general, targets are not to be reused. In formats of testing where a target is reused, a blank control needs to be run after each cleansing to assess the cleanliness of the target (demonstrating lack of peaks prior to testing).

Evidence of Compliance:
- ✓ QC records at defined frequency

CHM.21430 Mass Spectrometer Reagent Grade **Phase II**

Reagents and solvents are of HPLC-grade, MS-grade, or equivalent quality.

NOTE: HPLC-grade and MS-grade solvents with certification from the manufacturer (when available) are acceptable. If lesser grade reagents are used, the laboratory must document equivalent performance.

Evidence of Compliance:
- ✓ Reagent logs and test records

CHM.21435 Mass Spectrometer Consumables **Phase II**

Consumables appropriate to the instrument and assay are used.

NOTE: Consumables (eg, auto-pipettes and tips, solvents, target glass slides) utilized may be specified by the manufacturer. Other types of consumables must be validated.

Evidence of Compliance:
- ✓ Consumable logs **AND**
- ✓ Validation of alternative consumables not specified by the manufacturer

CHM.21440 Area of Analysis **Phase II**

A qualified pathologist selects or confirms the appropriate areas for analysis.

NOTE: The identity of the individual determining the areas for analysis must be recorded. For specific tissue types, a specialist in the related area may perform this duty (eg, dermatologist for skin biopsies).

Evidence of Compliance:
- ✓ Record of review by a qualified individual

CHM.21445 Analytical Data Analysis Procedure **Phase II**

The algorithms and steps that make up the data analysis process used to analyze, interpret, and report test results are defined.

NOTE: This data analysis process includes all algorithms, software, scripts, and reference databases, whether in-house, vendor-developed, or open source.

The written procedure must include:
- *Individual applications and databases used with versions and appropriate command line flags, or other configuration items needed to compile, install, and run the process*
- *Additional scripts or steps used to connect discrete applications in the process*
- *Name and version number of the source codes for algorithms used*
- *Description of input and output data files or information (eg, parameters/flags and values) in each process step*
- *Criteria and specific thresholds used*
- *Acceptance and rejection criteria for the results generated by the data analysis process. Criteria must be based on metrics and quality control parameters established during test*

optimization and utilized during validation. These should include criteria for determining when the data analysis process has failed and the data are either re-processed or not further processed.
- *Limitations in the test methodology for each test*
- *Written procedures for any portion of the data analysis process performed by a referral laboratory, if applicable*

REFERENCES
1) Jones EA, Deininger SO, Hogendoorn PC, Deelder AM, McDonnell LA. Imaging mass spectrometry statistical analysis. *J Proteomics.* 2012; 75(16):4962-89.
2) Kriegsmann J, Kriegsmann M, Casadonte R. MALDI TOF imaging mass spectrometry in clinical pathology: a valuable tool for cancer diagnostics (review). *Int J Oncol.* 2015; 46(3):893-906.

CHM.21450 Data Analysis Process Validation — Phase II

The laboratory validates the data analysis process on a control tissue sample and revalidates the entire process and/or confirms the performance of the components of the process as acceptable when modifications are made.

Evidence of Compliance:
- ✓ Records of validation and revalidation and/or confirmation studies, including metrics and QC parameters used to establish and assess performance **AND**
- ✓ Written approval of validations, revalidations and/or confirmation studies **AND**
- ✓ Records of review of referral laboratory, if applicable

REFERENCES
1) Jennings L, et al. Recommended practices and principles for validating clinical molecular pathology tests. *Arch Pathol Lab Med.* 2009; 133(5):743-755.

CHM.21455 Data Analysis Process - Updates — Phase I

The laboratory has a defined process for monitoring, recording, and implementing patch-releases, upgrades, and other updates to the data analysis process.

NOTE: The data analysis processes are composed of multiple components - open source or other software packages, additional scripts, and databases for managing content and aspects of analysis and reporting. Due to the ongoing evolution of the field, laboratories need to establish a procedure for regular monitoring of updates, patch-releases, and other upgrades for each component of the process. Congruent with the procedure, the laboratory must demonstrate that acceptable performance specifications are met when a change to the process is implemented. The extent of revalidation and/or confirmation is modification dependent. Revalidation/confirmation may cover all or a subset of steps in the data analysis process and must designate specific monitoring intervals and address when such updates will be implemented.

Evidence of Compliance:
- ✓ Records of monitoring activities **AND**
- ✓ Records of revalidation/confirmation data including the type of upgrade, metrics, and quality control (QC) parameters monitored to assess analytical run performance **AND**
- ✓ Approval of revalidation/confirmation data by the laboratory director **AND**
- ✓ Dates of implementation

CHM.21460 Data Storage — Phase I

The laboratory retains data necessary to support primary results generated and re-analysis for a minimum of two years and as required by national, federal, state (or provincial), and local laws and regulations.

NOTE: The data retained must include the files necessary to re-review cases as originally performed for original results reporting. Examples include specimen tacking and quality metrics

data/files, sequencing run quality metrics reports, log or configuration file information regarding data analysis process parameters, and exception log information. The retained files and records must also be structured to facilitate inter-laboratory replication of the original analyses, annotations and/or interpretation, whether initiated by the laboratory or at the request of the referring physician or patient tested.

The policy must be in accordance with national, federal, state (or provincial), and local requirements for storage of data, as applicable.

CHM.21465 Version Traceability **Phase I**

The specific version(s) of the data analysis process used to generate data files are traceable for each patient report.

NOTE: Data analyses processes are typically comprised of a combination of different software packages, scripts, and databases. The versions and configuration of each component in the process (eg, command line flags or other configuration items) must be traceable for each patient report. Records of each process component do not need to appear in the patient report. Rather, it is acceptable to refer to the process as a whole, using a laboratory-specific designation and/ or include log files if generated with each analysis of a patient dataset. Laboratory-specific designations must be unique to each version of process components and configurations. Any changes to software packages, scripts, databases, configuration files or other process components require tracking in the version control system and updating to a new version.

Evidence of Compliance:

- ✓ Records identifying software packages, scripts, and databases with associated version numbers and configuration items for a given patient report, as appropriate

ATOMIC ABSORPTION SPECTROPHOTOMETERS

Inspector Instructions:

	• Sampling of AA Spectrophotometer policies and procedures • Calibration records
	• How does your laboratory ensure optimal lamp performance?

CHM.21600 Burner Head/Aspirator **Phase II**

The burner head and aspirator are flushed thoroughly with water each day of use.

Evidence of Compliance:

- ✓ Record of burner head and aspirator maintenance

CHM.21700 Optical Beam Alignment **Phase II**

The optical beam alignment is checked at defined frequencies, and results are recorded.

NOTE: This should be done at least weekly, although daily checking is preferred.

CHM.21800 Atomizer **Phase II**

The atomizer is cleaned and flow rate optimized at regular, specified intervals, and the results recorded.

CHM.21900 Sampler System **Phase II**

Automatic sampler systems (eg, on graphite furnace) are checked for precision at specified periodic intervals.

Evidence of Compliance:

- ✓ Records of sampler system checks at defined frequency

CHM.22000 Graphite Furnace **Phase II**

If a graphite furnace is used, the blank value of a graphite tube is verified for each element tested.

NOTE: Residue from assayed samples may accumulate on the graphite tube, thus potentially resulting in false positive findings should the residue contain the element of interest. In addition, checking for the response of a blank may also serve as one of the indicators that the graphite tube may need replacement.

Evidence of Compliance:

- ✓ Record of graphite tube checks at defined frequency

CHM.22100 Calibration Curve **Phase II**

An adequate and appropriate calibration curve is established for quantitative testing.

CHM.22200 Lamp Energy **Phase II**

The lamp's energy is verified and recorded for each run.

NOTE: Atomic absorption spectrophotometric lamp energy must be verified and recorded for each run. Lamps lose performance characteristics over time. Decrements in lamp performance may be observed by a loss of sensitivity. Poor lamp performance may also serve as an indicator of another system failure, eg, loose connections.

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:

- Sampling of colorimeter/spectrophotometer policies and procedures
- Sampling of manufacturer required system checks

- How does your laboratory verify calibration curves?

CHM.22300 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

CHM.22400 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 should be followed.

Evidence of Compliance:

✓ Records of spectrophotometer checks at required frequency

CHM.22500 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 at required frequency

CHM.22600 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

FLAME PHOTOMETERS

Inspector Instructions:

- Sampling of flame photometer policies and procedures
- Sampling of system cleaning

- Filters (clean, not scratched, not deteriorated)

CHM.22700 Filter Photometers **Phase II**

Filters (filter photometers) are checked at least annually to ensure they are in good condition (eg, clean, free of scratches).

Evidence of Compliance:

✓ Records of filter checks at defined frequency

CHM.22900 Burner/Chimney **Phase II**

The burner, chimney and appropriate optical surfaces are checked for dirt and film and cleaned at defined intervals.

Evidence of Compliance:

✓ Record of maintenance at defined frequency

GENERAL CHEMISTRY

CHEMISTRY

CHM.28850 Ethanol Specificity **Phase II**

If the laboratory tests for ethanol, the method has been evaluated for ethanol specificity.

NOTE: Elevated lactic acid concentration and lactate dehydrogenase (LD) activity may falsely elevate enzymatically determined ethanol levels.

Evidence of Compliance:

✓ Records of ethanol specificity evaluation studies **OR** evaluation of information provided by the manufacturer **OR** evaluation of published literature

REFERENCES

1) Clinical and Laboratory Standards Institute. *Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline.* 3rd ed. CLSI Document C52-Ed3. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.
2) Clinical and Laboratory Standards Institute (CLSI). *Toxicology and Drug Testing in the Medical Laboratory.* 3rd ed. CLSI guideline C52. Clinical and Laboratory Standards Institute, Wayne, PA; 2017.
3) Frederick DL, King DS. Lactate Dehydrogenase Can Cause False-Positive Ethanols. *Clinical and Forensic Toxicology News (Quarterly AACC/CAP)*. June 2012:4-7.

CHM.28875 Urine Opiates Immunoassay Cutoff **Phase I**

The urine opiates immunoassay cutoff is appropriate for the clinical setting.

NOTE: Opiate class immunoassays are primarily designed to detect naturally occurring opiates (eg, morphine and codeine), and have varying cross-reactivity to the semisynthetic opioids (eg, oxycodone, hydrocodone). Therefore, when utilized for clinical care, including support of emergency departments and pain management clinics, the 300 ng/mL cutoff for the urine opiates immunoassays should be utilized. The 2000 ng/mL cutoff is more appropriate for workplace drug testing. As a class assay, the 300 ng/mL cutoff has better detection for lower

concentrations of naturally occurring opiates (morphine and codeine) and for the semisynthetic opioids (oxycodone, hydrocodone) when compared to the 2000 ng/mL cutoff.

It is recommended that the laboratory review the package insert for its opiates immunoassay for cross-reactivity with the semisynthetic opioids (eg, oxycodone, hydrocodone). Specific immunoassays for the detection of semisynthetic and synthetic (eg, buprenorphine, fentanyl) opioids are available and should be used when reliable detection of those drugs is required; alternative targeted methods such as mass spectrometry may also be appropriate.

If cutoff values different than those defined by the manufacturer are used, the laboratory must perform appropriate validation studies to support the modification.

Evidence of Compliance:

✓ Patient reports with cutoffs appropriate for the clinical setting

REFERENCES

1) Magnani BJ, Kwong TC, McMillin G, Wu AHB., eds Clinical Toxicology Testing: A Guide for Laboratory Professionals. 2nd ed. Northfield, IL: CAP Press; 2020.

THERAPEUTIC DRUG MONITORING

Inspector Instructions:

	• Sampling of TDM policies and procedures • Sampling of TDM patient reports (dosage, time of drug administration)
	• How is the clinician able to link TDM laboratory results to the dosage and time the patient received the drug?

CHM.28900 Specimen Collection/Drug Dosing **Phase I**

As applicable, information is available to clinical personnel for the optimal specimen collection time in relation to drug dosing.

Evidence of Compliance:

✓ Test reference guide **OR** other mechanism for providing guidance for specimen collection for therapeutic drug testing

REFERENCES

1) Nicholson PW, *et al.* Ideal sampling time for drug assays. *Br J Clin Pharm.* 1980;9:467470
2) Howanitz PJ, Steindel SJ. Digoxin therapeutic drug monitoring practices. A College of American Pathologists Q-Probes study of 666 institutions and 18679 toxic levels. *Arch Pathol Lab Med.* 1993;117:684-690
3) Schoenenberge RA, *et al.* Appropriateness of antiepileptic drug level monitoring. *JAMA.* 1995;274:1622-1626
4) Williamson KM, *et al.* Digoxin toxicity: an evaluation in current clinical practice. *Arch Intern Med.* 1998;158:2444-2499

CHM.29000 TDM Results **Phase II**

Where applicable, TDM results are reported in relation to patient dosing and/or timing information.

NOTE: The intent is to have a mechanism whereby the clinician can easily and accurately link TDM results from the laboratory to the dosage and time of drug administration. Ideally, the test result, dose and administration time would be reported in juxtaposition on the patient chart. This may be the responsibility of the laboratory, or an integrating function of reported laboratory analytic data with clinical information from other sources.

REFERENCES
1) Elin RJ. Computer-assisted therapeutic drug monitoring. *Clin Lab Med.* 1987;7:485-492
2) Howanitz PJ, Steindel SJ. Digoxin therapeutic drug monitoring practices. A College of American Pathologists Q-Probes study of 666 institutions and 18679 toxic levels. *Arch Pathol Lab Med.* 1993;117:684-690
3) Schoenenberge RA, *et al.* Appropriateness of antiepileptic drug level monitoring. *JAMA.* 1995;274:16221626
4) Williamson KM, *et al.* Digoxin toxicity: an evaluation in current clinical practice. *Arch Intern Med.* 1998;158:2444-2499
5) Steele BW, *et al*. An evaluation of analytic goals for assays of drugs. A College of American Pathologists therapeutic drug monitoring survey study. *Arch Pathol Lab Med.* 2001;125:729-735

CHM.29025 Immunosuppressive Drug Result Reporting **Phase II**

For the reporting of immunosuppressive drug results, the patient report contains all of the following:

1. **Appropriate therapeutic ranges based on the test method used**
2. **Analytical method (all tests) and method platform (immunoassays only)**
3. **Elements required in GEN.41096**

NOTE: For immunosuppressive drugs (eg, cyclosporine, sirolimus, tacrolimus, mycophenolic acid, everolimus), the therapeutic range may depend upon the test method, type of transplant, and length of time since the transplant procedure. Results from different types of samples and different methods are not interchangeable.

Evidence of Compliance:
- ✓ Patient results showing required report elements

TUMOR MARKER TESTING

Inspector Instructions:

READ

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

CHM.29050 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.

SWEAT TESTING FOR CYSTIC FIBROSIS

The laboratory diagnosis of cystic fibrosis includes SCREENING and CONFIRMATORY sweat testing. Screening tests include sweat conductivity. Confirmatory tests include quantitative analysis of sweat chloride.

SPECIMEN COLLECTION AND HANDLING

Inspector Instructions:

READ	• Sampling of sweat testing policies and procedures • Sampling of records/log of insufficient sweat samples • Sampling of iontophoresis unit maintenance records
OBSERVE	• An employee performing the sweat collection procedure, if possible
ASK	• How do you ensure effective disinfection of the sweat collection equipment? • What is your course of action if the patient is receiving oxygen from an open delivery system?

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

CHM.29100 Sweat Test/Appropriate Age **Phase I**

The sweat test is offered only to patients at an appropriate age.

NOTE: To increase the likelihood of collecting an adequate sweat specimen, sweat chloride testing can be evaluated in asymptomatic newborns with a positive newborn screen result or positive prenatal genetic test when the infant is at least ten days old, greater than 36 weeks gestation, and weighs >2kg. Sweat chloride testing should be performed as soon as possible at or after 10 days of life, ideally by four weeks of age. Sweat collections acquired in newborns >3.5kg (versus >2kg) improve specimen acceptability rates by 25% and should be considered when assessing the risk of a quality not sufficient (QNS) collection versus the need to obtain diagnostic information.

In symptomatic newborns (eg, those with meconium ileus), sweat chloride can be evaluated as early as 48 hours after birth if an adequate sweat volume can be collected; although, the likelihood of an inconclusive result may be greater at this age.

REFERENCES
1) Farrell PM, *et al*. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J. Pediatr.* February 2017;181S:S4-15.
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.
3) Eng W, LeGrys VA, Schechter MS, Laughon MM, Barker PM. Sweat-testing in preterm and full-term infants less than 6 weeks of age. *Pediatr Pulmonol.* 2005;40(1):64-7.

CHM.29150 Biennial Review Equipment Disinfection **Phase I**

The laboratory reviews the procedures employed for disinfection of equipment and facilities used for sweat collection at least biennially.

NOTE: The purpose of this review is to assure continued effectiveness. One suggested approach is biennial evaluation by the infection control department of the institution.

CHM.29200 Sweat Collection and Analysis Procedure **Phase II**

The laboratory follows generally accepted procedures for sweat collection and analysis, including steps to minimize sample evaporation or contamination.

NOTE: Because sample evaporation and contamination can have significant impact on the validity of test results, laboratories must incorporate the following steps into their procedure and/ or follow manufacturer's recommendations:

When using gauze or filter paper collection pads:

1. *Use gauze and/or filter paper that is low in electrolyte content*
2. *Wash the patient's skin thoroughly with distilled or deionized water, then dry before stimulation. Repeat after stimulation and before collection*
3. *Do not touch the weighing vial, wax film, collection site, or collection pad. Always use forceps or powder-free gloves*
4. *Use two pieces of waterproof adhesive tape on all sides of the paraffin wax film or wrap with a disposable stretch bandage to produce an airtight seal*
5. *Blot back into the collection pad any condensate that may have formed on the wax film during collection. Failure to collect the condensate can result in false positive test results*
6. *After collection, quickly transfer the specimen pad to the weighing vial and reweigh promptly*

When collecting sweat into Macroduct coils:

1. *Wash the patient's skin thoroughly with distilled, deionized water. Leave the skin slightly wet after washing the area where the electrode will be attached or add a drop of distilled or deionized water to either the skin or the Pilogel surface (after installation in the electrode). Repeat after stimulation and before collection*
2. *Avoid touching the collecting surface of the coil*
3. *Fasten the collector to the extremity with firm strap pressure. Test for proper attachment after sweat appears in the coils*
4. *Do not attempt to remove the entire collector assembly from the patient's extremity before separating the coil from the main body. Loss of specimen may occur*
5. *Do not contaminate the nippers or sweat dispensing needle with sweat sample*

When collecting and analyzing sweat using the Nanoduct system:

1. *Wash the patient's skin thoroughly with distilled, deionized water. Leave the skin slightly wet after washing the area where the electrode will be attached or add a drop of distilled or deionized water to either the skin or the pilogel surface (after installation in the electrode). Repeat after stimulation and before collection*
2. *Avoid touching the collecting surface of the device*

REFERENCES

1) Scott MG, *et al.* Electrolytes and blood gases, In Burtis C and Ashwood E (eds), Tietz textbook of clinical chemistry. Philadelphia: WB Saunders, 4th edition, 2006
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29300 Sweat Stimulation **Phase II**

Sweat is stimulated and collected from the patient's lower arm or upper leg, using a site that is free from diffuse inflammation or rash.

NOTE: Sweat must not be stimulated or collected from the head or trunk. Sweat must not be stimulated or collected from an area of diffuse inflammation, such as a rash or eczematous lesion, because of the likelihood of contamination by serous fluid.

REFERENCES
1) Liebke C, *et al.* Sweat electrolyte concentrations in children with atopic dermatitis. *Lancet.* 1997;350:1678
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29400 Pilocarpine Grade **Phase II**

If the laboratory prepares the pilocarpine solution for iontophoresis, the source of the pilocarpine is USP grade or equivalent.

CHM.29500 Electrode Placement **Phase II**

Electrodes used for stimulation are placed such that iontophoretic current never crosses the patient's trunk.

NOTE: The protocol must specify that electrodes used for stimulation be placed so that current does not cross the patient's trunk. This is to avoid the possibility of current crossing the heart, which results in cardiac depolarization.

CHM.29600 Iontophoresis Conditions and Equipment Maintenance **Phase II**

Iontophoresis conditions and maintenance requirements are defined.

NOTE: For safety reasons, the iontophoretic current source must be battery-powered, to avoid the possibility of patient exposure to line voltage. For manually controlled devices, iontophoresis must be performed for no more than five minutes at a current less than 4 mA, to prevent burns. The iontophoresis unit must be tested by qualified personnel (such as engineering personnel) for current leakage and current control at defined frequencies and records retained.

CHM.29700 Iontophoresis Oxygen **Phase II**

Iontophoresis is not performed on patients receiving oxygen by an open delivery system.

NOTE: While the possibility of an explosion due to the generation of an electrical spark is remote, it cannot be ignored. Often, these patients can temporarily receive oxygen via a facemask or nasal cannula, in which case sweat testing can be done.

CHM.29800 Iontophoretic Stimulation **Phase II**

The area of iontophoretic stimulation is equivalent to the area of sweat collection.

NOTE: Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases. The average sweat rate should exceed 1 g/m^2/min.

To ensure adequate sweat stimulation and accurately reflect sweat electrolyte concentration, a minimum acceptable sweat volume or weight is required. This requirement is based on the size of the electrode and stimulation area, the type and size of collecting media, and the duration of sweat collection. To standardize the process, the stimulation and collection area should be equivalent, and the time of collection consistent. For example, for the positive electrode, use a 1.5 x 1.5 inch (3.8 x 3.8 cm) electrode over a 2 x 2 inch (5.1 x 5.1 cm) gauze pad saturated with pilocarpine for stimulation, then collect sweat onto a 2 x 2 inch pre-weighed gauze pad.

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29850 Appropriate Sweat Collection Device **Phase II**

The sweat collection device is appropriate for the iontophoresis system.

NOTE: The sweat collection device must be designed for use with the appropriate iontophoresis system so that the stimulation and collection area are equivalent and the appropriate minimum acceptable sweat volume or weight can be achieved. Examples of acceptable combinations include:

- *Stimulation with Pilogel iontophoresis and collection into Macroduct coils*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into gauze/filter paper*
- *Stimulation and collection into Nanoduct conductivity cell*

Examples of unacceptable combinations include:

- *Stimulation with Pilogel iontophoresis and collection into gauze/filter paper*
- *Stimulation with Polychrome iontophoresis and collection into Macroduct coils, gauze or filter paper*
- *Stimulation with copper electrodes over gauze/filter paper pilocarpine pads and collection into Macroduct coils*

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.29900 Sweat Collection Time **Phase II**

For confirmatory sweat testing, collection time may not exceed 30 minutes.

NOTE: For quantitative testing, extending the collection time will not significantly increase the sweat yield and may lead to sample evaporation. Samples may be taken from less than maximally stimulated glands. This may lead to a false-negative result. In addition, altering the collection time will affect the minimum acceptable sweat weight or volume, because the time parameter of the rate equation has been changed. For screening kits, the manufacturer's instructions for collection times must be followed.

CHM.30000 Sweat Collection Parameters **Phase II**

The parameters of sweat collection are defined.

NOTE: These must include an established minimum acceptable sweat volume or weight based on the area of stimulation, area of collection and standardized time for collection. The average sweat rate should exceed 1 g/m^2/min, which in general corresponds to a minimum sample weight of about 75 mg of sweat collected on a 2 x 2 inch (5.1 x 5.1 cm) gauze or filter paper and about 15 µL of sweat collected in Macroduct coil in 30 minutes. Volume verification should be performed for any specimen that might be near the 15 µL threshold. Samples less than the required volume or weight must not be analyzed.

REFERENCES

1) Hjelm M, *et al.* Sweat sodium related to amount of sweat after sweat test in children with and without cystic fibrosis. *Acta Paediatr Scand.* 1986;75:652-656
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30100 Sweat Sample Rejection **Phase II**

Samples that do not meet the minimum sample size requirements are rejected and are not pooled for analysis.

NOTE: The average sweat rate of 1 g/m2/min is determined independently for each site. The requirement is a physiologic one, not an analytic one. Samples less than the required volume or

weight must not be pooled to achieve the weight/volume requirement. Measurement on samples from less than maximally stimulated sweat glands may lead to false-negative results.

Evidence of Compliance:
- ✓ Records of specimen rejection

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

CHM.30150 Sweat Rejection Incidence Rate **Phase I**

The incidence of insufficient sweat samples is routinely monitored.

NOTE: For quality monitoring, laboratories must collect data on the number of patients from whom an insufficient sweat sample has been obtained (QNS - quantity not sufficient). For patients older than three months of age, the annual insufficient rate should not exceed 5%. For patients up to three months of age, the rate should not exceed 10%. If these rates are exceeded, the collection procedure should be reevaluated for consistency with the CLSI guideline C34 4th ed.

For bilateral sweat collections, a QNS patient is a patient with an insufficient sample collected from both sites (eg, right arm and left arm). Each patient encounter is counted to determine the total number of sweat collections; thus, the same patient may appear repeatedly in the total population as well as the QNS population.

Evidence of Compliance:
- ✓ Records of insufficient collection **AND**
- ✓ Records of corrective action if rate exceeds the norm

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30250 Sweat Sample Storage **Phase I**

Appropriate storage conditions for collected sweat samples are defined.

NOTE: If there is a significant delay between collection and analysis, appropriate storage conditions must be followed: a) Sweat collected on gauze is stable at refrigerator temperatures for up to 72 hours once reweighed and secured in a vial with a tightly fitting cap; and b) Sweat collected in Macroduct coils is stable at refrigeration or room temperature for up to 72 hours in a 0.2 mL microcentrifuge tube with a tight fitting cap. Storage of sweat in microbore tubing is not recommended.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30300 Sweat Collection Skin Reaction **Phase II**

Processes to recognize and treat patient skin reactions (allergic or burns) to pilocarpine and/or other reagents used in iontophoresis are defined.

NOTE: Rarely, some patients may develop an area of, urticaria (hives) or small localized burns. In such cases, the procedure must be discontinued immediately and appropriate medical attention obtained. Sweat must not be collected over areas of urticaria or burns.

Evidence of Compliance:
- ✓ Records of follow-up treatment

ANALYTIC METHODS FOR SWEAT TESTING

Inspector Instructions:

READ

- Sweat testing method validation records
- Sampling of QC logs

CHM.30400 Sweat Test Validation **Phase II**

For sweat testing, the analytical method is validated by the laboratory prior to patient testing using specimens equivalent to the volume and concentration of patient sweat samples.

NOTE: Validation procedures must include studies of accuracy, precision, and upper/lower limits of the analytic measurement range. The laboratory should be aware that some instruments designed for serum or urine electrolyte determination may lack the sensitivity required for sweat testing.

Evidence of Compliance:

✓ Records of method validation

REFERENCES

1) Pillion DJ, Meczan E. Chloride measurement by microelectrode in cystic fibrosis and normal sweat. *Miner Electrolyte Metab.* 1987;13:196-200
2) Barnes GL, *et al.* Sweat testing by capillary collection and osmometry: suitability of the Wescor macroduct system for screening suspected cystic fibrosis patients. *Aust Paediatr J.* 1988;24:191-193
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):3707 [42CFR493.1255]
4) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.
5) Department of Health and Human Services, Centers for Medicare and Medicaid Services Center for Disease Control and Prevention. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register.* 2003 (Jan 24): Vol. 68, No. 16 [42CFR493]

CHM.30550 Sweat Chloride AMR **Phase II**

The lower limit of the sweat chloride analytical measurement range is less than or equal to 10 mmol/L.

NOTE: The lower limit of the sweat chloride analytical measurement range must be less than or equal to 10 mmol/L without any pretreatment that is not part of the usual assay procedure.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

CHM.30575 Confirmatory Sweat Test Report **Phase II**

If the test performed is a confirmatory test (ie, quantitative analysis of sweat chloride), the upper limit of AMR for sweat chloride results is less than or equal to 160 mmol/L.

NOTE: Even though the analytical instrument may have a higher upper limit of its AMR, sweat chloride concentrations > 160 mmol/L are not physiologically possible. Results of sweat chloride testing greater than 160 mmol/L must not be reported, and the patient must be retested.

Evidence of Compliance:
✓ Patient reports or worksheets

REFERENCES
1) Schultz IJ. Micropuncture studies of the sweat formation in cystic fibrosis patients. *J Clin Invest.* 1969;48:1470-1477

CHM.30600 Daily QC - Sweat Testing **Phase II**

The laboratory analyzes two levels of controls (one in the negative range and one in the positive range) at least once each day patient specimens are assayed.

NOTE: If sweat is collected from patients on gauze or filter paper, controls should be placed directly onto the same collection material, eluted, and treated in the same manner as a patient specimen.

For test systems with internal controls, the laboratory may limit daily quality control to the internal controls ONLY if all CAP requirements for internal controls are met, as listed in CHM.13900.

Evidence of Compliance:
✓ Records of QC results 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):7165 [42CFR493.1255]
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services Center for Disease Control and Prevention. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register.* 2003 (Jan 24): Vol. 68, No. 16 [42CFR493]

REPORTING OF RESULTS

Inspector Instructions:

- *Sampling of sweat analysis reports (appropriate reference intervals and disclaimer if applicable)*

***REVISED** 08/24/2023*
CHM.30700 Sweat Test Reporting **Phase II**

The laboratory report indicates the specific analytes measured in the sweat analysis, and applies the appropriate reference intervals and/or decision levels to patient results.

NOTE: The laboratory report must clearly indicate the analytes measured in the sweat test, and apply the appropriate reference intervals and/or decision levels to patient results.

The following table includes the Cystic Fibrosis Foundation reference intervals for chloride (Farrell PM et al 2017):

Test	Result	Interpretation
Sweat Chloride	≤29 mmol/L	CF unlikely
Sweat Chloride	30-59 mmol/L	Intermediate
Sweat Chloride	≥60mmol/L	Indicative of CF

Conductivity is a nonselective method for sweat analysis that has its own unique set of reference intervals. When sweat conductivity is expressed as units of aqueous sodium chloride solution,

the values are approximately 15 mmol/L higher than when chloride is measured directly. A patient having a sweat conductivity greater than or equal to 50 mmol/L should be referred to a specialized cystic fibrosis care center for a quantitative analysis of sweat chloride with or without sweat sodium.

REFERENCES
1) Farrell PM, *et al*. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J. Pediatr.* February 2017;181S:S4-15.
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

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

CHM.30800 Sweat Test Report Disclaimer **Phase II**

If the test performed is a screening test (eg, sweat conductivity), the report includes a statement regarding the limits of clinical interpretation.

NOTE: Suggested wording for such a disclaimer might be: "This result represents a screening test for cystic fibrosis. Patients having borderline or positive results should be referred for a quantitative sweat chloride concentration."

REFERENCES
1) Rosenstein BJ, Langbaum TS. Misdiagnosis of cystic fibrosis. Need for continuing follow-up and reevaluation. *Clin Pediatr.* 1987;26:78-82
2) Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, Wayne, PA, 2019.

PRENATAL SCREENING

TEST PANELS

Test panels include: 1) First trimester panel: total or free beta-hCG, pregnancy associated placental protein A (PAPP-A), and nuchal translucency (NT); 2) Second trimester quadruple panel: alpha-fetoprotein (AFP), unconjugated estriol (uE3), various forms of human chorionic gonadotropin (hCG), and dimeric inhibin-A (DIA); 3) Sequential and integrated panels: various combinations of first and second trimester tests. First trimester or second trimester panels may include additional markers to those listed if validated.

REQUISITIONS/CALCULATIONS/REPORTS

Requests for prenatal screening (neural tube defects, Down syndrome, etc.) must include specific information for meaningful interpretation of laboratory tests. For clinical screening purposes, analyte concentrations must be converted to multiple of the median (MoM) values, using gestational-age specific medians. The MoM value is used directly as the interpretative result for neural tube defect screening and for calculating risk for fetal trisomies. Gestational age-specific MoM values need to be adjusted for each patient, based on several variables. The laboratory must work cooperatively with the clinician to ensure that all necessary information is obtained.

A listing of published references is available with CHM.31150 (Prenatal Screen Risk Calculation) in the Master version of the checklist available for download by logging into cap.org and going to e-LAB Solutions Suite - Accreditation Checklists.

Inspector Instructions:

READ	• Sampling of prenatal screen policies and procedures • Sampling of prenatal screening requisitions • Sampling of records for adding or eliminating elements included in the risk calculation • Sampling of records of adjustment factors • Sampling of median value records • Sampling of records verifying calculated gestational age, maternal age and patient-specific risks • Sampling of maternal screen patient reports (demographics, clinical information, results reported in MoM, cut-off values)
ASK	• How does your laboratory verify or establish acceptable median values? • How does your laboratory monitor assay quality, appropriateness of medians and accuracy of gestational dating? • How does your laboratory monitor assay quality of nuchal translucency measurements? • What is your course of action if the requisition does not include all necessary information? • How does your laboratory verify or establish maternal weight adjustments?
DISCOVER	• Review runs that were accepted and rejected. Follow records to determine if the steps taken follow the written procedure for corrective action. • Review long term monitoring trends. Follow trends to determine if the steps taken follow the written procedure for corrective action. • Select a report with patient demographics requiring adjustment to MoM and review records for the use of correction factors for required markers

*****NEW** 12/26/2024***

CHM.31150 Prenatal Screen Risk Calculation **Phase II**

The laboratory determines which information and adjustments to include in the prenatal screening risk calculation.

NOTE: Expected elements in the prenatal risk calculation include:

- *Gestational age*
- *In vitro fertilization method, if applicable*
- *Initial or repeat testing*
- *Maternal age*
- *Maternal race or subpopulation as defined by the laboratory*
- *Maternal weight*
- *Medications to control diabetes*
- *Multiple gestation, if applicable*
- *Smoking status*

The rationale for exclusion of any expected element must be documented. Additional elements may be included in calculating the risk categorization. The rationale for additional elements must be documented.

REFERENCES

1) Wald NJ, *et al*. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural tube defects. *Lancet.* 1977;i:1323-1332.
2) Loughna, et al. Fetal size and dating: charts recommended for clinical obstetric practice. *Ultrasound.* 2009; 17.161-67.
3) Wald NJ, et al. Prenatal screening for open neural tube defects and Down syndrome: Three decades of progress. *Prenat Diagn.* 2010; 30:519-21.
4) Ioannou C, et al. Standardization of crown-rump length measurement. *BJOG.* 2013; 2(suppl):38-41.
5) Cuckle HS, *et al*. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Brit J Obstet Gynecol. 1987; 94:387-402.
6) Morris JK, Wald NJ. Estimating the risk of Down syndrome in antenatal screening and the gestation at which this applies. J Med Screen. 2008; 14(1):5-7.

7) Haddow JE, Palomaki GE, Canick JA, Knight GJ. Prenatal screening for open neural tube defects and Down's syndrome. In: Rodeck CH, Whittle MJ, ed. *Fetal Medicine: Basic Science and Clinical Practice.* 2nd ed. Churchill Livingstone: London;2009:243-264.
8) Watt HC, Wald NJ, Smith D, Kennard A, Densem J. Effect of allowing for ethnic group in prenatal screening for Down's syndrome. *Prenat Diagn.* 1996;16(8):691-8.
9) Benn PA, Clive JM, Collins R. Medians for second-trimester maternal serum alpha-fetoprotein human chorionic gonadotrophin, and unconjugated estriol; differences between races or ethnic groups. *Clin Chem* 1997 43(2):333-7
10) Cowans NJ, Spencer K. Effect of gestational age on first trimester maternal serum prenatal screening corrections for ethnicity and IVF conception. *Prenat Diagn.* 2013; 33(1):56-60.
11) Ball S, et al. Temporal effects of maternal and pregnancy characteristics on serum pregnancy-associated plasma protein-A and free beta human chorionic gonadotrophin at 7-14 weeks gestation. *Ultrasound Obstet Gynecol.* 2013; 41(1):33-9.
12) Burns NR, Kolarova T, Katz R, Ma K, Delaney S. Reconsidering race adjustment in prenatal alpha-fetoprotein screening. *Obstet Gynecol.* 2023; 141:438-44.
13) Haddow JE, Kloza EM, Knight GJ, Smith DE. Relation between maternal weight and serum alpha-fetoprotein concentration during the second trimester. *Clin Chem.* 1981; 27(1):133-4.
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15) Wright D, Papadopoulos S, Silva M, Wright A, Nicolaides KH. Serum free β-human chorionic gonadotrophin in the three trimesters of pregnancy: effects of maternal characteristic's and medical history. *Ultrasound Obstet Gynecol.* 2015; 46(1):51-9.
16) Wright D, Papadopoulos S, Silva M, Wright A, Nicolaides KH. Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristic's and medical history. *Ultrasound Obstet Gynecol.* 2015; 46(1):42-50.
17) Wald NJ, *et al.* Maternal serum alpha-fetoprotein and diabetes mellitus. *Br J Obstet Gynecol.* 1979;86:101-105
18) Sancken U, Bartels I. Biochemical screening for chromosomal disorders and neural tube defects (NTD): is adjustment of maternal alpha-fetoprotein (AFP) still appropriate in insulin-dependent diabetes mellitus (IDDM)? *Prenat Diagn*. 2001;21(5):383-6.
19) Hutley W, *et al.* Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. *Prenat Diagn* 2004;24:804-807
20) Savvidou MD, Syngelaki A, Muhaisen M, Emelyanenko E, Nicolaides K. First trimester maternal serum free- β human chorionic gonadotrophin and pregnancy-associated plasma protein-A in pregnancies complicated by diabetes mellitus. *BJOG*. 2012;119:410-6.
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22) Gurram P, Benn P, Campbell WA. Impact of diabetes on aneuploidy screening. *Clin Lab Med.* 2013;33(2):271-80.
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33) Madsen HN, et al. A reassessment of biochemical markers distributions in trisomy 21 affected and unaffected twin pregnancies in the first trimester. *Ultrasound Obstet Gynecol.* 2011; 37(1):38-47.
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43) Hackshaw AK, *et al.* Repeat testing in antenatal screening for Down syndrome using dimeric inhibin-A in combination with other maternal serum markers. Prenat Diagn, 2001;21:58-61
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46) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Standard - Second Edition.* CLSI document I/LA25-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
47) Messerlian G, Strickland SW, Wilbur J, et al. Use of maternal race and weight provides equitable performance in serum screening for open neural tube defects. *Clin Chem*. 2024; 70(7):948-56.

CHM.31800 Median Values **Phase II**

There is a record that the laboratory has established its own median values or verified that the medians from another source are appropriate for the population being screened.

NOTE: Systematic biases in maternal serum assay values of up to 30% can occur when kits from different manufacturers are used. In addition, between laboratory differences in equipment, reagents, and technique may introduce bias in assay results even when the same kit lot is used. These differences can be minimized by reporting results in multiples of the normal median (assuming that the medians are calculated using values measured on the population to be tested using the kit designated for screening). Ideally, day-specific medians would be established by testing approximately 100 patients per week of gestation. A second approach is to perform a split specimen study with another laboratory and transfer the other laboratory's medians using the comparison regression equation from the split specimen study. However, in practice the most practical method is to measure values on 300 consecutively collected specimens spread over the appropriate gestational age range, and perform weighted regression analysis using published models. It is not necessary to document that all specimens are collected from unaffected pregnancies because specimens from pregnancies affected with neural tube defects, Down syndrome etc., are infrequent. Smoothing data by weighted regression analysis allows median values to be calculated for weeks with limited data. Package insert medians may be outdated or inappropriate and should not be used even for a short time. Incorrect reference data may lead to inappropriate recommendations in the laboratory report.

Evidence of Compliance:

✓ Records establishing/verifying median values using in-house data

REFERENCES

1) Erickson JA, Ashwood ER, Gin CA. Evaluation of a dimeric inhibin-A assay for assessing fetal Down syndrome; establishment: comparison, and motoring of median concentrations for normal pregnancies. *Arch Pathol Lab Med.* 2004; 128(4):415-20.
2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Standard - Second Edition.* CLSI document I/LA25-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
3) Knight GJ. Quality assessment of a prenatal screening program. *Early Human Development.* 1996;47(Supp):S49-S53

CHM.31900 Median Value Review **Phase II**

Medians are reviewed at specified intervals or test volumes and when new reagent lots are introduced, and the medians are recalculated if necessary.

NOTE: Systematic shifts in analyte values observed with new reagent lots can cause significant deterioration in screening performance if not taken into account. One method for assessing a new lot is performing a split specimen comparison study between the new and old lot typically using 25 to 50 specimens. Bias between an existing and a new lot, if important (eg,>5%) can then be taken into account by adjusting the existing set of median values (or median equation).

In addition, review of medians at specified intervals is a valuable quality control mechanism to ensure validity of reported MoMs. Epidemiological monitoring of Down syndrome screening can be accomplished by determining the median MoM value at frequent intervals (eg, every 500-1000 patients, or weekly or monthly). If a persistent shift is noted (median MoM less than 0.90 or greater than 1.10), new medians should be determined. Records of the median MoMs must be retained.

Evidence of Compliance:

✓ Records of median value calculation or review at defined frequency or test volume

REFERENCES

1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda, Maryland. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural tube defects. *Clin Chim Acta.* 1980;105:9-24
2) Erickson JA, Ashwood ER, Gin CA. Evaluation of a dimeric inhibin-A assay for assessing fetal Down syndrome: establishment, comparison, and monitoring of median concentrations for normal pregnancies. *Arch Pathol Lab Med,* 2004 Apr;128(4):415-20.
3) Ashwood ER, Grenache DG, Lambert-Messerlian G. Pregnancy and its Disorders. *In: Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed.* Burtis CA, Ashwood ER, Bruns DE (eds), Elsevier Saunders: Philadelphia, PA, 2011.

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CHM.31950 Nuchal Translucency (NT) Measurement Quality **Phase I**

If screening panels are offered using nuchal translucency (NT) values, the laboratory has a process to ensure the quality of those measurements.

NOTE: The NT value is an important component of the test and may impact the results. Risk assessment can be used to determine if laboratories report the NT results.

Evidence of Compliance:

✓ Documentation of a quality assurance process

REFERENCES

1) American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No 77: Screening for fetal chromosomal abnormalities. *Obstet Gynecol.* 2007;109:217-27.
2) Ashwood ER, Grenache DG, Lambert-Messerlian G. Pregnancy and its Disorders. *In: Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed.* Burtis CA, Ashwood ER, Bruns DE (eds), Elsevier Saunders: Philadelphia, PA, 2011.
3) Schuchter K, et al. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn.* 2002; 22(3):211-5.
4) Malone FD, et al. Use of overall population, center-specific, and sonographer-specific nuchal translucency medians in Down Syndrome screening: which is best? (results from the faster trial). *Am J Obstet Gynecol.* 2003; 189(6):S232.
5) Palomaki GE, et al. Quality assessment of routine nuchal translucency measurements: A North American laboratory perspective. *Genet Med*, 2008;10(2):131-8.
6) Malone FD, D'Alton ME. First trimester sonographic screening for Down syndrome. *Obstet Gynecol.* 2003; 102(5):1066-79.

CHM.31960 Monitoring of Nuchal Translucency (NT) Measurements **Phase I**

If screening panels are offered using nuchal translucency (NT) values, the laboratory routinely performs epidemiological monitoring of these measurements.

NOTE: An example of such a monitoring procedure (with action limits) is provided below. For each sonographer with sufficient data (typically at least 30 to 50 measurements over six months), monitor and provide limits for three quality parameters.

- *Percent increase in NT measurements (in mm) by gestational age (eg, 15% to 35%)*
- *The NT median MoM (eg, 0.90 to 1.10) or the delta NT (eg, ±0.05 mm)*
- *The distribution of NT MoMs after a logarithmic transformation (log standard deviation), (eg, 0.08 to 0.13)*

Evidence of Compliance:

✓ Records of NT median data study(ies) **AND**

✓ Records of review at defined frequency

REFERENCES

1) Palomaki GE, Neveux LM, Donnenfeld A, Lee JE, McDowell G, Canick JA, Summers A, Lambert-Messerlian G, Kellner LH, Zebelman A, Haddow JE. Quality assessment of routine nuchal translucency measurements: A North American laboratory perspective. *Genet Med*, 2008 Feb;10(2):131-8
2) Ashwood ER, Grenache DG, Lambert-Messerlian G. Pregnancy and its Disorders. *In: Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed.* Burtis CA, Ashwood ER, Bruns DE (eds), Elsevier Saunders: Philadelphia, PA, 2011.
3) Palomaki GE, *et al*. Technical standards and guidelines: Prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements. *Genet Med,* 2009;11(9):669-681
4) Malone FD, et al. Use of overall population, center-specific, and sonographer-specific nuchal translucency medians in Down Syndrome screening: which is best? (results from the faster trial). *Am J Obstet Gynecol.* 2003; 189(6):S232.

CHM.32000 Screening Performance Monitoring **Phase II**

The percentages of women with screen-positive test results for neural tube defects (NTD), Down syndrome, and Trisomy 18 are calculated and reviewed at least biannually.

NOTE: Data from large studies provide guidelines for the percentage of pregnancies that will fall above specified AFP MoM levels (NTD screening) or with risks greater than specified risk cutoff levels (Down syndrome screening). Regular comparison of a laboratory's screen-positive rates with expected rates serves as a continuing measure of assay quality, appropriateness of medians, accuracy of gestational dating, and the distribution of maternal age. The frequency of

monitoring screen positive results will be dependent on the number of specimens analyzed per unit time, but it is recommended at least biannually, or optimally, quarterly.

Evidence of Compliance:

- ✓ Records of statistical analysis and evaluation of screen-positive test results at least biannually

REFERENCES

1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural tube defects. *Clin Chim Acta.* 1980;105:9-24
2) Ashwood ER, Grenache DG, Lambert-Messerlian G. Pregnancy and its Disorders. *In: Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed.* Burtis CA, Ashwood ER, Bruns DE (eds), Elsevier Saunders: Philadelphia, PA, 2011.
3) Palomaki GJ, *et al*. Risk based screening for trisomy 18 using alpha-fetoprotein, unconjugated estriol, and human chorionic. *Prenat Diagn* 1995;15:713-723
4) Knight GJ, *et al*. Epidemiologic monitoring of prenatal screening for neural tube defects and Down syndrome. *Clin Lab Med* 2003;22:531-551
5) Wald NJ, Hackshaw AK, George LM. Assay precision of serum alpha fetoprotein in antenatal screening for neural tube defects and Down's Syndrome. *J Med Screen*. 2000;7(2):74-7
6) Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Asses* 2003;7(11)

CHM.32200 Computer Calculations — Phase II

Gestational age, maternal age, neural tube defect and Down syndrome risk calculations are initially verified for accuracy and are reverified with any software updates or changes.

NOTE: Verification can be accomplished by interlaboratory comparisons, by comparison with results calculated or reported by proficiency testing programs, or by use of risk tables available on the CAP website (located in the CAP/ACMG Biochemical and Molecular Genetics Resource Committee Genetics Topic Center section). At a minimum, the accuracy of calculated gestational age, maternal age, and patient-specific risks must be verified.

Evidence of Compliance:

- ✓ Records of initial and subsequent calculation checks

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CHM.32300 Prenatal Screen Requisition and Report — Phase II

The prenatal screen requisition and report contain all information collected from the provider that is relevant to the clinical interpretation of the results.

NOTE: Prenatal screen risk calculation requisitions must include the information required by the laboratory to perform the risk assessment.

Evidence of Compliance:

- ✓ Requisitions with required elements **AND**
- ✓ Reports with required elements

CHM.32400 Multiple of Population Median — Phase II

Test results are reported as multiples of the population median (MoM).

NOTE: Reporting of results in terms of multiple of the population median (MoM) simplifies interpretation at various gestational ages, reduces possible systematic between-laboratory and between-kit bias in assay results, and facilitates comparison among laboratories. Laboratories can also compare their experiences with large-scale published studies more readily by using MoM as the reportable interpretive unit. The initial MoM is calculated as the measured analyte value divided by the median value for the appropriate gestational age. The MoM should also be adjusted for the other clinical variables known to influence the concentration of each analyte, generally by dividing by a factor specific for each variable.

REFERENCES
1) Report of a workshop sponsored by the National Institute of Child Health and Human Development (NICHD), Bethesda Maryland. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural tube defects. *Clin Chim Acta.* 1980;105:9-24
2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Standard - Second Edition.* CLSI document I/LA25-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

CHM.32500 Result Cut-off Values **Phase II**

The report classifies a pregnancy as screen-positive or screen-negative for open neural tube defects, based on the MSAFP test results.

NOTE: Cut-off levels based on AFP MoM values or risk have been established to determine the screening performance (initial positive rate, detection rate). Use of these cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES
1) Wald NJ, et al. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural tube defects. *Lancet.* 1977;i:1323-1332.
2) Cunningham MD, Tompkinson DG. Cost and effectiveness of the California triple-marker prenatal screening program. *Genet Med.* 1999;1(5):199-206.
3) American Society of Human Genetics. American of Human Genetics policy statement for maternal serum alpha-fetoprotein screening programs and quality control for laboratories performing maternal serum and amniotic fluid alpha-fetoprotein assays. *Am J Hum Genet.* 1987;40:5-82.

CHM.32600 Result Cut-off Values **Phase II**

The report classifies a pregnancy as screen-positive or screen-negative for fetal Down syndrome, based on the calculated risk.

NOTE: Cut-off values based on risk for Down syndrome have been established to determine the screening performance (initial positive rate, detection rate). Use of the cut-off values in the laboratory report can assist the physician in making clinical decisions about pregnancy management.

REFERENCES
1) Cunningham MD, Tompkinson DG. Cost and effectiveness of the California triple marker prenatal screening program. *Genet Med.* 1999;1(5):199-206.
2) American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No 77: Screening for fetal chromosomal abnormalities. *Obstet Gynecol.* 2007;109:217-27.
3) Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. *Lancet.* 2003;361:835-6.
4) Canick JA, et al. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol.* 2006;108(5):1192-9.

AMNIOTIC FLUID ALPHA-FETOPROTEIN (AFAFP)

Inspector Instructions:

	• Sampling of AFAFP policies and procedures • Sampling of AFAFP patient reports (results reported in MoM) • Sampling of QC logs
	• How does your laboratory verify or establish acceptable median AFAFP values? • What is your laboratory's course of action when you receive an amniotic fluid sample that is visibly contaminated with blood? • What is your laboratory's course of action when an amniotic fluid has an elevated AFP?

DISCOVER

- Select an abnormal AFAFP result and review records for the confirmatory testing performed, including QC for AChE testing

CHM.32700 Median AFAFP Values **Phase II**

There are records that the laboratory has established its own median AFAFP values or verified that medians provided from another source are appropriate for the population being screened.

NOTE: Systematic biases in AFAFP assay values of up to 30% can occur when kits from different manufacturers are used. In addition, between-laboratory differences in equipment, reagents, and technique may introduce bias in assay results even when the same kit is used. These differences can be minimized by reporting results in multiples of the median (assuming that the medians are calculated using values measured on the population to be tested using the kit designed for screening). Package insert medians may be outdated or inappropriate and should only be used as a general guide as to expected medians. When computing AFAFP medians from laboratory data, samples from pregnancies with known (or suspected) neural tube or ventral wall defects should be removed. A reasonable practice would be to trim all values over 2.5 or 3.0 MoM prior to computing medians using a log-linear model (for data between 15 and 22 weeks' gestation). Medians below 15 weeks do not follow the log-linear model and alternative curve fitting is required.

Evidence of Compliance:

✓ Records for median value determination using a reasonable number of in-house results (eg, 100 or more) **OR** records documenting the appropriateness of median values derived from package inserts or other sources for the laboratory's population of women tested

REFERENCES
1) Amniotic fluid alphafetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second report of the U.K. collaborative study on alphafetoprotein in relation to neural tube defects. *Lancet.* 1979;ii:651
2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

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CHM.32800 Median Value Calculation and Review **Phase II**

AFAFP medians are calculated and reviewed at specified intervals.

Evidence of Compliance:

✓ Records of median values calculation and review at defined frequency

CHM.32900 Multiple of Median **Phase II**

AFAFP results are reported in multiples of the median (MoM).

NOTE: Reporting of AFAFP results in terms of multiples of the median (MoM) simplifies interpretation at various gestational weeks, reduces the systematic between-laboratory and between-kit bias in results, and facilitates comparison of results between laboratories. Laboratories may compare their experiences with large-scale published studies much more readily when using MoM as the interpretive unit for AFP measurements.

REFERENCES
1) Wald NJ, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829
2) Clinical and Laboratory Standards Institute (CLSI). *Maternal Serum Screening; Approved Guideline—Second Edition*. CLSI document I/LA25-A2 (ISBN 1-56238-749-9). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011

CHM.33100 Dilution Control **Phase II**

If the analytical procedure for measurement of AFAFP requires specimen dilution, at least one amniotic fluid dilution control is processed with each analytic run of amniotic fluids.

Evidence of Compliance:

✓ Records of dilution control with each run

CHM.33200 Acetylcholinesterase Testing **Phase II**

Acetylcholinesterase (AChE) testing is performed on ALL amniotic fluids having elevated AFAFP concentrations.

NOTE: Acetylcholinesterase (AChE) testing is an essential confirmatory test for amniotic fluids with abnormal AFP results. The odds of having a fetus with a neural tube defect are considerably greater if both the AFAFP is elevated and the AChE is positive. The addition of AChE for the detection of neural tube defects will reduce the false positive rate while maintaining a high detection rate. This procedure may be performed in-house or by a referral laboratory. If fetal blood is present, acetylcholinesterase results must be interpreted with caution.

Evidence of Compliance:

✓ Patient reports showing AChE results, as applicable

REFERENCES

1) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet.* 1981;ii:321324
2) Wald N, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829
3) Crandall BF *et al.* Risks for fetal abnormalities after very and moderately elevated AFAFPS. *Prenat Diagn* 1997;17:837-841.

CHM.33300 Quality Control of Acetylcholinesterase Assays **Phase II**

Both positive and negative controls are included with each analytic run.

Evidence of Compliance:

✓ QC records for appropriate controls with each run

REFERENCES

1) Haddow JE, *et al.* Acetylcholinesterase and fetal malformations: modified qualitative technique for diagnosis of neural tube defects. *Clin Chem.* 1981;27:61-63
2) Report of the collaborative acetylcholinesterase study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet.* 1981;ii:321324
3) Wald NJ, *et al.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. *Prenat Diagn.* 1989;9:813-829

CHM.33400 Acetylcholinesterase Confirmation **Phase II**

Acetylcholinesterase-positive results are confirmed by addition of a specific inhibitor.

NOTE: Positive acetylcholinesterase results must be confirmed by the addition of a specific inhibitor of acetylcholinesterase, such as BW284C51.

Evidence of Compliance:

✓ Records of inhibitor testing for positive acetylcholinesterase results prior to reporting results

REFERENCES

1) Barlow RD, *et al.* A single method for amniotic fluid gelacetylcholinesterase determination, suitable for routine use in antenatal diagnosis of open neural tube defects. *Clin Chim Acta.* 1982;119:137-142.

ELECTROPHORESIS

Inspector Instructions:

	• Sampling of electrophoresis policies and procedures • Sampling of electrophoresis QC logs
	• Electrophoretic patterns (appropriate separations)

CHM.33500 Daily QC - Electrophoresis **Phase II**

Suitable control samples are run and reviewed with each batch of patient samples for all electrophoresis procedures for which controls are available.

Evidence of Compliance:

✓ Records of electrophoresis QC

CHM.33600 Electrophoresis Separations **Phase II**

Electrophoresis separations are satisfactory.

CHM.33700 Acceptable Limits - Controls **Phase II**

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

Evidence of Compliance:

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

HEMOGLOBIN SEPARATION

For purposes of diagnosing hemoglobinopathies, more than one test may be necessary. As an example, hemoglobin solubility testing alone is not sufficient for detecting or confirming the presence of sickling hemoglobins in all situations.

Inspector Instructions:

	• Sampling of abnormal hemoglobin policies and procedures • Sampling of patient reports (confirmatory testing, comments) • Sampling of QC records

	• Hemoglobin electrophoretic patterns (appropriate separations and controls) • Examine a sampling of medium (media) used to identify hemoglobin variants including alkaline/acid electrophoresis, isoelectric focusing, HPLC or other method
	• What is your course of action when the primary screening method appears to show Hb S? • What is your course of action when the primary Hb electrophoresis method shows Hb variants migrating in non-A/non-S positions?

CHM.33708 Hb S Primary Screen **Phase II**

For patient samples that appear to have Hb S in the primary screening by electrophoresis or other separation methods, the laboratory either: 1) performs a second procedure (solubility testing, or other acceptable method) to confirm the presence of Hb S, or 2) includes a comment in the patient report recommending that confirmatory testing be performed.

NOTE: For primary definitive diagnosis screening by electrophoresis or other separation methods, all samples with hemoglobins migrating in the "S" positions or peak must be tested for solubility or by other acceptable confirmatory testing for sickling hemoglobin(s). Known sickling and non-sickling controls both must be included with each run of patient specimens tested.

CHM.33716 Daily QC - Hgb Separation **Phase II**

Controls containing at least three known major hemoglobins, including both a sickling and a nonsickling hemoglobin (eg, A, F and S) are performed with the patient specimen(s) and separations are satisfactory.

NOTE: There are written procedures for instruments with multiple electrophoretic chambers or capillaries to ensure that QC is performed on each individual chamber or capillary.

Evidence of Compliance:

- ✓ QC records reflecting the use of appropriate controls **AND**
- ✓ Electrophoresis media/separation tracings demonstrating appropriate controls and separation

REFERENCES

1) Fairbanks VF. Hemoglobinopathies and thalassemias. Laboratory methods and case studies. New York, NY: BC Decker, 1980
2) Beuzard Y, *et al*. Isoelectric focusing of human hemoglobins, In Hanash, Brewer, eds. Advances in hemoglobin analysis. New York, NY: Alan R. Liss, 1981:177-195
3) Cossu G, *et al*. Neonatal screening of betathalassemias by thin layer isoelectric focusing. *Am J Hematol*. 1982;13:149
4) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
5) Honig GR, Adams JG III. Human hemoglobin genetics. Vienna, Austria: SpringerVerlag, 1986
6) Jacobs S, *et al*. Newborn screening for hemoglobin abnormalities. A comparison of methods. *Am J Clin Pathol*. 1986;85:713-715
7) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372
8) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
9) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
10) Armbruster DA. Neonatal hemoglobinopathy screening. Lab Med. 1990;21:815-822
11) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: Churchill Livingstone, 1991:1815-1827
12) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348- 352
13) Awalt E, *et al*. Tandem mass spectrometry (MS) – A screening tool for hemoglobinopathies. *Clin Chem*. 2001;47(suppl):A165
14) Bradley CA, Kelly A. Comparison of high performance liquid chromatography with electrophoresis for measurement of hemoglobins A, A2, S, F, and C. *Clin Chem*. 2001;47(suppl):A172
15) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A17315)

16) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

CHM.33732 Hemoglobin Variants — Phase II

All samples with hemoglobin variants migrating in "nonA, nonS" positions on alkaline electrophoresis, or other low resolution procedure are further defined with other acceptable methods where clinically and technically appropriate.

NOTE: If all clinically significant variants are not clearly separated by the primary method, additional testing must be performed to further characterize these hemoglobin variants. Examples include:

- *Performance by a complementary, separate methodology*
- *Increasing the duration of the assay (for HPLC) where the hemoglobins migrate/elute at different configurations*

Further workup of such variants, including referral to another laboratory, is dependent upon the patient's overall clinical situation, such as findings of erythrocytosis or a hemolytic anemia.

Evidence of Compliance:

✓ Patient reports and records reflecting further work-up, when appropriate

REFERENCES

1) Giordino PC. Strategies for basic laboratory diagnostics of the hemoglobinopathies in multi-ethnic societies: interpretation of results and pitfalls. *Int J Hematol.* 2013;35:465-79.
2) Sabath DE. Molecular diagnosis of thalassemias and hemoglobinopathies: an ACLPS critical review. *Am J Clin Pathol.* 2017;148:6-15.
3) Greene DN, Vaughn CP, Crews BO, Agarwal AM. Advances in detection of hemoglobinopathies. *Clinica Chimica Acta.* 2015;15:439-50.
4) Troxler H, Kleinert P, Schmugge M, Speer O. Advances in hemoglobinopathy detection and identification. *Adv Clin Chem.* 2012;57:1-28.
5) Bain BJ. Haemaglobinopathy diagnosis; algorithms, lessons and pitfalls. *Blood Rev.* 2011;25(5):2015-13.
6) Awalt E, *et al*. Tandem mass spectrometry (MS) – A screening tool for hemoglobinopathies. *Clin Chem*. 2001;47(suppl):A165
7) Bradley CA, Kelly A. Comparison of high performance liquid chromatography with electrophoresis for measurement of hemoglobins A, A2, S, F, and C. *Clin Chem*. 2001;47(suppl):A172
8) Bradley CA, Kelly A. Calibration verification of hemoglobins A, A2, S, and F with an automated chromatography system. *Clin Chem*. 2001;47(suppl):A173
9) Hoyer JD, *et al*. Flow cytometric measurement of hemoglobin F in RBCs: diagnostic usefulness in the distinction of hereditary persistence of fetal hemoglobin (HPFH) and hemoglobin S-HPFH from other conditions with elevated levels of hemoglobin F. *Am J Clin Pathol*. 2002;117:857-863

CHM.33764 Hb S Predominant Band — Phase II

All samples that appear to have Hb S as the predominant band by the primary screening and confirmed as sickling by appropriate methods are further examined to ascertain whether the "Hb S" band or peak contains solely Hb S or both Hb S and Hb D, Hb G or other variant hemoglobins.

NOTE: When the predominant hemoglobin component appears to be Hb S, it is necessary to determine whether this represents homozygous Hb S or a heterozygote for Hb S and another variant such as Hb D, Hb G, Hb-Lepore, or other hemoglobin variant(s). Given the clinical implications of homozygous Hb S (or Hb S/ß zero thalassemia) it is imperative to exclude other hemoglobin variants, however rare. Referral of these specimens to another laboratory for further workup is acceptable.

Evidence of Compliance:

✓ Patient records or worksheets showing the exclusion of hemoglobin variants **OR** referral for further work-up

REFERENCES

1) Black J. Isoelectric focusing in agarose gel for detection and identification of hemoglobin variants. *Hemoglobin*. 1984;8:117
2) Bunn HF, Forget BG. Hemoglobin: molecular, genetic and clinical aspects. Philadelphia, PA: WB Saunders, 1986
3) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part I. The introduction and thalassemia syndromes. *Lab Med*. 1987;18:368-372

4) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part II. The sickle cell disorders. *Lab Med*. 1987;18:441-443
5) Fishleder AJ, Hoffman GC. A practical approach to the detection of hemoglobinopathies: part III. Nonsickling disorders and cord blood screening. *Lab Med*. 1987;18:513-518
6) Adams JG III, Steinberg MH. Analysis of hemoglobins, In Hoffman R, *et al*, eds. Hematology: basic principles and practice. New York, NY: Churchill Livingstone, 1991:18151-827
7) Mallory PA, *et al*. Comparison of isoelectric focusing and cellulose acetate electrophoresis for hemoglobin separation. *Clin Lab Sci*. 1994;7:348-352

ANTI-NUCLEAR ANTIBODY TESTING

Inspector Instructions:

READ

- Sampling of ANA result reports

CHM.33780 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.

HIV PRIMARY DIAGNOSTIC TESTING

Inspector Instructions:

READ

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

CHM.33790 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.

BLOOD GAS ANALYSIS

The Chemistry and Toxicology Checklist is intended for inspection of laboratory sections performing testing in a dedicated space (eg, main laboratory, respiratory therapy). Laboratories performing testing at or near the patient bedside (eg, portable instruments) must use the Point-of-Care Testing Checklist.

The number of checklists needed for test sites under the same CLIA number and CAP number is determined as follows:

- *Blood gas testing performed in more than one area under the **same supervision** use one Chemistry and Toxicology Checklist (eg, main laboratory and stat lab);*
- *Blood gas testing performed in more than one area under **different supervision** use separate Chemistry and Toxicology Checklists for each separately supervised site (eg, main laboratory and respiratory therapy department);*

Testing sites within an institution with different CLIA and CAP numbers must submit separate applications and have separate full inspections.

SPECIMEN COLLECTION AND HANDLING

Inspector Instructions:

	• Blood gas collection policy and procedure • Sampling of records for performance of collateral circulation tests
	• How are personnel that perform arterial punctures made aware of possible complications?

Personnel performing arterial punctures are trained in the recognition and management of possible complications of this procedure.

Evidence of Compliance:

✓ Records of training in personnel files

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Blood Gas and pH Analysis and Related Measurements; Approved Guideline - Second Edition*. CLSI document C46-A2 (ISBN 1-56238-694-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA 2009.

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CHM.33900 Collateral Circulation — Phase II

For radial artery sampling, a test for collateral circulation is performed before arterial puncture if clinically indicated, with results recorded.

NOTE: Any of the various technologies evaluated in the published literature are acceptable. Consensus should be established between the laboratory and involved clinicians to define situations that require testing for collateral circulation, if any, to potentially avert patient injury.

Evidence of Compliance:

✓ Records of collection site and results of applicable collateral circulation testing

REFERENCES

1) Vaghadia H, *et al*. Evaluation of a postocclusive circulatory hyperaemia (PORCH) test for the assessment of ulnar collateral circulation. *Can J Anaesth.* 1988;35:591-598
2) Cheng EY, *et al*. Evaluation of the palmar circulation by pulse oximetry. *J Clin Monit.* 1989;5:1-3
3) Levinsohn DG, *et al*. The Allen's test: analysis of four methods. *J Hand Surg.* 1991;16:279-282
4) Fuhrman TM, *et al*. Evaluation of collateral circulation of the hand. *J Clin Monit.* 1992;8:28-32
5) Furhman TM, *et al*. Evaluation of digital blood pressure, plethysmography, and the modified Allen's test as a means of evaluating the collateral circulation to the hand. *Anaesthesia.* 1992;47:959-961
6) Fuhrman TM, McSweeney E. Noninvasive evaluation of the collateral circulation to the hand. *Acad Emerg Med.* 1995;2:195-199
7) O'Mara K, Sullivan B. A simple bedside test to identify ulnar collateral flow. *Ann Intern Med.* 1995;123:637
8) Starnes SL, *et al*. Noninvasive evaluation of hand circulation before radial artery harvest for coronary artery bypass grafting. *J Thorac Cardiovasc Surg.* 1999;117:261-266
9) Cable DG, *et al*. The Allen test. *Ann Thorac Surg.* 1999;67:876-877

CHM.34000 Ambient Air Contamination — Phase II

The laboratory has a process to prevent ambient air contamination of blood gas samples before analysis.

REFERENCES

1) Ishikawa S, *et al*. The effects of air bubbles and time delay on blood gas analysis. *Ann Allergy.* 1974;33:72-77
2) Mueller RG, *et al*. Bubbles in samples for blood gas determinations. *Am J Clin Pathol.* 1976;65:242-249
3) Madiedo G, *et al*. Air bubbles and temperature effect on blood gas analysis. *J Clin Pathol.* 1980;33:864-867
4) Biswas CK, *et al*. Blood gas analysis: effect of air bubbles in syringe and delay in estimation. *Brit Med J.* 1982;284:923-927
5) McKane MH, *et al*. Sending blood gas specimens through pressurized transport tube systems exaggerates the error in oxygen tension measurements created by the presence of air bubbles. *Anesth Analg.* 1995;81:179-182
6) Astles JR, *et al*. Pneumatic transport exacerbates interference of room air contamination in blood gas samples. *Arch Pathol Lab Med.* 1996;120:642-647
7) Clinical and Laboratory Standards Institute (CLSI). *Blood Gas and pH Analysis and Related Measurements; Approved Guideline - Second Edition*. CLSI document C46-A2 (ISBN 1-56238-694-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA 2009.

BLOOD GAS INSTRUMENTS

Inspector Instructions:

	• *Blood Gas analysis policy and procedure* • *Sampling of blood gas calibration records* • *Sampling of blood gas QC records*
	• *Is any testing performed on specimen types that are not FDA-cleared/approved on the blood gas instrument?*
	• *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 for corrective action*

CHM.34200 Calibration Materials — Phase II

The materials used for calibration of the pH, CO_2, and O_2 sensors are either in conformance with the instrument manufacturer's specifications or traceable to NIST Standard Reference Materials.

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Blood Gas and pH Analysis and Related Measurements; Approved Guideline - Second Edition*. CLSI document C46-A2 (ISBN 1-56238-694-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA 2009.

CHM.34300 Calibration - Blood Gas Instruments — Phase II

Blood gas instruments are calibrated according to manufacturer's specifications and at least as frequently as recommended by the manufacturer.

NOTE: Some instruments have built in calibration that is performed automatically by the instrument; however, there must be some defined procedure for verifying the reliability of this process. If appropriate, the calibration must compensate for the influence of barometric pressure.

Evidence of Compliance:

✓ Records for calibration 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):3709 [42CFR493.1267(a)]

CHM.34400 Daily QC - Blood Gas Instruments — Phase II

A minimum of one level of quality control for pH, pCO_2 and pO_2 is analyzed at least every eight hours of operation when patient specimens are tested, or more frequently if specified in the manufacturer's instructions or laboratory procedure, 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, or change of a critical instrument component, or with software changes, as appropriate.

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.* 2003(Jan 24) [42CFR493.1267(b)]
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. S & C: 16-20-CLIA: Policy Clarification on Acceptable Control Materials Used when Quality Control (QC) is Performed in Laboratories. April 8, 2016.

CHM.34500 Daily QC - Blood Gas Instruments — Phase II

The control materials for pH, pCO_2 and pO_2 represent both high and low values on each day of patient testing.

NOTE: If using internal controls, the electronic simulators should challenge at high and low values.

Evidence of Compliance:

- ✓ QC records reflecting the appropriate use of controls

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.* 2003(Jan 24) [42CFR493.1267(b)]
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) Ng VL, *et al*. The rise and fall of i-STAT point-of-care blood gas testing in an acute care hospital. *Am J Clin Pathol.* 2000;114:128-138

CHM.34600 QC - Blood Gas Instruments — Phase II

At least one level of quality control material for pH, pCO_2 and pO_2 is included each time patient specimens are tested, except for automated instruments that internally calibrate at least once every 30 minutes of use.

NOTE: An internal quality control process (eg, electronic/procedural/built-in) may be used to meet this requirement if an individualized quality control plan (IQCP) has been approved by the laboratory director.

Evidence of Compliance:

- ✓ QC results **OR** record of internal calibrator

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.* 2003(Jan 24): 3709 [42CFR493.1267(c)]
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.