Method Validation/Verification

CAP/CLIA regulated methods at Texas Department of State Health Services Laboratory

References

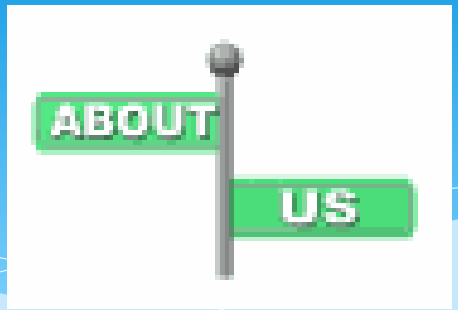
* Westgard J. O.: Basic Method Validation, Westgard Quality Corporation

* Sarewitz S.J.: CAP Accreditation Requirements for Validating Laboratory Tests, 7/9/13

Objectives

* Understand the validation process and perform appropriate validation/verification studies in accordance with CAP/CLIA requirements for both qualitative and quantitative methods including:
* FDA-cleared methods
* Non-FDA cleared methods, Methods developed in-House and FDA-cleared methods modified by the laboratory
* Instrument validation

Scope



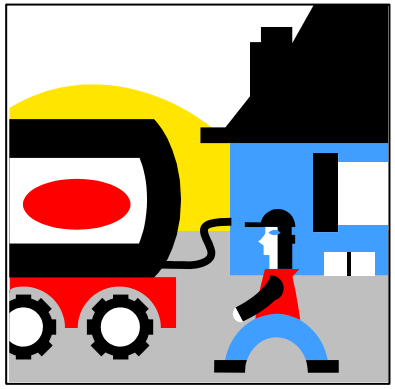
* Method Validations Required for:
  * All New Tests
  * Any Modification to Existing Procedures
* Equipment Validation/Verifications Required for:
  * All New Instruments
  * Any Moved Instruments
* All validation/verifications must be approved by the Laboratory Services Section Director prior to use.

Method Validation

* Method Validation is about Error assessment!
* Statistics don't tell you if the method is acceptable, they provide estimates of errors which allow you to judge the acceptability of a method.
* Method performance is judged acceptable when observed error is less than or equal to the defined allowable error.

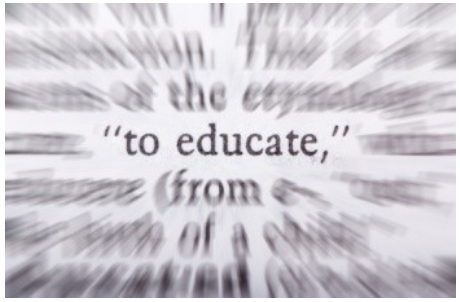
Preparation

* Definitions
* Reagents/Media/Standards
* Equipment

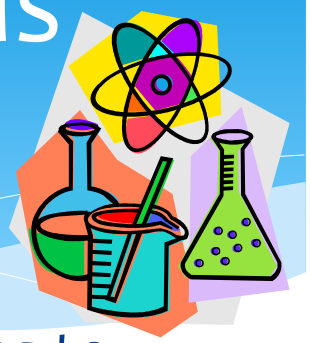


Definitions

* CAP/CLIA
* Accuracy, Bias, Systemic Error
* Precision, Reproducibility, Random Error
* Qualitative results
* Quantitative results
* Reportable Range, Analytic Measurement Range (AMR)
* Reference Range, Normal values
* Analytic Sensitivity
* Diagnostic Sensitivity
* Analytic Specificity
* Diagnostic Specificity
* Validation
* Verification



Reagents/Media/Standards



1. Must have sufficient and appropriate quantities to perform the verification study
2. Use the same lot throughout the entire verification study **(ideal)**
3. Ensure that expiration dates are long enough to complete the validation/verification study

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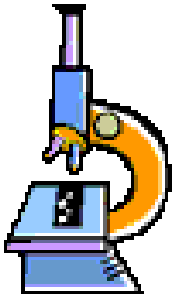
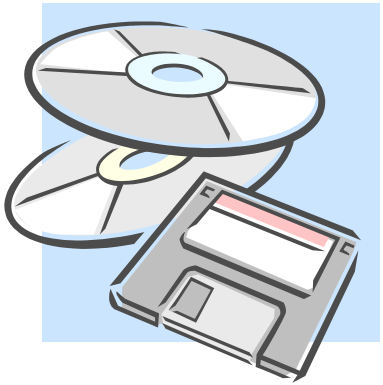
Communicate any needs or changes with the Media Prep Team and Consumer Micro QC related to the preparation of media and/or reagents

Equipment

1. Instrument to be used for method verification/validation
2. Software for Method Validation/Verification





Types of Validations

1. **Qualitative Methods**
   A. FDA cleared or approved methods
   B. Non-FDA cleared or approved tests
2. **Quantitative Methods**
   A. FDA cleared or approved methods
   B. Non-FDA cleared or approved tests
3. **Instrument Validation**
   A. Method Performance Specifications (CAP Requirements)

Qualitative Method – FDA cleared

1. Accuracy
2. Precision
3. Reportable Range
4. Reference Range (Normal Values)
5. Acceptance criteria
   A. 90% as compared to current/reference method
   B. Matches or exceeds manufacturer's information

Qualitative Method – Non-FDA cleared

1. Accuracy
2. Precision
3. Reportable Range
   A. Cut off Verification
4. Reference Range (Normal Values)

Qualitative Method – Non-FDA cleared

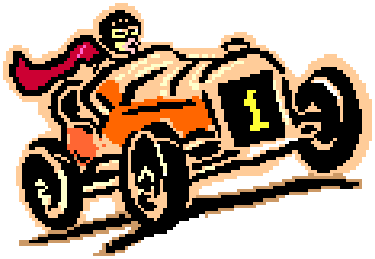
5. Sensitivity
   A. Analytical Sensitivity
   B. Diagnostic Sensitivity
6. Specificity
   A. Analytical Specificity
   B. Diagnostic Sensitivity
   C. Interfering Substances

7. Acceptance criteria
   A. 90% as compared to current/reference method
   B. Matches or exceeds manufacturer's information
   C. Observed Error is less than or equal to Acceptable Total Error

Quantitative Method – FDA cleared

1. Accuracy/Bias (Systematic Error)
   A. Comparison Experiment
      a. Comparison/Difference Plot
      b. Constant Systematic Error
      c. Proportional Systematic Error
   B. Recovery Experiment
   C. Statistics

2. Precision (Random Error)
   A. Replication Experiment
   B. Statistics



Quantitative Method – FDA cleared

3. Reportable Range
   A. Analytical Measurement Range
   B. AMR validation

4. Reference Range (Normal Values)
   A. Reference Range verification

5. Acceptance Criteria
   A. 90% as compared to current/reference method
   B. Matches or exceeds manufacturer's information

Quantitative Method – Non-FDA cleared

1. Accuracy/Bias (Systematic Error)
   A. Comparison Experiment
      a. Comparison/Difference Plot
      b. Constant Systematic Error
      c. Proportional Systematic Error
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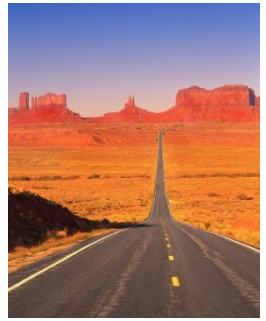
   C. Statistics

2. Precision (Random Error)
   A. Replication Experiment
   B. Statistics



Quantitative Method – Non-FDA cleared

3. Reportable Range (Analytical Measurement Range)
   A. Linearity Experiment

4. Reference Range (Normal Values)
   A. Reference Range verification

5. Specificity
   A. Interfering Substance Experiment

Quantitative Method – Non-FDA cleared

6. Sensitivity
   A. Detection Limit Experiment

7. Acceptance Criteria
   A. 90% as compared to current/reference method
   B. Matches or exceeds manufacturer's information
   C. Observed Error is less than or equal to Acceptable Total Error

Instrument Validation

METHOD PERFORMANCE SPECIFICATIONS

(CAP Requirements)

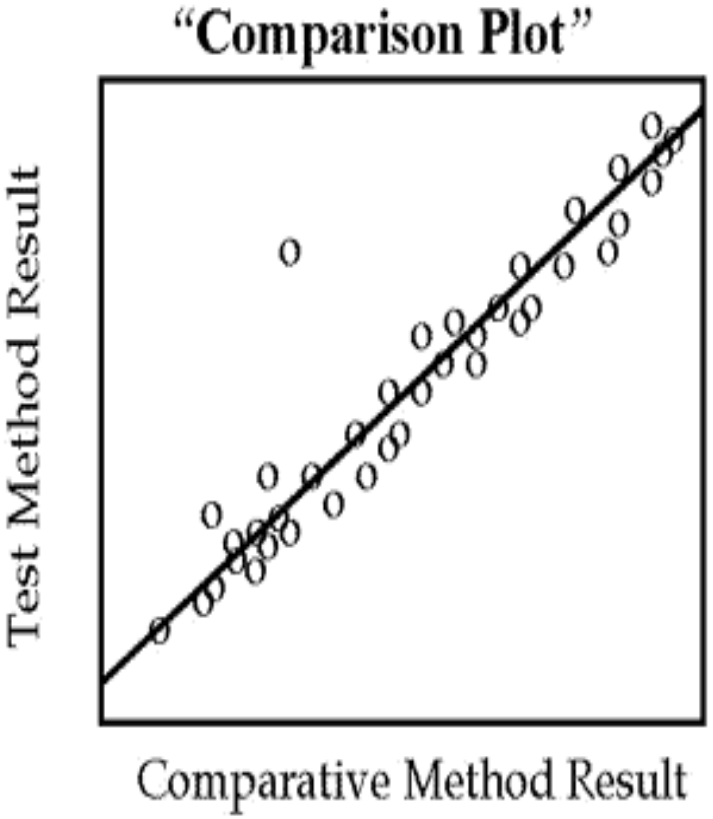
1. New Instrument of a different make or model of current instrument
2. Instruments of same make & model as the current instrument
3. Instruments that have been moved from one location to another in the laboratory

Experiment Section

* Comparison /Difference Plots
* Detection Limit Experiment for Sensitivity
* The Linearity or Reportable Range Experiment
* Regression Statistics for Comparison Experiment
* Allowable Total Error
* Interference Experiment
* Decision on Method Performance

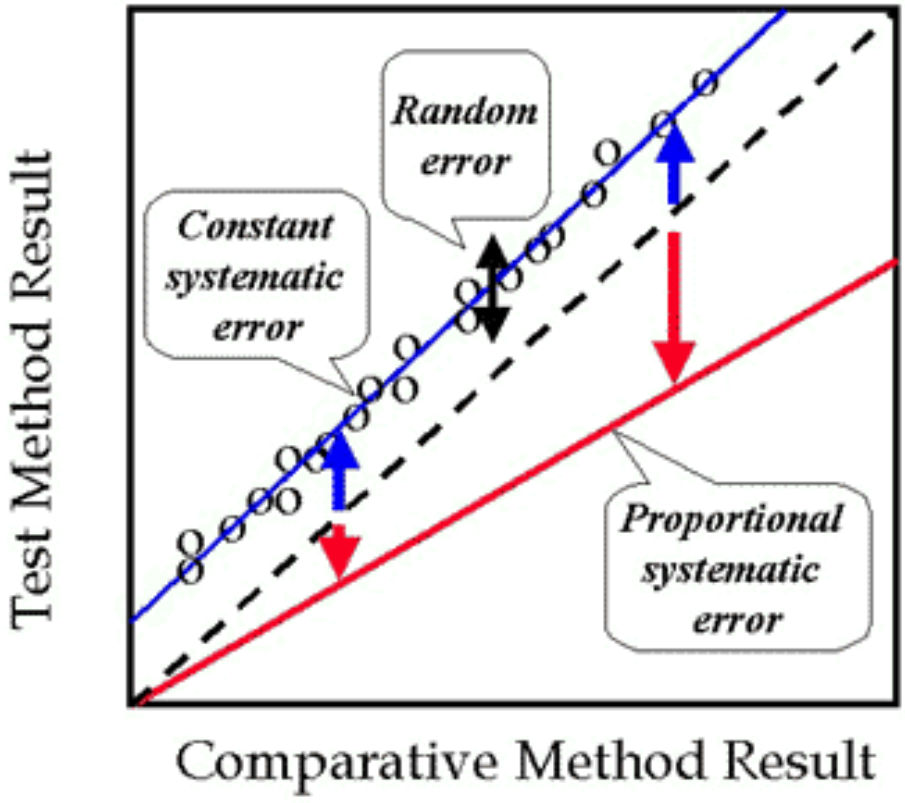
Comparison Plot

* Plot Test results on y-axis
* Plot Current or Comparison results on x-axis
* Show the general relationship
* Help identify discrepant results



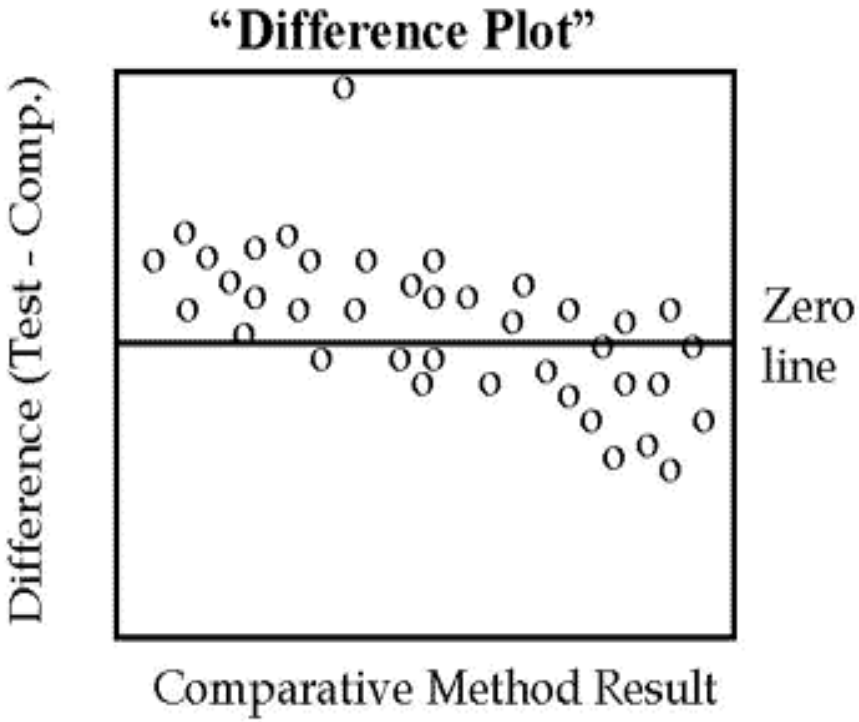
Comparison Plot

* Accuracy/Systematic Error- Two types:
* Constant Systematic Error
* Proportional Systematic Error



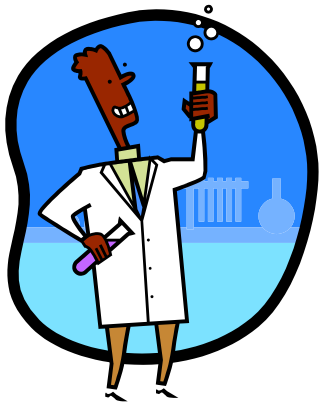
Difference Plot

* Also known as “Bias” Plot
* Test results minus comparative results on y-axis
* Current or Comparative results on x-axis
* Half of points above, half below zero line
* Help identify proportional/constant systematic error



Regression Statistics for Comparison Experiment

* Graph the data creating Comparison Plot
  * Identify outliers and repeat to confirm.
  * Line of best fit (visually or using statistics program) gives linear regression equation Y = a + bX
  * Calculate correlation coefficient "r" - measures how well the results from the 2 methods change together. A 1.000 indicates perfect correlation
  * If r is high (>=.99), use regression line to find bias at analyte concentrations corresponding to critical decision points (ex. glucose: 126 mg/dL)



Regression Statistics for Comparison Experiment

* If r < .975, regression equation not reliable; use paired t-test to determine if a bias is present at the mean of the data
* Analytes with wide range (cholesterol, glucose, enzymes, etc.) tend to have high r in comparison studies; analytes with narrow range (electrolytes) tend to have low r
* **r should not be used to determine the acceptability of a new method. It measures how well the results from the 2 methods change together**

Regression Statistics for Comparison Experiment

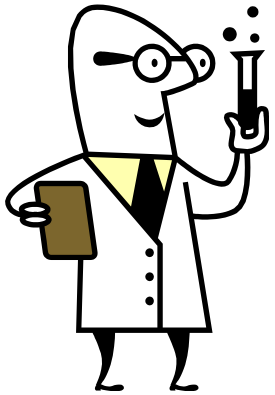
* t-Test used for **systematic error or inaccuracy**
  * Used to test two means and determine whether a difference exists between them.
  * Paired t-Test when every sample is analyzed by both the test and comparative method (two methods)
  * Does not address the acceptability of the method's performance, but only whether there is systematic error present.

Regression Statistics for Comparison Experiment

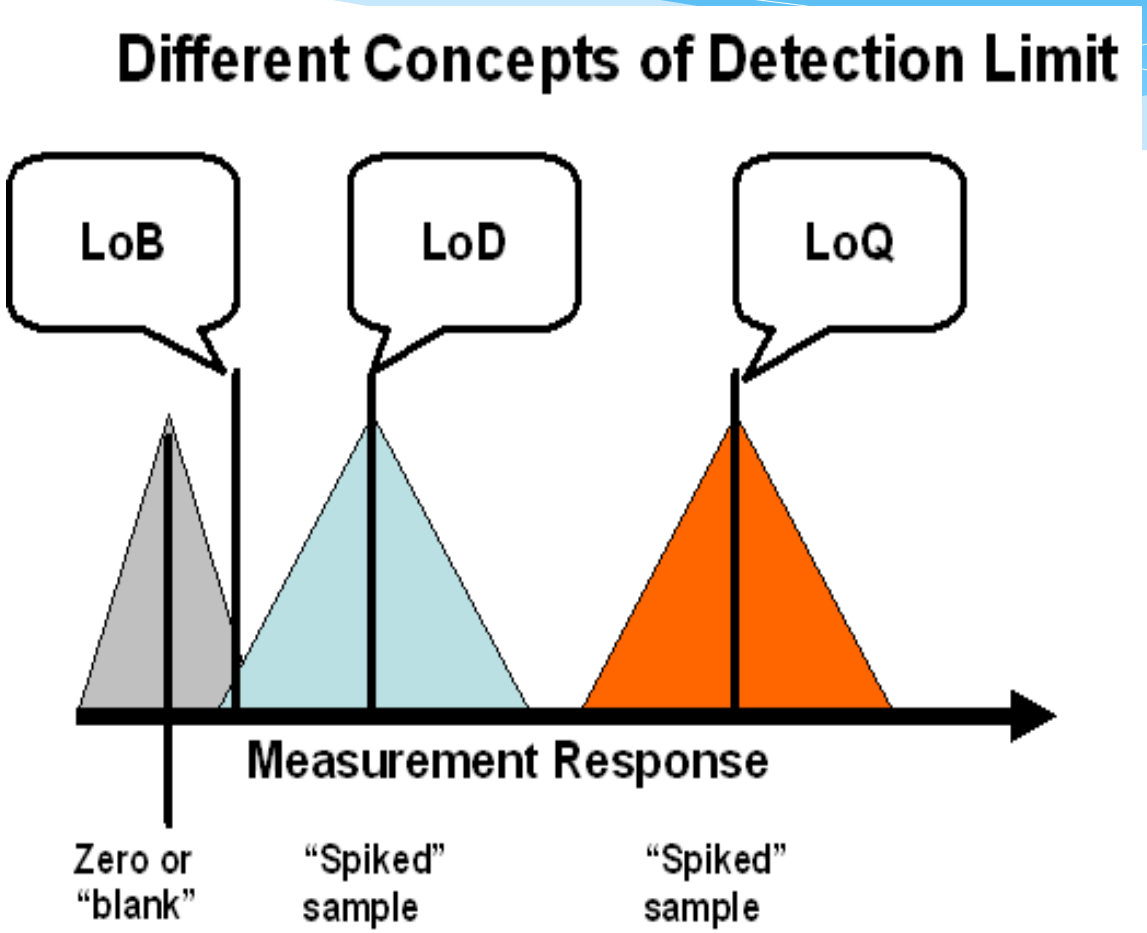
* F-Test- used for **random error or imprecision**
  * Tells whether the difference in variances is statistically significant
  * Compares the calculated F-value with a critical F-value
  * Says nothing about whether the random error of the test method is acceptable, but only whether it is different from that of the comparative method.

Detection Limit Experiment for Sensitivity

* Limit of Blank (LoB)
* Limit of Quantification (LoQ)
* Limit of Detection (LoD)
* Types of Samples
  * Blank Solution
  * Spiked Sample
  * Number of Replicate Measurements
  * Time Period of Study



Detection Limit Experiment

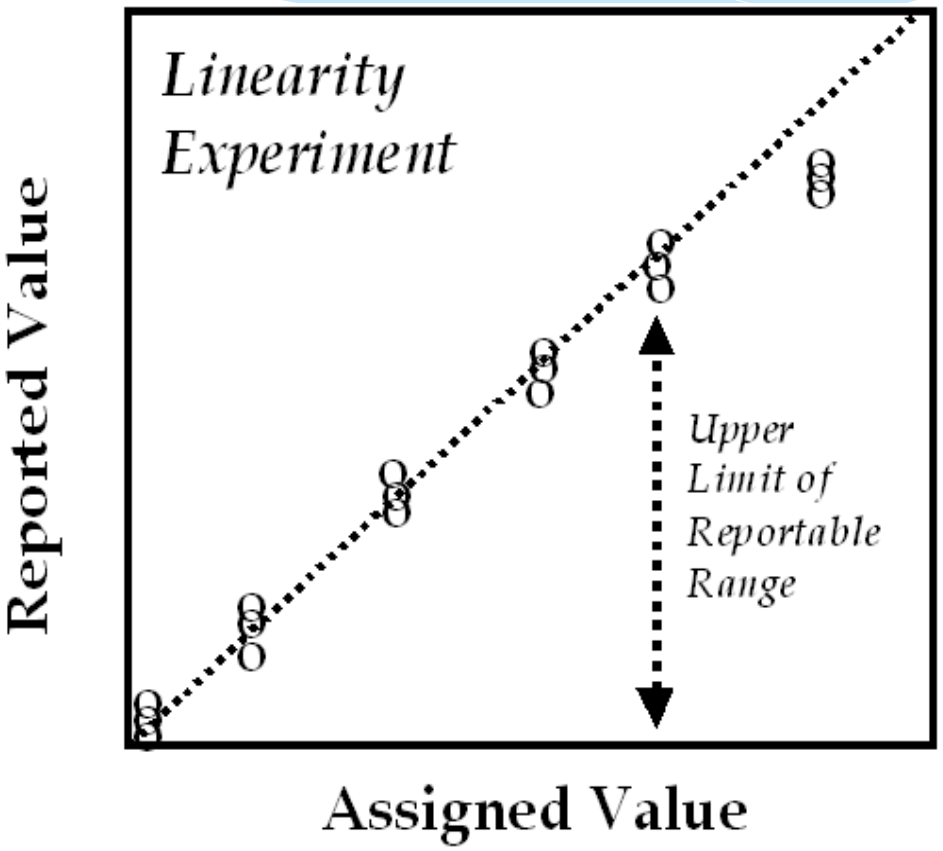


Reportable Range

* Analytical Measurement Range (AMR)
* Linearity Experiment
* 5 levels in triplicate

Reportable Range-Linearity Experiment

* Observed results on y-axis
* Known values on x-axis
* Create best straight line through as many points as possible, adhering to the lower points
* Assess Total Error where lines diverge to determine linearity



Allowable Total Error

* Allowable Total Error
  * Standards for Reporting Diagnostic Accuracy (STARD)
  * Analytical Quality Requirements- CLIA Proficiency Testing Criteria
* Observed Total Error = SE + RE
  * Systematic Error (SE)
    * Y= a +bx at medical decision concentration
    * SE = y – x
  * Random Error (RE)
    * RE = 3 x Standard Deviation from replication experiment

Allowable Total Error

CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register February 28, 1992;57(40):7002-186.

Test or Analyte	Acceptable Performance
Cholesterol, total	Target value ± 10%
Cholesterol, high dens. lipoprotein	Target value ± 30%
Glucose	Target value ± 6 mg/dL or ± 10% (greater)
Triglycerides	Target value ± 25%
Blood lead	Target value ± 10% or ± 4 mcg/dL (greater)
Hemoglobin	Target ± 7%
Rubella	Target value ± 2 dilution or (pos. or neg.)

Allowable Total Error

* Observed Total error = SE + RE must be less than the allowable Total Error

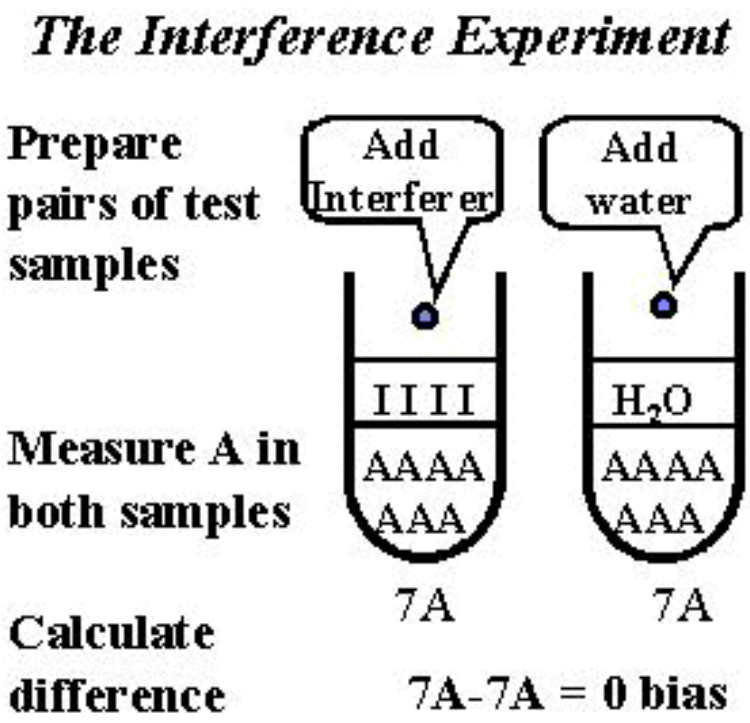
* To be used to judge method performance if there is no information for method performance from literature, or from manufacturer.

Interference Experiment

* For Qualitative Testing
* For Quantitative Testing
* Criteria for Acceptable Performance

Interference Experiment

* Test common interfering substances (interferer)
* Perform experiment for each substance in duplicate
* Acceptability is based on comparing the observed difference of readings and the manufacturer's information or the allowable error for the method.



Decision on Method Performance

* Method Decision Chart
* To be used when there is no documented information for acceptable performance
* The Method Decision Chart can help assess the acceptability of methods with marginal performance
* Provides objective assessment of performance relative to “standard” or quality requirement that defines the total allowable error

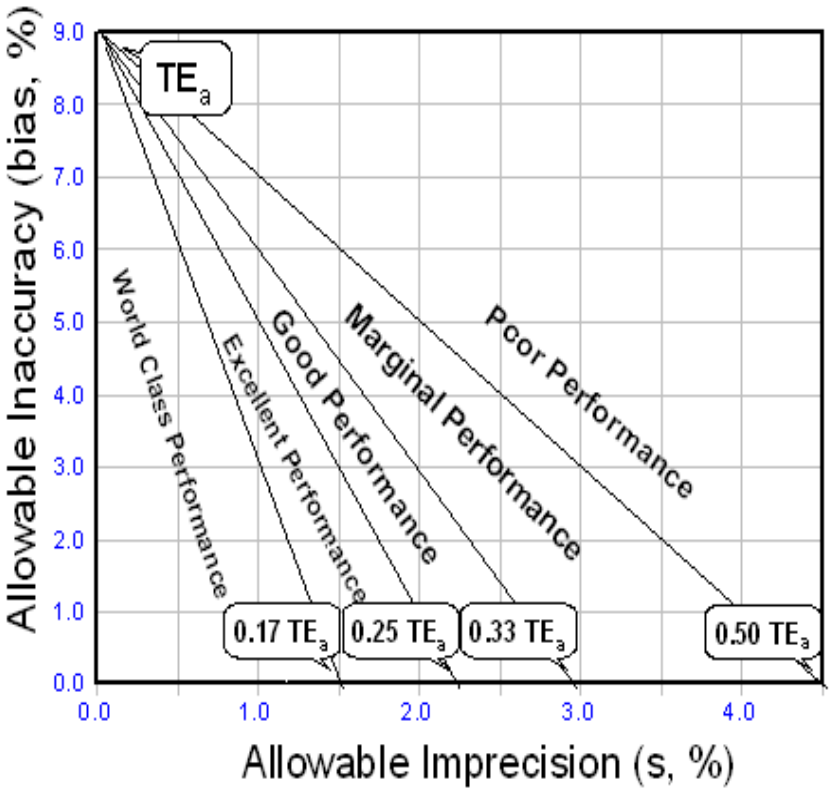
Method Decision Chart

* Express the allowable total error as a percentage of the medical decision concentration. Most CLIA allowable errors are already given in percent
* Express observed SD (s,%) and bias (bias,%) in percent
* Combine systematic and random errors in a graph showing ideal bias with differing levels of precision (random errors)

Method Decision Chart

* 1. Label the y-axis "Allowable inaccuracy, (bias,%)" and scale from 0 to TEa, e.g., if TEa is 10%, scale the y-axis from 0 to 10% in increments of 1%.

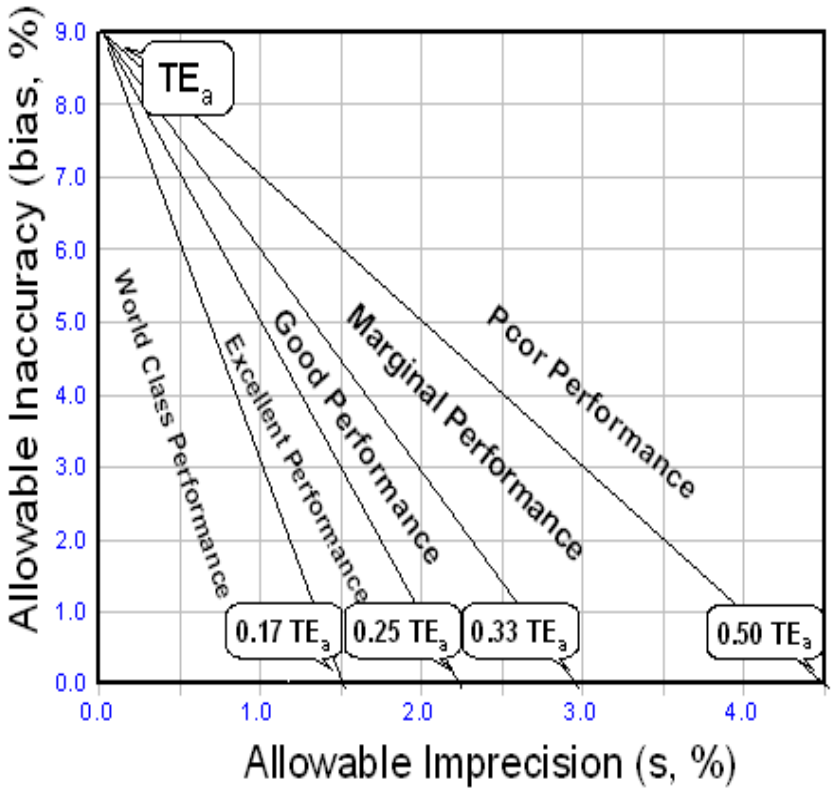
* 2. Label the x-axis "Allowable imprecision, (s,%) and scale from 0 to 0.5 TEa, e.g., if TEa is 10%, scale the x-axis from 0 to 5% in increments of 0.5%.



Method Decision Chart

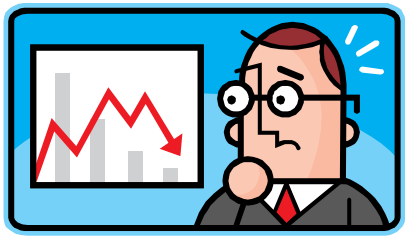
* 3. Draw a line for bias + 2 SD
* 4. Draw a line for bias + 3 SD
* 5. Draw a line for bias + 4 SD
* 6. Draw a line for bias + 5 SD
* 7. Draw a line for bias + 6 SD

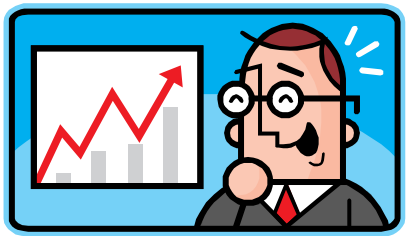
* 8.Label the regions "unacceptable," "poor," "marginal," "good," "excellent," and "world class" as shown in the figure.



Decision on Method Performance

* Method Decision Chart
  * Unacceptable Performance-
  * Poor Performance -Not acceptable
  * Marginal Performance- requires extra controls, well-trained operators and monitoring. Not acceptable
  * Good Performance -Acceptable
  * Excellent Performance -Acceptable
  * World Class Performance -Acceptable

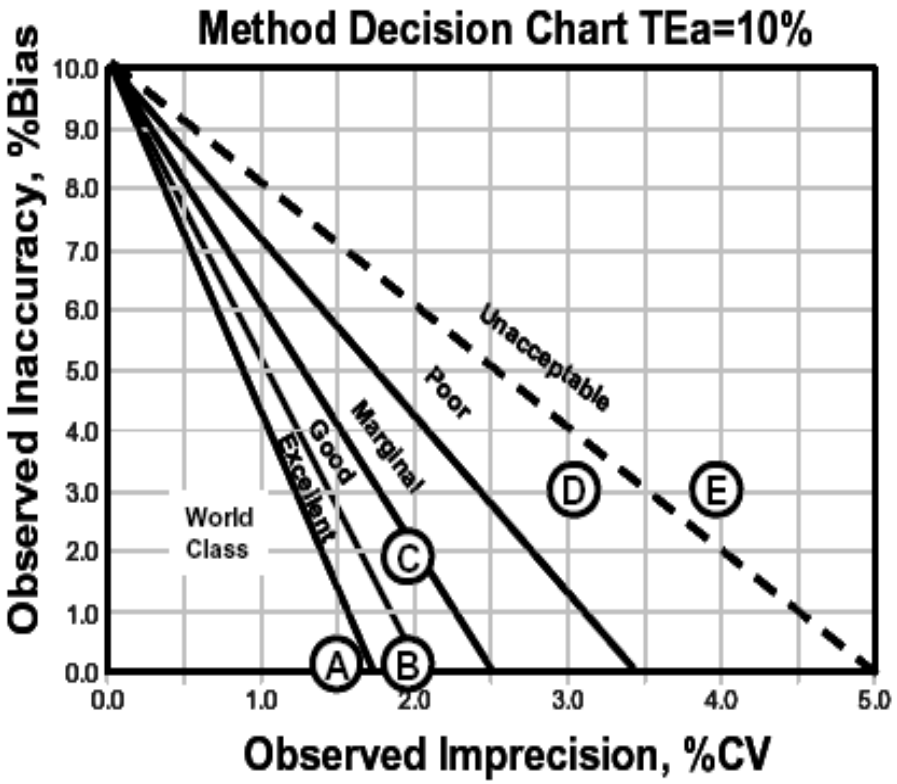




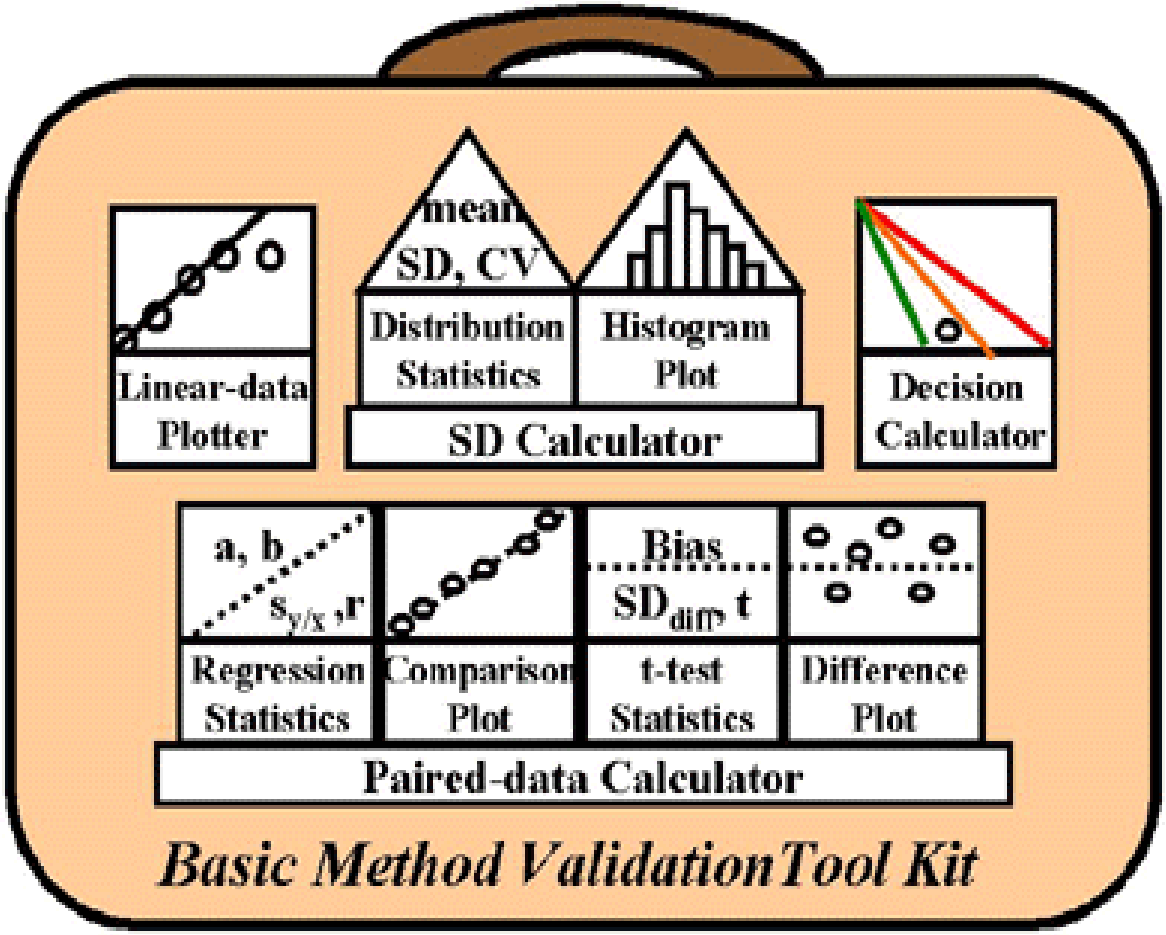
Method Decision Chart

* Express your observed bias and SD in percent and plot your observed results

* Methods A, B and C are acceptable because they demonstrate good performance compared to CLIA requirements for acceptable performance



Validation Tool Kit



Conclusion

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* Method performance is judged acceptable when observed error is less than or equal to the defined allowable error.