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**LEARNING OUTCOMES**

On completion of this exercise, the participant should be able to

- discuss the 1988 Clinical Laboratory Improvement Amendments standards and interpretive guidelines that apply to method and calibration verification of test systems.
- design and implement an acceptable procedure for determining the analytic measurement range of a test.
- explain the required frequency and conditions for performance of calibration verification procedures.

**Educational Commentary**

In 1988, in response to public concerns about the quality of laboratory testing, the US Congress passed the Clinical Laboratory Improvement Amendments (CLIA’88). This law continues to serve as the basis for the regulation of laboratories in the United States. The law is administered by the Centers for Medicare and Medicaid Services, which has published at least six revisions. The January 24, 2003, revision contained substantive changes in quality control procedures and also reorganized previous regulations into preanalytic, analytic, and postanalytic groupings, to mimic the flow of specimens through the laboratory. This revision also refers to moderate- and high-complexity testing as *nonwaived* testing, and specifies that quality control requirements for these two levels of testing are essentially the same.

The standards and guidelines include requirements that laboratories establish and verify performance specifications for all nonwaived test systems introduced into the laboratory on or after April 24, 2003. For most laboratories, this standard applies to all current tests unless the laboratory can prove that the test in question uses the same method, reagents, and instrument used before the April 2003 cutoff date or that the test is in some other way exempt.

The Centers for Medicare and Medicaid Services publishes the *State Operations Manual* (SOM) used by the state surveyors who perform CLIA inspections. Appendix C of the SOM includes interpretive guidelines that explain and interpret the standards used in the inspection of laboratories and laboratory services. Familiarity with the guidelines is essential for laboratories preparing for any inspection, particularly an inspection by an accreditation organization with CLIA-deemed status, such as The Joint

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Commission, the College of American Pathologists, or the Commission on Office Laboratory Accreditation. Several sections of Appendix C, particularly Subpart K, detail quality system standards that apply to the preanalytic, analytic, and postanalytic aspects of nonwaived testing¹.

This educational commentary will focus on selected parts of 2 standards:

- 493.1253. Establishment and verification of method performance specifications
- 493.1255. Calibration and calibration verification procedures

Performance Specifications

Standard 493.1253 specifies that the laboratory must verify the following performance specifications for all nonwaived tests approved by the US Food and Drug Administration (FDA):

- Accuracy
- Precision
- Reportable range
- Appropriateness of reference ranges (normal values) for the laboratory’s patient population

If a laboratory modifies an FDA-approved test system or uses a system not subject to FDA approval, including systems developed in house and standardized methods such as those listed in textbooks, additional performance characteristics must be verified. These include analytic sensitivity and specificity (to include interfering substances) and any other performance characteristics required for test performance. Typically, accuracy, precision, and reference range determinations are clear cut and involve (1) a comparison-of-methods experiment to determine accuracy, (2) a replication experiment to determine precision, and (3) an analysis of samples from a healthy representative population to document reference ranges.

The analytic measurement range (AMR), also known as the linear or dynamic range, is the range of test results from the lowest to the highest that are reliable and hence reportable. It is assumed that the test system produces a linear response between the lowest and highest reportable results. The interpretive guidelines state that determination of the AMR may be accomplished by assaying low and high calibration or control materials or by evaluating known samples of abnormal high and abnormal low values. If a dilution procedure is used when patient results exceed the reportable range, the laboratory must verify and document that the diluent is appropriate and that the results are accurate.

In practice, some laboratories perform a linearity experiment to verify the AMR of patient test results. A set of serially diluted samples or samples with known concentrations is assayed, and the range is determined by plotting measured results on the y-axis against the known or expected results on the x-axis. A best-fit line may be drawn manually or by computer using linear regression statistics available with any standard statistics software package. Frequently, the AMR is determined visually, by using the
best-fit line that includes the lowest points in the series and observing the highest point on the line before it deviates from linearity. Examples of criteria established by laboratories for the acceptable difference between the measured results and the expected results include percentages, absolute values determined by an unacceptably large coefficient of variation of the assay at similar concentrations, or other means. The upper limit of the AMR in these cases is the highest concentration that meets the acceptance criteria and falls on or near the best-fit line.

Ideally, the linearity study should consist of at least four samples supplied as a set or prepared in house either by dilutions of abnormally high patient samples or by spiking a pool with a standard for the analyte of interest. The diluents used should not substantially affect the sample matrix and may vary with the analyte to be measured. When maintaining a serum matrix is critical, acceptable diluents are analyte-free serum, and bovine or serum albumin. For some analytes and test systems, water or saline may be acceptable diluents. The diluent recommended by the manufacturer for diluting out-of-range specimens is also acceptable.

A convenient procedure for preparing a linearity series is to use a zero- or very–low-concentration pool and a pool containing the analyte at or above the anticipated upper limit of the reportable range. Mixing these two pools in proportions of 75:25, 50:50, and 25:75 gives three samples with analyte concentrations spaced between the low and high concentration pools. This set of five samples (two pools plus three mixtures) should be assayed at least in triplicate, and the mean of the measured values should be used for the graph of measured versus expected values.

Calibration and Verification

Calibration and calibration verification procedures are addressed in Standards 493.1253 (b), (3) and 493.1255. Interpretive guidelines for 493.1253 (b)(3) are stated in the SOM

“Through the verification/establishment process, the laboratory defines the frequency for calibration and control performance as well as the type, number, and concentration of calibration and control materials used to monitor, detect error, and evaluate method performance. The frequency for calibration and control performance must not be less than the frequency specified in the manufacturer's instructions.”

Standard 493.1255 addresses the calibration verification procedures. The interpretive guideline for this standard includes the following statement: "if the laboratory performs a calibration protocol using three or more levels of calibration materials that include a low, mid, and high value at least every six months, the calibration verification requirement is met." The assay must be done in the same manner as patient samples to substantiate the test system's calibration throughout the reportable range for patient test results. Calibration verification must also be performed whenever a complete change of reagents occurs, unless the laboratory can demonstrate that the AMR and control values are not affected by the reagent lot
number changes. Other situations requiring calibration verification include major preventive maintenance, replacement of critical instrument parts, and values for control materials that indicate a problem (out of range, shift, trend, and so on). In performing verification, the laboratory may use samples with known values (e.g., control materials, proficiency-testing samples) or calibration materials of a different lot number than is being currently used for calibration, but the laboratory must define acceptable limits for the values obtained. Although method and calibration verification standards constitute a small part of the CLIA’88 regulations, they are essential to ensuring the overall quality of the results reported. Performing and properly documenting these procedures is not only good laboratory practice; it is evidence that the laboratory is consistently reporting accurate results for all samples assayed.

References


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