TEST METHOD VERIFICATION AND VALIDATION

SWACM 2014

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OBJECTIVES

• Define validation and verification

• Describe components of the validation and verification processes

• List the verification requirements for unmodified FDA-cleared tests, modified (off-label) tests, and laboratory-developed tests

• Discuss verification and validation requirements for MALDI-TOF mass spectrometry
PROGRAM

Definitions (8:00-8:15 AM)

Validation—Overview and complicated issues (8:15-9:00 AM)

Overview of verification (9:00-9:45 AM)

Break (9:45-10:00 AM)

Verification cases (10:00-11:30 AM)
DEFINITIONS

Modified test

• Cleared or approved by the FDA. User changes
  • Pre-analytical
    • Intended use
    • Sample type
  • Analytical
    • Sample processing method
    • Change an incubation
    • Use a different instrument, such as thermal cycler
    • Change the cutoff, or implement a gray zone
DEFINITIONS

Laboratory-developed test (LDT)

• Built from individual reagents, optimized by laboratory (i.e. no commercial kit). Laboratory essentially writes its own package insert.
DEFINITIONS (CLIA, CLSI, CUMITECH 31A)

• Verification
  – “…one-time process performed to determine or to confirm a test’s expected performance prior to implementation in the clinical laboratory…“Does the test work?””

• Validation
  – “…ongoing process of monitoring a test, procedure, or method to ensure that it continuously performs as expected …“Does the test still work?””
DEFINITIONS
(COLL OF AMER PATHOL)

Microbiology checklist (molecular section only)
• FDA-cleared methods: verification (“validation” of different sample types)
• Lab-developed or modified tests: validation
DEFINITIONS
REAL WORLD?

Most use validation/verification interchangeably

• Process to confirm that a test performs as expected

Quality assurance

• Ongoing process - includes quality control - to ensure that test performs within expectations, and gives accurate and consistent results
COMPLICATED ISSUES IN ON-GOING QA (VALIDATION)

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WHAT IS VALIDATION
(OR ONGOING QA)?

Pre-analytic
Analytic
Post-analytic
NEW INSTRUMENT

If you acquire another instrument because your test volume increases, do you need to “validate” the new instrument?

• How do you assure the new instrument works?
“Internal controls may be used for daily quality control…” and “Daily controls may be limited to electronic/procedural/built-in…”

- Direct antigen test (MIC.14583); molecular test (MIC.63262)
- The package insert must allow it
- Assay cannot be modified (hence, the requirement above)
- Internal control must be compared to external controls for 20 consecutive testing days (lab director determines acceptance)
EQUIVALENT QC

When are external controls still required?
CMS TO IMPLEMENT IQCP

Individualized Quality Control Plan

• Reduced external QC frequency is important for tests with expensive reagents
• Individualized quality control plan (IQCP) will replace Equivalent quality control
  • Allows reduced external QC frequency as per EQC
  • Requires risk assessment for each test system
  • Requires ongoing monitoring of IQCP
So, if you decide to implement IQCP (reduced external QC frequency), you must evaluate risks for the following for each test, and each testing location

- Specimen (collection, transport, processing, etc.)
- Environment (temperature, humidity, water, etc.)
- Reagents (storage, expiration, etc.)
- Test system (interfering substances, mechanical, etc.)
- Testing personnel (training, competency, etc.)

Identify sources of potential errors; evaluate frequency and impact of errors

Evaluate frequency and impact of errors
INDIVIDUALIZED QUALITY CONTROL PLAN

Must be documented. Make part of the test procedure (QC section)

• In addition to risk assessment plan, QCP must
  • Describe nature and frequency of QC; criteria for acceptable performance
  • Describe process for immediate detection of errors
  • Describe process for ongoing review, evaluation of effectiveness
    • E.g. investigation in the event of a testing process failure
INDIVIDUALIZED QUALITY CONTROL PLAN

We are already doing much of the IQCP

The risk assessment may be the most novel part

IQCP transition period will end on Jan 1, 2016. Effective 1/1/2016 laboratories must

- Follow CLIA regulations as written (daily external QC)
- Implement IQCP

Sources:

NEW REAGENT
LOT/SHIPMENT QC

Often referred to as “parallel QC”, because as per CAP…

• Use patient specimens or external controls tested previously with current (old) lot/shipment
• Or test simultaneously with current lot/shipment
• For multiplexed molecular tests, must I test all analytes for
  • New shipment (MIC.63580)
  • New lot
  • Daily/wkly/monthly QC (MIC.63264)
How frequent must laboratory director (or designee) review QC (MIC.11020)?
New CAP Microbiology checklist standards

- MIC.16595. Calibration. Calibration control run each day of patient testing, change in target plate. Records are maintained
- MIC.16605. Controls. Appropriate control organisms are tested on daily basis
  - Bacteria
  - Yeast
| Date | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| E. coli ATCC 8739 Calibration | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acceptable Tolerance Limits Defined by Manufacturer | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| E. aerogenes ATCC 13048 External Control | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Initials | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acceptable Identification ≥ 85%; Performed Daily | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blank Control | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Initials | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Per Target Slide | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C. glabrata ATCC MYA 2950 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Initials | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acceptable Identification ≥ 85%; Performed on Days of Patient Testing | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Circle Discrepant Results; Note Corrective Action in Troubleshooting Log 7/10/2014

* - ATCC 19438

Pass (P)
Fail (F)
Not in use (N)
What’s AMR?

To what microbiology or infectious disease molecular tests does it apply?
AMR VERIFICATION

MIC.64832. AMR verification performed with matrix-appropriate materials; include low, mid, and high range of AMR

How frequently? (MIC.64834)
ANY ADDITIONAL QA ISSUES FROM AUDIENCE?
VERIFICATION OF MICROBIOLOGY TESTS

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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 as little as 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 2\textsuperscript{nd} run and 2\textsuperscript{nd} 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

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/odrh/CLIA/index.html (for a listing of waived, moderate complexity and high complexity tests)
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 functioning 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?

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

Beware of vendors telling you how to perform a verification/validation study. YOU are responsible for meeting regulatory requirements
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
  - MALDI-TOF? (example later)

- AST
  - Accuracy, reproducibility
  - Reportable range = both sensitive and resistant strains
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
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
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 TEST PERFORMANCE

**CAP**

- Perform validation study if test samples or use collection devices other than those listed in pkg insert (MIC.64770)
  - Validation studies include “reasonable” distribution of samples for each spec type
- Modified assay has at least equivalent performance (MIC.64956)
MODIFIED TESTS AND LDTS—
# 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; ASM Press*
VERIFICATION REPORT

Summarize performance

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
QUESTIONS?

BREAK
VERIFICATION CASE STUDIES

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CASE #1—VERIFICATION OF MULTIPLEX PCR

Multiplex PCR tests for respiratory, GI pathogens; positive blood cultures

How do you design a verification study for a multiplexed PCR test that isn’t prohibitively expensive, or take a long time?

How do you verify targets that are rare, or you can’t identify by other methods (parainfluenza virus 4, astrovirus, Entamoeba histolytica)?

• Shared samples from a lab that has already verified the test
• Commercially-available controls
  • Test mixtures of controls
Your lab acquires a MALDI-TOF mass spectrometer. How would you design a verification study?

Important issues

- FDA-cleared?
- Modifications? (agar, age of colonies)
CASE #3—NEW ANTIBIOTIC ON EXISTING PANEL

Do you need to perform verification study?

• Do CAP and CLIA describe both a “full” verification study and a “mini” verification study?

How would you design this study?
CASE #4—THROAT AND RECTAL SWABS FOR CT/NG NAAT

How would you design a study to verify (establish) performance characteristics of your CT/NG NAAT for throat and rectal swabs?
CASE #5—CHANGE PIECE OF EQUIPMENT

“Currently using Mitsubishi container system for anaerobes….switch to BD GasPak container system. What type of validation do I need?”
CASE #6—CHANGE FROM EIA TO MOLECULAR ASSAY FOR C. DIFFICILE TOXIN

Assumptions

- FDA-cleared nucleic acid amplification test
- Not modified by the lab (will follow package insert instructions for specimen requirements, performing the assay, interpreting results)

How would you design a study to verify (validate according to CAP) molecular test prior to reporting patient results?
CASE #7—NEW BLOOD CULTURE BOTTLES

Do we need to perform validation when we switch from glass to plastic blood culture bottles?
CASE #8—CHANGE SWABS

Do we need to perform a validation study if we switch from culturette swabs to E-swab (flocked swab with tube of Amies medium) for routine bacterial cultures?
CASE #8—CHANGE SWABS

What if you are changing to E-swab or a flocked swab, for a molecular or rapid antigen kit? Kit manufacturer specifies a particular swab type (not a flocked swab).
CASE #9—CHROMAGAR

Do I need to validate a new chromagar for ....?

• Is chromagar a test or a reagent?
• Are you reporting a result based solely on the chromagar?
• Does the manufacturer’s package insert provide sensitivity and specificity data?
ADDITIONAL CASES FROM AUDIENCE?