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Calibration Verification & Linearity: Regulatory Requirements and Application to Coagulation Assays

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Calibration

The process of establishing a correlation between the measurement signal generated by an instrument and the true concentration of analyte in the sample.

Calibration verification.

- The process of “testing materials of a known concentration in the same manner as patient specimens to assure the test system is accurately measuring samples throughout the reportable range.”

42 CFR 493.2

Linearity

Refers to the relationship between the final analytical result for a measurement and the concentration of the analyte being measured.

- Analyte concentration versus measurement signal is not always linear
- Not separately designated by CLIA

Killeen AA, Long T, Souers R et al. Verifying Performance Characteristics of Quantitative Analytical Systems. Arch Pathol Lab Med 2014;138:1173-1181.

Analytical measurement range (AMR)

The “range of concentrations of an analyte that a method can directly measure without any dilution, concentration, or other pretreatment.”

- Chemistry and Toxicology Checklist, CAP

AMR validation.

- A process used to verify the linear relationship between the analytical results of a method and the concentration of analyte over the entire measurement range

42 CFR 493.2

Regulatory requirements

Calibration verification is required by CLIA.

Laboratories which perform quantitative coagulation assays must verify:

- Calibration
- AMR validation (linearity)
- Whenever required by the method manufacturer

At least every 6 months.

42 CFR 493.1255

How to meet minimum requirements

Linearity experiment.

- Analyze 3 samples in duplicate
- Samples must span the AMR
- Include a minimal value, a mid-point value, and a maximum value near the upper limit
- Sec. 493.1255(b)(2)

Source of materials and acceptability criteria determined by laboratory director.

- Patient specimens
- Commercial kits
- Standard reference materials
- Calibrators

Please note:

Re-calibration of a test more frequently than every 6 months meets calibration verification requirements if the calibration includes samples with low, mid, and high values near the AMR.

Why is it important?

Required by CLIA.

If the calibration changes, patient test result values will change.

Can detect problems earlier than QC or PT.

- If linear range does not cover AMR, may be a problem with reagents, specimen handling, or analyzer
- Adjustments to reportable range to reflect the linear range

Why is it relevant to coagulation assays?

Coagulation testing has evolved.

- In the past, primarily clot-based testing
- Some tests and methods now measure a concentration of an analyte

Requirements apply to methods that are calibrated and directly measure concentration or activity of an analyte.

- EIA methods
- Immunoturbidity
- Chromogenic methods

http://www.captodayonline.com/Archives/1112/1112g_lap.html

Examples of applicable assays

EIA or immunoturbidity methods for:

- Coagulation factors
- Protein C and S antigens
- von Willebrand factor antigen
- Quantitative D-dimer

Chromogenic methods for:

- Antithrombin activity
- Protein C activity
- Heparins

Examples of exempt assays

Clot-based assays.

Platelet function tests.

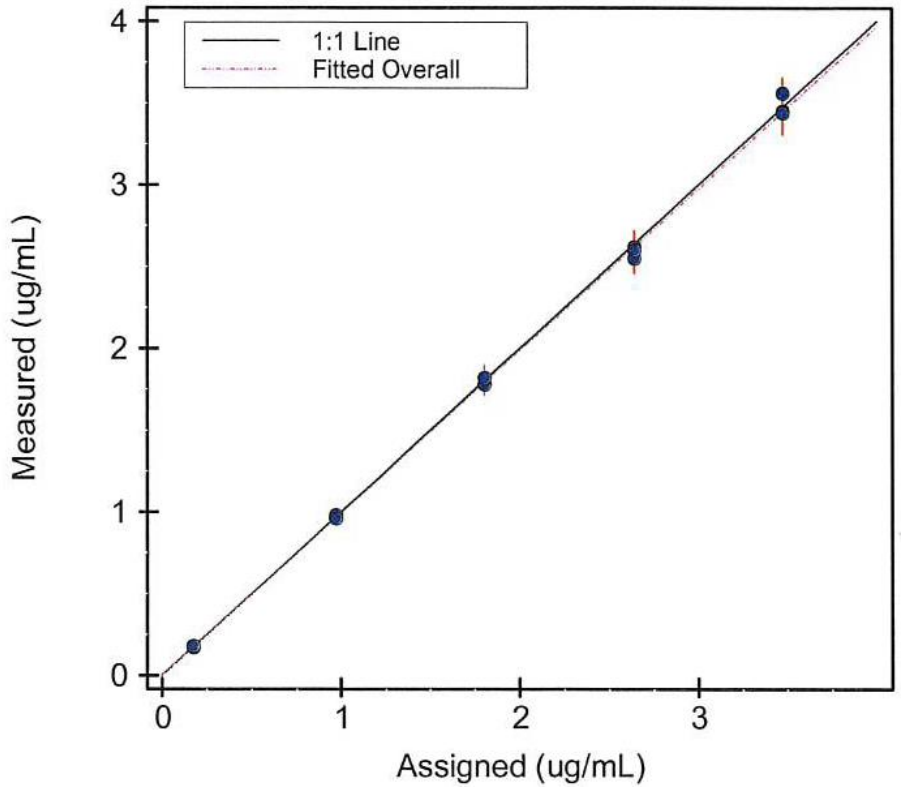
Example analyte

Quantitative D-dimer.

- AMR 0.27-4.0 µg/mL FEU
- 5 samples spanning the AMR measured in triplicate
- Slope and intercept calculated

Sample	Expected Value	Mean Observed
DDI-01	0.1771	0.177
DDI-02	0.973	0.973
DDI-03	1.807	1.807
DDI-04	2.641	2.590
DDI-05	3.475	3.483

D-dimer Scatter Plot



Slope 0.992
Intercept -0.001

Troubleshooting

Some content adapted from College of American Pathologists Calibration Verification/Linearity Participant Summary

Non-linearity

Consider sources of error:

- Specimen handling
- Analytical phase of testing
- Clerical errors

Problems with high or low specimens

Possible manifestations.

- Observed value different than expected
- Samples don't adequately challenge the upper or lower AMR

How to investigate.

- Assess for recovery issues near the limits of the AMR
- Review dilution protocols
- Assess specimen handling and possible degradation
- Were samples within the AMR for the instrument?
- May need to add samples to adequately challenge the limits

Bias

Evidence of bias.

- Slope not equal to 1
- Non-zero intercept
- Non-zero percent difference on a bias plot (not shown)

How to investigate.

- Instrument maintenance needed?
- Review QC results for acceptability
- Review recent calibration for error or need for recalibration
- Review reagent handling
- Reagent lot-to-lot comparisons
- Confirm written procedures were followed
- Consider sample mixing or reconstitution problems or improper storage

Imprecision

Possible manifestations.

- Large difference between replicates for a single specimen
- Standard deviation exceeds allowable random error

How to investigate.

- Exclude clerical error in recording of results
- Review specimen handling (reconstitution, storage, mixing, etc.)
- Review quality control data
- Perform simple precision study

References

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Disclosures/Potential Conflicts of Interest

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- **Employment or Leadership**:
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