Welcome!

This is a self-paced online course which will include examples from current laboratory best practices and offers real-world, tangible knowledge that can be immediately applied in your laboratory.

All learning objectives are based on Unites States federal regulatory standards for calibration verification / linearity testing. At the end of this session, you will be able to answer:

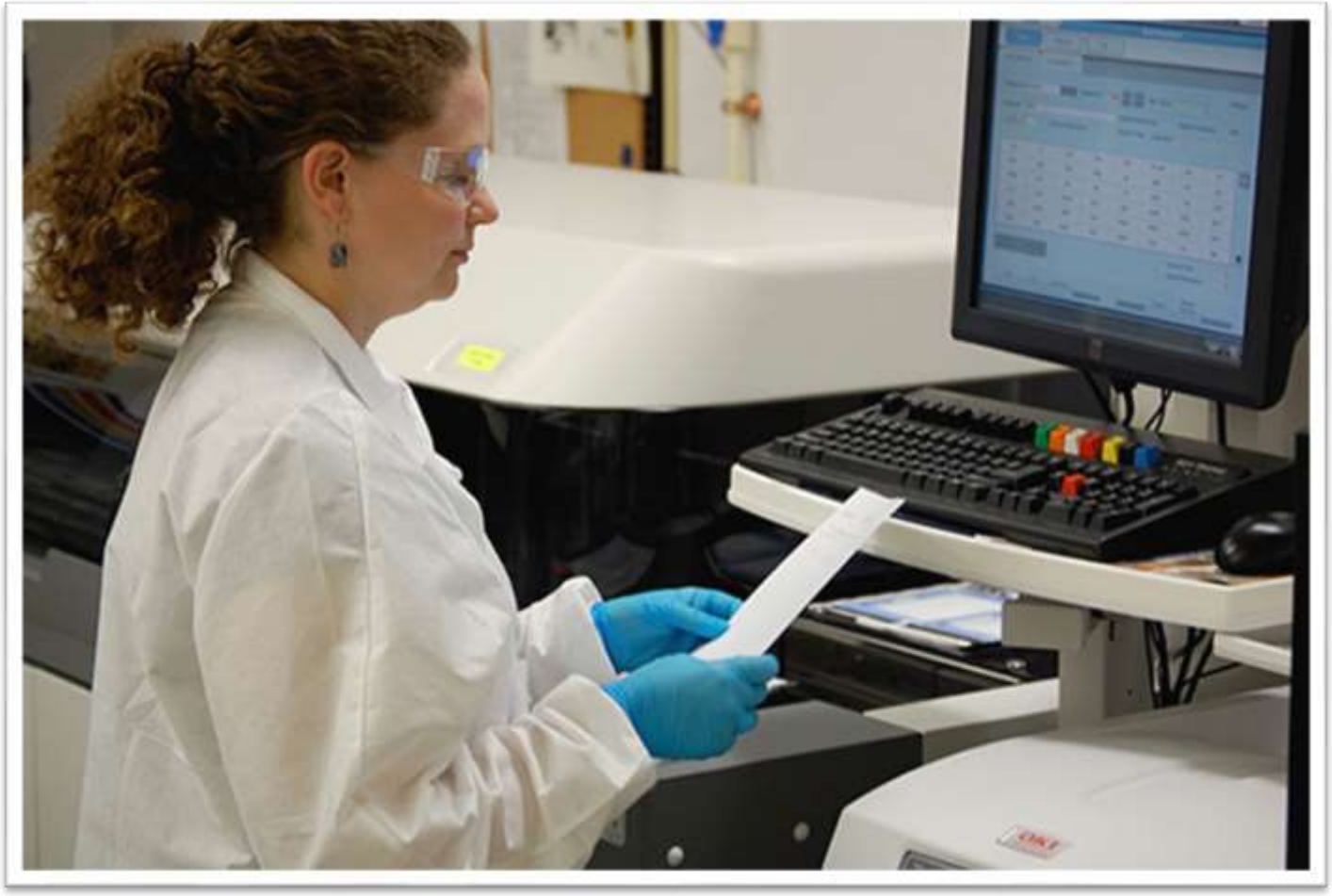
- What is Calibration?
- What is Calibration Verification?
- What is Reportable Range?
- What is Analytical Measurement Range (AMR)?
- Why perform Calibration Verification testing?
- How often is Calibration Verification required?
- Is Calibration Verification required when installing a new method or instrument?
- How do I perform Calibration Verification?
- What should I do if Calibration Verification fails?

Laboratory testing plays a large role in clinical decisions, providing physicians, nurses, and other health care providers with information that aids in the prevention, diagnosis, treatment, and management of disease.

The **Clinical Laboratory Improvement Amendments (CLIA)** of 1988 are United States federal regulatory standards that apply to all clinical laboratory testing performed in the United States, except clinical trials and basic research.

The **Centers for Medicare and Medicaid Services (CMS)** has the primary responsibility for the operation of the CLIA Program. Within CMS, the program is implemented by the Center for Medicaid and State Operations, Survey and Certification Group, Division of Laboratory Services.

The primary purpose of CLIA '88 is to ensure the accuracy, reliability and timeliness of patient test results because - when it comes to patient care - there is little room for error.



What happens when laboratories begin to report inaccurate patient results?

The foundation of quality healthcare begins to crumble. It's why quality control testing and calibration verification / linearity testing are key elements and a central part of all good laboratory practices. Calibration verification / linearity is especially important because it validates that a testing system continues to work properly throughout the entire reportable range versus only a small portion of the range.

- This course begins by defining key terms and explaining some of the concepts surrounding calibration verification.
- It then discusses the value of calibration verification / linearity testing.
- The course then focuses on detailed information about selecting appropriate calibration verification / linearity materials, testing your instrument's full reportable range and interpreting calibration verification / linearity results to help you understand the process and meet regulatory requirements.

The following key terms are very important in understanding calibration verification / linearity testing. In this section we highlight both CLIA and CAP definitions because of slight variances in terms. Other accrediting agencies have adopted CLIA's definitions.

What is Calibration?

CLIA

Calibration is a process of testing and adjusting an instrument or test system to establish a correlation between the measurement response and the concentration or amount of the substance that is being measured by the test procedure.[1]

CAP

CAP and CLIA define calibration in a similar manner.

Calibration is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures are typically specified by a method manufacturer, but may also be established by the laboratory.[2]

1. Centers for Medicare & Medicaid Services Standards and Certification: CLIA '88 Laboratory Requirements (42 CFR 493) and detailed CLIA definitions (42 CFR 493.2)
2. CAP definitions are sourced from the 2012 CAP Chemistry and Toxicology Checklist

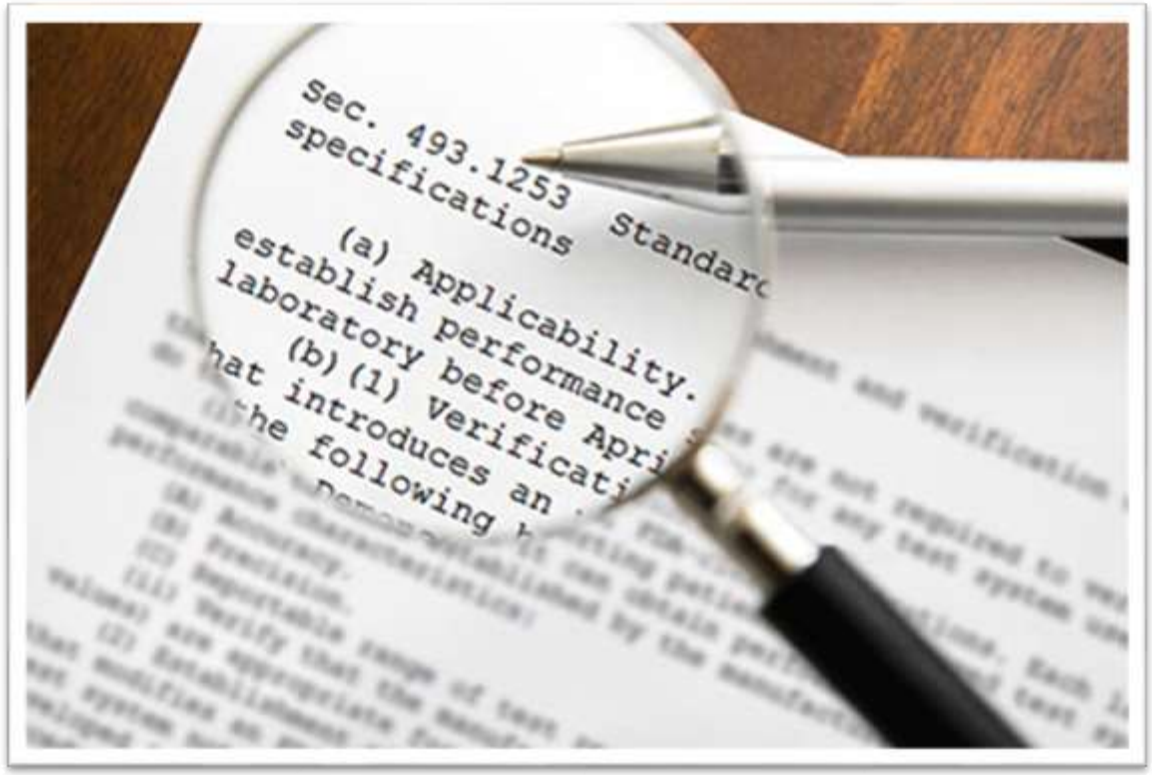
What is Reportable Range / Analytical Measurement Range?

CLIA

Reportable range is the span of test result values over which the laboratory can establish or verify the accuracy of the instrument or test system measurement response.[1]

CAP

CAP uses the term Analytical Measurement Range (AMR) rather than CLIA's Reportable Range, however, they carry the same meaning. CAP defines AMR as the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.[2]

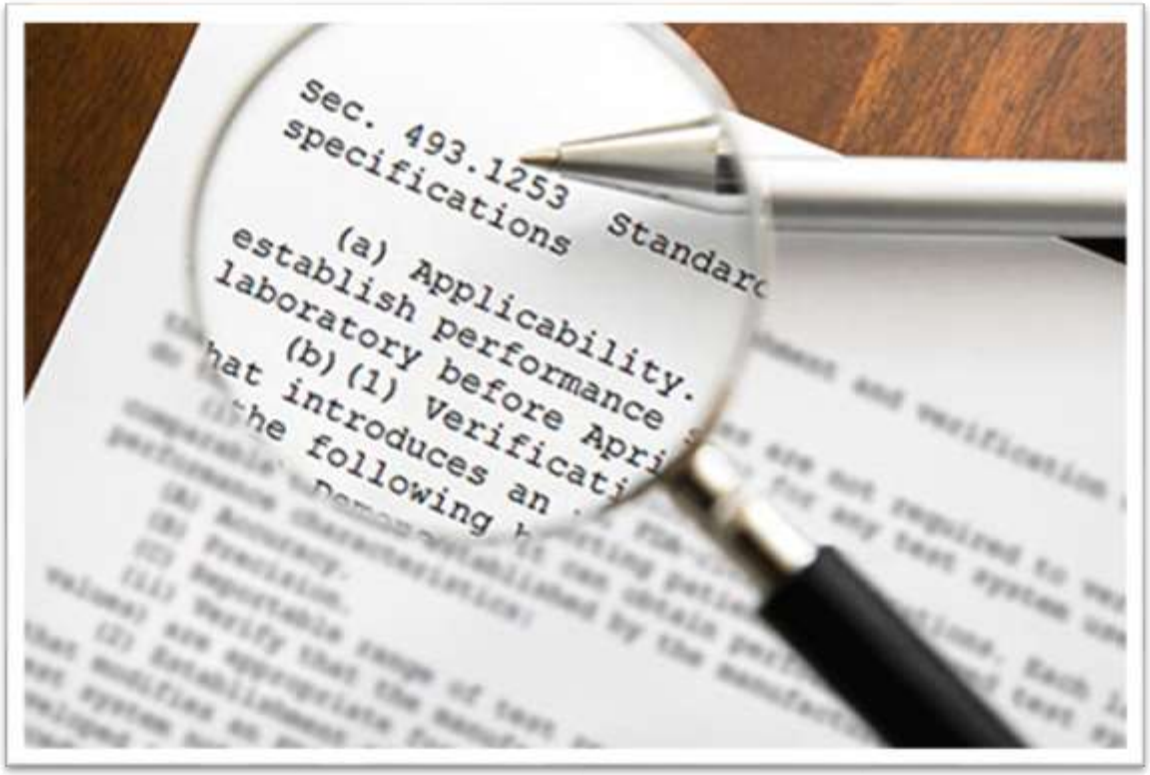


1. Centers for Medicare & Medicaid Services Standards and Certification: CLIA '88 Laboratory Requirements (42 CFR 493) and detailed CLIA definitions (42 CFR 493.2)
2. CAP definitions are sourced from the 2012 CAP Chemistry and Toxicology Checklist

What is Calibration Verification?

CLIA

Calibration Verification is the assaying of materials of known concentration in the same manner as patient samples to substantiate the instrument or test system's calibration throughout the reportable range for patient test results.[1]



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2. CAP definitions are sourced from the 2012 CAP Chemistry and Toxicology Checklist

What is Calibration Verification?

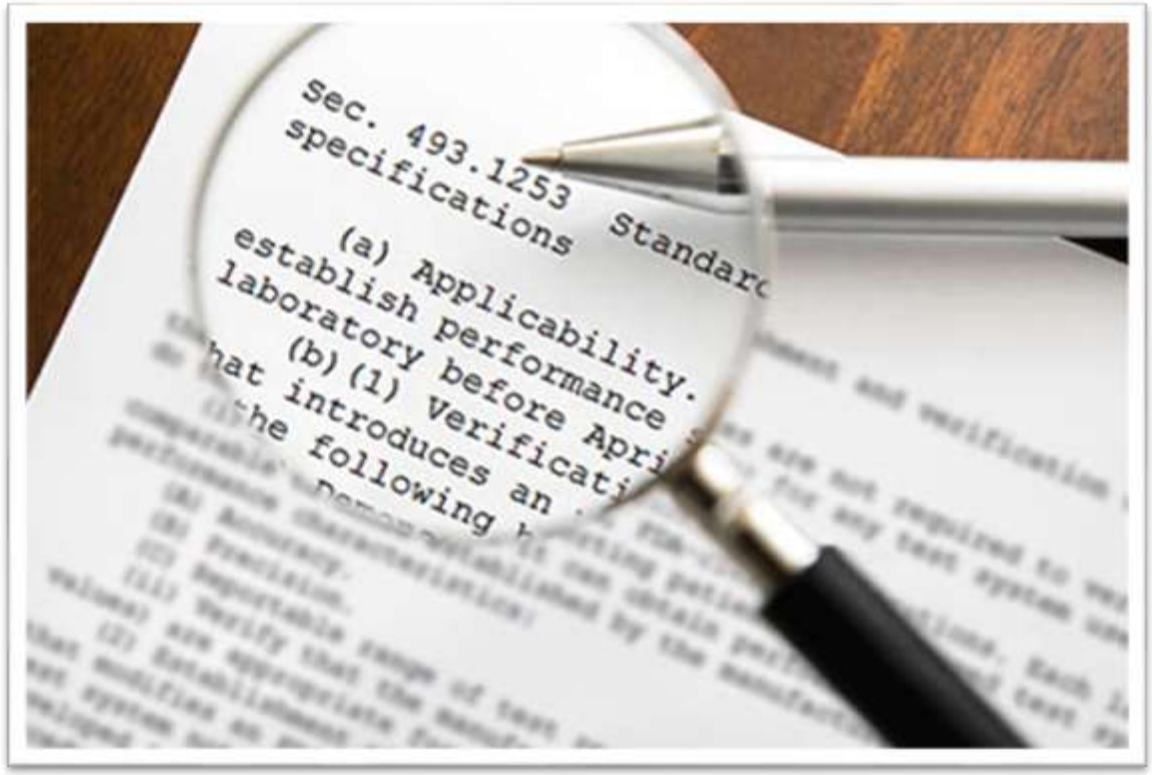
CAP

Calibration verification, as interpreted by CAP, carries a more restrictive meaning versus when interpreted by CLIA. As defined by CLIA, calibration verification refers to two distinct processes: to substantiate and verify the correct method calibration; and to validate the reportable range for patient test results. CAP limits the use of the term calibration verification to the first process and uses the term analytical measurement range (AMR) validation to refer to the second process.

Calibration verification is the process of confirming that the current calibration settings remain valid for the method.[2]

Analytical measurement range (AMR) validation is defined as the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. Validation of the AMR is accomplished by demonstrating a linear relationship for appropriate set of samples that cover the AMR.[2]

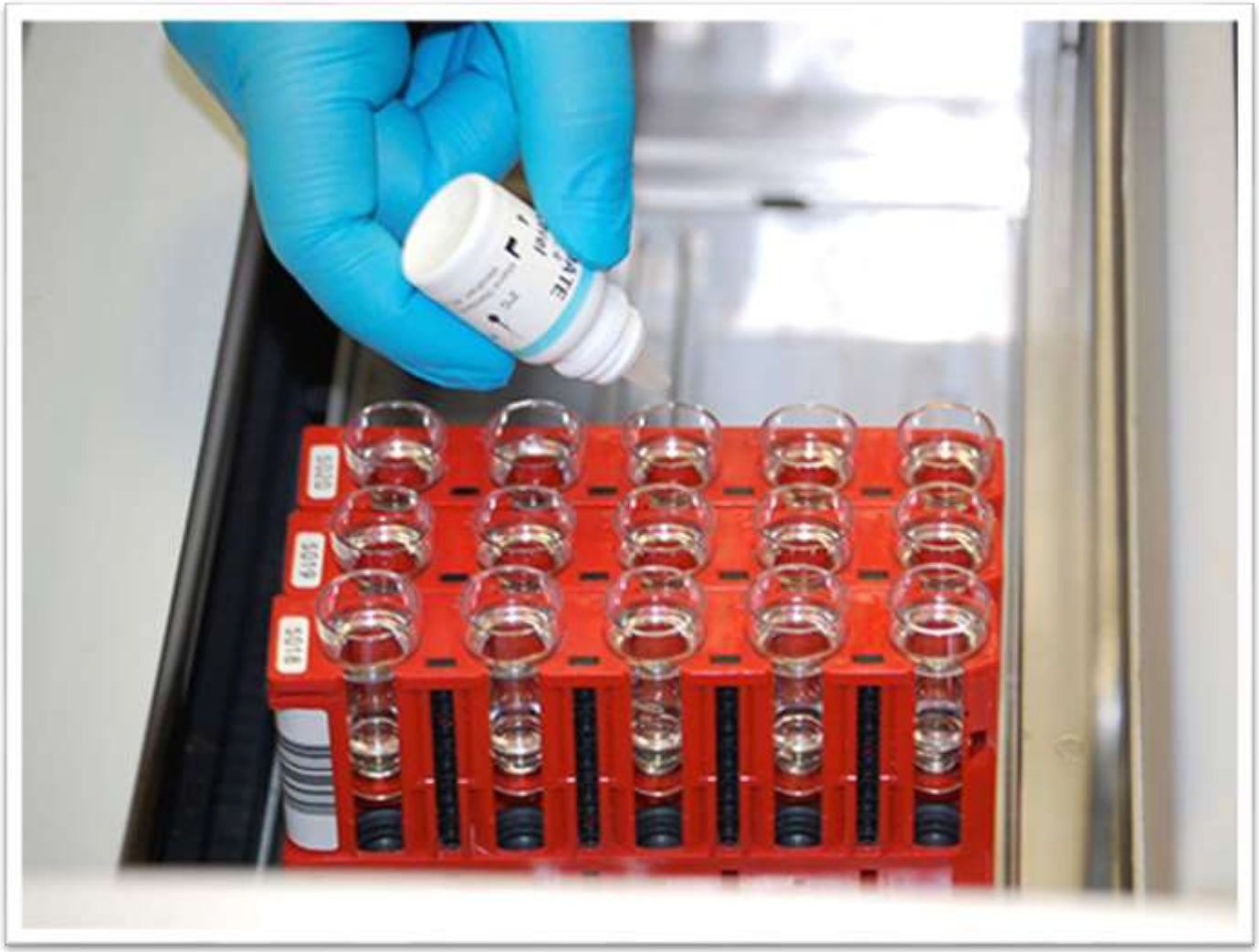
In order for CAP accredited laboratories to meet CLIA calibration verification / linearity requirements, they must perform **calibration verification** and **analytical measurement range (AMR) validation.**[2]



1. Centers for Medicare & Medicaid Services Standards and Certification: CLIA '88 Laboratory Requirements (42 CFR 493) and detailed CLIA definitions (42 CFR 493.2)
2. CAP definitions are sourced from the 2012 CAP Chemistry and Toxicology Checklist

Calibration verification / linearity testing substantiates the continued performance of a laboratory's testing systems by:

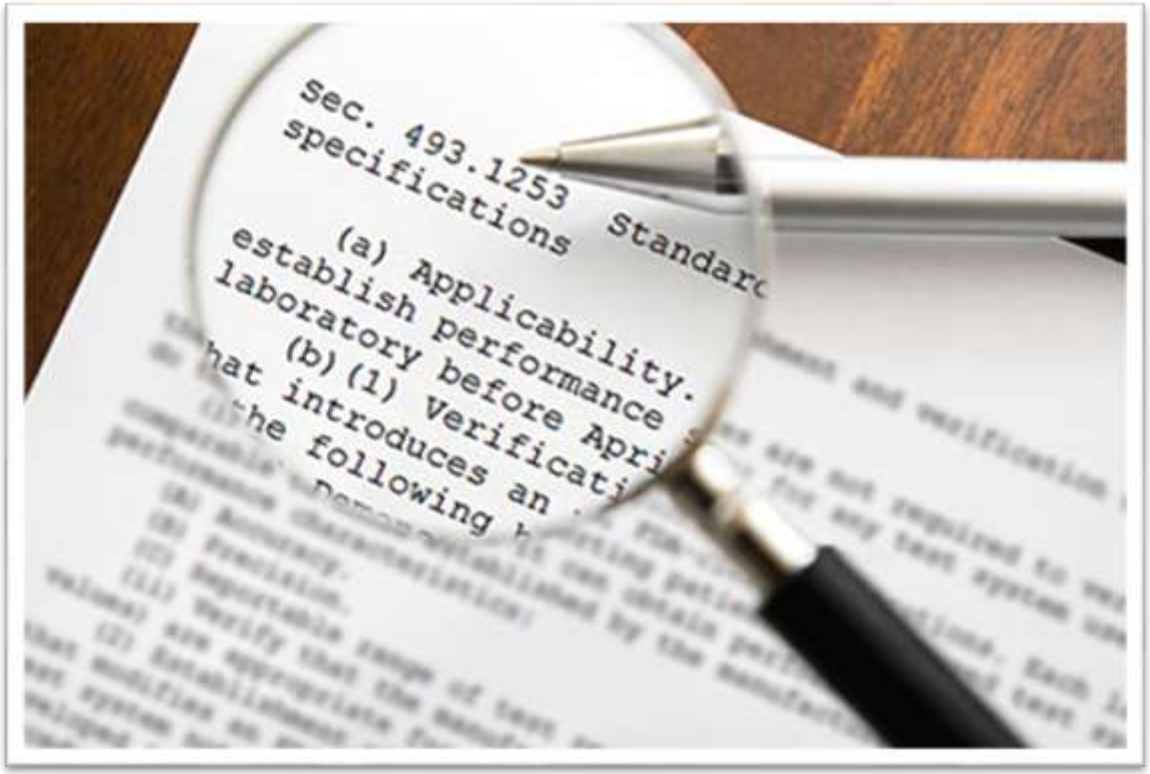
- Checking the test system's calibration to verify that it is still valid
- Challenging laboratory instruments by testing if a method is giving a linear response across the full reportable range
- Supplementing quality control (QC) and proficiency testing (PT) because it typically challenges a larger portion of the reportable range



How often is Calibration Verification required?

According to CLIA's regulation 42 CFR 493 section 493.1255, calibration verification / linearity testing must be performed and documented at least once every 6 months and/or whenever the following occur:

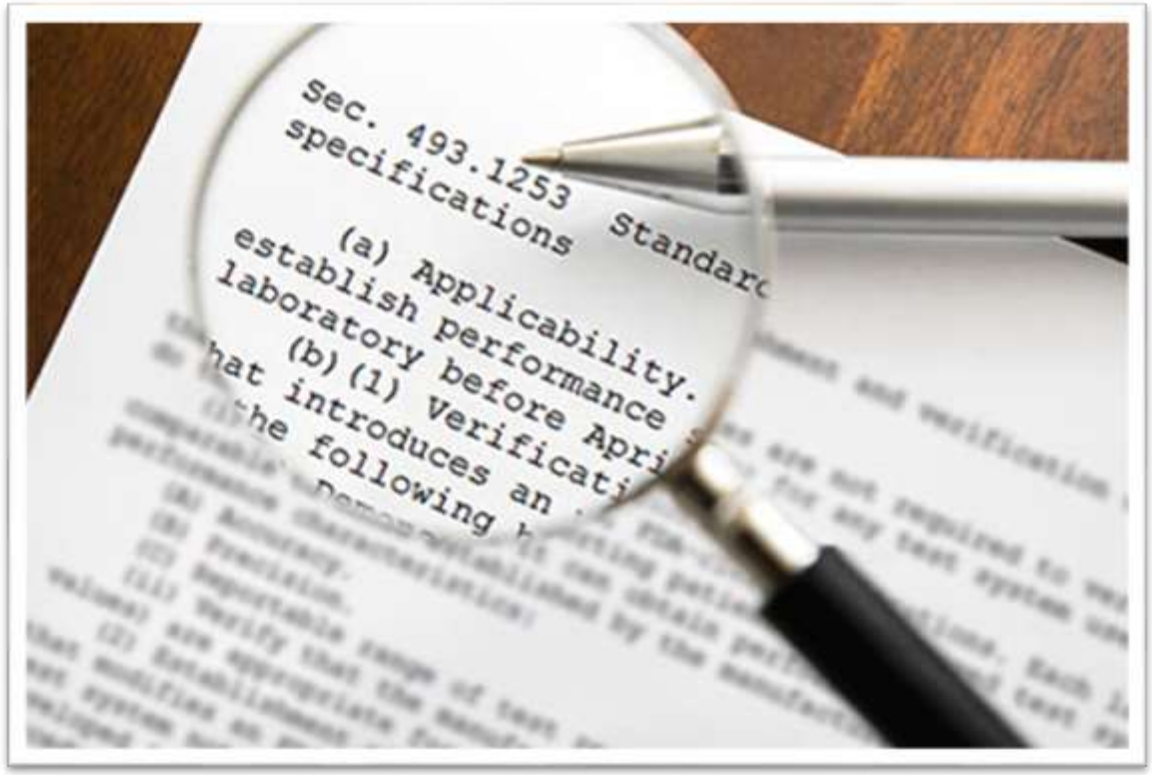
- A complete change of reagents for a procedure is introduced, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.
- There is major preventive maintenance or replacement of critical parts that may influence test performance.
- 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.
- The laboratory established procedures that requires calibration verification / linearity to be run more frequently.
- New instrument performance validation.



What is new instrument performance validation?

Per 42 CFR 493 in section 493.1253, for a new unmodified, FDA-cleared or approved test system, the standard requires that the lab demonstrate it can obtain performance specifications comparable to those established by the manufacturer for the following performance characteristics:

A. Accuracy
B. Precision
C. Reportable range of test results for the test system

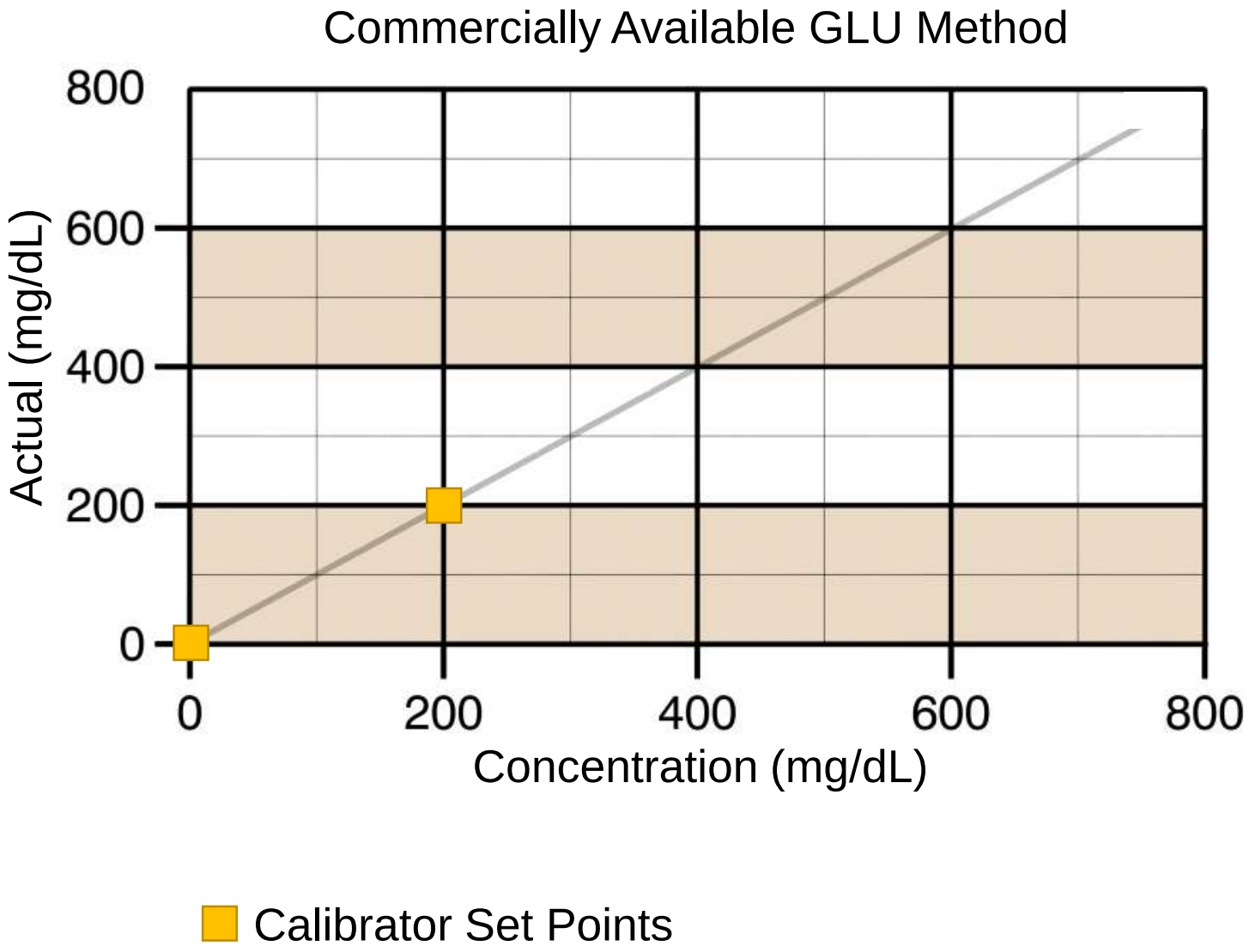


The following examples demonstrate how calibration verification / linearity testing can substantiate the continued performance of a laboratory's testing systems.

Example 1:

Commercially Available GLU Method

Sensitivity: 2 mg/dL
Reportable Range: 750 mg/dL
Calibrator Set Points: 0 and 198 mg/dL

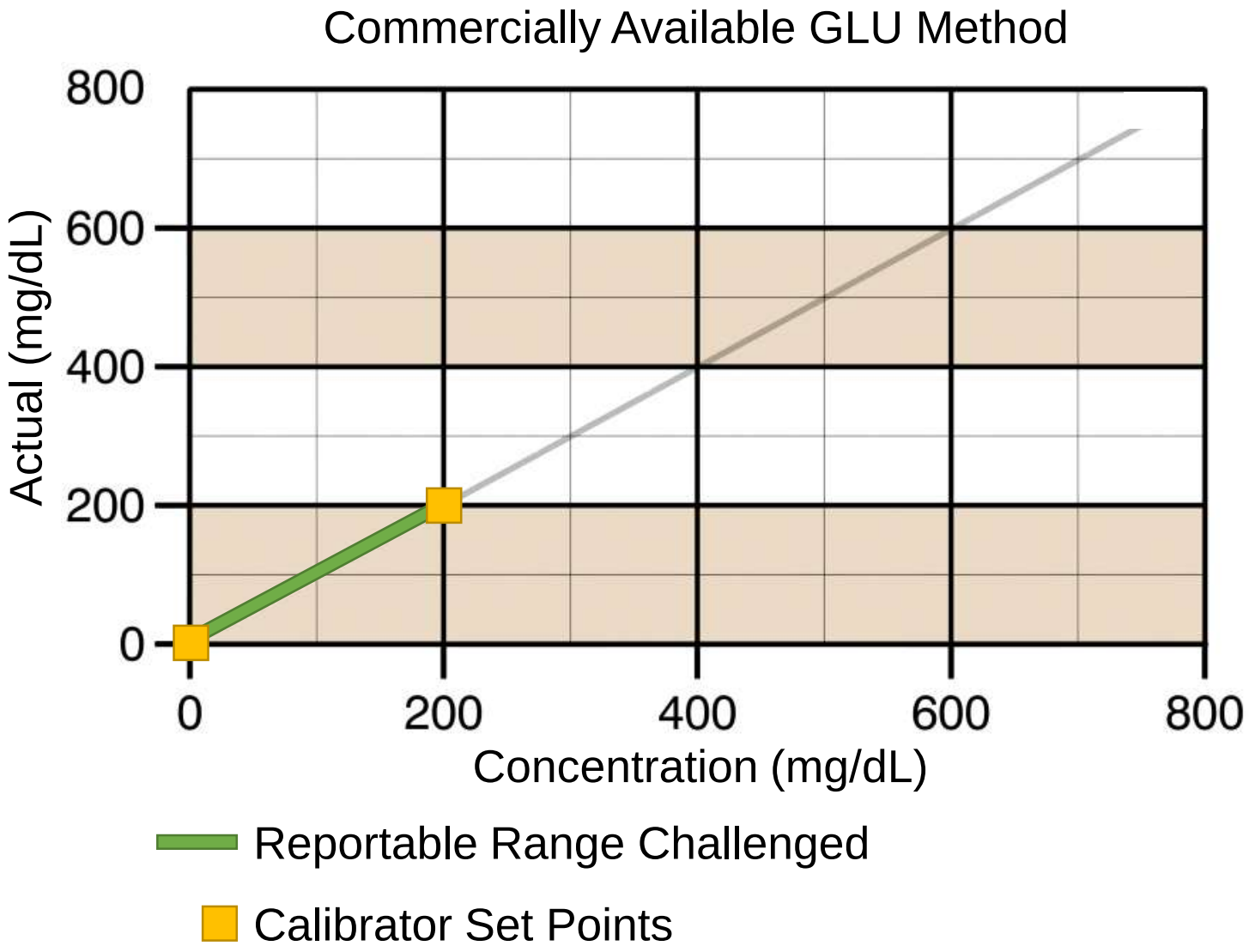


Example 1:

Commercially Available GLU Method

Sensitivity: 2 mg/dL
Reportable Range: 750 mg/dL
Calibrator Set Points: 0 and 198 mg/dL

With the act of calibration, approximately 26% of the method range has been set.

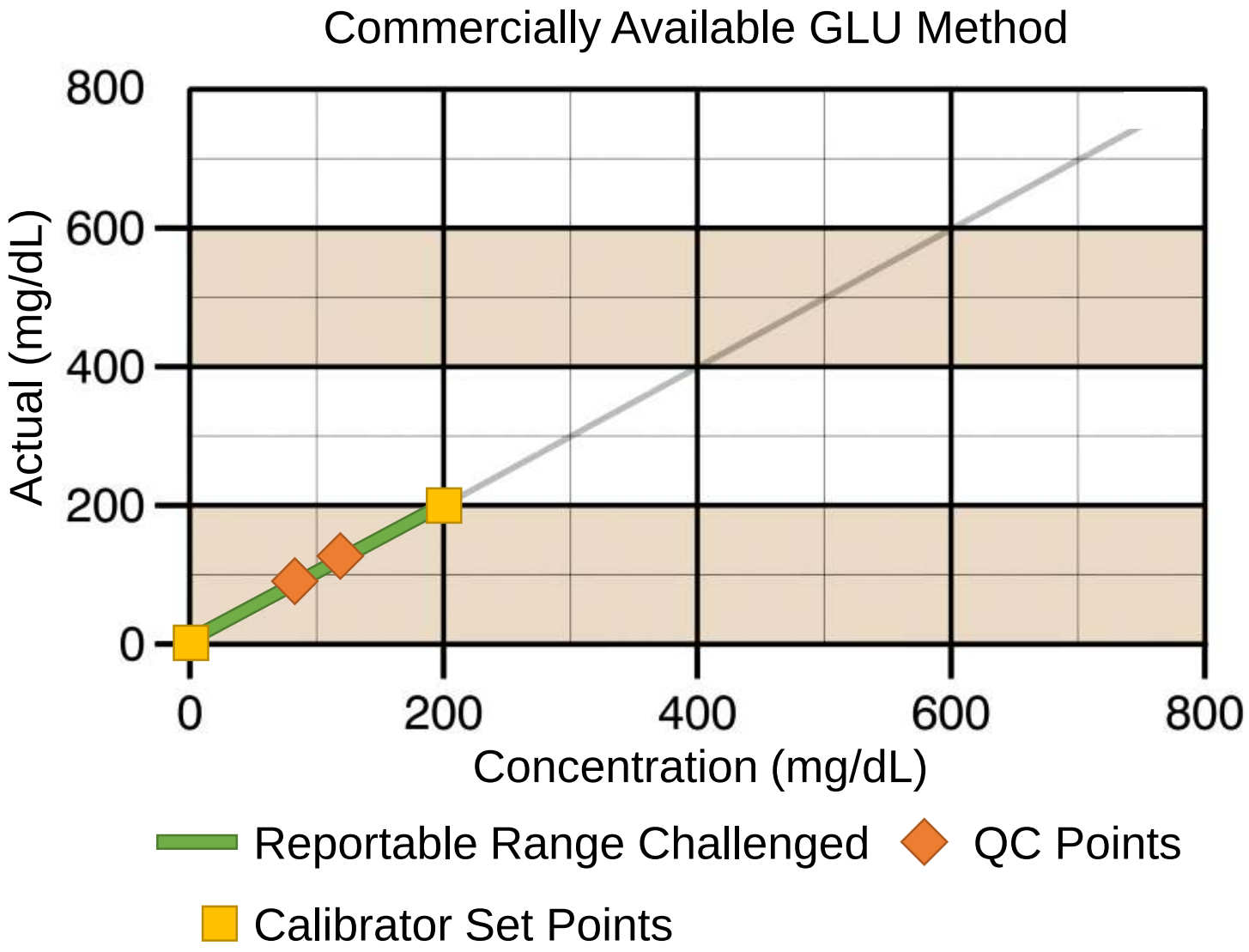


Example 1:

Commercially Available GLU Method

Sensitivity: 2 mg/dL
Reportable Range: 750 mg/dL
Calibrator Set Points: 0 and 198 mg/dL
Two Point QC Material: 86 and 116 mg/dL

Approximately 4% of the method range has been challenged by running QC material.



Example 1:

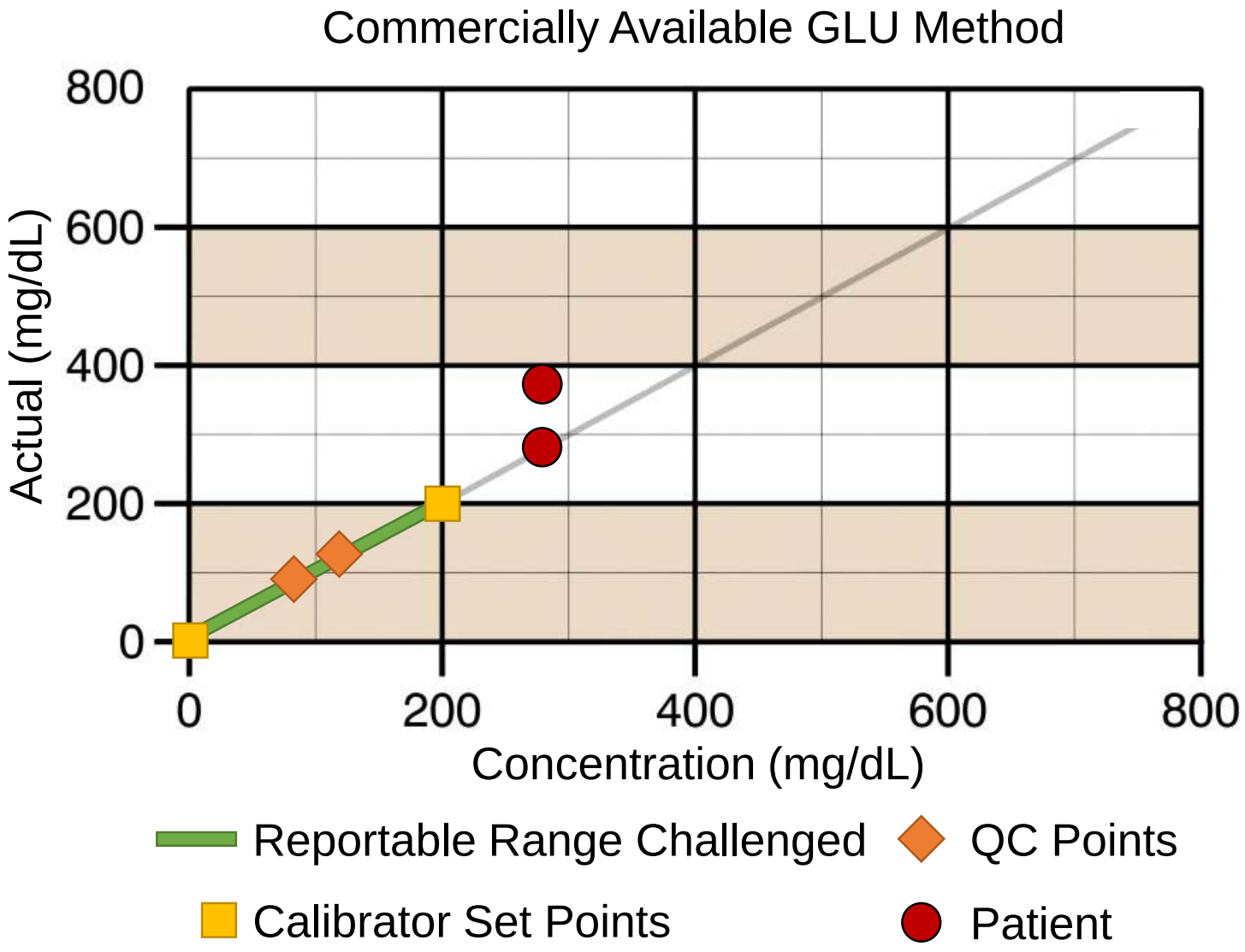
Commercially Available GLU Method

Sensitivity: 2 mg/dL
Reportable Range: 750 mg/dL
Calibrator Set Points: 0 and 198 mg/dL
Two Point QC Material: 86 and 116 mg/dL

If a laboratory reports a patient GLU sample with a concentration of 275 mg/dL as a 350 mg/dL would it impact a clinical decision?

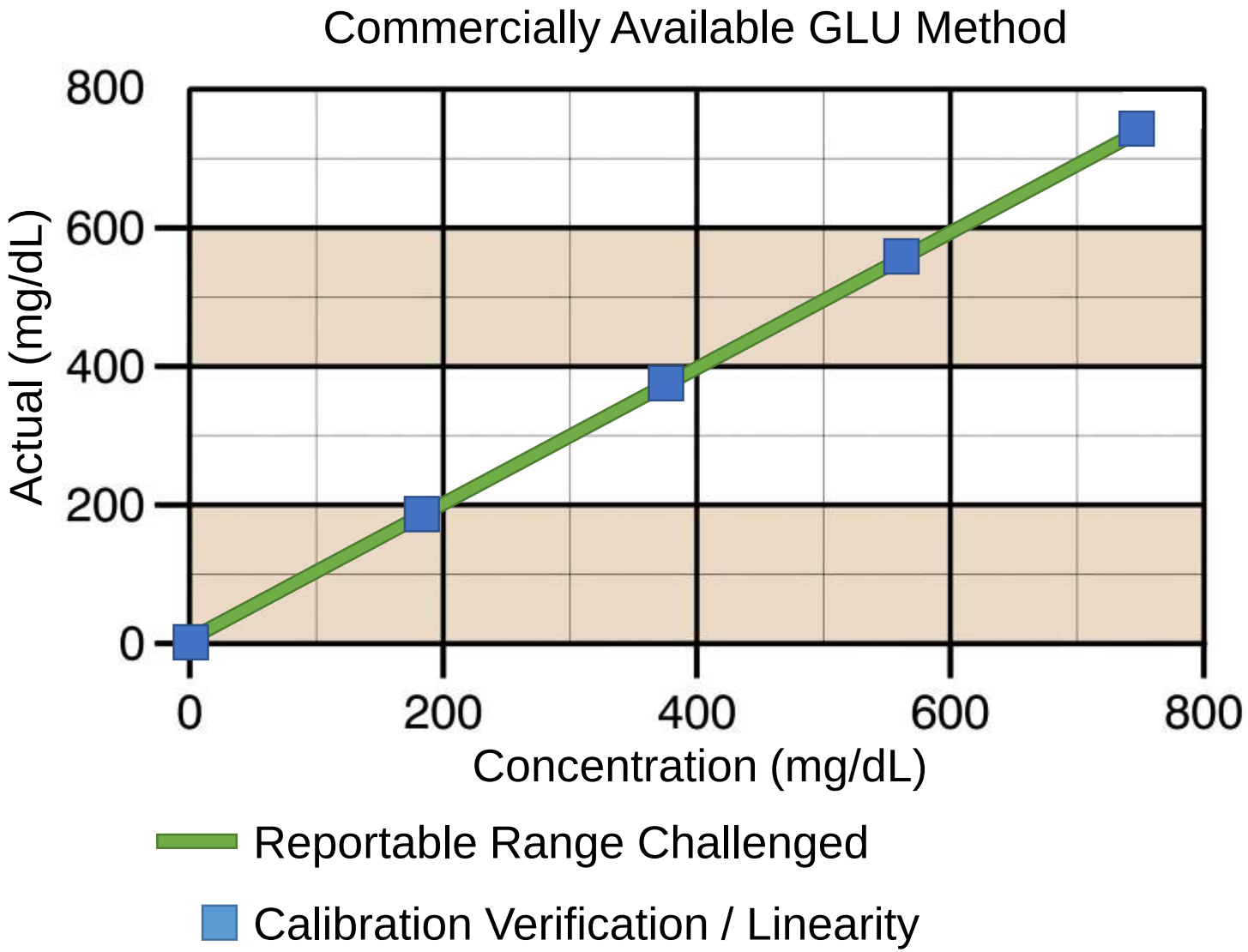
If a laboratory depends solely on Calibration and QC, in this example, they have only calibrated their instrument system to 198 mg/dL or challenged the method with QC material to 116 mg/dL.

If the laboratory runs a patient GLU sample with a concentration of 275 mg/dL, how does the lab know they will get 275 mg/dL?



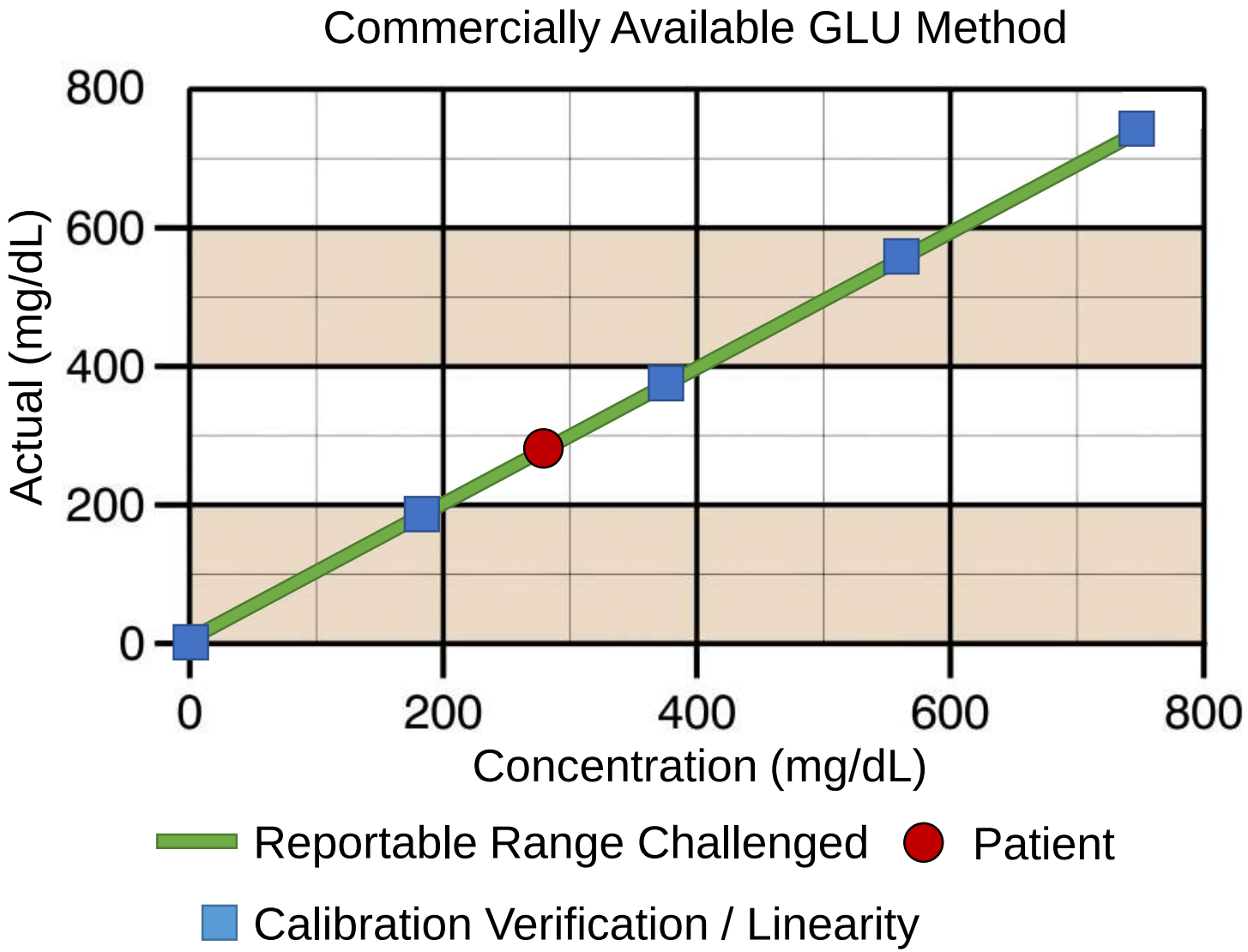
Example 1:

The **ONLY** way a laboratory can know if they are getting consistent results across the full reportable range is to **perform the calibration verification / linearity experiment.**



Example 1:

After running the calibration verification / linearity experiment, if the instrument gives a linear response, a laboratory knows that a patient sample with a concentration of 275 mg/dL will give a response of 275 mg/dL.

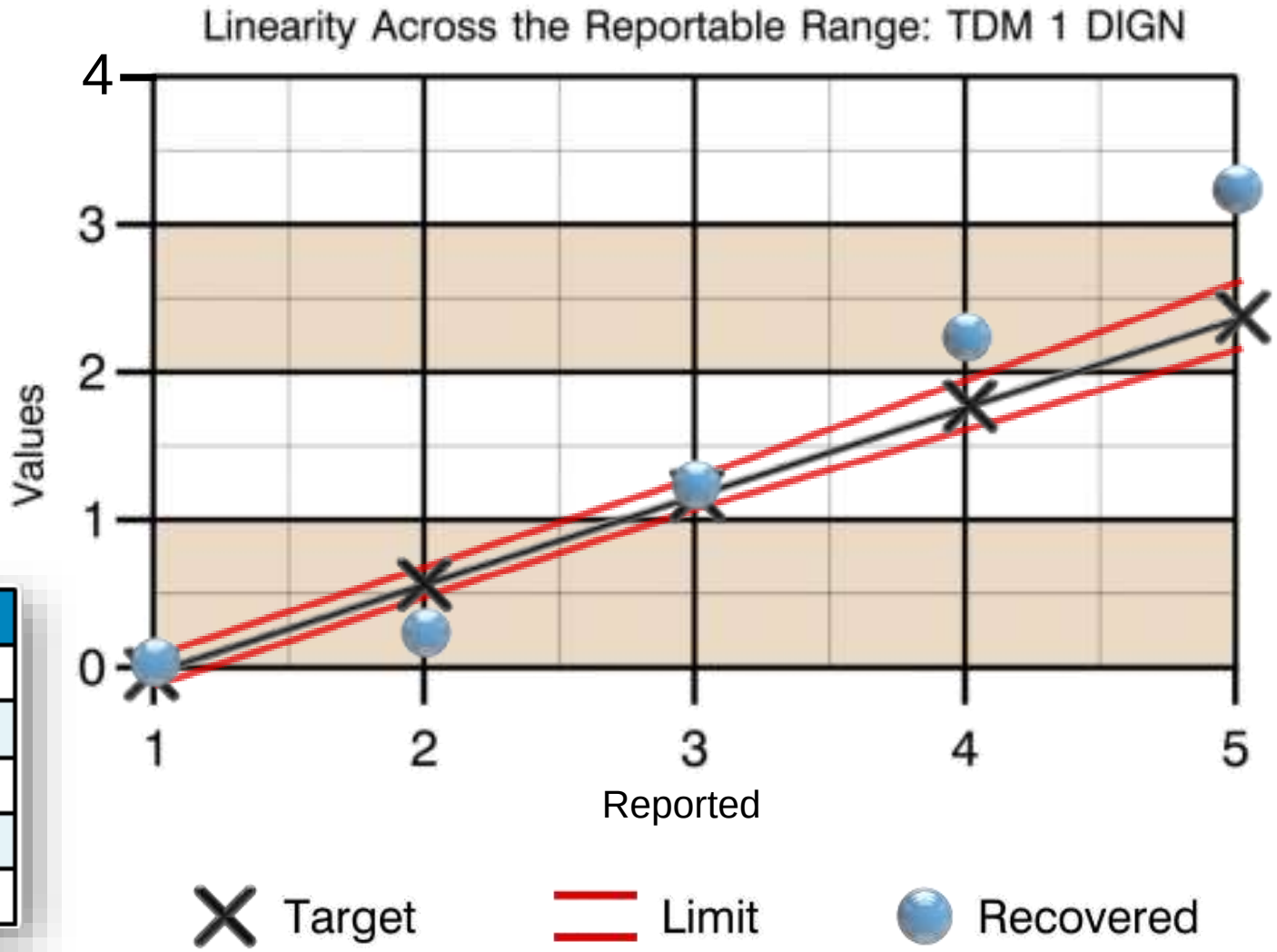


Example 2:

Even if the full reportable range is covered by calibrators, there is value in running calibration verification / linearity testing. In this example, after calibration and re-calibration, an instrument continues to deliver non-linear results. Calibration verification / linearity testing shows all levels outside total allowable error limits.

Initial Results: A laboratory performed routine calibration verification / linearity testing using VALIDATE® TDM1. The following was the linearity report for Digoxin (DIGN) generated using a free Data Reduction software:

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	0.133	0.000	**0.133	100%	0.100	N/A
2.0	0.467	0.200	**0.267	57.2%	0.100	N/A
3.0	1.067	1.200	0.133	** 12.5%	0.107	10%
4.0	1.667	2.200	0.533	** 32.0%	0.167	10%
5.0	2.267	3.200	0.933	** 41.2%	0.227	10%

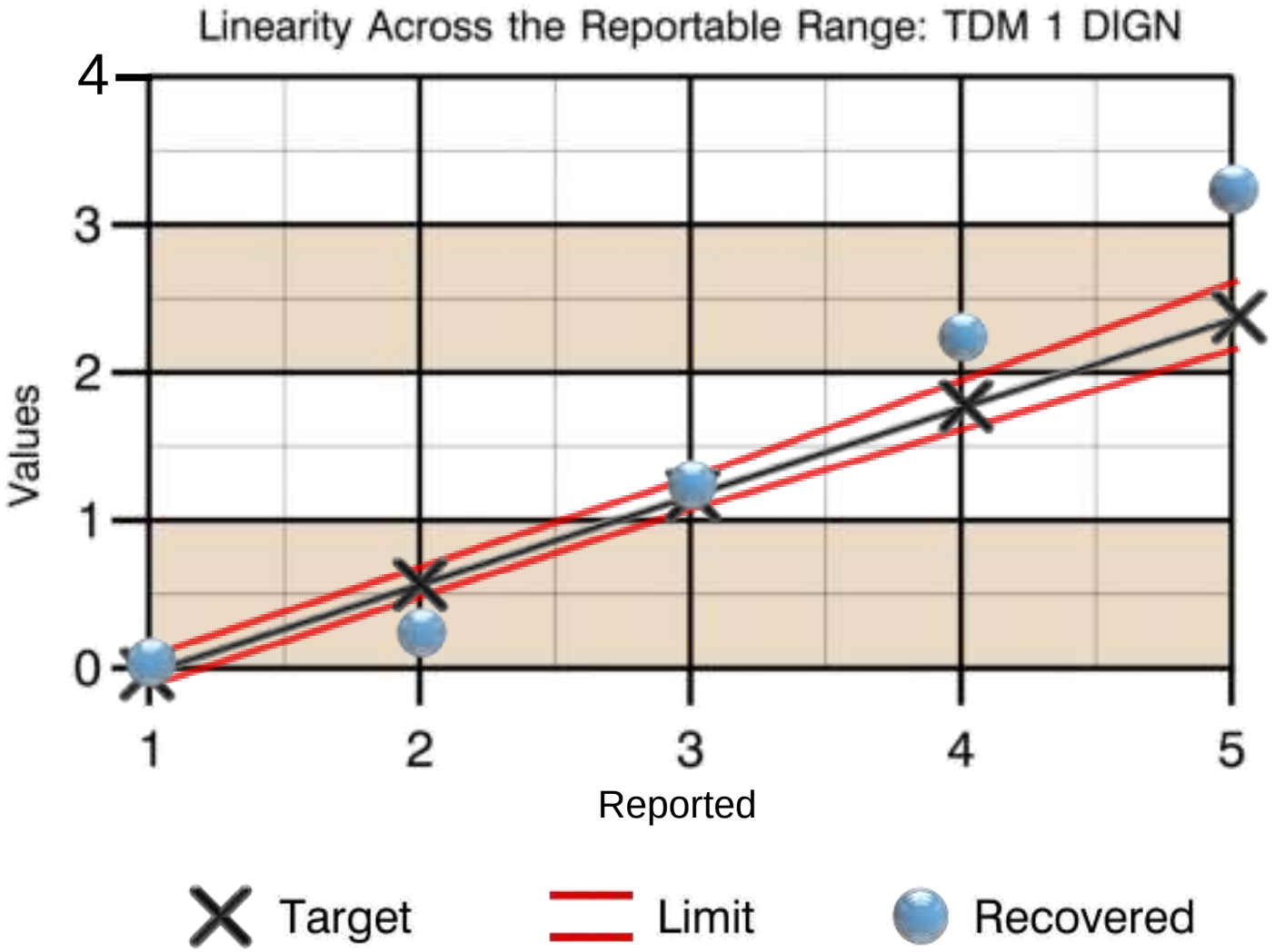


Example 2:

Troubleshooting: The results were not consistent with Peers or with typical product performance. The laboratory took the troubleshooting step of recalibrating their DIGN assay. Calibration set points covered the method range of 0.3 – 5.0 ng/mL (calibration set points were 0, 0.5, 1, 2, 3 an 5 ng/mL).

After re-calibration, calibration verification / linearity testing was performed and results continued to be nonlinear. The laboratory requested service from the instrument manufacturer.

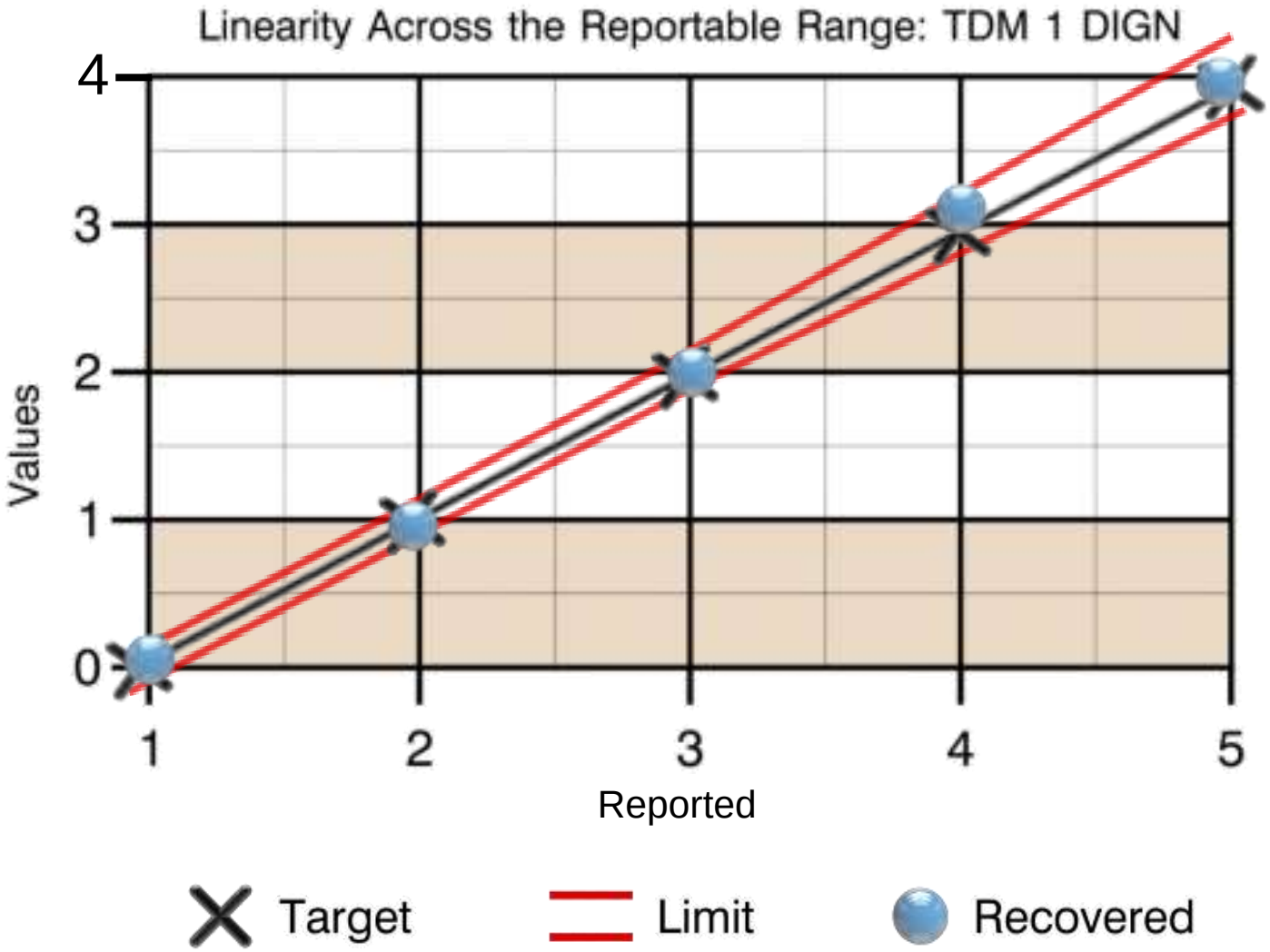
During the service call, **a probe alignment issue was discovered and corrected.**



Example 2:

To confirm that correcting the probe alignment issue also corrected the nonlinear response, the laboratory re-ran the calibration verification / linearity testing. The updated graph shows that all Levels are within the statistical limits.

Summary: As calibration is not intended to identify instrument issues, in this case, if the laboratory depended on calibration alone, the laboratory could have reported inaccurate patient results. Calibration verification / linearity testing is the only way to test if a method is giving a nonlinear response.



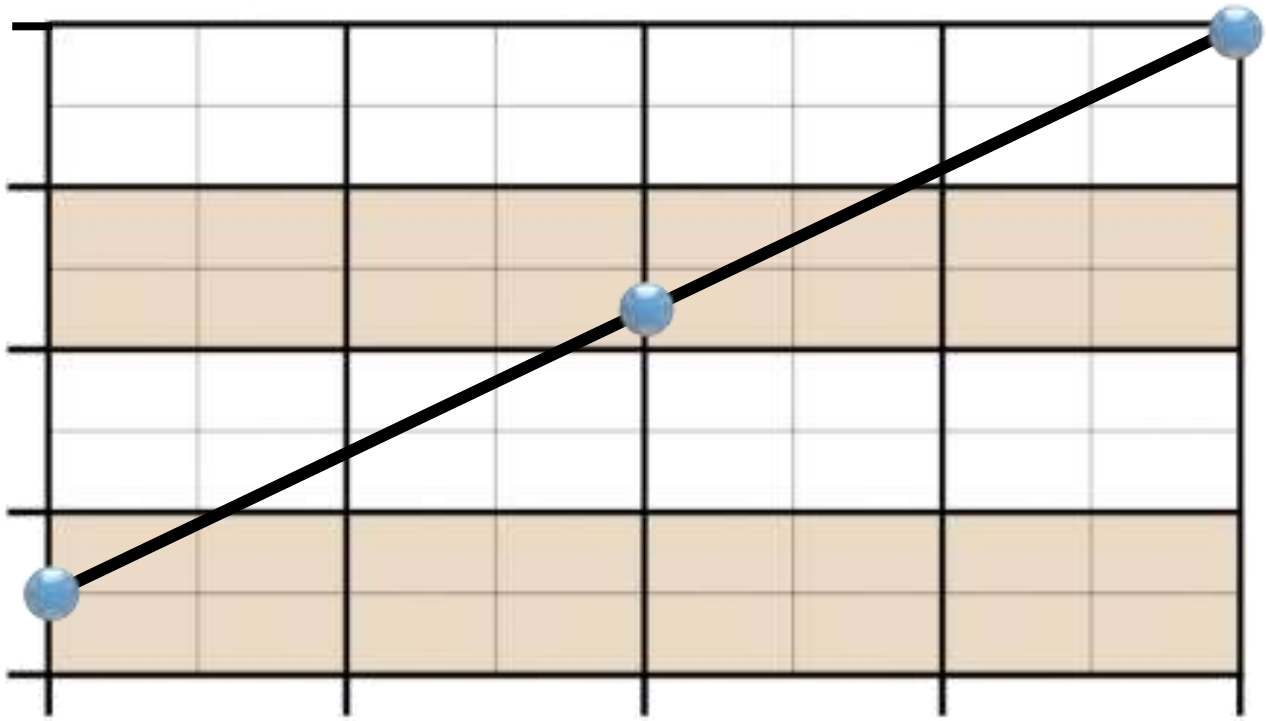
This section discusses how to perform calibration verification / linearity testing. Steps involved in performing the experiment are as follows:

1. **Choose materials for the experiment**
2. **Establish acceptance limits**
3. **Run the samples just like a patient or control sample**
4. **Interpret Results**
5. **Review, Approve and File documentation for future reference**

Step 1. Choose materials for the experiment

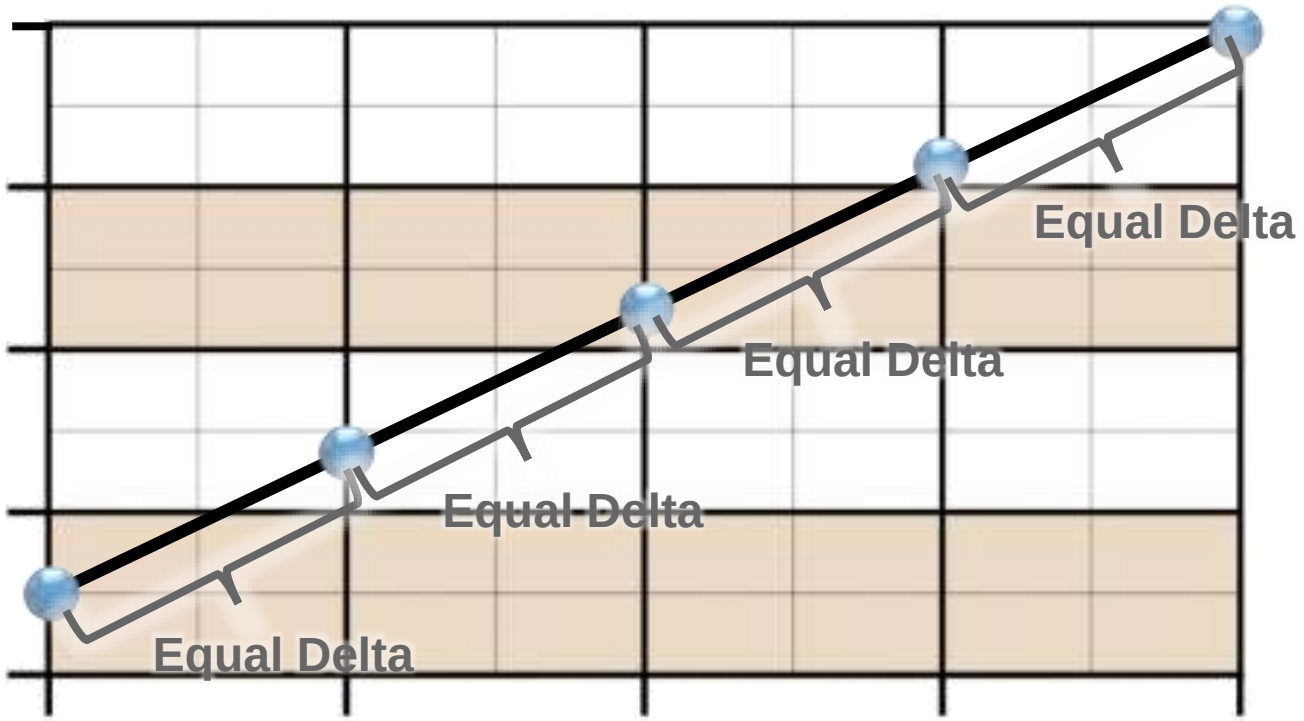
Since the purpose of calibration verification / linearity testing is to substantiate the instrument or test system's calibration **throughout the entire reportable range**
for patient test results, CLIA requires a minimum of three levels be tested:

- One at the low end of the reportable range
- One near the midpoint of the reportable range
- One at the high end of the reportable range



Step 1. Choose materials for the experiment

White CLIA only requires three levels be tested, **CLSI EP6-A** and other industry experts such as James Westgard[3], recommend at least 5 levels with equal deltas between them be used when performing calibration verification / linearity testing. The more levels with equal deltas tested the better your understanding will be of how the method is performing.



3. Westgard, James O., *Basic Method Validation,* 3rd ed, Madison, WI: Westgard QC Inc., 2008.

Step 1. Choose materials for the experiment

To create 5 levels with equal deltas, CLSI recommends to first take a **Low** sample and a **High** samples. The **Low** sample will be considered Level 1 and the High sample will be Level 5. Using these 2 samples, make 3 additional levels as follows:

- Level 2 = 3 parts of the low sample mixed with 1 part of the high sample
- Level 3 = 1 part of the low sample mixed with 1 part of the high sample
- Level 4 = 1 part of the low sample mixed with 3 parts of the high sample

Step 1. Choose materials for the experiment

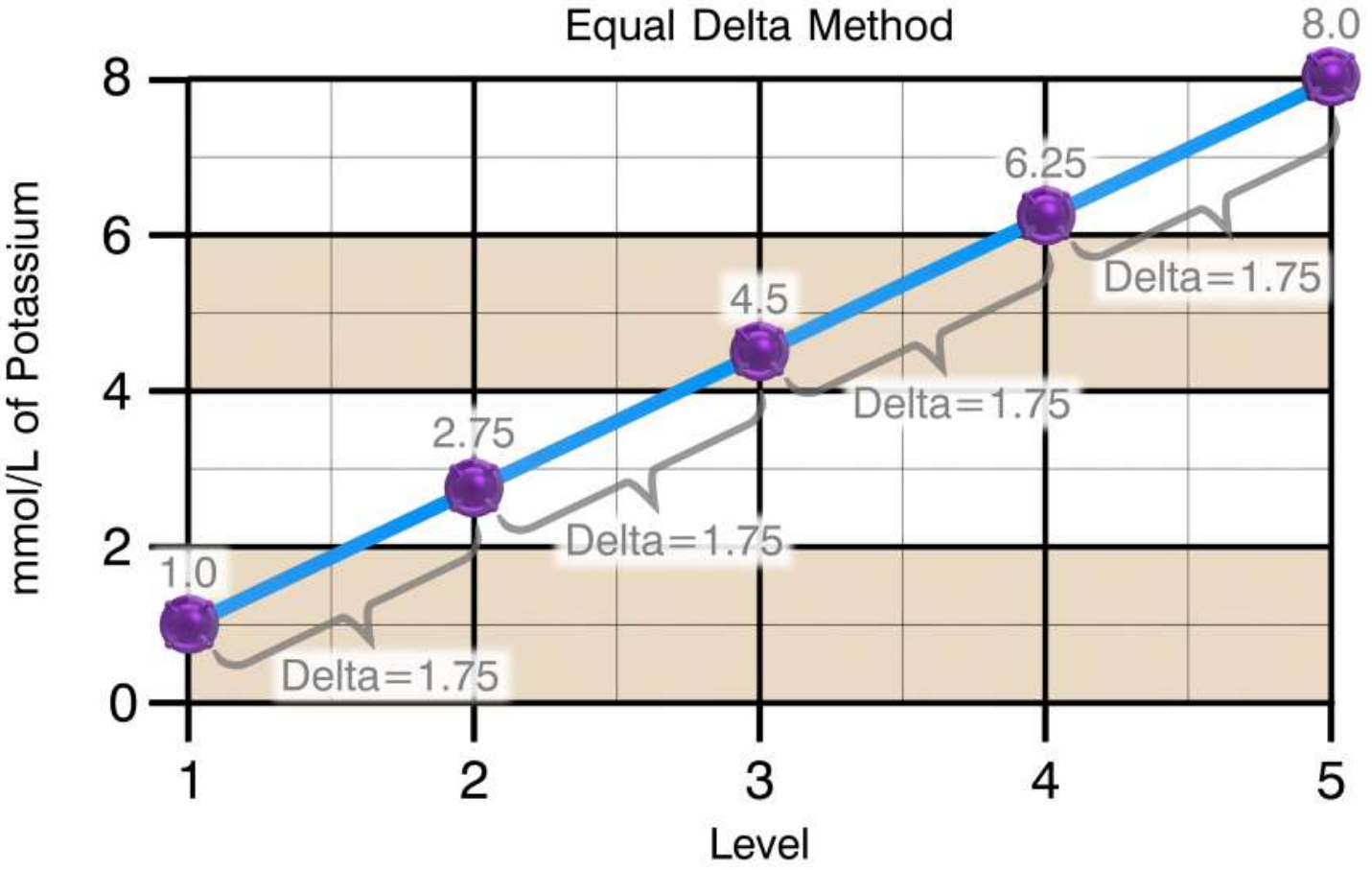
When calibration verification / linearity material is prepared with equal deltas, it allows you to calculate Theoretical Values using the following formulas:

- Level 1 = low sample recovery
- Level 2 Theoretical = (0.75 * Level 1) + (0.25 * Level 5)
- Level 3 Theoretical = (0.50 * Level 1) + (0.50 * Level 5)
- Level 4 Theoretical = (0.25 * Level 1) + (0.75 * Level 5)
- Level 5 = high sample recovery

Step 1. Choose materials for the experiment

For example: If a **Low** sample has an assayed value of 1.0 mmol/L of Potassium, a **High** sample assayed value of 8.0 mmol/L, and a five level set was created using CLSI EP6-A equal delta method, the following theoretical values would apply:

- Level 1 = low sample recovery of 1.0 mmol/L
- Level 2 Theoretical = (0.75 * 1) + (0.25 * 8) = 2.75
- Level 3 Theoretical = (0.50 * 1) + (0.50 * 8) = 4.50
- Level 4 Theoretical = (0.25 * 1) + (0.75 * 8) = 6.25
- Level 5 = high sample recovery of 8.0 mmol/L



Step 1. Choose materials for the experiment

A variety of materials may be used to perform calibration verification / linearity experiments, for example:

- Commercially available calibration verification / linearity materials
- Proficiency testing samples
- Patient specimens
- Control materials, if they span the full reportable range
- Calibrators used to calibrate the analytic measurement system that are from a different lot than the one used for calibration. If using the current lot of calibrators, you are only performing a precision check

Step 1. Choose materials for the experiment

Note: When evaluating calibration verification / linearity materials, you want to make sure the material you select meets all of your labs needs. To help ensure a positive end result, ask yourself these questions:

- Is the entire reportable range covered from Low to High? Is the material manufactured for your system's specific ranges?
- Are there 5 levels, including a low, mid and high point?
- Is there enough volume available to run duplicates or triplicates? Troubleshooting?
- Is prep time quick and easy before assaying?
- Are samples liquid ready-to-use, potentially eliminating reconstitution or admixture mistakes?
- Will samples remain stable if the experiment needs repeating?
- Are the dilutions pre-made according to EP6-A guidelines and have known values and/or equally-spaced deltas?
- If using calibrators, is a different lot number being used?
- Is data reduction and graphing available?
- How quickly can the data be processed? Instantly?
- Is peer analysis available for comparison to other users?
- Is there live Technical Support available for questions?

Step 2. Establish acceptance limits

For each test performed, the laboratory is responsible for establishing performance specifications for calibration verification / linearity and for reportable range verification for each analyte tested. Ideally, the amount of error allowed due to non-linearity should take into account clinical significance and be independent of error that results from bias and other analytical error (imprecision).

Bias error is typically error that is the result of calibration and the element of accuracy conveyed by calibration set points.

Imprecision error can be influenced by a number of variables such as system maintenance and sample integrity.

Total allowable error is an analytical quality requirement that sets a limit for both the imprecision (random error) and bias (systematic error) that are tolerable in a single measurement or single test result.[4]

4. Westgard, James O., *The Meaning and Application of Total Error,* http://www.westgard.com/essay111.html, retrieved August 1, 2012

Step 2. Establish acceptance limits

For many analytes, CLIA has published total error limits, which includes all sources of error. Each lab should establish what portion of the total error budget is allowed for non-linearity. Commercial calibration verification / linearity data reduction formats typically allow for 25 – 50% of the Acceptable Performance (Total Allowable Error) limits for non-linearity.

This table shows CLIA's Acceptable Performance for several analytes and the result of using 50% of CLIA's Acceptable Performance to create limits for acceptance of calibration verification / linearity experiments.

For more detail, the CLIA '88 criteria for acceptable performance can be found on the internet in two locations: Routine Chemistry or Toxicology.

Test or Analyte	Acceptable Performance (Total Allowable Error)	Set at 50% of CLIA Total Error
Alanine aminotransferase (ALT)	Target value ± 20%	± 10%
Albumin	Target value ± 10%	± 5%
Alkaline phosphatase	Target value ± 30%	± 15%
Amylase	Target value ± 30%	± 15%
Aspartate aminotransferase (AST)	Target value ± 20%	± 10%
BIlirubin, total	Target value ± 0.4 mg/dL or ± 20% (greater)	± 0.2 mg/dL or ± 10%
Calcium, total	Target value ± 1.0 mg/dL	± 0.5 mg/dL
Glucose	Target value ± 6 mg/dL or ± 10% (greater)	± 3 mg/dL or ± 5%

Step 3. Run the samples just like a patient or control sample

Laboratories should test a minimum of 2 replicates at each level; 3 replicates at each level is considered best practice.

Why? Triplicates will aid to differentiate outliers. For example, if a lab runs duplicates and receives outputs of 1 and 10, which one is the outlier? On the other hand, if run in triplicate, then 1, 10 and 10 shows that the result of 1 may be the outlier.

Step 4. Plot the data and perform data reduction or statistical analysis

- Simple graph paper of Microsoft Excel® regression data analysis can be used
- Calculate the theoretical values
- Plot theoretical values on the x-axis
- Plot theoretical values on the y-axis
- Compare theoretical values to recovered values based on previously established acceptance limits

Note: Many commercial data reduction services are available. Peer group data, which compares your data results to others with the same instrument and reagent systems – giving you a better understanding of methods and troubleshooting needs, may also be available from providers of commercial calibration verification / linearity material.

Step 5. Review, Approve and File documentation for future reference

- Be sure to have an authorized person review and accept / approve results.
- For results that exceed acceptance limits, look at the absolute difference between the target value and the mean value. So degree of nonlinearity may be acceptable.
- If the degree of nonlinearity is deemed acceptable by your laboratory, accept the results and document your reasoning.
- If the degree of nonlinearity is deemed un-acceptable:
  - Repeat calibration procedure, if the test system is factory calibrated, consult with the manufacturer of the test system.
  - Re-run calibration verification / linearity experiment to see if the re-calibration corrected the problem.
  - If re-calibration does not correct the non-linearity, refer to peer data or contact the instrument manufacturer for further troubleshooting.

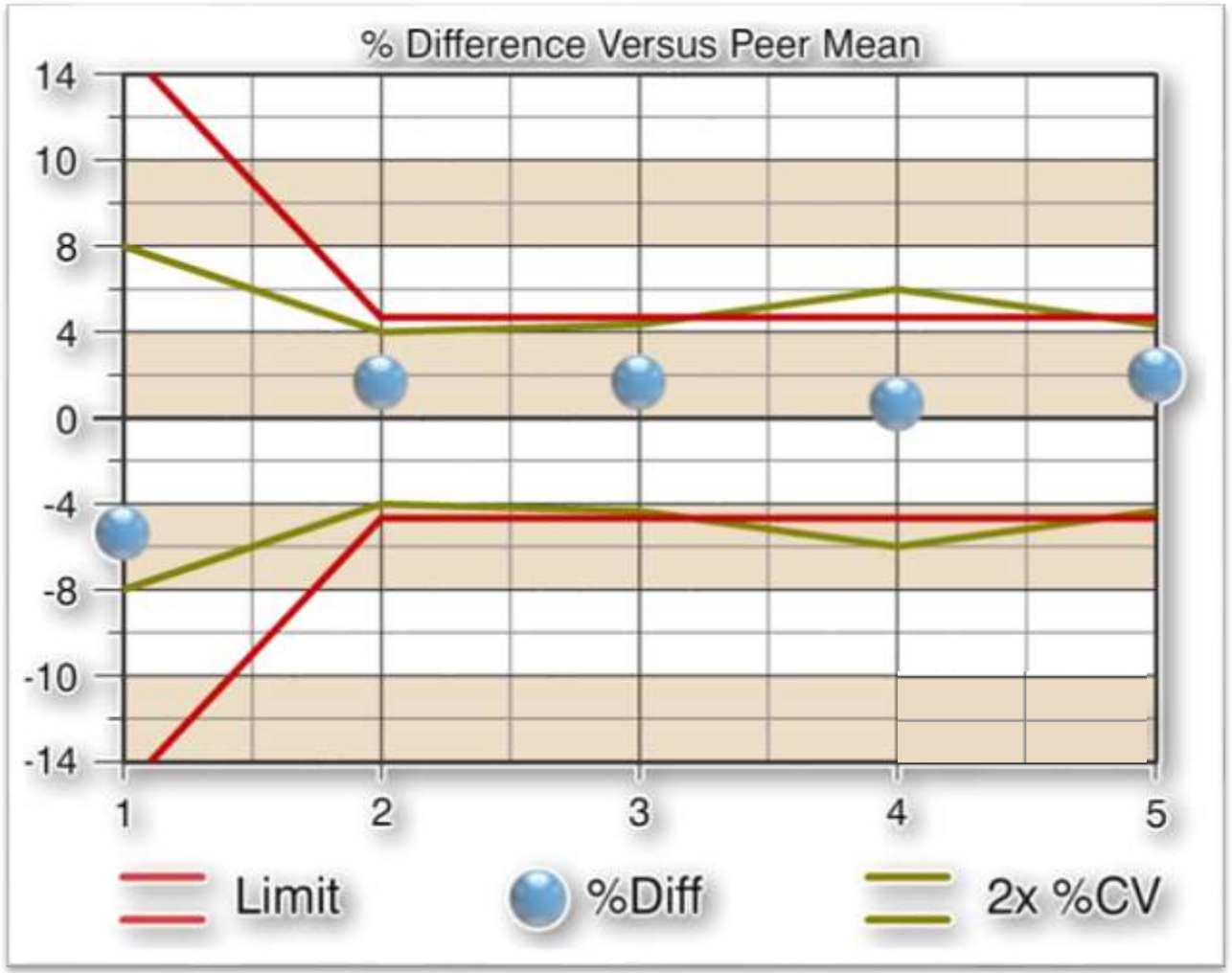
Step 5. Review, Approve and File documentation for future reference

What should I do if I get results that fall outside of the applied limits, but I am consistent with my Peer Group?

- If your results are nonlinear but consistent with your peers, this indicates that the nonlinearity is not limited to your specific analyzer and that the method itself may truly be nonlinear. Any result that falls outside the allowable error limits should be evaluated by the laboratory for clinical significance.

- If nonlinearity seen is deemed acceptable, or not clinically significant, you could accept the result and document your reasoning.

- If the nonlinearity seen is determined to be unacceptable or clinically significant, you could choose to limit the upper end of your range to the mean of the highest level tested that was within the statistical limits or contact the instrument provider to discuss troubleshooting.

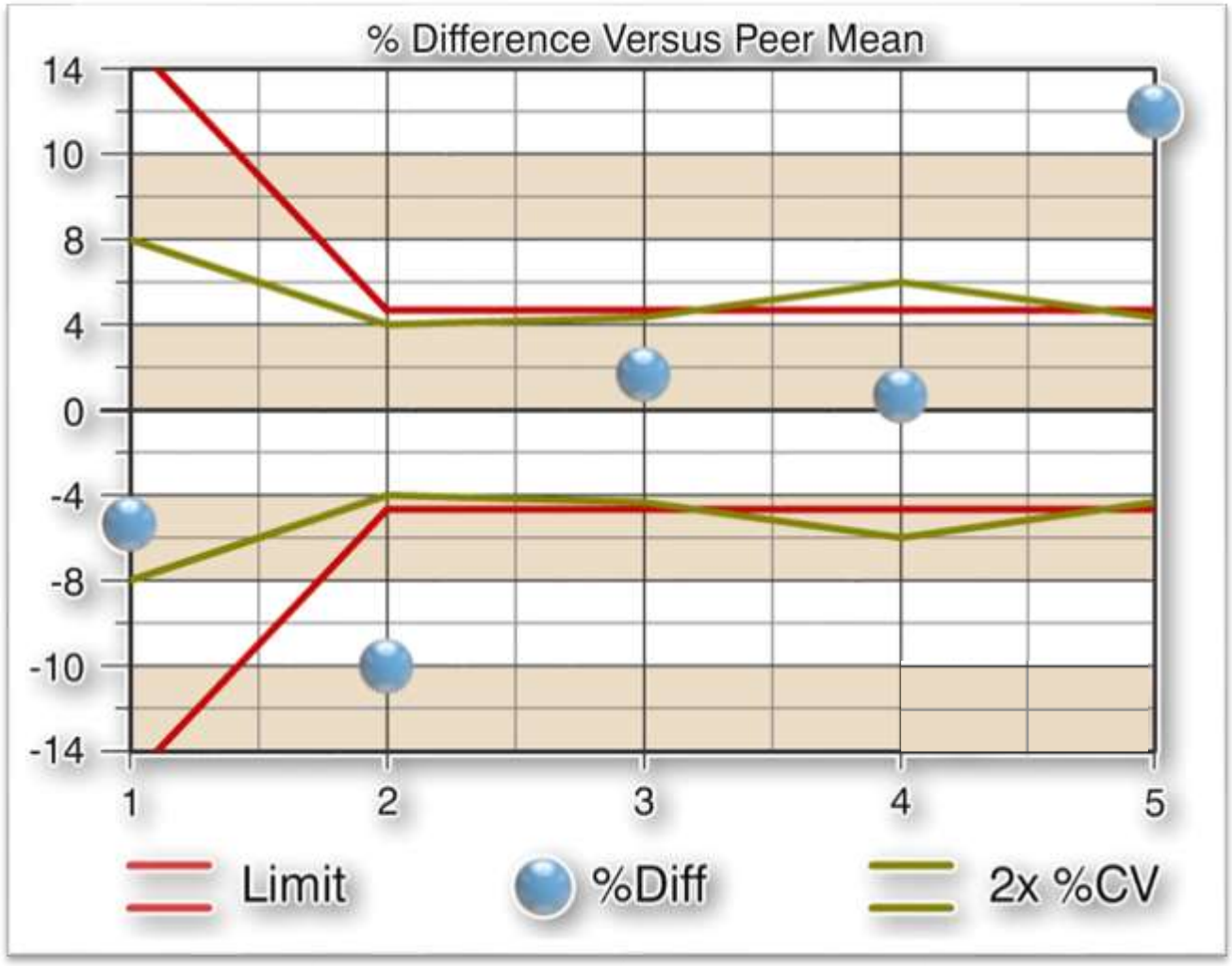


Step 5. Review, Approve and File documentation for future reference

What should I do if I get results that fall outside of the applied limits, but I am different from my Peer Group?

- Any result that falls outside the allowable error limits should be evaluated by the laboratory for clinical significance. If the nonlinearity seen is deemed acceptable, or not clinically significant, you could accept the result and document your reasoning.

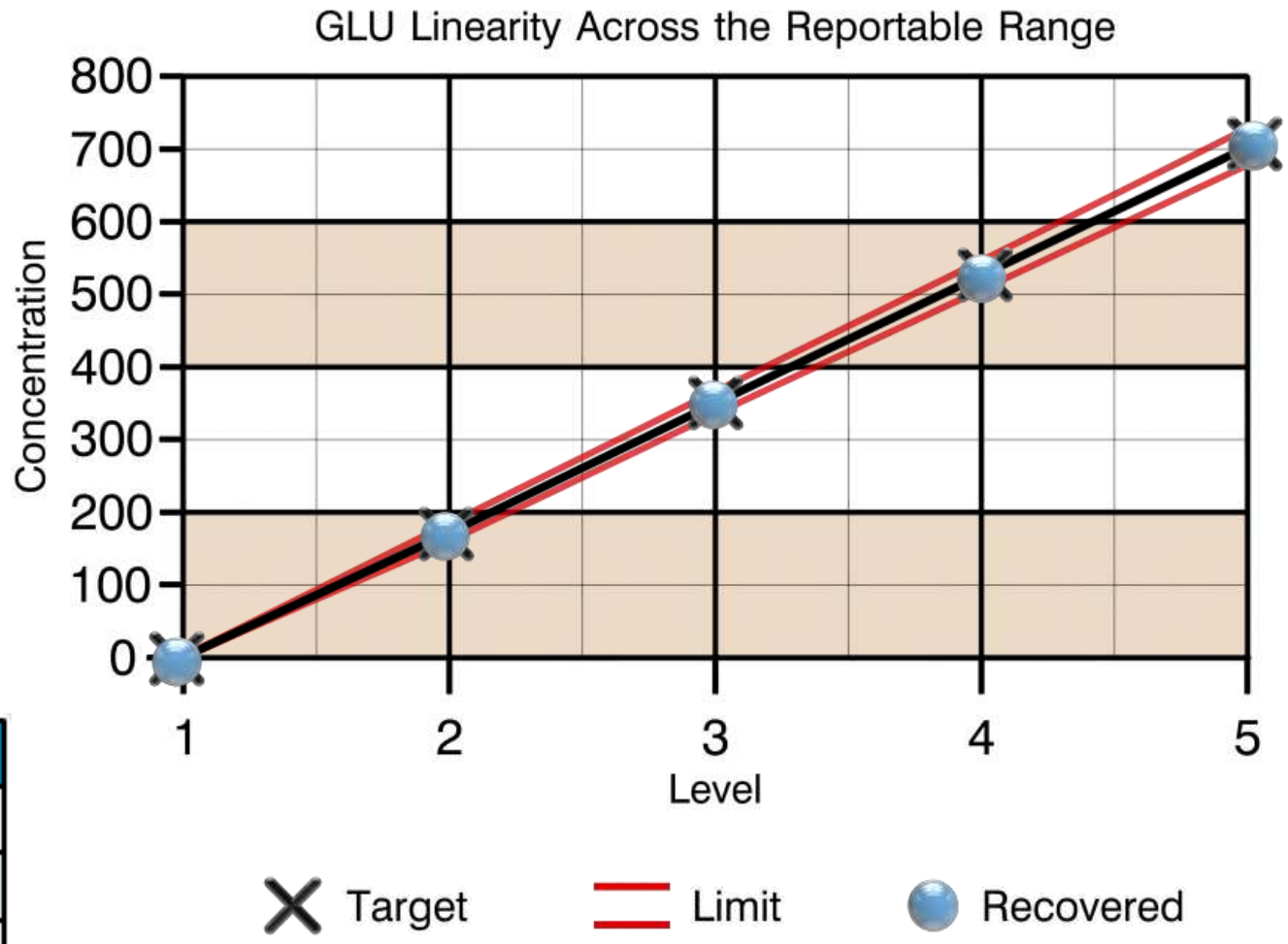
- If the nonlinearity seen is determined to be unacceptable, or clinically significant, you could choose to limit the upper end of your range to the mean of the highest level tested that was within the statistical limits of proceed with troubleshooting.



Sample Experiment 1 is a Five level calibration verification / linearity material for glucose. Levels were created using the CLSI EP6-A equal delta protocol for preparing linearity sets. The lab chose to use 50% of CLIA's suggested total allowable error as their acceptance limits. Results were run in triplicate as recommended to identify outliers in the data set.

Since the set has levels with equal-deltas, the target values can be generated based on the results. In this case all levels met the lab's acceptance criteria of +/- 3 mg/dL or 5%, whichever is greater.

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	2.89	0.000	2.89	100%	3.00	N/A
2.0	180.39	180.33	0.06	0.0%	9.02	5%
3.0	357.89	358.00	0.11	0.0%	17.89	5%
4.0	535.39	535.33	0.06	0.0%	26.77	5%
5.0	712.89	708.33	4.56	0.6%	35.64	5%

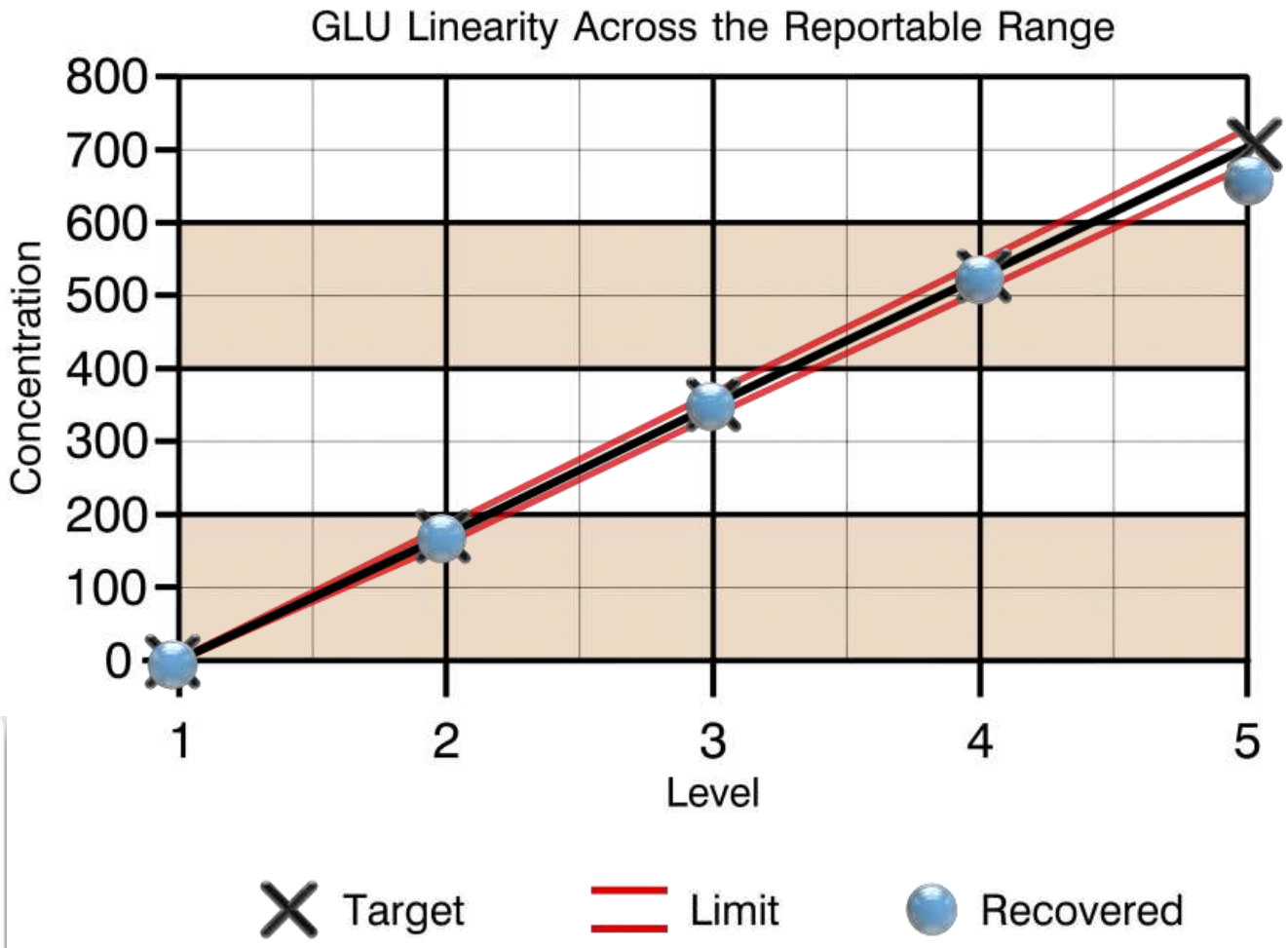


Sample Experiment 2 is a Five level calibration verification / linearity material for glucose. Levels were created using the CLSI EP6-A equal delta protocol for preparing linearity sets. The lab chose to use 50% of CLIA's suggested total allowable error as their acceptance limits. Results were run in triplicate as recommended to identify outliers in the data set.

Since the set has levels with equal-deltas, the target values can be generated based on the results. In this case, the statistical analysis of Level 5 was outside the lab's acceptable criteria of +/- 3 mg/dL or 5%, whichever is greater.

The lab must determine the clinical significance of the difference between target value and recovered value.

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	0.89	0.67	0.22	24.7%	3.00	N/A
2.0	176.89	177.33	0.44	0.2%	8.84	5%
3.0	352.89	352.67	0.22	0.1%	17.64	5%
4.0	528.89	527.33	1.56	0.3%	26.44	5%
5.0	704.89	659.67	45.22	**6.4%	35.24	5%



Where can I find additional information about the CLIA requirements pertaining to calibration and calibration verification?

Click here to refer to “State Operations Manual,” Appendix C-Interpretive Guidelines, Calibration and Calibration Verification Procedures (§492.1255) available on the CMS website.

Links to other laboratory-related resources can be found at these websites:

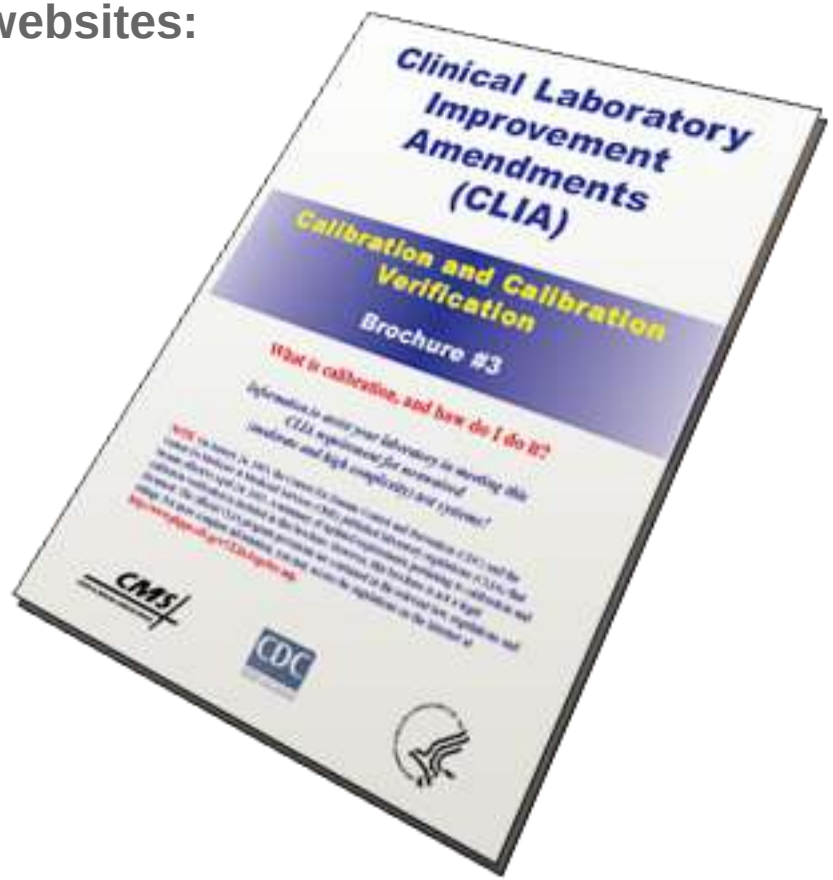
Centers for Disease Control and Prevention (CLIA Regulations)

CLIA Section 493.1253 Regulation

CLIA Brochure #3

CLIA Definitions

Centers for Medicare and Medicaid Services (General CLIA Info)



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