Implementing a New POC Test or a Lab Test? A Crash Course on Method Evaluation

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EMORY
UNIVERSITY

Learning Objectives

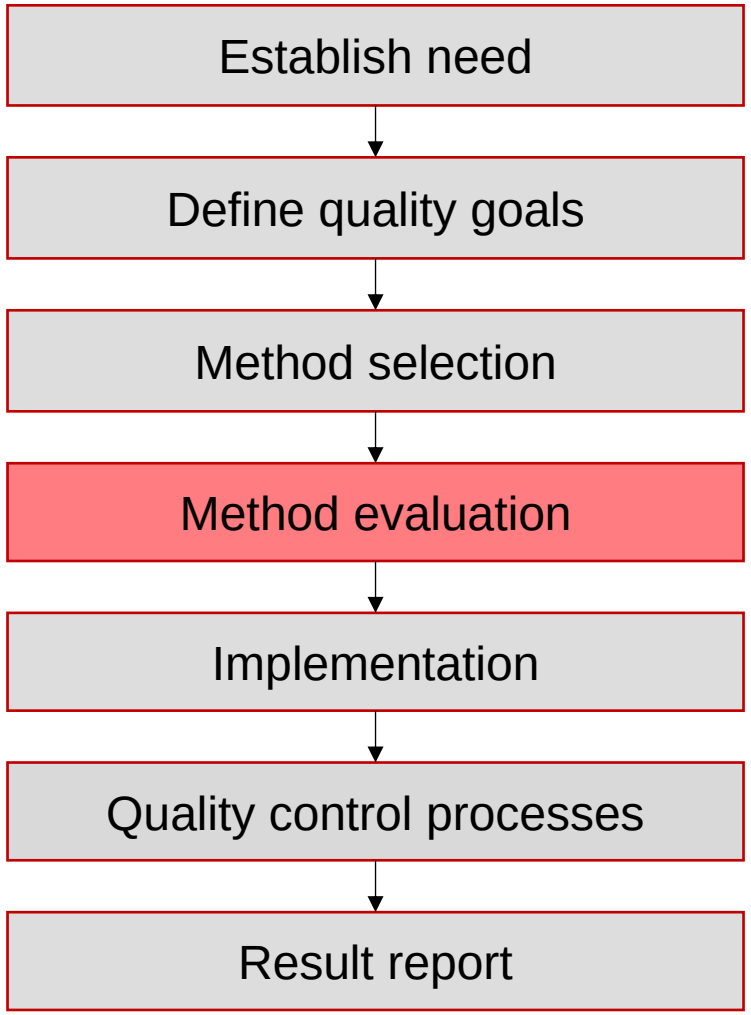
1. Describe studies that are required in evaluating Point Of Care or Laboratory tests

2. Define acceptability criteria and analyze the data in terms of allowable total error

3. Discuss solutions for issues that may occur during method evaluation

Nothing to disclose

Implementing a new test?



Why do we do it?

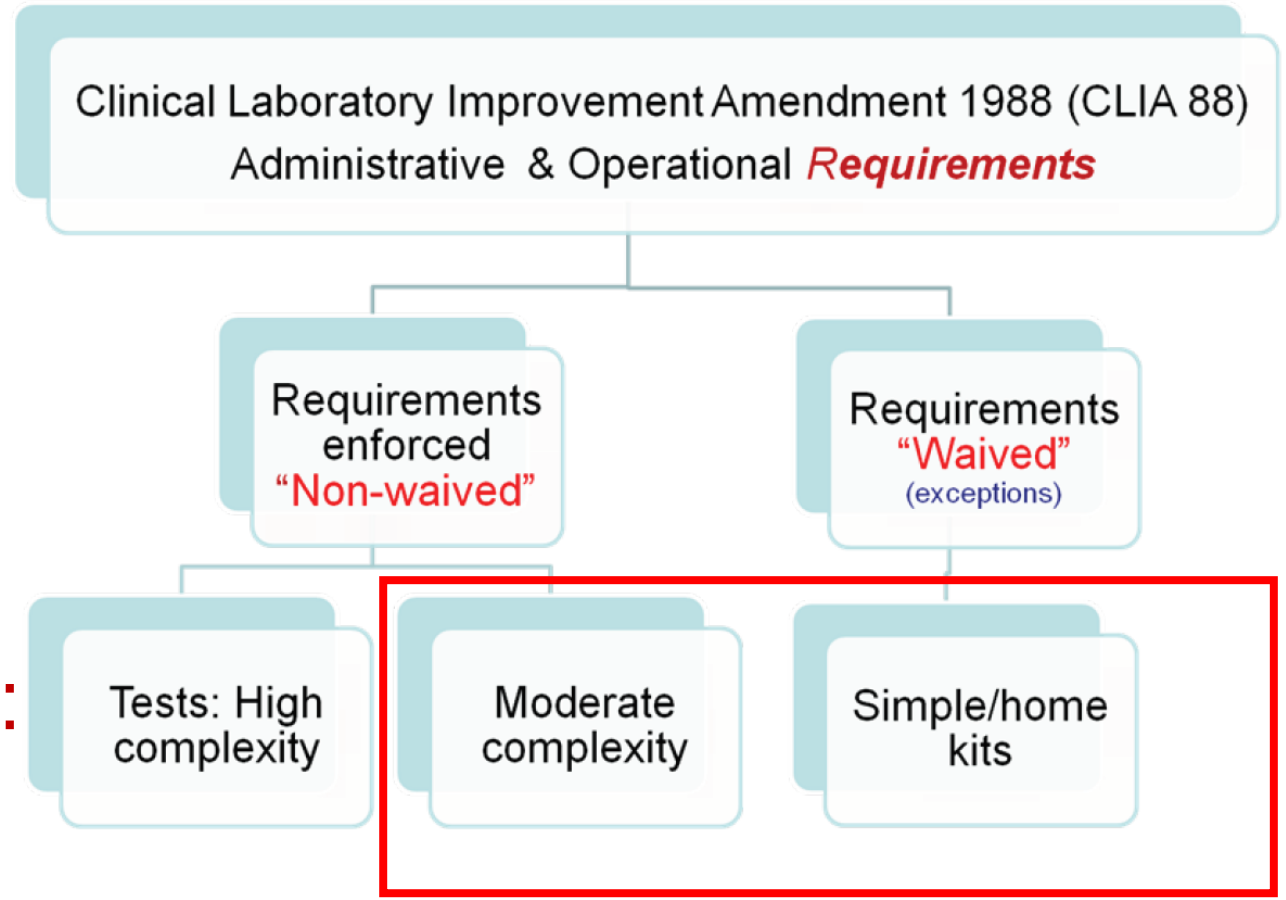
- Error assessment
  - How much error is present in the result
  - What type of analytical error is present
- Required by CLIA and accrediting agencies
- Pass Proficiency Testing
- Improvements over existing methodology

Method validation requirements vary:

Non-FDA approved > FDA approved > Waived tests

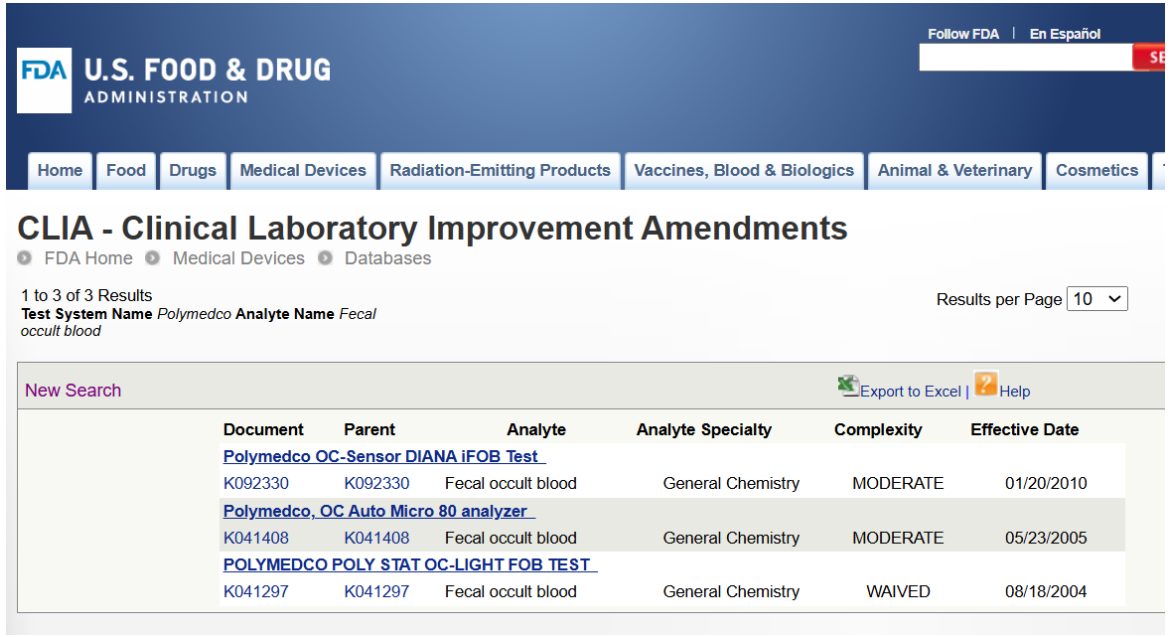
Waived tests versus nonwaived

FDA:



POC testing

Check FDA website to see a POC test is waived or non waived (moderate complexity or high complexity)



https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/search.cfm

Method evaluation requirements vary

WAIVED TESTS

Requirement	CMS (CLIA)	COLA Accreditation	TJC Accreditation	CAP Accreditation
Verification of method's performance	No	No, unless required by manufacturer or organization	No, unless required by manufacturer or organization	No, unless required by manufacturer or organization

The New Poor Lab's Guide to the Regulations by S. Ehrmeyer

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NONWAIVED TESTS

Requirement	CMS (CLIA)	COLA Accreditation	TJC Accreditation	CAP Accreditation
Verification of method's performance	Yes, follow as appropriate **§493.1235**	Same as for CLIA	Same as for CLIA	Same as for CLIA

§493.1235 Standard: Establishment and verification of performance specifications

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Important points about POCT

- POCT means testing performed near patient by non-laboratorians
- Simple, portable but more expensive than tests in the laboratory

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- POC test does not mean it is waived (example p2y12)

Important points about POCT

- POCT means testing performed near patient by non-laboratorians
- Simple, portable but more expensive than tests in the laboratory
- Most POCT are CLIA waived
- POC test does not mean it is waived (example p2y12)
- Some POC type devices are not CLIA regulated (example- jaundice meter)

CLIA applies to laboratories that examine "materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings"

Method evaluation plan of action

1

- Check the assay's complexity and FDA approval (USA)
- Define quality goals (Allowable Total Error, ATE): units or %
- List and describe experiments

EVALUATION PLAN

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- Collect and analyze data (EP evaluator, Excel, R, etc.)

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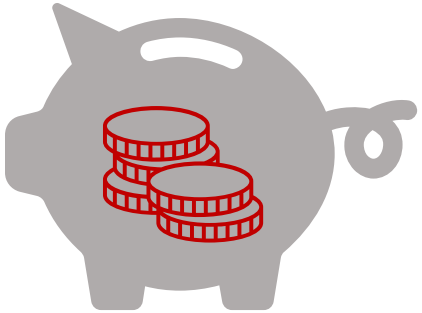
3

- Determine acceptability of the assay
  - Calculate Error and compare to Allowable Error

EVALUATION SUMMARY

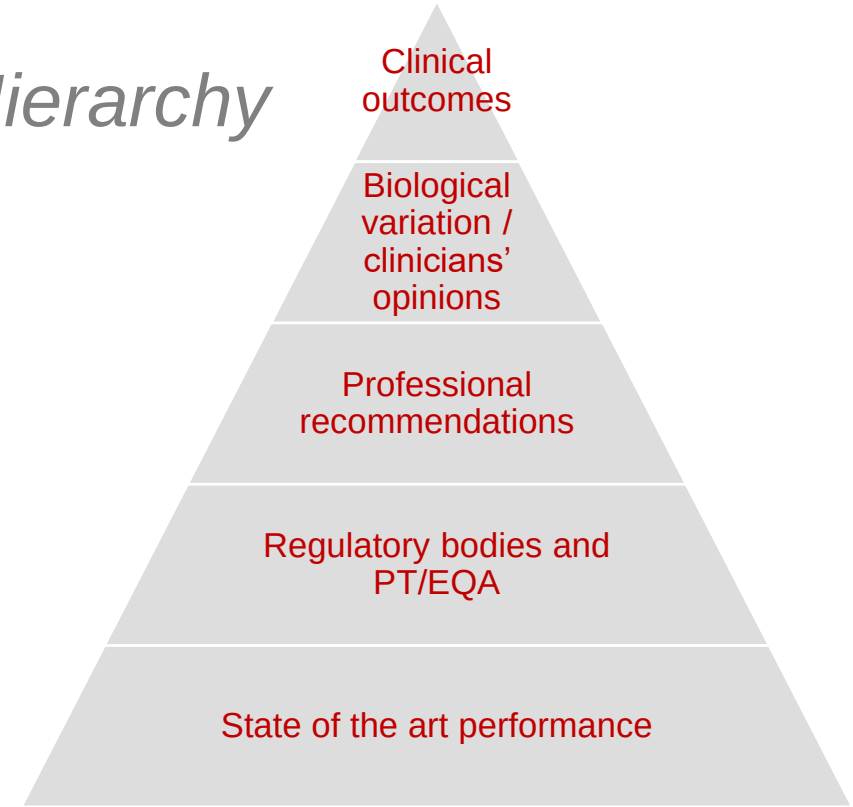
Quality goals: Allowable Total Error (ATE/TE_a)

- ATE is the error budget that is permitted for the test
- ATE used in:
  - Method evaluation
  - Reagent lot-to-lot comparisons
  - QC troubleshooting
  - Method correlations
  - Results corrections



ATE/TE_a: where to find them?

Hierarchy



The 1999 Stockholm Consensus Conference on Quality Specifications

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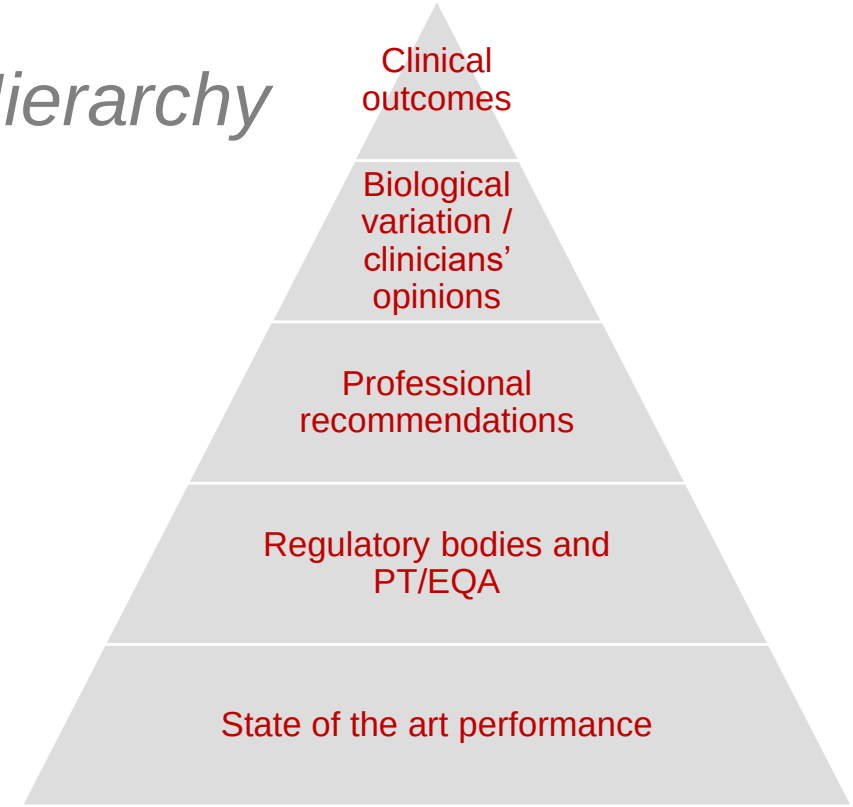

The 1999 Stockholm Consensus Conference on Quality Specifications

Models

1. Clinical outcomes
2. Biological variation
3. State of the art performance

2014 Milan Strategic Conference

Pros and cons of each resource

ATE Resources		Strengths	Weakness
Clinical Outcomes	Direct	Based on clinical data	Studies difficult to perform Uncommon/few examples
	Indirect	Easy to perform (e.g. survey) Based on experience	Subjective decision

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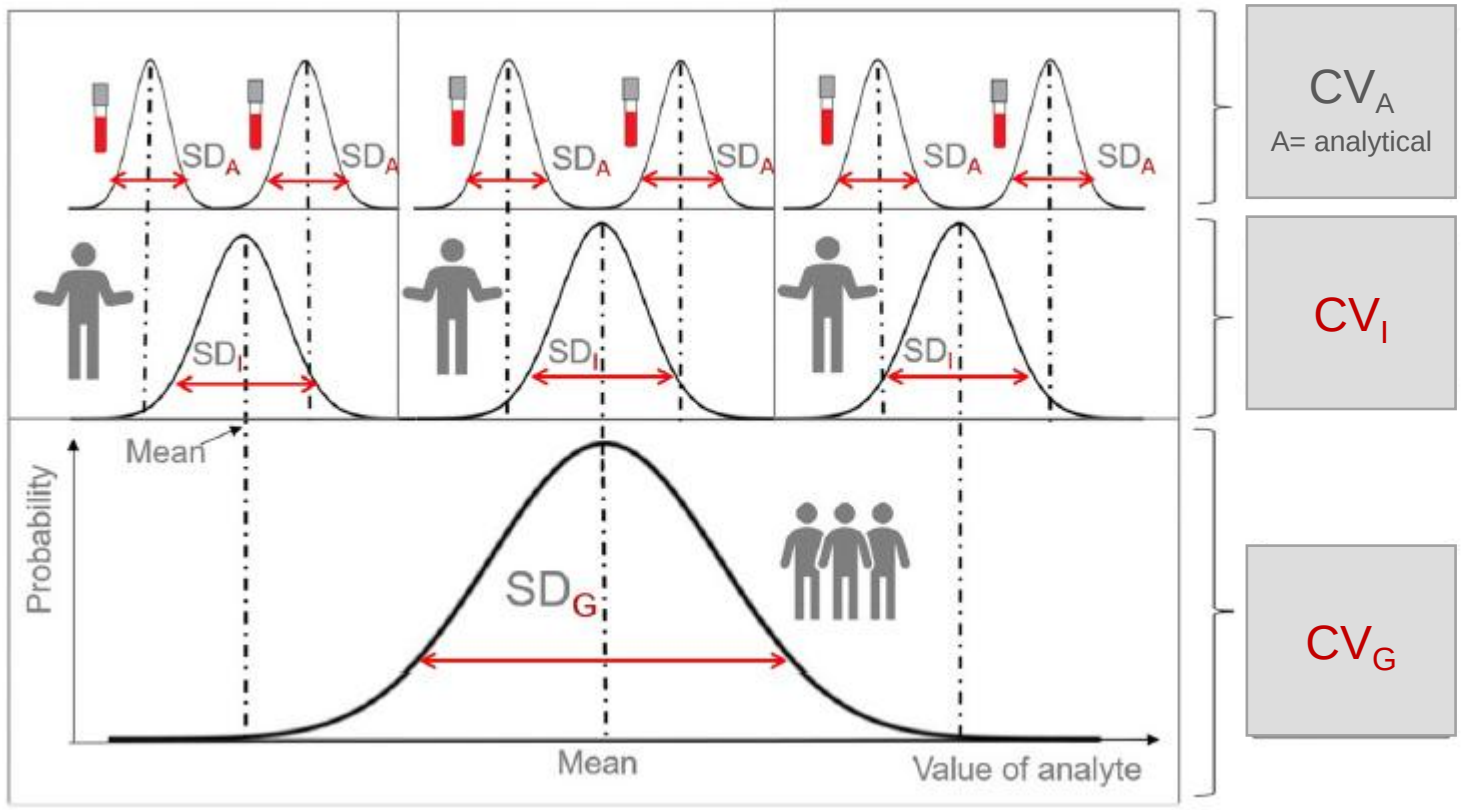
Biological variation		Up-to-date and widely available Database https://biologicalvariation.eu/ 3 models: minimum, desirable and optimum	Often performed on healthy population Rigorous statistical analysis on BV study is imperative

Components of Biological Variation (BV)

- Two components of BV:
  - **CV_I %**: within-person BV (**i**ndividual variation)
  - **CV_G %**: between-person BV (**g**roup variation)

Recall:
CV (%) = SD/mean *100
CV = coefficient of variation
SD = standard deviation



Roraas et al. 2012 Clinical Chemistry

ATE/TE_a derived from biological variation (BV)

- ATE = Allowable Bias + 1.65 X (Allowable Imprecision)

ATE (%) = B + (1.65 X I)

  - Allowable Bias is based on CV_I and CV_G
  - Allowable Imprecision is based on CV_I

The 1.65 multiplier is the one-sided 95% significance probability level

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$$ATE\ (\%) = B + (1.65\ X\ I)$$

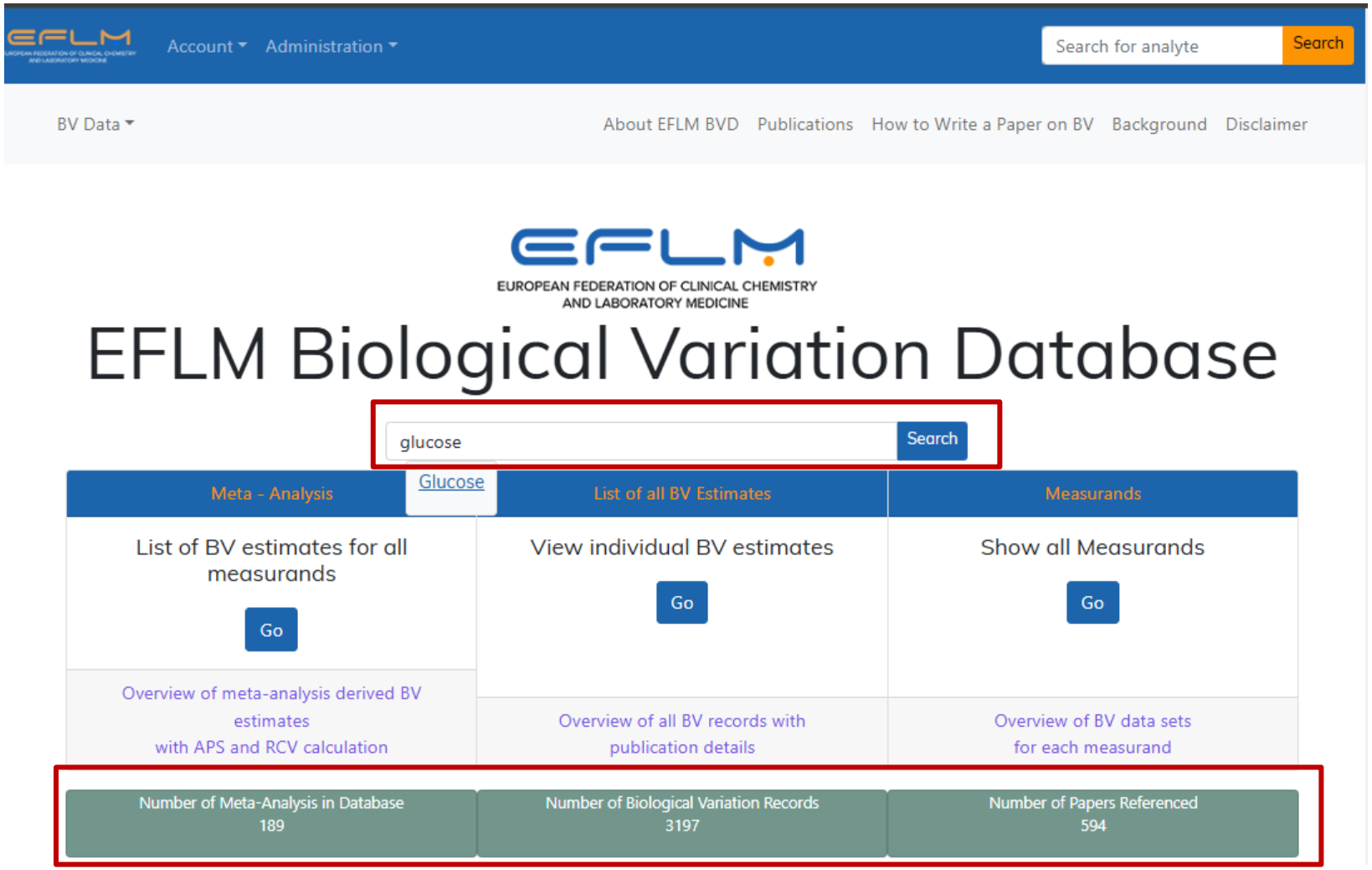
  - Allowable Bias is based on CV_I and CV_G
  - Allowable Imprecision is based on CV_I

- Three sets of analytical performance specifications:

1. Minimum $I\ (\%) = \mathbf{0.75} \times Cv_i$ $B\ (\%) = \mathbf{0.375} \times \sqrt{(CV_i^2 + CV_g^2)}$
2. Desirable $I\ (\%) = \mathbf{0.5} \times Cv_i$ $B\ (\%) = \mathbf{0.25} \times \sqrt{(CV_i^2 + CV_g^2)}$
3. Optimum $I\ (\%) = \mathbf{0.25} \times Cv_i$ $B\ (\%) = \mathbf{0.125} \times \sqrt{(CV_i^2 + CV_g^2)}$

The 1.65 multiplier is the one-sided 95% significance probability level

Biological variation database



Biological variation database

ATE

Glucose

Analytical Performance Specification | RCV Calculation

Matrix	BV Estimate	median CV estimate	lower CI limit	higher CI limit	Comments	Date Updated
Serum/plasma		4.7	3.0	5.4		5/3/2025
Serum/plasma		8.0	2.7	10.8		5/3/2025

Colour Key for References

Included In Meta Analysis | Not Included In Meta Analysis

Reference	Dataset	Estimate of CVI	Estimate of CVG	Gender	Age	State of Well Being	Matrix	Sampling Interval
Variability of capillary plasma glucose in healthy i ndividuals in repeated 75g oral glucose tolerance tests Cummings ST and Fraser CG, Ann Clin Biochem, 634-7, 25, 1988 View Edit Delete	View	4.7	6.1	Mixed		Healthy	Capillary plasma	1 per Week
The EuBIVAS: within- and between-subject biological variation data for electrolytes, lipids, urea, uric acid, total protein, total bilirubin, direct bilirubin and glucose Aarsand AK, Diaz-Garzón J, Fernández-Calle P, et al, Clin Chem, 1380-93, 64(9), 2018	View	4.7	8.1	Mixed		Healthy	Serum	1 per Week

ATE

Specification	CVa	BIAS	MAU	Total Error
Minimum	3.5	3.5	7.1	9.3
Desirable	2.4	2.3	4.7	6.2
Optimal	1.2	1.2	2.4	3.1

Cva: BV based imprecision
MAU: measurement of uncertainty

Pros and cons of each resource

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	Indirect	Easy to perform (e.g. survey) Based on experience	Subjective decision
Biological variation		Up-to-date and widely available Database 3 models: minimum, desirable and optimum	Often performed on healthy population Rigorous statistical analysis on BV study is imperative
State-of-the-art		Readily accessible	Not desirable but what is currently achievable

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Professional Organizations		Based on clinical data and professional experience	Only available for few analytes No consensus among groups
Accrediting and regulatory organizations		Ensures passing of EQA/PT Based on state-of-the-art, with input from industry and professional organizations	Set to identify poor performers and not clinically needed accuracy

TE_a comparison across different sources

Analyte	Clinical outcomes/ Expert opinion	Biological variation (desirable)	CLIA (‘25) / CAP
pH, B			
pO2, B			
pCO2, B			
K (mmol/L), B			
K (mmol/L), S/P			
Glucose (mg/dL), B			
Glucose (mg/dL), S/P			
HbA1c (NGSP), B			
Hemoglobin, B			
Prothrombin time (seconds or INR), B			
Cholesterol			

B: whole blood – BGAS analyzers, ISTAT, S/P: serum/plasma

TE_a comparison across different sources

Analyte	Clinical outcomes/ Expert opinion	Biological variation (desirable)	CLIA (‘25) / CAP
pH, B	N/A		
pO2, B	N/A		
pCO2, B	N/A		
K (mmol/L), B	±4.8-9.6% (indirect, questionnaire)		
K (mmol/L), S/P			
Glucose (mg/dL), B	±11.2-15.9%(indirect, questionnaire)		
Glucose (mg/dL), S/P			
HbA1c (NGSP), B	±6.7% (indirect)		
Hemoglobin, B	±3.6- 5.2 (indirect, que)		
Prothrombin time (seconds or INR), B	N/A		
Cholesterol	± 9% (NCEP)		

B: whole blood – BGAS analyzers, ISTAT, S/P: serum/plasma

TE_a comparison across different sources

Analyte	Clinical outcomes/ Expert opinion	Biological variation (desirable)	CLIA (‘25) / CAP
pH, B	N/A	N/A	
pO2, B	N/A	N/A	
pCO2, B	N/A	±5.7% (n=1)	
K (mmol/L), B	±4.8-9.6% (indirect, questionnaire)	N/A	
K (mmol/L), S/P		±4.9%	
Glucose (mg/dL), B	±11.2-15.9%(indirect, questionnaire)	N/A	
Glucose (mg/dL), S/P		±6.2%	
HbA1c (NGSP), B	±6.7% (indirect)	±2.4%	
Hemoglobin, B	±3.6- 5.2 (indirect, que)	±3.9%	
Prothrombin time (seconds or INR), B	N/A	±3.6%	
Cholesterol	± 9% (NCEP)	±8.6%	

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TE_a comparison across different sources

Analyte	Clinical outcomes/ Expert opinion	Biological variation (desirable)	CLIA ('25) / CAP
pH, B	N/A	N/A	±0.04
pO2, B	N/A	N/A	±15% or ±15 mmHg
pCO2, B	N/A	±5.7% (n=1)	±8% or ±5 mmHg
K (mmol/L), B	±4.8-9.6% (indirect, questionnaire)	N/A	±0.3 mmol/L
K (mmol/L), S/P		±4.9%	
Glucose (mg/dL), B	±11.2-15.9%(indirect, questionnaire)	N/A	±8% or ±6 mg/dL
Glucose (mg/dL), S/P		±6.2%	
HbA1c (NGSP), B	±6.7% (indirect)	±2.4%	±8% / ±6%
Hemoglobin, B	±3.6- 5.2 (indirect, que)	±3.9%	±4%
Prothrombin time (seconds or INR), B	N/A	±3.6%	±15%
Cholesterol	± 9% (NCEP)	±8.6%	±10%

B: whole blood – BGAS analyzers, ISTAT, S/P: serum/plasma

Example of TE_a for Glucometers

Purpose	Organization	Glucose Value	TEa	Percentage of Data
Home-use meters	ISO 15197 standard	< 100 mg/dL	± 15 mg/dL	95%
		≥ 100 mg/dL	± 15%	
	FDA 2020 standard	Reportable range of meter	± 15%	95%
Hospital-use meters	CLSI POCT12	< 100 mg/dL	± 12 mg/dL	95%
		≥ 100 mg/dL	± 12.5%	
		< 75 mg/dL	± 15 mg/dL	98%
		≥ 75 mg/dL	± 20%	
	FDA 2020 standard	< 75 mg/dL	± 12 mg/dL	95%
		≥ 75 mg/dL	± 12%	95%
		< 75 mg/dL	± 15 mg/dL	98%
		≥ 75 mg/dL	± 15%	98%

Clin Chem. 2023;69(8):808-868

Method evaluation plan of action

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- Check the assay's complexity and FDA approval (USA)
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- **List and describe experiments**

EVALUATION PLAN

2

- Collect and analyze data (EP evaluator, Excel, R, etc.)

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- Determine acceptability of the assay
  - Calculate Error and compare to Allowable Error

EVALUATION SUMMARY

What is required in a method evaluation?

FDA approved (moderate or high complexity

Verify

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals

Non-FDA (Modified FDA or Lab Developed Test)

Establish

1. Analytical measuring range (AMR)
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5. Analytical Sensitivity
6. Analytical Specificity
7. Any additional studies

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CLIA Waived

Method evaluation not required

What is required in a method evaluation?

POC testing

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AMR study verifies linear relationship

- Only applies to tests that produce quantitative numerical results
  - not required for clot-based coagulation tests, platelet function tests, and other tests where output is a unit of time or arbitrary reporting (f.ex. Ratio for AKI risk score)
  - check with manufacturer

AMR: analytical measuring range

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- Only applies to tests that produce quantitative numerical results
  - not required for clot-based coagulation tests, platelet function tests, and other tests where output is a unit of time or arbitrary reporting (f.ex. Ratio for AKI risk score)
  - check with manufacturer
- Samples
  - Commercial material
  - Patient pool (mix of very high with very low concentrations)
- At least 3-5 different concentration covering the AMR, equally spaced is ideal
- Assay ideally in triplicate
- Goal: slope of the line 0.9 – 1.1 and values should fall in within 10% of the upper and lower limit of the AMR

AMR: analytical measuring range

Analytical vs reportable range

- Analytical measurement range (AMR)
  - the maximum range of values that can be assayed accurately without dilutions - provided by the manufacturer

    - Example: Glucose, S/P: 10 – 800 mg/dL
      Glucose, Capillary (POCT): 10 – 600 mg/dL

If value is lower that AMR: report as <X
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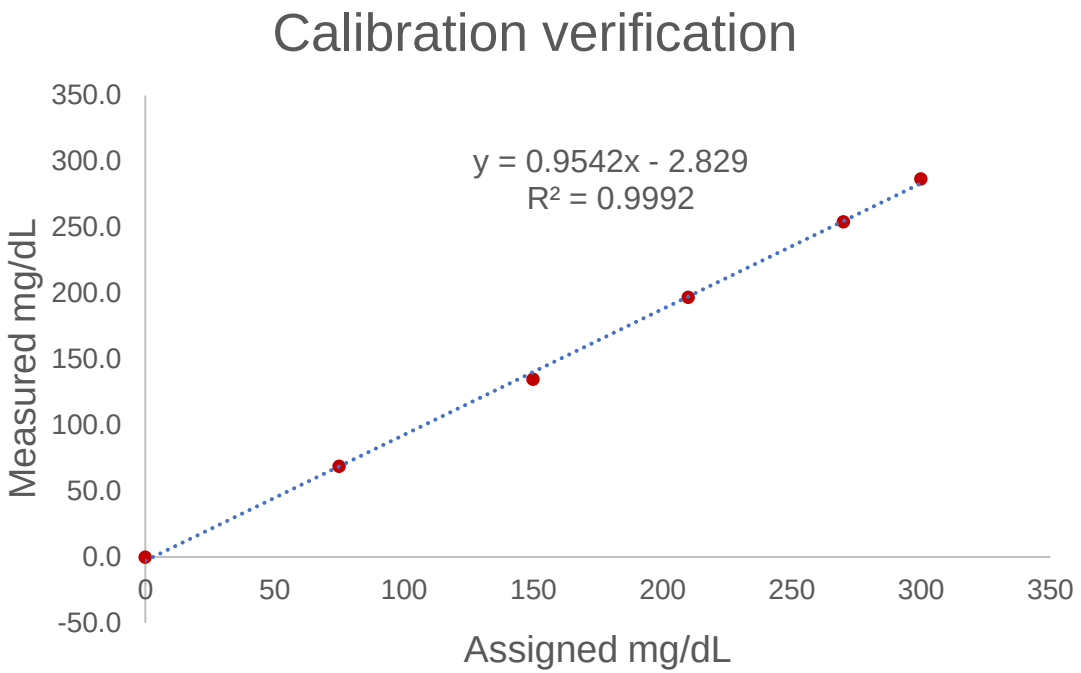
- Reportable range (RR)
  - can be reported following dilutions - provided by the manufacturer or established by the lab (hard to apply on POCT instruments)
    - Example: Glucose, S/P: 10 – 2400 mg/dL

Example of the AMR

Target	Rep 1	Rep 2	Rep 3	Average	%CV
0	0.0	0.0	0.0	0.0	0.0
75	68.1	69.8	68.7	68.9	1.3
150	136.6	133.3	134.6	134.8	1.2
210	195.8	196.8	198.4	197.0	0.7
270	254.1	257.0	252.2	254.4	0.9
300	285.9	286.4	288.2	286.8	0.4

AMR: 0 – 300 mg/dL
Observed range: 0 – 287 mg/dL
Slope: 0.95
R=0.99



Goal= slope 0.95-1.05, R > 0.975
cover 10% low and high AMR

Solutions for issues with linearity

- Repeat the study
- Use saline to lower the observed range
- Look for a new kit (various vendors, different calibrator lot)
- Use a patient sample
- Truncate the AMR

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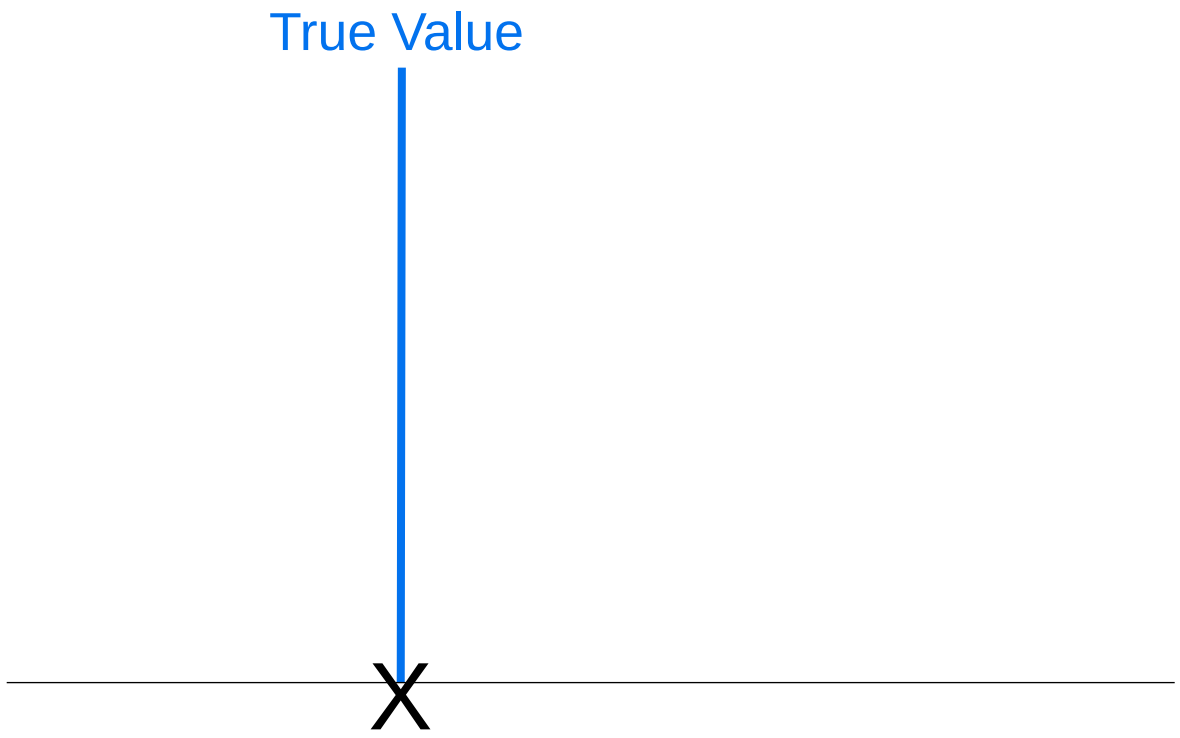
Accuracy and precision in terms of error



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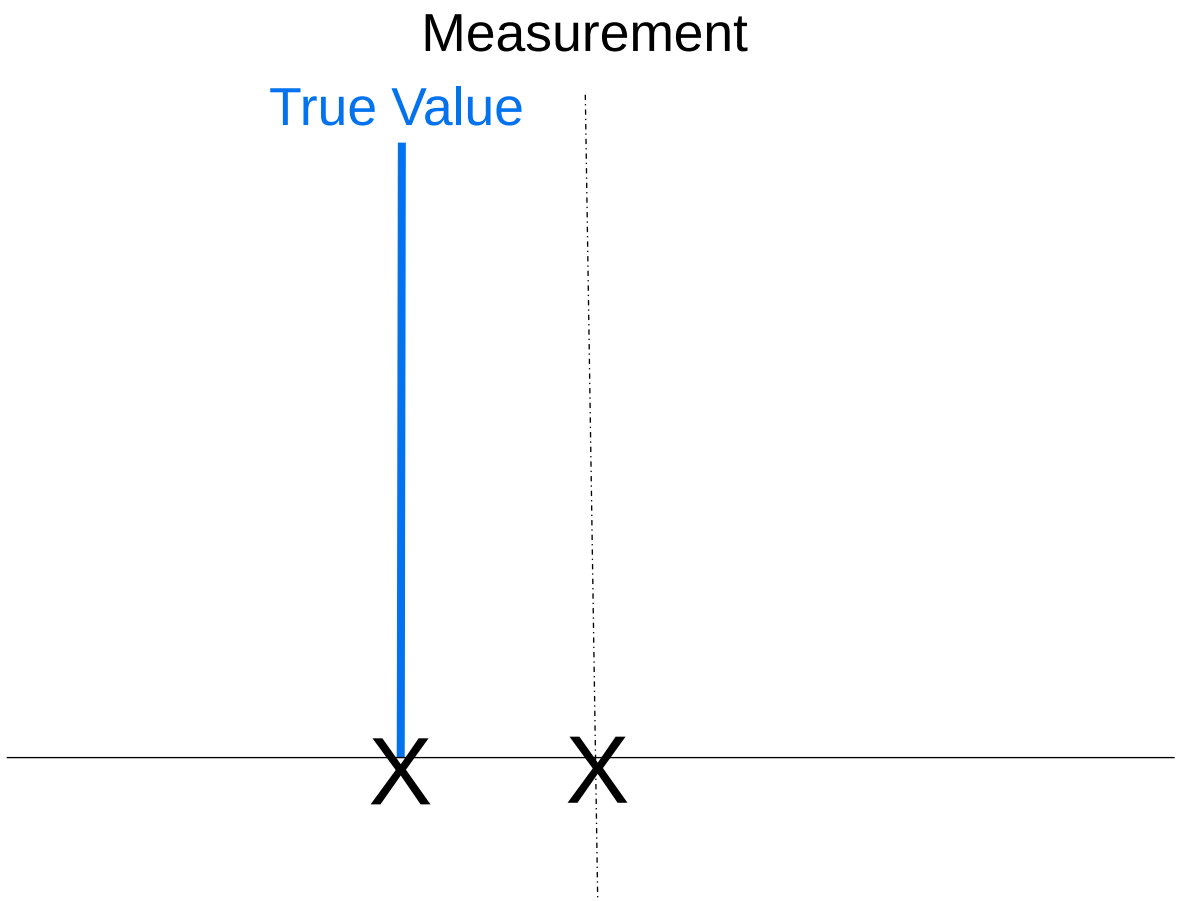

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    - Slope indicates proportional error
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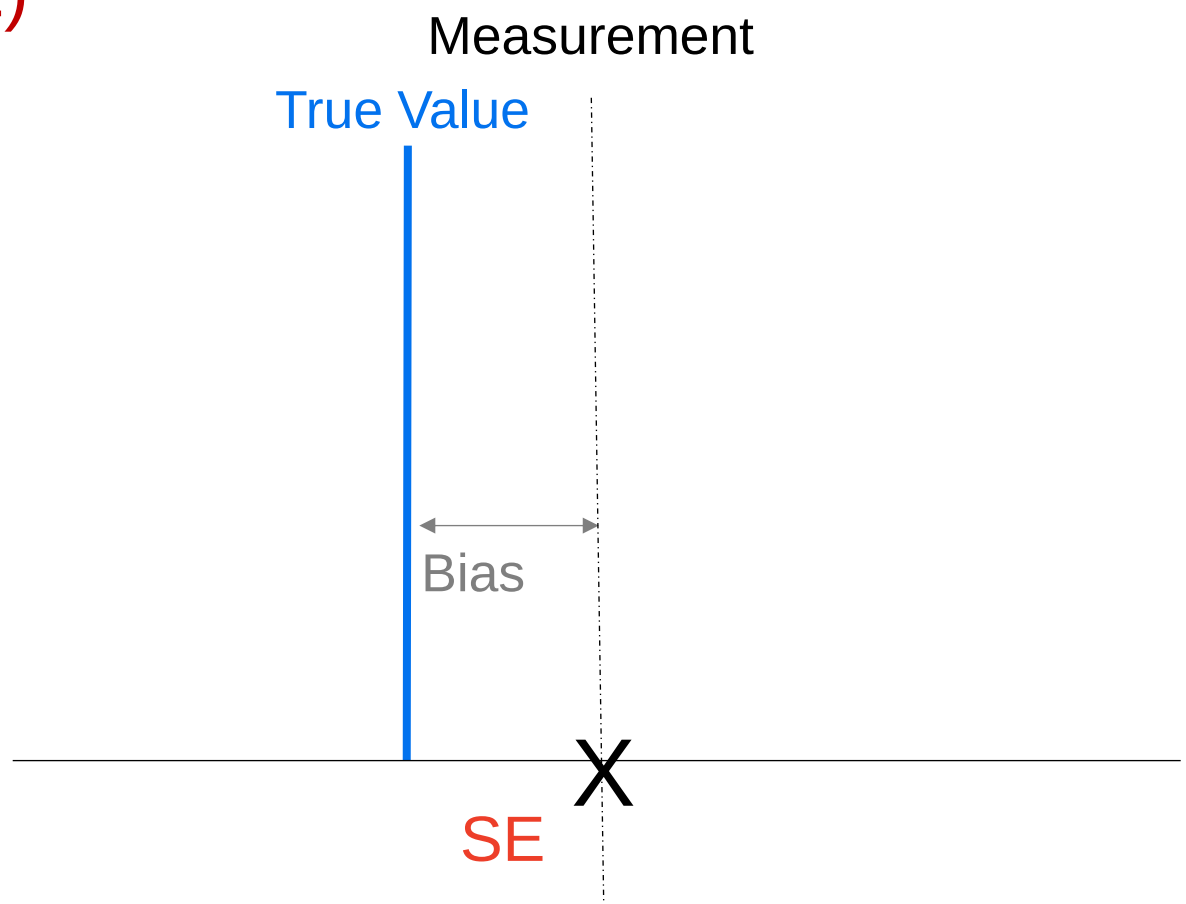

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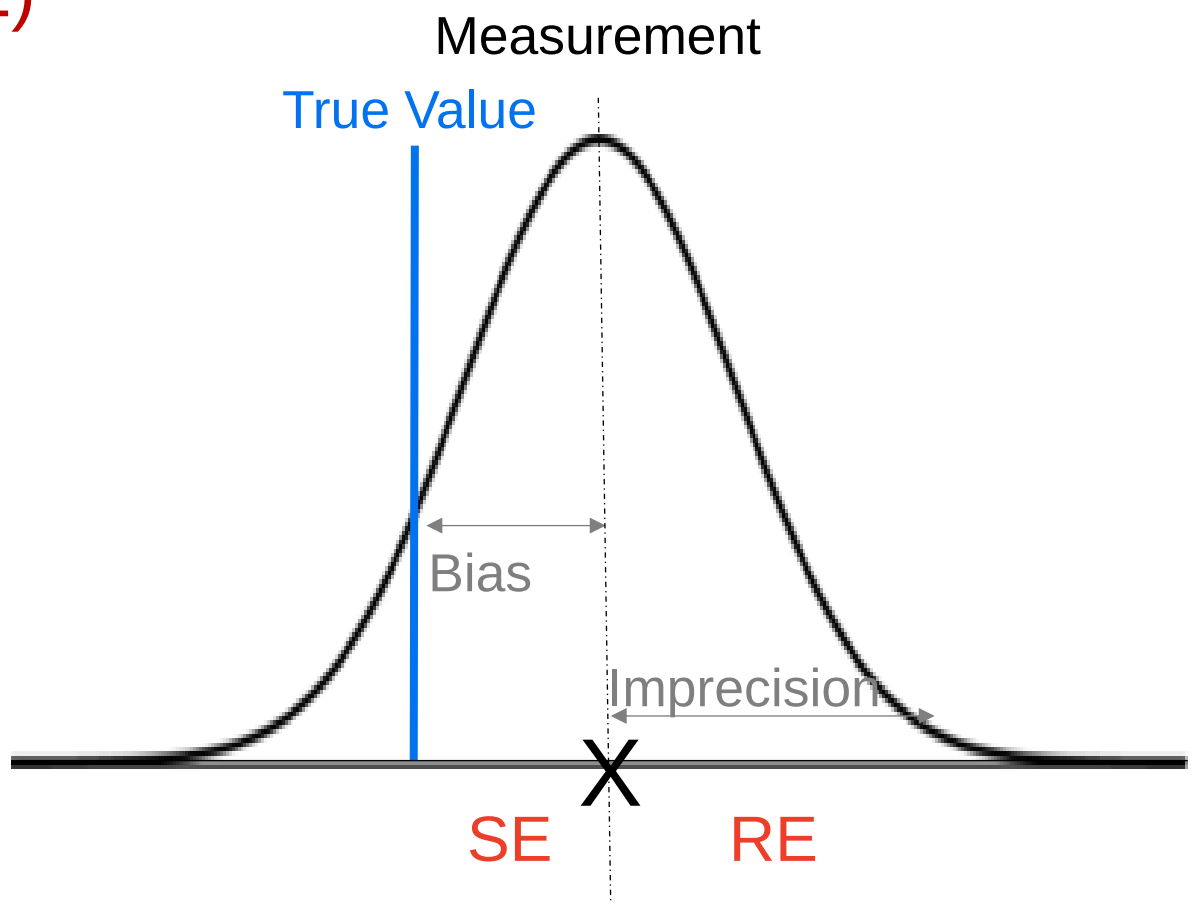
    - Slope indicates proportional error
    - y-intercept indicates constant error
- Precision = random error (RE)
  - Estimated by
    - Standard deviation (SD)
    - Coefficient of variation (CV)
      - CV = SD/mean*100
    - Correlation coefficient
    - Standard error of the estimate



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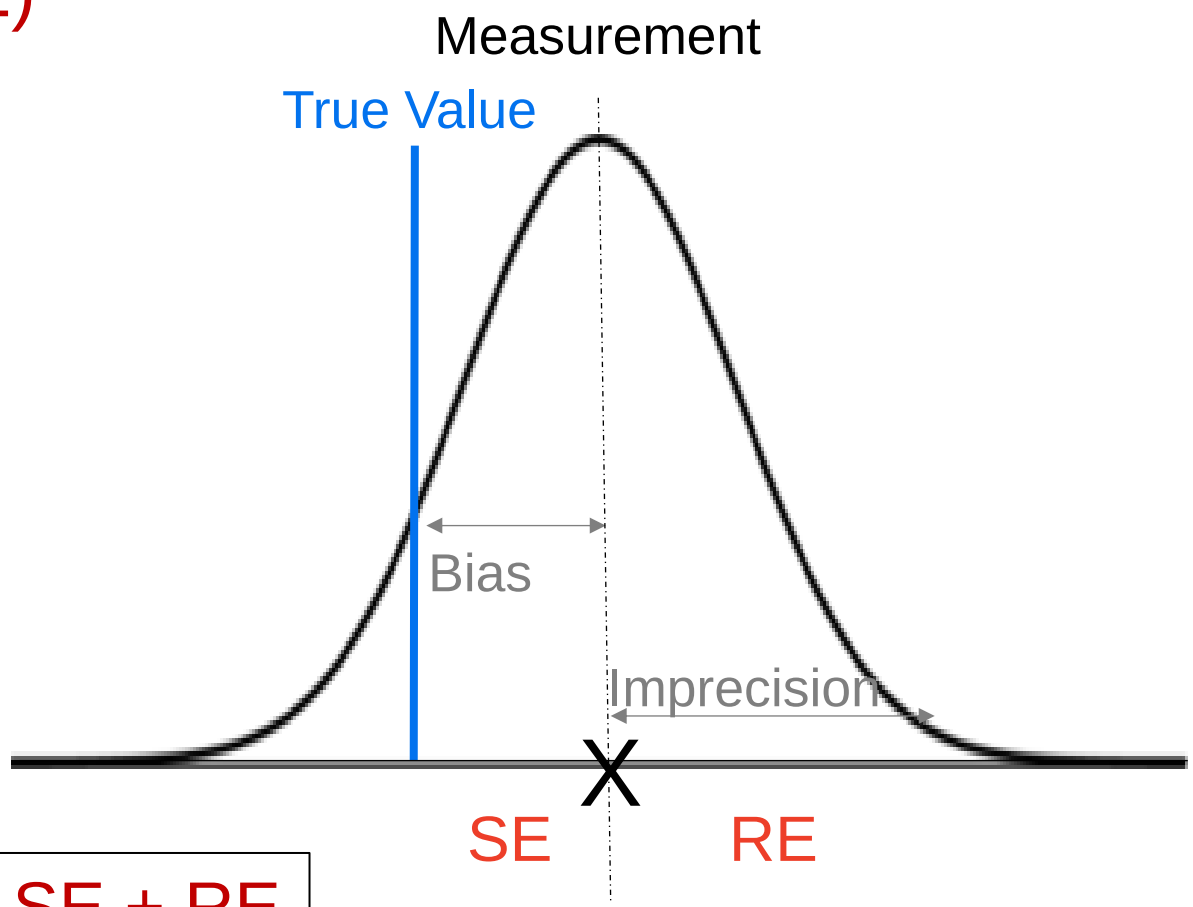

Total analytical error = SE + RE

Precision experiments estimate Random Error

Intra-assay

- Within run (day)
  - Short-term variation
- N = 10 or 20
  - 2 or 3 concentrations
- Assayed in one run
- Goal:
  - CV < ATE/4 *(acceptable)*
  - CV < ATE/6 *(desirable)*
  - CV ≤ manufacturer’s claims

ATE: allowable total error

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Inter-assay

- Between run (day)
  - Long(er)-term variation
- N = 20
  - 2 or 3 concentrations
- Assayed over
  - 1 x 20 days
  - 2 x 10 days
  - 4 x 5 days
- Goal:
  - CV < ATE/4
  - CV < ATE/3
  - CV ≤ manufacturer's claims

ATE: allowable total error

Example of precision study

Intra-assay

	QC L1	QC L2
1	60.6	238.5
2	59.3	237.5
3	59.8	238.7
4	59.4	233.5
5	58.4	239.2
6	58.4	242.8
7	59.0	232.3
8	57.1	242.7
9	59.3	242.4
10	59.0	233.7
Mean	59.0	238.1
SD	1.0	3.9
CV	1.6%	1.6%

Goal:

CV < ATE/4

ATE = 20%

CV < 5%

Inter-assay

	Date	QC L1	QC L2
1	2/14/2024	55.3	245.7
2	2/14/2024	51.3	244.3
3	2/15/2024	53.5	241.8
4	2/16/2024	56.6	253.9
5	2/16/2024	55.6	249.2
6	2/18/2024	55.6	238.0
7	2/18/2024	55.3	243.6
8	2/18/2024	57.1	243.5
9	2/19/2024	51.8	236.5
10	2/19/2024	56.0	244.4
11	2/20/2024	50.4	249.4
12	2/20/2024	55.2	262.9
13	2/21/2024	51.8	247.6
14	2/21/2024	53.8	250.6
15	2/22/2024	50.8	245.8
16	2/22/2024	50.7	238.6
19	2/25/2024	56.7	252.0
20	2/25/2024	54.4	261.3
21	2/26/2024	54.1	257.5
22	2/26/2024	54.1	247.3
23	2/27/2024	51.7	240.3
24	2/27/2024	52.4	242.4
25	4/30/2024	54.5	250.5
	Mean	53.9	247.3
	SD	2.1	7.0
	CV	3.9%	2.8%

Goal:

CV < ATE/3

ATE = 20%

CV < 6.7%

Solutions for issues with precision study

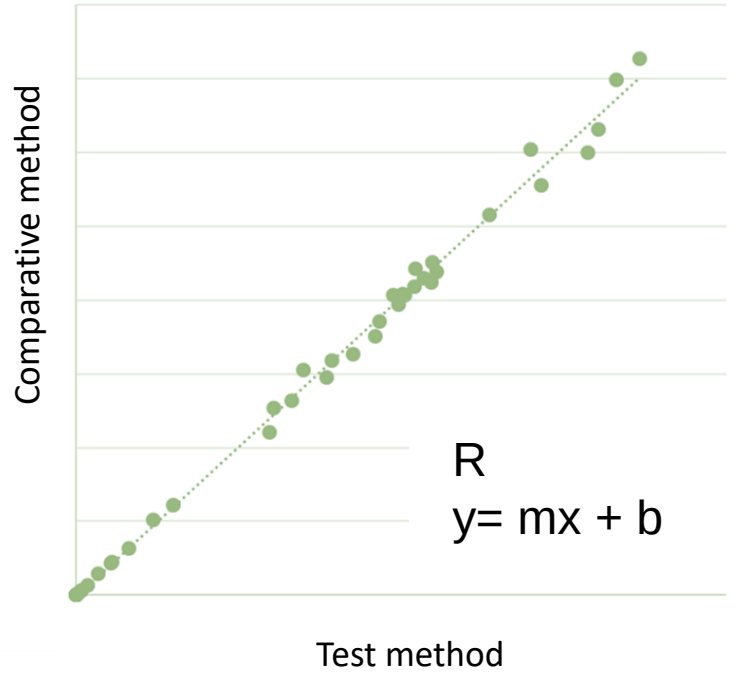
- Look for outliers
- Repeat precision study
- Check manufacturer specifications
- Check CV from the current QC (if applicable)
- Use a different QC product
- Reassess ATE (rarely)

Accuracy experiments estimate Systematic Error

- Inaccuracy is determined by comparison of a test method to a comparison method
- 40 patient specimens (preferred) spanning the AMR
  - 20 patients acceptable if replacing method with like method
- Time frame (recommended minimum of 5 days)
  - Multiple calibration
  - Spiking and dilutions are possible
- Evaluate data
  - Graphically
  - Statistically

Analysis of comparison data

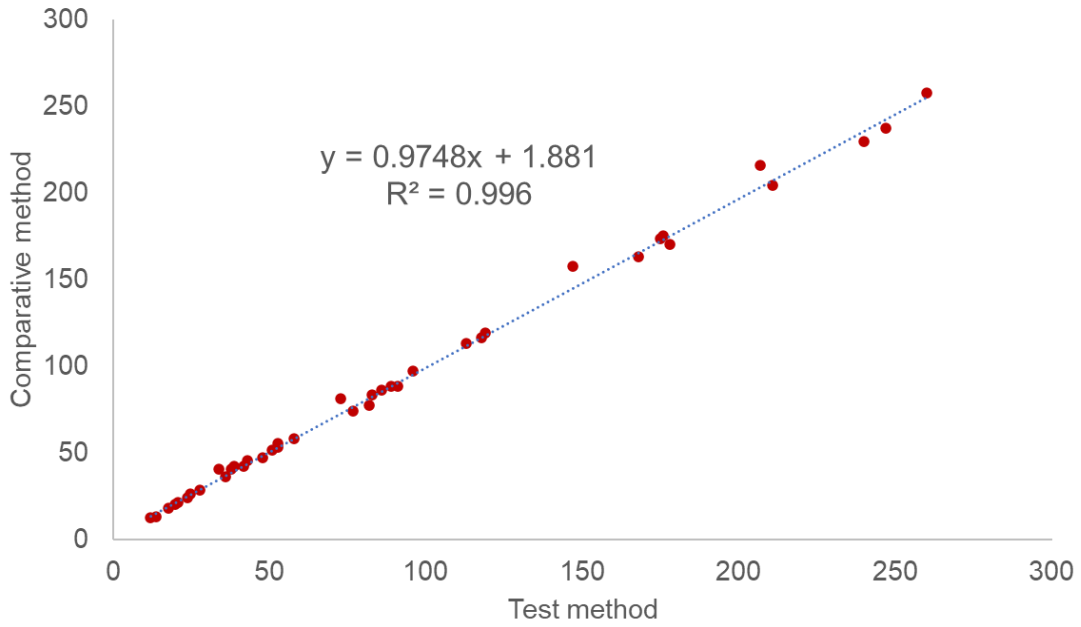
1. List results from two method pairs
    1. X – results of reference method
    2. Y – results of new method
2. Create a scatter plot
3. Determine the correlation coefficient “R”
4. Generate a “linear best fit line”
    - Least Squares, if R ≥ 0.975
    - Deming Regression, if R < 0.975
        - Affected by outliers
    - Passing Bablok, if R < 0.975
        - Not affected by outliers



m = slope (indicates a proportional error)
goal => 0.9-1.1
b = intercept (indicates constant error)

Accuracy data

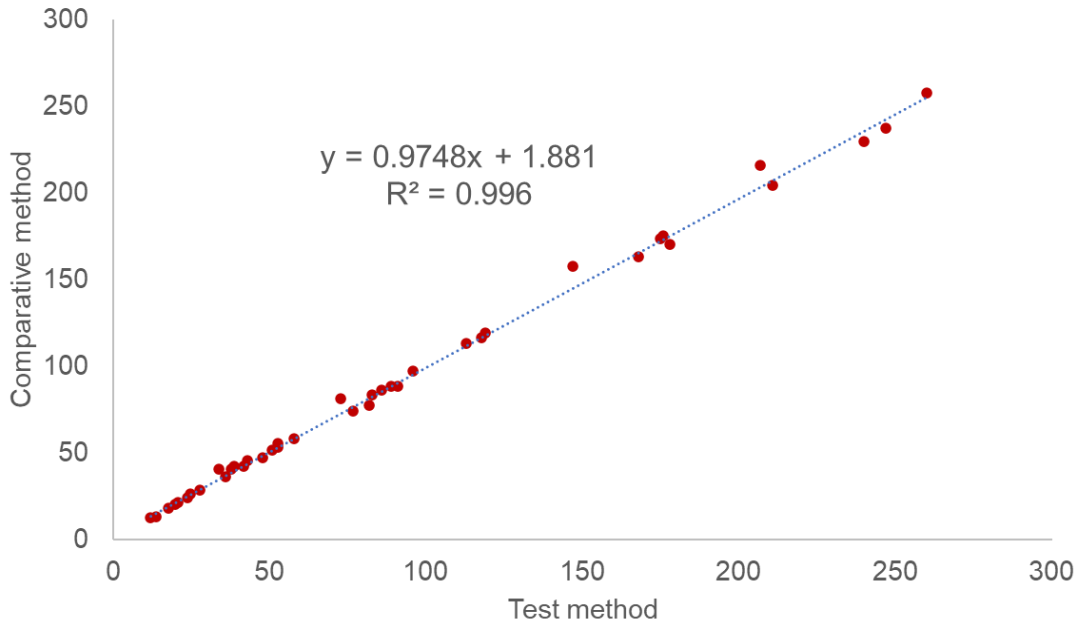
Goal= slope 0.9-1.1, span the AMR



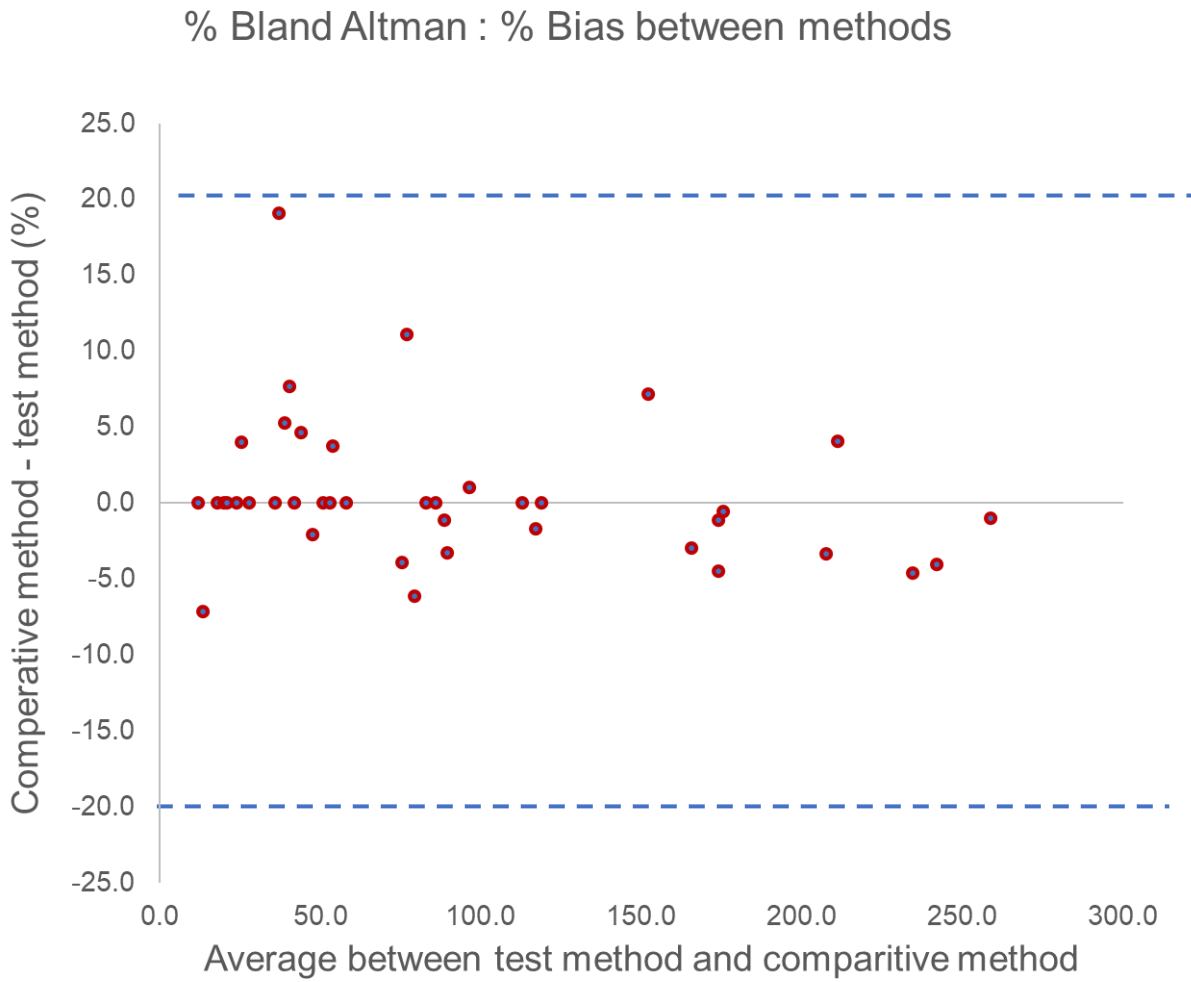
Scatter plot

Accuracy data

Goal= slope 0.9-1.1, span the AMR



Scatter plot



% Bland-Altman

ATE = 20%

Correlation Coefficient ≠ Bias

- “R” – statistical term
- Indicates linear relationship between methods
- Small R indicates inadequate results range
- Systematic error has no effect on R
- “R“ is influenced by random errors only

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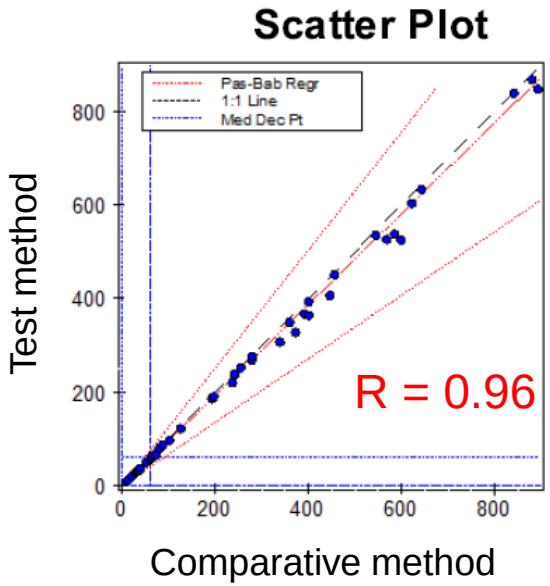
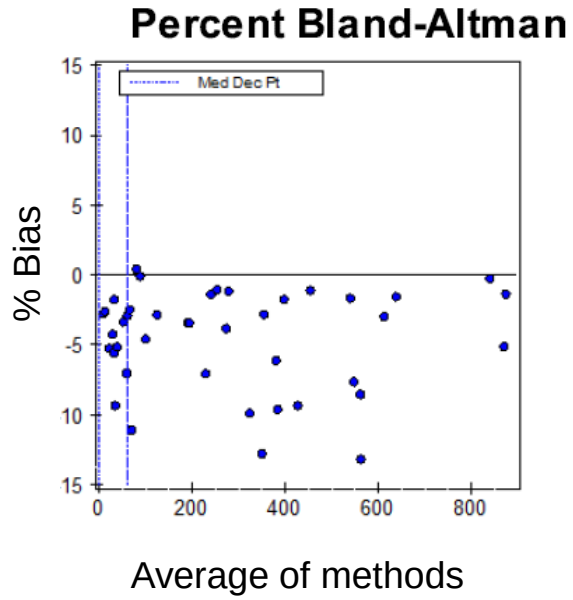


y= 0.716x + 2.3



Solutions for issues with accuracy

- Repeat study using fresh samples
- Recalibrate both assays (if applicable)
- Change reagent lots
- Run PT samples
- Use spiked samples to cover upper end of the AMR

What is required in a method evaluation?

FDA approved (moderate or high complexity

Verify

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. **Reference intervals**

CLIA Waived

Method evaluation not required

Non-FDA (Modified FDA or Lab Developed Test)

Establish

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals
5. Analytical Sensitivity
6. Analytical Specificity
7. Any additional studies

Reference interval evaluation

- Results observed in a “healthy” population (generally central 95%)
- Criteria for evaluation of reference intervals include:

1. Introduction of a new analyte to the test repertoire
2. Change of analytic methodology
3. Change in patient population

Reference interval studies

- Maintain current reference interval
  - Method comparison meets acceptance criteria

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  - Calculate the % of results outside the proposed reverence range (10% outside verifies the proposed range)

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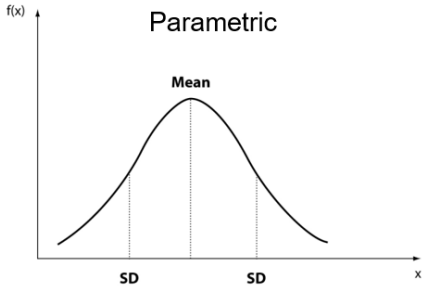
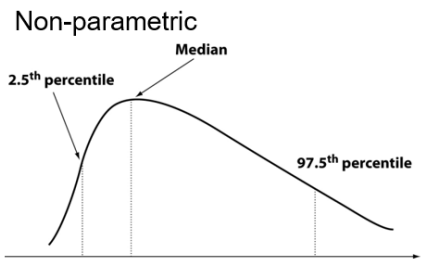
  - Calculate the % of results outside the proposed reverence range (10% outside verifies the proposed range)

- Establishing a reference range
  - Create a histogram of the results
  - Minimum N = 120
    - If subclasses, 120 per subgroup (gender, age)



- Assumes normal distribution
- Calculate the mean ± 2SD



- Arrange test results in ascending order
- Discard the highest 2.5% and the lowest 2.5%

Reference interval studies

- Maintain current reference interval
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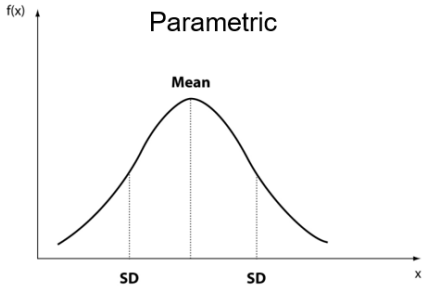

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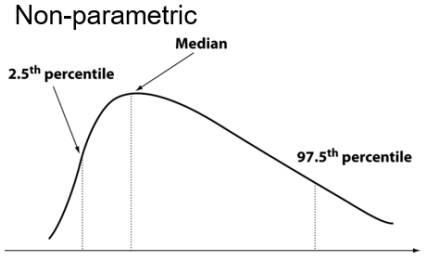


- Arrange test results in ascending order
- Discard the highest 2.5% and the lowest 2.5%

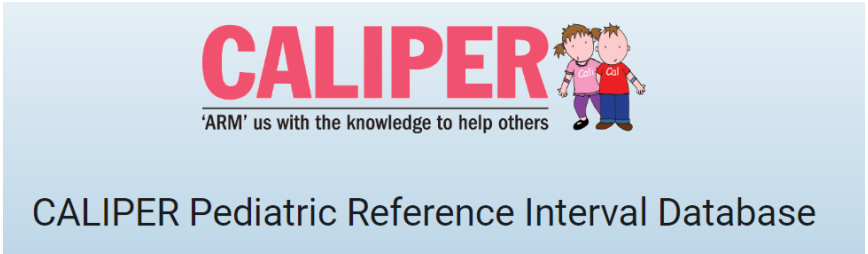
- Transferring a reference range
  - Acceptable but not recommended

$Y_{lower} = (X_{lower} * \text{slope}) + \text{y-intercept}$
$Y_{higher} = (X_{higher} * \text{slope}) + \text{y-intercept}$

Considerations on reference ranges

- Pediatric ranges – difficult to obtain

  CALIPER for pediatric reference ranges
  https://caliper.research.sickkids.ca/#/



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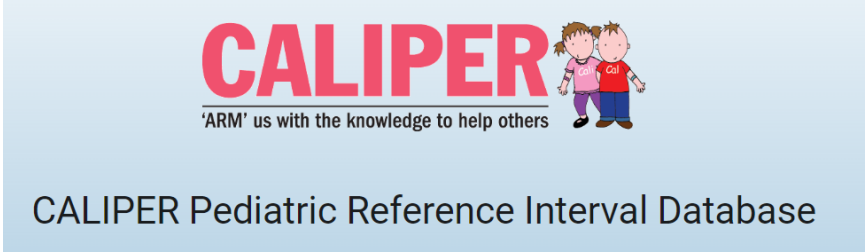

- Venous blood is the reference sample for most laboratory testing
  - Easy to obtain (compared to arterial)
  - Reflects circulating concentration of cells, analytes, proteins in blood
  - Defined by ADA as reference type for diabetes diagnosis

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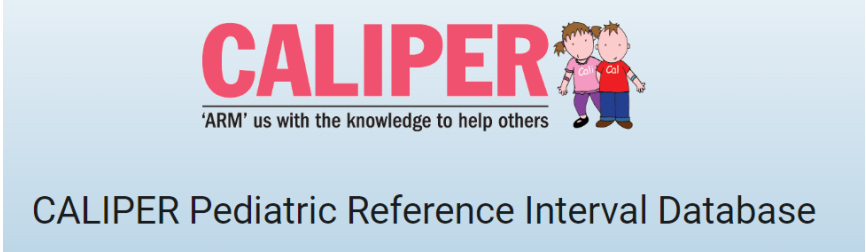

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  - Easy to obtain (compared to arterial)
  - Reflects circulating concentration of cells, analytes, proteins in blood
  - Defined by ADA as reference type for diabetes diagnosis

- Capillaries: cannot be directly accessed (too small)
  - Capillary blood" is mixture of arterial blood, venous blood and interstitial fluid
  - Tests offered in capillary: A1C, hemoglobin, Bilirubin, Glucometer, PT/INR, blood gases
  - K, AST, LDH several fold higher

Method evaluation plan of action

1

- Check the assay's complexity and FDA approval (USA)
- Define quality goals (Allowable Total Error, ATE): units or %
- List and describe experiments

EVALUATION PLAN

2

- Collect and analyze data (EP evaluator, Excel, R, etc.)

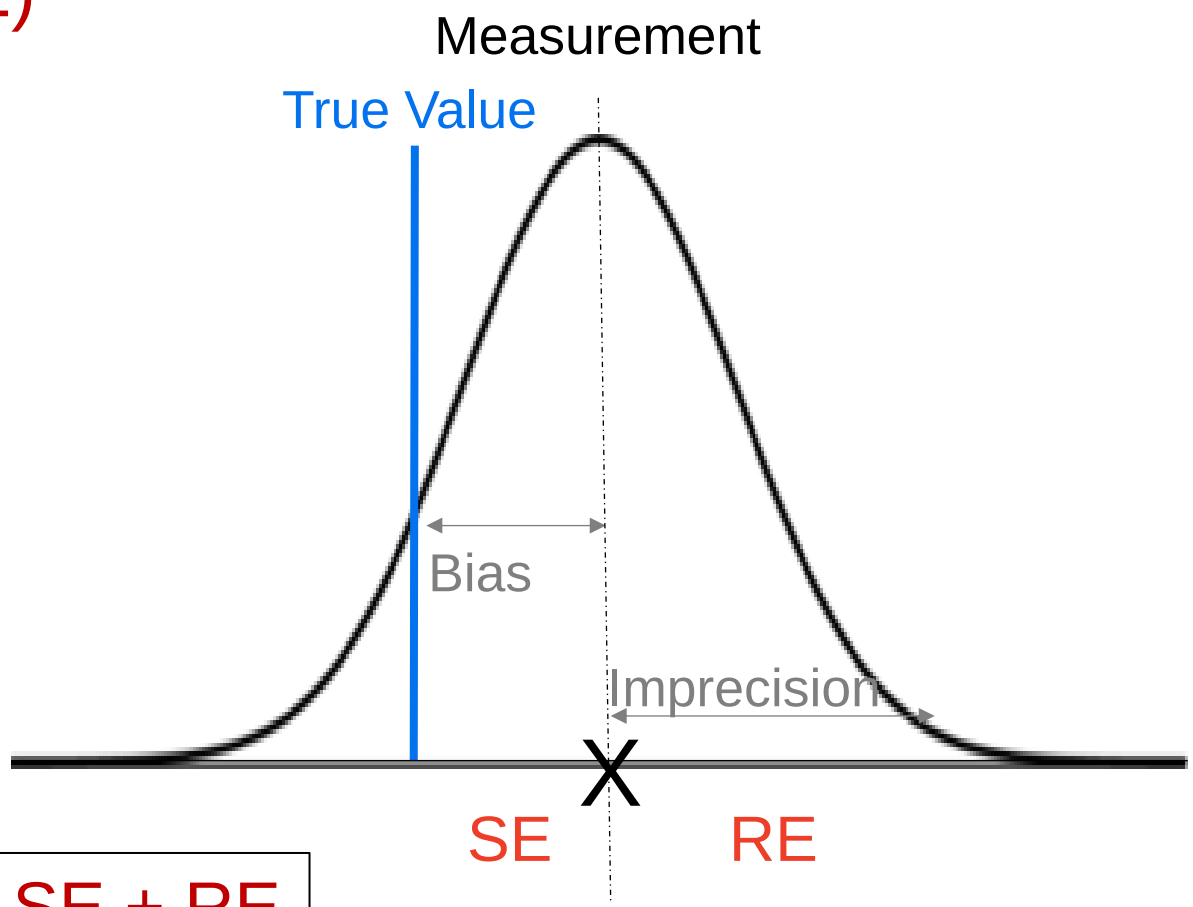
3

- Determine acceptability of the assay
  - Calculate Error and compare to Allowable Error

EVALUATION SUMMARY

Accuracy and precision in terms of error

- Accuracy = bias, systematic error (SE)
  - Estimated from regression analysis
    - Slope indicates proportional error
    - y-intercept indicates constant error
- Precision = random error (RE)
  - Estimated by
    - Standard deviation (SD)
    - Coefficient of variation (CV)
      - CV = SD/mean*100
    - Correlation coefficient
    - Standard error of the estimate



Total analytical error = SE + RE

Using Quality Goals in Method Evaluation

1. Define Quality Goals = Total Allowable Error (TE_a)
2. Error Assessment = Total Analytical Error (TAE)

TAE= Bias (SE) + Imprecision (RE)

3. Compare TAE vs ATE (TE_a)
   - When TAE < TE_a, test method is considered acceptable for patient care use

Error analysis at medical decision limits

Target value
40
99
126
200
400

TAE= Bias + Imprecision

Error analysis at medical decision limits

Target value	Slope	Y-int	New method value	%Bias
40	0.96	1.91	40.3	0.8
99	0.96	1.91	96.9	2.1
126	0.96	1.91	122.9	2.5
200	0.96	1.91	193.9	3.0
400	0.96	1.91	385.9	3.5

TAE= Bias + Imprecision

%Bias = ABS(Bias/target value) x 100

Error analysis at medical decision limits

Target value	Slope	Y-int	New method value	%Bias	CV (Day to Day QC)
40	0.96	1.91	40.3	0.8	1.2
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Error analysis at medical decision limits

Target value	Slope	Y-int	New method value	%Bias	CV (Day to Day QC)	Total Analytical Error (%)
40	0.96	1.91	40.3	0.8	1.2	2.8
99	0.96	1.91	96.9	2.1	1.2	4.1
126	0.96	1.91	122.9	2.5	1.2	4.5
200	0.96	1.91	193.9	3.0	1.2	5.0
400	0.96	1.91	385.9	3.5	1.2	5.5

TAE= Bias + Imprecision

Total Analytical Error = I%BiasI + 1.65 x %CV
*1.65 for one sided estimate
%Bias = ABS(Bias/target value) x 100

Error analysis at medical decision limits

Target value	Slope	Y-int	New method value	%Bias	CV (Day to Day QC)	Total Analytical Error (%)	Total Allowable Error (%)	Total Error Observed < Total Error Allowed
40	0.96	1.91	40.3	0.8	1.2	2.8	8	YES
99	0.96	1.91	96.9	2.1	1.2	4.1	8	YES
126	0.96	1.91	122.9	2.5	1.2	4.5	8	YES
200	0.96	1.91	193.9	3.0	1.2	5.0	8	YES
400	0.96	1.91	385.9	3.5	1.2	5.5	8	YES

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What is required in a method evaluation?

FDA approved (moderate or high complexity

Verify

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals

CLIA Waived

Method evaluation not required

Non-FDA (Modified FDA or Lab Developed Test)

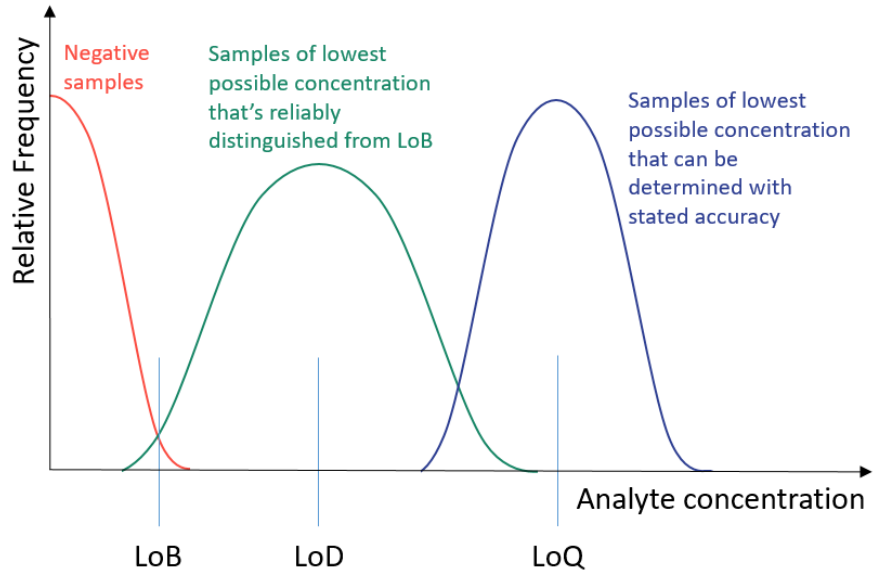
Establish

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals
5. **Analytical Sensitivity**
6. **Analytical Specificity**
7. Any additional studies

Analytical sensitivity

- Purpose: to determine the lowest concentration of an analyte
  - For FDA approved tests documentation from the manufacturer may be used
  - For non-FDA or FDA modified, analytical sensitivity must be established

- Evaluated parameters:
  - Limit of blank (LOB)
  - Limit of detection (LOD)
  - Limit of quantification (LOQ)



LOQ study on Et Glycol

Ethylene Glycol spiked samples

		~2		~3		~4		~5		~10	
Patient pool	SD	Low	SD	Low	SD	Low	SD	Low	SD	Low	SD
0.85				4.96		5.61		6.99		13.10	
-0.89		2.80		4.43		5.41		6.81		12.81	
-0.74	0.788	2.56	0.120	3.96	0.418	5.38	0.205	6.20	0.354	12.43	0.477
-0.19		2.69		4.63		5.11		6.88		12.85	
										13.72	
-0.68		1.20		2.87		3.91		5.11		11.02	
-1.34	0.343	1.12	0.247	2.01	0.650	3.16	0.473	4.63	0.305	10.59	0.446
-0.56		1.34		3.60		4.24		4.67		10.61	
-0.85		1.68		2.87		4.05		5.23		11.54	
-0.08		1.43		2.79		4.20		5.21		10.83	
-0.85	0.557	0.95	0.661	2.79	0.135	4.01	0.149	5.13	0.115	10.71	0.221
-0.48		2.32		2.61		3.93		5.40		10.69	
-1.39		0.89		2.52		4.24		5.21		10.32	
-0.15		1.92		3.35		4.71		5.03		11.75	
-0.14	0.375	1.30	0.338	2.69	0.446	4.38	0.405	5.54	0.527	11.31	0.643
-0.29		1.70		3.43		4.47		6.16		11.41	
-0.93		1.20		2.56		3.76		5.07		10.26	
Total Mean		1.67		3.25		**4.41**		5.58		11.53	
Total SD		0.64		0.85		0.67		0.78		1.08	
Total CV		38.36		26.16		15.29		13.93		9.33	
LOB	**LOD**	1.42		1.77		1.48		1.64		2.14	

- The lowest concentration that can be quantified reliably with predefined goals for bias, imprecision or total allowable error
  - Example: analyte lowest concentration where imprecision is ≤ 20%

LOQ study on Et Glycol

Ethylene Glycol spiked samples

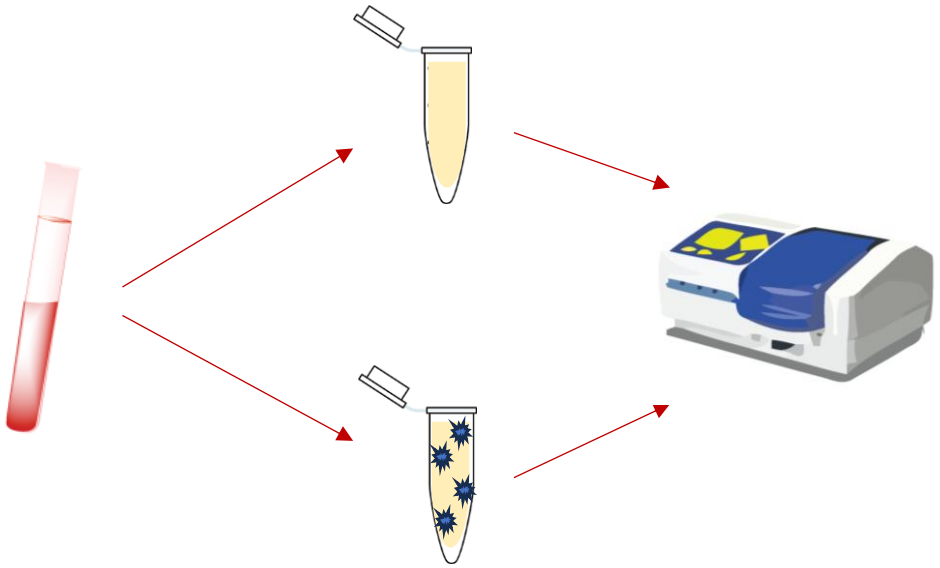
		~2		~3		~4		~5		~10	
Patient pool	SD	Low	SD	Low	SD	Low	SD	Low	SD	Low	SD
0.85				4.96		5.61		6.99		13.10	
-0.89		2.80		4.43		5.41		6.81		12.81	
-0.74	0.788	2.56	0.120	3.96	0.418	5.38	0.205	6.20	0.354	12.43	0.477
-0.19		2.69		4.63		5.11		6.88		12.85	
										13.72	
-0.68		1.20		2.87		3.91		5.11		11.02	
-1.34	0.343	1.12	0.247	2.01	0.650	3.16	0.473	4.63	0.305	10.59	0.446
-0.56		1.34		3.60		4.24		4.67		10.61	
-0.85		1.68		2.87		4.05		5.23		11.54	
-0.08		1.43		2.79		4.20		5.21		10.83	
-0.85	0.557	0.95	0.661	2.79	0.135	4.01	0.149	5.13	0.115	10.71	0.221
-0.48		2.32		2.61		3.93		5.40		10.69	
-1.39		0.89		2.52		4.24		5.21		10.32	
-0.15		1.92		3.35		4.71		5.03		11.75	
-0.14	0.375	1.30	0.338	2.69	0.446	4.38	0.405	5.54	0.527	11.31	0.643
-0.29		1.70		3.43		4.47		6.16		11.41	
-0.93		1.20		2.56		3.76		5.07		10.26	
Total Mean		1.67		3.25		**4.41**		5.58		11.53	
Total SD		0.64		0.85		0.67		0.78		1.08	
Total CV		38.36		26.16		15.29		13.93		9.33	
LOB	0.36	**LOD**	1.42		1.77		1.48		1.64		2.14

LOQ

- The lowest concentration that can be quantified reliably with predefined goals for bias, imprecision or total allowable error
  - Example: analyte lowest concentration where imprecision is ≤ 20%

Analytical Specificity

- An analytical specificity experiment is performed to determine the effect of potential interferences on the assay results



+ Add interferant

		Alcohol concentration	Replicate 1	Replicate 2	Mean, mg/dL	Bias	% Bias	ATE	Acceptable ?
Sample	Et Glycol	**Methanol**							
1	pos	0	68.72	66.48	67.60	-8.70	-12.9	2 or 20%	YES
2	pos	2000 mgdL	60.84	56.96	58.90				
3	pos	0	64.71	59.62	62.17	-0.83	-1.3	2 or 20%	YES
4	pos	2000 mgdL	62.72	59.95	61.34				
5	pos	0	60.58	58.14	59.36	-4.82	-8.1	2 or 20%	YES
6	pos	2000 mgdL	56.37	52.71	54.54				
		Ethanol							
7	pos	0	58.43	55.5	56.97	-0.97	-1.7	2 or 20%	YES
8	pos	2000 mgdL	56.62	55.37	56.00				
9	pos	0	66.66	61.37	64.02	-4.00	-6.2	2 or 20%	YES
10	pos	2000 mgdL	60.52	59.52	60.02				
11	pos	0	58.99	56.35	57.67	-6.53	-11.3	2 or 20%	YES
12	pos	2000 mgdL	53.2	49.09	51.15				
		Isoproponal							
13	pos	0	58.22	56.45	57.34	-2.21	-3.8	2 or 20%	YES
14	pos	2000 mgdL	56.98	53.28	55.13				
15	pos	0	52.04	47.16	49.60	1.26	2.5	2 or 20%	YES
16	pos	2000 mgdL	53.04	48.68	50.86				
17	pos	0	58.34	56.03	57.19	-4.49	-7.8	2 or 20%	YES
18	pos	2000 mgdL	54.34	51.06	52.70				
		Acetone							
19	pos	0	52.22	49.21	50.72	3.22	6.3	2 or 20%	YES
20	pos	2000 mgdL	55.27	52.59	53.93				
21	pos	0	54.38	51.73	53.06	4.12	7.8	2 or 20%	YES
22	pos	2000 mgdL	59.12	55.23	57.18				
23	pos	0	53.54	50.41	51.98	-2.29	-4.4	2 or 20%	YES
24	pos	2000 mgdL	51.16	48.22	49.69				

Additional experiments- proficiency testing

Proficiency Results:

Survey	CAP ID	Result	Mean	Low	High	Acceptable?
AL2-01	1	≤4.0	0.00	0.00	0.00	YES
AL2-02	2	103.92	98.65	73.99	123.31	YES
AL2-03	3	≤4.0	0.00	0.00	0.00	YES
AL2-04	4	42.50	39.55	29.66	49.44	YES
AL2-05	5	≤4.0	0.00	0.00	0.00	YES
AL2-06	6	≤4.0	0.00	0.00	0.00	YES
AL2-07	7	76.70	76.27	57.20	95.34	YES
AL2-08	8	≤4.0	0.00	0.00	0.00	YES
AL2-09	9	≤4.0	0.00	0.00	0.00	YES
AL2-10	10	33.98	142.00	106.50	177.50	YES
AL2-11	11	≤4.0	0.00	0.00	0.00	YES
AL2-12	12	≤4.0	0.00	0.00	0.00	YES
AL2-13	13	≤4.0	0.00	0.00	0.00	YES
AL2-14	14	243.54	251.75	188.81	314.69	YES
AL2-15	15	≤4.0	0.00	0.00	0.00	YES
ALC-01 (2024)	1	≤4.0	0.00	0.00	0.00	YES
ALC-02	2	167.32	169.58	127.19	211.98	YES
ALC-3	3	≤4.0	0.00	0.00	0.00	YES
ALC-4	4	≤4.0	0.00	0.00	0.00	YES
ALC-5	5	≤4.0	0.00	0.00	0.00	YES
ALC-6	6	87.12	83.32	62.49	104.15	YES
ALC-7	7	≤4.0	0.00	0.00	0.00	YES
ALC-8	8	≤4.0	0.00	0.00	0.00	YES
ALC-9	9	≤4.0	0.00	0.00	0.00	YES
ALC-10	10	111.19	108.25	81.19	135.31	YES

Additional experiments- carryover

L: Low [Et Glycol]

H: High [Et Glycol]

Sample	Result
L1	13.34
L2	13.01
L3	12.86
H1	208.72
H2	206.85
L4	12.54
H3	214.11
H4	207.72
L5	12.67
L6	13.37
L7	12.67
L8	12.54
H5	199.96
H6	207.57
L9	12.45
H7	207.32
H8	205.20
L10	12.88
H9	208.44
H10	211.09
L11	11.84

High-Low Mean: 12.5 mg/dL
Low-low Mean: 12.9 mg/dL
Carryover: 0.4 mg/dL
Grady Error limit (3*SD for low-low): 1.0
Passes: YES

Example of Evaluation Summary

Analyte: Glucose
Analyzer and Serial #: Chemistry, #
FDA approved/FDA non-approved, Complexity: FDA approved, Moderate Complexity
Units of Measure: mg/dL
Specimen(s): ☒ Serum ☒ Plasma ☐ Urine ☐ CSF ☐ Body Fluid

Reagent Risk Evaluation: Reagents were evaluated for the following risks: reproductive toxicity, acute toxicity, and carcinogenic potential. No risks were identified.

Quality Requirement/Goal (include units): 8% or ±6 if <60 mg/dL

A. **Initial Precision** (Within-run)

Within Run Precision

Manufacturer's Criteria:	5	UW Criteria (1/4 Total Error):	2.5

	MQ1	MQ3	Serum/ Plasma Pool Female	Serum/ Plasma Pool Male
Observed %CV	1.1	0.9	1.2	0.7
Pass UW Criteria?	YES	YES	YES	YES
Pass Manufacturer's Criteria?	YES	YES	YES	YES

B. **Analytical Measurement Range** (AMR/Linearity)
Goal: Confirmation within 10% of both ends of the stated range. **Slope is 0.90 – 1.10**; R^2 value is **>0.98**

AMR

Manufacturer Stated Range	UWHC Confirmation	Slope	Intercept	Corr. Coefficient (R^2)	Recommended AMR	Acceptable?
5 - 800	0.018 - 733.8	0.997	-4.003	0.9997	5 - 800	YES

Dilution Study

C. **Dilution Studies (Reportable Range)** – List dilutions performed and % bias observed

D. **Day to Day Precision**

Day to Day precision

Manufacturer's Criteria: <	5	UW Criteria (1/3 TE(a)): <	3.3

Product/Level	N (X/X)	Mean	SD	CV	Acceptable (UW)	Acceptable (Manufacturer)
MQ Level 1	20/20	56.218	0.6781	1.2	YES	YES
MQ Level 3	20/20	354.6262	4.1403	1.2	YES	YES

E. **Method Comparison** (Accuracy)

Method Comparison

X-Method	N (X/X)	Mean of X	Mean of Y	Est. Bias	(Regular) Regression Statistics		
					Slope	Intercept	Correlation Coefficient (R)
UWHC Chemistry Analyzer	40/40	333.620	321.154	-12.496	0.957	1.909	0.9997
Enter X-Method (2)							
Enter X-Method (3)							

F. **Additional Accuracy** (PT Results) Survey Name/shipment:

CAP PT

CAP ID	Observed value	Peer Mean	SD	CV	Low	High	Acceptable?

G. **Final Acceptance (Sigma value and Total Error Assessment)**
Assay Sigma = TEa(%) - Bias (%)/CV
Total Error = 1.65(CV) + %Bias

Analytical Error Assessment

Target value	Slope	Y-Int	%BIAS (y=mx+b)	EST bias	CV (Day to Day QC)	Sigma Value	Total Error Observed (%) %Bias + 1.65(CV)	Method ATE	Total Error Allowed > Total Error Observed
40	0.957	1.91	0.4725	-12.496	1.2	6.3	2.5	8	PASS
99	0.957	1.91	2.371717	-12.496	1.2	4.7	4.4	8	PASS
126	0.957	1.91	2.784921	-12.496	1.2	4.3	4.8	8	PASS
200	0.957	1.91	3.3455	-12.496	1.2	3.9	5.3	8	PASS

Discussion:

I have reviewed the verification/validation data above and the performance of the method is **considered acceptable** for patient testing.

Approved by
Faculty Director: _____ Date: _____

Approved by
Laboratory Manager: _____ Date: _____

Approved by
Quality Section: _____ Date: _____

What about CLIA waived?

FDA approved (moderate or high complexity

Verify

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals

Non-FDA (Modified FDA or Lab Developed Test)

Establish

1. Analytical measuring range (AMR)
2. Precision (*Im*precision)
3. Accuracy
4. Reference intervals
5. Analytical Sensitivity
6. Analytical Specificity
7. Any additional studies

CLIA Waived

Method evaluation not required

Conclusion

- Developing a detailed validation plan with pre-determined acceptability criteria is an important step in method evaluation
- There are several resources and guidelines that can be used when defining ATE for a clinical test
- Accuracy and precision studies can be evaluated separately or together by estimating total analytical error at various medical decision levels and comparing the result to ATE

Thank you for listening!

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Analytical sensitivity - analysis

Limit of Blank (LoB):

- The lowest concentration that can be distinguished from background
- Mean conc. of blank + 2SD (>20 replicates)

Limit of Detection (LoD):

- The lowest number that will almost always have a non-zero result
  (1) Mean conc. of blank + 3 SD
  (2) LOD = LOB + 1.645(SD low concentration)

Limit of Quantification (LoQ): “functional sensitivity”

- The lowest concentration that can be quantified reliably with predefined goals for bias, imprecision or ATE
  - Example: Analyte lowest concentration where CV ≤ 20%

Useful tools: CLSI guidelines

CLSI Evaluation Protocol Documents

Performance Claim	CLSI Documents
Precision	CLSI EP05 | *Evaluation of Precision of Quantitative Measurement Procedures* CLSI EP12 | *Evaluation of Qualitative, Binary Output Examination Performance*[a] CLSI EP21 | *Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures*
Accuracy	CLSI EP09 | *Measurement Procedure Comparison and Bias Estimation Using Patient Samples* CLSI EP12 | *Evaluation of Qualitative, Binary Output Examination Performance*[a] CLSI EP21 | *Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures*
Reportable interval	CLSI EP06 | *Evaluation of Linearity of Quantitative Measurement Procedures* CLSI EP17 | *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures* CLSI EP34 | *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking*
Reference interval	CLSI EP28 | *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*
Analytical sensitivity	CLSI EP17 | *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*
Analytical specificity	CLSI EP07 | *Interference Testing in Clinical Chemistry* CLSI EP37 | *Supplemental Tables for Interference Testing in Clinical Chemistry*
Clinical validation	CLSI EP12 | *Evaluation of Qualitative, Binary Output Examination Performance*[a] CLSI EP24 | *Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves* CLSI EP27 | *Constructing and Interpreting an Error Grid for Quantitative Measurement Procedures*
Fundamental	CLSI EP19 | *A Framework for Using CLSI Documents to Evaluate Medical Laboratory Test Methods* CLSI QSRLDT | *Quality System Regulations for Laboratory-Developed Tests | A Practical Guide for the Laboratory*
Total analytical error	CLSI EP21 | *Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures*

Thanks to Paula Ludwig, Tabby Kern and CLSI!

Example of acceptable criteria per study

Name of the study	Time frame	Number of samples	Number of replicates	Possible Performance Goals
Precision within-run	Same day	2-3 QC or patient samples	10-20	CV < 1/4 ATE or CV <1/6 ATE*
Precision day-to-day	5-20 days	2-3 QC materials	20	CV < 1/4 ATE (using 6 sigma) or CV < 1/3 ATE (University of Wisconsin goal)*
Accuracy	5-20 days, run samples simultaneously on the old and new method	40 patient samples spanning the AMR	1	Slope 0.9-1.1
Analytical measuring range (AMR)	Same day	5	3	Choose samples across the AMR with the lowest and the highest sample being within 10% of low and 10% of high AMR Slope 0.9-1.1
Analytical Sensitivity	3 days	2 or more	10-20	LOQ: CV ≤ ATE or LOQ: CV ≤ 20%
Analytical Specificity	Same day	5 and more	2-3	≤ ½ ATE
Carryover	Same day	2	N/A	≤ ½ ATE
Dilution	Same day	3 or more	2-3	≤ ½ ATE

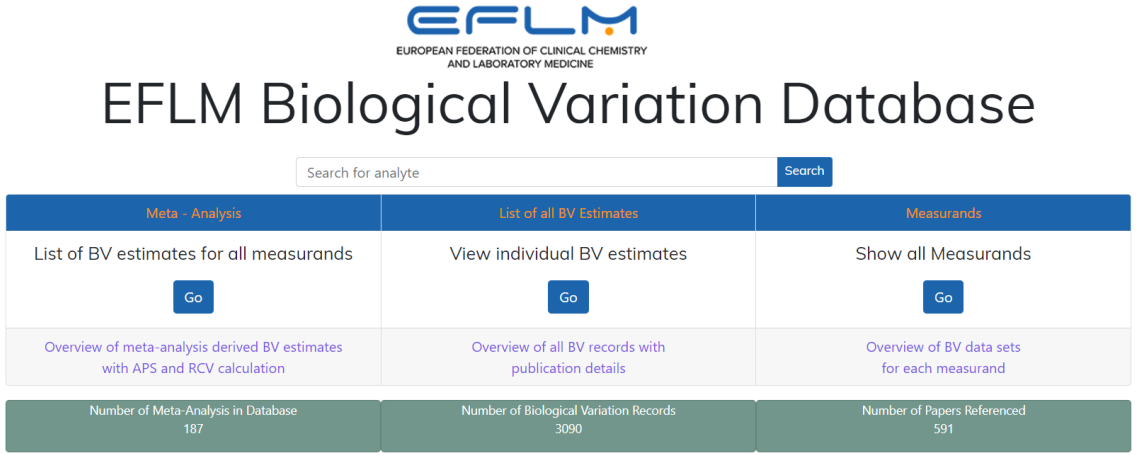
QC: Quality control, CV: coefficient of variation (use CV when ATE is defined in % and standard deviation (SD) when ATE is defined in concentration units), LOQ: limit of quantification

*Or within manufacturer's specification

https://www.myadlm.org/CLN/Articles/2024/MayJune/Navigating-method-evaluation-in-clinical-laboratories

Useful tools:

- EFLM database for ATE
https://biologicalvariation.eu/



- CALIPER for pediatric reference ranges
https://caliper.research.sickkids.ca/#/

- Total Analytical Error calculation
https://www.myadlm.org/CLN/Articles/2013/september/Total-Analytic-Error