Verification of Microbiology Tests

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Definitions*

- "**Verification** is the *one-time* process performed to determine or to confirm a test's expected performance *prior* to implementation in the clinical laboratory; simply put "Does the test work?""
- "**Validation** is an *ongoing* process of monitoring a test…to ensure that it continuously performs as expected; simply put "Does the test still work?"... validation may include personnel competency assessment, quality control,…proficiency testing….an integral part of the laboratory's quality assurance program.""

* Definitions used by CLIA, CLSI

Source: *Cumitech 31A*. 2009. ASM Press

Definitions

- **Modified test**: FDA-cleared or approved test performed outside of package insert instructions
- **Laboratory-developed test**: procedures developed in-house that have not been cleared by FDA. May incorporate commercial reagents not cleared by the FDA for in vitro diagnostic use (e.g., RUO or ASRs) or reagents produced in-house

Verification

- Confirm that test performs as per manufacturer's specifications
  - What? Any FDA cleared/approved diagnostic, identification, or antibiotic susc test
  - When? A new test procedure, or different manufacturer
  - How? Several analyses using at least 20 specimens. With a well designed panel, these analyses can be completed in a couple days

Verification Components

- Accuracy (CAP requires sensitivity and specificity)
- Reproducibility
- Reportable range
- Reference (normal) range
- Other test characteristics, as applicable (precision, analytical measurement range)

Verification of Unmodified FDA-Cleared Test

- Accuracy
  - At least 20 specimens (mix of positive and negative)
  - Depends on reference method; ≥90%
- Reproducibility
  - At least several members of 20-spec panel
  - Run in duplicate; rpt 2nd run and 2nd operator
  - Same or comparable results

Verification of Unmodified FDA-Cleared Test

- Reportable range
  - Include positives (from 20-spec panel) with low and high values
  - Test should detect both weak and strong positives
- Reference (normal) range
  - May use negative specimens (from 20-spec panel)
  - Values should be negative, or produce values below a cutoff
  - May use manufacturer's reference range (pkg insert) if same patient population
  - May use published reference range

Sources: *Cumitech 31A;* http://www.cms.hhs.gov/clia/downloads/6064bk.pdf.

How are the final regulations being implemented?

CMS is allowing each laboratory that it inspects to have one educational survey following the April 24, 2003, effective date of the regulations. This will give laboratories time (2 years) and the opportunity to receive the technical assistance that may be needed to meet the updated requirements.

Where can I find additional information and guidance?

Assistance for meeting the requirements is provided in Appendix C of the State Operations Manual (CMS Publication 7), which is posted on CMS's CLIA Website. Information about CLIA and links to other laboratory-related resources can be found on the following Websites:

CDC: www.phppo.cdc.gov/clia/default.asp
CMS: www.cms.hhs.gov/clia/default.asp
FDA: www.fda.gov/cdrh/CLIA/index.html (for a listing of waived, moderate complexity and high complexity tests)

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Department of Health & Human Services

CMS
Centers for Medicare & Medicaid Services

Clinical Laboratory Improvement Amendments (CLIA)

Verification of Performance Specifications
Brochure #2

What is it and how do I do it?

The CLIA regulations now include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA.

Information to assist your laboratory in meeting this CLIA requirement!

NOTE: On January 24, 2003, the Centers for Disease Control and Prevention (CDC) and the Centers for Medicare & Medicaid Services (CMS) published laboratory regulations (CLIA) that became effective April 24, 2003. A summary of the updated requirements pertaining to performance specification verification are included in this brochure. However, this brochure is not a legal document. The official CLIA program provisions are contained in the relevant law, regulations and rulings. For more complete information, you may access the regulations on the Internet at http://www.phppo.cdc.gov/CLIA/regs/toc.asp.

CDC

BACKGROUND

The CLIA Quality System Regulations became effective on April 24, 2003. Now the laboratory is required to check (verify) the manufacturer's performance specifications provided in the package insert--for accuracy, precision, reportable range, and reference ranges--for each new unmodified, moderate complexity test that the laboratory performs before reporting patient test results. The verification process helps to assure that the test, when used in your laboratory by your testing personnel for your patient population, is performing as the manufacturer intended.

This requirement applies when the laboratory **REPLACES** a test system or instrument (with the same model or a different model); **ADDS** a new test; or **CHANGES** the manufacturer of a test kit.

The requirement does not apply to tests performed by the laboratory before April 24, 2003.

TIP! ***While the laboratory's technical consultant or director should be involved in the planning and evaluation of the performance specification checks, the test system manufacturer may also assist by providing a verification protocol and appropriate samples for the evaluation.***

ACCURACY

Are your test results correct?

The laboratory needs to compare the accuracy of the test results it obtains when using a test system with the manufacturer's accuracy claims. This can be done by testing commercially available calibrators/calibration and quality control materials with known values, proficiency testing materials that have established values, and previously tested patient specimens with established values. If test results for these samples fall within the manufacturer's stated acceptable limits, accuracy is verified.

PRECISION

Can you obtain the same test result time after time?

The laboratory is responsible for verifying that it can repeatedly test the same samples on the same day, and on different days and get the same or comparable results (reproducible), regardless of which member of the laboratory's testing personnel performs the test (operator variance). Several of the laboratory's testing personnel should participate in this evaluation to help determine overall laboratory variance. Exception: For fully automated test systems that are not operator dependent, operator variance should not affect the test's precision and may not need to be evaluated by more than one person.

REPORTABLE RANGE

How high and how low can test result values be and still be accurate?

To verify the manufacturer's established reportable range for the test, choose samples with known values at the highest and lowest levels the manufacturer claims accurate results can be produced by the test system. The laboratory may only report patient test results that fall within the verified levels. The laboratory director and/or the technical consultant will need to decide how the laboratory will report results that are greater than the highest verified level or less than the lowest verified level.

REFERENCE RANGES/INTERVALS (NORMAL VALUES)

Do the reference ranges provided by the test system's manufacturer fit your patient population?

You may begin patient testing using the manufacturer's suggested reference range(s) or you may use other published reference ranges from a textbook or a journal publication. Reference ranges can vary based on the type of patient (e.g., pediatric, male, female). Over time, you may need to adjust your reference range(s) to better fit the patient population(s) you routinely test. When you test known normal patients, the results should be within your reference range and with abnormal patients, you should expect results outside the reference range.

How many samples do I need to test?

While testing 20 samples is considered the "rule of thumb" for statistical purposes, this is not a magic number. Depending on the test system and the laboratory's testing volume, the actual number of specimens needed for each part of the verification study may vary.

Once the laboratory director has reviewed and approved the results of the verification studies, the laboratory may begin using the test system for routine testing and reporting patient test results. Conversely, if the study results indicate that the test is not accurate or results cannot be consistently reproduced, the laboratory's technical consultant and the test system manufacturer should be consulted regarding steps to resolve the problem.

TIPS! ***With planning, verifying a test system's accuracy; precision, including operator variance; and reportable range may be performed using the same samples. For example, you may test samples with known values at the upper and lower end of the manufacturer's reportable range along with samples that are in the normal range for your patient population, in different runs, on different days, using several of the personnel who will normally perform the testing. The activities of the personnel verifying the test system will also facilitate meeting CLIA's personnel competency requirements for these employees. In addition, the laboratory director may use the verification process to meet the CLIA requirements for establishing the test system's quality control protocol, an essential component of the laboratory's overall quality system.***

Where can I find additional information about the CLIA requirements pertaining to the verification of performance specifications?

You may refer to the State Operations Manual, Appendix C-Interpretive Guidelines, §493.1253, available on the CMS website at: www.cms.hhs.gov/clia.

Verification of Unmodified FDA-Cleared Test

- Verification specimen panel
  - Own patient specimens
    - Current test serves as reference method
    - Split and send to outside lab
  - Patient specimens from another lab (or vendor)
  - Old proficiency samples, QC or calibrators
    - Should be in appropriate matrix, and have analyte in clinically relevant concentrations
  - Spiked samples (own lab, or provided by vendor)
    - Appropriate matrix, and analyte in clinically relevant concentrations

Verification of Unmodified FDA-Cleared Test

- Operators who would perform routine patient testing should perform verification study
- Vendors often offer to perform/assist
  - OK only for fully automated test systems where inter-operator variability not an issue
  - Preferred assistance: free reagents/kits and data analysis (for complex systems that produce a lot of data, such as AST or serology platform), discrepant analysis

Test Verification

- Accuracy, reproducibility, reportable and reference range best describe a diagnostic test
- What about an identification test, or AST?
  - Organism identification test
    - Accuracy (species, genus) and reproducibility
  - AST
    - Accuracy, reproducibility
    - Reportable range = both sensitive and resistant strains

Verification of AST

- At least 30 isolates per panel
- Acceptance criteria
  - If reference method is not used, no very major errors
  - Less than 5% major errors (one test S or R, other test opposite result)
  - Overall essential agreement (+/- 1 twofold dilution) and categorical agreement (SIR) ≥90%

Source: *Cumitech 31A*

Test Verification

- What about blood culture system?
  - Sensitivity, specificity, reportable & reference ranges are not applicable
  - Seeded bottles
    - At least 20 representative isolates spiked at low CFU
    - Detection of all isolates within expected time
  - Parallel study
    - Collection of both bottle sets; compare both systems

Modified Tests and Laboratory-Developed Tests (LDTs)

Modification Examples

- Change in specimen handling, incubation time, temperature
- Change in specimen or reagent dilution
- Using a different calibration material (or changing the manufacturer's set-points)
- Change or elimination of a procedural step

Source: CLIA Subpart K, 493:1253

Modification Examples

- Change in the cutoff or method of calculating the cutoff for semi-quantitative assays
- Any change in intended use
  - Different sample matrix (e.g. plasma vs. urine)
  - Using test for another purpose (e.g. screening vs. diagnostic)
  - Changing the type of analysis (e.g. qualitative results reported as quantitative)
- Etc.

Source: CLIA Subpart K, 493:1253

Verification vs. Establishment of Performance Characteristics

- Under CLIA, labs must establish performance characteristics of modified tests or LDTs
  - Requires more analyses and more rigorous studies
    - CLIA: verification studies, plus analytical sensitivity, analytical specificity/interfering substances, others as applicable (e.g. for quantitative methods)

Source: CLIA Subpart K, 493:1253

Verification vs. Establishment of Performance Characteristics

- CAP
  - Perform validation study if test samples or use collection devices other than those listed in pkg insert
  - Validate modified cut-off value for positive result
  - Modified assay has at least equivalent performance
  - Validation studies include “reasonable” distribution of samples for each spec type

Source: CAP Microbiology Checklist

Modified Tests and LDTs—Number of Specimens to Test

- *Cumitech 31A:* recommended number of specimens
  - ≥ 50 positive specimens
  - ≥ 100 negative specimens
  - Rationale: scientifically justified, and laboratories performing LDTs or modified tests have resources to perform larger studies
  - Number of samples may depend on extent of modification
  - This is a recommendation. Neither CLIA nor CAP specify number of specimens

Source: *Cumitech 31A*

Verification Report

- Summarize performance—did it meet your acceptance criteria (as defined in a verification protocol)?
- Attach raw data
- Director (or designee) must sign the report, documenting that the test does indeed perform as per manufacturer's specifications
- Keep report for life of assay, plus 2 years

Recent CAP Checklist Changes

- All Common checklist
  - Section on test method validation (verification)
- Microbiology checklist (Molecular Microbiology)
  - MIC.63262. Daily QC
    - "Daily controls may be limited to electronic/ procedural / built-in…" if meeting several criteria, including 20 consecutive days of validation against external controls
  - MIC.63575. New reagent lot validation
    - Includes at least 1 positive and 1 negative patient specimen (by prior reagent lot), and 1 weakly positive pt specimen if results are reported as such

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