Hemostasis Test Validation, Performance and Reference Intervals

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DISCLOSURE
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- **Relevant Financial Relationships**
  None

- **Off Label Usage**
  None
Chemistry and Hematology

Lab Values

- Results expressed as amounts or participles
  - Albumin: 3-4 g/dL
  - Sodium: 135-145 mEq/L
  - RBC: 4.3-5.9 x10^6/ mm³

International Standards

- Seamless values between methods
- Excellent reproducibility
Coagulation
Lab Values

- Results expressed as arbitrary values
  - PTT: 32 sec
  - Factor VIII: 34%
  - FVIII Inhibitor: 12 Bethesda units

- Few International Standards
- Values vary between methods
  - Heparin Therapeutic range:
    - Reagent A: 65-85 sec
    - Reagent B: 85-120 sec

- Moderate reproducibility
Coagulation Testing Accuracy
Performance and Reference Intervals

Outline

- Validation of New Assay
  - Components required for validation
  - Protocol for validation
- Assay Method Performance
  - Internal QC performance
  - External Proficiency Testing
- Reference Intervals
  - Basic principles
  - Common methods
Bottom-line for Hemostasis Testing

- All aspects of hemostasis testing leads to Post Medical Decision Making
- Testing parameters should be based on “Evidence Based Medicine”, however Tradition is more common
- Must compromise between statistical number and minimal number for evaluation of a test
Coagulation Laboratory
Assays and Procedures

- Evaluation of a pathophysiological condition
  - Assist in the diagnosis of the cause of bleeding or thrombosis
  - Monitoring Therapy

- To have clinical value as an assay:
  - Total error must be low to reflect the biological condition
New Test Implementation and Validation

Range of tests:
- FDA approved
- Analyte Specific Reagent (ASR)
- Research Use Only (RUO)
- “Home Brew”

All new tests must be systematically validated and regulated
- Level of evaluation varies
- All new tests must be validated
- All tests must meet a defined level of reliability
- All tests must continue to be validated on a regular basis
Coagulation Laboratory Analysis

Assay Error

For an analysis to have value:

- Total error must be low to differentiate between normal and abnormal

- General types of error:
  - Individual biological variability
  - Pre-Analytical variability
  - Analytical variability in test performance
  - Statistical error
  - Interfering substances
New Test Implementation and Validation

Assay Validation Protocol

- Purpose of validating is to insure the generation of high quality data for accurate diagnosis of disease
- To ensure a proper validation, a pre-approved protocol must be used
- Protocol must include:
  - All administrative aspects of test
  - Statistical validation of assay
  - All test parameters evaluated
Coagulation Test Implementation and Validation Protocol

Responsibilities

- **Ultimate responsibility:**
  - Laboratory Director
  - Departmental Director

- **Specific responsibilities:**
  - Department Supervisor
  - Laboratory Information Manager (LIM)
  - Quality Assurance Technologist
Coagulation Test Implementation and Validation Protocol

Supervisor’s Responsibilities

- Existence of patents
- Written procedure
  - Proper format
  - Not final draft
  - Usually provided by manufacturer
Coagulation Test Implementation and Validation Protocol

Supervisor’s Responsibilities

- Statistical Validation study
  - Varies depending on type of test
- Included Parameters
  - Accuracy
  - Precision
  - Analytical Sensitivity and Specificity
  - Interferences
  - Reference Range and AMR
  - Limits of Detection
  - Correlation
Coagulation Test Implementation and Validation Protocol

Supervisor’s Responsibilities

- Quality Control procedure
  - Control materials
  - Frequency
  - Criteria for acceptability
- MSDS Information
- Complete test information for LIS
- Necessary information to clinicians
- Cost analysis and test charge
Coagulation Test Implementation and Validation Protocol

Supervisor’s Responsibilities

Training Plan:

- Training methods and materials
- List of staff requiring training
- Documentation of completion
- Documentation of competency assessment
- New employee training checklist
Coagulation Test Implementation and Validation Protocol

LIM’s Responsibilities

- Add test information to LIS files
- Verify report format
  - Lab Information System
  - Hospital Information System
- Establish CPT coding and other coding necessary for cost recovery
Coagulation Test Implementation and Validation Protocol

QA Technologist’s Responsibilities

- Add procedure to Document Inventory Log
  - Assign document number
- Add signed procedure to SOP manual
- Add to Specimen Collection manual
- Add to Activity Menu
  - For accrediting organization
- Implement Proficiency Testing procedure
Validation Parameters

Coagulation's Dilemma

- Validation varies on type of test
  - FDA approved
  - Research Use Only
  - “Home Brew”
- Accuracy
- Precision
- Interferences
- Reference Range
- AMR
- Limits of Detection
- Correlation
Performance of Hemostasis Tests

- Necessary to ensure accurate results over time
- Must have on-going evaluation of the method
- Must be able to compare results
  - From validation until current run
- Internal QC:
  - On-going periodic analysis
  - Required by regulatory agencies
  - Set of rules to determine acceptance or failure
- External Quality Assessment
  - Periodic comparison with other labs
  - Best measure of accuracy
  - Set of rules to determine acceptance or failure
Reference Interval in Coagulation and Hemostasis

- INTERPRETATION OF COAGULATION DATA IS A COMPARATIVE DECISION-MAKING PROCESS!
- Compare patient result to reference interval
- Reference interval set up for:
  - “Normal” or healthy
  - Physiologic conditions
  - Pathologic conditions
- Therapeutic reference interval is determined in similar manner
Establishing Reference Intervals for Coagulation Assays

<table>
<thead>
<tr>
<th>Ref Interval</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Analyte</td>
<td>Determination is difficult</td>
</tr>
<tr>
<td></td>
<td>Lack data on physiology, pathology and medical aspects</td>
</tr>
<tr>
<td>New Method</td>
<td>Determination somewhat easier</td>
</tr>
<tr>
<td></td>
<td>Physiology and medical aspects known</td>
</tr>
<tr>
<td>Transference</td>
<td>Easier to determine</td>
</tr>
<tr>
<td></td>
<td>Must use caution in establishing</td>
</tr>
</tbody>
</table>
Use of “Transference” for Establishing Reference Interval

- Applying previously established ranges (by lab, others or literature) to new assay methods.
- Methods to accept transference:
  - Use of inspection and subjectivity of method procedure to determine similar range
  - Validation of range with small number of reference samples
- Methods not well established for coagulation assays
Validation of Coagulation Tests

Summary

- Validation of a coagulation test can be difficult
- Set up protocol for validation:
  - Protocol written
  - Approved before starting
- Protocol to Include:
  - Administrative issues
  - Validation with statistical analysis
Performance Criteria of Coagulation Tests

Summary

- Continued evaluation of assay
- Internal Quality Control
  - Periodic testing of same sample
  - Maintain precision and accuracy over time
  - Established rules for acceptability
- External Quality Control
  - Measure of accuracy and precision
Reference Intervals Determinations for Coagulation Tests

Summary

- Reference interval is critical for clinical decision making
- Different types of reference intervals:
  - Normal
  - Physiologic
  - Pathologic
  - Therapeutic
- Reference interval determinations:
  - New analyte
  - New method
  - Transference
Reference Literature for Validation, Performance and Ref Intervals

- **Guidelines for Validation of Assays:**

- **Guidelines for Reference Intervals:**
  - CLSI Document on Reference Intervals in the Clinical Laboratory (C28-A2)

- **Guidelines for Specific Coagulation Tests:**
  - CLSI Document on PT and APTT (H47-A2)
  - Numerous CLSI Documents on Coagulation Testing (H series)
Questions?
## Testing Validation Parameters

Coagulation's Compromise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimum</th>
<th>Practical</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>100</td>
<td>10-20</td>
<td>±5%</td>
</tr>
<tr>
<td>Precision</td>
<td>10 within runs</td>
<td>5 within runs</td>
<td>%CV</td>
</tr>
<tr>
<td></td>
<td>10 between runs</td>
<td>5 between runs</td>
<td></td>
</tr>
<tr>
<td>Interferences</td>
<td>25 of each</td>
<td>~5 of each</td>
<td></td>
</tr>
<tr>
<td>Reference Range</td>
<td>100 normals</td>
<td>20-40 normals</td>
<td>Mean ±2 SD</td>
</tr>
<tr>
<td>AMR</td>
<td>50-100</td>
<td>20-40</td>
<td></td>
</tr>
<tr>
<td>Limits of Detection</td>
<td>25 undetectable</td>
<td>5-10 undetectable</td>
<td>+3 SD of zero</td>
</tr>
<tr>
<td>Correlation</td>
<td>200</td>
<td>20-25</td>
<td>R &gt; 0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slope ~1.0</td>
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