pSMILE
SAFETY·QUALITY·RESULTS

Validation of Qualitative and Quantitative Assays

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Objectives

- Determine the essential components required for the validation of qualitative and quantitative assays
- Understand each component of validation including precision, accuracy, linearity, and reference ranges
- Work through practical examples of point-of-care, hematology, and chemistry validations
- Navigate the Resources sections of the pSMILE website for validation tools and templates

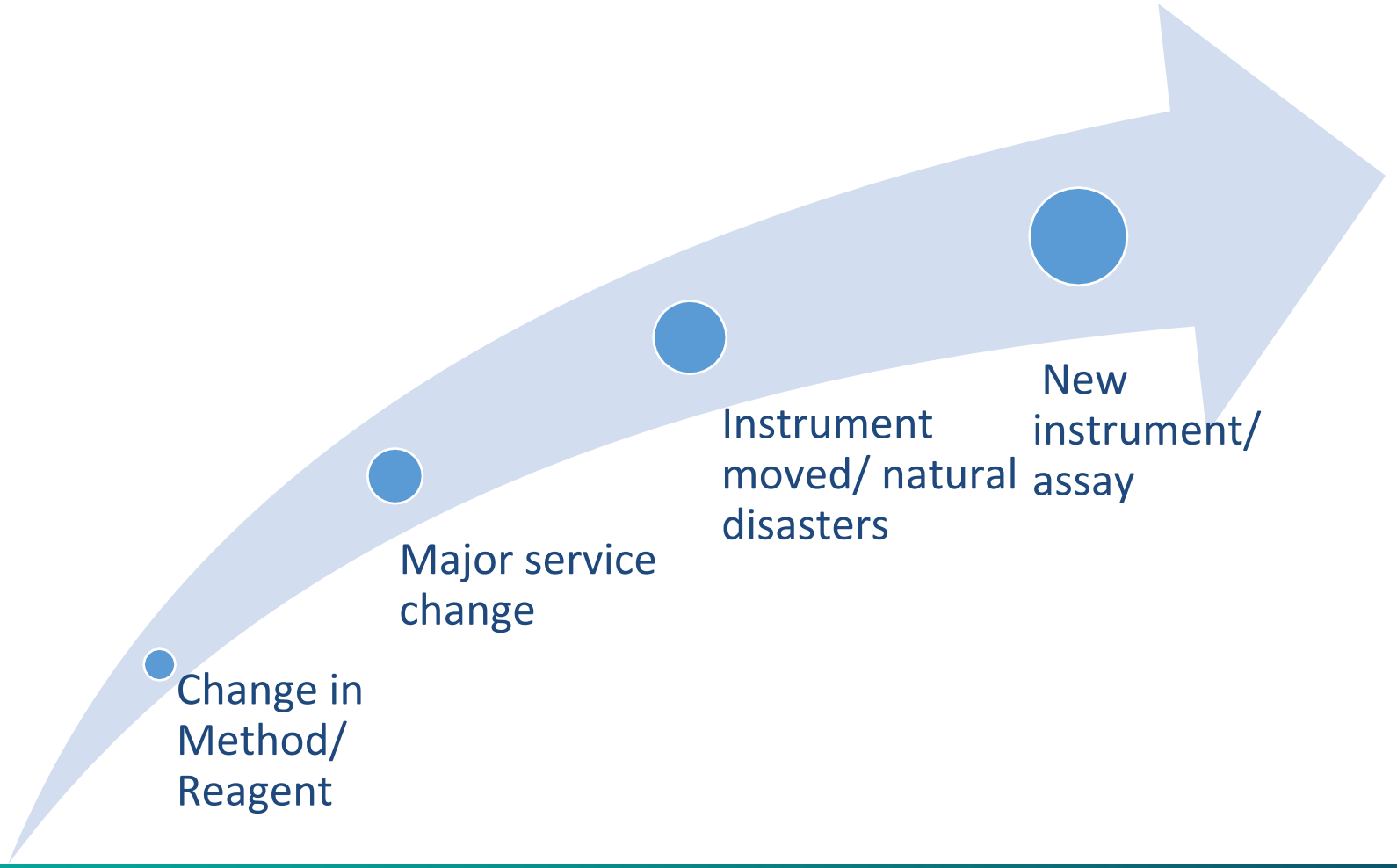
Agenda

- Introduction/Precision
- Accuracy
- Linearity, AMR, CRR
- Reference Ranges
- Questions and comments

Main Elements of Validation

- Validation is the **verification** of:
  - Precision
  - Accuracy
  - Linearity
  - Analytical Measurement Range (AMR) & Clinical Reportable Range (CRR)
  - Sensitivity & Specificity
    - FDA-approved/ non FDA-approved
  - Reference Ranges

Validate whenever there is a major change to the testing system



What is the first step?

- Make a plan for each instrument/method to be validated that includes:
  - What exactly is being validated: instrument, method, kit name, analytes, etc.
  - What type of samples will be used
  - Reference method used
  - Acceptability criteria for each type of testing

SMILE Validation Org
Hospital Complex
123 Big Road
City-Township, Country

Validation Plan for Vitros 250 Chemistry Analyzer

I. **Overview**
1. Precision
2. Accuracy
3. Linearity
4. Analytical measurement range (AMR) and Clinical reportable range (CRR)
5. Sensitivity
6. Specificity
7. Reference Range
8. Method Approval

II. **Plan:** The validation will be conducted on the Vitros 250 analyzer (serial number 25012919) for the following analytes and methods: Albumin, Alkaline Phosphatase, ALT, Amylase, AST, BUN, Calcium, Chloride, Cholesterol, CK, CO2, Creatinine, Direct Bilirubin, Glucose, HDL Cholesterol, Lactate, Lipase, Phosphorous, Potassium, Sodium, Total Bilirubin, Total Protein, Triglycerides, Uric Acid

1. Precision

a. Precision is reproducibility - the agreement of the measurements of replicate runs of the same sample. It is the process of determining the range of random error. The precision is measured in terms of coefficient of variation (CV).

b. Random Error will be evaluated by running between day and within day precision using normal and abnormal control samples. Between-day will be tested by running each sample once per day for 20 days or 4 samples per day for 5 days. Within day will be tested by running each sample 20 times in one day. The mean, standard deviation (SD) and CV will be calculated of the replicates.

c. Acceptability criteria: The % CV for each assay is expected to be equal to or less than the manufacturer's performance specifications for precision. In the event that an assay does not perform as expected, the %CV will be compared to the allowable random error (33% of SMILE Total Allowable Error Limits for between day and 25% of SMILE Total Allowable Error Limits for within day). Refer to SMILE Chemistry TE Limits table (Appendix 1).

Example Validation Plan

Templates available at psmile.org

Precision

Precision is reproducibility: the ability of a measurement to be consistently reproduced

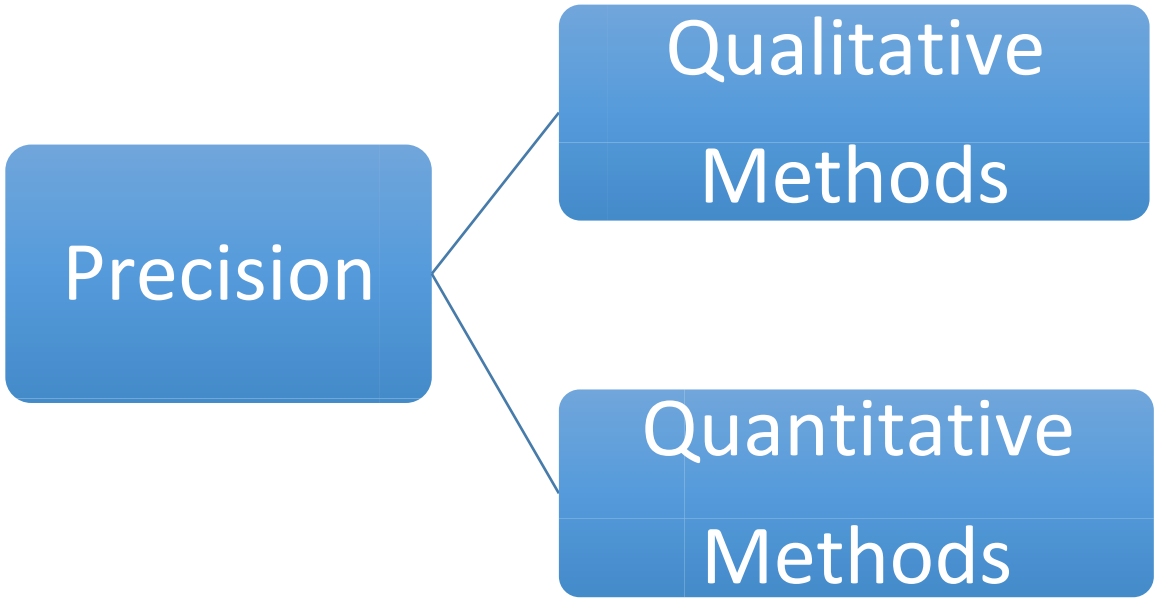
How is precision measured?

- **Coefficient of variation (CV)**
  - A statistical measure of the dispersion of data points in a data series around the mean.

$$CV=\left(\frac{SD}{Mean}\right) x\ 100$$

  - Expressed as a percentage (%)
- Replication experiments are performed to estimate **imprecision** or **random error**

Different Method Requirements



When is Precision verification required for **qualitative** assays?

1. If the qualitative results are derived from a **quantitative value such as an OD.**

AND

2. Manufacturer's **package insert** describes precision specifications for the assay.

Finding precision in package insert- **Qualitative** assays

TABLE III

ABBOTT PRISM HBsAg Assay Reproducibility

Panel Member or Control	Number of Replicates	Mean S/CO*	Intra-assay SD	Intra-assay %CV	Inter-assay[a] SD	Inter-assay[a] %CV
1	440	6.98	0.283	4.1	0.390	5.6
2	440	4.06	0.160	3.9	0.222	5.5
3	440	1.39	0.068	4.9	0.077	5.6
4	439[b]	8.86	0.513	5.8	0.596	6.7
5	438[c]	4.62	0.162	3.5	0.244	5.3
6	439[b]	1.37	0.078	5.7	0.083	6.1
7	440	0.34	0.036	10.6	0.039	11.6
Negative Control	439[b]	0.26	0.038	14.6	0.041	15.6

How to verify precision for **qualitative** assays

Short Term/ Within Run/Intra-assay Precision

- Samples: positive and negative controls
- Testing: Run each level of control 20 times on the same run, if possible

Long term/Between Run/Inter-assay Precision

- Samples: positive and negative controls
- Testing: Run each level of control at least once per day, but not more than 5 times per day, for a total of 20 runs

Calculation and Acceptability – **Qualitative** Methods

1. Calculate the mean, SD, and CV of the **numerical result (OD)** for each level of control
2. Compare your CV to the manufacturer's CV
3. Lab CV should be ≤ manufacturer's CV

Qualitative Precision Example

ABBOTT murex

E

Murex HIV Ag/Ab Combination

REF 7G79-01 / 02

C14GE41GB

GE41/42

Read Highlighted Changes

Revised June 2009

Murex HIV Ag/Ab Combination

Table 4

Murex HIV Ag/Ab Combination - Assay Reproducibility

Specimen	Number of Assays	Number of Replicates	Mean Absorbance/ Cut-off ratio	Intra-assay %CV	Inter-assay %CV
Negative Control	4	10	0.266	8.7	11.3
HIV-1 Positive Control	4	10	8.287	4.3	4.7
QA01	4	10	3.672	4.6	7.3
QA02	4	10	4.696	5.6	12.9
QA03	4	10	3.006	3.9	4.2
QA04	4	10	1.663	6.8	9.2

Results- Acceptable or Not?

NOM_PRENOM	Abs	RESULTAT	DAT_TEST
	0.29	neg	05-Feb-09
	0.259	neg	05-Feb-09
	0.308	neg	05-Feb-09
	0.323	neg	05-Feb-09
	0.298	neg	05-Feb-09
	0.298	neg	05-Feb-09
	0.282	neg	05-Feb-09
	0.285	neg	05-Feb-09
	0.296	neg	05-Feb-09
	0.287	neg	05-Feb-09
	0.265	neg	05-Feb-09
	0.326	neg	05-Feb-09
	0.29	neg	05-Feb-09
	0.303	neg	05-Feb-09
	0.31	neg	05-Feb-09
	0.277	neg	05-Feb-09
	0.317	neg	05-Feb-09
	0.291	neg	05-Feb-09
	0.27	neg	05-Feb-09
	0.29	neg	05-Feb-09
Mean	**0.293**		
SD	**0.018**		
CV	**6.17%**		

NOM_PRENOM	Abs	RESULTAT	DAT_TEST
	3.628	POS	05-Feb-09
	3.588	POS	05-Feb-09
	3.59	POS	05-Feb-09
	3.547	POS	05-Feb-09
	3.498	POS	05-Feb-09
	3.533	POS	05-Feb-09
	3.595	POS	05-Feb-09
	3.68	POS	05-Feb-09
	3.528	POS	05-Feb-09
	3.644	POS	05-Feb-09
	3.485	POS	05-Feb-09
	3.558	POS	05-Feb-09
	3.521	POS	05-Feb-09
	3.659	POS	05-Feb-09
	3.6	POS	05-Feb-09
	3.642	POS	05-Feb-09
	3.651	POS	05-Feb-09
	3.54	POS	05-Feb-09
	3.566	POS	05-Feb-09
	3.654	POS	05-Feb-09
Mean	**3.585**		
SD	**0.058**		
CV	**1.63%**		

Precision for **quantitative** methods

- Precision verification is required for **all quantitative methods**

Finding precision in package insert- **Quantitative** assays

SYNCHRON® System(s)
Chemistry Information Sheet

BUN
Urea Nitrogen
Kit Reorder # 442750

PRECISION

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

TABLE 8 PRECISION VALUES

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[1]		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Within-run	Serum/Plasma	2.0	0.71	66.7	24.0	3.0
	Urine	3.0	1.07	100.0	35.7	3.0
Total	Serum/Plasma	3.0	1.07	66.7	24.0	4.5
	Urine	4.5	1.61	100.0	35.7	4.5

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[15] Each laboratory should characterize their own instrument performance for comparison purposes.

For **Hematology instruments**- also see operator's manual

How to verify precision for **quantitative** assays

- Two Levels:
  - Normal/Abnormal
  - Hematology: Normal/High Abnormal
- Patient Samples *or* Quality Control

Hematology Controls- Low level control is not recommended

How to verify precision for **quantitative** assays

Short Term/ Within Run/Intra-assay Precision

- Testing: run each level of control 20 times on the same run, if possible, or at a minimum within the same day

Long term/Between Run/Inter-assay Precision

- Testing: run each level of control at least once per day, not more than 5 times per day, for a total of 20 runs

Calculation and Acceptability – **Quantitative** Methods

1. Calculate mean, SD, and CV for each level using the 20 data points
2. Compare your CV to the manufacturer's CV
3. Lab CV should be ≤ manufacturer's CV
4. *If Lab CV > manufacturer's CV, compare to 25% or 33% of Total Allowable Error (TEa)*

Total allowable error (TEa)

- Allowable error- the amount of error that can be tolerated without invalidating the medical usefulness of the analytic result

- pSMILE Recommendations for TEa are based on CLIA and are the same criteria used to evaluate EQA

pSMILE Recommendations for TEa

pSMILE Minimum Recommended Validation requirements for

Chemistry Total Allowable Error (TEa)

Analyte	pSMILE Total Error Limits (whichever is greater)		Precision	
	Percentage	Minimum detectable difference or absolute	Short Term 25% TE (1)	Long Term 33% TE (1)
Albumin	± 10% (1)	±0.2 g/dL 2.0 g/L	2.5%	3.3%
Alk. Phos	± 30% (1)	±5.0 U/L	7.5%	9.9%
ALT	± 20% (1)	±5.0 U/L	5.0%	6.6%
Amylase	± 30% (1)	±5.0 U/L	7.5%	9.9%
AST	± 20% (1)	±5.0 U/L	5.0%	6.6%
Bilirubin, Direct	± 20% (1)	± 0.4 mg/dL	5.0%	6.6%
Bilirubin, Total	± 20% (1)	± 0.4 mg/dL	5.0%	6.6%
Calcium	± 8% (2)	± 1.0 mg/dL 0.25 mmol/L	2.0%	2.64%
Chloride	± 5% (1)	± 2.0 mmol/L	1.25%	1.65%
Cholesterol	± 10% (1)	±3.0 mg/dL 0.08 mmol/L	2.5%	3.3%
CO2	± 20% (2)	±4.0 mmol/L	5.0%	6.6%
Creatinine	± 15% (1)	± 0.3 mg/dL 26.52 µmol/L	3.75%	4.95%

Quantitative Precision Example

Package insert

Precision
Reproducibility was determined using human samples and controls in an internal protocol (within run: n = 21, between run: n = 10). The following results were obtained:

	Within-run			Between-run		
Sample	Mean		CV	Mean		CV
	U/L	µkat/L	%	U/L	µkat/L	%
Human serum	58	0.97	1.8	58	0.97	3.2
Precitrol-N	32	0.53	2.1	32	0.53	3.2
Precitrol-A	124	2.07	1.1	124	2.07	1.8

Analytical sensitivity (lower detection limit)
4 U/L (0.07 µkat/L)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

	Normal	Abnormal
n1	37	206
n2	39	210
n3	38	210
n4	36	209
n5	38	206
n6	38	206
n7	42	205
n8	44	200
n9	41	204
n10	44	200
n11	41	204
n12	42	202
n13	41	199
n14	40	210
n15	38	203
n16	38	205
n17	36	204
n18	38	205
n19	37	211
n20	38	209

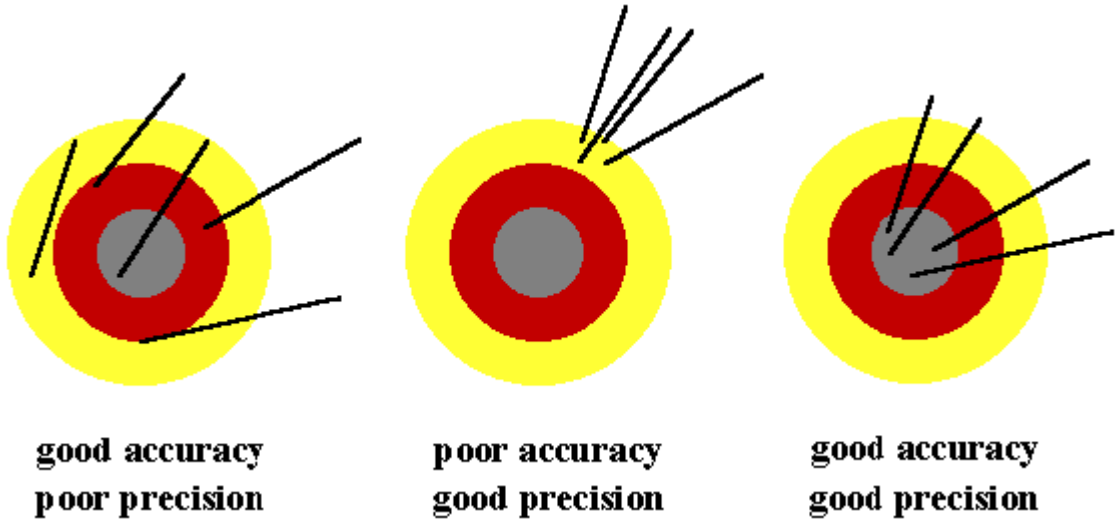
AST- Roche Cobas

Between Day

Normal CV: 6.2%
Abnormal CV: 1.7%

Important Note

- **Precision experiments are performed to verify manufacturer's claims**



- **Remember to verify precision <u>first!</u>**

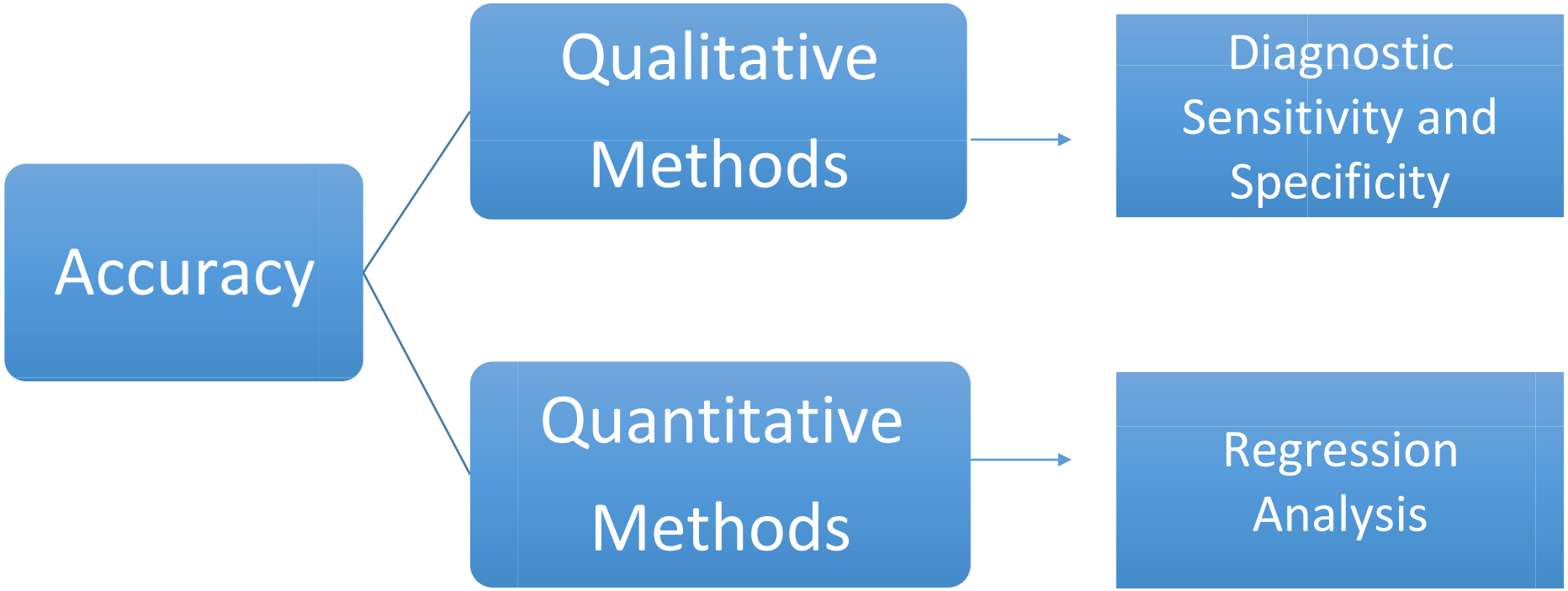
QUESTIONS?

Accuracy

Accuracy is the true value of a substance being measured.

Verification of accuracy is the process of determining that the test system is producing true, valid results.

Different Method Requirements



Determining Your **Qualitative** Reference Method

- The ideal reference method is a similar instrument/method
- Choose between:
  - An in-house reference method that has been previously validated and performing successfully on EQA
    - ➢ *Patient samples will be used*
  - EQA panels with known results
    - ➢ *EQA samples will be used*
  - Or a combination of both!

Sample Criteria

Sample Number

- 10 positive and 10 negative (or 10 of each expected result)

Other Considerations

- Rapid HIV tests: if using patient samples and method is non-FDA approved, may have additional confirmation requirements
- For urine hCG, manufacturer's stated cut-off limit should be considered

Diagnostic Sensitivity and Specificity

- The performance of qualitative tests is most commonly described in terms of **diagnostic sensitivity** and **specificity**

- Not to be confused with **analytic sensitivity** (lower limit of detection) and **analytic specificity** (interfering substances) that are another part of validation testing

Compiling the Results

- Once testing is complete develop a contingency table that compares the results of the qualitative test being validated with the results of the reference method

Abbott Murex anti-HCV (IDCP results)	Diagnostic Accuracy Criteria (Peer Results from CAP Surveys)		**Total**
	Positive	Negative	
Positive	***16 (True Positive)***	***0 (False Positive)***	***16 (TP+FP)***
Negative	***0 (False Negative)***	***19 (True Negative)***	***19 (FN+TN)***
Total	***16 (TP+FN)***	***19 (FP+TN)***	***35 (N)***

Calculations and Acceptability

- Use the table to calculate the following parameters and compare them to the manufacture's package insert

Diagnostic Sensitivity
100 x [TP/(TP+FN)]

Diagnostic Specificity
100 x [TN/(FP+TN)]

Positive Agreement
100 x [TP/(TP+FP)]

Negative Agreement
100 x [TN/(TN+FN)]

	Lab Result (%)	Expected Result	Acceptability
Sensitivity= 100 x [TP/(TP+FN)]	100%	99%	Acceptable
Specificity= 100 x [TN/(FP+TN)]	100%	99%	Acceptable
Positive Agreement (Positive Predictive Value) =100 x TP/(TP+FP)	100%	99%	Acceptable
Negative Agreement (Negative Predictive Value)= 100 x TN/(TN+FN)	100%	99%	Acceptable

Where to find the Manufacturer's Claims

PERFORMANCE CHARACTERISTICS

A multi-center clinical study was conducted to establish the performance of the QuickVue One-Step hCG-Urine test compared to results obtained from another commercially available hCG test. A quantitative method was used to resolve any discrepant results between the two test methods. In this multi-center field trial, 499 urine specimens, collected from patients presenting for pregnancy testing, were evaluated. A concordance of >99% was determined.

Urine Correlation
hCG Comparative Test

QuickVue hCG-Urine	+	−
+	252	0
−	0	247

Sensitivity: >99%
Specificity: >99%
Agreement: >99%

Determining Your **Quantitative** Reference Method

- The ideal reference method is a similar instrument/method
- Choose between:
  - An in-house reference method that has been previously validated and performing successfully on EQA
    - ➢ *Patient samples will be used*
  - EQA panels or commercial standards with known results
    - ➢ *EQA or standard samples will be used*
  - Or a combination of both!

Sample Criteria

- Sample Number
  - At least 20 specimens, 40 is preferable
  - Tested in duplicate

- *For quantitative testing it is important that your accuracy specimens span the AMR of the instrument*

Statistics used for Accuracy

Coefficient Correlation

- The correlation coefficient (R) must be >0.975

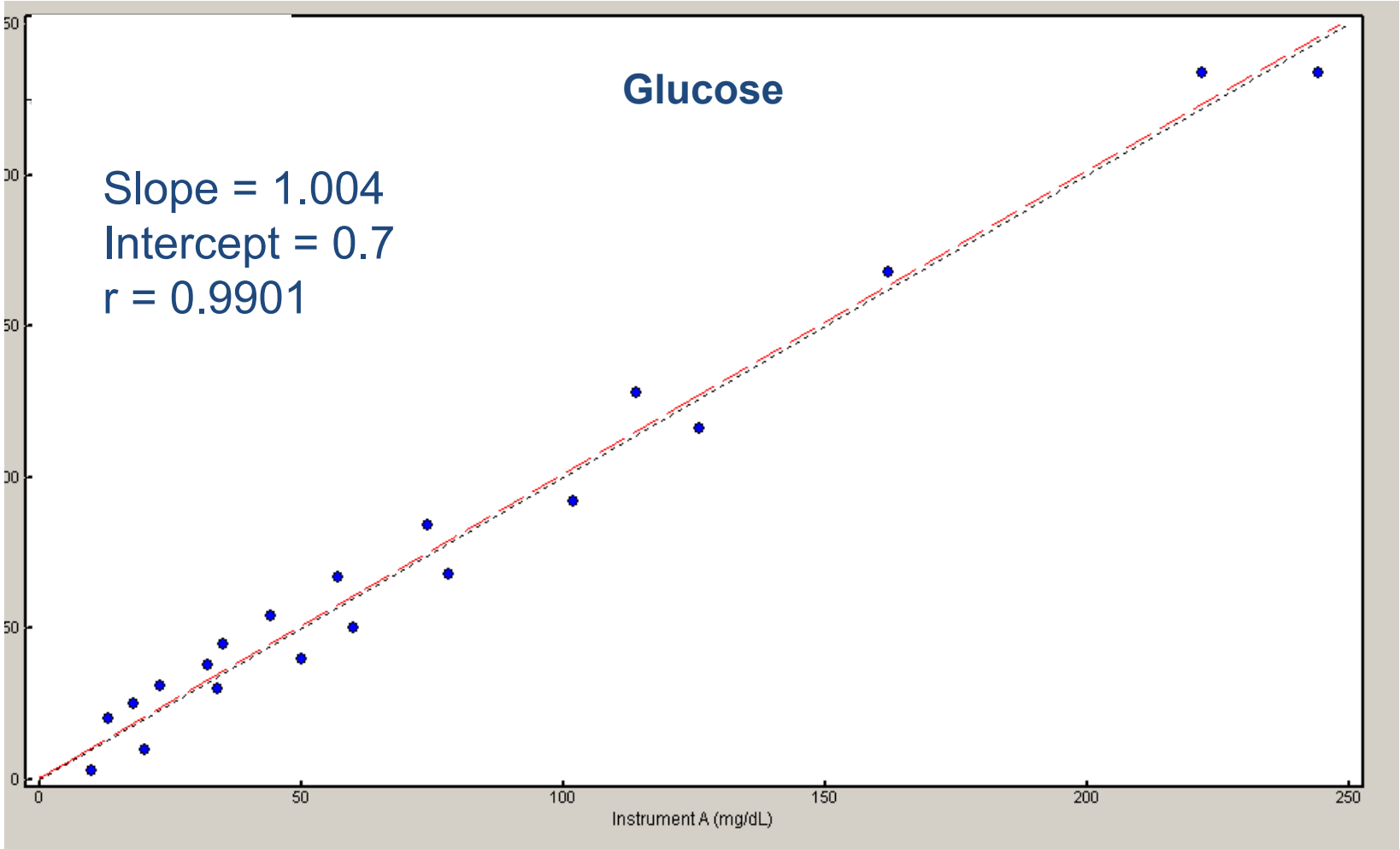
Slope

- The slope should be close to one

Intercept

- The intercept should be close to zero

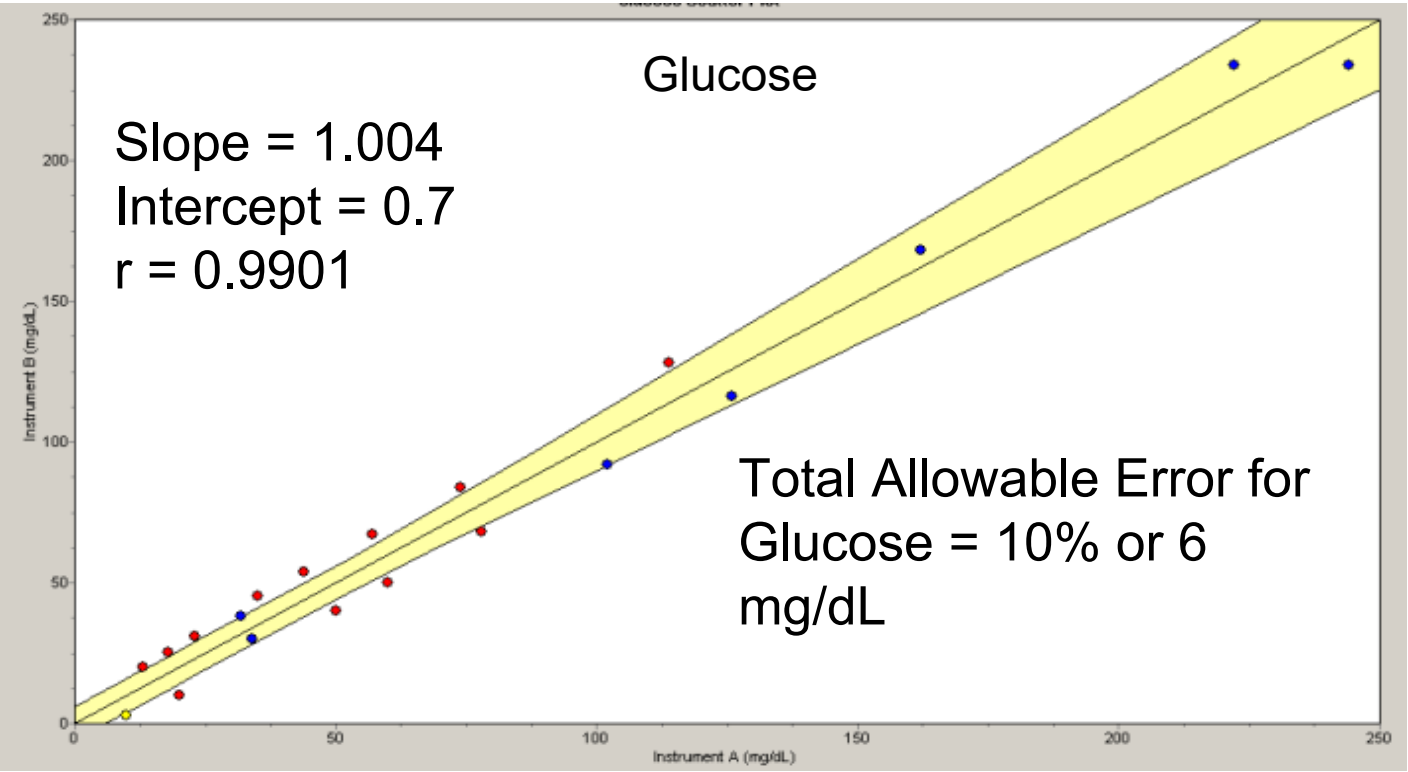
Accuracy Data Evaluation



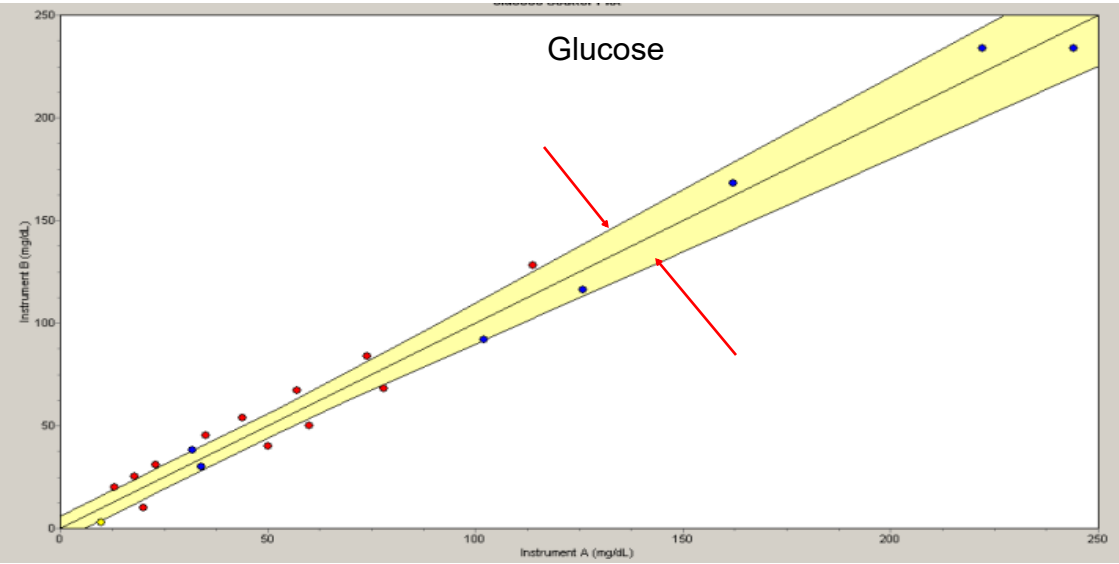
There is no acceptable range, only ideals!

Slope should be “close” to 1.000
Intercept should be “close” to 0.0

Obviously not close enough…



Total Error (TEa) limit lines



- Error Index- Should be between -1.0 and 1.0

$$\frac{X - Y}{TEa}$$ For EACH X-Y pair

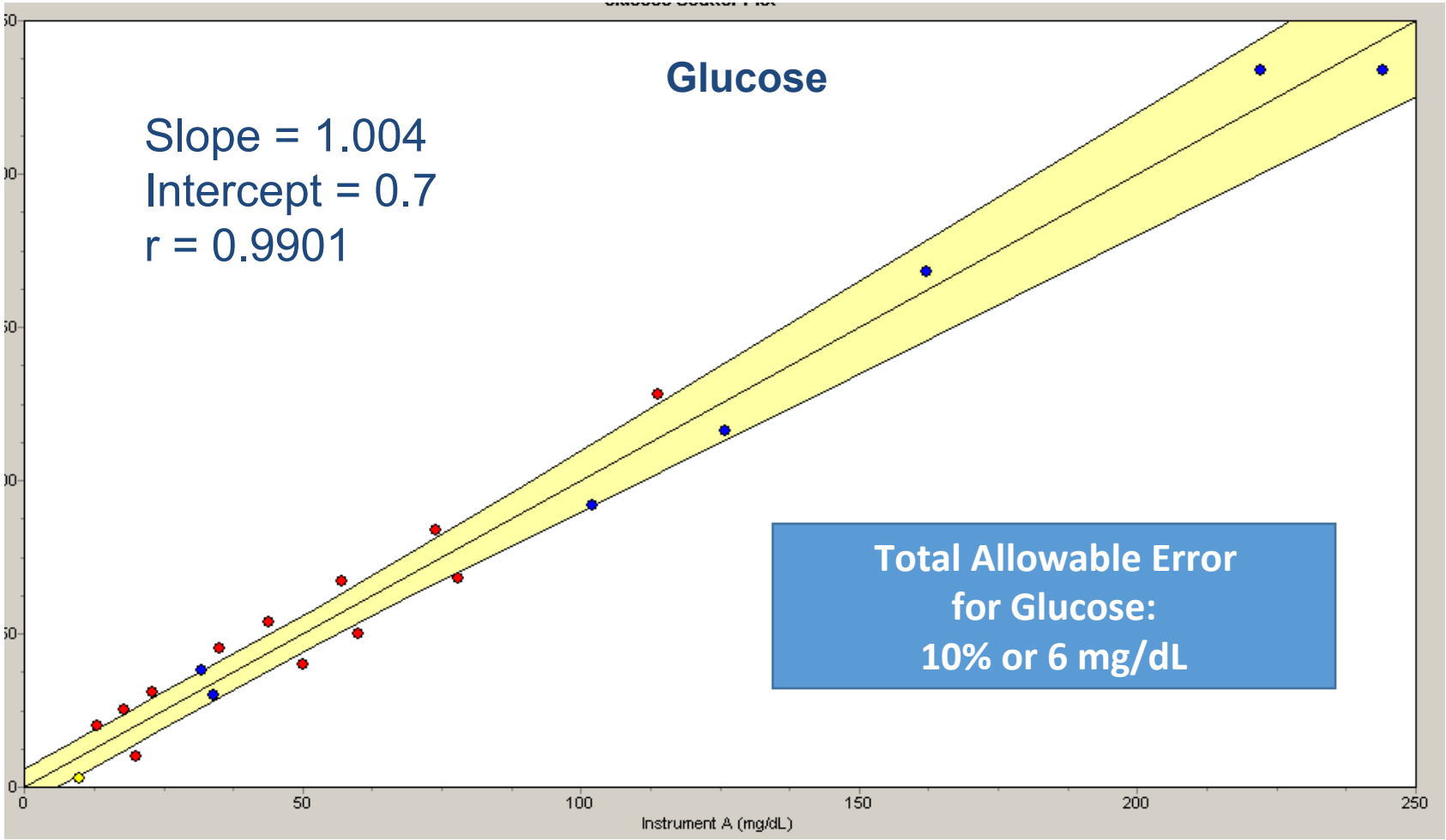
More statistics used for Accuracy

Error Index

- The “Error Index” measures the difference between the two methods as a ratio of the Total Allowable Error.
  - **Y** = New method
  - **X** = Comparison method

- Acceptability Criteria - The Error Index is measured for each X-Y pair.
  - The Error Index must fall within -1 and 1
  - For 95% of the specimens

Accuracy Data Evaluation with Total Allowable Error Limits



pSMILE Minimum Recommended Validation requirements for

Chemistry Total Allowable Error (TEa)

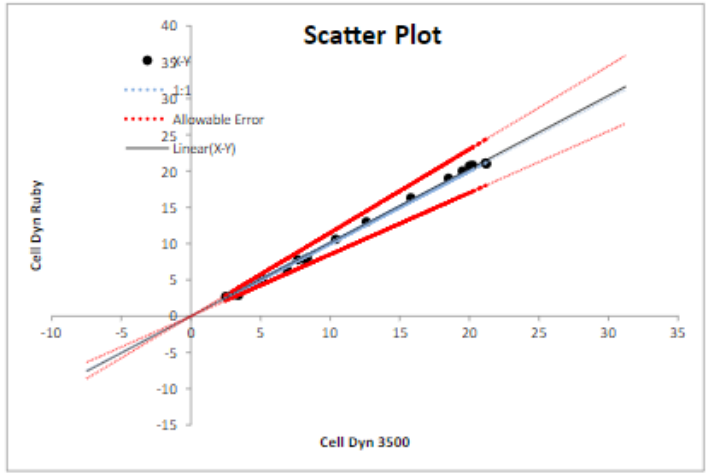
Analyte	pSMILE Total Error Limits (whichever is greater)		Precision	
	Percentage	Minimum detectable difference or absolute	Short Term 25% TE (1)	Long Term 33% TE (1)
Albumin	± 10% (1)	±0.2 g/dL 2.0 g/L	2.5%	3.3%
Alk. Phos	± 30% (1)	±5.0 U/L	7.5%	9.9%
ALT	± 20% (1)	±5.0 U/L	5.0%	6.6%
Amylase	± 30% (1)	±5.0 U/L	7.5%	9.9%
AST	± 20% (1)	±5.0 U/L	5.0%	6.6%
Bilirubin, Direct	± 20% (1)	± 0.4 mg/dL	5.0%	6.6%
Bilirubin, Total	± 20% (1)	± 0.4 mg/dL	5.0%	6.6%
Calcium	± 8% (2)	± 1.0 mg/dL 0.25 mmol/L	2.0%	2.64%
Chloride	± 5% (1)	± 2.0 mmol/L	1.25%	1.65%
Cholesterol	± 10% (1)	±3.0 mg/dL 0.08 mmol/L	2.5%	3.3%
CO2	± 20% (2)	±4.0 mmol/L	5.0%	6.6%
Creatinine	± 15% (1)	± 0.3 mg/dL 26.52 µmol/L	3.75%	4.95%

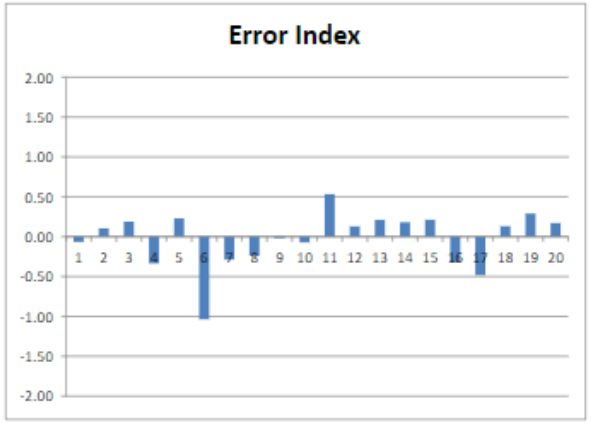
Quantitative Method Comparison

Method being evaluated (Y):	Cell Dyn Ruby	Date:	7/19/2023
Reference Method (X):	Cell Dyn 3500	Total Allowable Error (TEa):	
Analyte:	WBC	Conc.	Pct.
Units:	10E3/uL	0.12	15

Experiment: PASSED

Spec#	X	Y	Δ	Error Index
1	3.03	3	-0.03	-0.07
2	7.66	7.78	0.12	0.10
3	20.23	20.8	0.57	0.19
4	3.18	3.02	-0.16	-0.34
5	20.01	20.7	0.69	0.23
6	3.41	2.88	-0.53	-1.04
7	8.35	7.99	-0.36	-0.29
8	3.35	3.23	-0.12	-0.24
9	21.16	21.1	-0.06	-0.02
10	21.23	21	-0.23	-0.07
11	2.5	2.7	0.2	0.53
12	10.4	10.6	0.2	0.13
13	15.8	16.3	0.5	0.21
14	18.5	19	0.5	0.18
15	12.6	13	0.4	0.21
16	8.2	7.8	-0.4	-0.33
17	6.9	6.4	-0.5	-0.48
18	5.1	5.2	0.1	0.13
19	4.6	4.8	0.2	0.29
20	19.5	20	0.5	0.17
Mean	10.7855	10.865	0.0795	-0.02
Min	2.5	2.7	-0.53	-1.04
Max	21.23	21.1	0.69	0.53
	Slope	1.027		
	Intercept	-0.217		
	Correl. Coef. (R)	0.999		





Quantitative Method Comparison

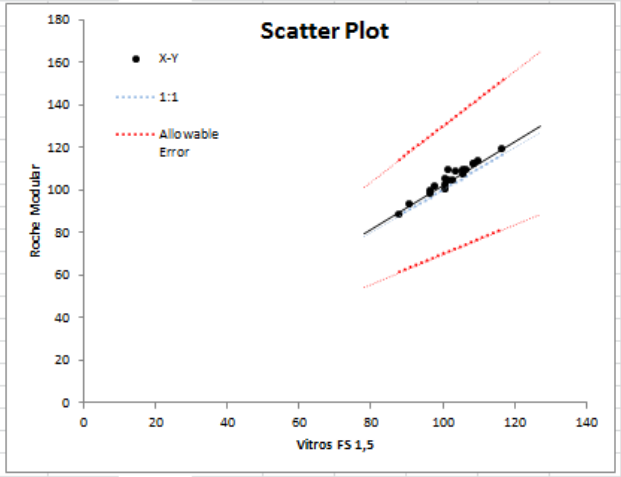
Method being evaluated (Y):	Roche Modular		Date:	
Reference Method (X):	Vitros FS 1,5		Total Allowable Error (TEa):	
Analyte:			Conc.	Pct.
Units:	U/L		5	30

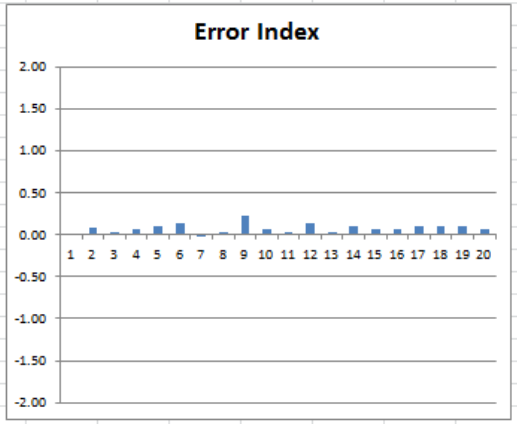
Experiment: FAILED (See Note Below)**

Spec#	X	Y	Δ	Error Index
1	88	88	0	0.00
2	91	93	2	0.07
3	97	98	1	0.03
4	97	99	2	0.07
5	98	101	3	0.10
6	101	105	4	0.13
7	101	100	-1	-0.03
8	101	102	1	0.03
9	102	109	7	0.23
10	102	104	2	0.07
11	103	104	1	0.03
12	104	108	4	0.13
13	106	107	1	0.03
14	106	109	3	0.09
15	106	108	2	0.06
16	107	109	2	0.06
17	109	112	3	0.09
18	109	112	3	0.09
19	110	113	3	0.09
20	117	119	2	0.06
Mean	102.75	105	2.25	0.07
Min	88	88	-1	-0.03
Max	117	119	7	0.23

Slope	1.062
Intercept	-4.143
Correl. Coef. (R)	0.974

**Correlation Coefficient is too low. Troubleshoot Assay and re-analyse specimens





How to Capture Accuracy Results

Document acceptability by filling in the table in your Validation Summary

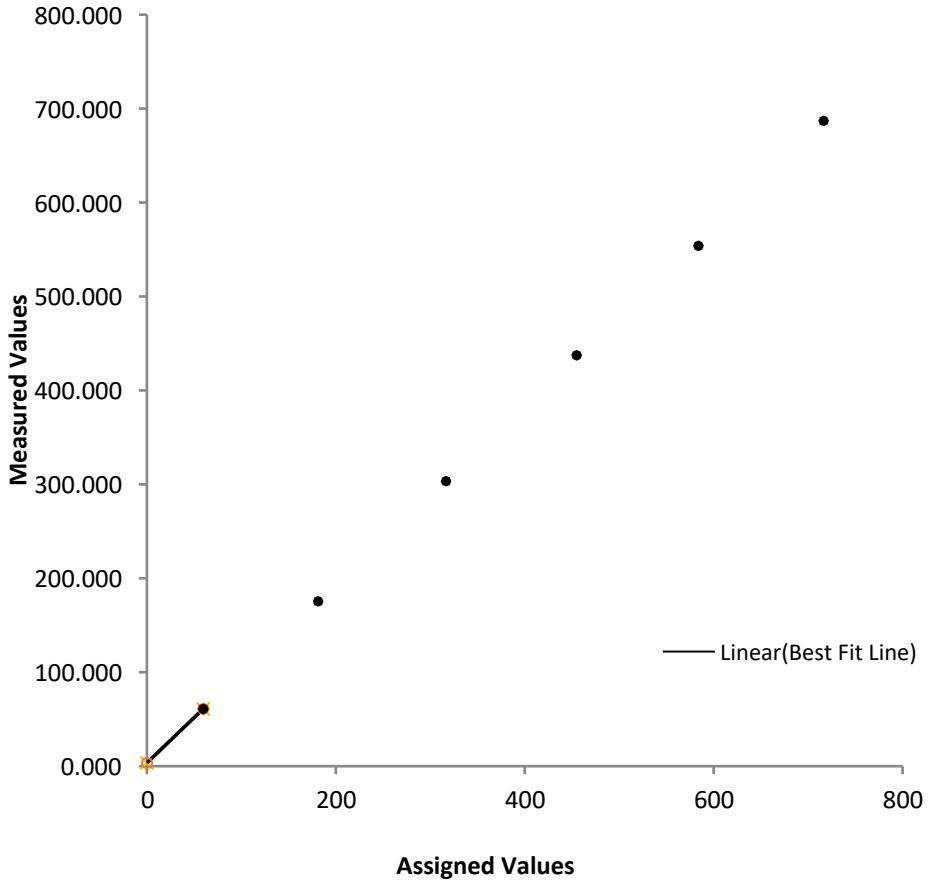
- The correlation coefficient (R) must be >**0.975**
- The Error Index must be between **-1 and 1 for 95%** (19/20) of specimens
- Slope and intercept data should be reviewed for appropriateness

Analyze	Total Allowable Error	Correlation Coefficient (R)	Linear Regression Statistics	Linear Regression Statistics	Error Index Range	% of Error Indices -1.0 to 1.0	Acceptability
.		Expected >0.975	Slope	Intercept	Expected -1.0 to1.0	Expected ≥ 95%	
ALT	5.0 U/L or 20%	0.999	1.039	1.299	-0.14 to 1.20	**90%**	**Unacceptable**
AST	5.0 U/L or 20%	0.987	-1.009	0.083	-0.24 to 0.35	100%	Acceptable

Linearity

A quantitative analytical method is said to be LINEAR when measured results from a series of sample solutions are directly proportional to the concentration or activity in the test specimens

LINEARITY



This means that a straight line can be used to characterize the relationship between measured results and the concentrations or activity levels

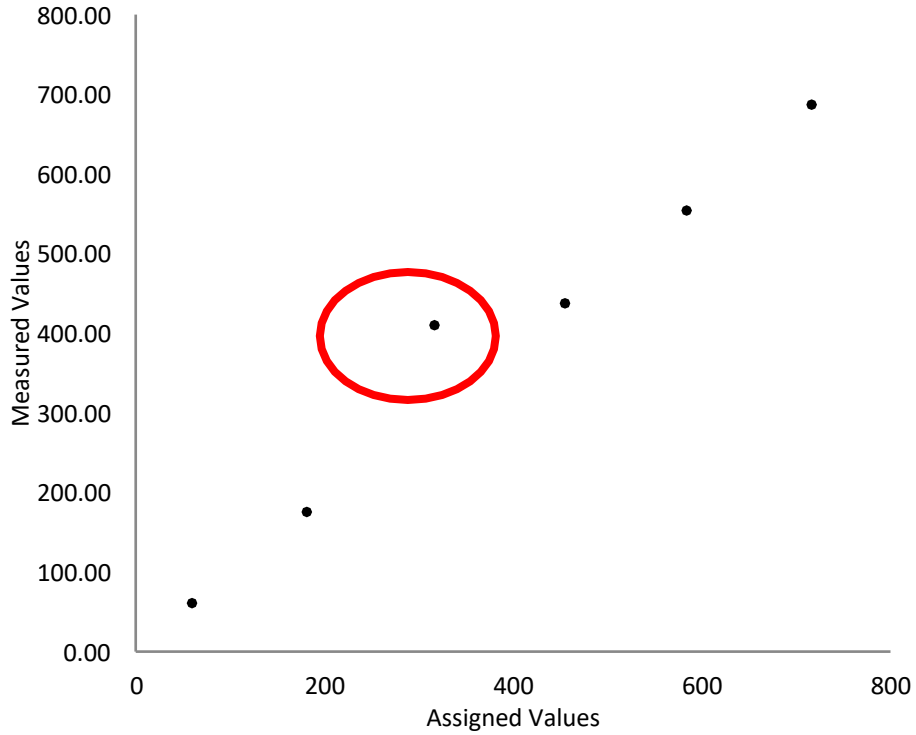
Sample Criteria

- At least 5 samples that cover the reportable range
- The values should be equidistant from each other
- Material:
  - Quality control
  - Calibrators
  - Commercial Linearity Standards

Contact pSMILE for Sources

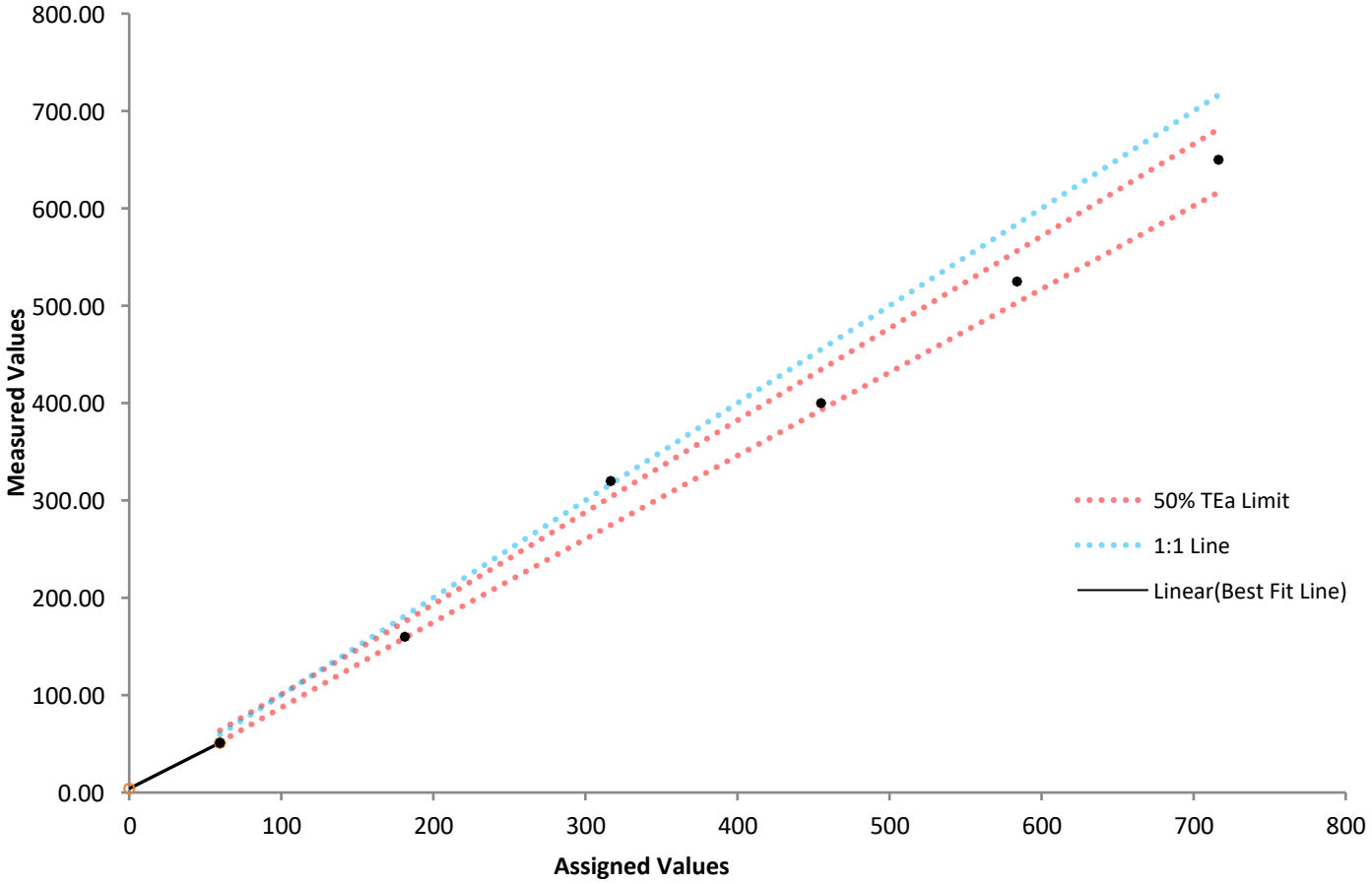
Testing

- Test each sample in duplicate and average results
- Plot data immediately
  - pSMILE Linearity Worksheet
  - EP Evaluator
  - Any Regression Analysis Program
- Visually evaluate and correct any outliers!



Data evaluation

Intercept -3.587
Slope 0.907
Correlation Coefficient 0.9997



Acceptability Criteria

- The method is linear if the difference between the **predicted** Y and the **measured** Y is ***less than the allowable error*** for <u>each specimen point</u>

- The pSMILE Linearity spreadsheet and EP Evaluator will indicate “Pass” or “Fail” based on the above criteria

Use 50% of TE LIMITS for Linearity

SMILE Minimum Recommended Validation requirements for
Chemistry Total Allowable Error (TEa)

Analyte	SMILE Total Error Limits (whichever is greater)		Precision	
	Percentage	Minimum detectable difference or absolute	Short Term 25% TE	Long Term 33% TE
Albumin	± 10% (1)	±0.2 g/dL 2.0 g/L (4)	2.5%	3.3%
Alk. Phos	± 30% (1)	±5.0 U/L (4)	7.5%	9.9%
ALT	± 20% (1)	±5.0 U/L (4)	5.0%	6.6%
Amylase	± 30% (1)	±5.0 U/L (4)	7.5%	9.9%
AST	± 20% (1)	±5.0 U/L (4)		
Bilirubin, Direct	± 20% (2)	± 0.4 mg/dL 6.84 umol/L (2)		
Bilirubin, Total	± 20% (1)	± 0.4 mg/dL 6.84 umol/L (1)	5.0%	6.6%
Calcium	± 8.3% (4)	± 1.0 mg/dL 0.25 mmol/L (1)	2.08%	2.74%

50% of TE =
15% or 2.5 U/L

Data Evaluation using pSMILE Worksheet

Method Being Evaluated: Vitros 950/Glucose Oxidase - Color Date: 25-Mar-10

Analyte: Glucose Total Allowable Error (TEa):

Units: mg/dL Conc.: 6 Percent: 10%

Enter values in ascending order
(lowest value data in top cells, highest value data in bottom cells)

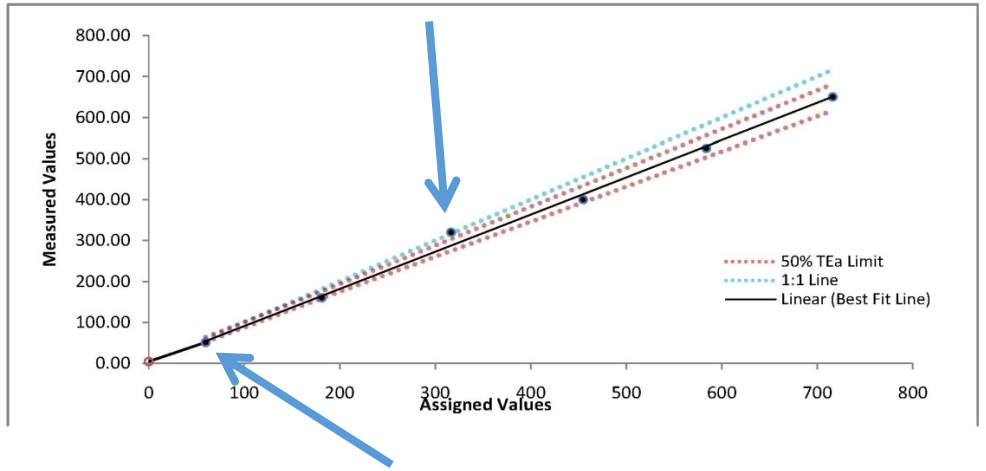
Manufacturer's stated analytical measurement range:
Low: 10 High: 1000

Measured										
Rep 1	Rep 2	Rep 3	Avg	Assigned	Target	± Diff	% Diff	± Limit	% Limit	Grade
51	51		51.00	59.6	57.67	-6.67	-11.6%	6.00	5%	Unacceptable
160	160		160.00	181.2	167.15	-7.15	-4.3%	8.36	5%	Acceptable
320	320		320.00	316.6	289.06	30.94	10.7%	14.45	5%	Unacceptable
400	400		400.00	454.9	413.57	-13.57	-3.3%	20.68	5%	Acceptable
525	525		525.00	583.8	529.63	-4.63	-0.9%	26.48	5%	Acceptable
650	650		650.00	716.3	648.92	1.08	0.2%	32.45	5%	Acceptable

	Coefficients	*Std Err*
Intercept	4.010	14.327
Slope	0.900	0.032
Correl. (r)	0.9975	

Your Linearity Evaluation: LINEAR 400 to 650 PARTIAL RANGE

Verified AMR: 400 to 650 mg/dL



How to Capture this in your Validation Report

3. Linearity and Reportable Range-refer to tab C

i. Linearity

Analyte	Linear Regression Statistics		Allowable Systematic Error	Linearity Pass/Fail	Visual Evaluation	Acceptability
	Slope (Ideal=1.0)	Intercept (Ideal=0.0)	50% of CLIA	As evaluated by EP Evaluator		
ALT	0.970	0.282	10%	Pass	Linear	Acceptable
AST	0.931	0.42	10%	Pass	Linear	Acceptable
Albumin	1.018	0.36	5%	Pass	Linear	Acceptable

Analytical Measurement Range (AMR)

The AMR is 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

AMR Validation

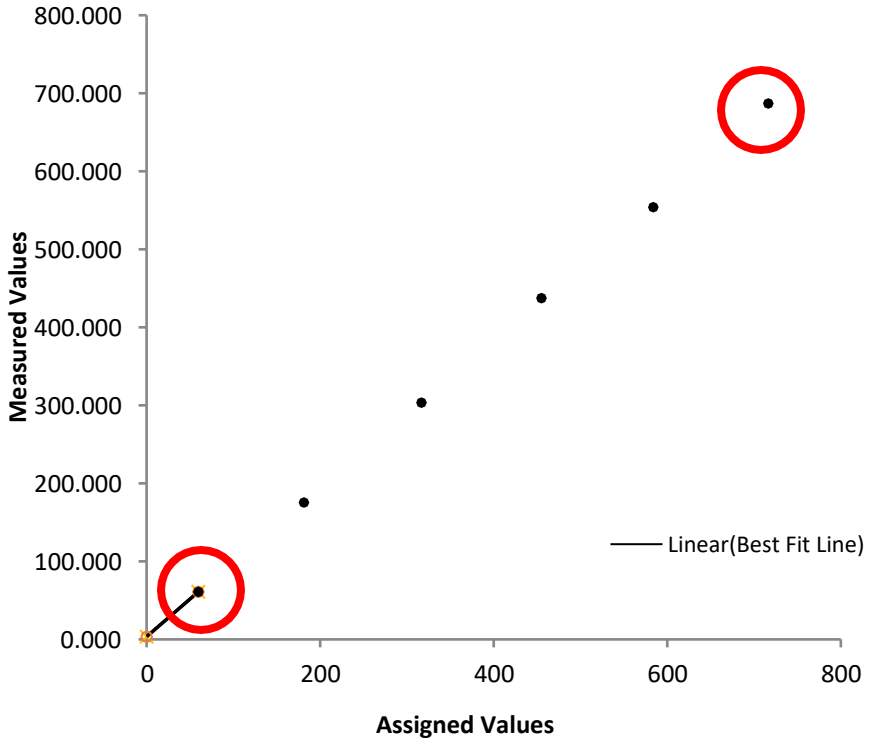
- **AMR validation** is the process of confirming that the assay system will ***correctly recover*** the concentration or activity of the analyte over the AMR
- The manufacturer defines the AMR
- It is the laboratory's responsibility to verify it

Sample Criteria

- Samples with an assigned or known value
  - Quality control
  - Calibrators
  - Commercial linearity standards

Sample Preparation

- Dilute the lowest sample to verify the low end of the AMR
- The high end of the AMR will only be as high as the highest sample



Working Example

SMILE Minimum Recommended Validation requirements for
Chemistry Total Allowable Error (TEa)

Analyte	SMILE Total Error Limits (whichever is greater)		Precision	
	Percentage	Minimum detectable difference or absolute	Short Term 25% TE	Long Term 33% TE
Albumin	± 10% (1)	±0.2 g/dL 2.0 g/L (4)	2.5%	3.3%
Alk. Phos	± 30% (1)	±5.0 U/L (4)	7.5%	9.9%
ALT	± 20% (1)	±5.0 U/L (4)	5.0%	6.6%
Amylase	± 30% (1)	±5.0 U/L (4)	7.5%	9.9%
AST	± 20% (1)	±5.0 U/L (4)	5.0%	6.6%
Bilirubin, Direct	± 20% (2)	± 0.4 mg/dL 6.84 umol/L (2)	5.0%	6.6%
Bilirubin, Total	± 20% (1)	± 0.4 mg/dL 6.84 umol/L (1)	5.0%	6.6%
Calcium	± 8.3% (4)	± 1.0 mg/dL 0.25 mmol/L (1)	2.08%	2.74%

- Total Bilirubin
  - Manufacturer AMR 0-25 mg/dL
- Allowable Error:
  - 20% or 0.4 mg/dL

Lower Limit Verification

Manufacturer's
AMR: 0 - 25

- Need to verify **0 mg/dL**
  - (Lower Limit AMR)
- TE is 20% or **0.4 mg/dL**
  - (Whichever is greater)
- Need a standard within an assigned value from:

0 – 0.4 mg/dL

Lower Limit Verification

Manufacturer's AMR: 0 - 25

- Bilirubin Lowest Standard Available
  - Assigned Value: 0.6 mg/dL
  - Subtract Total Error:

0.6 – 0.4 = 0.2 mg/dL

If you use this standard **without dilution**, this would be the lowest limit that could be accepted (after verification).

- If possible, **dilute the standard** to get within TE of the Lower Limit AMR

Lower Limit Verification

Manufacturer's AMR: 0 - 25

- Dilute Standard 1:2
  - 0.6/2 = 0.3 mg/dL Assigned Value
- Determine the Acceptable Criteria:
  - 0.3 ± 0.4 TE = 0–0.7 mg/dL
- Test the Standard:
  - Example Test Result = 0.40 mg/dL
- Evaluate Acceptability
  - 0.40 is within 0-0.7

Acceptable! Verified lower limit AMR is 0 mg/dL

Upper Limit Verification

Manufacturer's AMR: 0 - 25

- Need to verify **25 mg/dL**
  - (Upper Limit AMR)
- TE is **20%** or 0.4 mg/dL
  - (Whichever is greater)
- Need a standard within an assigned value from: **20 - 25 mg/dL**

25 x 0.2 = 5.0 mg/dL

25 – 5 = 20 mg/dL

Upper Limit Verification

Manufacturer's AMR: 0 - 25

- Bilirubin Highest Standard Assigned Value:
  - 22.5 mg/dL
- Determine the acceptable Criteria:
  - 22.5 ± 20% TE =

22.5 x 0.2 = 4.5 mg/dL

22.5 ± 4.5 =

18 – 27 mg/dL

Acceptable Limits for 22.5 Standard: 18 - 25

Your acceptability limit shouldn't exceed the manufacturer's limit!

Upper Limit Verification

Manufacturer's AMR: 0 - 25

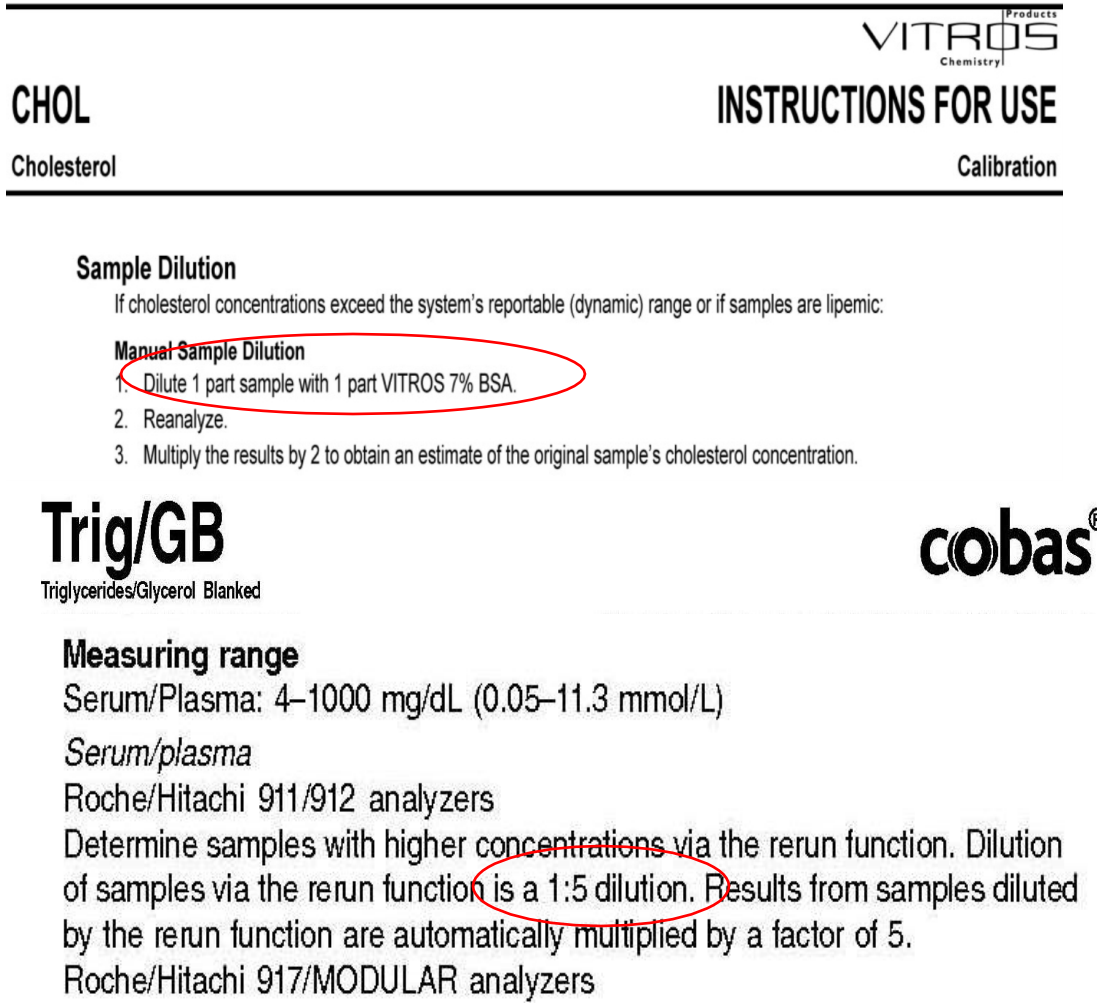
- Test the Standard:
  - Example Test Result = 21.0 mg/dL
- Evaluate Acceptability
  - 21.0 is within 18 – 25 mg/dL

Acceptable! Verified Upper limit AMR is 25 mg/dL

Clinical Reportable Range (CRR)

The CRR is the range of analyte values that a method can report as a quantitative result allowing for specimen dilution, concentration or other pretreatment used to extend the direct AMR

CLINICAL REPORTABLE RANGE



VITROS Products Chemistry

CHOL

Cholesterol

INSTRUCTIONS FOR USE

Calibration

Sample Dilution

If cholesterol concentrations exceed the system's reportable (dynamic) range or if samples are lipemic:

Manual Sample Dilution

1. Dilute 1 part sample with 1 part VITROS 7% BSA.
2. Reanalyze.
3. Multiply the results by 2 to obtain an estimate of the original sample's cholesterol concentration.

Trig/GB

Triglycerides/Glycerol Blanked

cobas®

Measuring range

Serum/Plasma: 4–1000 mg/dL (0.05–11.3 mmol/L)

Serum/plasma

Roche/Hitachi 911/912 analyzers

Determine samples with higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Roche/Hitachi 917/MODULAR analyzers

- How to determine what is appropriate:
  - Manufacturer's Recommendations
  - Literature References
  - Clinical Significance

Determining a CRR

- The laboratory should establish a CRR that covers a range inclusive of Grade 4 Adverse Events on the DAIDS Toxicity Table**without exceeding** the manufacturer's recommendations for dilution.

LABORATORY					
PARAMETER		**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
CHEMISTRIES	***Standard International Units are listed in italics***				
Bilirubin (Total)					
	Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
	Infant*†, ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	25.1 – 30.0 mg/dL *429 – 513 µmol/L*	> 30.0 mg/dL *> 513.0 µmol/L*
	Infant*†, ≤ 14 days (hemolytic)	NA	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	> 25.0 mg/dL *> 428 µmol/L*

How to Capture this in your Validation Report

4. Analytical Measurement Range (AMR) and Clinical Reportable Range (CRR)- refer to tab D

Analyte	Mfg's AMR	Low Value Verified	High Value Verified	Reportable Range	Dilutions	CRR	DAIDS Toxicity Grade 4
ALT	5-700 U/L	2.5	770	5-700	1:10	5-7000	>381
Total Bilirubin	0 – 25 mg/dL	0	25	0 - 25	1:10	0-250	>125

Analytical Sensitivity and Specificity

Analytical Sensitivity *is the lowest concentration of an analyte that can be measured (also called the Lower Limit of Detection).*

Analytical Specificity *is the determination of the effect of interfering substances*

Analytical Sensitivity and Specificity

- Unmodified/FDA approved method:
  - Refer to test package insert
- Modified/non FDA approved method:
  - The laboratory must establish the lowest concentration that the method can accurately measure that is distinguishable from zero
  - The laboratory must determine the effect of interfering substances
  - Consult with the Networks for requirements and recommendations

Reference Ranges

The range of test values expected for a designated population where 95% of the individuals are presumed to be healthy (or normal)

How do you validate reference ranges?

1. Transference of reference ranges (with verification)
2. Establishment of reference ranges
3. Transference of reference ranges (without verification)

Transference with Verification

1. Select an established reference range from a population similar to your patient population
2. Select a pool of willing donors from your local area
3. Screen the donors with a questionnaire to ensure that you are selecting healthy individuals
4. Collect samples from 20 donors in each age/gender partition
5. Test samples immediately and evaluate

Manufacturer's ranges may not be suitable for international laboratories

Transference with Verification

	If	Then
1	≥ 90% of samples are within the reference range	• The reference range is verified.
2	< 90% of samples are within the reference range	• Re-evaluate the range being verified. • Re-evaluate the healthy volunteer qualifications. • Collect and evaluate 20 additional samples.
3	≥ 90% of the additional samples are within the reference range	• The reference range is verified.
4	< 90% of the additional samples are within the reference range	• Proceed with step II below (Establishment of Reference Ranges)

Reference Interval Analysis

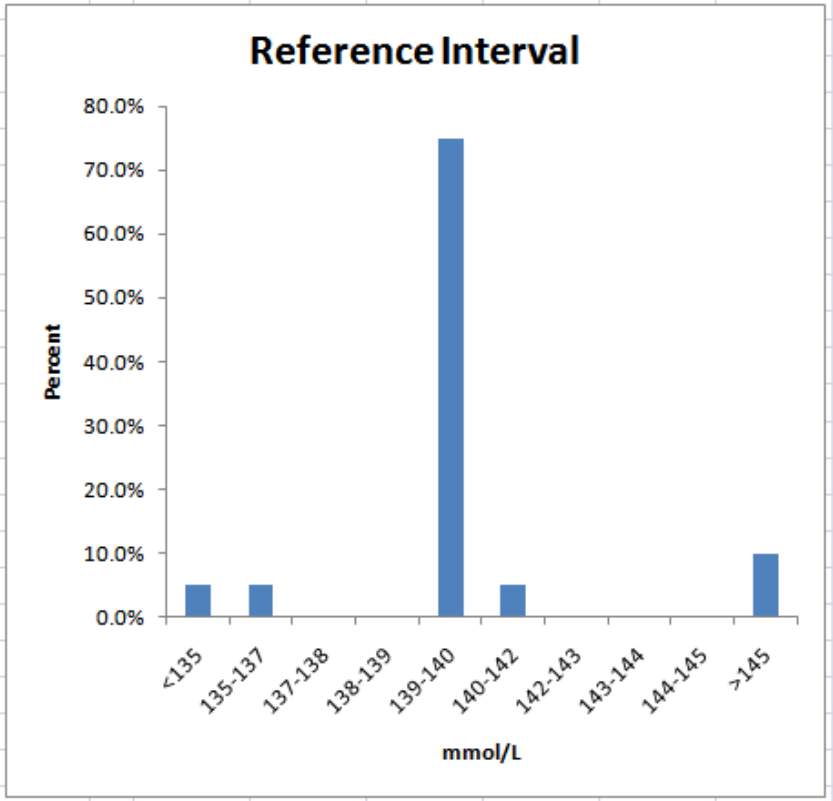
Method being evaluated:	Vitros 250
Date:	
Analyte	Sodium
Proposed Reference Range:	135 to 145 mmol/L

ENTER RESULTS IN CELLS AT RIGHT

146	135	134	140	140
140	140	140	150	140
140	140	140	140	140
140	140	141	140	140

mmol/L	*Frequency*	%
<135	1	5.0%
135-137	1	5.0%
137-138	0	0.0%
138-139	0	0.0%
139-140	15	75.0%
140-142	1	5.0%
142-143	0	0.0%
143-144	0	0.0%
144-145	0	0.0%
>145	2	10.0%

Mean:	140.3
SD:	3.21
Median:	140
Range:	134-150
Obs Outside:	15.0%
Grade:	Unacceptable



Establishment of Reference Ranges

1. Qualify healthy volunteers. This can be done through a questionnaire or health assessment.

2. Obtain samples from 120 healthy participants for each range to be established.

3. Test each sample immediately after collection and evaluate.

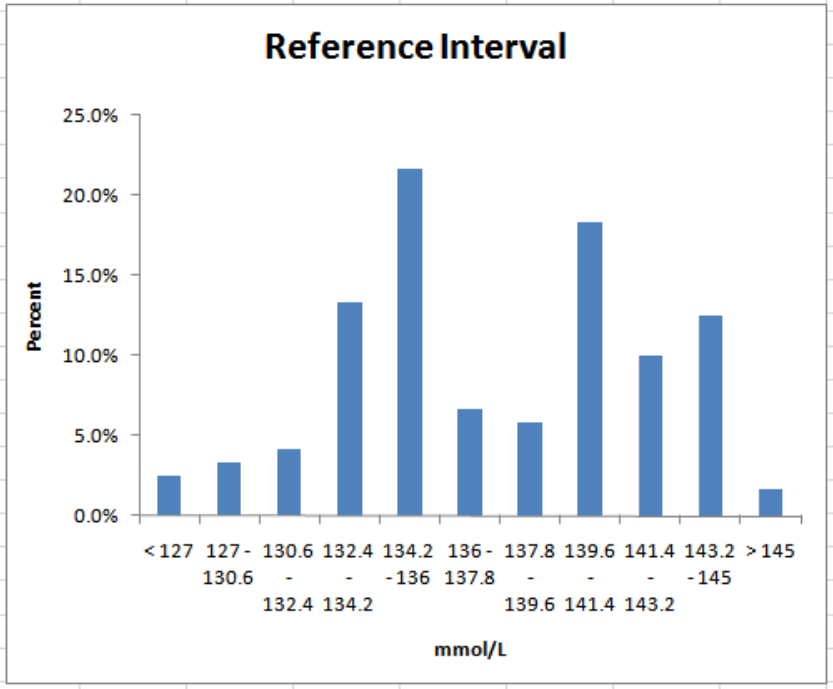
Method being evaluated:			Vitros 250					Date:				
Analyte			Sodium									
Reference Range:	135-145					Units:	mmol/L					

ENTER RESULTS IN CELLS BELOW

	132	130	137	131	129	127	138	135	135	135	135	135
	130	136	136	138	138	136	137	137	137	137	137	137
	133	133	133	133	133	133	133	133	133	133	133	133
	136	134	138	132	131	132	133	130	138	137	125	125
	140	140	140	140	140	140	140	140	140	136	138	140
	144	144	144	144	144	144	144	144	144	135	138	144
	135	135	135	135	135	135	133	134	135	146	146	135
	136	136	136	136	136	136	145	145	145	145	145	136
	142	142	142	142	142	142	142	142	142	142	142	142
	141	141	141	141	141	141	141	141	141	141	141	141

Mean:	137.68
SD:	4.64
Minimum:	125
Maximum:	146
Median:	137

mmol/L	*Frequency*	Percent
< 127	3	2.5%
127 - 130.6	4	3.3%
130.6 - 132.4	5	4.2%
132.4 - 134.2	16	13.3%
134.2 - 136	26	21.7%
136 - 137.8	8	6.7%
137.8 - 139.6	7	5.8%
139.6 - 141.4	22	18.3%
141.4 - 143.2	12	10.0%
143.2 - 145	15	12.5%
> 145	2	1.7%



Reference Range Verification | Reference Range Establishment

Transference of Reference Ranges **without** verification

- CLSI guidelines permit the “transference” of established reference intervals without verification.

- Things to consider:
  - Similarity of geographics and demographics.
  - Similarity of test methodology.
  - Sound clinical judgment and consultation with local medical professionals.
  - Approval by the laboratory medical director is required and must be documented.

Important points to consider when using this approach

- The Medical Director is charged with the approval of reference ranges.

- Documentation is required and needs to include at least:
  - 1) source and reasons for range adoption
  - 2) written plan of review—including possible verification over time

- Usually only recommending for pediatric populations

Example Validation Summary

ABC Laboratory
Hospital Complex
123 Big Road
City-Township, Country

Validation Summary Report

Purpose: Validation

Description of Equipment/Process:
Equipment/Process: **Vitros 950 Chemistry Analyzer**
Serial Number: **999435**
Location: **ABC Lab, City-Township, Country**
Date: **18 June – 20 August 2008**
FDA Approval Status: Approved

Procedure:

Refer to the **ABC Lab** Validation Plan for **Vitros 950 Chemistry Analyzer**

Results: All raw data reports and statistical analysis can be found in the **Vitros 950 Chemistry** Validation binder.

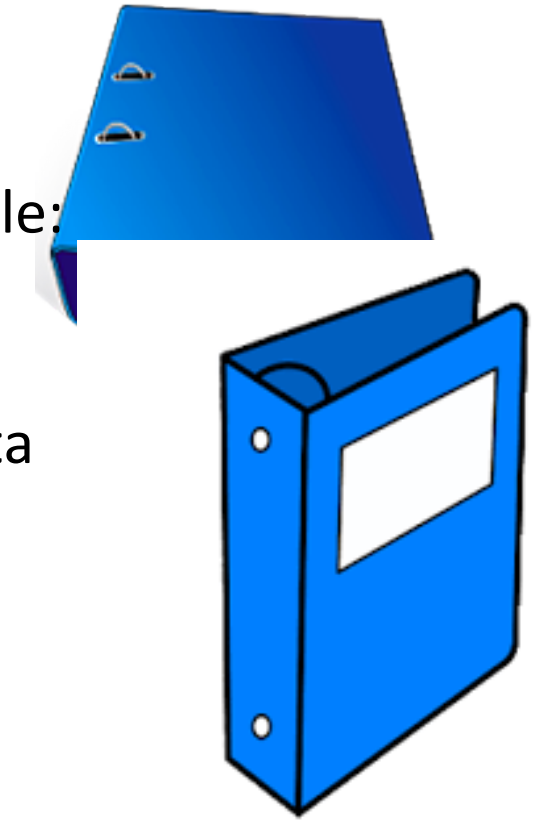
1. Precision- refer to tab A

Analyte	Expected Results		Observed Results		Acceptability
			Between Day		
	Manufacturer's Precision	33% of CLIA	Normal Control CV%	Abn Control CV%	
ALT	3.3%	6.6%	3.8%	4.3%	Acceptable
AST	3.1%	6.6%	3.1%	2.1%	Acceptable
Albumin	1.5%	3.3%	2.8%	2.6%	Acceptable

Analyte	Expected Results		Observed Results		Acceptability
			Within Run		
	Manufacturer's Precision	25% of CLIA	Normal Control CV%	Abn Control CV%	
ALT	2.6%	5%	1.0%	0.9%	Acceptable
AST	2.4%	5%	1.7%	0.4%	Acceptable
Albumin	1.0%	2.5%	0.6%	0.8%	Acceptable

Organize & File:

- Plan
- Report
- Raw Data



QUESTIONS?

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