Calibration Verification and Linearity Information Guide

On January 24, 2003, CDC and CMS issued revised CLIA regulations, effective April 24, 2003, establishing requirements for calibration verification applicable to moderate- and high-complexity testing. Full regulations are available at: https://www.cdc.gov/clia/php/about/index.html.[1]

In accordance with CMS, calibration verification must be performed at least every six (6) months and whenever specific events occur that may impact analytical performance. The laboratory must ensure calibration verification is repeated when:

- All of the reagents used for a test procedure are changed to new lot numbers, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are also not adversely affected by reagent lot number changes.
- There is major preventive maintenance or replacement of critical parts that may influence the test's performance. This includes when the laboratory sends a test system to the manufacturer for repairs. The laboratory must check the calibration of a repaired test system before resuming patient testing and reporting results.
- Control 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.
- The laboratory has determined that the test system's reportable range for patient test results should be checked more frequently (CMS Calibration and Calibration Verification).[2]

Verification materials must provide known target values and must span the analytical measurement range (AMR). Materials may be supplied as liquid, frozen, or lyophilized; preparation must conform strictly to manufacturer instructions. The laboratory must document the source, lot numbers, storage conditions, and preparation of all materials used.

However, there are exceptions to calibration verification requirements as stated within CMS Calibration and Calibration Verification brochure (2006):

- *Instruments that are factory or manufacturer calibrated do not require calibration verification*
- *Tests that are considered non-quantitative (e.g., Prothrombin Time (PT) and Activated Clotting Time (ACT), which are measured in units of time) do not require calibration verification*
- *For automated cell counters, the calibration verification requirements are considered met if the laboratory follows the manufacturer's instructions for instrument operation, and tests two levels of control materials each day of testing, provided the control results meet the laboratory's criteria for acceptability.*
- *For automated chemistry analyzers, the calibration verification requirements are considered met if the laboratory follows the manufacturer's instructions for instrument operation and routinely tests three levels of control materials (lowest level available, mid-level, and highest level available) more than once each day of testing, the control material results meet the laboratory's criteria for acceptability and the control materials are traceable to National Institute of Standards and Technology (NIST) reference materials.*

- *If the test system's calibration procedure includes three or more levels of calibration material, and includes a low, mid, and high value, and is performed at least once every six months, then the requirement for calibration verification is also met.*[2]

All levels must be processed in the same manner as patient specimens. The number of replicates must follow laboratory-defined procedures. The laboratory must compare measured values to expected values and determine acceptability using predefined criteria (e.g., slope, intercept, percent recovery, or other laboratory-defined limits).

Documentation must include:

- Verification materials used, lot numbers, and preparation records
- Test dates, personnel performing the testing, and instrument identification
- Expected vs. observed results for each level
- Statistical evaluations or graphs used to assess linearity and accuracy
- Final determination of acceptability and supervisory review

All records must be available for inspection and retained in accordance with CAP and CLIA retention requirements.

AUDIT's Auditor™ QC Program (www.auditmicro.com) may be used to streamline the review and documentation of calibration verification data. By allowing laboratories to input instrument results and automatically generate graphical assessments, the program promotes uniform evaluation practices and maintains required records in an organized, retrievable format consistent with accreditation expectations and fulfills the instrument comparison requirements: COM.04250 Comparability of Instruments and Methods

Q. What is Calibration Verification?

A. "Calibration Verification" is defined in the Clinical Laboratory Improvement Amendments of 1988 (CLIA) by the Centers for Medicare and Medicaid Services (CMS) and the Centers for Disease Control (CDC) as "…the assaying of materials of known concentrations in the same manner as patient samples to substantiate the instrument or test system's calibration throughout the reportable range for patient test results." [3]

Q. Is Calibration Verification necessary for every test that is being run in my laboratory?

A. The final CLIA ruling concerning certain quality control provisions took effect April 24, 2003 and includes calibration verification. This final ruling states that at a minimum, laboratories must check three points in the reportable range to verify calibration – a low, mid and high point. This must be performed for every laboratory performing unmodified moderate and high complexity testing at least once every six (6) months and whenever any of the following occurs:

1. A complete change of reagents for a procedure is introduced.
2. There is major preventive maintenance or replacement of critical parts that may influence test performance.
3. Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits,
4. The laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.

Q. What is linearity?

A. *Linearity* is a fundamental characteristic of good 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 final analytic results and not to the relationship between instrument signal output and analyte concentration, which may be nonlinear; for example, a competitive immunoassay may show a sigmoidal relationship between signal and analyte concentration.[4]

Q. What is AMR Validation, should I be concerned with it, and is this in any way associated with calibration verification?

A. AMR Validation, or analytical measurement range (aka reportable range), is the process of confirming that an assay system will accurately measure the concentration or activity of a given analyte over the AMR. The materials used for validation must be known to have matrix characteristics appropriate for the method. The test specimens must have analyte values that, at a minimum, are near the low, midpoint, and high values of the AMR. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparative method values, and by dilution ratios of one or more specimens with known values. Each laboratory must define limits for accepting or rejecting validation tests of the AMR. The AMR must be revalidated at least every six (6) months and following changes in lots of analytically critical reagents of major system components.[5]

[1]

[1] Centers for Disease Control and Prevention. Clinical Laboratory Improvement Amendments (CLIA) program. https://www.cdc.gov/clia/php/about/index.html. Published n.d.

[2] Centers for Medicare & Medicaid Services. CLIA Brochure #3: Calibration and Calibration Verification. U.S. Department of Health & Human Services; 2006. https://www.cms.gov/files/document/clia-brochure-calibration-and-calibration-verification-april-2006.pdf

[3] Federal Register 42 CFR Part 493, Department of Health and Human Services, January 24, 2003; §493.1255, (b) (1) (ii).

[4] Pritt BS, Barth RF. Verifying performance characteristics of laboratory tests: A practical approach. *Arch Pathol Lab Med.* 2014;138(9):1173-1179. doi:10.5858/arpa.204740

[5] College of American Pathologists (CAP), Calibration and Standards guidelines.