The Virtues and Pitfalls of Implementing a New Test

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Objectives

1. Identify regulations governing laboratory quality

2. Recognize what needs to be done to validate a new test

3. Describe test performance characteristics

Case Study

• A neurology faculty member, Dr. Johnson, approaches you as laboratory director with a question.
• He is starting a research study for a new drug to treat epilepsy that is refractive to other treatments.
• Patients will be recruited from the neurology clinic in the hospital.
• The study sponsor requests that all women of child-bearing age have a negative urine pregnancy test before enrolling.
• The sponsor requires that study participants use a specific point-of-care pregnancy test to screen female patients.
Audience Poll

• Which of the following best describes your response to this request for a new POC pregnancy test?
  A. Allow Dr. Johnson to perform the test in the neurology clinic, since it is research and not for clinical purposes.
  B. Require Dr. Johnson to submit test performance data, operator training, evidence of QC, a draft procedure manual, and enrollment in proficiency testing for your Chair of Pathology to sign-off
  C. Request that Dr. Johnson use the hospital's current POC kit or send the test to the core laboratory
  D. Nothing is required as urine pregnancy is CLIA waived, which means it is waived of all regulations

CLIA ‘88

• Clinical Laboratory Improvement Amendments of 1988
• Federal Regulatory Standards that apply to all clinical laboratory testing performed on humans in the United States
• Sets minimum guidelines for accuracy and reliability of laboratory testing
• Enforced through CMS – Centers for Medicare and Medicaid Services
• Noncompliant labs can be sanctioned
  – Lose ability to perform testing for up to 2 yrs
  – Lab Directors cannot direct any other lab while sanctioned
  – Cannot bill or be reimbursed by Medicare for 2 years
• Other state and accreditation agencies (Joint Commission and CAP) may have additional requirements

CLIA ‘88: Definitions

• CLIA does not apply to components or functions of:
  – Research laboratories that test human specimens but do not report patient-specific results for the diagnosis, prevention or treatment of any disease or impairment of, or the assessment of the health of individual patients.
  – Determination of CLIA applicability depends on who has access to the result and how it may be utilized in the management of the patient
  – CLIA also doesn’t apply to forensic testing or labs certified by NIDA for drug testing performed to NIDA guidelines
Test Complexity Under CLIA ‘88

Tests are categorized as **waived**, **moderate complexity**, or **high complexity** depending on the following criteria:

1. Scientific and technical knowledge needed
2. Training and experience needed
3. Reagent and material preparation
4. Characteristics of operational steps
5. Calibration, quality control, and proficiency testing materials
6. Test system troubleshooting and equipment maintenance
7. Interpretation and judgment needed

CLIA Waived Complexity Testing

- Approved for home use
- So simple, no patient mismanagement even if performed incorrectly
- Lay person can get results comparable to laboratory technologist
- Labs have only 3 requirements!
  1. Pay biennial fee (every 2 years) for CLIA certificate renewal
  2. Follow manufacturers instructions for use
  3. Allow the laboratory to be inspected - Generally, for cause (patient complaint), Random state survey, Periodic inspections not required!
- Note: No method evaluation required!

Moderate/High Complexity Tests

- Labs performing moderate or high complexity testing also have requirements for:
  - Minimum education training and competency for lab director, supervisory and technical staff
  - Test verification and periodic correlation of instrumentation
  - Establishment of a quality control and assurance plan
  - Enrollment in an external quality control (proficiency testing) program
  - Maintenance, calibration and calibration verification
  - Test ordering and reporting documentation
Validation vs Verification

• Validation – establishing the performance specifications of a new diagnostic tool such as a new test, internally developed test or modified method – Manufacturers or LDT’s
• Verification – A one-time process to determine performance characteristics of a test before use in patient testing – Verifying an already validated test!

Joint Commission Regulations

• Joint Commission PA5.3.3 - Validation of methods
• Before a new test is used to report individual results, it must be verified that the testing system will produce accurate results on a consistent and reliable basis.
• Joint Commission PA 5.3.3.1 -
• As required, a system exists to evaluate/correlate the relationship between results for the same test performed with different methodologies or instruments or at different testing sites.

CAP Regulations

• Method evaluation spread throughout checklist questions, examples:
  – Equipment is approved by lab director.
  – Acceptable limits set for daily QC
  – Comparability of instrument/method for more than one method checked twice a year
  – Criteria for calibration verification established
  – AMR validation with matrix-appropriate materials
Method “Validation/Verification” to CLIA

- Moderate Complexity
  - Accuracy
  - Precision
  - Reportable Range
  - Verify Reference Range

- High Complexity
  - Accuracy
  - Precision
  - Analytical Sensitivity
  - Analytical Specificity
  - Reportable Range
  - Reference Range(s)
  - Establish calibration and control procedures
  - Other performance criteria

Laboratory Regulations

- General and open to some interpretation
- Direct what must be done, not “how” it is accomplished.
- How to meet the regulations:
  - There is no one right way.
  - Bottom line - the institution will need to back their reasoning for what is done
    - Consensus - CLSI protocols
    - Literature - do what others have done
    - Manufacturer’s recommendations
  - Balance cost and what is reasonable

Precision

- Within-run (Intra-assay)
- Between-run
- Day-to-day (Interassay or total)
CLSI EP-5

- **Familiarization (Days 1 - 4)**
- **Within run (Day 5) 20 tests**
  - 20 consecutive replicates/single run
- **Total (Days 6 - 25) 80 tests**
  - 2 replicates/concentration level/run
  - 2 runs/day x 20 days
- **Calculate Mean, SD and CV**
  - \( CV = \frac{SD}{\text{Mean}} \times 100 \)

Audience Poll
Which Best Describes the Precision Example?

A. Good precision, acceptable
B. Borderline acceptable, repeat study with another control
C. Unknown, need more data
D. Poor precision, unacceptable

Manufacturer Specifications

<table>
<thead>
<tr>
<th>Clinical Chemistry</th>
<th>SD</th>
<th>Sample Concentration</th>
<th>% CV Guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alburnin (BCG)</td>
<td>1.8</td>
<td>≤ 2 mg/dL</td>
<td>≤ 2%</td>
</tr>
<tr>
<td>ABH (HCC)</td>
<td>2.5</td>
<td>≤ 2 U/L</td>
<td>&lt; 2%</td>
</tr>
<tr>
<td>ALT</td>
<td>2.9</td>
<td>≤ &lt; 10 U/L</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>AST, P - P</td>
<td>5.0</td>
<td>≤ &lt; 10 U/L</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.5</td>
<td>≤ &lt; 1 g/dL</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5</td>
<td>≤ 150 mg/dL</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Hemoglobin (Hb)</td>
<td>1.0</td>
<td>≤ &lt; 15 g/dL</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Platelet (PLT)</td>
<td>2.0</td>
<td>≤ 400 x 10^9/L</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>LDH (LDH)</td>
<td>0.3</td>
<td>≤ &lt; 200 U/L</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Na</td>
<td>0.4</td>
<td>≤ &lt; 150 mEq/L</td>
<td>≤ 5%</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>0.4</td>
<td>&lt; 5 mEq/L</td>
<td>≤ 5%</td>
</tr>
</tbody>
</table>
Reportable Range (Linearity)

- Must establish reportable limits (undiluted) and maximum dilution
- Two pools (mixed)
  - Spike low sample with known amount of analyte
  - Dilute high sample with a blank
  - Mix high and low sample to create a curve
- Standard reference materials

CLSI EP-6

- Familiarization (Days 1 - 5)
- Linearity (Day 6) 16 tests
  - Dilute high sample with low (1:3, 1:1, 3:1)
  - 4 reps at ≥ 4 levels/single run
- Evaluate linear fit
  - XY plot
  - Compare variance to ensure equivalence across levels
  - Calculate slope, intercept, tolerance (Sy/x)
  - Calculate lack of fit

Forcing a Line on a Curve
Linearity and Spurious Points

Linearity and Imprecision

Audience Poll
Which of the following is TRUE about the linearity figure?

A. Linear, full range of data
B. Linear, partial range of data
C. Requires dilution study to make conclusion
D. Non-linear, unacceptable
Which of the following is TRUE about the linearity figure?

**Albumin**

A. Linear, full range of data  
B. Linear, partial range of data  
C. Requires dilution study to make conclusion  
D. Non-linear, unacceptable

**Reference Range**

- Verify existing ranges
  - Literature
  - Manufacturer’s
  - Current method
- Construct new reference range
- Considerations
  - Statistically relevant numbers
  - Well vs sick patients

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Galen & Gambino Beyond Normality: The Predictive Value and Efficiency of Medical Diagnoses. 1975 John Wiley & Sons, New York

Accuracy

- Bias to a “reference” method
  - Absolute - recovery to a known amount
  - Relative - match another method
- Reference method free of interferences
  - Rarely happens in the “real” world

CLSI EP-9

- Familiarization (Days 1-5)
- Accuracy (Days 6 - 10) 160 tests
  - Analyze >40 specimens in duplicate by both test and reference method over 5 days (8/day)
- Estimate bias
  - Calculate regression statistics and plot
    - slope (bias)
    - intercept
    - \( r \) (adequacy of range)
    - standard error \( (Sy/x) \)
    - confidence interval
Audience Poll
Which of the following is TRUE about this HgbA1c Correlation?
A. Good correlation, no issues
B. Too much bias, question whether method will meet medical standards.
C. Too much imprecision, could cause physician confusion if results mixed with core lab HgbA1c
D. Poor correlation, generally bad correlation, consider repeating or picking new POC method

Audience Poll
Which of the following is TRUE about this Sodium Correlation?
A. Good correlation, no issues
B. Too much bias, question whether method will meet medical standards.
C. Too much imprecision, could cause physician confusion if results mixed with core lab Sodium
D. Poor correlation, generally bad correlation, consider repeating or picking new POC method

Sodium Correlation

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>CONCENTRATION RANGE</th>
<th>N</th>
<th>AVERAGE DIFFERENCE</th>
<th>S'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>100-150</td>
<td>2</td>
<td>5.000</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>39</td>
<td>5.347</td>
<td>2.845</td>
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<tr>
<td></td>
<td>150-180</td>
<td>28</td>
<td>2.101</td>
<td>1.982</td>
</tr>
</tbody>
</table>
Other Considerations

- Method dependent
- Carry-over - CLSI EP-3
- Analytical Sensitivity – CLSI EP17
  - Absolute Limit of Detection
  - Practical operating - 2-3 x LOD (or > 2-10 SD)
- Interference - CLSI EP-7
  - Paired Difference - 2 pools (high 10x toxic)
  - Dose-Response - linear increase to titrate interferent and determine tolerance

K Hemolysis Interference

K Icterus Interference
Summary

- Method evaluation is important to document initial performance, quality improvement and for future troubleshooting
- CLSI, the manufacturer and peer literature can all be resources for verification and validation protocols
- Precision, accuracy, reportable range and reference (normal) range must be evaluated, at a minimum, for all non-waived tests before patient use
- No single “how to” manual for validation - adapt standards as necessary for your lab
- Performance specifications are documented in Laboratory Procedure Manual for future reference
- In addition, new tests require a written procedure, documentation of training, QC, proficiency testing and ongoing periodic re-evaluation necessary
  - New reagent, control, calibrator lots/shipments
  - Semi-annually (calibration verification and correlation of instruments)

References

- HCFA 42 CFR; Final Rule; Medicare, Medicaid and CLIA Programs; Regulations implementing Clinical Laboratories Improvement Amendments of 1988 (CLIA. Fed. Regist. 57;7001-288 (28 February 1992)
- Joint Commission on Accreditation of Healthcare Organizations CAMH Comprehensive Accreditation Manual for Hospitals. 2012 JCAHO, Oakbrook Terrace, IL.
- College of American Pathologists Commission on Laboratory Accreditation. Inspection Checklist. 2012 CAP, Northfield, IL.