Calibration Verification
Linearity in the Clinical Lab.
Did I Pass or Fail?

2017 ASCLS New Jersey
Presentation Topics & Objectives

- Calibration Verification
  - Key Definitions
  - Why do I need to perform CV?
  - How do I perform CV?
  - When do I perform CV?
  - Did my CV Pass Or Fail? (My organic chemistry final nightmare all over again)
  - Does a failed CV mean I need to retake the class? (Troubleshooting)
Calibration Verification Definition

• **Calibration** tests the accuracy of the instrument.
  • Est. relationship between analyte content & instr. Measurement sig.

• **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 laboratory’s reportable range of the instrument.

• **Calibration verification** tests the accuracy of the instrument *throughout the instrument’s entire reportable range*.
Reportable Range

- **Reportable Range** is the span of test result values over which *the lab can establish or verify* the accuracy of the instrument or test system’s measurement response.

- It is the range of results for an analyte, from minimum to maximum, which *the instrument’s test system can actually measure*.

- The laboratory sets the Reportable Range.

- After you run your calibration verification, this is what you will report.
Analytical Measurement Range (AMR)

- Defined by CAP as the range of numerical results a method can produce without any special specimen pre-treatment, such as dilution, that is not part of the usual analytical process (same as reportable range in CLIA terminology).

- AMR can be different from Reportable Range

- AMR, unlike Reportable Range, comes from the manufacturer and should show linearity over its entire AMR
Why Perform Calib Verification

- Per CLIA Sec. 493.1255 calibration verification procedures are required to substantiate the continued accuracy of the test system throughout the laboratory’s reportable range.

- Non-waived moderately complex quantitative tests must have calibration verification performed!
  - Non-Waived
    - Moderately Complex
    - High Complexity
  - Waived
  - Quantitative
  - Semi-quantitative
How To Perform Calibration Verification

- Run at least three levels of material (CLIA requirement)
- Run a low, middle and high level as if running patient samples
- CLIA does not specify the number of replicates (recommend at least two replicates as GLP to pick up potential outliers)
- Record data
- Document results and determine if the results meet the laboratory’s acceptable criteria

Quality control made easy.
Acceptable Cal Ver Material

- Materials of known concentration whose known upper and lower values are near the upper and lower values of the instrument. Must also include a mid-range and have enough volume of sample to complete the run
  - Calibration Material
  - Proficiency testing samples with known results
  - Controls with known values
  - Previously tested patient samples
  - Commercially available material
When To Run Calib Verification

Per CLIA 493.1255(b)(3)

- Labs must perform every six months (or more frequently if specified in the test system’s instructions) or if:
  - There is a complete change of reagents for a procedure – unless the lab can show that the new lot of reagents does not affect the range of the patient’s test results and that daily quality control results are not adversely affected by changing reagent lots
  - CLIA’s probe for 493.1255(b) ‘If a laboratory does not perform CV after a complete change of reagents, what data does the lab have to document that changing the reagent lot numbers does not affect the reportable range of patient test results, and does not adversely affect control results?’
  - Use your routine QC data (5 controls before and 5 after the reagent change) to show a shift has not occurred
When To Run Calib Verification

CLIA 493.1255(b)(3) cont.

• Major preventative maintenance or replacement of critical instrument components which may affect patient test results

• Control materials reflect an unusual trend or shift, or are outside of the laboratory’s acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem
  • Did analyzer get moved?
  • Different technician on the analyzer?
  • Change in analyzer protocol?

• The laboratory’s established schedule for verifying the RR for patient test results requires more frequent calibration verification

Quality control made easy.
Why Calib Verification Matters

- Performing QC does not generally challenge the upper and lower limits of the instrument
- It is possible to have a passing QC and a failing CV
- The instrument’s test system may have a limited number of calibrators
- Test method calibrators may not span the entire reportable range of the instrument, even with three calibrators
- Can detect Hook Dose Effect
- Patient presents significantly high or low
- Want to give results to physician with confidence
So, Did I Pass? What Are Acceptable Results?

- Plot data on linear graph
  - Individualized Custom Computer program
  - Commercial third party/manufacturer program

- Values obtained from running the test samples as patient samples are compared to the known values (peer data) of the material.

- If the values are acceptable, calibration of the analyte is verified
So, Did I Pass?
What Are Acceptable Results?

• 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 ($R^2$): 0.98 – 1.00
    • Tells data is linear
  • Slope: 0.90 – 1.10 (+/- 10%)
    • Tells how well you match up to the expected data
Sample Worksheet for Calibration Verification

Worksheet and Documentation Form for Calibration Verification

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

Calibration Verification Results

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

Performed by ____________________________ Date ____________________

Reviewed by ____________________________ Date ____________________
So, Did I Pass? #1

Quality control made easy.
So Did I Pass?  #2

![Quality control made easy.](image_url)
So, Did I Pass?   #3
Quality control made easy.

So Did I Pass?  #4

Linearity/Calibration Verification Report

Company Name
Lab Name: Primary
Analyzer Model
Analyzer Name
Date Of Run
Technician

AUDIT MicroControls

USER DATA SUMMARY

Slope Y-Intercept R-Squared

USER VS. PEER COMPARISON

(305 Peers)

Approved By:  Date:

Each lot of product is manufactured such that a linear relationship exists among levels. Actual results obtained may vary depending on instrumentation, methodology, and analyst temperature. Results may also be dependent on the accuracy of the instrument and method calibration. The degree of acceptable linearity is an individual judgment based on methodology, clinical significance, and knowledge of the test analyst. AUDIT MicroControls, Inc. assumes no responsibility for the validity or interpretation of the above data.
What Does It All Mean?

- A successful calibration verification confirms that the test system is providing accurate results for the analyte throughout the reportable range of the test.
- You need to challenge the entire range of what the manufacturer claims, to the best of your ability.
- Clinical significance, the patient population.
- Peer Data helps!
What If Calibration Verification Fails

- If CV fails, it means the instrument calibration for this analyte may no longer be valid and corrective action is needed
  - Review QC Results
    - Patterns/values + or – mean?
    - Shifts/trends over time?
    - Are Accuracy & precision acceptable?
  - Check CV Material for OVS & CLS
  - Check Reagents
    - Reagent change?
    - New Lot#?
    - Manufacturer change?
    - New formulation of reagent?
  - Review Instrument Maintenance Log
    - Missing data/doc’s; Problems? Changes?
Additional Troubleshooting

- **Environmental changes**
  - Instrument moved?

- **Service Records**
  - Serviced of late?
  - Software/hardware upgrades or changes?

- **Instrument Operation**
  - New instrument operators?
  - All operators following est. protocol?

- **Check Comparative Method**
  - Another lab nearby to compare results?

- **Rerun Cal Ver**

- If instrument is factory calibrated, call mfg.
# Troubleshooting Checklist

**Troubleshooting Checklist for Calibration Verification**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Serial Number</td>
</tr>
</tbody>
</table>

## 1. Check your quality control (QC) results for the analyte
- Are there any patterns seen in the control results? [Yes/No]
  - Are all values below the mean? [Yes/No]
  - Are all values above the mean? [Yes/No]
- Are there any noticeable shifts or trends over time? [Yes/No]
- Are accuracy and precision acceptable? [Yes/No]

**Comments:**

## 2. Check your calibration verification material
- Are the materials used appropriate and in-date? [Yes/No]
- Have you properly determined the acceptable limits for the calibration verification material? [Yes/No]

**Comments:**

## 3. Check your reagents
- Have there been any reagent changes? [Yes/No]
- Is there a new lot number of reagent? [Yes/No]
- Has there been a change in manufacturer? [Yes/No]
- Has there been a new formulation (check the package insert) of current reagent? [Yes/No]
- Are the reagents in date? [Yes/No]

**Comments:**

## 4. Check instrument maintenance
- Review the daily, weekly, monthly, quarterly, etc. logs. Is there any missing maintenance, problems, or changes? [Yes/No]

**Comments:**

## 5. Check the environment
- Has the instrument been moved recently? [Yes/No]
- Have there been any changes to the environment or surroundings of the instrument? [Yes/No]

**Comments:**

## 6. Check the service record
- Has the instrument been serviced recently? [Yes/No]
- Has there been any software or hardware upgrades or changes? [Yes/No]

**Comments:**

## 7. Check instrument operation
- Are there new instrument operators? [Yes/No]
- Are all operators following established procedures for instrument operation? [Yes/No]
- Has there been any recent modification to the technique used to run the test? [Yes/No]

**Comments:**

## 8. Check a comparative method
- Is there another laboratory nearby that can run your calibration verification material so you can compare the results? [Yes/No]

**Comments:**

## 9. Will recalibration be performed for the analyte?
[Yes/No]

**Comments:**
Thank You!!

If you have any questions or comments please feel free to contact me:
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