Calibration Verification - Guidelines and Regulations
Presentation Topics & Objectives

- Define difference between Calibration and Calibration Verification

- What are acceptable Calibration Verification materials

- How and when Calibration Verification is to be performed

- Troubleshooting for a failed Calibration Verification
Let’s Start From the Beginning

- January 24th, 2003 Centers for Disease Control and Prevention (CDC) and the Centers for Medicare & Medicaid (CMS) publish laboratory regulations (CLIA) to become effective April 24th, 2003

  - Include requirements for Calibration and Calibration Verification for nonwaived tests
  - Nonwaived tests include moderately and high complexity tests
Discussion of Terminology

Definitions and applications of following:

- Calibration
- Calibration Verification
- Reportable Range
- Analytical Measurement Range (AMR)
Definitions

- **Calibration:** is the process of testing and adjusting the instrument or test system readout to establish a correlation between the instrument’s measurement of the substance being tested and the actual concentration of the substance.

  - Basically “Tells” the instrument how to read a certain level of the analyte being tested.
Calibration Verification: means testing materials of known concentration in the same manner as patient specimens to assure the test system is accurately measuring samples throughout the reportable range.
Reportable Range: the span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response.
Analytical Measurement Range (AMR): Defined by CAP (College of American Pathologists) as the range of numeric results a method can produce without any special specimen pre-treatment, such as dilution, that is not part of the usual analytic process. (Same as reportable range in CLIA terminology.)
Definitions Continued

- **Linearity**  As defined by CLSI’s (formerly NCCLS) EP06-A guideline: The ability (within a given range) to provide results that are directly proportional to the concentration (amount) of the analyte in the test sample.

  Note: “Linearity” does not appear in CLIA regulations.
Calibration Verification essentially checks the Calibration of the analyzer by confirming that the test system is able to provide accurate results throughout the Analytical Measurement Range of the instrument for that particular analyte.
In a Nutshell

- Calibration “tells” the instruments how to read certain analyte concentrations
- Calibration Verification tests to make sure the calibrators are accurate across the instrument's reportable range
Calibration Overview
Calibration Requirements

- Laboratory is responsible for performing calibration as directed by the manufacturer’s test system instructions AND when Calibration Verification does not produce acceptable results.

- Make sure to document each time calibration is performed.

- More frequent calibration may be required if the test system’s calibration is less stable than instrument manufacturer’s suggested frequency.
Exceptions to Calibration

- Manual procedures in which an instrument is not used
  - Microbiology cultures
  - Tilt-tube prothrombin times
  - ABO group and D (Rho) typing
Exceptions to Calibration Continued

- **Microscopic procedures**
  - Urine sediment examination
  - KOH and pinworm preparations
  - All manual cell differential procedures
  - Manual Cytology screening procedures

- **Procedures which involve instruments in which calibration is not practical**
  - Prothrombin procedures
How to Perform Calibration

- The manufacturer’s instructions of the test system should explain the process for calibration

- Frequency
- Replicates
- Analyte concentration
- Acceptable material
Calibration Materials

- *Calibration materials, also called calibrators are solutions of known analyte concentrations*

- *May contain more than a single analyte*

- *The term “standards” generally used to refer to calibration material*
Reasons to Perform Calibration Verification

- Can Detect Hook Dose Effect

**Equation:**

\[ y = 0.4024x + 17.346 \]

**Coefficient of Determination:**

\[ R^2 = 0.8768 \]
Test method may have limited number of calibrators
  - May have only one or two calibrators

Test method calibrators may not span the instrument’s reportable range
  - Even if the test method has three calibrators (minimum number of calibrators required to be exempt from Calibration Verification, may not adequately span AMR)
Reasons to Perform Calibration Verification

Continued

- *Daily Quality Controls generally do not challenge lower and upper limits*

- *Daily QC normally targets Normal and Abnormal patient ranges only*

- *It is possible to have “Passing” Daily QC, but “Failing” Calibration Verification since controls may not span entire analyzer range*

- *Especially important in Therapeutic Drug Monitoring*
Calibration Verification Requirements

- Labs must perform every 6 months (or more frequently if specified in the test system’s instructions) or if:
  - There is a change in reagent lot numbers for a particular analyte—unless the laboratory can show that the new lot of reagent does not affect the range of the patient test results and that daily quality control results are not adversely affected by changing reagent lots.
Calibration Verification Requirements Continued

- There is major preventative maintenance or replacement of critical instrument components which may affect test patient results
  - Includes analyzer being sent to manufacturer for repairs
Calibration Verification Requirements Continued

- Quality control materials begin to shift or are out of the laboratory’s acceptable limits and other methods of checking the accuracy of the control results are not able to ascertain and resolve the problem.

- The individual laboratory has determined the reportable range for patient test results are to be monitored more frequently.
Calibration Verification Requirements Continued

- **Document each time Calibration Verification is performed**

- **IMPORTANT NOTE**: The laboratory must also be able to verify calibration on factory-calibrated instruments that cannot be calibrated by the end user
What Are Acceptable Calibration Verification Materials?

- Materials of Known Concentrations
  - Calibration Materials
  - Proficiency Testing samples with known results
  - Commercially available material
  - Controls with known values
  - Patient samples with known values
Calibration Verification Exceptions

- **For Automated Cell Counters:**
  - Follow manufacturer’s instructions for analyzer operation and tests two levels of control each day of patient testing and results are within lab’s acceptable range.

- **Calibration Verification is met if:**
  - Running 3 or more levels of calibrator and includes a low, mid, and high value, and is performed at least once every 6 months.
If laboratory follows manufacturer’s instruction for analyzer operation and runs three levels of control material:
- on a routine basis (low, mid, and high)
- more than once each day patient results are reported
- and the control results meet the lab’s criteria for acceptability
- and the controls are NIST traceable, the calibration verification requirements have been met
Calibration Verification Review

When: Every 6 Months or whenever the following occurs:

- New lot of reagent used (unless lab can verify that there is no change in results)
- Major preventative maintenance or replacement of critical test method components
- Controls indicate shift or are out of the laboratory’s acceptable range
- Laboratory determines more frequent verification required
How to Perform Calibration Verification – Tools and Resources
How To Perform Calibration Verification

- **Document**
  - Date, Analyte, and Calibration Verification material used

- **Run 3 levels of material**
  - Run low, mid, and high levels as if running patient samples
  - Record data
  - CLIA does not state minimum number of replicates

- **Compare results to expected values**

- **Document results and determine if results meet laboratory’s acceptable criteria**
Sample Calibration Verification Results Worksheet

Worksheet and Documentation Form for Calibration Verification

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Date</th>
<th>Instrument</th>
<th>Serial Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent/Strip/Cassette Lot#</td>
<td>Expires:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Materials used</td>
<td>Source of Acceptable Limits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low Level</th>
<th>Mid Level</th>
<th>High Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expiration Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceptable Limits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calibration Verification Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results Obtained Repetition 1</td>
</tr>
<tr>
<td>Optional: Repetition 2</td>
</tr>
<tr>
<td>Repetition 3</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Results Acceptable?</td>
</tr>
<tr>
<td>Comments and/or Corrective Actions</td>
</tr>
</tbody>
</table>

Performed by ________________________ Date ____________

Reviewed by ________________________ Date ____________
How To Perform Calibration Verification
Continued

- **Plot data on Linear Graph**
  - Computer program - spreadsheet
  - Commercial Programs
  - Manufacturer material and programs
  - Third Party control manufacturer and programs
Sample Calibration Verification Results

Spreadsheet and Graph

<table>
<thead>
<tr>
<th>Technician</th>
<th>Analyte</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terry Smith</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Run</th>
<th>Instrument</th>
<th>Method</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/2/2007</td>
<td></td>
<td></td>
<td></td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level</th>
<th>Units</th>
<th>Predicted Value</th>
<th>Actual Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mg/dL</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>mg/dL</td>
<td>202</td>
<td>200</td>
</tr>
<tr>
<td>C</td>
<td>mg/dL</td>
<td>397</td>
<td>401</td>
</tr>
<tr>
<td>D</td>
<td>mg/dL</td>
<td>591</td>
<td>597</td>
</tr>
<tr>
<td>E</td>
<td>mg/dL</td>
<td>785</td>
<td>783</td>
</tr>
</tbody>
</table>

SLOPE   1.0016
INTERCEPT 0.6843
COEFFICIENT R 0.9999

Glucose

\[ y = 1.0016x + 0.6843 \]

\[ R^2 = 0.9999 \]
Sample Calibration Verification Results
Spreadsheet and Graph

Linearity/Calibration Verification Report

Company Name: General Hospital
Lab Name: Chemistry Lab
Analyzer Model: AnyChem Analyzer
Analyzer Name: AnyChem S/N #12345
Date Of Run: Dec 31, 2014
Technician: Jane Doe

USER DATA SUMMARY

<table>
<thead>
<tr>
<th>Level</th>
<th>Theoretical*</th>
<th>User Mean</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.904</td>
<td>0.935</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8.473</td>
<td>8.475</td>
<td>5.95, 5.96, 5.99</td>
</tr>
<tr>
<td>C</td>
<td>11.983</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>17.515</td>
<td>18.22</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>23.935</td>
<td>23.050</td>
<td></td>
</tr>
</tbody>
</table>

*Theoretical values are determined by generating a line from the most and least observed values.

USER VS. PEER COMPARISON (200 Peers)

<table>
<thead>
<tr>
<th>Level A</th>
<th>Level B</th>
<th>Level C</th>
<th>Level D</th>
<th>Level E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.012</td>
<td>0.523</td>
<td>2.612</td>
<td>18.206</td>
<td>23.120</td>
</tr>
</tbody>
</table>

Approved By: __________________________ Date: __________________________

Each kit of product is manufactured such that a linear relationship exists among levels. Actual results obtained may vary depending on instrumentation, methodology, and assay technique. Results may also be dependent on the accuracy of the instrument/assay system calibration. The degree of association, non-linearity, or an individual judgment based on methodology, clinical significance, and medical decision levels of the test analyst. AUDIT MicroControls, Inc. assumes no responsibility for the reading and interpretation of the above data.
So, Do I Pass???
What are Acceptable Results?
So What Are Acceptable Results?

The Million Dollar Question (drum roll please.....):

How do I know if my results are “Good Enough”?
What is Acceptable Calibration Verification?—The Ultimate Question

- Each laboratory is responsible for establishing acceptable parameters
- Some labs use CLIA ’88 Proficiency Testing Limits
- General Rule of Thumb used by some inspectors and laboratory managers:
  - **R Coefficient (Also R²): 0.98 – 1.00**
    - Tells you if the data is linear
  - **Slope: 0.90 – 1.10 (+/- 10%)**
    - Tells you how well you match up to the expected data
Goal of Calibration Verification

- Confirm that the test system is reporting accurate results throughout the test system’s reportable range
What if Calibration Verification Fails?
How to Address Failed Cal Ver

- As per CLIA guidelines:
  - Repeat calibration procedure
  - Good Laboratory Practice to run controls after recalibrating and before reporting patient results
  - If the instrument is factory-calibrated, contact instrument manufacturer
Additional Troubleshooting

- Calibration Verification Troubleshooting

- Number of variables to be considered
  - QC Material
  - Reportable Range of Instrument
  - Changes in Reagent
  - Maintenance Logs
  - Environmental Conditions
  - Instrument Servicing
  - Changes in Operators
  - Compare on similar instrument
# Troubleshooting Checklist for Calibration Verification

**Analyte** 
**Date**

**Instrument** 
**Serial Number**

<table>
<thead>
<tr>
<th>1. Check your quality control (QC) results for the analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Are there any patterns seen in the control results?</td>
</tr>
<tr>
<td>• Are all values below the mean?</td>
</tr>
<tr>
<td>• Are all values above the mean?</td>
</tr>
<tr>
<td>• Are there any noticeable shifts or trends over time?</td>
</tr>
<tr>
<td>• Are accuracy and precision acceptable?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>2. Check your calibration verification material</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Are the materials used appropriate and in-date?</td>
</tr>
<tr>
<td>• Have you properly determined the acceptable limits for the calibration verification material?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>3. Check your reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Have there been any reagent changes?</td>
</tr>
<tr>
<td>• Is there a new lot number of reagent?</td>
</tr>
<tr>
<td>• Has there been a change in manufacturer?</td>
</tr>
<tr>
<td>• Has there been a new formulation (check the package insert) of current reagent?</td>
</tr>
<tr>
<td>• Are the reagents in date?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>4. Check instrument maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Review the daily, weekly, monthly, quarterly, etc. logs. Is there any missing maintenance, problems, or changes?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>5. Check the environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Has the instrument been moved recently?</td>
</tr>
<tr>
<td>• Have there been any changes to the environment or surroundings of the instrument?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>6. Check the service record</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Has the instrument been serviced recently?</td>
</tr>
<tr>
<td>• Has there been any software or hardware upgrades or changes?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>7. Check instrument operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Are there new instrument operators?</td>
</tr>
<tr>
<td>• Are all operators following established procedures for instrument operation?</td>
</tr>
<tr>
<td>• Has there been any recent modification to the technique used to run the test?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>8. Check a comparative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Is there another laboratory nearby that can run your calibration verification material so you can compare the results?</td>
</tr>
</tbody>
</table>

**Comments:**

<table>
<thead>
<tr>
<th>9. Will recalibration be performed for the analyte?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes / No</td>
</tr>
</tbody>
</table>

**Comments:**

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Moving Forward – Current and Future Calibration Verification Topics
Point-of-Care Testing and Calibration Verification

- **If a lab is running nonwaived tests on POCT instruments, Calibration Verification may be required**

- **Some analyte panels on the Abbott i-STAT® require Calibration Verification**
Excerpt from Advance for Administrators of the Laboratory November 22, 2010:

- **Fourth most common reason for lab non-compliance citation:**

  #4 - “not performing calibration verification according to the manufacturer's instructions including: the number, type and concentration of materials to be used; use of materials at low, medium and high values within the reportable range as determined by the laboratory; acceptable limits for calibration verification, once every 6 months or more often, if required by laboratory procedures;”
References and Resources
References

- CLIA Brochure #3: www.cms.hhs.gov/clia
- COLA LabGuide, 5/07
- www.cygnustechologies.com/Hook Effect.pdf
Resources

- **Article by Kathryn Connolly Quality Management Systems Accreditation Manager for COLA:**


- **dgrhoads website:** http://www.dgrhoads.com/

- **CMS website:** http://www.cms.hhs.gov/CLIA/
Thank you for your time and interest in Audit MicroControls. We hope that you find our product offering helpful in meeting your Calibration Verification / Linearity requirements.

Please feel free to contact us by phone at: (866) 252-8348 or via email at: sales@auditmicro.com if you should have any questions.