C27 – "How-Should-I" Guide to Laboratory Method Validation

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DECRIPTION:

The Final CLIA-88 Rules require that all labs certified under the CLIA program validate method performance prior to placing assays into service, but there is considerable confusion as to exactly how this should be accomplished. While instrumental system vendors typically perform method validations studies on new equipment assays, lab personnel will need to perform the required studies themselves when additional tests are added or when manual tests are to be performed.

Participants will learn what method validations studies must be done based on CLIA and accreditation entity requirements and how these studies may be competently and efficiently accomplished. Some of the items covered will be how studies for precision, accuracy, analytical sensitivity, functional sensitivity, limit of detection, linearity, recovery, interferences, and reference range validation can be accomplished. Spreadsheets to perform the needed calculation assessments will be utilized and supplied to participants following the presentation for use in their laboratories. The presentation will enable participants to create a systematic approach for accomplishing the initial and on-going method validation studies that might be required to support their laboratory services.

OBJECTIVES:

At the end of the session, the participant will be able to:

- Conduct the method validation studies required by regulatory entities and good laboratory practice.
- Understand what validation studies must be performed for different types of tests and how often they must be done.
- Create a good method validation program that is sustainable and yields good value for the resources required.

How-Should-I

Guide to Laboratory Method Validation

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The LabCorp-Dynacare Tennessee laboratory at the University of Tennessee Medical Center in Knoxville provides laboratory services for the medical center and for LabCorp clients within a 100 mile radius of the laboratory.

Presentation Overview

This presentation on Method Validation will enable attendees to:

- **Identify the specific validation studies required prior to placing assays into clinical service.**
- **Identify the validation studies required to be done while an assay is in clinical service.**
- **Perform the validation studies required before and during the offering of an assay for patient sample testing.**

Producing a Quality Laboratory Product

The product produced by clinical laboratories is INFORMATION

Clinical Laboratory Method Validation Systems and Practices Should Be Designed To Assist in Assuring that the Information Reported On Testing of Patient Samples is Reliable

CLIA Method Validation Requirements

Validation Studies Must Be Done To Validate IVD Vendor Claims And Establish Assay Performance Parameters

- **Initial Validation Studies: must be completed and acceptable to the medical director before the assay can be used for reporting results from patient sample testing**

- **Continual Revalidation Studies: must be performed at a specified frequency until the assay is taken out of clinical service.**

Suitability of assays for clinical use is the responsibility of the laboratory's Medical Director under the CLIA regulations.

CLIA Content

- CLIA
- Chronology
- Complexities
- Demographics
- CLIA Law
- Regulations

Related Content

- DLS Home
- Genetics
- International
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- MASTER
- MPEP
- NLTN
- Publications
- Training

Current CLIA Regulations (including all changes through 01/24/2004)

While every effort is made to ensure the accuracy of this hyperlinked version of the regulations, the 2004 Codification is the definitive document. A linked version of the offical regulation may be found on the Government Printing Office (GPO) site.

Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services (Appendix C)

PART 493--LABORATORY REQUIREMENTS

Subpart A--General Provisions

Section	
493.1	Basis and scope
493.2	Definitions
493.3	Applicability
493.5	Categories of tests by complexity
493.15	Laboratories performing waived tests
493.17	Test categorization
493.19	Provider-performed microscopy (PPM) procedures
493.20	Laboratories performing tests of moderate complexity
493.25	Laboratories performing tests of high complexity

 http://www.phppo.cdc.gov/clia/default.aspx 6

How are the final regulations being implemented?

CMS is allowing each laboratory that it inspects to have one educational survey following the April 24, 2003, effective date of the regulations. This will give laboratories time (2 years) and the opportunity to receive the technical assistance that may be needed to meet the updated requirements.

Where can I find additional information and guidance?

Assistance for meeting the requirements is provided in Appendix C of the State Operations Manual (CMS Publication 7), which is posted on CMS's CLIA Website. Information about CLIA and links to other laboratory-related resources can be found on the following Websites:

CDC: www.phppo.cdc.gov/clia/default.asp
CMS: www.cms.hhs.gov/clia/default.asp
FDA: www.fda.gov/cdrh/CLIA/index.html (for a listing of waived, moderate complexity and high complexity tests)

February 2004

DEPARTMENT OF HEALTH & HUMAN SERVICES

CMS

Clinical Laboratory Improvement Amendments (CLIA)

Verification of Performance Specifications
Brochure #2

What is it and how do I do it?

The CLIA regulations now include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA.

Information to assist your laboratory in meeting this CLIA requirement!

NOTE: On January 24, 2003, the Centers for Disease Control and Prevention (CDC) and the Centers for Medicare & Medicaid Services (CMS) published laboratory regulations (CLIA) that became effective April 24, 2003. A summary of the updated requirements pertaining to performance specification verification are included in this brochure. However, this brochure is not a legal document. The official CLIA program provisions are contained in the relevant law, regulations and rulings. For more complete information, you may access the regulations on the Internet at http://www.phppo.cdc.gov/CLIA/regs/toc.asp.

CDC

BACKGROUND

The CLIA Quality System Regulations became effective on April 24, 2003. Now the laboratory is required to check (verify) the manufacturer's performance specifications provided in the package insert--for accuracy, precision, reportable range, and reference ranges--for each new unmodified, moderate complexity test that the laboratory performs before reporting patient test results. The verification process helps to assure that the test, when used in your laboratory by your testing personnel for your patient population, is performing as the manufacturer intended.

This requirement applies when the laboratory **REPLACES** a test system or instrument (with the same model or a different model); **ADDS** a new test; or **CHANGES** the manufacturer of a test kit.

The requirement does not apply to tests performed by the laboratory before April 24, 2003.

TIP! ***While the laboratory's technical consultant or director should be involved in the planning and evaluation of the performance specification checks, the test system manufacturer may also assist by providing a verification protocol and appropriate samples for the evaluation.***

ACCURACY

Are your test results correct?

The laboratory needs to compare the accuracy of the test results it obtains when using a test system with the manufacturer's accuracy claims. This can be done by testing commercially available calibrators/calibration and quality control materials with known values, proficiency testing materials that have established values, and previously tested patient specimens with established values. If test results for these samples fall within the manufacturer's stated acceptable limits, accuracy is verified.

PRECISION

Can you obtain the same test result time after time?

The laboratory is responsible for verifying that it can repeatedly test the same samples on the same day, and on different days and get the same or comparable results (reproducible), regardless of which member of the laboratory's testing personnel performs the test (operator variance). Several of the laboratory's testing personnel should participate in this evaluation to help determine overall laboratory variance. Exception: For fully automated test systems that are not operator dependent, operator variance should not affect the test's precision and may not need to be evaluated by more than one person.

REPORTABLE RANGE

How high and how low can test result values be and still be accurate?

To verify the manufacturer's established reportable range for the test, choose samples with known values at the highest and lowest levels the manufacturer claims accurate results can be produced by the test system. The laboratory may only report patient test results that fall within the verified levels. The laboratory director and/or the technical consultant will need to decide how the laboratory will report results that are greater than the highest verified level or less than the lowest verified level.

REFERENCE RANGES/INTERVALS (NORMAL VALUES)

Do the reference ranges provided by the test system's manufacturer fit your patient population?

You may begin patient testing using the manufacturer's suggested reference range(s) or you may use other published reference ranges from a textbook or a journal publication. Reference ranges can vary based on the type of patient (e.g., pediatric, male, female). Over time, you may need to adjust your reference range(s) to better fit the patient population(s) you routinely test. When you test known normal patients, the results should be within your reference range and with abnormal patients, you should expect results outside the reference range.

How many samples do I need to test?

While testing 20 samples is considered the "rule of thumb" for statistical purposes, this is not a magic number. Depending on the test system and the laboratory's testing volume, the actual number of specimens needed for each part of the verification study may vary.

Once the laboratory director has reviewed and approved the results of the verification studies, the laboratory may begin using the test system for routine testing and reporting patient test results. Conversely, if the study results indicate that the test is not accurate or results cannot be consistently reproduced, the laboratory's technical consultant and the test system manufacturer should be consulted regarding steps to resolve the problem.

TIPS! ***With planning, verifying a test system's accuracy; precision, including operator variance; and reportable range may be performed using the same samples. For example, you may test samples with known values at the upper and lower end of the manufacturer's reportable range along with samples that are in the normal range for your patient population, in different runs, on different days, using several of the personnel who will normally perform the testing. The activities of the personnel verifying the test system will also facilitate meeting CLIA's personnel competency requirements for these employees. In addition, the laboratory director may use the verification process to meet the CLIA requirements for establishing the test system's quality control protocol, an essential component of the laboratory's overall quality system.***

Where can I find additional information about the CLIA requirements pertaining to the verification of performance specifications?

You may refer to the State Operations Manual, Appendix C-Interpretive Guidelines, §493.1253, available on the CMS website at: www.cms.hhs.gov/clia.

COLA has put together comprehensive guidance information for the laboratories it accredits

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FAST FACTS 33

LABORATORIES, START YOUR INSTRUMENTS

Finally! Your new instrument has been delivered and you can hardly wait to get it up and running. Just as you're about to plug it in, you stop to think about what you need to have in place before you can start testing patient specimens . . .

The Clinical Laboratory Improvement (CLIA) amendments of 1988 as well as the COLA Criteria for Quality Laboratory Performance list specific requirements for new instrument startup. These requirements are based upon the complexity level of the instrument and the tests it performs. The possible complexity levels are waived, moderate, and high.

Your sales representative or the manufacturer should be able to provide you with this information. You may also determine the complexity of a test by using the searchable CLIA test complexity database at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/search.cfm.

Note: Deviation from the manufacturer's written procedure changes the complexity of any waived or moderate instruments. It automatically becomes high complexity and is subject to high complexity requirements.

Waived Instruments

- Follow manufacturer's instructions for installation and operation. This includes calibration, performance of quality control (QC), maintenance and any other function checks, etc., as outlined in the instrument manual.

Moderate Complexity Instruments

- Determine reportable and patient normal ranges (manufacturer's stated ranges may be used).
- Calibration - COLA criteria #50-52 state the minimum requirements for calibration. Manufacturer's requirements must be followed if they are more stringent than COLA's.
- Quality Control - QC requirements differ based upon the specialty/subspecialty of testing. See COLA criteria #139-260. Adhere to the manufacturer's QC requirements if they are more stringent than COLA's requirements.
- Enroll in proficiency testing for regulated analytes, or perform split specimen analysis for all unregulated analytes.

High Complexity Instruments

Subject to the same requirements as moderate instruments outlined above with these additions.

- Calibration - Follow additional COLA criteria #56-60 specific for high complexity instruments.
- Quality Control - See additional COLA criteria #261-278. You must verify and document accuracy, precision, reportable patient range, linearity, sensitivity, specificity and other performance characteristics required for test performance.

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Method Validation

Lab Method Validation Mainly Concerns

Identification of the sources of potential errors

Quantification of the potential errors in the method.

Assay Validation describes in mathematical and quantifiable terms assay performance characteristics

Validation of Analytical Procedures is the process of determining the suitability of a given methodology for providing useful analytical data. A method that is valid in one situation could well be invalid in another.

Sec. 493.1253 Standard: Establishment and verification of performance specifications

(a) Applicability. Laboratories are not required to verify or establish performance specifications for any test system used by the laboratory before April 24, 2003.

(b)(1) Verification of performance specifications. Each laboratory that introduces an unmodified, FDA-cleared or approved test system must do the following before reporting patient test results:

(i) Demonstrate that 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.

(ii) Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

(2) Establishment of performance specifications. Each laboratory that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures, Gram stain, or potassium hydroxide preparations), or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable:

(i) Accuracy.

(ii) Precision.

(iii) Analytical sensitivity.

(iv) Analytical specificity to include interfering substances.

(v) Reportable range of test results for the test system.

(vi) Reference intervals (normal values).

(vii) Any other performance characteristic required for test performance.

(3) Determination of calibration and control procedures. The laboratory must determine the test system's calibration procedures and control procedures based upon the performance specifications verified or established under paragraph (b)(1) or (b)(2) of this section.

(c) Documentation. The laboratory must document all activities specified in this section.

CLIA Final Rule

Performance Verification

NonWaived

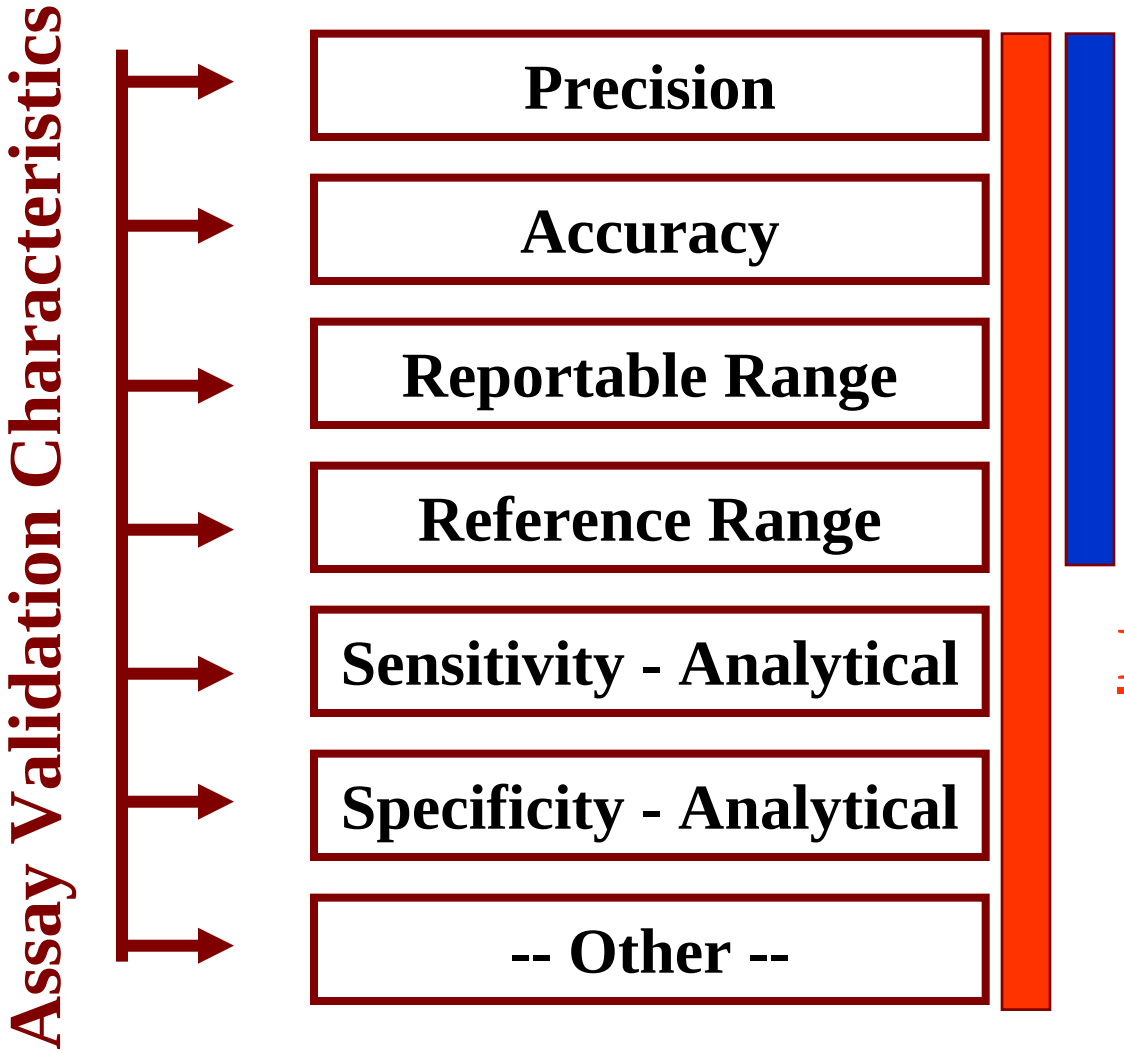
- **Accuracy – Systematic Error**
- **Precision – Random Error**
- **Reportable Range**
- **Reference Interval**

Modified or non-FDA Cleared

Additional Requirements

- **Analytical sensitivity**
- **Analytical specificity**
- **Any other characteristics needed for validation**

CLIA Final Rule Assay Validation Characteristics



NonWaived Method Validation

Typically Satisfied Through Four Studies

Replication study

Estimates imprecision

Linearity study

Estimates imprecision

Determines the reportable range

Comparison of methods study

Estimates inaccuracy or bias

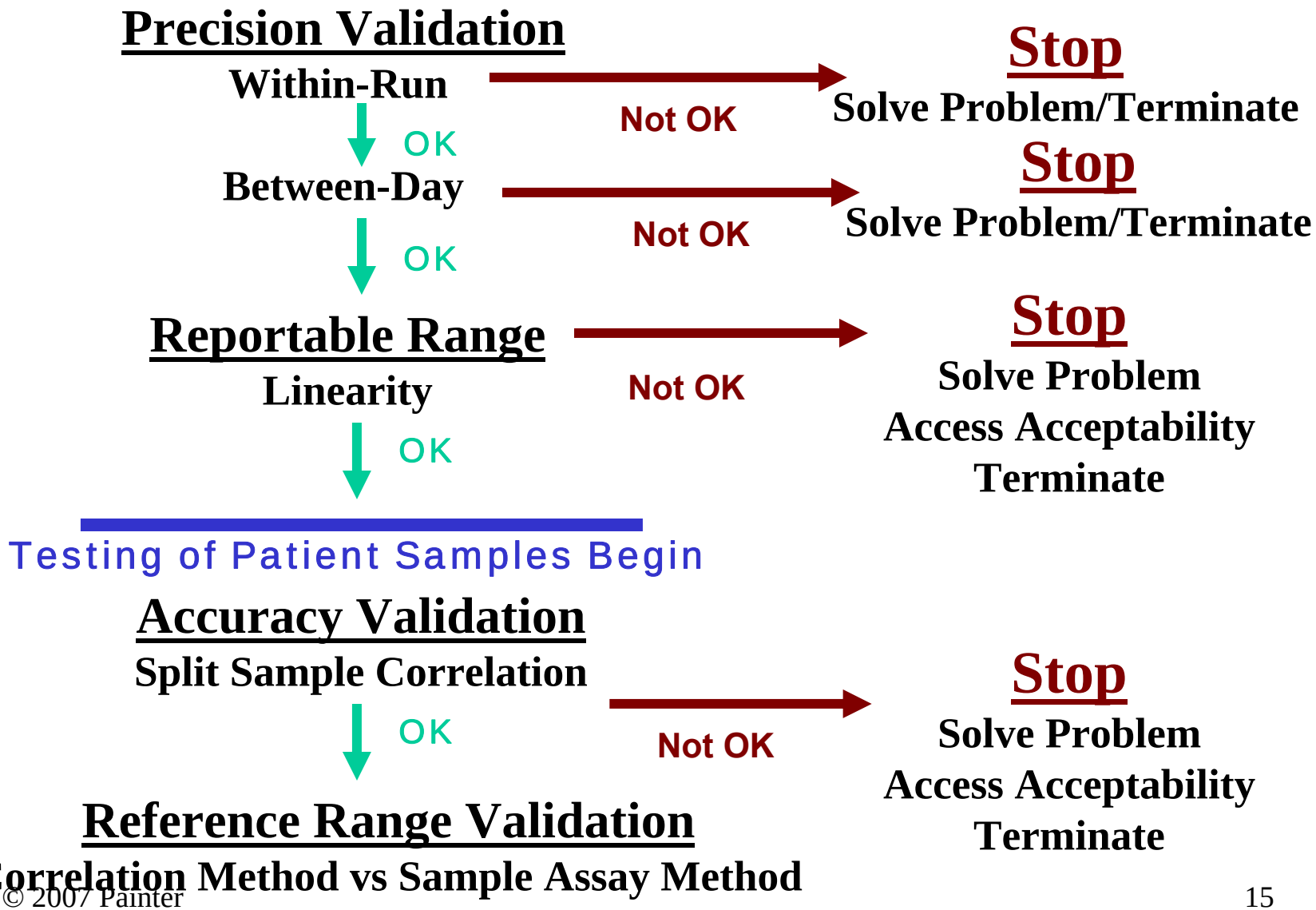
Reference Range Validation

Test patient samples to verify the reference range

Assay Evaluation Task Practicality for Clinical Labs

Evaluation Task	Example of How It is Done	Doable By
Within-run Precision	Run 3 levels 20 X each	All labs
Within and Total Precision	Run 2 levels/ on 2 runs/20 days	All labs
Detection Limit	Means +/- 3SD of a blank	All labs
Analytical Sensitivity	Determine from Cal Curve slope	
Linearity	Sequential high sample dilution	All labs
Recovery	Different analyte levels spiked	All labs
Bias	Absolute and percent difference between expected vs test results	Most labs
Split-sample Correlation	40 – 100 samples over 5 days	Most labs
Expected Range	Hundreds to thousands samples from people of appropriate sexes and ages	Some labs
Interferences	Spiking with different levels of probable interfering compounds	Some labs
Diagnostic Cutoff	Score test results as TP, FP, TN, FN for different cutoff points as determined by predicate assay, clinical diagnosis or an accepted "gold standard"	Few labs

Validation Sequence Prior To Patient Testing



Precision

Defined is the degree of agreement among individual test results obtained when the procedure is applied repeatedly to multiple samplings of a homogeneous sample.

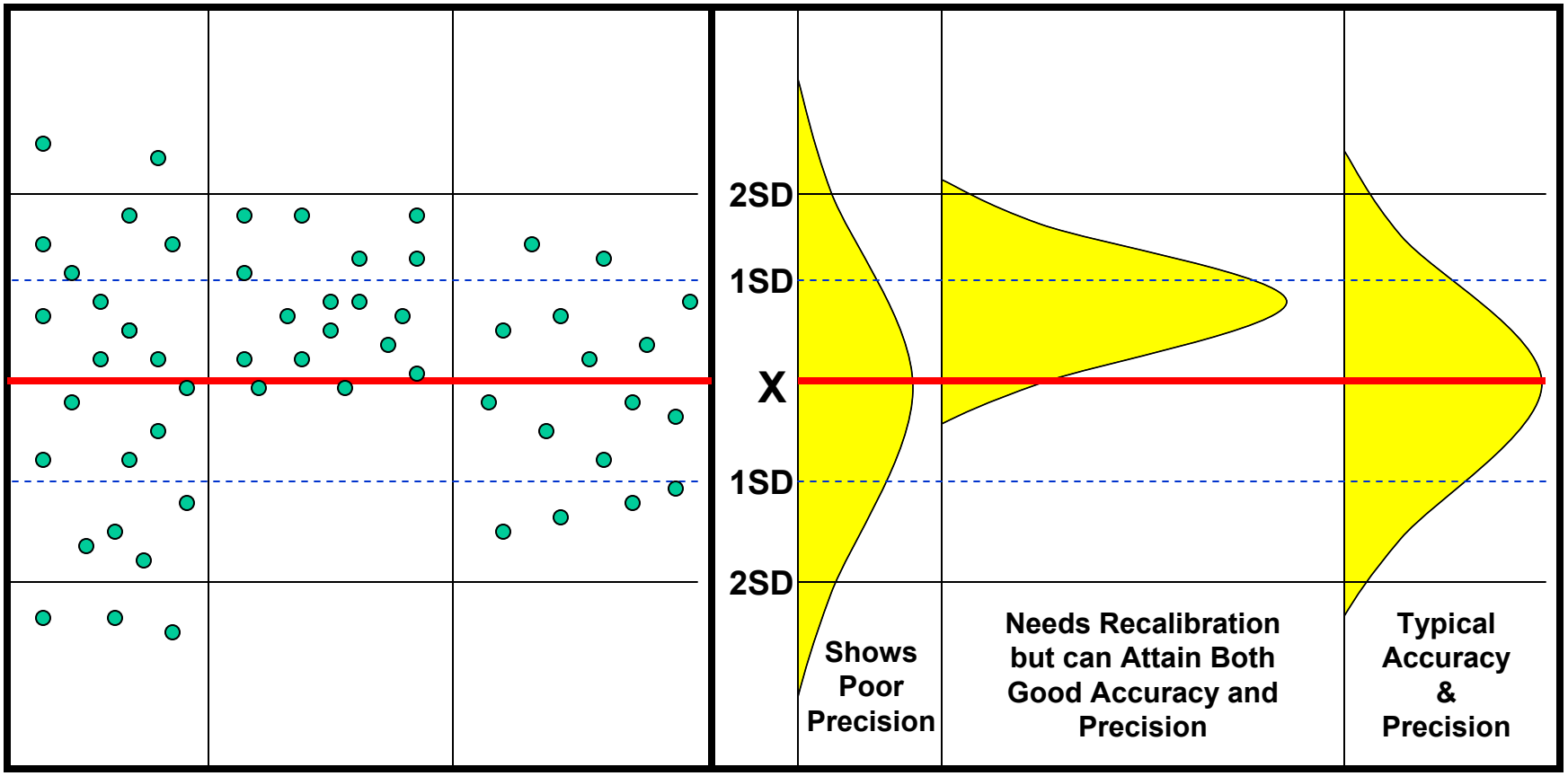
Imprecision = Random Error (RE)

Accuracy

A measurement of the exactness of an analytical method, or the closeness of agreement between the measured value and the true value.

Inaccuracy = Systematic Error

L-J Charts Show Current Accuracy and Precision



Inaccuracy = Systematic Error

- Usually quantified by comparing a method to a "gold standard"
- Compare value between the "test method" and the "gold standard" to estimate the SE
- Systematic error may stay the same over a range of values or may change as concentration changes.

Imprecision = Random Error

- Defined as an error that can either be positive or negative, whose direction and exact magnitude cannot be predicted.
- Usually quantified by the standard deviation (SD).
- SD usually increases as concentration Increases
- Therefore it is useful to calculate the coefficient of variation (CV%), which expresses the error as a percentage of the mean concentration.

Total Error (TE)

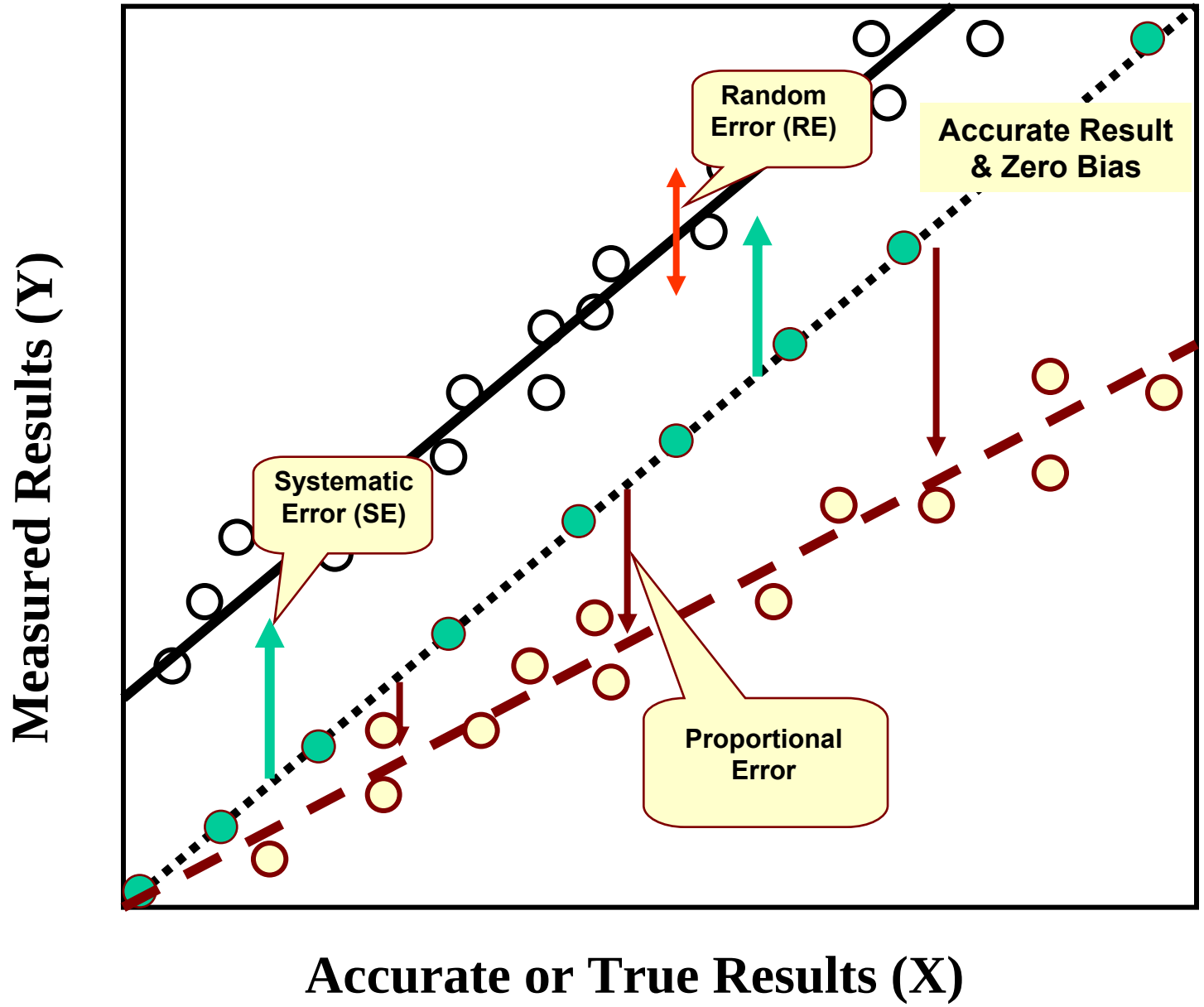
Defined as the net or combined effect of random and systematic errors:

TE = RE + SE

Total Error

Regulatory agencies define acceptable error in terms of "total allowable error" (TEa) – e.g., CLIA:

- **ALT: target value +/- 20%**
- **Potassium target value +/- 0.5mM/L**
- **Albumin target value +/- 10%**
- **Hemoglobin target value +/- 15%**
- **Magnesium target value +/- 25%**
- **Leukocyte count target value +/- 15%**



Before You Start A Method Validation

- Define a quality requirement for the test in the form of the amount of error that is allowable.
- Make a plan and write an outline for each validation study
- Schedule ample time to perform the studies
- Familiarize the techs doing the testing with the validation studies to be done
- Make sure the instrument/method is functioning properly. i.e. is passing QC and calibration.
- Have enough reagents and supplies in stock.

Precision Validation

Does the Assay Yield a Reproducible Result

Good Accuracy Requires Good Precision

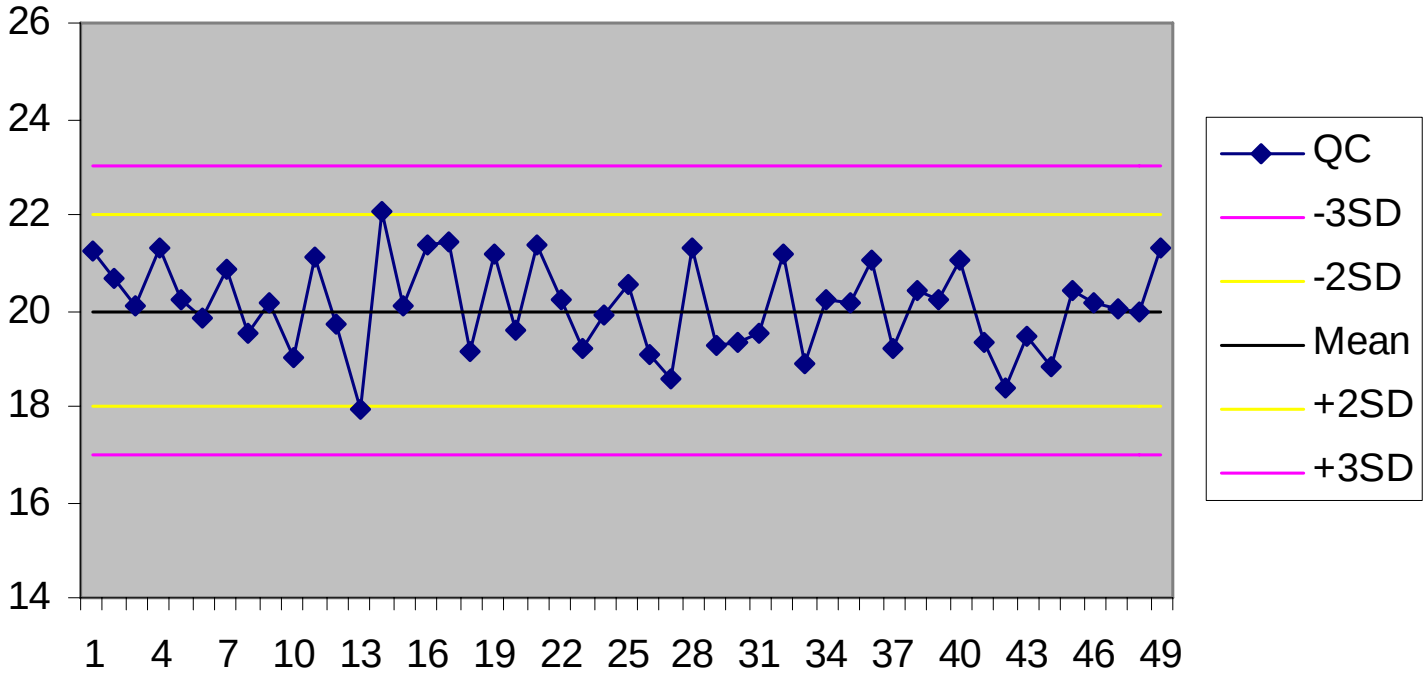
Step #1: Validate within-run precision

Run 20 samples at two or more (e.g., H & L) levels.
Pick levels of clinical significance (e.g., Cholesterol of 200 mg/dL; Glucose of 126 mg/dL; CKMB of 6 ng/mL; Troponin of 0.156 pg/mL; BNP of 100 ng/mL.

Step #2: Validate between-run precision

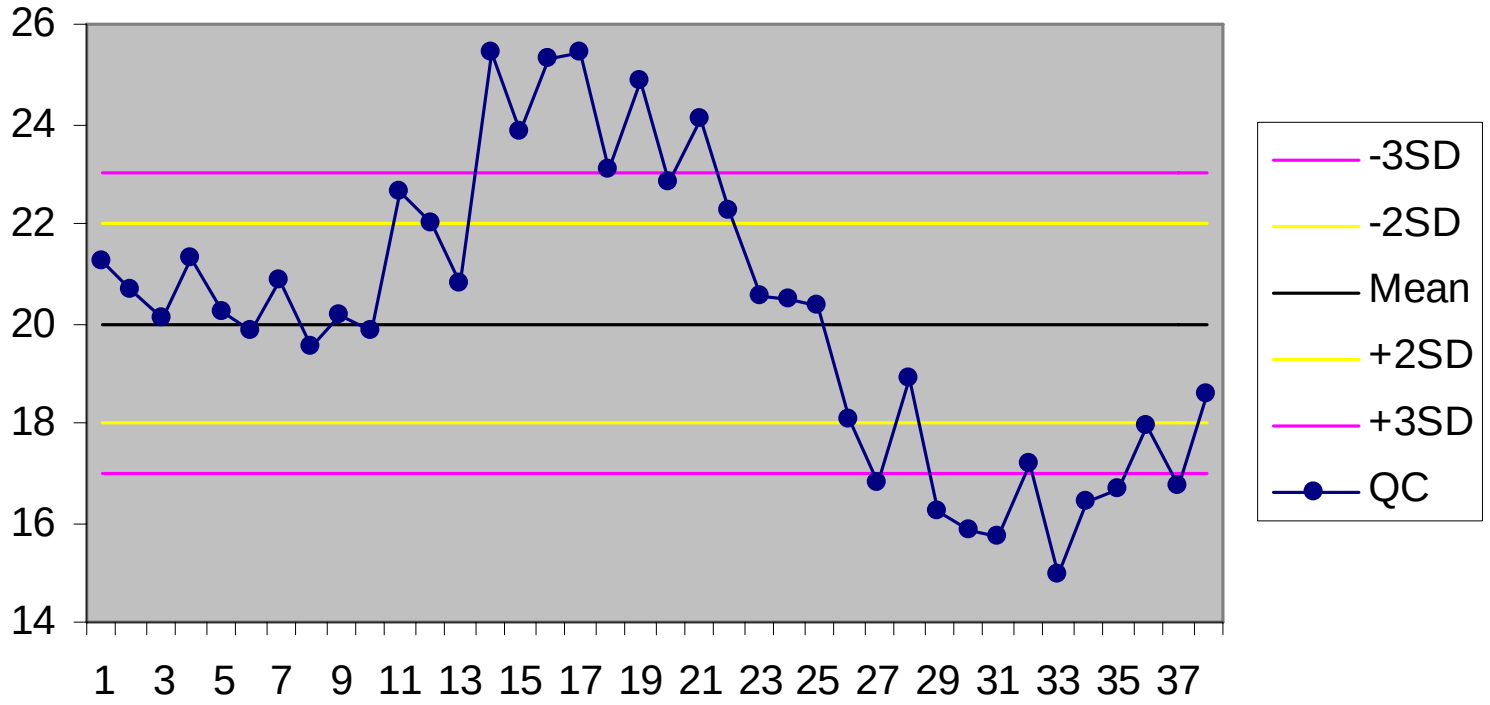
Run 20 samples of two levels across 20 days

A Stable Assay Shows A Random Distribution of Results Around the Mean



Mean = 20, SD = 1 or %CV of 5% [%CV= (SD/Mean)x100]
95% of QC results between 18 and 22 as expected with 2SD limits
So a result of 20 has a 95% confidence interval of 18 to 22.

An Unstable Assay Does Not Show A Consistent Random Distribution of Results Around the Mean



Mean = 20, SD = 1 or CV% of 5% Plus fluctuating mean. Interpretation: Result of 20 has 95% confidence limit of 18 – 22, PLUS a significant and variable bias

Replication Experiment

Factors to consider:

– Time period

- Within-run/ within day measurements
- Between-day measurements (over ≥ 20 days).

– Sample selection

- Standard solutions
- Control Solutions
- Pools of fresh patient samples

– Number of samples (e.g., 3 levels)

Replication Experiment – Minimum Studies

- Select at least 2 different control/standard materials or patient specimens that represent low and high medical decision concentrations for the test of interest.

  - Analyze each material 20 times within a run or within a day

- Short-term imprecision/random error

  - Analyze each material once per day for 20 days

- Long- term imprecision/random error

Replication Experiment Calculations

For each of the 20 test results obtained from a single source material:

- **Calculate the Mean, SD, and CV%**

Replication Experiment

The CLIA criteria for optimal assay performance:

- **Short term = "within-run" or "within-day" experiment. SD < 0.25 TEa**
- **Long- term = "between-day" experiment SD < 0.33 TEa**

	LOW	MID	HIGH
D1	3.1	6.1	18.4
D1	2.9	5.9	18.6
D1	3	6.2	18.3
D2	3.2	6.1	18.4
D2	2.9	6	18.2
D2	2.8	5.9	18.5
D3	3	5.8	18.6
D3	3.2	6	18.7
D3	3.1	6.1	18.3
D4	2.9	6.1	18.1
D4	3.1	6	18.3
D4	2.9	5.8	18.2
D5	3	6.2	18.4
D5	3.1	6.1	18.6
D5	3.2	6.1	18.4
D1 Mean	3.00	6.07	18.43
D1 SD	0.10	0.15	0.15
D1 %CV	3.33	2.52	0.83
D2 Mean	2.97	6.00	18.37
D2 SD	0.21	0.10	0.15
D2 %CV	7.02	1.67	0.83
D3 Mean	3.10	5.97	18.53
D3 SD	0.10	0.15	0.21
D3 %CV	3.23	2.56	1.12
D4 Mean	2.97	5.97	18.20
D4 SD	0.12	0.15	0.10
D4 %CV	3.89	2.56	0.55
D5 Mean	3.10	6.13	18.47
D5 SD	0.10	0.06	0.12
D5 %CV	3.23	0.94	0.63
Grand Mean	3.03	6.03	18.40
Grand SD	0.128	0.128	0.173
Grand %CV	4.23	2.12	0.94
Target	3	6	18
Bias (D)	0.03	0.03	0.40
CLIA PT Allowed TEa (30%)	0.90	1.80	5.40
Mean of SD's for 5 Days	0.125	0.123	0.146
SQR of SD	0.016	0.015	0.021
Corrected SD	-0.026	-0.026	-0.027
Total Imprecision	0.099	0.097	0.118
Total %CV	10.380	5.176	1.871

Precision Study for FDA Approved Assays

Vendor Claim Validation Protocol

- **Enables estimate of precision both within run and between day, carry-over and linearity.**
- **Select 2 samples having concentrations in the upper and lower 10% of the stated analytical range (L & H) and a MID level sample, all of similar matrix.**
- **Test samples in sequence (M, M, H, L, M, M, L, L, H, H, M) where the first two M samples check the system before testing 9 samples in study.**

Example Table for Precision Claims Validation Study

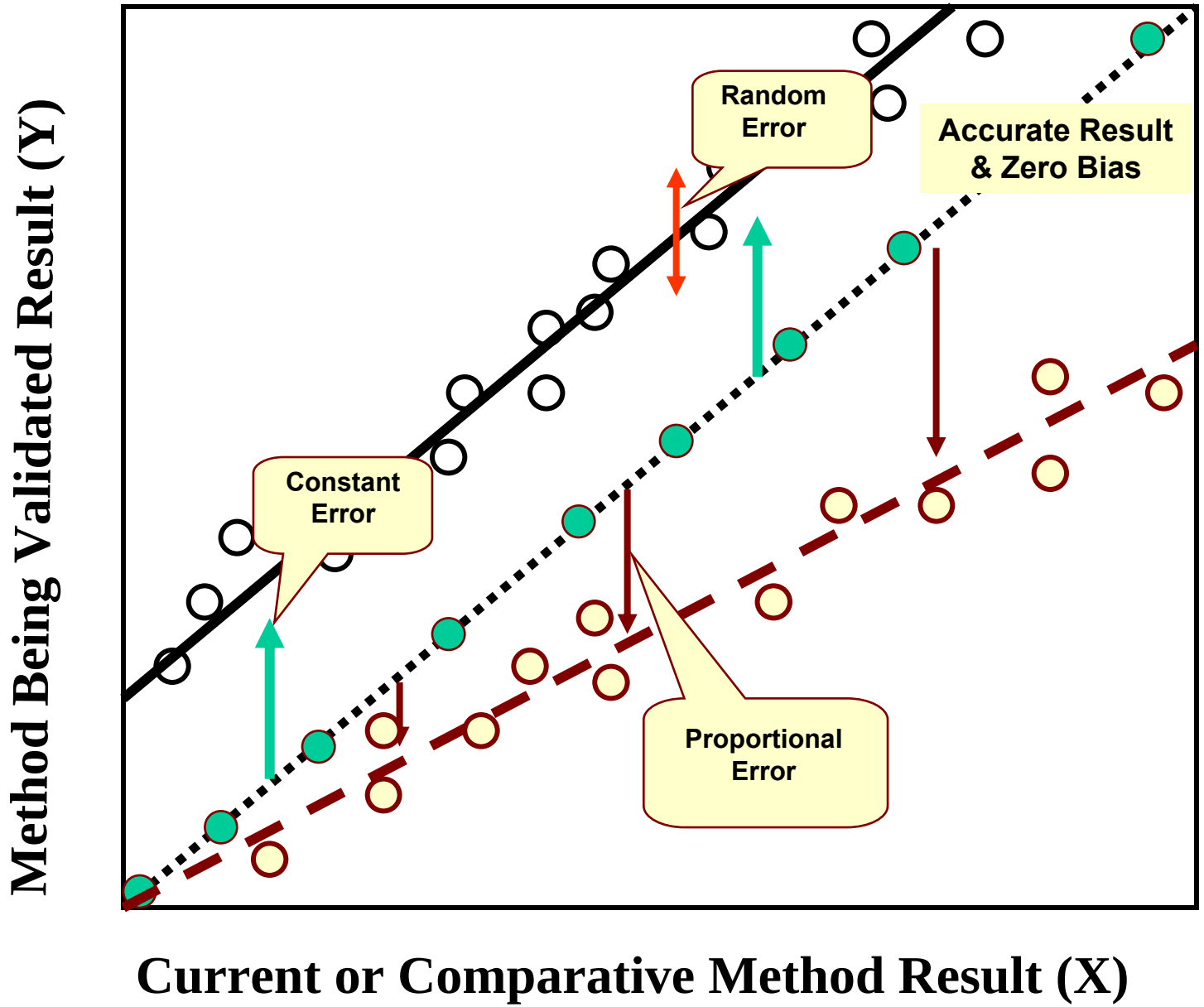
Within-Run Data Analysis Table (Day 1)					Between-Run Data Analysis Table			
Tube	Level	Low Meas	Mid Meas	High Meas	Within-Run Assay Days	Low WR Mean/SD	Mid WR Mean/SD	High WR Mean/SD
1	Mid		---		1	0.23/0.057	1.23/0.05	3.33/0.087
2	Mid		---		2	0.19/0.061	1.31/0.051	3.16/0.092
3	High			3.3	3	0.21/0.052	1.18/0.049	3.19/0.097
4	Low	0.2			4	0.31/0.058	1.20/0.047	3.23/0.083
5	Mid		1.2		5	0.16/0.055	1.25/0.052	3.36/0.088
6	Mid		1.3					
7	Low	0.3			Mean	0.22/------	1.23/-----	3.25/-----
8	Low	0.2			Target (C)	0.20/------	1.20/-----	3.20/-----
9	High			3.4	Bias			
10	High			3.3		0.02/------	0.03/-----	0.05/-----
11	Mid		1.2		Allowed Bias	0.03/------	0.03/-----	
	SUM	0.7	3.7	10.0	Mean of SD's (R)	---/0.057	--/0.0498	--/0.089
	WR Mean	0.23	1.23	3.33	(S) WR Mean's SD^2	--/0.0032	--/0.0025	--/0.0076
	WR SD				Corrected SD T = S – (R/3)	/ - 0.0157	/ -0.163	/ - 0.022
	% CV				Total Imprecision U = (R +T)	--/0.041	--/0.334	--/0.111
					Total % CV	-----/92%	----/47%	---/10%
					Allowable % CV			

Regulated CLIA88 Analytes	Decision Level (DL)	CLIA88 Total Error Limits	Max CLIA ± Error Limit	Desired ± Total Error & Estimated (%CV)
Routine Chemistry Analytes				
Cholesterol, total	200 mg/dL	± 10%	20 mg/dL	5 mg/dL (2.5%)
Cholesterol, high density lipoprotein (HDL)	35 mg/dL 65 mg/dL	± 30%	10.5 mg/dL 19.5 mg/dL	2.6 mg/dL (7.5%) 4.9 mg/dL (7.5%)
Creatinine	1.0 mg/dL 3.0 mg/dL	± 0.3 mg/dL or ± 15%	0.3 mg/dL 4.5 mg/dL	0.08 mg/dL (8.0%) 0.11 mg/dL (3.7%)
Glucose	50 mg/dL 126 mg/dL 200 mg/dL	± 6 mg/dL or ± 10%	6 mg/dL 12 mg/dL 20 mg/dL	1.5 mg/dL (3.0%) 3.15 mg/dL (2.5%) 5.0 mg/dL (2.5%)
Potassium	3.0 mmol/L 6.0 mmol/L	± 0.5 mmol/L	0.5 mmol/L	0.13 mmol/L (2.2%)
Sodium	130 mmol/L 150 mmol/L	± 4 mmol/L	4 mmol/L	1.0 mmol/L (0.67%)
Thyroid stimulating hormone	0.3 mIU/L 5.0 mIU/L	± 3 SD	3 SD	0.75 SD
Antinuclear antibody	POS	± 2 dilution or (pos. or neg.)		1 dilution
Anti-Human Immunodeficiency virus	POS	Reaction or nonreactive		
Complement C3	100 mg/dL	± 3 SD	3 SD	0.75 SD
Hematology Analytes				
Erythrocyte count	4.5 M/uL 5.9 M/uL	± 6%	0.27 M/uL 0.35 M/uL	0.07 M/uL (1.5%) 0.09 M/uL (1.5%)
Hematocrit	35% 50%	± 6%	2.1% 3.0%	0.53% (1.5%) 0.75% (1.5%)
Hemoglobin	12 g/dL 17 g/dL	± 7%	0.84 g/dL 1.19 g/dL	0.21 g/dL (1.7%) 0.30 g/dL (1.8%)
Leukocyte count	3.5 K/uL 11.0 K/uL	± 15%	0.52 K/uL 1.65 K/uL	0.13 K/uL (3.7%) 0.41 K/uL (3.7%)
Platelet count	50 K/uL 500 K/uL	± 25%	12.5 K/uL 125 K/uL	3.12 K/uL (6.2%) 31.2 K/uL (6.2%)
Fibrinogen	150 mg/dL	± 20%	30 mg/dL	7.5 mg/dL (5.0%)
Partial thromboplastin time	40 seconds	± 15%	6.0 Sec	1.5 Sec (3.7%)
Prothrombin time	INR 3.6	± 15%	0.54 INR	0.14 INR (3.9%)

Optimal
Between-Day Precision

33% of TEa with no Bias

25% of TEa with Bias



Accuracy Validation

Does the Assay Yield the Correct Result

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the measured value and the value that is accepted as a conventional true value or an accepted reference value.

The determination of Accuracy usually requires a "gold standard" or an accepted method to which a new method can be compared

External Proficiency Testing (PT) and PT validated samples can check method accuracy

Accuracy Validation

Comparison of Methods

- Performed to estimate inaccuracy or systematic error of the new method.
- Experiment is performed by analyzing patient samples by the new method (test method) and a comparative method, then estimate the systematic errors on the basis of the differences observed between the methods.

Comparison of Methods

Comparative method

- Must be carefully selected, assumed to yield the correct results.
- Any differences between a test method and a comparative method are assigned to the test method, because the correctness of the comparative method is well documented

Comparison of Methods-Measuring Inaccuracy

Factors to consider

- Comparative method (Ideal is reference method)
- # of specimens to test
  - At least 40 patient samples
  - Cover the entire reportable range
  - One third in the low abnormal range, one third in the normal range and one third in the high abnormal range
- Use controls, standards or CAP survey material for spiking to make higher level samples

Comparison of Methods-Measuring

Inaccuracy

- Single vs duplicate measurements

  Sufficient Volume of specimen

- Time period

  Test specimens on different days

  Minimum 5 days, could extend to 20 days

  Test the specimens on both methods simultaneously or within 2 hours of each other.

Comparison of Methods – Data Analysis

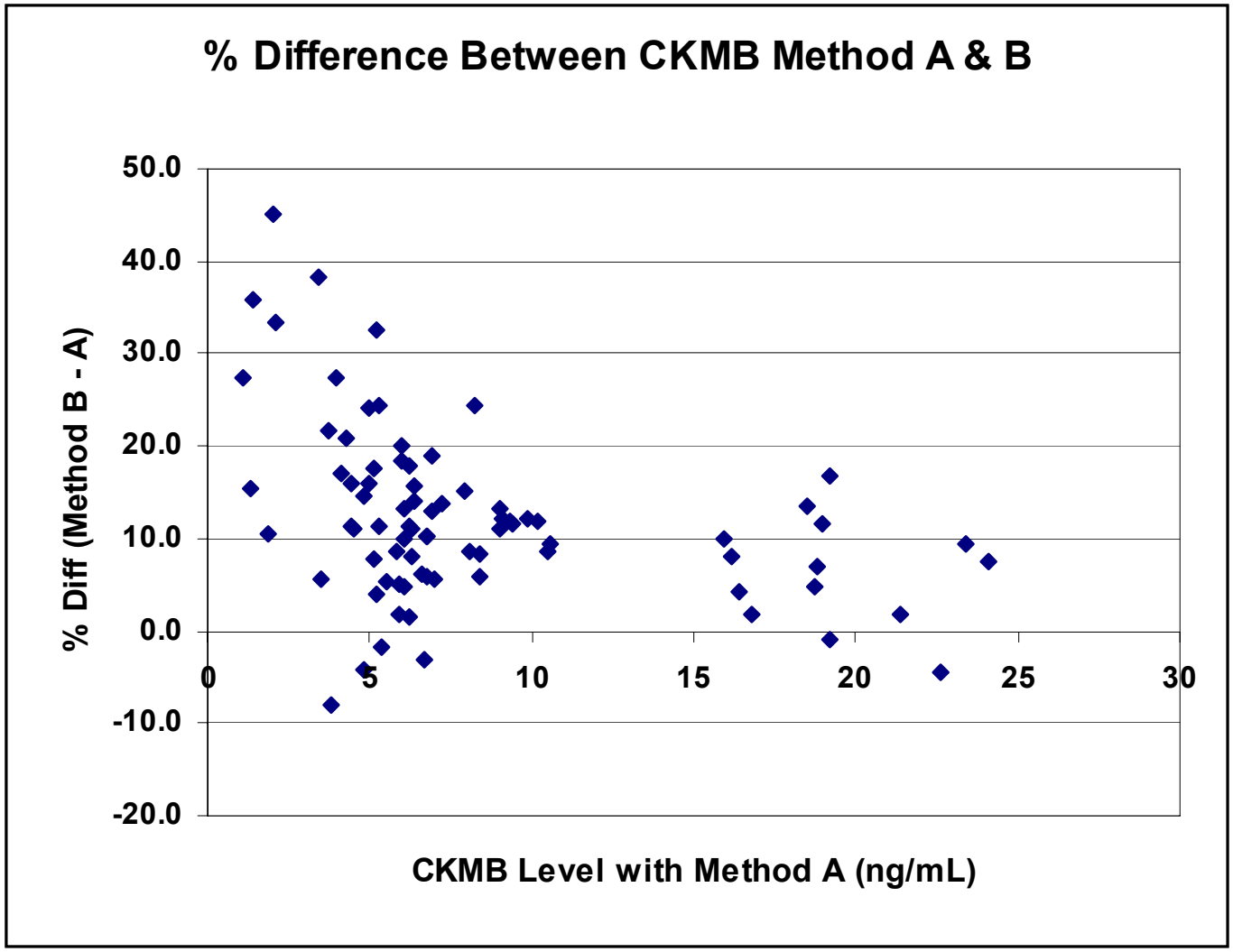
Graph the data

Difference plot

- Difference between the test results minus comparative results on y axis vs. comparative results on the x axis
- Differences should scatter around the zero line.
- Look for outliers and repeat the measurement.

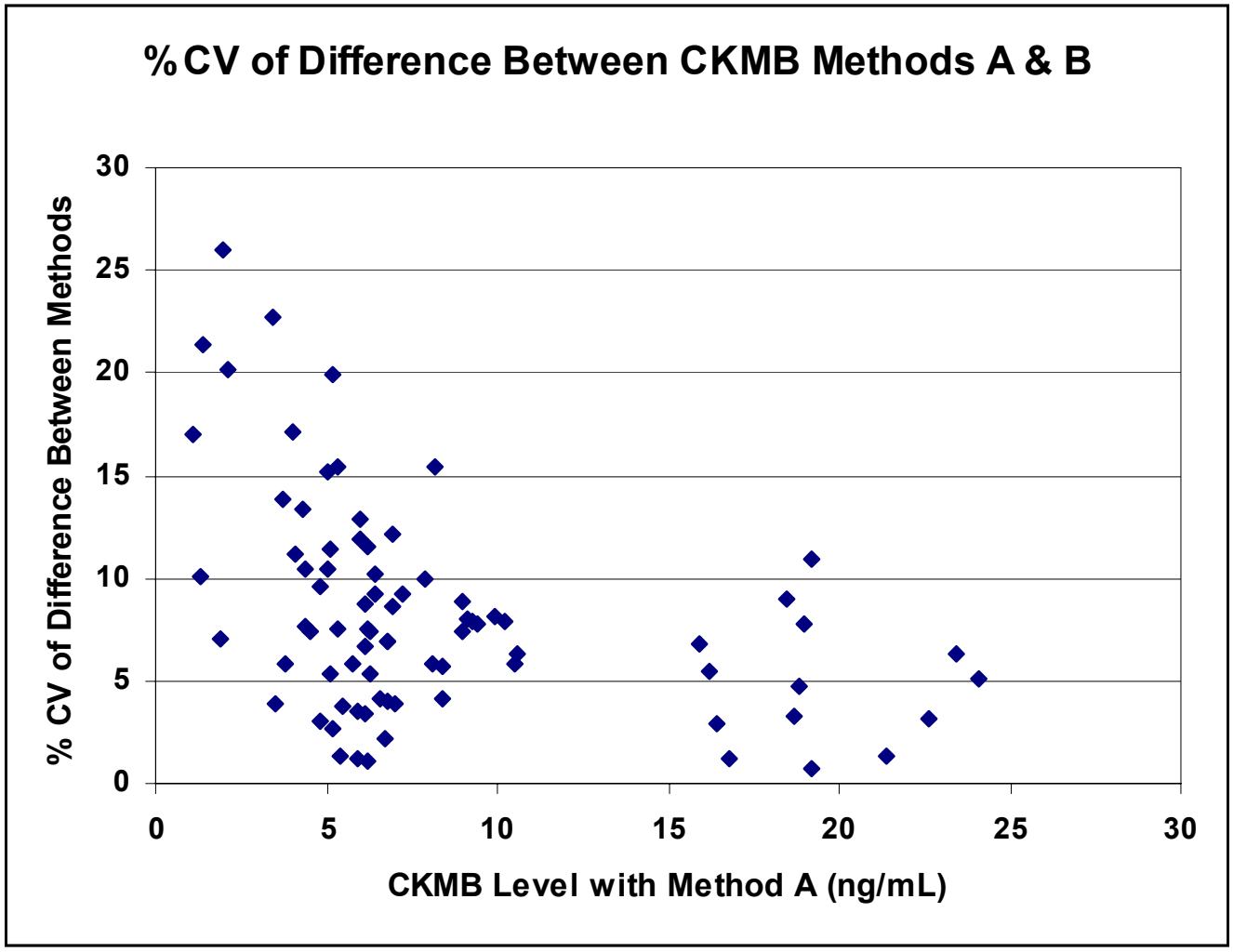
Comparison plot

- Plot the test values on the y axis vs the comparison values on the x axis.
- Inspect for outliers and repeat.



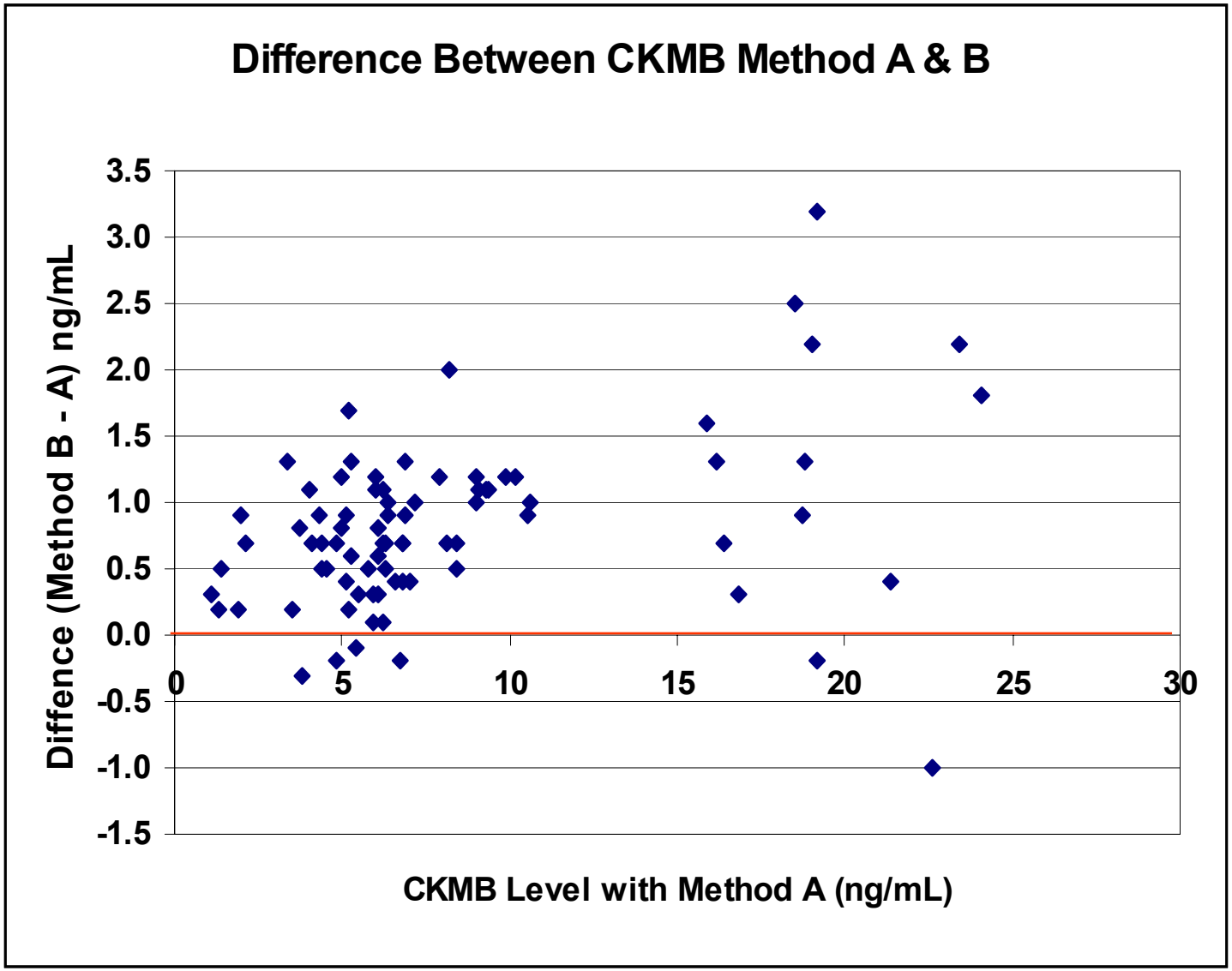
% Difference = ((Method B – Method A)/Method A) x 100

% Diff = ((11 – 10)/10) x 100 = 10%

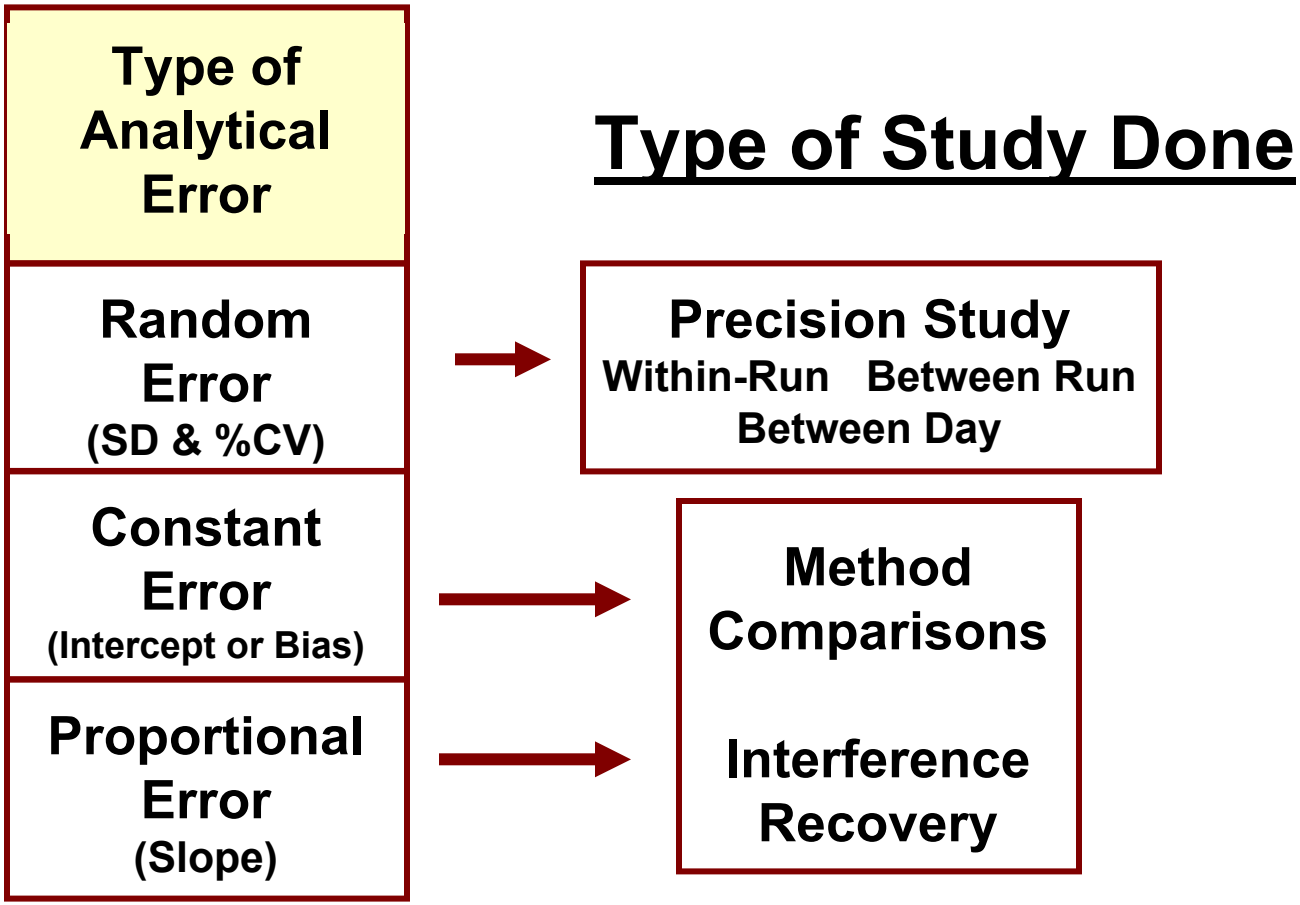


% CV = (SD of Differences/Mean of Differences) x 100

% CV = (0.1/5) x 100 = 2%



Studies to Detect Different Types of Error



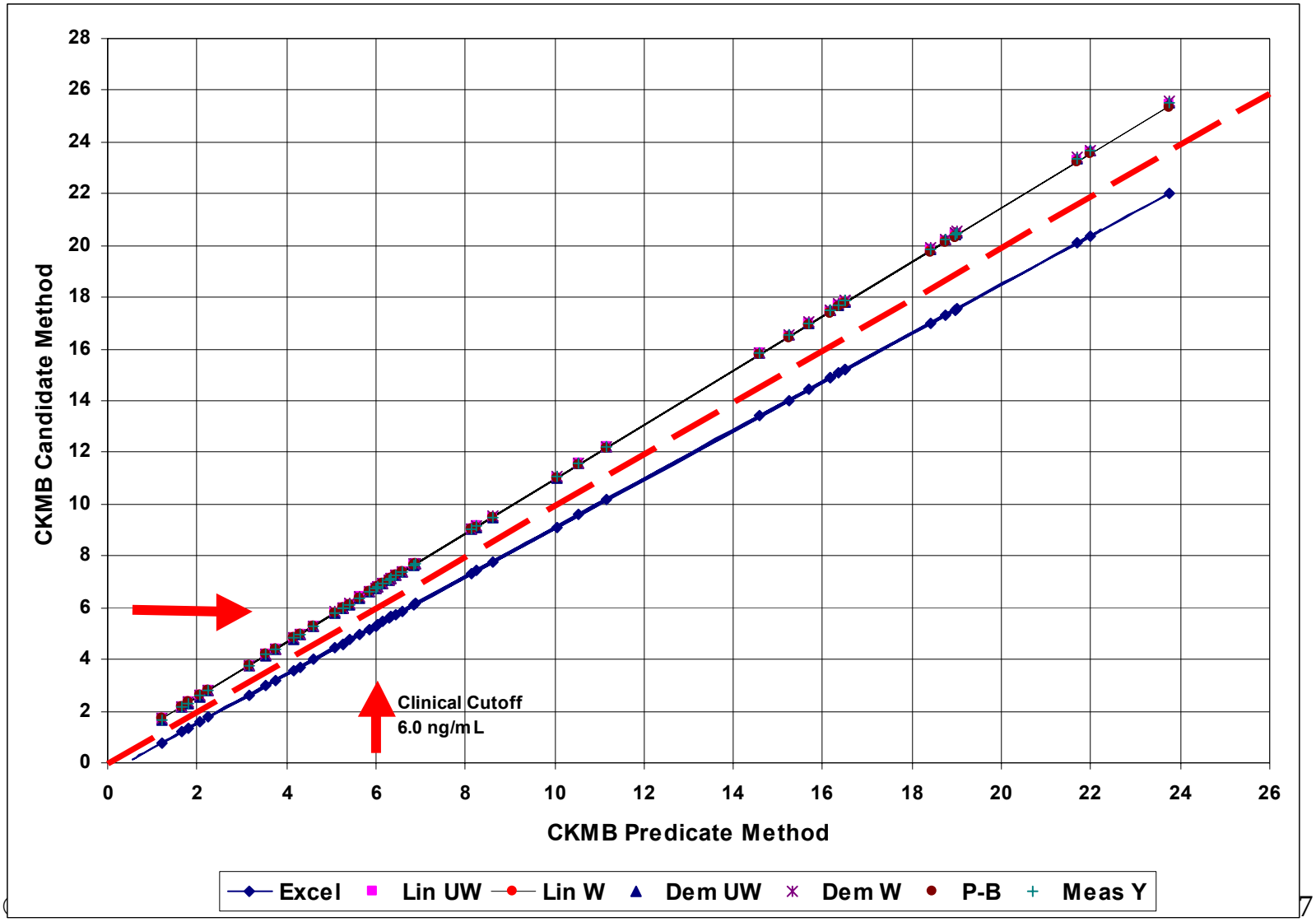
Comparison of Methods – Data Analysis

Calculate statistics

Different statistical tools are available for calculating the systematic error or bias.

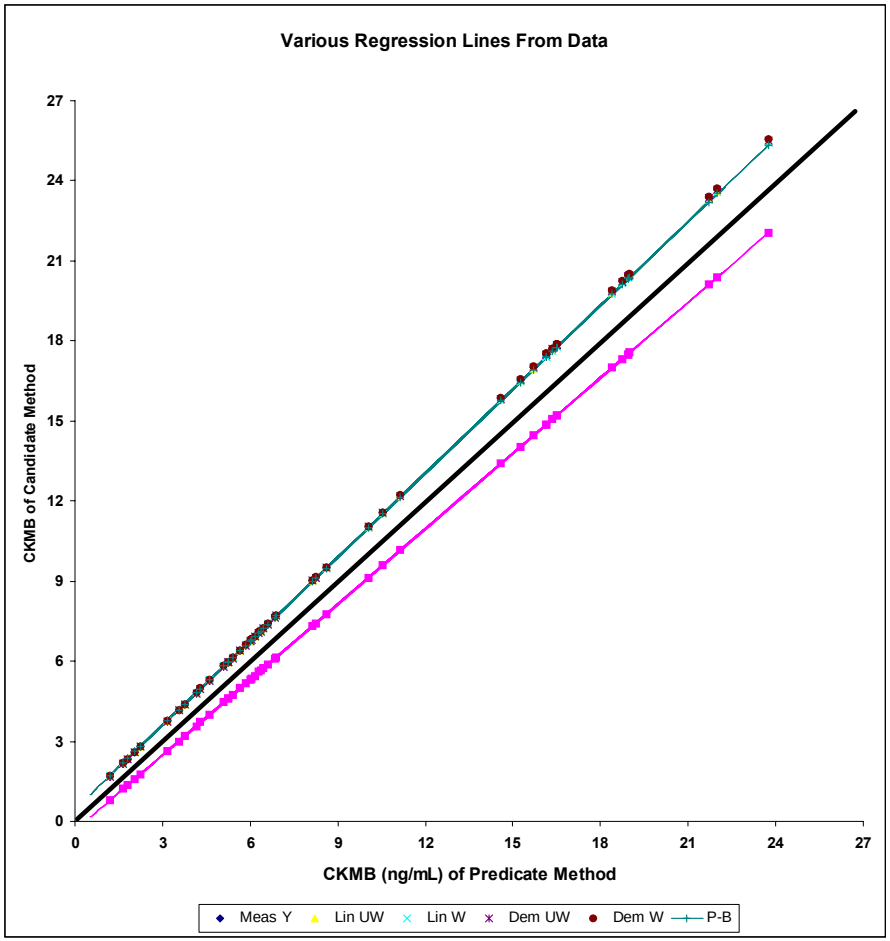
- **Linear regression analysis**
- **Paired t-test**
- **Deming's regression**
- **Passing-Blalock regression**
- **Correlation Coefficient - r**

All Regression Line Calculation Methods Do Not Yield the Same Line



Comparison of Methods - Statistics

- If you select to use linear regression statistics, you must evaluate the correlation coefficient (r):
- Correlation coefficient estimates the degree of association between two variables.
- If r> 0.99, use linear regression statistics
- If r < 0.99, use the paired t-test or another method.



Regression Method	Slope	Slope Range	Y-Intercept	Intercept Range
	Proportonal Bias	Slope 95% Confidence Range	Constant Bias	Bias 95% Confidence Range
Linear Unweighted	1.053	1.024 – 1.061	0.450	0.119 – 0.782
Linear Weighted	1.051	1.017 – 1.095	0.449	0.299 – 0.599
Deming Unweighted	1.057	1.013 – 1.102	0.406	0.067 – 0.725
Deming Weighted	1.058	0.996 – 1.117	0.428	0.001 – 0.854
Passing-Bablok	1.048	1.014 – 1.096	0.465	0.210 – 0.710
MS EXCEL Spreadsheet	0.942		-0.345	

Comparison of Methods

Criteria for acceptable performance:

- Must combine calculated random error (from the replication experiment) with the systematic error (from the comparison of methods experiment) to calculate

TOTAL ERROR

– TEcalc = SE + RE

– TEcalc = bias + 3SD

– TEcalc < TEa

- Method performance is judged acceptable when the observed error (TEcalc is smaller than the defined allowable error (TEa)

Detection of Interferent Bias

- Duplicates are not sensitive for detection of interferent bias unless the effect is four times greater than the SD at that analyte concentration.

- Replicates of 5 can detect a bias about 2.3 times larger than the SD.

- When the change due to the interferent is less than the SD over 27 replicates are required.

Method Validation

Validation of Reportable Range or Linearity

- It is essential to assess the analytical range of a method, i.e., the lowest and highest test results that are reliable and can be reported.
- It is important to validate the manufacturer's claims for reportable range of their system/method.

Reportable Range Validation

Validates Upper & Lower Limits of Reliable Results

Synonym Studies
Analytical Range & Linear Range

- The Analytical Range of a quantitative assay is defined as; "the range of concentration or other quantity in the sample over which the method is applicable without modification".
- Once the Analytical Range is defined it can be used as the CLIA Reportable Range.
- For linear assays the Reportable Range is generally equivalent to the Linear Range.

Reportable Range

Results produced within an assay's Reportable Range are considered to have acceptable precision and accuracy

They Are RELIABLE

Reportable Range Validation

Validates Upper & Lower Limits of Reliable Results

The analytical range is determined by measuring levels of analyte ranging in concentration from zero (blank) through the highest level of clinical interest without sample dilution

- **Linear assays can use simple calibrations of one sample of known level and math (e.g., UNK = (Abs UNK/Abs Known) x Conc. Known)**

- **Non-linear assays (e.g. immunoassays) require 5 to 7 calibrator points to reliably describe the reportable range of analyte being measured.**

Reportable Range

Factors to consider

– Sample selection

- Standard solutions
- Dilutions of a concentrated specimen
- Proficiency Testing specimens for linearity

– Use preferably 5 different levels of concentrations

- May require more than 5 levels to determine where linearity “falls out”

Reportable Range Experiment

Step 1: Prepare samples

– Commercial samples or patient samples.

– Choose at least 5 different concentrations

– One near the zero level or estimated lower level of detection limit, and one slightly above the upper limit of the manufacturer's reportable range.

Reportable Range

Step 2: Perform measurements

- NCCLS – 4 measurements on each specimen.
- Westgard – 3 measurements are sufficient.
- Calculate the mean of the measurements for each concentration level.

Reportable Range

Step 3: Plot data

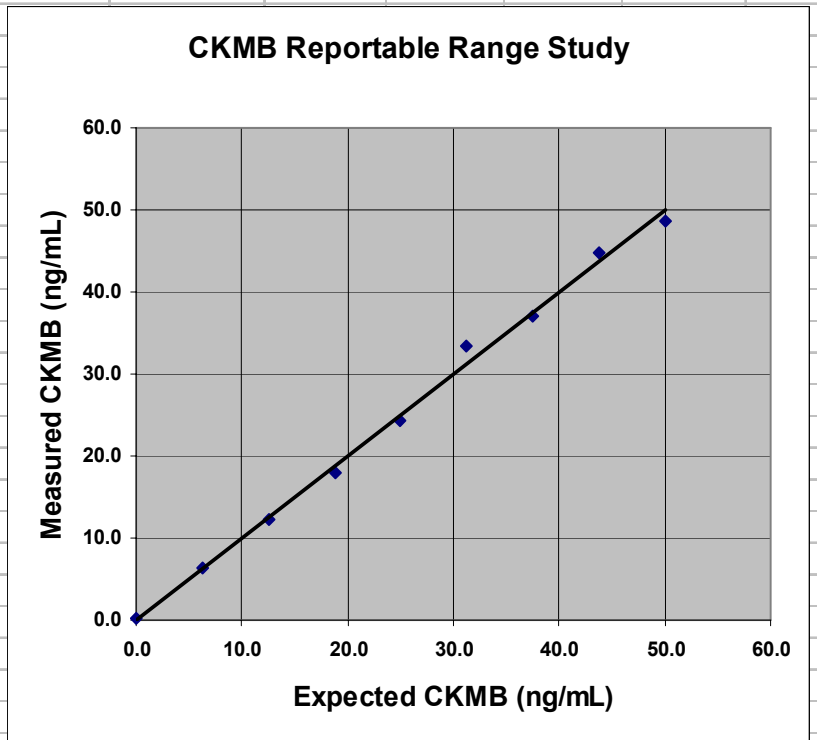
- Measured mean values on y axis vs the known or assigned values on the x axis.
- Manually draw the best straight line through data points. (Do not use the computer)

– Give more weight to the lowest points in the series.

- Inspect for linearity
- Make visual decision as to the acceptable reportable range.

Example CKMB Reportable Range Verification Study

			Measured					
		Expected	Rep1	Rep2	Rep3	Mean	Diff	% Diff
Blank	A	0.0	0	0.5	0	0.2	0.17	
	B=A+C	6.3	6	6	7	6.3	0.08	1.33
	C=A+E	12.5	13	12	12	12.3	-0.17	-1.33
	D=A+E	18.8	19	18	17	18.0	-0.75	-4.00
	E = A+F	25.0	22	27	24	24.3	-0.67	-2.67
	F=E+G	31.3	32	35	33	33.3	2.08	6.67
	G=E+I	37.5	36	38	37	37.0	-0.50	-1.33
	H=G+I	43.8	45	46	43	44.7	0.92	2.10
High CAL	I	50.0	48	51	47	48.7	-1.33	-2.67

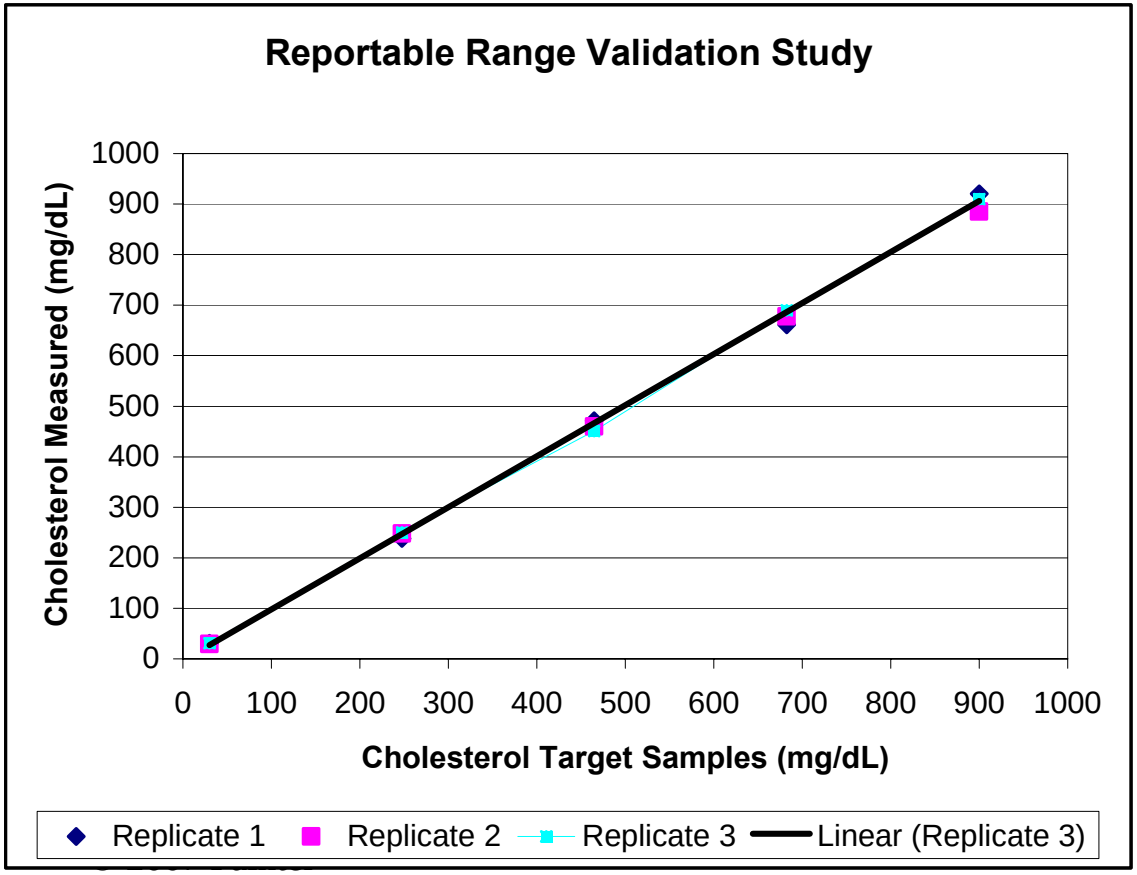


	2.0 mL P1	1.5 mL P1 0.5 mL P5	1.0 mL P1 1.0 mL P5	0.5 mL P1 1.5 mL P5	2.0 mL P5
	Pool-1	**Pool-2**	**Pool-3**	**Pool-4**	**Pool-5**
	30	**248**	**465**	**683**	**900**
Replicate 1	31	238	471	661	920
Replicate 2	29	248	460	678	885
Replicate 3	31	250	451	690	910
Mean	30.3	245.3	460.7	676.3	905.0
SD	1.2	6.4	10.0	14.6	18.0
%CV	3.8	2.6	2.2	2.2	2.0

r = 0.99992

Slope = 1.00245

Intercept= -2.606897



Reportable Range Validation Study

(A)

Get sample with what is believed to be highest level that can be assayed without dilution

(B)

Get sample with lowest level that is believed to be measurable

(C)

Perform the mixing study shown with 5 pools, graph the results and determine if reportable range is acceptable

Assay Reference Intervals

An Essential Element to Assay Clinical Utility

- Central 95% of results from a reference population - IFCC/NCCLS definition
- Excludes 2.5% above and below interval
- For healthy population are “Health-associated Reference intervals”
- Can be any population, but must be defined
  – eg, pregnant, premature, hospitalised, treated.

Method Validation

Reference Intervals or Normal Reference Range:

- Verification of the manufacturer-supplied reference intervals for the population being served by the laboratory must be assessed.
- It should be the last experiment to be studied in the method validation process.

Creating A Reference Interval

- Define and select reference population
- Define collection conditions and numbers
- Collect samples
- Analyze samples
- Perform statistical evaluation
- Put into practice

Define Reference Population

- Source
  - eg blood bank, lab volunteers, students
- Numbers
- Exclusions
- Likely Partitioning
  - Age
  - Sex
  - Other
- **Difficult to get extremes of age and high numbers**

Reference Intervals

- Several different ways to validate the transfer of the manufacturer's reference intervals to your individual lab.
- "divine judgment"
- Verification with 20 samples
- Estimation with 60 samples
- Full reference interval study

Reference Intervals

Divine judgment

- If there is consistency in demographics of the manufacturer's study population and the population served by the local lab, then the manufacturer reference intervals may be subjectively transferred to your lab.
- Decision should be made by Lab Medical Director or equivalent.

Reference Intervals

Verification with 20 samples

To transfer the manufacturer's reference intervals to your lab:

- Test 20 samples from healthy individuals representing your local population.
- If < 3 values fall outside the vendors reference interval, you may consider the reference interval verified.

Reference Intervals

Estimation with 60 Samples

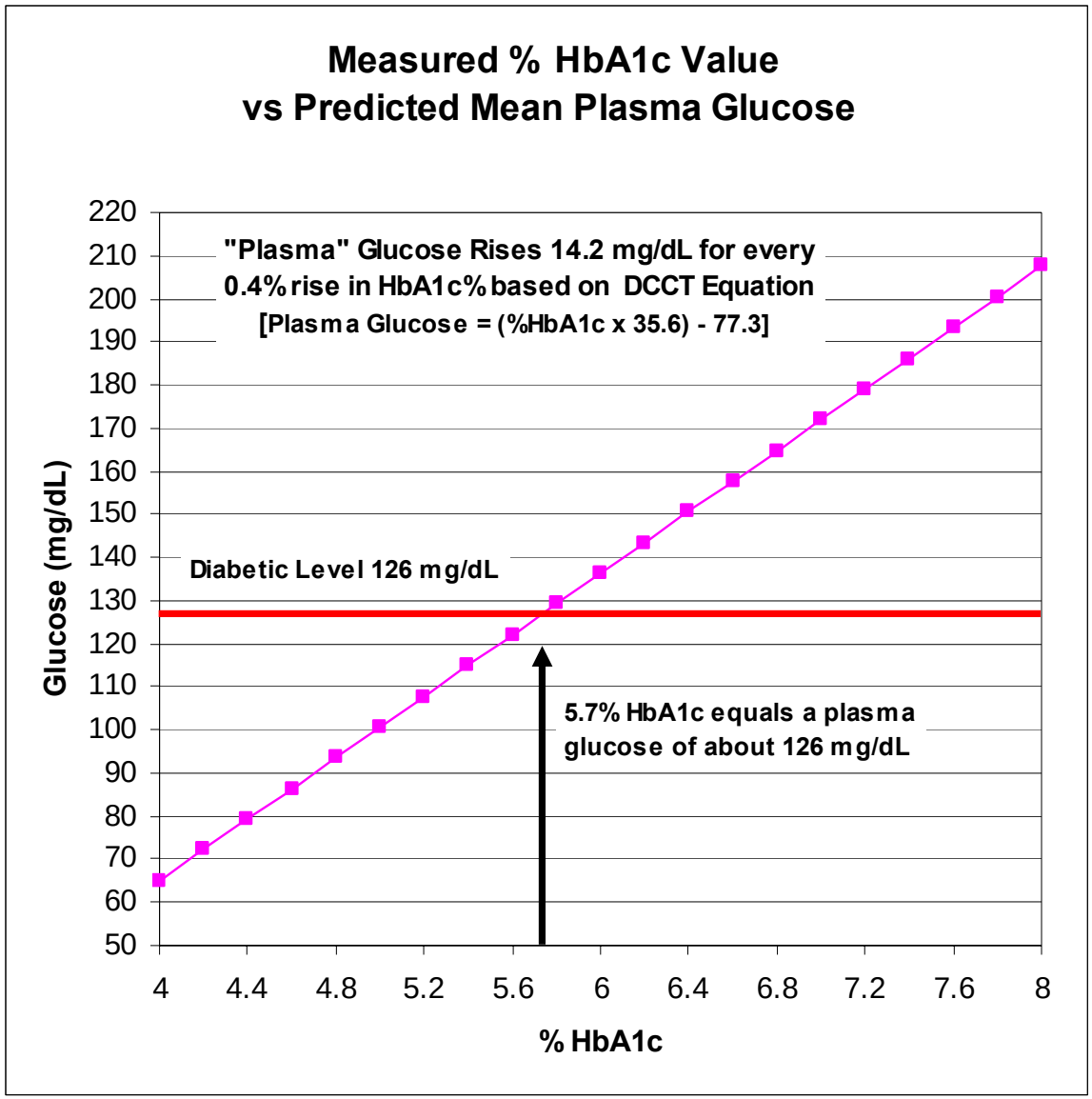
- Collect and analyze samples from 60 healthy individuals from your local population.
- Estimate the reference intervals from the 60 samples and compare it with the reported manufacturer's intervals.

Reference Intervals

Full Reference Interval Study

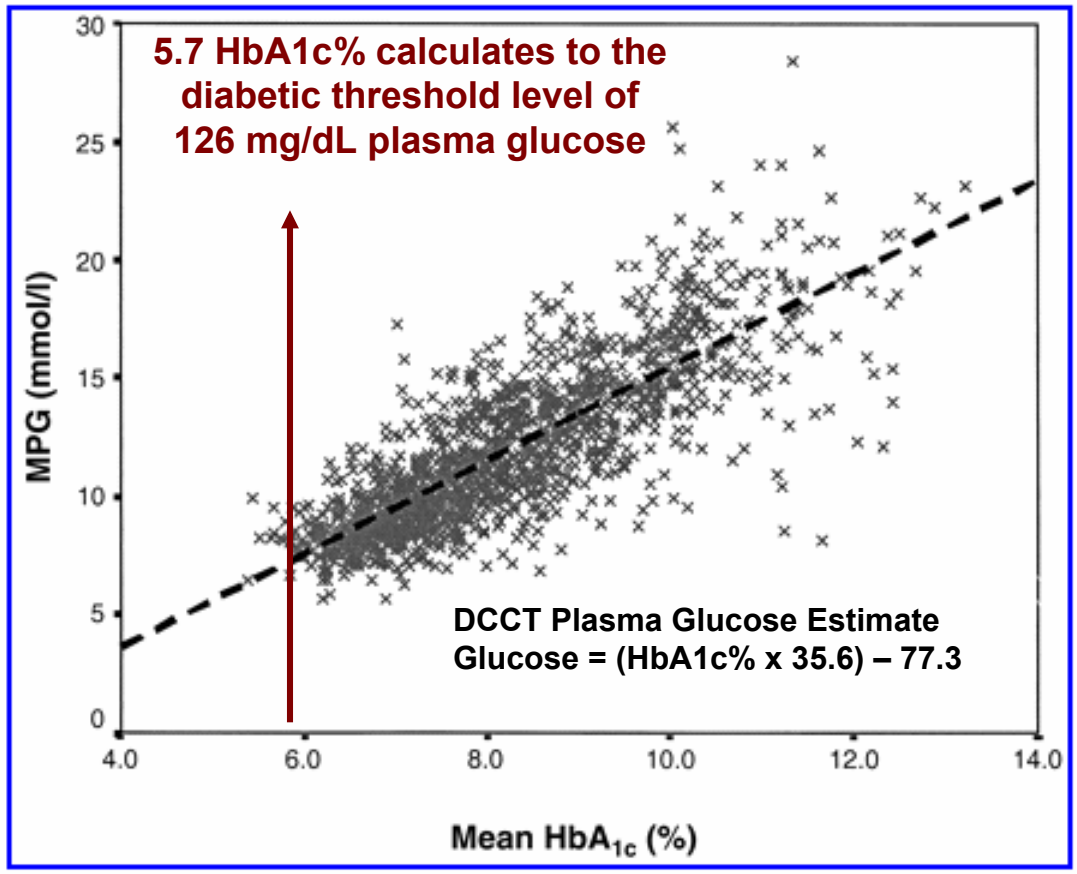
- Recommended when the demographics of the populations are different.
- Minimum requirement = 120 individuals from each group i.e. 120 men and 120 women.

Graph showing the general relationship between plasma glucose and HbA1c% level that is based on the formula derived from the Diabetes Control and Complication Trial (DCCT) published in Diabetes Care 2002 Feb;25(2):275-8.



HbA1c%	Glucose Plasma
4	65
4.1	69
4.2	72
4.3	76
4.4	79
4.5	83
4.6	86
4.7	90
4.8	94
4.9	97
5	101
5.1	104
5.2	108
5.3	111
5.4	115
5.5	119
5.6	122
5.7	**126**
5.8	129
5.9	133
6	136
6.1	140
6.2	143
6.3	147
6.4	151
6.5	154
6.6	158
6.7	161
6.8	165
6.9	168
7	172

The actual Diabetes Control and Complication Trial (DCCT) patient result data published in Diabetes Care 2002 Feb;25(2):275-8 shows a lot more variation in patient glucose levels compared to HbA1c% measured than most people realize. This is the exact data set used to derive the DCCT formula for estimating mean plasma glucose from HbA1c% level. Significantly, virtually no non-diabetic patients were tested.



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4.9	97
5	101
5.1	104
5.2	108
5.3	111
5.4	115
5.5	119
5.6	122
5.7	**126**
5.8	129
5.9	133
6	136
6.1	140
6.2	143
6.3	147
6.4	151
6.5	154
6.6	158
6.7	161
6.8	165
6.9	168
7	172

Generating Reference Intervals

Is hard to do well

Requires time, effort and money

But any local data may be very useful

"Impractical" Intervals

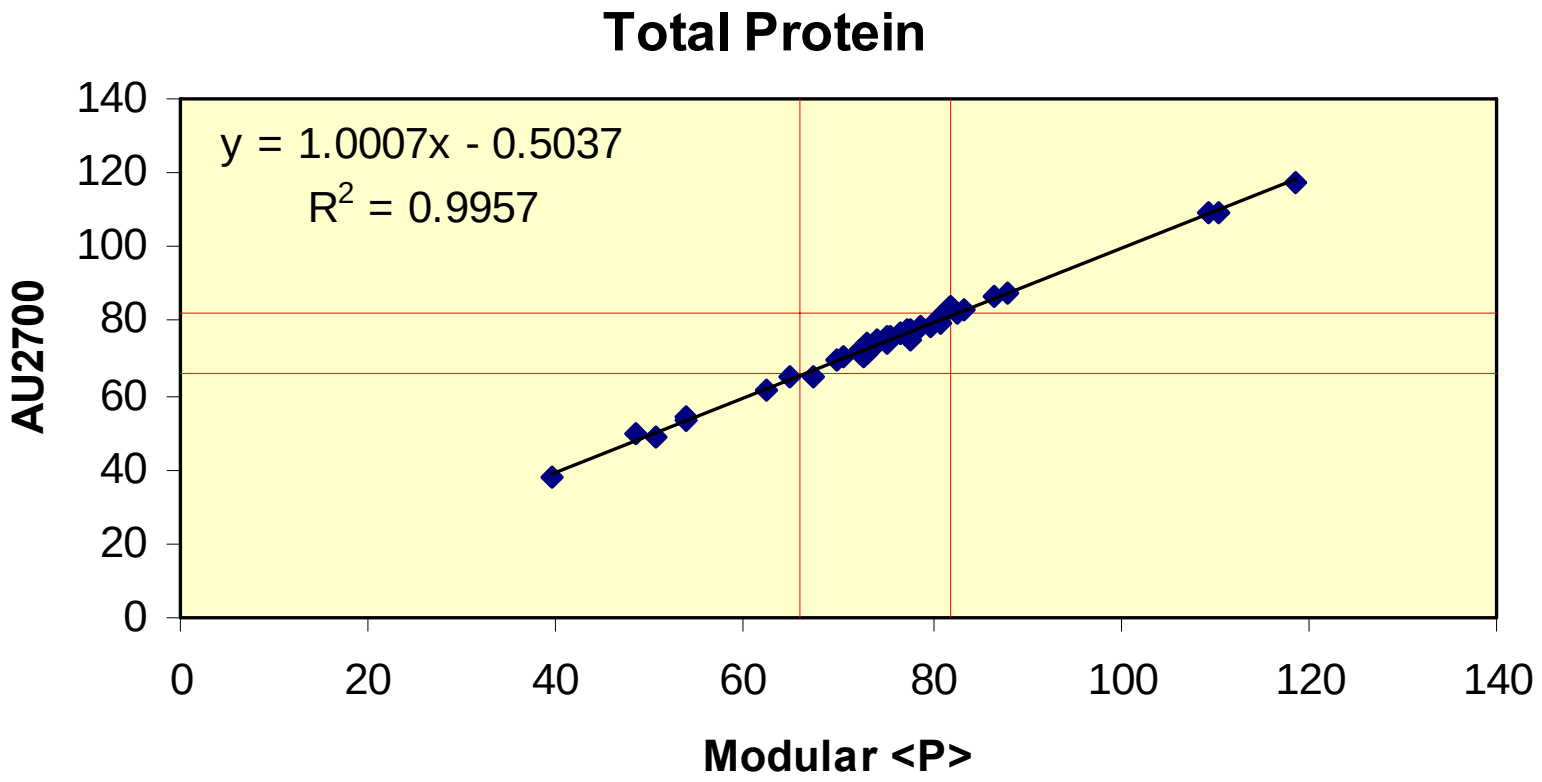
Some reference intervals are essentially impossible to produce from local studies:

- **Pediatric intervals**
- **Stages of pregnancy (eg hCG in 5th week)**
- **Stages of menstrual cycle**
- **Nutritional parameters**
  - **Reflects local diet**
  - **May normalize deficiency state**

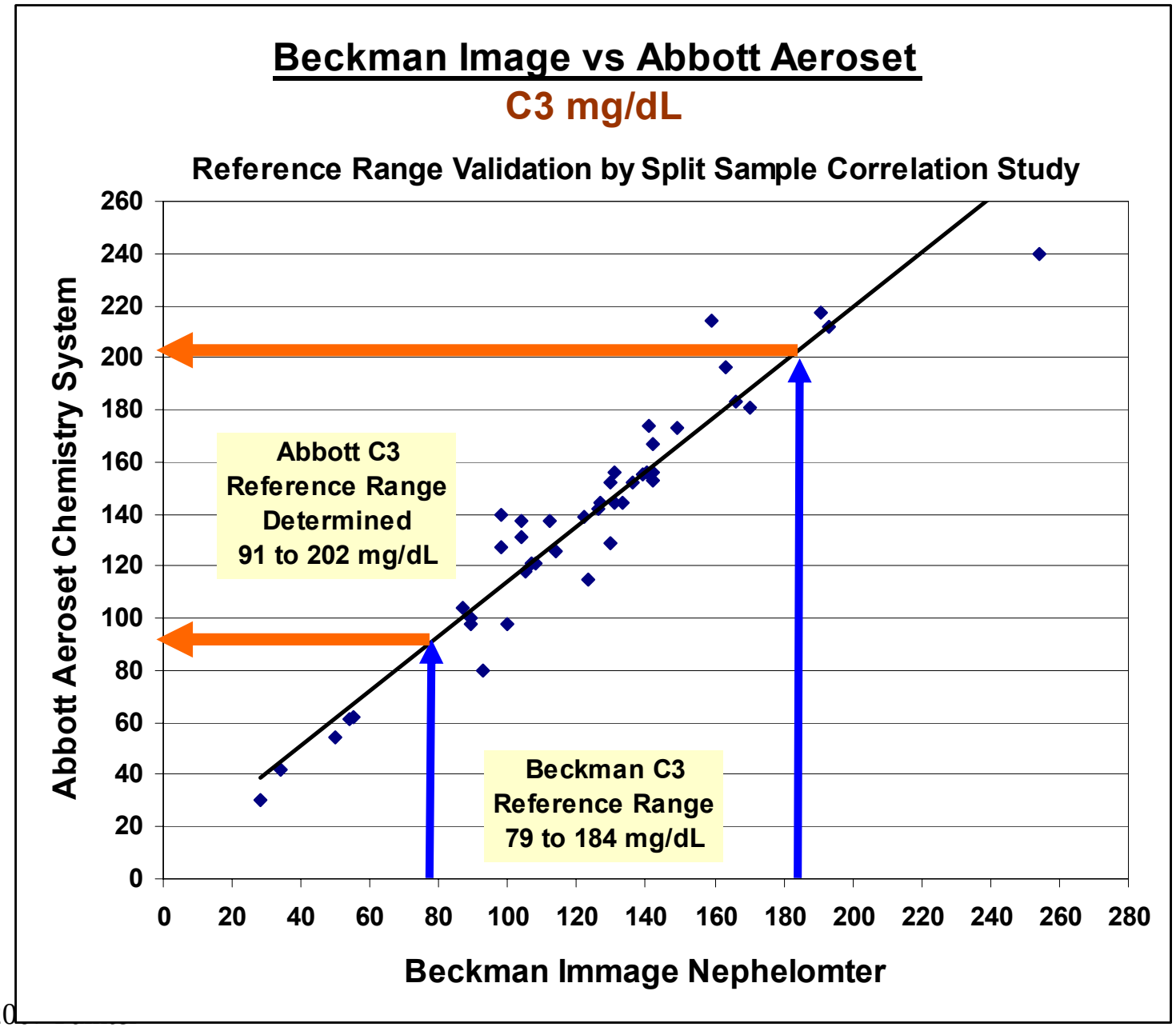
Transfer Intervals from Previous Method

- Implies previous intervals are good
  - Check source and validity
- Transfer requires good correlation
- Advantage is clinical acceptance

Transferring Reference Intervals



Wide range of results, assayed over several days, excellent correlation And linearity. Transfer with no problem



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Validation of Reference Intervals

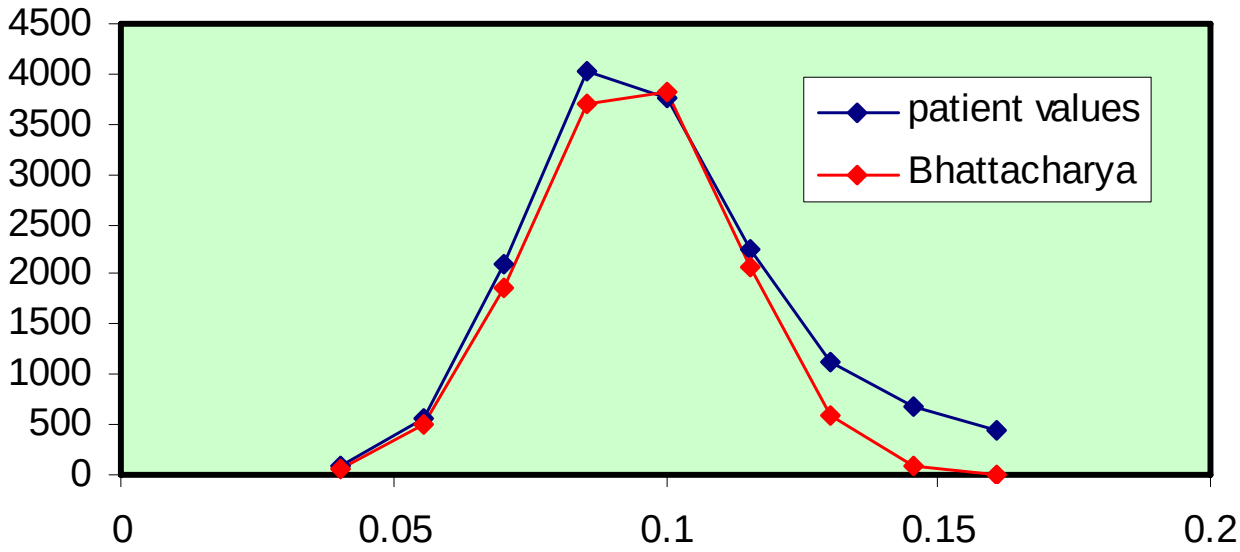
- CLSI (NCCLS) protocol
- Measure 20 samples appropriate for reference interval on new method
- Exclude outliers
- If 2 or fewer are outside proposed inetrvals
  - Accept intervals
- If >2 are outside proposed intervals
  - Measure another 20
  - If 2 or fewer are outside – accept intervals
- Cannot detect overly wide intervals

Review Previous Method

- Previous method may have significant amounts of data (information)
- For many assays many of the results will be on “normal” patients
- For all assays will allow assessment of previous reference intervals
- Methods:
  - Inspection
  - Frequency histograms (all data, some data)
  - Formal methods (Bhattacharya)

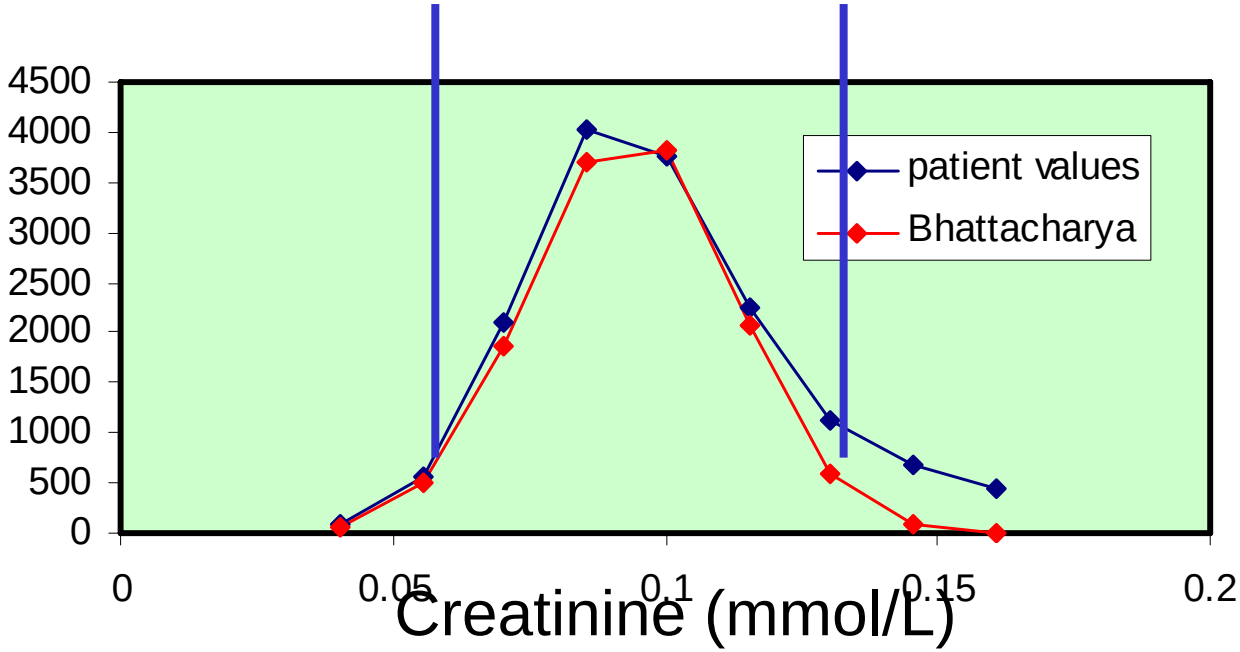
Bhattacharya

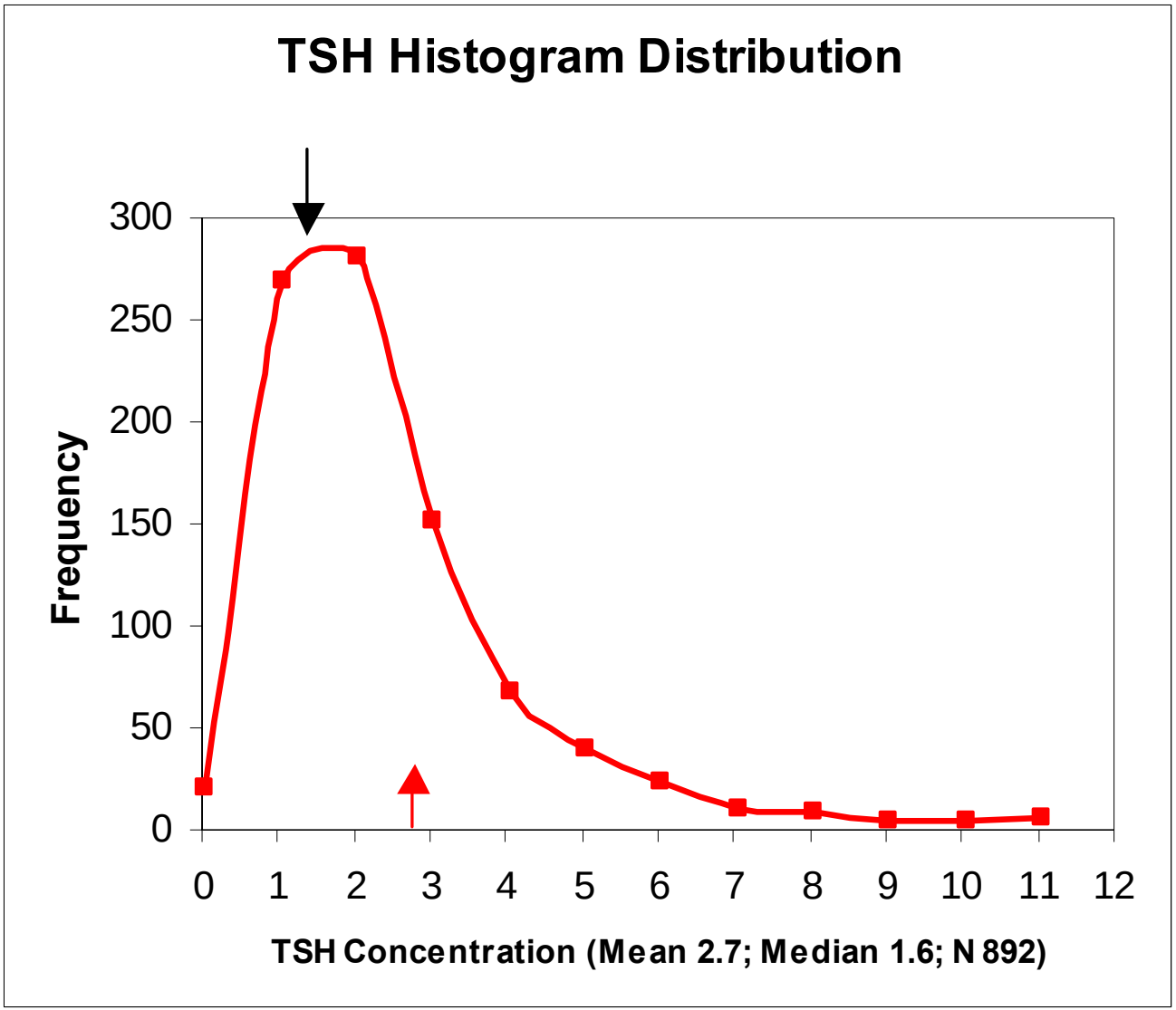
- Assumes Gaussian (or Log Gaussian) distributions
- Assumes a significant proportion of requests are on unaffected individuals

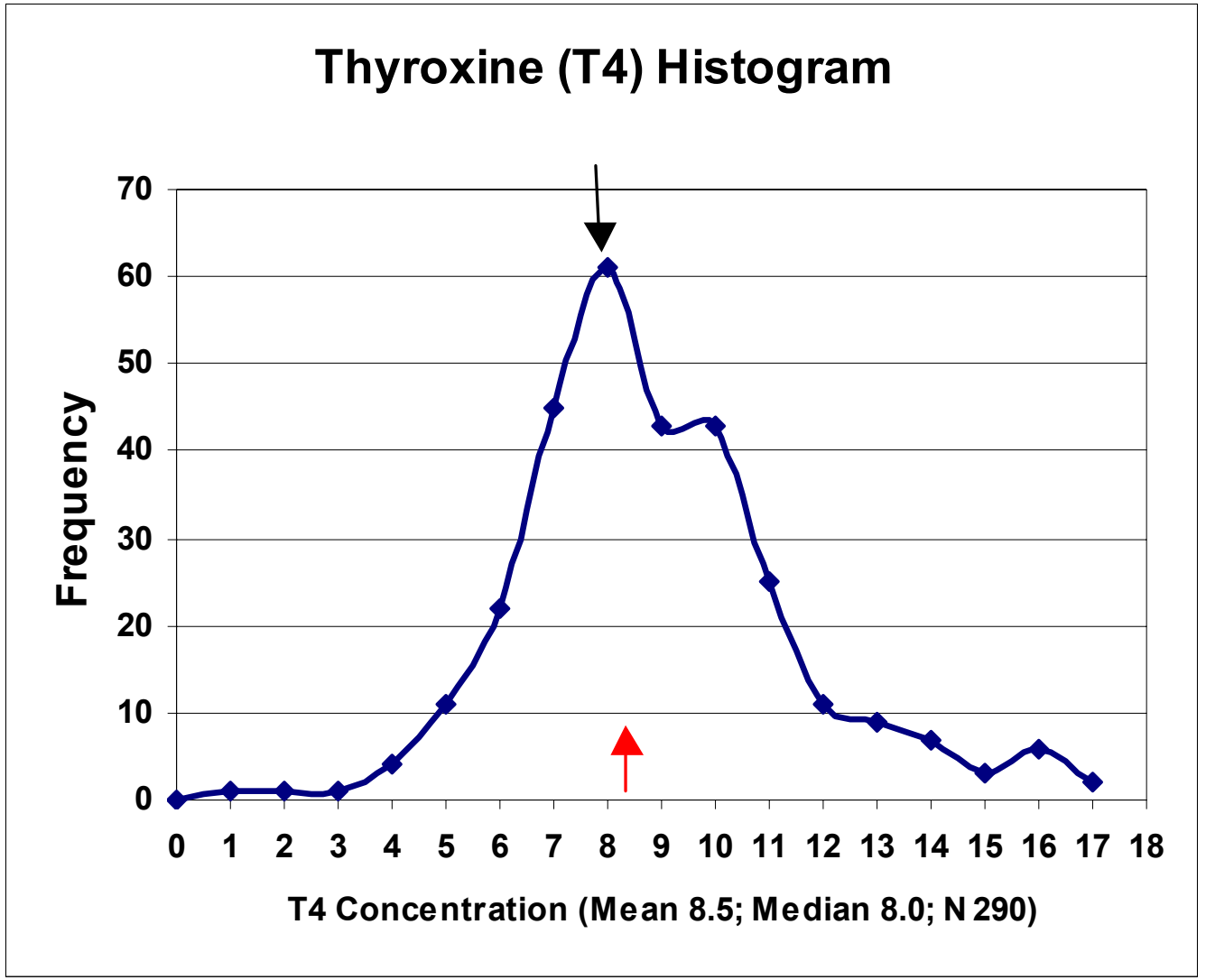


Data Mining

- Bhattacharya ignores effects of outliers and samples not part of majority distribution.
- Reference intervals based on majority.

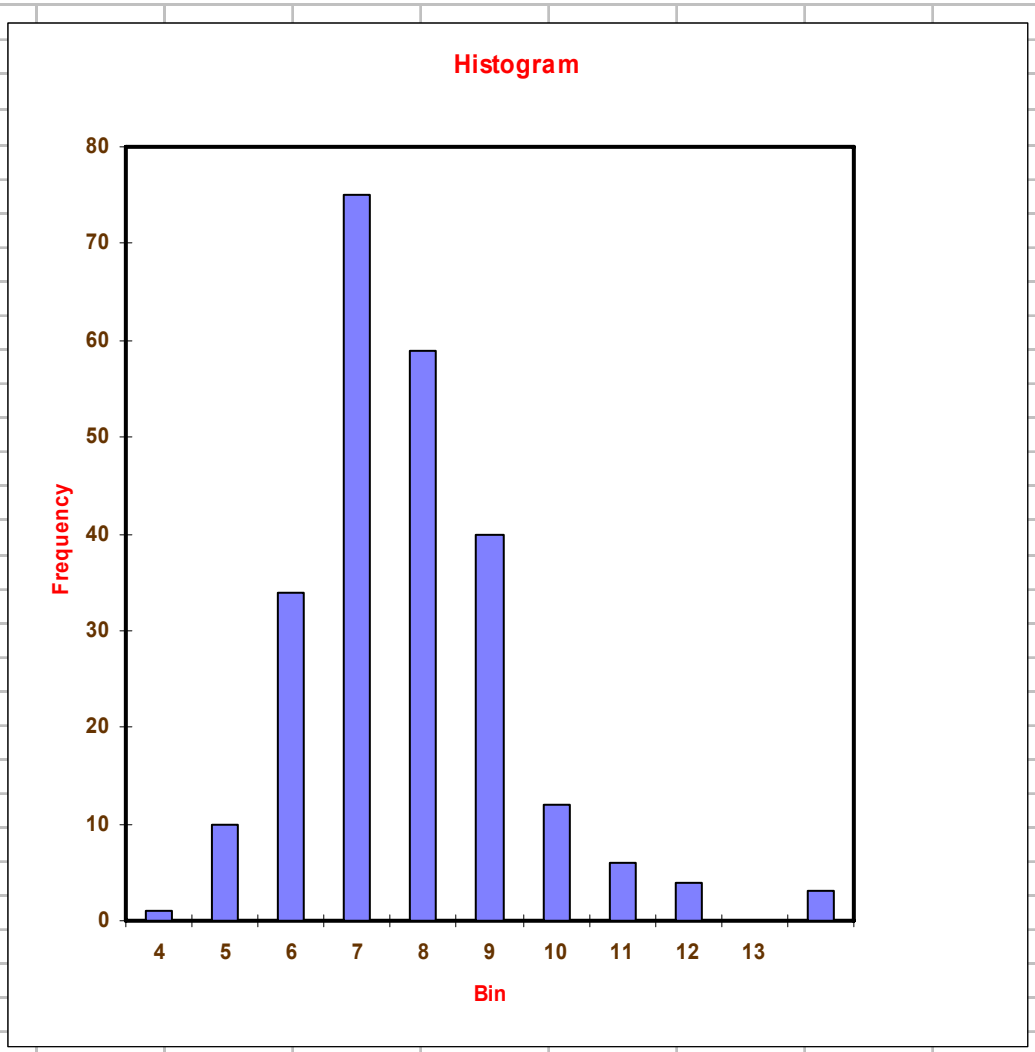






Tracking Physician % HbA1c Patient Management

Average HbA1c% =		7.4	6 Mo Count
Std. Dev. HbA1c% =		1.7	243
Median HbA1c% =		7.1	
4	*Bin*	*Frequency*	% in Range
5	4	1	0.41
6	5	10	4.12
7	6	34	13.99
8	7	75	30.86
9	8	59	24.28
10	9	40	16.46
11	10	12	4.94
12	11	6	2.47
13	12	4	1.65
	13	0	0.00
	More	3	1.23



Modified Method Validation Requirements

Comparison of methods study
Estimates inaccuracy or bias

Replication study
Estimates imprecision

Detection Limit study
Estimates constant interferences

Recovery study
Estimates proportional interferences

Linearity study
Estimates imprecision
Determines the reportable range

Reference Range Validation
Generally requires a more extensive patient sample testing study to validate the appropriate reference range(s) to be used for the test

Section §493.1253(c) requires that the laboratory must have documentation of the verification or establishment of all applicable test performance specifications.

CKMB Assay Diagnostic Cutoff for AMI Detecton Study

Cutoff value for positive test:----> **6**
(Used to calculate Sensitivity and Specificity below)

Range of cutoff values to look at for ROC:
Maximum test cutoff value:-> **23**
Minimum test cutoff value:-> **3**

Statistics for Study Population:

Prevalence	**82.5** %	If	**Prevalence =**	**7**	%
Sensitivity	**87.88** %				
Specificity	**43** %	then:			
Pos Pred Value	**88** %		**Pos Pred Value =**	**10.4**	%
Neg Pred Value	**43** %		**Neg Pred Value =**	**97.9**	%

1 = no disease
2 = disease

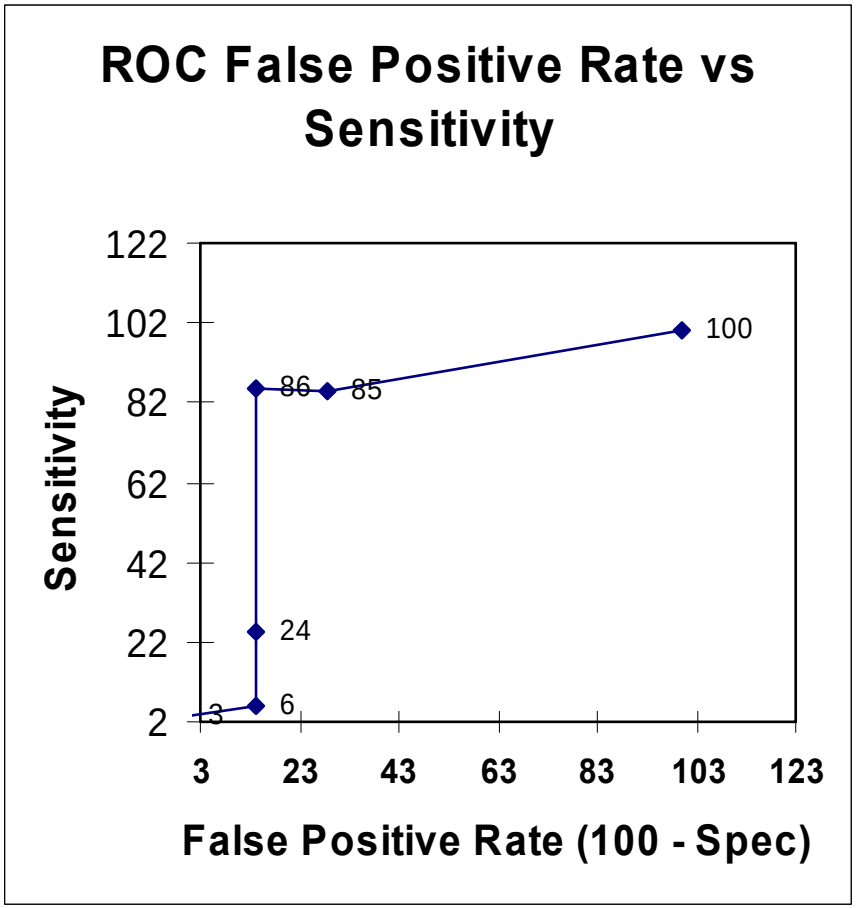
Pt ID #	Result	Dis	Cutoff = 6	True Pos	False Pos	True Neg	False Neg	Cutoff = 3	True Pos	False Pos	True Neg	False Neg
ID #	7	2	6	1	0	0	0	3	1	0	0	0
ID #	11	2	6	1	0	0	0	3	1	0	0	0
ID #	3	1	6	0	0	1	0	3	0	1	0	0
ID #	9	2	6	1	0	0	0	3	1	0	0	0
ID #	15	2	6	1	0	0	0	3	1	0	0	0
ID #	5	2	6	0	0	0	1	3	1	0	0	0
ID #	7	2	6	1	0	0	0	3	1	0	0	0
ID #	21	1	6	0	1	0	0	3	0	1	0	0
ID #	12	2	6	1	0	0	0	3	1	0	0	0

Impact of Prevalence on Assay Clinical Utility			
Medical Cutoff	**6 ng/mL**	6 ng/mL	6 ng/mL
High Value Plotted	**4 ng/mL**	4 ng/mL	4 ng/mL
Low Value Plotted	**10 ng/mL**	10 ng/mL	10 ng/mL
Prevalence (Population)	**64.6%**	2% theoretical	6% theoretical
MI's Number	**31 (of 48)**	20 (per 1,000)	60 (per 1,000)
Normal Number	**17 (of 48)**	980	940
True Pos	**31**	17.2	51.7
False Pos	**0**	0	0
True Neg	**12**	980	940
False Neg	**0**	2.8	8.3
Sensitivity	**100%**	100%	100%
Specificity	**71%**	71%	71%
Predictive Value Pos	**86%**	100%	100%
Predictive Value Neg	**100%**	99.7%	99.1%

Diseased=	40
No dis.=	960
TP =	35.2
FP =	548.6
TN =	411.4
FN =	4.8
PPV =	6.0
NPV =	98.8

-- ROC PLOT #1---

Cutoff	False (+) 100-Spec	Sensitivity
3	100	100
7	29	85
11	14	86
15	14	24
19	14	6
23	0	3



Method Validation- Additional Experiments

- Interference Experiment
- Detection Limit Experiment

These are required for the modified or non-FDA approved nonwaived tests.

Detection Limit – Analytical Sensitivity

Detection Limit Experiment

- Estimates the lowest concentration of an analyte that can be measured.
- Experiment performed by preparing a

  "blank" sample that has zero conc. of analyte

  "spiked" samples of low concentrations of analyte.
- Samples are measured repeatedly (replication),then the Means and SDs are calculated from the values obtained.

Sensitivity

Validates How Low the Assay Can Measure

(Synonyms: Limit of Detection; Limit of Quantification)

The Limit of Detection (LOD) of a method may be defined as the concentration of analyte which gives rise to a signal that is significantly different from the negative control or blank.

The LOD is the lowest concentration of analyte that can be distinguished from background.

The results obtained at the Limit of Detection are not necessarily Precise or Accurate

Sensitivity

Validates How Low the Assay Can Measure

Assays should have both high analytical sensitivity and a low limit of detection to truly discriminate between a very low level of analyte and zero level of analyte.

Analytical Sensitivity: is the slope of the analytical calibration curve and is therefore not significantly impacted by the assay's precision.

Limit of Detection (LOD): is the smallest single result which, with stated probability, can be distinguished from a suitable blank so it is highly impacted by the precision of the assay.

Sensitivity

Example Limit of Detection Study

1. Measure a sample with low but measurable level of analyte (LAL) 10 – 20 times and a zero analyte level sample (ZAL)

2. Determine the Mean and SD of "raw absorbance" values

3. Construct a slope using the Mean values of LAL and ZAL [Slope=(Conc. LAL – Conc. ZAL)/(Mean LAL – Mean ZAL)] the subtract the slope conc. from the LAL sample concentration.

Example: LOD of CKMB Assay using a 2 ng/mL LAL sample assayed 20 times gave Mean +/- absorbance units of 0.1 +/- 0.03 for the zero ZAL sample and 0.5 +/- 0.04 for the 2ng/mL LAL sample. [slope = (2-0)/(0.5-0.1) = 5.0 ng/ml per Abs unit] A 2SD upper limit of the zero sample is 0.16 abs units (0.03 x 2) plus the mean of 0.1) times 5 ng/mL per abs unit equals 0.8 ng/mL for the slope line. Subtracting 0.8 from the 2.0 ng/mL non-zero sample give the assays LOD of 1.2 ng/mL.

Sensitivity

More Rigorous Example Limit of Detection Study

1. Measure a sample with zero analyte level (ZAL) 5 times per day for 5 days to get 25 data points

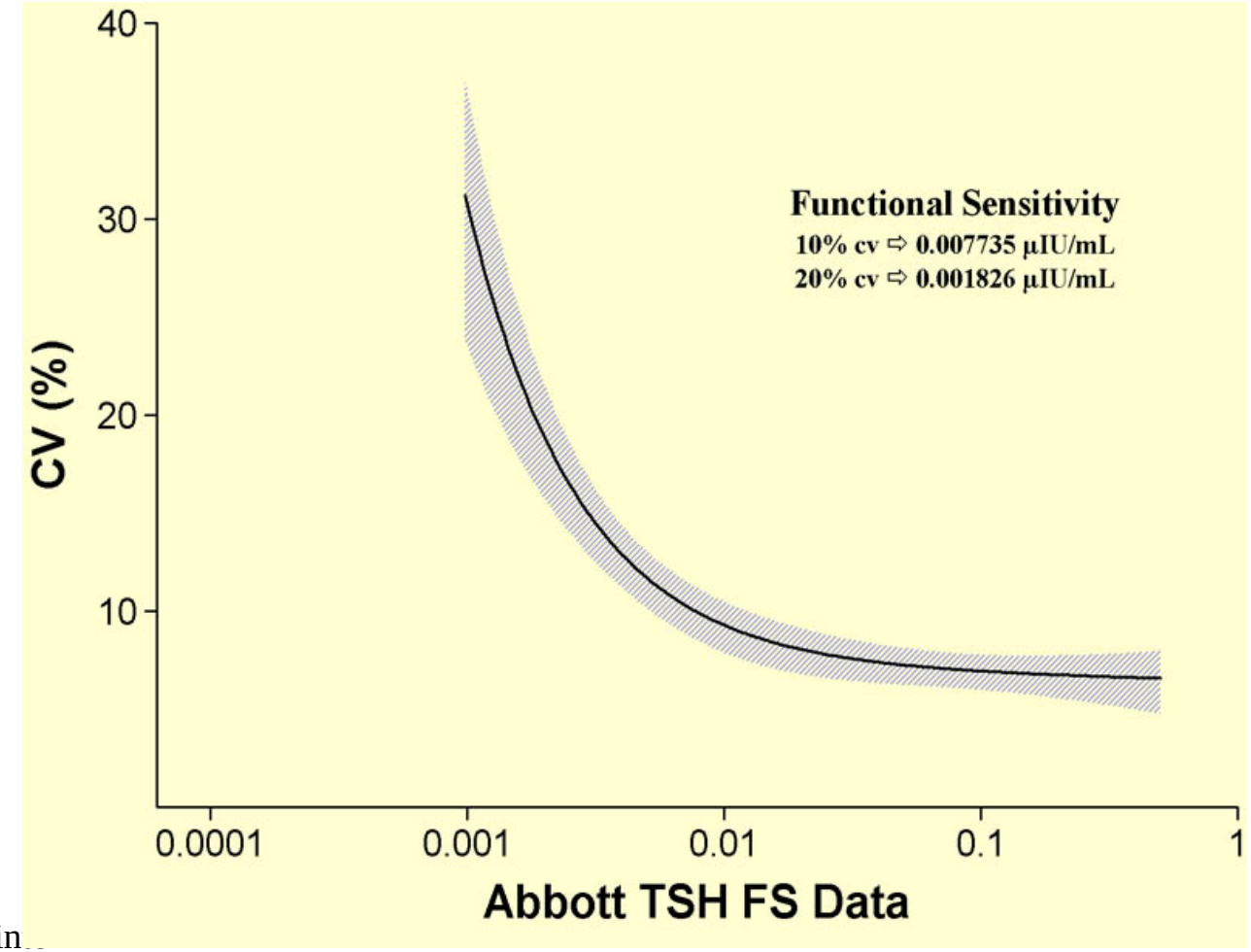
2. Determine the Mean and SD of "raw absorbance" values

3. The LOD is set at the Mean plus 3 SD's for a 99% probability or 2 SD's for 95% probability. Then the LOD concentration is determined using the slope line determined of raw absorbance units vs concentration.

Example: LOD of CKMB Assay using a 0.0 ng/mL ZAL sample assayed 5 times each for 5 days gave Mean +/- absorbance units of 0.1 +/- 0.03 for 3SD upper limit of the zero sample is 0.19 abs units (0.03 x 3) plus the mean of 0.1) times 5 ng/mL per abs unit equals 0.95 ng/mL for the slope line. Subtracting 0.95 from the 2.0 ng/mL non-zero sample give the assays LOD of 1.05 ng/mL.

Functional Sensitivity Study

Shows Assay Sensitivity At an Acceptable Precision Level



Analytical Specificity Validation

Validates Interference to Assay Performance

The Specificity of a method defines the ability of the method to measure the analyte of interest to the exclusion of other relevant components.

Selectivity describes the ability of an analytical method to differentiate various substances in a sample.

Interference Experiment

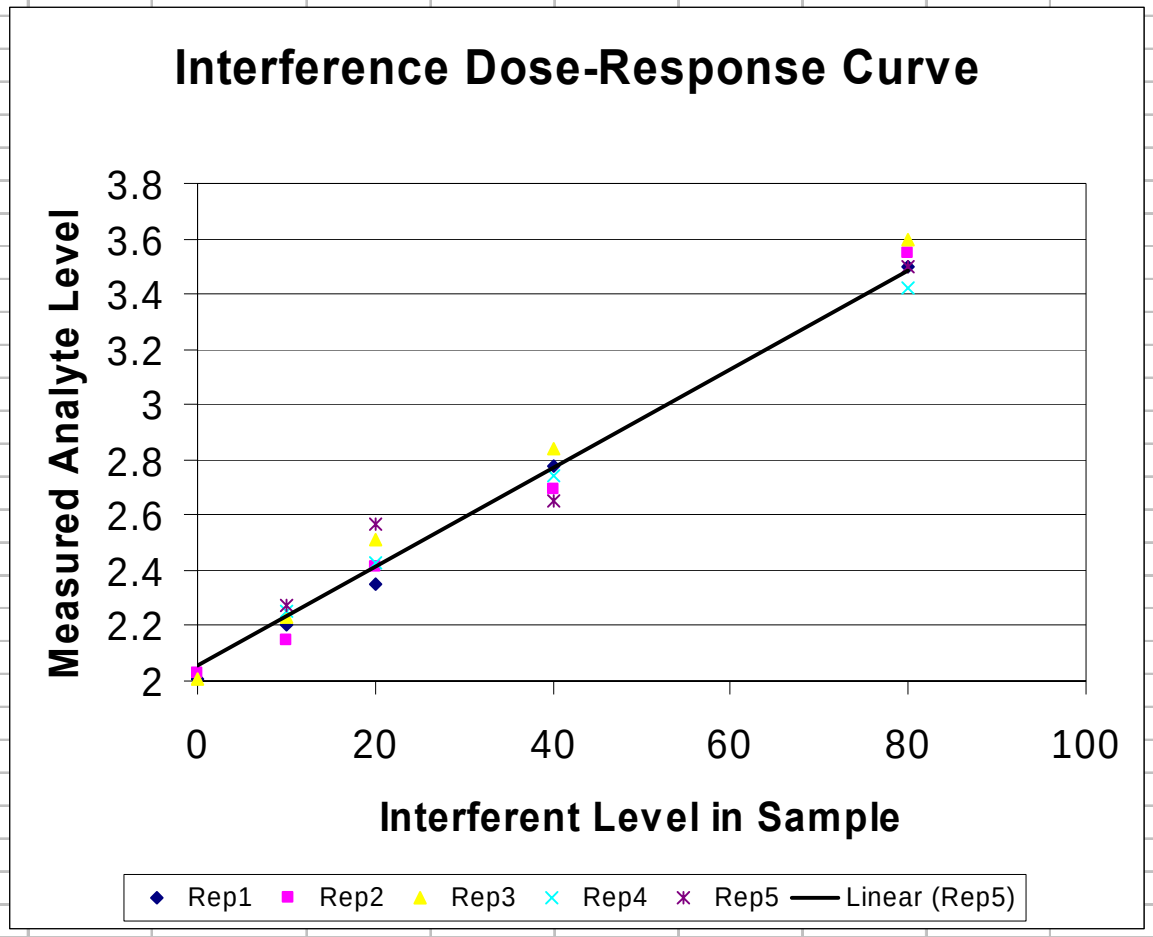
Analytical Specificity

Interference Experiment

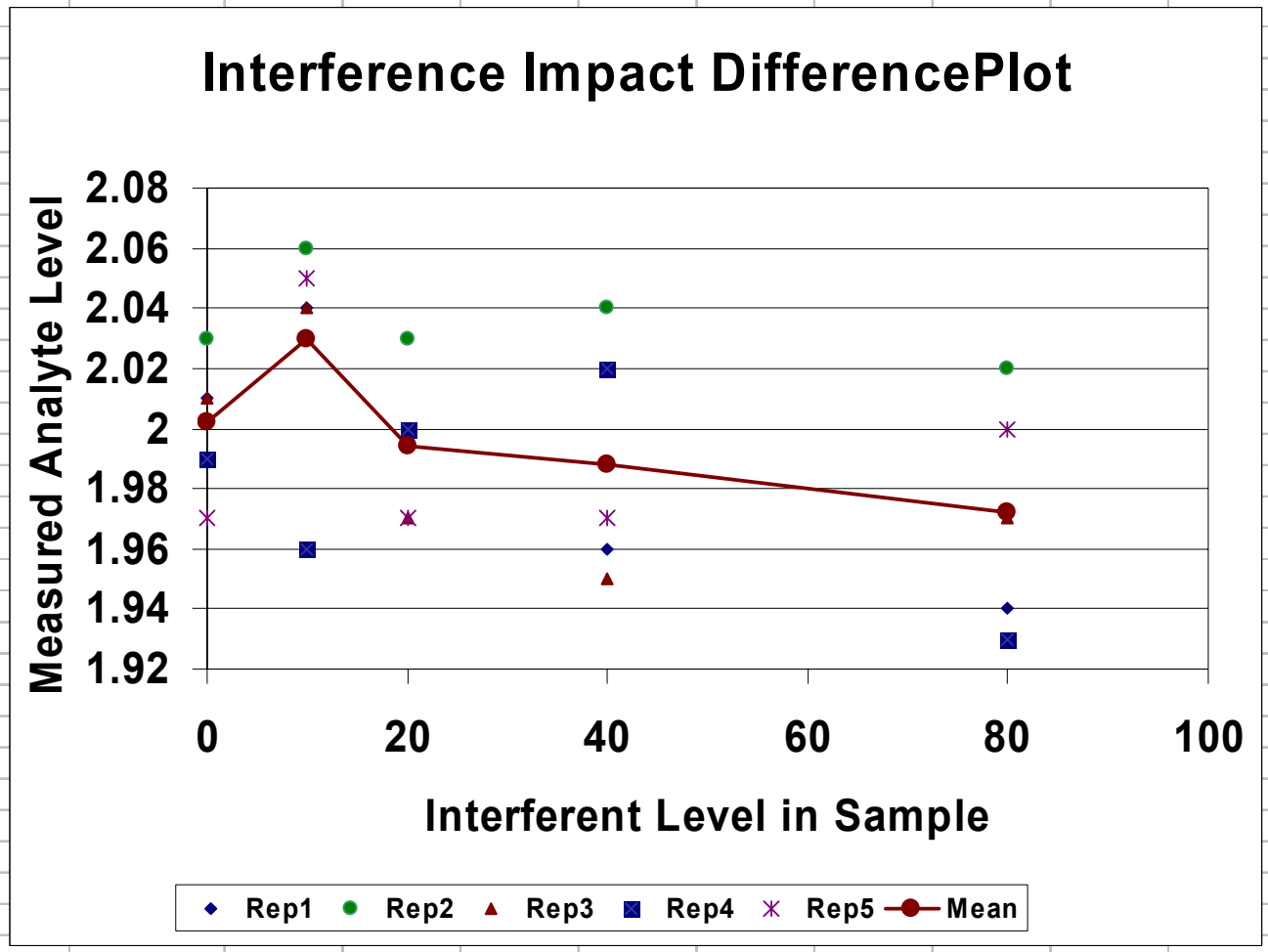
- Estimates systematic error caused by other materials that may be present in the specimen being analyzed.
- e.g. lipemia, bilirubin, hemolysis etc

Compare the results between the neat specimen and the specimen with the added substance.

Interferent	Analyte	Interferent	Rep1	Rep2	Rep3	Rep4	Rep5	Mean	M - L	SD	% Diff	95% Conf	
0	2	0	2.01	2.03	2.01	1.99	1.97	2.002	0	0.023	#DIV/0!	0.028268	2.0673
10	2	10	2.2	2.15	2.23	2.25	2.27	2.220	0.218	0.047	-77.800	0.058143	2.0673
20	2	20	2.35	2.41	2.51	2.43	2.57	2.454	0.452	0.086	-87.730	0.10721	2.0673
40	2	40	2.78	2.69	2.84	2.74	2.65	2.740	0.738	0.074	-93.150	0.092349	2.0673
80	2	80	3.5	3.55	3.6	3.42	3.5	3.514	1.512	0.067	-95.608	0.082971	2.0673
											StdError		0.067272
											Slope	0.018525	
											Intercept	2.03025	
										0	2.01		
										0	2.03		
										0	2.01		
										0	1.99		
										0	1.97		
										10	2.2		
										10	2.15		
										10	2.23		
										10	2.25		
										10	2.27		
										20	2.35		
										20	2.41		
										20	2.51		
										20	2.43		
										20	2.57		
										40	2.78		
										40	2.69		
										40	2.84		
										40	2.74		
										40	2.65		
										80	3.5		
										80	3.55		
										80	3.6		
										80	3.42		
										80	3.5		

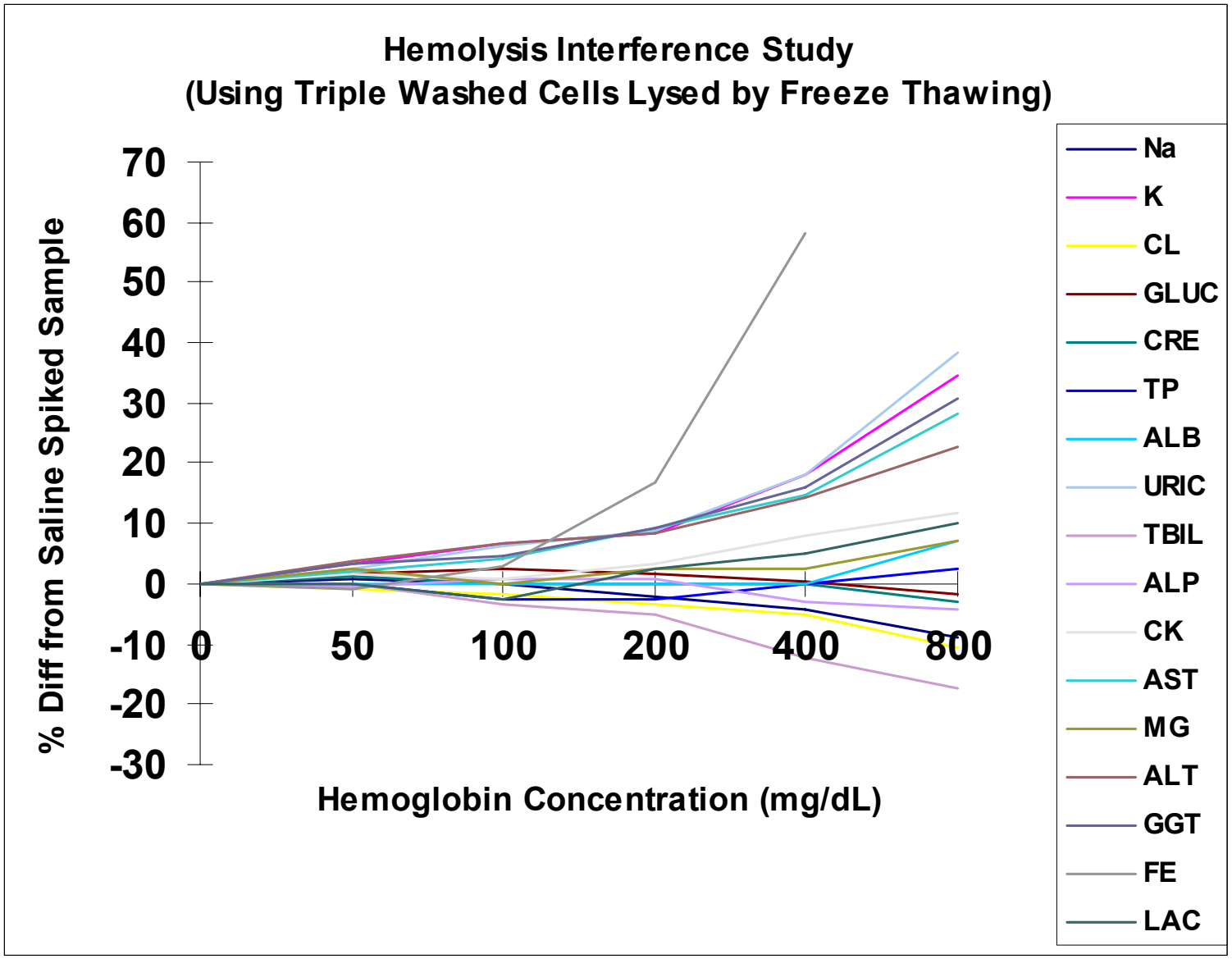


	Interferent	Analyte	Interferent	Rep1	Rep2	Rep3	Rep4	Rep5	Mean	M - L	SD	% Diff	95% Conf
L	0	2	0	2.01	2.03	2.01	1.99	1.97	2.002	0	0.023	0.100	0.028
LM	10	2	10	2.04	2.06	2.04	1.96	2.05	2.030	0.028	0.040	1.500	0.050
M	20	2	20	2	2.03	1.97	2	1.97	1.994	-0.008	0.025	-0.300	0.031
HM	40	2	40	1.96	2.04	1.95	2.02	1.97	1.988	-0.014	0.040	-0.600	0.049
H	80	2	80	1.94	2.02	1.97	1.93	2	1.972	-0.03	0.038	-1.400	0.048



The Number of Samples Required to Detect an Interferrent Induced Bias Increases as the Bias Error Decreases and/or Precision Worsens

A	Concentration of Analyte for Bias detection=	0.2
B	The 1 SD precision of assay at (A) concentration =	0.04
C	Amount of Bias wanting to be detected=	0.05
D	Calculation of (d = C/B)	1.25
E	Samples required to be tested in order to detect a Bias at the specified level with a 95% Probability	17



levels should be tested–one at the high end of the reportable range, one at the low end of the reportable range, and one near the midpoint of the reportable range.

Are there exceptions to calibration verification requirements?
Yes, there are exceptions:

- Control activities routinely used to satisfy the CLIA requirements at §493.1256 do not satisfy the calibration verification requirements. However, there is an exception for automated cell counters. For automated cell counters, the calibration verification requirements are considered met if the laboratory follows the manufacturer's instructions for instrument operation, and tests two levels of control materials each day of testing, provided the control results meet the laboratory's criteria for acceptability.
- If the test system's calibration procedure includes three or more levels of calibration material, **and** includes a low, mid, and high value, **and** is performed at least once every six months, then the requirement for calibration verification is also met.

What should I do if calibration verification fails?
If calibration verification results are unacceptable, you must repeat the test system's calibration procedure. After repeating the calibration procedure, it is good laboratory practice to run controls before resuming patient testing.

If the test system is factory-calibrated, consult with the manufacturer of the test system.

Is there a difference in the requirements for calibration and calibration verification based on the complexity of the test system?
No. The CLIA calibration and calibration verification requirements are the same for all nonwaived test systems.

Where can I find additional information about the CLIA requirements pertaining to calibration and calibration verification?
Refer to "The State Operations Manual," Appendix C–Interpretive Guidelines, Calibration and calibration Verification Procedures (§493.1255) available on the CMS website at: **www.cms.hhs.gov/clia**.

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

CDC: **www.phppo.cdc.gov/clia/default.asp**
FDA: **www.fda.gov/cdrh/CLIA/index.html** (for a listing of waived, moderate complexity and high complexity tests).

Clinical Laboratory Improvement Amendments (CLIA)

Calibration and Calibration Verification

Brochure #3

What is calibration, and how do I do it?

Information to assist your laboratory in meeting this CLIA requirement for nonwaived (moderate and high complexity) test systems!

NOTE: On January 24, 2003, the Centers for Disease Control and Prevention (CDC) and the Centers for Medicare & Medicaid Services (CMS) published laboratory regulations (CLIA) that became effective April 24, 2003. A summary of updated requirements pertaining to calibration and calibration verification is included in this brochure. However, this brochure is not a legal document. The official CLIA program provisions are contained in the relevant law, regulations and rulings. For more complete information, you may access the regulations on the Internet at **http://www.phppo.cdc.gov/CLIA/regs/toc.asp.**

What is the difference between calibration and calibration verification?

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.

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.

Calibration

Is there a new requirement for calibration?

No, the CLIA requirements for calibration have not changed. The laboratory is responsible for performing calibration as directed by the manufacturer's test system instructions, and when calibration verification of the test system (see below) does not produce acceptable results.

Reminder: Be sure to document in the laboratory's records **each** time you perform calibration.

Is calibration required for every procedure my laboratory performs?

No, calibration is not required for the following:

- Manual procedures—such as microbiology cultures and tilt-tube prothrombin time test systems.
- Microscopic procedures—such as KOH preparations, pinworm preparations, urine sediment analysis, all manual cell differential procedures, and manual cytology screening procedures.
- Procedures involving an instrument in which calibration is not practical—such as prothrombin procedures.

How do I perform calibration?

The test system's instructions should describe the process for performing calibration, as well as when and how often it is to be performed.

What materials should I use to perform calibration?

The test system's instructions should specify the number, type and concentration of the calibration material to use.

Calibration material is a solution that contains a known amount of analyte. In the past, the term "standard" was generally used to mean calibration material.

Calibration Verification

Is there a new requirement for calibration verification?

No, the laboratory has always been responsible for calibration verification or "checking" calibration. However, the process for checking a moderate complexity test system's calibration was not defined. The regulations now describe how and when calibration verification is to be performed for nonwaived (moderate and high complexity) tests.

Reminder: Be sure to document in the laboratory's records **each** time you perform calibration verification.

When must I check a test system's calibration (perform calibration verification)?

Once every 6 months (or more frequently if specified in the test system's instructions) and whenever any of the following occur:

- All of the reagents used for a test procedure are changed to new lot numbers, **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 the test's performance. This includes when the laboratory sends a test system to the manufacturer for repairs. The laboratory must check the calibration of a repaired test system before resuming patient testing and reporting 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.
- The laboratory has determined that the test system's reportable range for patient test results should be checked more frequently.

Reminder: The laboratory **is** responsible for verifying calibration on factory-calibrated test systems that cannot be calibrated by the user.

What materials should I use to perform calibration verification?

A variety of materials with known concentrations may be used to verify calibration, for example, commercially available standards or calibration materials, proficiency testing samples with known results, control materials with known values, or patient specimens with known values.

Since the purpose of calibration verification is to check whether the test system is providing accurate results throughout the reportable range, three

Sec. 493.1255 Standard: Calibration and calibration verification procedures

Calibration and calibration verification procedures are required to substantiate the continued accuracy of the test system throughout the laboratory's reportable range of test results for the test system. Unless otherwise specified in this subpart, for each applicable test system the laboratory must do the following:

(a) Perform and document calibration procedures--

(1) Following the manufacturer's test system instructions, using calibration materials provided or specified, and with at least the frequency recommended by the manufacturer;

(2) Using the criteria verified or established by the laboratory as specified in Sec. 493.1253(b)(3)--

(i) Using calibration materials appropriate for the test system and, if possible, traceable to a reference method or reference material of known value; and

(ii) Including the number, type, and concentration of calibration materials, as well as acceptable limits for and the frequency of calibration; and

(3) Whenever calibration verification fails to meet the laboratory's acceptable limits for calibration verification.

(b) Perform and document calibration verification procedures--

(1) Following the manufacturer's calibration verification instructions;

(2) Using the criteria verified or established by the laboratory under Sec. 493.1253(b)(3)--

(i) Including the number, type, and concentration of the materials, as well as acceptable limits for calibration verification; and

(ii) Including at least a minimal (or zero) value, a mid-point value, and a maximum value near the upper limit of the range to verify the laboratory's reportable range of test results for the test system; and

(3) At least once every 6 months and whenever any of the following occur:

(i) 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.

(ii) There is major preventive maintenance or replacement of critical parts that may influence test performance.

(iii) 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.

(iv) The laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.

Calibration Verification

CLIA Requirements

What: **Requires testing of samples at upper and lower reporting range limit as well as a mid-range sample**

When: **Every 6 months or change in assay or assay performance**

This requirement is satisfied if all assays are routinely calibrated using 3 or more levels that span the reportable (analytical) range

Comparison of Results Between Analyzers

Sec. 493.1281 Standard: Comparison of test results

(a) If a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple testing sites, the laboratory must have a system that twice a year evaluates and defines the relationship between test results using the different methodologies, instruments, or testing sites.

(b) The laboratory must have a system to identify and assess patient test results that appear inconsistent with the following relevant criteria, when available:

(1) Patient age.

(2) Sex.

(3) Diagnosis or pertinent clinical data.

(4) Distribution of patient test results.

(5) Relationship with other test parameters.

(c) The laboratory must document all test result comparison activities.

Producing a Quality Laboratory Product

The product produced by clinical laboratories is INFORMATION

Clinical Laboratory Method Validation Systems and Practices Should Be Designed To Assist in Assuring that the Information Reported On Testing of Patient Samples is Reliable