Verification and Validation in Clinical Microbiology

Carolinas Clinical Connection
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Objectives
- Define the processes associated with microbiology test verification and validation
- Understand the regulatory requirements for test verification/validation
- Describe the design of a microbiology instrument verification study
- Demonstrate the development of a validation process for clinical microbiology instrumentation

Available Resources
- Although required by CLIA, there are currently no concise guidelines from CLIA or CAP for verification and validation of microbiological procedures.
- ASM Clinical Microbiology Procedures Handbook, 3rd edition
- Cumitech 31A: Verification and Validation of Procedures in the Clinical Microbiology Laboratory
- CLSI M52-P: Validation and Verification of ID and AST Systems (not yet published)

Definitions (as used by CLIA / CLSI)
- Verification – a one-time process completed before a test or instrument is used for patient testing. The performance characteristics being verified can include sensitivity, specificity, precision, accuracy, and predictive value.

For unmodified, FDA-cleared or -approved tests, CLIA requires the laboratory to verify the manufacturer’s performance specifications provided in the package insert before reporting patient test results. This applies when the laboratory replaces an instrument (with the same model or a different model) adds a new test to the instrument, or changes instrument manufacturers.

Definitions (as used by CLIA / CLSI)
- Verification – a one-time process completed before a test or instrument is used for patient testing. The performance characteristics being verified can include sensitivity, specificity, precision, accuracy, and predictive value.

Technologists who would perform routine patient testing should perform the verification study.

Vendors may offer their assistance:
- Free reagents
- Data analysis
- Resolution of discrepant results
The results of a verification study should indicate one of three possibilities:

- The test is acceptable for routine use.
- Further verification studies are required.
- Immediate corrective action is required by the manufacturer, the user, or both. The test is unsuitable for routine use until its performance parameters can be verified.

**Definitions**

- **Validation** – once an instrument or test system has been verified, validation demonstrates that it repeatedly continues to give the expected results as performed over time and continues to meet the manufacturer’s claims.

Validation may include:

- Internal and external proficiency testing
- Maintenance and calibration records
- Personnel training and competency assessments
- Parallel testing of duplicate instruments
- Correlation with clinical findings

Validation therefore becomes part of the overall laboratory QA program.

It should cover the complete analytical process — preanalytical, analytical, and postanalytical.

Note that these definitions for validation and verification are accepted by CLSI, CLIA, and the International Organization for Standardization, but the definitions accepted by the Joint Commission and CAP are the inverse.

**Verification of Automated Blood Culture Instruments**
• One of the more difficult tasks facing the clinical microbiologist
• Parallel testing would require collection of additional blood from each patient and may not be possible in some patients and institutions
  ◦ Low level of positivity means that most specimens will be of little value in the comparison
  ◦ Incidence of contamination and predominance of a limited number of pathogens may skew the evaluation

**BLC system verification studies should answer these questions**

• Will the media used by the system support the growth of organisms (including yeasts, anaerobes, and fastidious organisms) commonly seen in the user’s patient population?

• Will the instrument detect, in a timely fashion, the majority of pathogenic organisms from blood cultures which contain these microorganisms?

Implementing a newer version of a blood culture system

Per Cumitech 31A, a new verification study may not be necessary

"If the differences between the current and new systems are limited to the blood culture instrument (hardware and software) and the blood culture bottles are not changed, then an instrument function check by a vendor technical representative is sufficient to verify adequate performance of the complete blood culture system in the user’s laboratory."

**Verification Method #1**

**Seeded Blood Culture Studies**

• Select a minimum of 15 to 20 isolates representative of blood culture isolates normally seen in the institution

• Prepare seeded blood cultures with isolates of each of the above species
  ◦ The minimum amount of sterile, antibiotic-free human blood recommended by the manufacturer should be placed in each bottle.
  ◦ The numbers of organisms placed in each bottle should approximate those found in cases of septicemia. This can be done with serial dilutions of the organisms before inoculation to achieve approximately 5 to 30 CFU per bottle.

**Methodology**

• Colonies of each test strain were placed into separate tubes of sterile saline to achieve a McFarland 1.0 Standard (equivalent to \( \sim 3 \times 10^8 \) CFU/ml)

• From these tubes, a series of three 1:100 dilutions was made, resulting in a final concentration of \( \sim 3 \times 10^2 \) CFU/ml or 30 CFU / 0.1ml of inoculum.

**Serial dilutions for blood culture inoculation**

- Colonies of each test strain were placed into separate tubes of sterile saline to achieve a McFarland 1.0 Standard (equivalent to \( \sim 3 \times 10^8 \) CFU/ml)
- From these tubes, a series of three 1:100 dilutions was made, resulting in a final concentration of \( \sim 3 \times 10^2 \) CFU/ml or 30 CFU / 0.1ml of inoculum.
Each test and reference blood culture vial was inoculated with 0.1ml of the final dilution of each test organism.

To determine the CFU count, 0.1ml of the final dilution was inoculated onto the agar medium appropriate for each organism, using the spread plate technique.

Uninoculated sterility control vials, and vials with added human blood and no organism of both the test and reference blood culture vials were included.

### Data

<table>
<thead>
<tr>
<th>Organism</th>
<th>Culture Method</th>
<th>Actually Pos</th>
<th>Predicted</th>
<th>Positive Time</th>
<th>Predicted Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>Yes</td>
<td>3.9</td>
<td>3.7</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>E. coli</td>
<td>Yes</td>
<td>2.9</td>
<td>3.6</td>
<td>3.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Staph.</td>
<td>Yes</td>
<td>3.8</td>
<td>3.6</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>S. pyogen</td>
<td>No</td>
<td>2.8</td>
<td>3.4</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>B. fragil</td>
<td>No</td>
<td>1.3</td>
<td>3.1</td>
<td>3.4</td>
<td>3.4</td>
</tr>
</tbody>
</table>

If all isolates are detected within a time to positivity consistent with the old instrument (and with the literature for given organisms), the method can be considered to be verified.

Any problems with detection should be investigated by repeating the tests with the same patient strains.

### Verification Method #2
Parallel Blood Culture Studies

- Allows evaluation of all aspects of the new system under actual patient and laboratory conditions.
- Duplicate sets of blood cultures inoculated with equivalent blood volumes should be obtained until a minimum of 20 positive blood cultures (not including contaminants) representative of the blood culture isolates normally seen in the institution are evaluated.
- The new method is considered verified if the sensitivity is at least 95%, relative to the reference method, and times to detection are not significantly different.

Source: Cumitech 31A, 2009 ASM Press
Planning the study

- Verification plan and acceptance criteria should be established prior to beginning the study
- Accurately characterize or detect the analyte of interest

One approach to defining acceptance criteria is to establish the total allowable error for a test. This is generally used for quantitative testing.

This same approach may be used for AST testing by defining agreement between methods as:
- A result within one doubling dilution
- No difference in the interpretation of resistance

Verification samples

- Well-characterized clinical specimens or culture isolates
- Proficiency samples and/or commercially prepared reference panels are also good sample choices, but may not reflect the user institution’s patient population

FDA permits the use of these clinical specimens/isolates for verification studies provided the specimens were collected for routine patient care, would otherwise have been discarded, and are not individually identifiable.

The distribution of organisms used for the study should
- be similar to those commonly isolated at the institution
- represent resistant phenotypes observed in the institution
- Staph. aureus (MRSA) D-test positive Staph. aureus
- VRE Coagulase negative staphylococci
- ESBLs KPC, Amp-C producers
- MDR Pseudomonas aeruginosa and Acinetobacter

Test five isolates in triplicate for 3 to 5 days

Use isolates with known antibiotic resistance phenotypes such as ATCC QC strains

At least two isolates should be antibiotic resistant – MRSA, KPC, etc

Acceptable results are ≥95%, including both essential and categorical agreement

Precision (reproducibility)
**Precision essential agreement (PEA)**

- Precision within ±1 twofold dilution of the test antibiotic

\[
\text{PEA} = \frac{\# \text{ within } \pm 1 \text{ well of known MIC}}{\text{total results}} \times 100
\]

**Precision categorical agreement (PCA)**

- Agreement with the interpretive results (susceptible/intermediate/resistant [SIR]) of the precision test isolate

\[
\text{PCA} = \frac{\# \text{ of categorical result matches}}{\text{total results}} \times 100
\]

The total tested can be calculated for all organisms and drugs combined or for each drug.

**Accuracy**

- Larger labs can compare the test AST system against a reference method as well as the current system and are able to do studies of 100 or more isolates per panel.

- Detailed FDA recommendations for these types of evaluations are available at [www.fda.gov](http://www.fda.gov)

**Accuracy**

- Due to limited resources, most hospitals evaluate the test AST system against the current AST system only.

- The test system may not be incorrect when discrepant results occur — it cannot be assumed that the current system is correct when results differ. Because of this, for this type of evaluation, there are no very major errors (VME) where the test system indicates a susceptible result and the current system indicates resistance.

- **MinE**
  - Minor discrepancy
  - One AST is I and the other is S or R

- **ME**
  - Major discrepancy
  - One AST is S and the other is R
  - Should be less than 5%

- MinE + ME should be <10%
Accuracy

- **EA**
  - Agreement within ±1 twofold dilution of the test AST system with the current AST system
  - Should be ≥90%

- **CA**
  - Agreement of interpretive results (SIR) between the test AST system and the current AST system
  - Should be ≥90%

Results summary

- **ME** = # of ME discrepancies / X 100 = 3.4% acceptable
  - Total # of test results from both systems = 457
- **MinE** = # of MinE discrepancies / X 100 = 2.0% acceptable
  - Total tested
- **EA** = # of comparisons within ±1 twofold / X 100 = 99.9% acceptable
  - Total tested
- **CA** = # of categorical result matches / X 100 = 96.3% acceptable
  - Total tested

Evaluation of results

- If the specified limits are exceeded, the test is unverified and should not be used. Corrective action with assistance from the manufacturer should be undertaken, after which a minimum of 20 appropriate isolates should be tested to demonstrate that the problem has been corrected.
- Error rates may not be significant with certain drug/bug combinations.
- If a significant number of organisms have MICs near the breakpoint, the categorical agreement may be less than 90%.

20/30 day QC

- Should be run concurrent with the rest of the verification testing using QC organisms designated by the AST system manufacturer.

Validation of instrumentation

- Weekly QC/new lot QC
- Proficiency testing
- Training and competency testing
- Preventive maintenance
- Correlation with clinical findings
- Review of Appendix A in the CLSI M100-S21 standards concerning resistant, intermediate and nonsusceptible isolates.
- Upload to LIS interface
- Review of expert system interpretations
Summary

- Although required, still have only guidelines for verification and validation
- Work with laboratory director to determine best approach for your microbiology lab
- Goal is to ensure good laboratory practice with accurate patient results

Thank you!