Laboratory Developed Tests

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Objectives

• Compare and contrast new test validation requirements for CAP and CLIA for non-FDA approved tests
• Describe approaches for analytical validation of non-FDA approved tests
• Review test management expectations for non-FDA approved tests
Conflicts

No conflicts to report
Sec. 493.1253 Standard: Establishment and verification of performance specifications

- (a) Applicability (Grandfather clause not recognized by CAP)
- (b)(1) Verification of performance specifications (FDA-approved method)
- (b)(2) Establishment of performance specifications. (Modified or not subject to FDA approval)
Section 493.1253(b)(2)

Each laboratory that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures, or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable:

• (i) Accuracy.
• (ii) Precision.
• (iii) Analytical sensitivity.
• (iv) Analytical specificity to include interfering substances.
• (v) Reportable range of test results for the test system.
• (vi) Reference intervals (normal values).
• (vii) Any other performance characteristic required for test performance.
CAP Checklist

The primary guidance is given in the note to the section Method Performance Specifications:

“...For tests that are not FDA-cleared or approved (including tests developed in-house), or for FDA-cleared/approved tests modified by the laboratory, the laboratory must establish accuracy, precision, analytic sensitivity, interferences and reportable range, as applicable; data on interferences may be obtained from manufacturers or published literature, as applicable. (Note that testing matrices other than those listed in manufacturer instructions constitutes a modification of an FDA cleared/approved test.)
CAP Checklist

Specific requirements are:

• GEN.42020: Analytic accuracy and precision
• GEN.42030: Analytic interferences
• GEN.42085: Reportable range
  • Analytical measurement range
  • Clinical reportable range
• GEN.42162: Reference range
What does the FDA do for you?

- Ensure that the indications for use (IFU) are justified
- Evaluate initial test performance (manufacturer’s claims)
- Ensure continued performance at the stated manufacturer’s claims
- Establish a floor for “state of the art”
What does the FDA *NOT* do for you?

- **Ensure that your patient needs are met**
  - Although a common level of performance is typically ensured across different manufacturers’ products that have the same indications for use, this may not provide adequate performance in specific situations
- **Implementation of a method is a director’s decision**
Approaching Laboratory Developed Tests

Nature of the test result
- Qualitative vs. Quantitative

Source of the method
- Modification of an FDA-approved method
- Implementation of a published method
- Completely developed in-house
  - Just another way of getting the answer
  - First and only version method for the test
Primary Approach

• Use as much information as you already have and fill in the blanks
• Identify a similar test or system and use its validation as a guideline
• Evaluate the stability of the samples
• Remember: YOU are responsible for EVERYTHING
**Terminology**

**Validate**
- Establish from first principles how a method performs
  - Determine criteria for performance de novo

**Verify**
- Document that the method performs as expected
  - Ensure that published criteria for performance are met
Fundamental Principles

When using samples/material to evaluate method performance:

- Determine which samples are meaningful
- Establish a target value for each sample
  - This may be a numeric result, positive/negative, or staining characteristic
- Determine a range of acceptability around that target
  - Include an acceptable number of outliers
- Do this before running your samples
Qualitative Tests

• Most common application: Anatomic pathology special stains
• Clinical performance
  • Sensitivity and specificity
  • The actual number of test samples is dependent on the director’s assessment of critical specimens/cases that need to be evaluated
  • A battery of specimens with known reactivity facilitates this evaluation
Qualitative Tests

In the clinical laboratory, need to consider:

- Cutoff
- Detection limits

- There is no requirement that a positive/negative test be able to determine “zero” from “any at all”

- The laboratory must be able to state at what level a test will reliably produce a positive result (detection limit)
Quantitative Tests

Modification of an FDA-approved method

• Examples:
  • Different matrix/specimen
  • Altered linear range
• Underlying test method verified to perform as per manufacturer’s claims
• Laboratory must determine and validate those aspects that are affected by the desired modification
Modification of an FDA-approved Method

Different Matrix

• Most often: Body fluids using serum or urine chemistries
  • Clinical utility commonly requires less accuracy than standard samples (a semi-quantitative result to show presence or absence of contamination of one fluid by another)
  • The clinical utility should be used to establish the performance characteristics necessary for the matrix
Body fluid matrix validation of an FDA-approved method

Obtaining samples to test

• Admixing high and low fluid samples to get (calculated) intermediate values
• Using a surrogate blank (water/saline) to make dilutions of high samples
• Adding known amounts of pure analyte as a spike (if the pure substance is available)
• Spiking blank/low level fluid with serum having a high concentration of analyte (1:100 serum/fluid, assuming that this will not alter the fluid matrix significantly)
Body fluid matrix validation of an FDA-approved method

Assumptions to assess

- If the response curve on the body fluid samples is linear on the method, then no significant matrix effect is demonstrated.
- Very broad acceptance limits (20% or more around the target) may be acceptable if the values are used only semi-quantitatively.
- No valid reference range because the expected concentration depends on how the fluid accumulates.
Modification of an FDA-approved method

Different specimen type

• Most often: non-approved collection container
  • In this situation, the desired result is to be able to use the alternate collection container in lieu of the manufacturer’s approved specimen type
  • Altered response may be seen (a shift of the slope of the method either upwards or downwards)
  • This may require the establishment of a “new” test specific for the alternate container and, especially, validation of an appropriate reference range for the specimen type
Modification of an FDA-approved method

Altered linear range

• Most often: Adjusting (“tuning”) a secondary analyzer to produce results similar to the primary instrument for a given method
  • Although this doesn’t seem to be a “modification,” it has been treated by CMS as such
  • Sufficient patients throughout the analytical range of the secondary instruments must be used to demonstrate both a linear response and a constant association with the primary instrument
    – This is usually the data set used to establish the adjustment factors
Quantitative Tests

Implementation of a published method

- Performance criteria are stated but there is no claim of consistency
- Critical components of the method must be determined by the laboratory
  - Quality control of these components is the laboratory’s responsibility
- Method validation depends on the information given in the published method
Implementation of a Published Method

Clinical performance (“indications for use”)
• Appropriate guidelines for analytical performance must be used to determine suitability of the method

Traceability
• A mechanism for ensuring that the results produced by a method can be directly correlated to clinical decision points must be in place
Implementation of a Published Method

In-house developed tests

• Raw reagents are purchased and combined in an analytical system determined and evaluated by the laboratory

• Although the individual reagents may have claims made by the manufacturer(s), there is no guarantee that the combination will work consistently
Implementation of a Published Method

Peer-reviewed publications in the medical literature typically provide analytical characteristics capable of providing medically significant results:

- These characteristics can be used to verify performance of the laboratory’s method.
- Note that the specific literature method may not necessarily match the laboratory’s version; the ability to match the literature performance analytically may serve as the criterion for acceptance.
Implementation of a Published Method

Publications outside of the medical literature often leave the medical significance unstudied

- The initial assessment by the laboratory must include an evaluation of suitability for medical decision-making (indications for use)
  - Most commonly through clinical studies in the literature
  - Although clinical studies that demonstrated utility may not use the method being implemented, a direct relationship between the results given in the studies and the in-house method must be established
In-house Developed Test

Just another way of getting the answer
  • Based on available instrumentation
  • Considered an improvement over currently available methods

First and only method for producing results
  • Based on clinical studies showing the medical utility
  • The method is developed by the laboratory as a way of producing results based on these studies
In-house Developed Tests

Validation should ensure that the results can be interpreted using information from clinical studies or currently-available methodology. The specific method must be validated completely:

- It is the laboratory’s responsibility to determine the analytical performance that can be reliably expected from the method.
- It is the laboratory’s responsibility to assess whether this analytical performance is sufficient to provide meaningful information.
Implementation of a New Method

The clinical relevance of the test is determined by the laboratory (often in association with a clinical research group)

• All method performance characteristics must be established by the laboratory
• In addition, the criteria for interpretation must be established
  • Reference range
  • Decision points
Implementation of a New Method

Validation establishes the analytical characteristics of the test

- No prior assumption or claims on how the test should perform can be used as verification criteria

- Clinical and Laboratory Standards Institute provides several guidance documents for these determinations

- Once these performance characteristics have been established, the laboratory must make a clinical assessment of suitability for use
Implementation of a New Method

For “first and only” methods, the clinical relevance also needs to be established

- In my experience, this is done either in preparation for or at a level comparable to peer-review clinical study publication
Lot-to-lot Validation

Although the manufacturer may make some statements as to how they quality control their method or material that they provide, the suitability for the laboratory’s application is not ensured.
Lot-to-lot Validation

- The validation of new lots of reagents must be sufficiently rigorous to assess whether the performance of the method has been maintained.
- If the system fails to maintain the desired level of performance, the laboratory needs to re-evaluate the method to determine what has changed and how to correct the deviation.
Calibrator Lot Changes

Calibrator values should be determined by rigorous, standardized methods

- Established by national standards laboratories, either by comparison to certified reference material or by direct analysis of the new calibrator lot
- Calibrator values may be determined by first principles (gravimetric, spectrophotometric extinction coefficient, etc)
Calibrator Lot Changes

Cross-check of new calibrators against old lot is typically rigorous

- New calibrator is run as patient sample against the old calibrator
  - Verifying the assigned new calibrator value
- Patients are tested in parallel runs using old vs. new calibrator
- Standard samples and controls are assessed
  - Ideally, the standard samples should include reference material if available
Summary

The laboratory must determine:

• Suitability for clinical use
• Performance characteristics expected from the test

The laboratory must ensure:

• Continued performance at the level that was originally documented

Maintain records of the initial assessment and validation of new lots of reagents
Summary

• Compare (as much as possible) against known/published information
• Prove the performance characteristics of any aspect of the test that the laboratory has implemented on its own
  • Recognize that establishing performance characteristics requires more data than verifying these same characteristics
Summary

Determine acceptance criteria for ongoing evaluation of the method

- Verify each change of reagent material as if the method is new to the lab
- Ensure traceability of the method through assessment of the calibrator (if in-house source)

Although we have not discussed records here, recall that the laboratory has the only proof of method performance

- CAP Checklist states “The laboratory must retain records of method performance specifications while the method is in use and for at least two years after discontinuation of a method” for all methods