COLLEGE of AMERICAN PATHOLOGISTS

Master

Clinical Biochemical Genetics Checklist

CAP Accreditation Program

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Clinical Biochemical Genetics 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
Clinical Biochemical Genetics 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
CBG.17150	08/24/2023

REVISED Checklist Requirements

Requirement	Effective Date
CBG.12250	08/24/2023
CBG.12300	08/24/2023
CBG.17100	08/24/2023
CBG.17500	08/24/2023
CBG.17600	08/24/2023

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
CBG.17200	08/23/2023

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a clinical biochemical genetics laboratory section or department.

The Clinical Biochemical Genetics Checklist covers aspects of clinical biochemical genetic testing performed for the diagnosis of inborn errors of metabolism (IEM), including, but not limited to, the analysis of amino acids, organic acids, enzymes involved in intermediary metabolism, carnitine and acylcarnitines, acylglycines, CSF neurotransmitters, sugars, glycosaminoglycans and glycoproteins. Biochemical tests for the identification of heterozygotes of IEMs and newborn screening for IEMs are also covered.


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).*

CLINICAL BIOCHEMICAL GENETICS GENERAL ISSUES

QUALITY MANAGEMENT

GENERAL ISSUES

Inspector Instructions:

- Sampling of patient results with review by the laboratory director or qualified designee within the turnaround time specified

CBG.10900 Result Review **Phase I**

The laboratory director or qualified designee reviews test results within the turnaround time specified by the laboratory's written procedure.

NOTE: In the case of clinically significant abnormal results, there must be a procedure for rapid communication to and receipt by the laboratory director or qualified designee.

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
	• 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?
	• Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration, and unacceptable calibration verification

This introduction discusses the processes of calibration, calibration verification, and analytical measurement range (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 test system.

In some instances, suitable calibration materials may not be available, eg, when the analyte is unstable. In these cases, calibration and calibration verification may not be possible, and therefore at least two samples from normal patients should be analyzed along with the patient samples submitted for testing, to verify that the normal patient samples have results in the normal range.

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 CBG.12500 & CBG.12600). 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.

CBG.11700 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.

The calibration procedure must define the limits of acceptable variation, eg, +/- 20% of the expected value. These limits should be applied to all standard and control samples run after the calibration is performed. The procedure should also specify the actions to be taken if a control or standard sample falls outside the defined range. For some analytes (eg, enzymes) calibration is limited to the product of the reaction, rather than the enzyme concentration or activity itself.

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,p 99-111

CBG.11800 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.
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): [42CFR493.1255]

CBG.12100 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*

Evidence of Compliance:

✓ Records of calibration verification documented 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.

CBG.12200 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*

CBG.12250 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*

CBG.12300 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 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.

It is also not necessary for every analyte in a multiple-analyte procedure to be verified in this way; it is acceptable to verify a clinically important subset of analytes, or one or more analytes representing groups with the same chemical characteristics. For example, in automated amino acid analysis, the laboratory may verify a single amino acid eluted with each buffer.*

**A laboratory test that detects or measures multiple similar compounds, such as organic acids or amino acids. It does not refer to a multiple-test chemistry panel.*

Evidence of Compliance:

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

REFERENCES

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

CBG.12500 Diluted or Concentrated Samples — Phase II

If a result is greater than or less than the AMR, a numeric value 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, 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

CBG.12600 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

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.

CONTROLS – NONWAIVED TESTS

Inspector Instructions:

	• Sampling of quality control policies and procedures • Sampling of QC records
	• 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 change 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?
	• 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 procedures for corrective action

CBG.12800 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 external controls must be run as follows:

- *For quantitative tests, two controls at two different concentrations must be run daily or with each batch of samples/reagents, unless a different requirement is specifically required by this checklist. Analytes selected are based on availability of materials.*
- *For qualitative tests, a negative control and a positive control (when available) must be run daily or with each batch.*

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

Evidence of Compliance:

✓ Records of QC results

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) (i, ii)], [42CFR493.1256(d)(6)].
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) Ye JJ, *et al*. Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol.* 2000;113:240-248
4) 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.

CBG.12900 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.

CBG.13000 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)]

CBG.13100 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)]

CBG.13200 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.

In clinical biochemical genetics laboratories calibrators and control materials are not available for some of the analytes detected in complex metabolic profiles. As these analytes often have critical clinical significance, it is acceptable to use surrogate calibrations (using compounds with similar structure) to generate quantitative results used in the context of pattern recognition and profile interpretation. When surrogate calibrators are used, their use and the basis of their use must be validated and recorded.

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)].

CBG.13300 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.

CBG.13400 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, CV) 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) 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) Brooks ZC, *et al*. Critical systematic error supports used of varied QC rules in routine chemistry. *Clin Chem.* 2000;46:A70

CBG.13500 QC Corrective Action — Phase II

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

NOTE: In the case of complex metabolic profiles as seen in clinical biochemical genetics laboratories, controls of analytes of clinical significance should meet the laboratory's overall criteria for acceptability.

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.

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)]

CBG.13600 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.

For newborn screening testing, good laboratory practice is to punch controls and patient blood specimens with the same equipment.

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)

CBG.13700 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)]

CBG.13800 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.

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

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

ENZYME ASSAYS

Inspector Instructions:

- Sampling of enzyme assay policies and procedures
- Sampling of control, calibration curve records
- Sampling of patient reports for completeness

CBG.14100 Control for Interference **Phase II**

Appropriate blanks are included in each run.

NOTE: Blanks are used to control for interference from two sources: background activity related to the reagents and non-enzymatic conversion of substrate to product.

CBG.14200 Calibration Curve **Phase II**

Standards are used to create a calibration curve for each run unless the calibration has been validated to remain stable over a defined period of time.

Evidence of Compliance:
- ✓ Records for calibration of each run or at the defined interval **AND**
- ✓ Records for validation of calibration stability if calibration is not performed with each run

CBG.14300 QC - Enzyme Assays **Phase II**

Controls are analyzed with each run.

NOTE: Ideally at least one affected control and one normal control sample are analyzed with each run. However, samples from affected patients may not always be available, and the use of inactivated samples (ie, samples that have been heated or treated in some other way to inactivate the enzyme of interest) is an acceptable alternative.

CBG.14400 Reference Intervals **Phase II**

Reference intervals for normal, disease, and if appropriate, carrier reference intervals are defined for each assay.

NOTE: Reference intervals must be established by the laboratory based on its own analysis of samples from multiple individuals, when appropriate. For rare diseases, it may not be possible for the laboratory to establish its own disease ("affected") reference interval. In this case, it is permissible to use reference intervals from the literature or other laboratories performing the test, as long as these are based on the same analytic method.

Evidence of Compliance:
- ✓ Records of establishment of reference intervals **OR**
- ✓ Literature to support reference intervals

REFERENCES

1) Clinical and Laboratory Standards Institute (CLSI). *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory - Approved Guideline-Third Edition.* CLSI Document EP28-A3c. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.

CBG.14500 Report Content **Phase II**

Laboratory reports include an interpretation of the result that reflects the presence or absence of the disease (or carrier state), possible limitations of the test, and, if appropriate, recommendations for additional testing.

CHROMATOGRAPHY AND MASS SPECTROMETRY

THIN LAYER CHROMATOGRAPHY (TLC)

Inspector Instructions:

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

CBG.14600 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 solution, previously tested positive patient samples, 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.

CBG.14700 Daily QC - TLC **Phase II**

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

NOTE: Appropriate positive controls must include compounds that test the extraction, chromatographic range of the TLC plate, and the staining/development system. Negative and positive controls (when available) must be extracted and carried through the entire procedure with each plate or card.

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)]
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.

CBG.14800 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 must be followed.

GAS CHROMATOGRAPHY (GC)

Inspector Instructions:

	• Sampling of GC policies and procedures • Sampling of control, calibration/standards records • Sampling of column verification records
	• How does your laboratory evaluate potential carryover? • How have you determined the limit of detection and the AMR?

CBG.14900 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.

CBG.15000 Daily QC - GC **Phase II**

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

NOTE: Controls used in GC 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 inappropriate. 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*

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.

CBG.15100 Sample Run Order **Phase II**

A record of sample run order is maintained for review.

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

CBG.15200 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

CBG.15300 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. *Gas Chromatography/Mass Spectrometry Confirmation of Drugs; Approved Guideline.* 2nd ed. CLSI Document C43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2010.
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 (CLSI). *Preliminary Evaluation of Quantitative Medical Laboratory Measurement Procedures.* 4th ed. CLSI guideline EP10. Clinical and Laboratory Standards Institute, Wayne, PA; 2024.

CBG.15400 Column Verification **Phase II**

New columns are verified for performance before use.

Evidence of Compliance:
✓ Records of column verification

CBG.15500 Instrument Calibration **Phase II**

The laboratory calibrates GC equipment and reviews calibration records for acceptability.

CBG.15700 Gas Leakage **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

CBG.15800 Reagent Grade **Phase II**

Reagents, solvents and gases are of appropriate grade.

CBG.15900 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.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Inspector Instructions:

READ	• Sampling of HPLC policies and procedures • Sampling of control, calibration/standards records • Sampling of column verification records
ASK	• How does your laboratory evaluate potential carryover? • How have you determined the limit of detection and the AMR?

CBG.16000 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.

Quality control materials in the appropriate concentration range may be used for calibration verification, providing that the linear response is verified by periodic multipoint calibration verification and AMR verification.

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]

CBG.16100 Quality Control - HPLC **Phase II**

Appropriate controls are extracted and run through the entire procedure.

NOTE: Controls used in HPLC 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*

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]

CBG.16200 Sample Run Order **Phase II**

A record of sample run order is maintained for review.

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

CBG.16300 Chromatographic Characteristics/Column Performance **Phase II**

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

Evidence of Compliance:
- ✓ Records of review and approval

CBG.16400 Column Verification **Phase II**

New columns are verified for performance before use.

Evidence of Compliance:
- ✓ Records of column verification

CBG.16500 Reagent Grade **Phase II**

Reagents and solvents are of appropriate grade.

CBG.16600 Instrument Calibration **Phase II**

The laboratory calibrates HPLC equipment and reviews calibration records for acceptability.

CBG.16700 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 for reassessment of samples with potential carryover

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) Society of Forensic Toxicologists/American Academy of Forensic Sciences. *Forensic Toxicology Laboratory Guidelines.* 2002; 8.2.8:13

CBG.16900 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

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?

CBG.17000 Instrument Calibration **Phase II**

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

REFERENCES

1) 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.
2) 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***

CBG.17100 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.

Tandem mass spectrometers require tuning at the time of maintenance that requires shutdown of the vacuum.

Evidence of Compliance:
- ✓ Records of tuning

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

CBG.17150 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.

CBG.17300 Identification Criteria - Single Stage Mass Spectrometry Phase II

The identification criteria for single stage mass spectrometry (ie, GC/MS, LC/MS) are in compliance with recommendations.

NOTE: One acceptable criterion for compound identification by GC/MS using ion ratios is that the unknown result must have ion ratios within a predefined tolerance limit. 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.

Identification using ion ratios typically requires the use of at least two ion ratios. However, one ion ratio of two characteristic ions may be acceptable if there are only a few characteristic ratios AND if there are other identifying characteristics, eg, retention time. The internal standard's identification should be monitored with at least one ion ratio. An acceptable criterion for compound identification using total spectra is that the unknown result must have a "spectral

match" quality or fit that is within the defined limits that the laboratory has set and validated. Ion ratios determined from total spectra analysis are an acceptable identification method, and should fulfill the same criteria as given above for ion ratio identification.

Laboratories using mass spectrometric methods for quantitative purposes based on total ion current measurements (without ion ratios) should have ancillary information and assay characteristics that validate this process, eg, known compound of interest, retention times, potential interferences by endogenous compounds or other drugs/metabolites, etc.

Evidence of Compliance:
✓ QC and test records

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.

CBG.17400 Identification Criteria - Tandem Mass Spectrometry **Phase II**

The identification criteria for tandem mass spectrometry (MS/MS) are validated and recorded.

NOTE: 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

***REVISED** 08/24/2023*
CBG.17500 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 trends 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 define appropriate acceptance criteria for each reportable analyte during routine testing of patient samples.

REFERENCES
1) Clinical and Laboratory Standards Institute. *Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline.* CLSI Document C50-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
2) Clinical and Laboratory Standards Institute (CLSI). *Liquid Chromatography-Mass Spectrometry Methods.* 2nd ed. CLSI document C62. Clinical and Laboratory Standards Institute, Wayne, PA; 2022.
3) Annesley TM. Ion Suppression in Mass Spectrometry. *Clin. Chem.* 49, pp. 1041-1044 (2003).

***REVISED** 08/24/2023*
CBG.17600 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.

COLORIMETERS, SPECTROPHOTOMETERS, AND FLUORIMETERS

Inspector Instructions:

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

CBG.17700 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

CBG.17800 Spectrophotometer Checks **Phase II**

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

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

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

CBG.17900 Stray Light **Phase II**

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

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

CBG.18000 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

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]

ELECTROPHORESIS

Inspector Instructions:

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

CBG.18025 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

CBG.18050 Electrophoresis Separations **Phase II**

Electrophoresis separations are satisfactory.

CBG.18075 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 verification of each lot

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

CBG.18080 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

CBG.18085 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

CBG.18090 Counting Times **Phase II**

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

NEWBORN SCREENING SPECIMEN COLLECTION, HANDLING AND TESTING

This section applies to laboratories that perform testing on newborns from whole blood heel stick samples collected on filter paper collection devices for the routine screening of congenital disorders. Additional specimen collection requirements for newborn screening specimens are found in the Laboratory General Checklist.

Inspector Instructions:

	• Specimen collection instructions, including handling, transport, and submission • Sampling of procedures and records for follow up of positive or invalid results • Records for the monitoring of the quality of specimens, completeness of collection records, and transportation time • Sampling of patient reports
	• How is follow-up tracked for patients requiring additional testing?

CBG.20110 Specimen Collection Instructions — Phase II

Instructions for the proper collection, handling, transport, and submission of newborn screening specimens are provided to locations submitting specimens for analysis.

NOTE: It is acceptable for this information to be electronically available to users rather than in paper format. Instructions must describe the proper application and drying of blood spots and submission of patient information needed for interpretation of the data. The collection instructions must be in compliance with the current edition of the CLSI Standard NBS01, Blood Collection on Filter Paper for Newborn Screening Programs, and state or local regulations for collection of specimens.

REFERENCES

1) Clinical and Laboratory Standards Institute. *Blood Collection on Filter Paper for Newborn Screening Programs.* 7th ed. CLSI standard NBS01. Clinical and Laboratory Standards Institute. Wayne, PA; 2021.

CBG.20120 Specimen Quality Monitoring — Phase II

The laboratory monitors specimen quality, completeness of patient records, and transportation time for specimens submitted for newborn screening.

NOTE: The patient records submitted with newborn screening specimens must include information for patient identification and proper interpretation of the data including all required elements defined in the most recent edition of CLSI Standard NBS01. If problems with specimen quality or missing collection information are identified, the laboratory must record appropriate corrective actions that lead to continuous quality improvement.

Specimens should be transported after they are dry and no later than 24 hours after collection or following the instructions provided by the designated newborn screening laboratory. Delays in specimen transportation from the collection facility to the testing laboratory may compromise the integrity of the specimen and results. Ultimately, the delay could critically impact the newborn.

Evidence of Compliance:

- ✓ Records of monitoring for poor quality specimens, incomplete collection information submitted, and specimen transport problems **AND**
- ✓ Records of communications with clients that submit specimens with quality issues

CBG.20130 Consent Procedure — Phase II

In cases where an indication of consent is required on the newborn screening collection device, either for collection or for later use (research), there is a process for review and action to ensure appropriate use of the specimen.

NOTE: Records must demonstrate that this procedure is followed.

CBG.20140 Out-of-Range/Invalid Results **Phase II**

The laboratory reports positive (out of range) or invalid results to the submitting location and other appropriate entities to allow for patient follow-up within a timeframe appropriate to ensure maximum health benefit.

NOTE: Positive results include those results that are outside of the expected range of testing results established for a particular condition. Invalid results include situations where the laboratory is unable to complete the screening process due to an unsuitable specimen, test, or incomplete information. The findings must be communicated in a manner consistent with the urgency of the intervention needed. For situations requiring repeat screening or confirmatory testing, the laboratory must clearly communicate the timing of the actions to be taken.

Results must be reported to the submitting location within seven days of specimen receipt and within three days for specimens received for tests requiring additional action (eg, invalid or positive). The records must indicate when results were reported and who received the results. In cases where the testing laboratory is responsible for ensuring that a replacement specimen has been received and analyzed, appropriate records must attest to specimen receipt, testing and result reporting.

CBG.20150 Results Reporting **Phase II**

Newborn screening results are reported to the submitting location and include all required result reporting elements from the Laboratory General Checklist.

CBG.20160 Follow-up Procedures **Phase I**

In cases where the testing laboratory is responsible for testing and follow-up (including patient tracking), all follow-up procedures are "closed loops" consistent with the CLSI Guideline NBS02, Newborn Screening Follow-up, or appropriate local policy.

NOTE: The laboratory's written procedures should include:

1. *Cases requiring notification*
2. *Roles and responsibilities of all individuals in the follow up system, as appropriate (laboratory staff, physicians, and birthing centers)*
3. *Method and timing of notifications (eg, phone call, fax or letter)*
4. *Monitoring of follow-up to track the actions taken until resolution - specimen monitoring, follow-up calls/letters, nurse visits, etc.*
5. *Case Resolution - follow-up actions, including the extent of actions required before closing a case without resolution or lost to follow up*

The procedures must follow local laws and regulations. "Lost to follow up" occurs when a notification cannot be made.

REFERENCES

1) Clinical and Laboratory Standards Institute. *Newborn Screening Follow-up;* 3rd ed. CLSI Guideline NBS02. Clinical and Laboratory Standards Institute, Wayne, PA; 2023.

HEMOGLOBIN SEPARATION

This section is intended for laboratories that are performing screening tests on newborns from whole blood heel stick specimens collected on filter paper for the routine screening for abnormal hemoglobin variants.

Inspector Instructions:

	• Sampling of abnormal hemoglobin policies and procedures • Sampling of patient reports (confirmatory testing, comments) • Sampling of QC records
	• Hemoglobin separation 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 screening method shows Hb variants migrating in non-A/non-S positions?

CBG.20165 Hb S Primary Screen **Phase II**

For samples that have screening results demonstrating the presence of a hemoglobin consistent with Hb S and suggesting a possible clinically significant condition, reporting of the screening results includes a recommendation 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 sickling hemoglobin(s). Known sickling and non-sickling controls both must be included with each run of patient specimens tested.

REFERENCES
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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
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CBG.20170 Daily QC - Hgb Separation **Phase II**

Controls containing at least three known major hemoglobins, including Hb F and both a sickling and a nonsickling hemoglobin (eg, A, F and S) are applied 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**
- ✓ Data, tracings, or other appropriate testing results (eg, photographs, gels, cards with banding patterns) demonstrate appropriate controls and separation

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CBG.20175 Hemoglobin Variants — Phase II

All samples with hemoglobin variants not appearing to be Hb A, Hb F, or Hb S by the separation procedure in use, are reported with a recommendation to obtain confirmatory testing consistent with local screening program recommendations.

NOTE: The laboratory must have a defined process for the reporting of abnormal hemoglobin variants, developed in consultation with hematology advisors. The procedure must address the confirmatory testing recommendations, if confirmatory testing is not performed on-site.

Evidence of Compliance:

- ✓ Patient reports and records reflecting adherence to laboratory reporting procedures

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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.
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