UPDATE AND ESSENTIALS OF VERIFICATION/VALIDATION

SWACM 2016

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OBJECTIVES

• Describe components of the validation and verification processes

• List the verification requirements for unmodified FDA-cleared tests

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

Intro and Definitions (1:00-1:15 PM)

Validation/QA—Overview and complicated issues (1:15-2:00 PM)

Overview of verification (2:00-2:45 PM)

Break (2:45-3:00 PM)

Verification cases (3:00-4:30 PM)
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)

• Verification
  • FDA-cleared methods

• Validation (outside of package insert)
  • Lab-developed or modified tests
  • Different specimen types
DEFINITIONS
REAL WORLD?

Validation/verification used interchangeably

• Process to confirm that a test performs as expected

Quality assurance

• Ongoing process to ensure that test performs within expectations, and gives accurate and consistent results. Includes
  • Quality control
  • Personnel training and competency
  • Proficiency testing
  • Etc.
# TEST MODIFICATIONS

<table>
<thead>
<tr>
<th>Change</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-analytical</strong></td>
<td></td>
</tr>
<tr>
<td>Intended use of test</td>
<td>Viral load tests for diagnosis</td>
</tr>
<tr>
<td>Specimen type</td>
<td>BAL for influenza tests; different blood culture bottle for rapid ID test</td>
</tr>
<tr>
<td><strong>Analytical</strong></td>
<td></td>
</tr>
<tr>
<td>Sample processing method</td>
<td>Unextracted samples for influenza PCR</td>
</tr>
<tr>
<td>Change an incubation</td>
<td>Decreased, to save time</td>
</tr>
<tr>
<td>Use a different instrument</td>
<td>Thermal cycler</td>
</tr>
<tr>
<td><strong>Post-analytical</strong></td>
<td></td>
</tr>
<tr>
<td>Change cutoff, or implement gray zone</td>
<td>AST breakpoint; equivocal zone for Ct/Ng DNA</td>
</tr>
</tbody>
</table>
DEFINITIONS

Laboratory-developed test (LDT)

- Built from individual reagents, optimized by laboratory (i.e. no commercial kit). Laboratory essentially writes its own package insert.
  - Examples: “home-brewed” PCR tests
- “Research Use Only” kit validated for diagnostic use. Laboratory adds diagnostic/clinical performance criteria to an existing insert.
  - Examples: RUO kits for Zika PCR, IgM ELISA
WHY DO WE NEED TO VALIDATE/VERIFY?

Accurate and reliable (reproducible) test results
- Before reporting patient results
- Ongoing
ACCURATE VS. RELIABLE

(a) Low accuracy
   Low precision

(b) Low accuracy
   High precision

(c) High accuracy
   Low precision

(d) High accuracy
   High precision
COMPLICATED ISSUES IN ONGOING QA (aka VALIDATION)

SWACM 2016

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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 that the new instrument performs as expected?
QC (DEFAULT)

When (how often)?

COM.30450. New reagent lot confirmation of acceptability. New reagent checked against old.

- Qualitative- at least positive and negative
- Quantitative- should include patient spec (less likely to have matrix interference than manufactured materials)
IQCP

Individualized Quality Control Plan

- Applies to
  - Tests with built-in controls (membrane tests, molecular)
  - AST
  - Media
- Reduced external QC frequency is important for tests with expensive reagents
- Individualized quality control plan (IQCP) replaces Equivalent Quality Control
  - Allows reduced external QC frequency. However, CAP requires no less frequently than every 31 days
  - 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

COM.50200. Test list; must use CAP forms

COM.50300. Risk assessment

- Evaluate potential sources of error to include all:
  - Pre-, analytical, post-analytical
  - Clinical impact of inaccurate results
  - Reagents, environ, specimen, personnel, test system
  - Lab’s environmental, instrument monitoring data (PM and QC logs)
  - Manufacturer’s instructions

COM.50400. QCP approval (by lab director, **not** a designee)

COM.50500. QCP elements (except for QC frequency, already doing this)

COM.50600. Quality assessment monitoring (except for annual re-approval of QCP by director **or** designee, already doing this)
Test system:
Lyra HSV 1+2/VZV Direct PCR Test (Quidel)

Test system Primary SOPs include:
11.08.10 Lyra HSV 1+2/VZV Direct

Historical Quality Review:
CLIA ’88 requires testing of external controls with each day of testing, or monthly QC if the test has a built-in procedural control, and the laboratory has sufficiently demonstrated comparability of internal and external controls over 20 consecutive days of testing. In addition, external controls are tested on each new lot or shipment. UTMB has been performing the Lyra HSV/VZV test since May 2015 with rare QC problems. Test results from external and internal controls have been very reliable. Processes to mitigate patient reporting errors and delayed reports are addressed in this IQCP.

Information Used to Conduct Risk Assessment
Regulatory and Accreditation Requirements:

Checklist from Accrediting Agency:
MIC.63262 Daily QC. Controls are run daily for quantitative and qualitative tests. For qualitative tests a positive and negative control must be run daily.

Method verification:
Test system verification completed in 2015. Documentation filed with printed copies of SOPs or separately in Microbiology supervisors office.

Training of personnel:
Completion of training documented in training records
Summary of in-house data from routine QC testing:
QC testing was performed according to SOP 11.08.10. Review of QC records for the past 3 months (since testing began in May 2015) that contained approximately 38 external control results and 53 internal control results demonstrated:
• No negative control failures. No HSV1 or HSV2 positive control failures. Two VZV controls not detected in June 2015 due to incorrect assay (HSV only) programmed for run. We discontinued daily external controls on July 16, 2015 to incorporate Equivalent QC program.
• Only one internal control failure (1/53, 2%). Repeat test was OK.

Summary of in-house data from routine instrument performance checks:
Instrument (Smartcycler) checks performed according to SOP 11.08.10. There has been no instrument performance issues noted that would impact patient results.

Summary of corrected reports and physician complaints:
Corrected reports are stored in the LIS. QA reports are located in the Microbiology Laboratory. Review of reporting errors identified prior to report release, corrected reports and physician complaints for the past 3 months revealed:
• No corrected reports
• No physician complaints

Risk Assessment and Determination of Risk Level

<table>
<thead>
<tr>
<th>Frequency of occurrence</th>
<th>Severity of harm to patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlikely (once every 2-3 years)</td>
<td>Negligible (temporary discomfort)</td>
</tr>
<tr>
<td>Occasional (once per year)</td>
<td>Minor (temporary injury; not requiring medical intervention)</td>
</tr>
<tr>
<td>Probable (once per month)</td>
<td>Serious (impairment requiring medical intervention)</td>
</tr>
<tr>
<td>Frequent (once a week)</td>
<td>Critical (life threatening consequences)</td>
</tr>
</tbody>
</table>

Risk Level:
### Risk Acceptability Assignment

<table>
<thead>
<tr>
<th>Risk Factor (Possible Sources of Error)</th>
<th>Frequency of occurrence</th>
<th>Severity of harm to patient</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-analytical</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Specimen:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient identification</td>
<td>Occasional</td>
<td>Serious</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Collection/container/volume</td>
<td>Occasional</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Integrity</td>
<td>Occasional</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Transport</td>
<td>Probable</td>
<td>Minor</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Storage</td>
<td>Occasional</td>
<td>Negligible</td>
<td>Acceptable</td>
</tr>
<tr>
<td><strong>Analytical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Testing Personnel:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td>Unlikely</td>
<td>Serious</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Competency</td>
<td>Occasional</td>
<td>Serious</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Proficiency Testing</td>
<td>Occasional</td>
<td>Serious</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Staffing</td>
<td>Occasional</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td><strong>Reagents:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shipping/receiving/storage</td>
<td>Occasional</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Expiration dates</td>
<td>Unlikely</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Preparation/use</td>
<td>Unlikely</td>
<td>Minor</td>
<td>Acceptable</td>
</tr>
<tr>
<td><strong>Environment:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature/airflow/humidity/ventilation</td>
<td>Occasional</td>
<td>Negligible</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Utilities</td>
<td>Occasional</td>
<td>Negligible</td>
<td>Acceptable</td>
</tr>
<tr>
<td><strong>Test System:</strong></td>
<td></td>
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</tbody>
</table>
## Risk Assessment

<table>
<thead>
<tr>
<th>Possible Sources of Error</th>
<th>How can identified sources of error be reduced?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-analytical</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Specimen</strong></td>
<td>Improper specimen collection/handling/processing</td>
</tr>
</tbody>
</table>
|                           | • Adhere to procedure 11.08.10 that addresses specimen collection, transport, receiving and storage.  
|                           | • During initial training and competency assessment, emphasize proper specimen handling/processing is most critical part of any test. |
| Patient identification    | See above (Specimen)                          |
| Collection/container/ volume | See above (Specimen)                     |
| Integrity                 | See above (Specimen)                          |
| Transport                 | See above (Specimen)                          |
| Storage                   | See above (Specimen)                          |
| **Analytical**            |                                               |
| **Testing Personnel:**    | Incompletely trained                          |
|                           | During initial training and competency assessment, emphasize all steps in procedure  
|                           | • Emphasize pipetting techniques to avoid carry-over contamination |
Final QCP for Lyra HSV 1+2/VZV Direct PCR Test

Based on risk assessment and Quality Assessment, the QCP consists of following the instructions in the Quality Control section of SOP 11.08.10, and are summarized here.

**Perform Quality Control with each new lot number, each new shipment or every 30 days, whichever is more frequent.** Controls are provided by Quidel.

Daily internal controls and external control results are recorded in the Quality Control Log Book. Criteria for acceptable performance are provided in SOP 11.08.10.

If the correct control results are not obtained, patient results are not reported. Supervisor or lead technologist is contacted, as well as manufacturer technical support if necessary.

Testing environment is monitored daily, and recorded on the instrument log (stored in testing area).

Specimen quality is assessed upon receipt in the Clinical Microbiology laboratory. Specimen requirements are described SOP 11.08.10.

Training and competency of testing personnel are performed according to Laboratory Services SOP 1.01.5 “Education & Competency Assessment”. Documentation is stored in employee files.
### Quality Assessment: Ongoing Monitoring for QCP Effectiveness (performed by supervisor/manager)

- Reasons for QC failures, PT failures, and patient isolate reporting errors will be examined and addressed as needed in a new/updated risk assessment: 1) Has a new risk factor been identified? 2) Does this change the frequency of risk? 3) Does the risk factor change the potential severity of harm to patient?
- Daily review of patient results for reporting errors and clinician complaints. Take corrective action and revise QCP as needed.
- Monthly review of QC results and Troubleshooting Log. Take corrective action and revise QCP when unexpected QC failures indicate adjustment to the QC plan defined herein is needed.
- Regular review of Proficiency Testing results. Take corrective action and revise QCP if necessary when PT results are not acceptable.
- Monthly review of all equipment maintenance/monitoring logs according to standard laboratory protocols. Take corrective action and revise QCP as needed.
- Regular training and competency assessment according to standard laboratory protocols. Modify training and revise QCP as needed.
- Continual participation in this institution’s quality program that addresses specimen handling and erroneous specimen labeling. Take corrective action and revise QCP as needed.

<table>
<thead>
<tr>
<th>This QCP has been prepared by Michael Loeffelholz, PhD</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

| This QCP has been reviewed and is approved by the laboratory director | Signature | Date |
Individualized Quality Control Plan Summary

Complete a separate form for each IQCP in use and present to the inspector during the on-site inspection.

<table>
<thead>
<tr>
<th>Laboratory Name:</th>
<th>University of Texas Medical Branch</th>
<th>Laboratory Section/Department:</th>
<th>Microbiology</th>
<th>CAP Number:</th>
<th>2125101</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>1) Instrument/Device Include name, manufacturer, and model</th>
<th>2) Tests List all tests included under the IQCP</th>
<th>3) Number of Devices In Use</th>
<th>4) List of Test Sites* If used in more than one area</th>
<th>Date of Director Approval</th>
<th>Date Implemented</th>
<th>Date Retired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyra; Quidel HSV 1, HSV 2, VZV (multiplex PCR)</td>
<td></td>
<td>1</td>
<td>N/A</td>
<td>Click here to enter a date.</td>
<td>Click here to enter a date.</td>
<td>Click here to enter a date.</td>
</tr>
</tbody>
</table>

5) Process Used to Monitor Risk

List control processes put in place based on risk assessment – define the monitor and frequency evaluated.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Environment</th>
<th>Specimen</th>
<th>Test System</th>
<th>Testing Personnel</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>During initial training and competency assessment (annually), emphasize procedures to</td>
<td>During initial training and competency assessment, (annually) emphasize procedures for</td>
<td>Adhere (daily) to procedure 11.08.10 that addresses specimen collection, transport and storage.</td>
<td>During initial training and competency assessment (annually), emphasize standard rules to take responsibility of test system problems (correct/report).</td>
<td>During initial training and competency assessment (annually), emphasize all steps in procedure.</td>
<td>Supervisor maintains log of test problems including incorrect results. Log is reviewed monthly by supervisor and Director.</td>
</tr>
<tr>
<td>Take responsibility for reagents/supplies</td>
<td>Temperature documentation</td>
<td>Understand temperature/environment requirements for test system</td>
<td></td>
<td></td>
<td>During initial training and competency assessment (annually), emphasize pipetting techniques to avoid carry-over contamination.</td>
</tr>
<tr>
<td>Maintain reagents at proper storage conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>During initial training and competency assessment (annually), emphasize need for timely results.</td>
</tr>
<tr>
<td>Check expiration dates Perform required QC at frequency defined in procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Designated staff members assigned to

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Environment</th>
<th>Specimen</th>
<th>Test System</th>
<th>Testing Personnel</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>During initial training and competency assessment</td>
<td>During initial training and competency assessment</td>
<td>PT samples are circulated among all</td>
<td>Review of electronic system interfaces</td>
</tr>
</tbody>
</table>

* Test sites with different CLIA and CAP numbers must complete separate forms.

NOTE: The form is intended to be used as an inspector tool and does not meet the checklist requirements for documenting the IQCP risk assessment.
List of Individualized Quality Control Plans

Complete the fields below for each IQCP in use and present to the inspector during the on-site inspection. Fill out a separate Individualized Quality Control Plan Summary form for each IQCP listed.

<table>
<thead>
<tr>
<th>Laboratory Name:</th>
<th>University of Texas Medical Branch</th>
<th>CAP Number:</th>
<th>2125101</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>1) Laboratory Section/Department</th>
<th>2) Instrument/Device</th>
<th>Include name, manufacturer, and model</th>
<th>3) Tests</th>
<th>List all tests included under the IQCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiology</td>
<td>Affirm, Becton Dickinson</td>
<td>Vaginal Pathogens Panel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>BD Max, Becton Dickinson</td>
<td>Clostridium difficile toxin, MRSA, Group B Streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>BinaxNOW urine antigen, Alere</td>
<td>Legionella, Streptococcus pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>Cryptococcus Antigen Lateral Flow Test, Immuno-Mycologics</td>
<td>Cryptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>Quik Chek, Alere</td>
<td>Giardia/Cryptosporidium panel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>ICON H. pylori IgG, Beckman Coulter</td>
<td>H. pylori IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>Lyra HSV 1+2/VZV Direct, Quidel</td>
<td>HSV1, HSV2, VZV panel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology</td>
<td>QuickVue+ Strep Test, Quidel</td>
<td>Strep A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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
  - New lot
  - Daily/wkly/monthly
<table>
<thead>
<tr>
<th>Run Name</th>
<th>Initials</th>
<th>Lot Number</th>
<th>Expiration Date</th>
<th>Process Ctrl</th>
<th>Monthly</th>
<th>New Lot</th>
<th>New Ship</th>
<th>Neg</th>
<th>HSV-1</th>
<th>HSV-2</th>
<th>VZV</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.21.15</td>
<td>072114</td>
<td>7-9-16</td>
<td></td>
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</tbody>
</table>

Acceptance criteria:
- Process control (in NC) = pass (✓)
- Negative Control (NC) = HSV-1, HSV-2, VZV viral DNA negative (neg)
- Positive Control = HSV-1, HSV-2, VZV viral DNA positive (pos)

Document any out of range QC on Troubleshooting Log
*Notify director or supervisor if QC fails to perform as expected*
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<tr>
<th>Run Name</th>
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</tbody>
</table>

Acceptance criteria:
- Process control (in NC) = pass (V)
- Negative Control (NC) = HSV-1, HSV-2, VZV viral DNA negative (neg)
- Positive Control = HSV-1, HSV-2, VZV viral DNA positive (pos)

Document any out of range QC on Troubleshooting Log
*Notify director or supervisor if QC fails to perform as expected*
# Troubleshooting Log

**Location:** McC 5-11D

<table>
<thead>
<tr>
<th>Date/Tech</th>
<th>Problem</th>
<th>Solution</th>
<th>Reference #</th>
<th>Repeat#</th>
<th>Probable Cause</th>
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<tr>
<td>12/4/15 JB</td>
<td>HSV/Varicella</td>
<td>Repeat pt sample Internal control failed again, report pt as no result due to inhibitors</td>
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<td>variation</td>
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<td>12/10/15 UF</td>
<td>PoS CT fail DAD ABR</td>
<td>Repeat with new control</td>
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<td>12/19/15 HIT</td>
<td>DAD FABR fail - ERROR</td>
<td>Removed tip - Repeated Run</td>
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<td>mechanical debris on disk</td>
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<td>HSV DAD PCR ON FOCUS</td>
<td>Repeat POS CTL</td>
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<td>variation</td>
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</table>
How frequent must laboratory director (or designee) review QC (MIC.11020)?
MALDI-TOF MASS SPEC QC

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 (if tested that day)
  - AFB (if tested that day)
  - For FDA-approved systems, must use QC organisms specified by manufacturer
|                      | 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** |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

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**Acceptable Tolerance Limits Defined by Manufacturer**

|                      | 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. aerogenes**      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ATCC 13048            |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| External Control      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Acceptable Identification ≥ 85%; Performed Daily**

|                      | 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 |
|----------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Blank Control**     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Acceptable Identification ≥ 85%; Performed on Days of Patient Testing**

|                      | 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 |
|----------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **C. glabrata**       |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ATCC MYA 2950         |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

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Circle Discrepant Results; Note Corrective Action in Troubleshooting Log

7/10/2014

- **E. coli ATCC 19453**

- PASS (P)
- **FAIL (F)**
- Not IN USE (N)
AMR VERIFICATION

What’s AMR?

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

MIC.64884. AMR verification performed with matrix-appropriate materials; include low, mid, and high range of AMR

How frequently? (MIC.64886)
ANY ADDITIONAL QA ISSUES FROM AUDIENCE?
VERIFICATION (aka VALIDATION)

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? A panel of at least 20 specimens. With a well designed panel, accuracy (sensitivity/specificity), reproducibility can be completed in as little as a couple days
VERIFICATION COMPONENTS

Accuracy
Reproducibility
Reportable range
Reference (normal) range
Other test characteristics, as applicable (precision, analytical measurement range)

COM.40300. Accuracy and precision. Lab verifies or establishes analytical accuracy and precision

- Accuracy - comparing results to reference method
- Precision - repeat testing at varying concentrations (quantitative test) or “activities” (pos/neg qualitative test)
VERIFICATION
COMPONENTS

Other test characteristics

COM.40500. Analytical interferences. Lab understands analytical interferences (copy from package insert to SOP)
VERIFICATION OF UNMODIFIED FDA-CLEARED TEST

Accuracy

- At least 20 specimens (mix of positive and negative)
- Depends on reference method; ≥90%

Reference, or “Truth”

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<tbody>
<tr>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
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</table>

Test being verified

Accuracy = 19/20 (95%)
Sensitivity = 9/10 (90%)
Specificity = 10/10 (100%)
VERIFICATION OF UNMODIFIED FDA-CLEARED TEST

Reproducibility (precision)

- 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)

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/sec.asp.

February 2004
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—each in a 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?

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)

• Change in the cutoff or method of calculating the cutoff for semi-quantitative assays
  • MIC.64825. Modified cut-off for a positive result has been validated (e.g. call PCR “blips” negative)

• Etc.

Source: CLIA Subpart K, 493:1253
VERIFICATION VS. ESTABLISHMENT OF TEST PERFORMANCE

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)
- COM.40450. Analytical specificity established for modified tests and LDTs

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. CLIA doesn’t specify number
- CAP (COM.40350): minimum of 20 samples

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

COM.40000. Method validation/verification approval.
VERIFICATION CASE STUDIES

SWACM 2016

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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?
“Currently using ABC container system for anaerobes....switch to XYZ 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 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 liquid transport (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).
Do I need to validate a new chromagar?

- 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?