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Method Validation

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Learning Objectives

After this presentation, you should be able to:

1. Define method evaluation.
2. List the steps needed to complete a method evaluation study.
3. Define total allowable error (TEa).
4. Apply TEa to method evaluation.
5. Describe recommendations for Sigma values.

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Looking to implement a clinical test?

- Establish the need
- Clinical performance
  - Clinical sensitivity
  - Clinical specificity
- Define the performance standards
  - Costs/efficiencies/space
  - Turn around times/sample requirements
  - Analytical Quality (from kit insert, references)
- Select the new method
- ➢ Evaluate the new method
- Implement the new method

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What is method evaluation?

- Determination of:
  - analytical performance characteristics
  - clinical performance characteristics
- Validation
  - Objective evidence that requirements for a specific intended use can be fulfilled consistently
- Verification
  - Objective evidence that requirements have been fulfilled

What do you do?

- FDA approved?
  - Clinical Laboratory Improvement Amendments (CLIA) requirements
  - Match performance specs established by the manufacturer
    - Accuracy — Should be comparable to manufacture's
    - Precision — Should be smaller than CLIA requirement
    - Reportable Range — Appropriate for patient care
    - Verify manufacturer's reference intervals
    - Determine test system calibration and control procedures based on specs above
    - Document all activities

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Experiments to Validate?

- FDA approved?
  - Reportable Range
    - Linearity
  - Precision
    - Within-run precision
    - Total precision and QC ranges
  - Accuracy
    - Comparison of methods
  - Reference Intervals

Why?

- Clinical significance - leads to accurate medical decisions
- Required by CLIA*, CAP, and The Joint Commission
  (*Clinical Laboratory Improvements Amendments of 1988)
- Pass proficiency testing
- Improvements over existing methodology

- Assay validation requirements vary:

  Non-FDA approved > FDA approved > Waived tests

  Today we are going to focus on

  FDA approved, non-waived tests

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Steps in Method Validation

1) Define Goals
2) Error Assessment
3) Compare error vs. analytical goal

1st Step in Method Validation

Define Goals

- Accept that all lab measurements contain experimental error
- What is an acceptable performance for:
  - Precision?
  - Accuracy?
  - Sensitivity?
  - Analytical measurement range?

Define Goals

- Lab error should be:
  - smaller than CLIA (or other regulatory) requirement:
    - CLIA / 2?
    - CLIA / 3?
    - CLIA / 4?
    - CLIA / 6?
  - consistent with manufacturer's claims
  - compatible with patients' care

2nd Step in Method Validation

Error Assessment

- Method validation assesses
  - Type of error
  - Magnitude of error
  - Clinical Significance of error
    - Literature guidelines
    - Physician input
    - Professional judgment

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3rd Step in Method Validation

Compare error vs. analytical goal

Accept or reject your new method

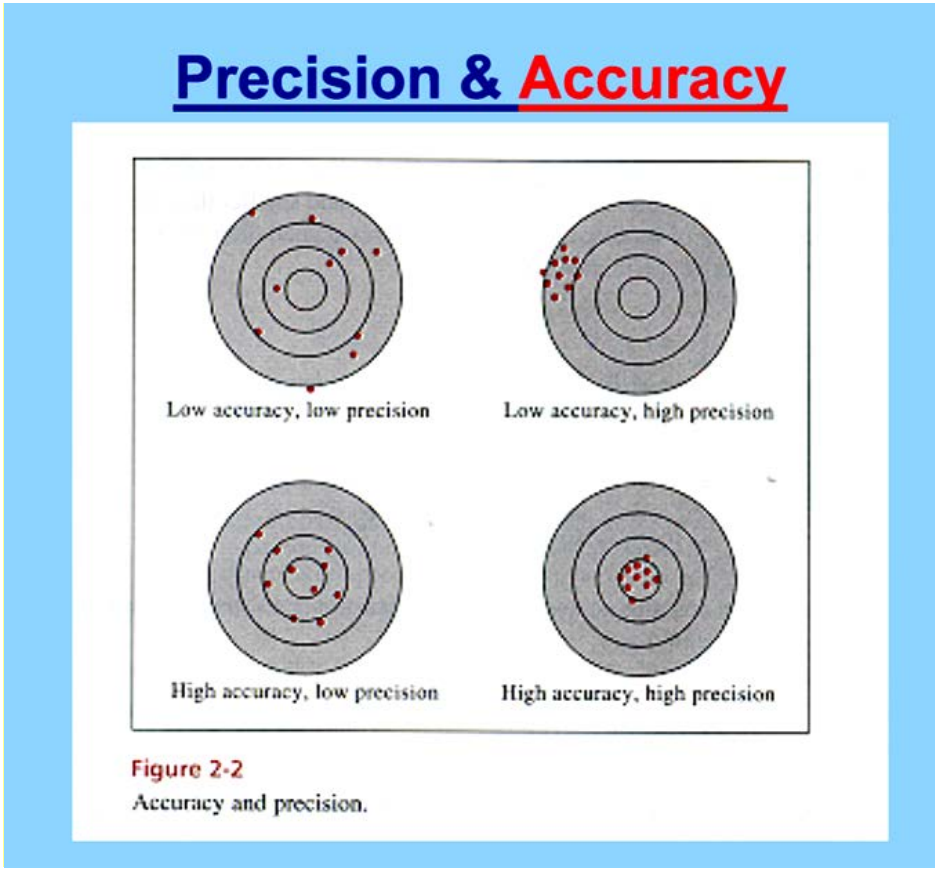
Accuracy and Precision

Accuracy – closeness of measured value to the "true" value – bias

Precision – dispersion of repeated measurements about the mean – reproducibility

Reliability –

Accuracy + Precision



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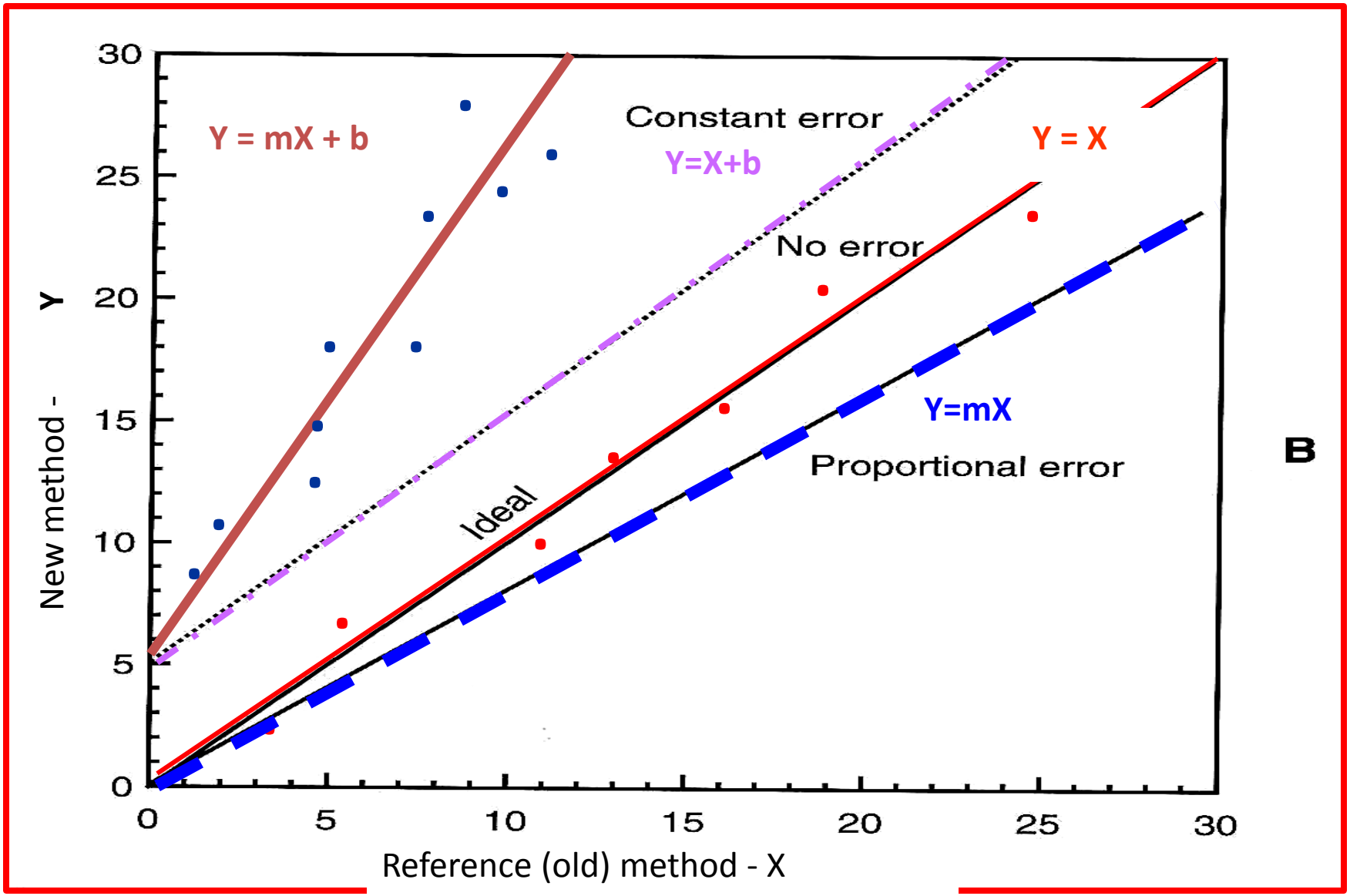
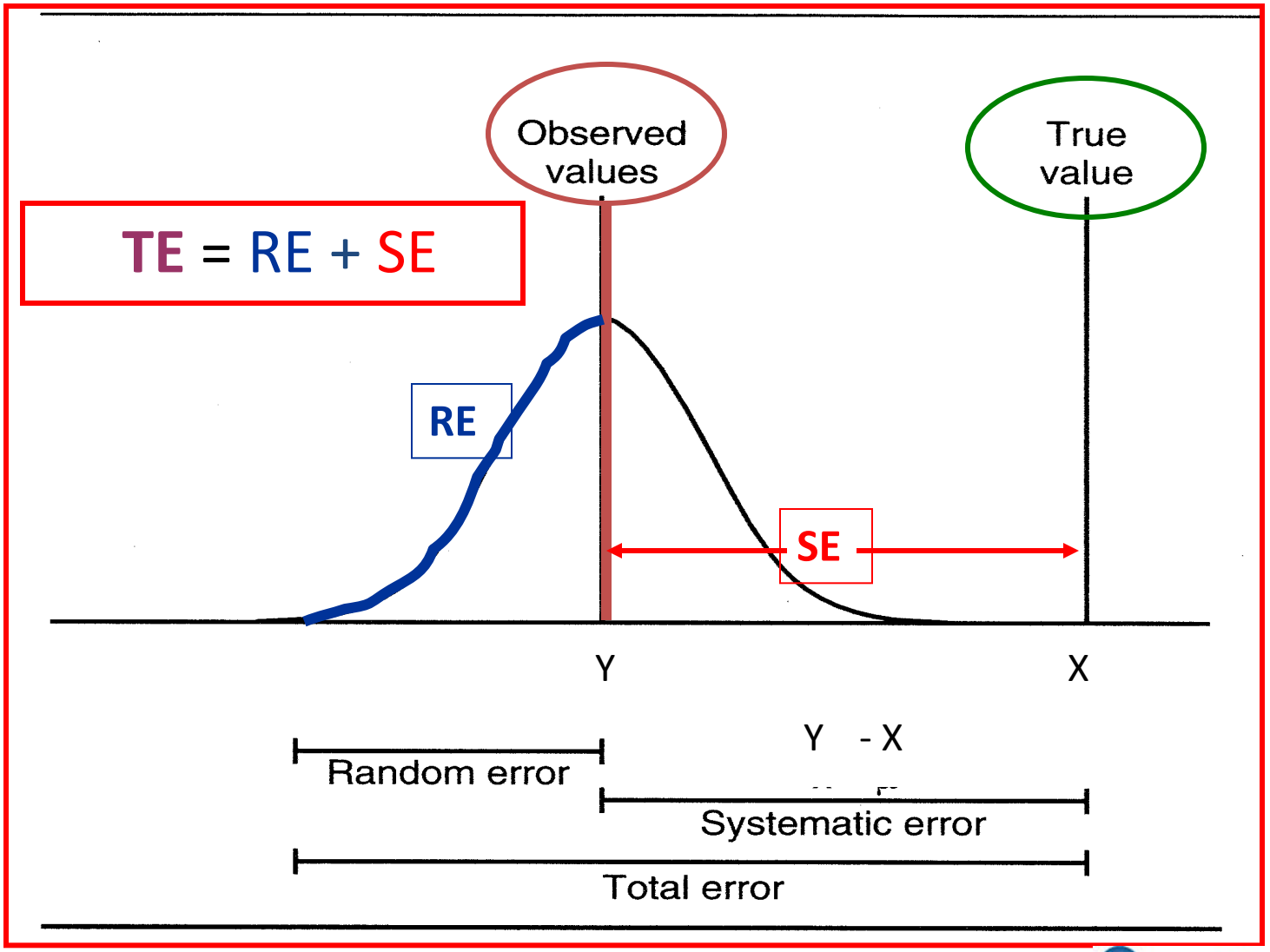
Systematic and Random Errors



Total Analytical Error - TE



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Systematic Error - Affects accuracy

Systematic error (SE) - Bias

- Types of systemic errors:
  - Proportional (indicated by slope)
  - Constant (indicated by intercept)
  - Proportional + Constant (Combination of both)
  - Caused by (examples): bad calibrators, bad reagents, bad pipettes, interference

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Random Error (RE) - Affects precision

- May be caused by (for example):
  - Variability in volume of sample or reagent delivered
  - Changes in environment
  - Inconsistent handling of materials
- Estimated by:
  - Standard deviation (SD)
  - Coefficient of variation (CV)
  - Correlation coefficient (r)

Magnitude of Error – **TE**

- TE is the total maximum error of a test as measured in the lab

- TE is the sum of: random + systemic errors

$$\mathbf{TE} = RE + SE$$

- Determined
  - For each given method
  - At various medical decision levels (X_c)

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Total **A**llowable **E**rror - TE_A

TE_A is the total error permitted by CLIA, based on

- Medical requirements
- Best available analytical method
- Compatible with proficiency testing expectations

Goal: Total Analytical Error < Total Allowable Error

$$TE < TE_A$$

Determined

- Method specific
- Measured at various Medical decision levels (X_c)

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Ready to Validate?

- FDA approved?
  - Reportable Range
    - Linearity
  - Precision
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  - Accuracy
    - Comparison of methods
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AMR: Linearity Study

- Analytical Measurement Range (AMR)
  - Range of analyte where results are proportional to the true concentration of analyte in the sample
  - Range over which the test can be performed w/o modification (e.g. no dilution)
- Also called: Dynamic Range, and Reportable range
- Determined in the lab by linearity experiments

AMR vs. MD/C

- **A**nalytical **M**easurement **R**ange – **AMR**
  - Range of analyte values that a method can directly measure w/o modification (no dilutions, concentrations, other pretreatments that are not part of the usual assay process)
- Maximum Dilution/Concentration (formerly **C**linically **R**eportable **R**ange – **CRR**)
  - Range of analyte values which are clinically significant
  - Can be reported following modification (such as dilutions)

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AMR vs. MD/C

Measurement range should be medically useful if:

- MD/C > AMR
  - Value higher than AMR: report as > X or dilute
  - Value lower than AMR : report as < X or concentrate

If: MD/C < AMR - Limit AMR

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Linearity Study – **"to do" list**

- Samples:
  - Ideal: Use "**traceable" standards** in matrix matched sample
  - Mix of very high with very low pt.'s samples are OK if conc. are known
  - Dilute high samples in acceptable matrix diluent
- At least 5-7 different conc. points within the reportable range (5 – 95% of AMR), equally spaced is ideal
- Testing is performed in duplicate
- Run from lowest to highest (to avoid carryover)
- Pipetting accuracy and precision is critical

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Limit of Detection

- Limit of Blank (LoB):
  - The lowest concentration that can be distinguished from background (blank, zero) noise
  - Sometimes called limit of absence.
  - Calculated as: Mean conc. of blank zero (>20 replicates) **+** 2SD
  - This is the number provided in most kit inserts
- Limit of Detection (LoD):
  - The lowest number that will almost always have a non-zero result (mean conc. of blank + 3 SD)
- Limit of Quantification (LoQ):
  - The lowest concentration that can be quantified reliably
  - Analyte lowest concentration where CV $\leq$ 20% (or other error goal)
  - Results with higher CV% have large random error, thus are not useful for clinical interpretation

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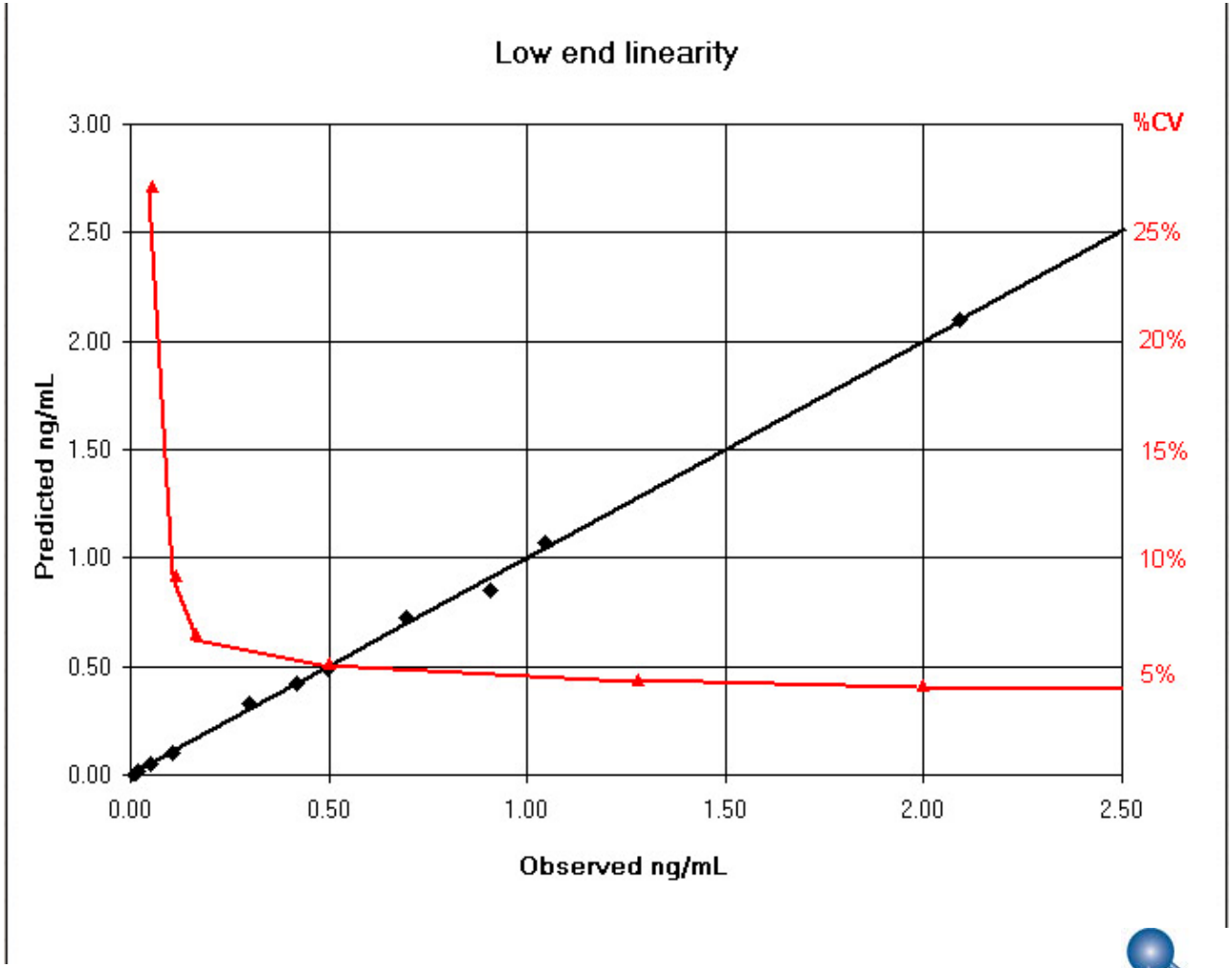
LOQ Experiments

- Only needed if MD/C begins
  - At or near zero
  - At or below the manufacturer's stated AMR
  - Not necessary for most assays
- Start with low end linearity study
  - Determine the low end AMR
- Follow up with precision study
  - Calculate the precision (CV) at low end concentrations

LOQ study example



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Experiments to Validate?

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Reproducibility Studies for Precision
Random Error

- Use matrix matched samples
- Intra-Assay (within-run) Precision > 20x
- Inter-Assay (between-run) Precision > 20x
- Select specimens near medical decision levels
  - At least 2 control levels
- Calculate: mean, SD, CV%

Note: If you don't have established control limits, and they are being established during the experiment, revise limits every 5 days and look for evidence of unacceptable runs.

CLSI EP5

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Method Comparison
What do I do?

1. List results from two methods in pairs
   - Each pair represents the same sample
     X – results of reference method
     Y – results of new method
2. Create a scatter plot (plot the means of duplicates) if done in duplicate)
   - May also use a difference plot to analyze data
3. Look for outliers and data gaps
   - Repeat both methods for outliers
   - Try to fill in gaps or eliminate highest data during analysis

Method Comparison
What do I do?

4. Determine the correlation coefficient

   Check if "r" > 0.975

   Note - Linear regression analysis may not be valid if the correlation coefficient is low.

The correlation coefficient - r

- “r” – a statistical term
- It indicates the extent of linear relationship between the methods
- Ideally, r should be 1.00
- “r” can ranges from +1 to –1

Characteristics of r

- "r" influenced by range of values
  - r < 0.975 may indicate that the range of data is too limited
- "r" is influenced by random errors only
- Systematic error has no effect on r
  - r is only used to assess linear relationship between methods
  - Method accuracy should not be based on r

Method Comparison
What do I do?

5. Generate a “linear best fit line”

$$Y = mX + b$$

m = slope (indicates a proportional error)

b = intercept (indicates constant error)

Method Comparison
What do I do?

6. Evaluate linear regression line:

   Evaluate slope

   Slope = 0.900 = -10% proportional error

   Slope = 1.100 = +10% proportional error

   Intercept should be close to zero (indicating very small constant bias)

   May need to evaluate separate areas of the graph independently.

Method Comparison
What do I do?

7. Calculate systematic error at medical decision levels

   Use slope and intercept to calculate systematic error:

   $Yc = mX + b$ $SE = Y - X$

   Yc = Calculated result on new method

   X = Result from existing method

   m = Slope observed in method comparison experiment

   b = Intercept observed in method comparison experiment

Method Comparison
What do I do?

8. Compare result tracking over time. May be needed if:

   Results are monitored over long intervals (trends)

   The method comparison shows significant differences between the two methods

Experiments to Validate?

- FDA approved?
  - Reportable Range
    - Linearity
  - Precision
    - Within-run precision
    - Total precision and QC ranges
  - Accuracy
    - Comparison of methods
  - Reference Intervals
    - Normal Range

The concept of reference values as recommended by the IFCC

Reference Interval

reference individuals

constitute a

reference population

from which is selected a

reference sample group

on which are determined

reference values

on which is observed a

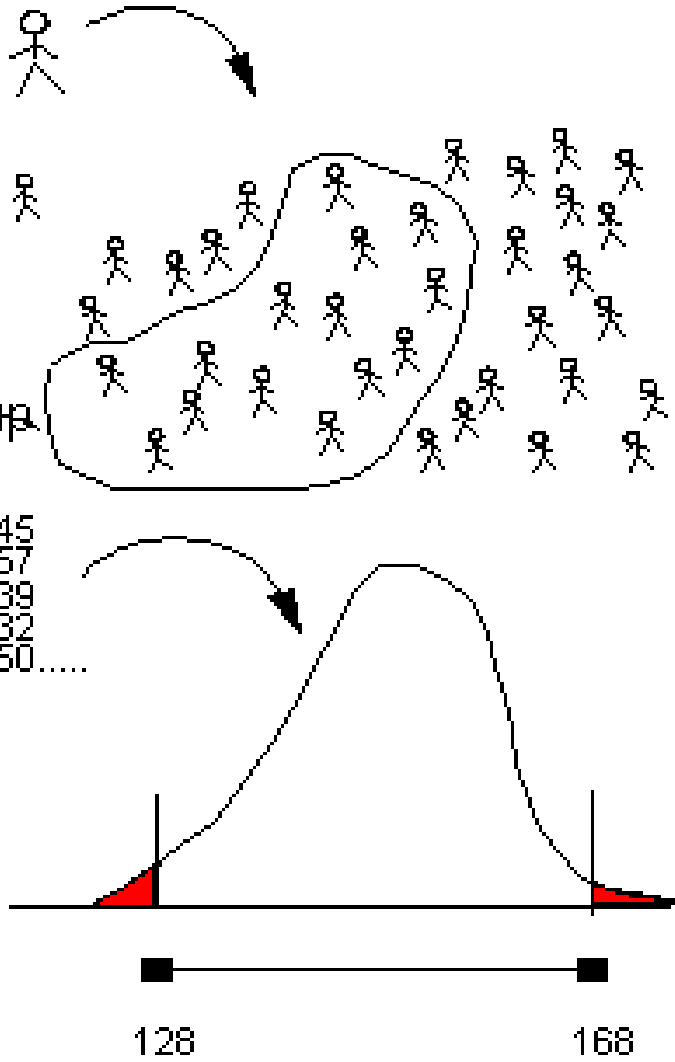
reference distribution

on which is calculated

reference limits

that define a

reference interval



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Reference Range Studies

- CLIA ‘88 requires verification of FDA approved manufacture’s reference range
- Reference range study should reflect the laboratory’s patient population
- Reference interval itself doesn't enter into the decision on method acceptability
- Usually done last, but testing should be done over several days.
- Data analysis will depend upon the distribution of the results.

Reference Range Studies

- Validating a reference range: The number of samples needed if age/sex not a factor:
  - Verification of manufacturer's range N $\geq$ 20
    - Used if using the manufacturer's range and the test will be used in the exact manner described by the manufacturer.
  - Estimating a reference range N = 40-60
    - Used if the manufacturer's range is not adequate or if the use of the test not conform exactly to the manufacturer's intended use.
  - Establishing a reference range N $\geq$ 120
    - Non-FDA approved tests or if there will be significant changes to the use of the method.

Reference Range Studies

- Transferring a reference range:
  - New reference range is calculated based on the systematic analytical differences between the two methods.
  - Can be done if the lab has previously established a reference range and is changing methodology
  - Acceptable, but not recommended method.
  - Should be verified by running at least 20 samples.
  - To reduce errors introduced by drift, transference calculations should be limited to one method change.

Reference Range Studies

- "Divine judgment" of the Lab Director
  - Use only when all other options are unavailable.
  - May be needed for sub-population ranges.
  - Use published data from respected sources.

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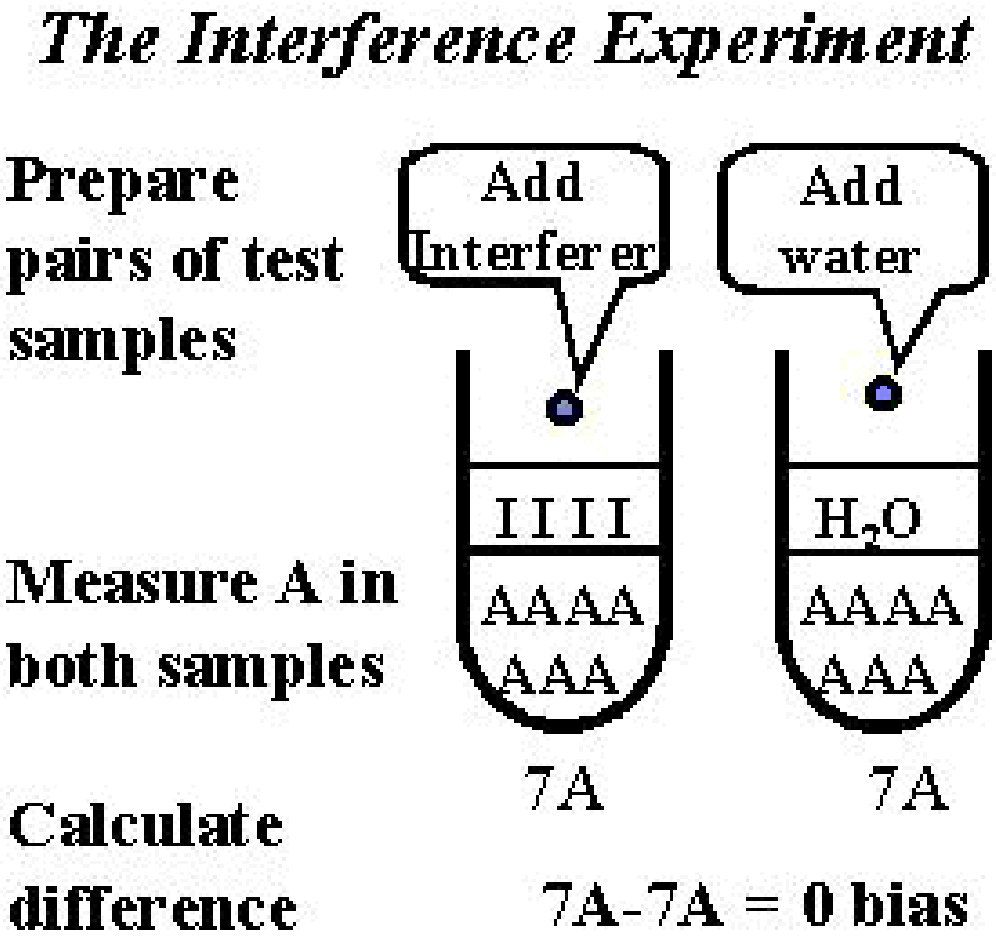
Interference Studies

Materials in patient specimen that cause errors which are independent of analyte concentration

- Include substances commonly found in serum or plasma, such as:
  - Lipids (Lipemia)
  - Hemoglobin (Hemolysis)
  - Bilirubin (Icterus)
- Less common substances:
  - Drugs
- **Immunoassay Interferences**:
  - HAMA and other heterophile antibodies
  - Specific antibodies
  - Rheumatoid Factors
  - Non-specific binding of immunoglobulins (sticky serum, “anti-plastic”)
- Anticoagulants

Interference Studies



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Interference Studies – **“to do” list**

- The interfering substance is “spiked” into a known sample (no analyte added)
- Added volume < 10%
- Run in duplicates
- Calculate interference (bias):

Bias = (sample + interference) **-** baseline sample

(sample + buffer/water)

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Interferences in Immunoassays

- Non-specific binding
  - High levels of immunoglobulins
  - Immune complexes
- Interfering antibodies
  - Rheumatoid factor
  - Specific antibodies to the analyte
  - Heterophile antibodies (antibodies to reagent non-human proteins)
- High concentrations of these types of substances may be difficult to obtain. Interference studies may require “mixing experiments”.

Put Method On Line

- Write and test a procedure!
  - CLSI protocol (GP2)
  - Maintenance
  - Calibration
  - Control system
- Staff training
- Document Method Evaluation experiments according to appropriate regulations
- Start routine service
- Monitor performance

Self Assessment Questions

1. Which of the following is a step in method validation?
   a) **Error assessment**
   b) Vendor consultation
   c) FDA approval
   d) Dissociative statistics

2. The lower limit of quantitation is defined as:
   a) The lowest number that will almost always have a non-zero result
   b) The lowest concentration that can be distinguished from background
   c) The lowest concentration that can be quantified reliably
   d) None of the above

3. The range of analyte where results are proportional to the true concentration of analyte in the sample without modification defines which of the following?
   a) Clinical reportable range
   b) Precision
   c) Analytical measurement range
   d) Accuracy

4. When evaluating a linear regression line (y = mx + b), which of the following denotes the lowest level of proportional and constant bias?

a) y = .28x + .94

b) y = 1.15x + .25

c) y = 1.05x - .04

d) y = .34x + 1.00