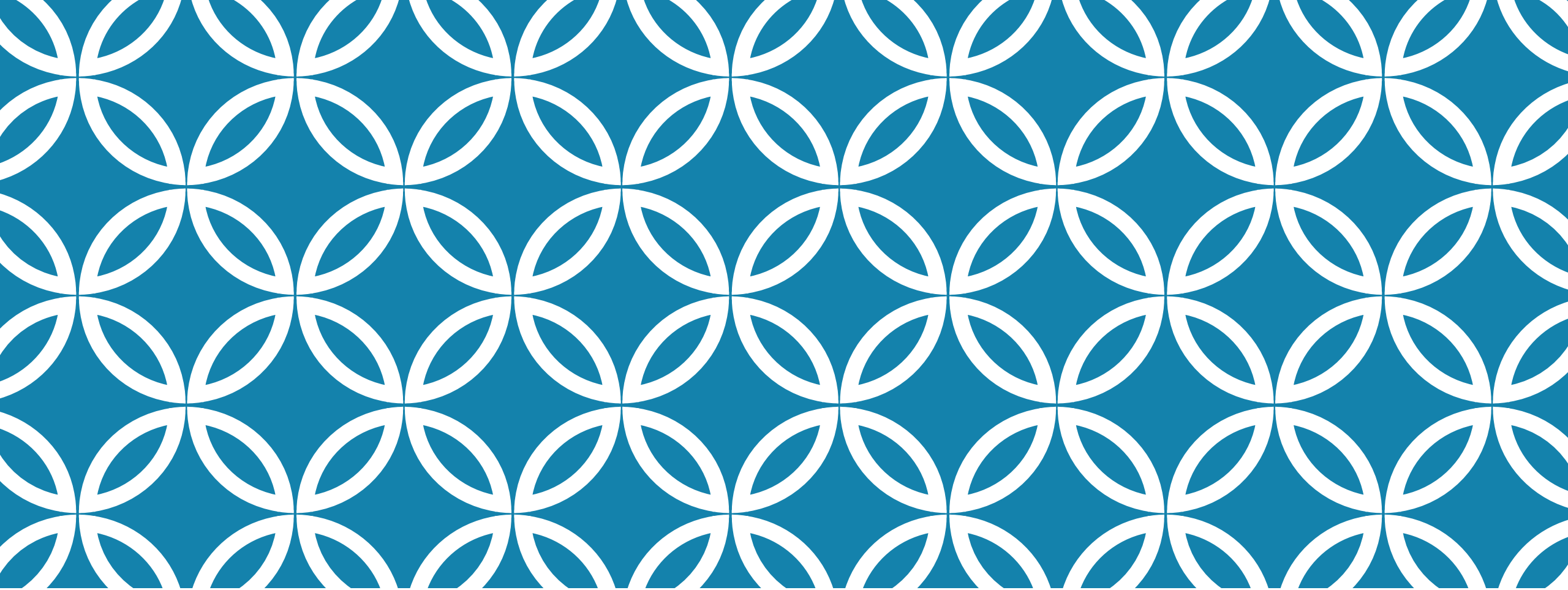

METHOD VALIDATION CASE

METHOD VALIDATION PROTOCOL

CLIA Regulation 493.1253 (2)

1. Accuracy – (closeness to true/comparative method)
2. Precision – (reproducibility)
3. Reference Interval
4. Reportable range – (linearity, AMR= Analytical Measurement Range, CRR= clinical reportable range)
5. Analytical sensitivity- (lower limit)
6. Analytical specificity – (interferences)

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1. ACCURACY

- Agreement between test result and "true" result → Bias
- Bias: a measure of trueness- Systematic error
- 2 ways:
  - CLSI-EP15-A2
    - Run 20 samples within testing range both new and comparative method
    - Compare with Deming Regression model or Passing Bablock Regression Model
  - CLSI-EP09-A2
    - Analyze minimum 40 samples by both new and comparative method over the clinically meaningful range
    - Duplicate measurement by test method and comparative method
    - Difference between the test method & comparative method measured as BIAS
    - Compare the average bias between the two methods with manufacturer's claims for allowable limit
    - Otherwise check CLIA limits/reference books for clinically allowable limits

Systematic error – Affects Accuracy

Caused by (examples): bad calibrators, bad reagents, bad pipettes, interference

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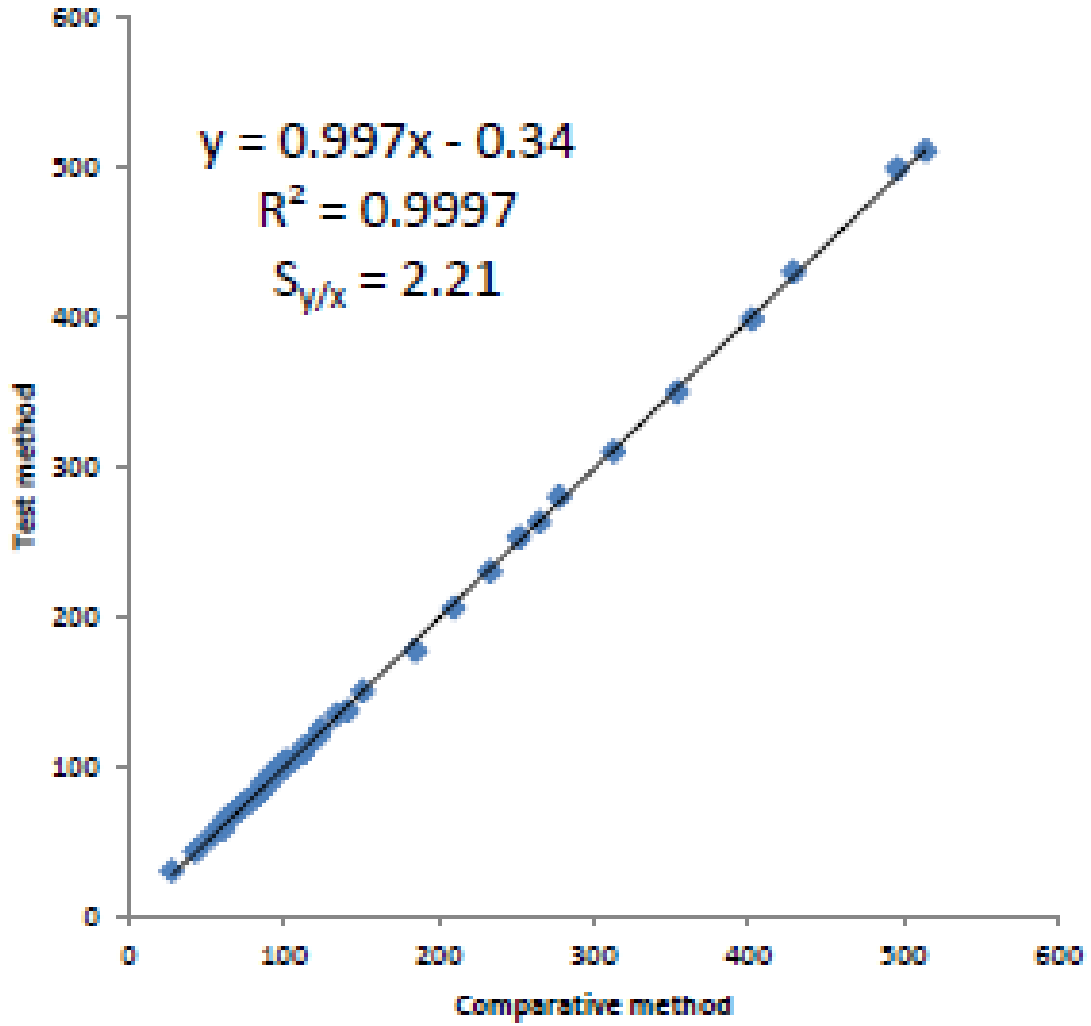

Professional Practice in Clinical Chemistry

1. ACCURACY

Glucose

Nomor	Nilai	Nilai	Selisih	% bias
1	96	100	(4)	-3.75
2	100	99	1	1.20
3	103	101	2	1.94
4	105	100	5	4.76
5	101	101	0	0.20
6	101	100	1	0.89
7	99	98	1	0.61
8	100	98	2	2.40
9	104	101	3	3.08
10	99	99	-	0
11	100	97	3	2.80
12	100	99	1	1.40
13	101	96	5	4.85
14	99	98	1	1.41
15	99	98	1	1.21
16	100	101	(1)	-0.90
17	99	100	(1)	-1.11
18	101	100	1	0.59
19	102	102	(0)	-0.20
20	99	99	(0)	-0.30
21	99	101	(2)	-1.82
22	100	101	(1)	-1.20
23	99	99	(0)	-0.40
24	97	101	(4)	-3.81
25	103	100	3	3.01
26	101	100	1	1.29
27	97	99	(2)	-2.27
28	100	99	1	1.20
29	98	99	(1)	-1.22
30	101	101	1	0.50
31	101	99	2	1.88
32	99	100	(1)	-0.51
33	98	100	(2)	-2.35
34	100	100	1	0.50
35	100	98	2	2.30
36	106	100	6	5.38
37	102	103	(1)	-0.49
38	99	101	(2)	-2.42
39	100	102	(2)	-1.70
40	99	103	(4)	-4.24

Level 1
AVG % bias = 0.37

Nomor	Nilai	Nilai	Selisih	% bias
1	233	240	(7)	-2.83
2	237	239	(2)	-0.68
3	251	244	7	2.91
4	245	238	7	2.86
5	234	236	(2)	-1.03
6	238	237	2	0.63
7	244	240	5	1.84
8	244	243	1	0.45
9	253	244	9	3.68
10	242	242	-	0
11	241	241	(0)	-0.12
12	240	239	1	0.38
13	243	234	9	3.83
14	240	235	5	2.25
15	239	233	6	2.68
16	238	242	(4)	-1.55
17	241	243	(2)	-0.87
18	241	240	1	0.41
19	245	241	4	1.51
20	240	244	(4)	-1.58
21	238	243	(5)	-2.23
22	238	236	2	0.84
23	237	233	4	1.60
24	234	239	(5)	-2.18
25	237	237	(0)	-0.17
26	239	242	(3)	-1.42
27	241	245	(4)	-1.54
28	239	237	2	0.88
29	236	241	(5)	-1.91
30	242	239	3	1.07
31	239	237	2	0.67
32	240	242	(2)	-0.67
33	238	239	(1)	-0.29
34	238	242	(4)	-1.85
35	243	240	3	1.40
36	240	231	9	3.92
37	249	241	8	3.29
38	240	239	1	0.33
39	240	243	(3)	-1.25
40	245	240	5	2.08

Level 2
AVG % bias = 0.43

CLIA Allowable Bias = 10%

2. PRECISION

- Express in SD or CV of the set of replicates
- Within run precision
  - Patient or QC samples assayed 20 times on the same day within the same run
  - If precision poor, no need to do further evaluation
- Between run precision
  - Patient or QC samples once per day for 20 days

Samples at least 2-3 levels –medical decision points

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Table 1. Data on imprecision.

Sample	Glucose mg/dL (mmol/L) Mean	SD	CV, %
Within run (n = 20 replicates)			
I	50.4 (2.8)	1.4 (0.08)	2.8
II	200.6 (11.14)	2.7 (0.15)	1.4
Between run (n = 20 runs)			
I	51.2 (2.84)	2.1 (0.12)	4.1
II	202.3 (11.24)	3.5 (0.19)	1.7

2. PRECISION

- What's good precision?
  - Depends on the analyzer and the analyte
    - 5% CV considered good
- Random error – affects precision
- May be caused by (for example):
  - Variability in volume of sample or reagent delivered
  - Changes in environment
  - Incosistent handling of materials

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3. REFERENCE INTERVAL

Adopted Reference Limits

- Manufacturer suggested
- Reference laboratory
- Reference text Books / Articles

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3. REFERENCE INTERVAL

- Validating a refence range: The number of samples needed if age/sex not a factor:
  - Verification of manufacturer's range N ≥ 20
    - Used if using the manufacturer's range and the test will be used in the exact manner described by the manufacturer

    CLSI C28-A2:
    - Test 20 healthy individuals: If ≤ 2 samples outside proposed limits, validated
    - If > 2 samples outside, can repeat with another 20, and accept if ≤ 2 samples outside
    - Not worth repeat if > 5 samples outside proposed limits
    - If not validated, will need to establish own reference limits
  - Estimated a reference range, N=40-60
    - Used if the manufacturer's range is not adequate or if the use of the test not conform exactlt to the manufacturer's intended use
  - Establish a reference range, N ≥ 120
    - Non FDA approved test or if there will be significant changes to the use of method
    - Briefly, need 120 individuals (200 if does not follow Gaussian distribution) to be confident in accuracy
    - Need to have criteria for inclusion and exclusion

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4. REPORTABLE RANGE

Validation of reportable range

- Minimum of 3 test specimens (4-5 better), measured in duplicate or triplicate
- Use appropriate matrix / “traceable” standards
- Well established target concentrations
- Concentration near the low, midpoint and high values of the Analytical Measurement Range (AMR)
- Run from lowest to highest (to avoid carryover)
- Pipetting accuracy and precision is critical

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AMR VS MD/C OR CRR

- Analyticial Measurement Range (AMR)
  - Range of analytes that a method can directly measure w/o modification (no dilutions, concentrations, other pretratements)
- Maximum Dilution/ Concentration (MD/C) formerly Clinical Reportable Range (CRR)
  - Range of analyte values which are clinically significant
  - After modification (dilution, concentration, etc)
  - Value higher than AMR: report as > X or dilute
  - Value lower than AMR: report as < X or concentrate

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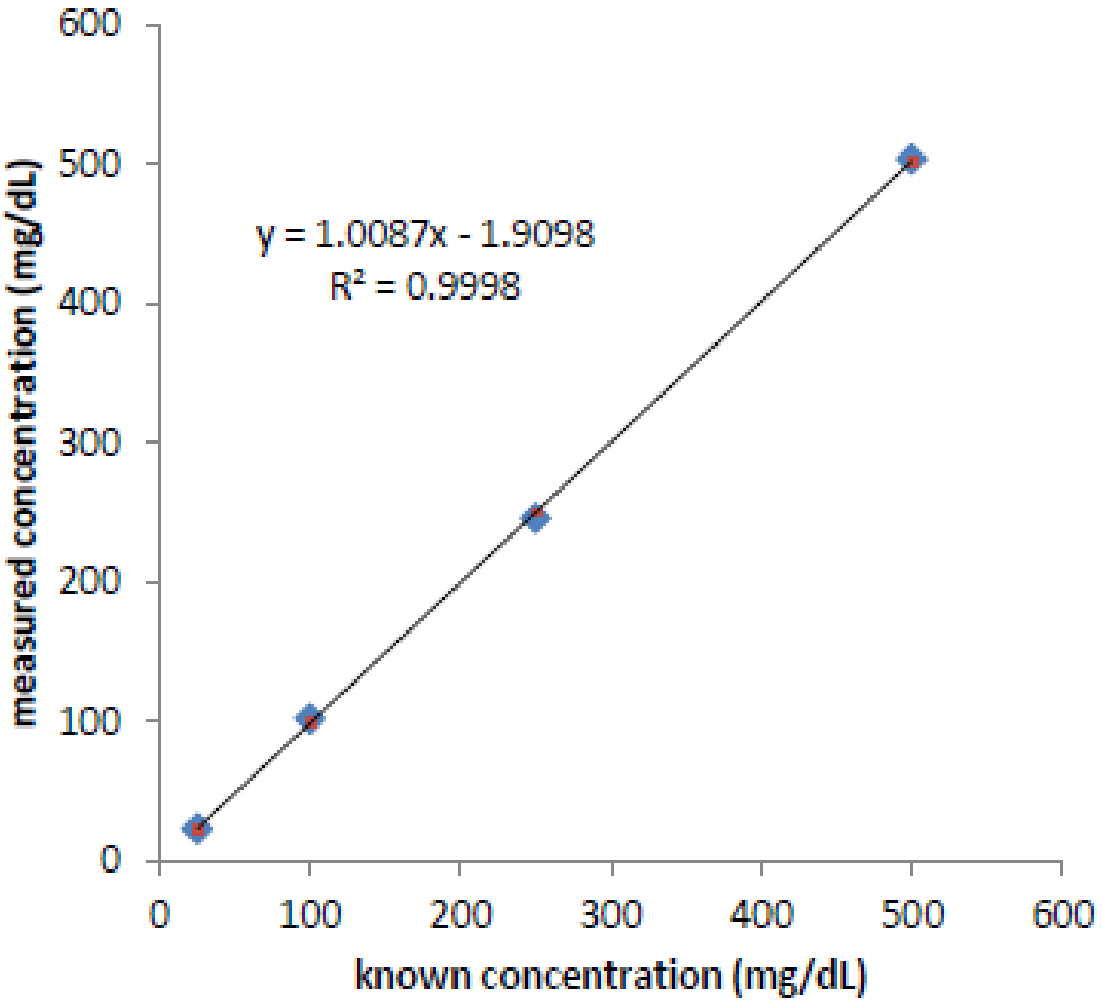

known	Average measured
25	23
100	102
250	246
500	504

linearity = 25 – 500 mg/dL

CAP "AMR"

5. ANALYTICAL SENSITIVITY

- Also called lower detection limit
- 2 steps: determination of values obtained with blank samples and values obtained with low level positive samples
- Blank samples often use the zero calibrator for an assay
- Low level positive samples identified at or only slightly above the manufacturer's stated analytical sensitivity
- CLSI EPI 17-A describes:
  - Run 20 blanks or low level samples; if < 3 exceed stated blank value, accept that value

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	Expected range	< 1 mg/dL
Sampel no	Sample	Glucose
1	BLANK	0
2	BLANK	0
3	BLANK	0
4	BLANK	0
5	BLANK	0
6	BLANK	0
7	BLANK	0
8	BLANK	0
9	BLANK	0
10	BLANK	0
11	BLANK	0
12	BLANK	0
13	BLANK	0
14	BLANK	0
15	BLANK	0
16	BLANK	0
17	BLANK	0
18	BLANK	0
19	BLANK	0
20	BLANK	0

6. ANALYTIC INTERFERENCES

- Most commonly, involves listing stated interferences from manufacturer and evaluating samples in correlation studies for differences (outliers), investigation of causes of interferences
- If any outliers during method comparison study with test method, laboratory can check the interfering substances

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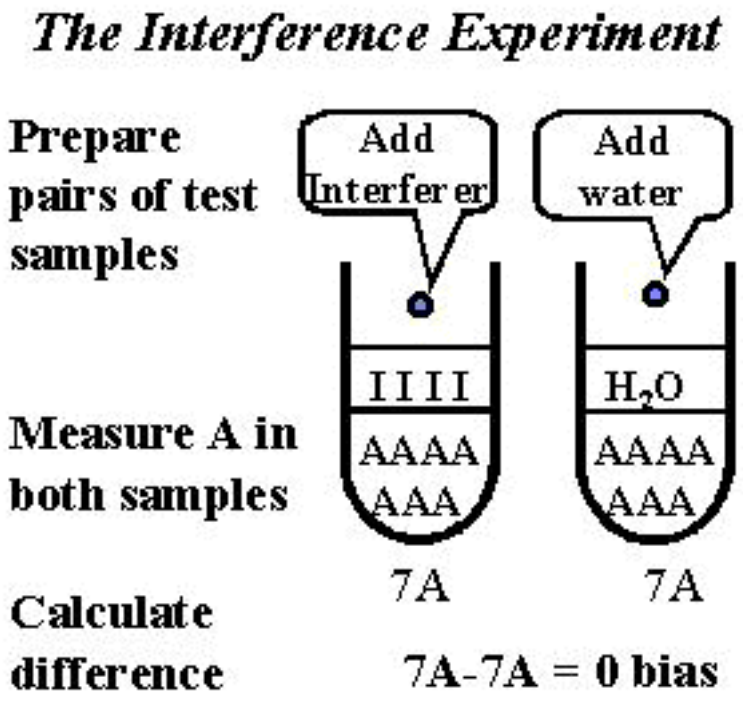
6. ANALYTIC INTERFERENCES

- Added volume < 10%
- Run in duplicates
- Calculate the bias:

Bias = (sample +interference) – baseline samples (sample+buffer/water)



Professional Practice in Clinical Chemistry

Method evaluation should also ensure that the magnitude of the errors affecting the results are acceptable

Evaluate the Total Error (TE) in the assay

TE = Random Error (RE) + Systematic Error (SE)

Example: Glucose

- Acceptable performance = 10% (CLIA)
- Xc= 200 mg/dL → Ea (Allowable Error) = 20 mg/dL

Table 1. Data on imprecision.

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II	202.3 (11.24)	3.5 (0.19)	1.7

Random Error (RE) → From an inter assay precision study, SD= 200 mg.dL was 3.5 mg/dL

RE = 4 SD = 4(3.5) = 14 mg/dL

Systematic Error (SE) → From linear regression for glucose

$y = 0.997x - 0.34$

$R^2 = 0.9997$

$S_{y/x} = 2.21$

Yc = 0.997(200)-0.34 = 199.06

SE = [Yc-Xc} = [200.00-199.06] = 0.94 mg/dL

TE = RE+ SE = 14+0.94 = 14.94 mg/dL

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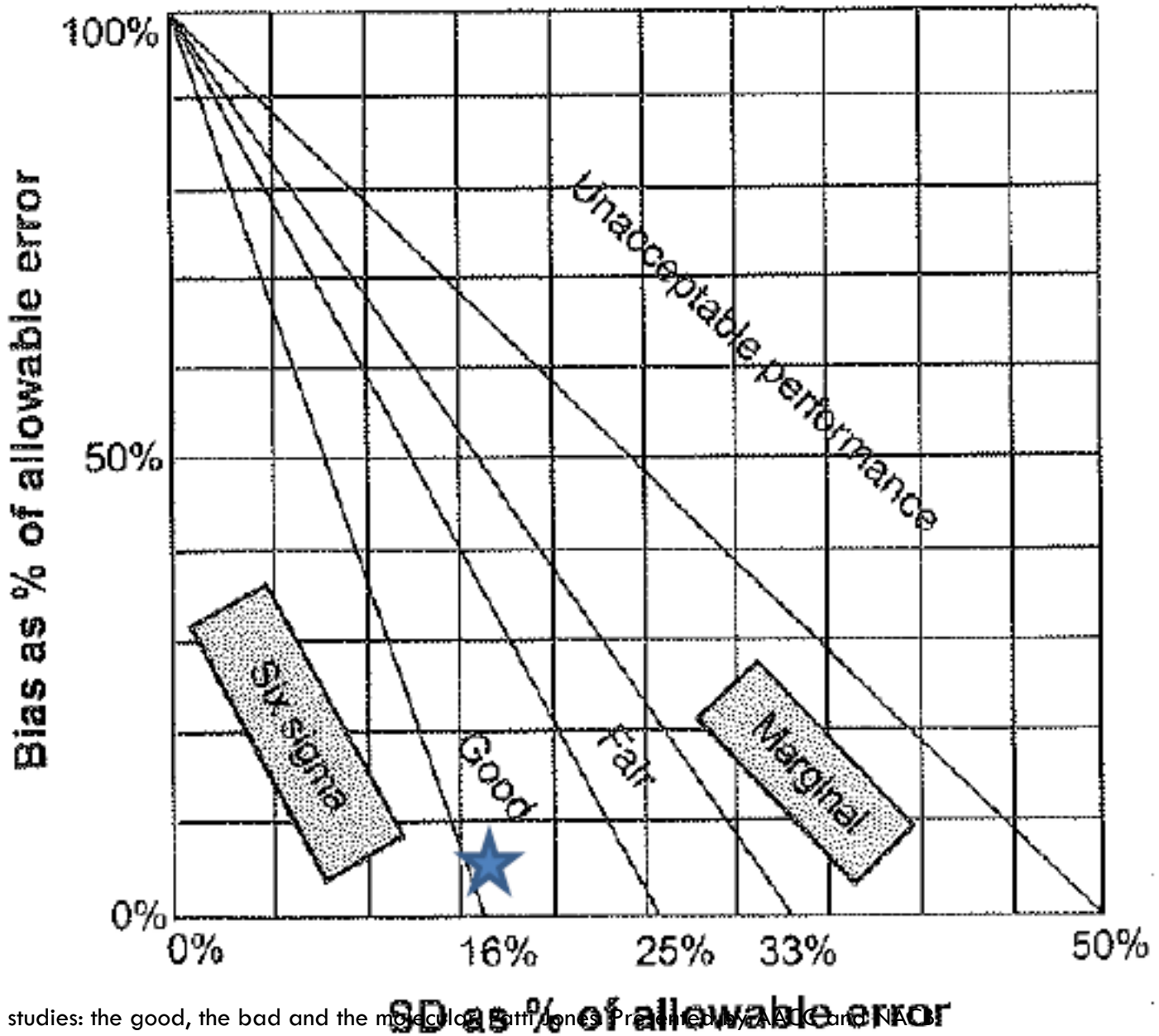

Bias = 0.94 = 4.5%

SD = 3.5= 17.5%


THANK YOU