EDUCATIONAL COMMENTARY – METHOD VALIDATION: CALIBRATION AND LINEARITY

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- List and discuss the CLIA ‘88 standards and interpretive guidelines applying to method and calibration verification of test systems.
- Design and implement an acceptable procedure for determining the reportable range of a test.
- List the required frequency and/or conditions for performance of calibration verification procedures.

In response to public concerns about the quality of laboratory testing, Congress in 1988 passed the Clinical Laboratory Improvement Amendments (CLIA). That 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 (CMS), which has published at least 6 revisions to the “final” rule. The January 24, 2003, revision to the final rule contained substantive changes in quality control procedures and also reorganized previous regulations into preanalytical, analytical, and postanalytical 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 the quality control requirements for these 2 levels of testing are essentially the same.

The CMS also publishes the State Operations Manual (SOM) used by state surveyors (inspectors, auditors) who perform CLIA inspections. Appendix C of the SOM includes interpretive guidelines that explain and interpret the standards used in the survey (inspection, audit). 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 Commission, CAP, and COLA. Several sections of the SOM, including Subpart K (parts 1 and 2), detail quality system standards applying to the preanalytical, analytical, and postanalytical aspects of nonwaived testing.¹

Included in the standards and guidelines are 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 being performed is using the same method, reagents, and instrument used before the April 2003 cutoff date or that it is in some other way exempt. 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
EDUCATIONAL COMMENTARY – METHOD VALIDATION: CALIBRATION AND LINEARITY (cont.)

Performance Specifications
Standard 493.1253 specifies that the laboratory must verify the following performance specifications for all nonwaived tests approved by the FDA:

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

Also, 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 that must be verified include analytical sensitivity and specificity (to include interfering substances) and any other performance characteristics required for test performance.

Typically, accuracy, precision, and reference interval determinations are clear-cut and involve 1) a comparison-of-methods experiment to determine accuracy, 2) a replication experiment to determine precision, and 3) analyzing samples from a healthy representative population to document reference intervals.

The reportable range (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. The assumption is made that the test system produces a linear response between the lowest and highest reportable results. The interpretive guidelines state that determination of a reportable range may be accomplished by assaying low and high calibration or control materials or by evaluating known samples of abnormal high and abnormal low values. In the guidelines associated with this requirement, probes or questions for the inspectors address concerns that 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, laboratories may (and some often do) perform a linearity experiment to verify the reportable range of patient test results. A set of serially-diluted samples or samples with known concentrations is assayed, and the reportable range is determined from a plot of measured results on the y-axis versus the known or expected results on the x-axis. A best-fit line may be drawn manually or via computer using linear regression statistics available with any standard statistics software package. Frequently, the reportable range 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.\(^2\) Examples of criteria established by laboratories for the acceptable difference between the measured and expected results include percentages (10%, 20%, etc), absolute values determined by an unacceptably large coefficient of
variation of the assay at similar concentrations, or some other means. The upper limit of the reportable range in these cases would be the highest concentration meeting the acceptance criteria and falling on or near the best-fit line.

Ideally, the linearity study should consist of at least 4 or 5 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 (such as a drug that is therapeutically monitored). The diluents used for dilutions 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. One way to choose an acceptable diluent is to use the diluent recommended by the manufacturer for diluting out-of-range specimens.

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 2 pools in proportions of 75:25, 50:50, and 25:75 gives 3 samples with analyte concentrations spaced between the low and high concentration pools. This set of 5 samples (2 pools plus 3 mixtures) should be assayed at a minimum in triplicate, and the mean of the measured values should be used for the graph of measured versus expected values.2

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) state, “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.”1

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 3 or more levels of calibration materials that include a low, mid, and high value at least every 6 months, the calibration verification requirement is met.”1 Calibration verification must also be performed whenever a complete change of reagents occurs, unless the laboratory can demonstrate that the reportable range and control values are not affected by the reagent lot number changes.1 Other situations requiring calibration verification include major preventive maintenance, replacement of critical instrument parts, or if values for control materials indicate a problem (out of range, shift, trend, etc). 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 when the verification is performed.

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 but is an essential component of the evidence that the laboratory is consistently reporting accurate results for all samples assayed including patient and proficiency testing samples.

References

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