TEHRAN UNIVERSITY OF MEDICAL SCIENCES

Total allowable error, verification and validation

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Type of Error

Type of Error	Stage	Example in Hematology	Possible Consequence
Pre-analytical Error	sample collection, handling, transport	❖ Collecting blood in a tube **without EDTA**, leading to clot formation. ❖ Drawing blood from a site with **IV infusion**, causing sample dilution. ❖ **Delayed transport** of CBC sample at room temperature → increased MCV and false results.	❖ Clotted sample → inaccurate CBC results. ❖ False decrease in cell counts. ❖ Misleading interpretation of anemia or cell indices.

Type of Error	Stage	Example in Hematology	Possible Consequence
Analytical Error	instrumental or methodological phase	❖ **Incorrect calibration** of the hematology analyzer → false WBC count. ❖ **Expired or degraded reagents** → inaccurate Hb or Hct results. ❖ **Instrument malfunction** (e.g., clogged aperture or sensor issue).	❖ Systematic or random measurement errors. ❖ False increase/decrease in blood cell counts. ❖ Misdiagnosis or inappropriate clinical decision.

Type of Error	Stage	Example in Hematology	Possible Consequence
Post-analytical Error	After analysis (reporting, interpretation, communication)	**Typing error** in report: Hb = 15 instead of 5 g/dL.	Wrong clinical decisions.
		Results entered into the wrong patient's file.	Delay in treatment.
		Failure to notify critical results (e.g., platelets <10,000/µL).	Potential patient harm.

- **Random Error (Imprecision):** The scatter of repeated results around the mean, usually expressed as %CV.
  - RE= $X_i - \bar{X}$

- **Systematic Error (Bias):** The deviation of the laboratory mean from the true/reference value.
  - SE= $\bar{X} - \mu$

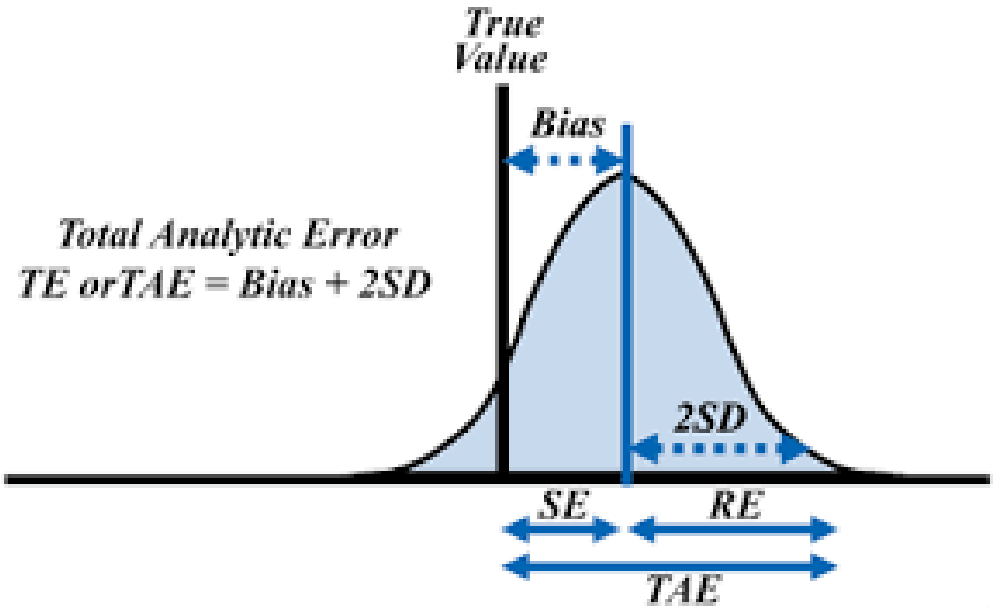
Total Allowable Error (TEa)

- **Total Allowable Error (TEa)** is the maximum error (including both **systematic error [bias]** and **random error [imprecision]**) that can be tolerated in a clinical laboratory test result **without affecting medical interpretation and clinical decision-making**.

- TEa values are defined by **international guidelines (CLIA, CAP, etc.)** or national standards, and are based on **clinical needs, biological variation, and analytical performance**.

Components of TEa

- **Systematic Error (Bias)**
- **Random Error (Imprecision)**
- TEa is essentially a **threshold value** that combines both.



TEa= Bias + ZSD

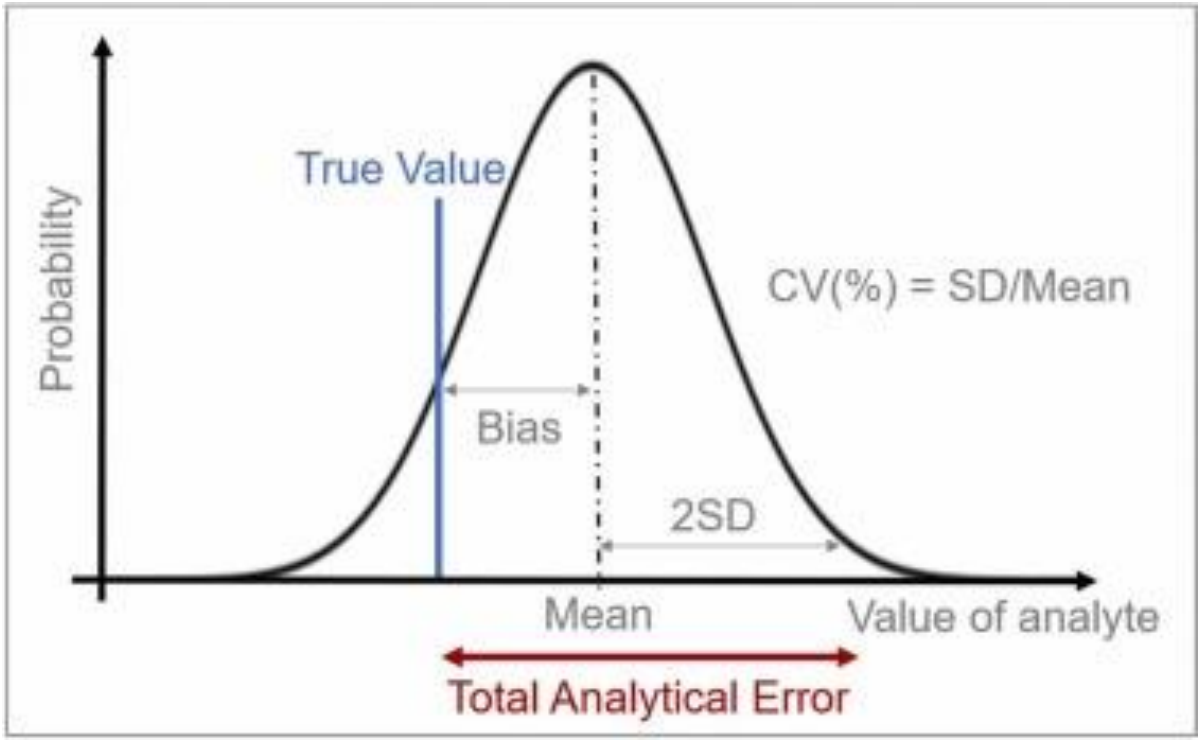
TEa= Bias + 1.65SD (90% Cl)

TEa= Bias + 1.96SD (95% Cl)

TEa= Bias + 2SD (95.45% Cl)

TEa= Bias + 3SD (99.7% Cl)

Total Allowable Error (TEa)



Similarities with other errors

- Like bias and CV, TEa is used to assess test performance.
- All three relate directly to accuracy and precision of laboratory testing.

Differences from Other Errors

- **Bias** and **CV** describe the *actual* performance of a method, while **TEa is an acceptance criterion (a benchmark)**.

- TEa is **clinical and quality-oriented**, while bias and CV are strictly statistical.

- TEa is usually greater than the actual analytical errors and serves as the **limit for acceptability** in QC programs.

Example: Blood Glucose

- **TEa (CLIA standard):** ±10% or ±6 mg/dL (whichever is greater)

Laboratory data:

True value = 100 mg/dL

Laboratory mean = 105 mg/dL

SD = 2 mg/dL

Calculations:

Bias: (105 – 100) = 5 mg/dL = 5%

CV: (2 / 105) × 100 ≈ 1.9%

Sigma Metric: $$\sigma = \frac{TEa - Bias}{CV} = \frac{10 - 5}{1.9} \approx 2.6$$

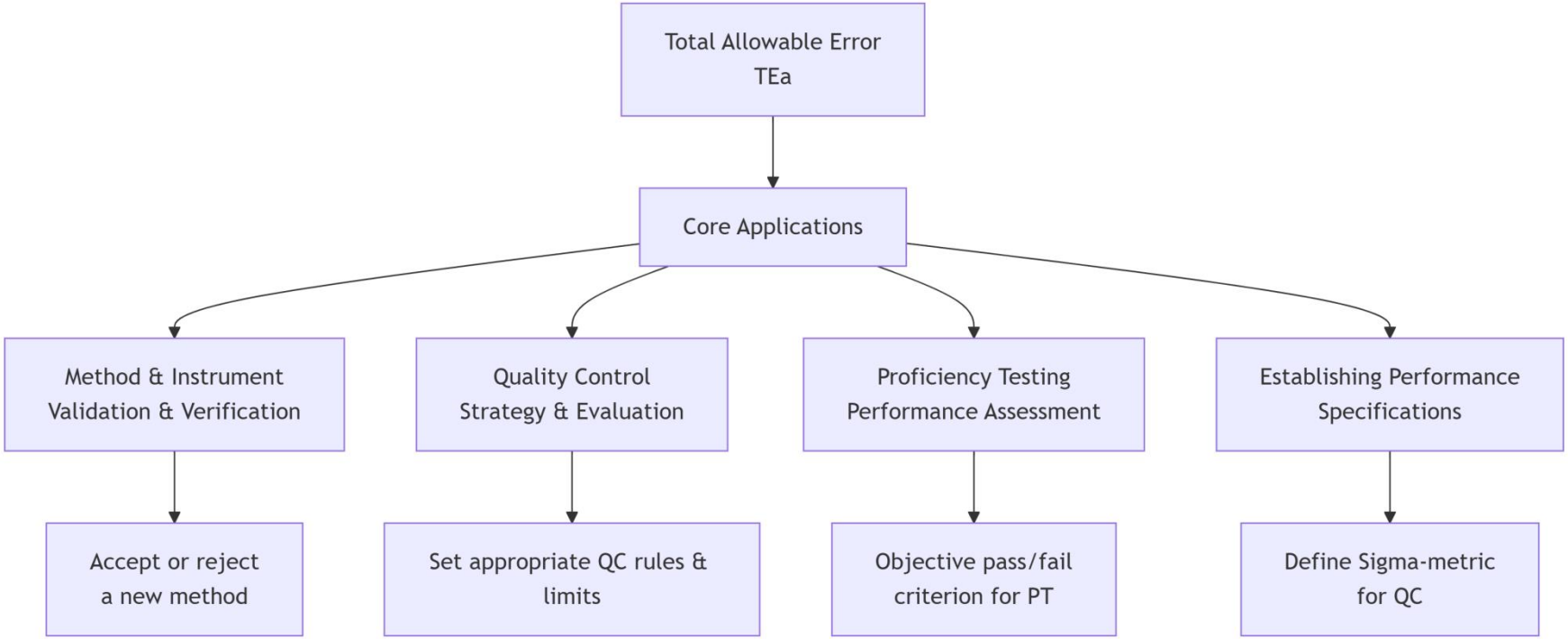
Interpretation: Since σ < 3, the method does not meet quality standards and needs improvement.

Methods of Expressing Total Allowable Error (TEa)

- Percentage Expression (% of target value)
- Absolute Value Expression (Fixed amount)
- Combination of Percentage and Fixed Value
- Base of SD (target value ±3SD)

Situations Requiring Determination of Total Allowable Error (TEa) in the Clinical Laboratory

- During Method Validation or Verification
- In Internal Quality Control (IQC)
- In Method Comparison and External Quality Assessment (EQA/PT)


Total Allowable Error
TEa
Core Applications
Method & Instrument
Validation & Verification
Quality Control
Strategy & Evaluation
Proficiency Testing
Performance Assessment
Establishing Performance
Specifications
Accept or reject
a new method
Set appropriate QC rules &
limits
Objective pass/fail
criterion for PT
Define Sigma-metric
for QC

Theories and Approaches for Defining Total Allowable Error (TEa) Goals in Clinical Laboratories

- Clinical Outcome–Based Theory
- Biological Variation–Based Theory
- Analytical Performance–Based Theory
- Regulatory and Professional Guideline–Based Theory

Biological Variation–Based Theory

- Pre analytical variation
- Analytical variation
  - Random error
  - Systematic error
- Biological Variation

Biological Variation

- **Within subject**
- **Between subject**

Biological Variation for Hematology Parameters

Parameter	Biological Variation (CV~I~ %)
WBC Count	10.2%
RBC Count	2.9%
Hemoglobin (Hb)	2.5%
Hematocrit (Hct)	2.6%
MCV	1.6%
MCH	1.4%
MCHC	1.0%
Platelet Count	8.3%
Neutrophil Count	19.9%
Lymphocyte Count	13.1%
Monocyte Count	20.0%
Eosinophil Count	22.9%
Reticulocyte Count	15.0%

Analytical Performance–Based Theory

Concept:
The Total Allowable Error (TEa) is determined based on the actual and achievable performance of existing laboratories or analyzers.

Basis of Determination:
TEa values are derived from External Quality Assessment (EQA/PT) programs, inter-laboratory studies, or manufacturer performance specifications.

Advantage:
Practical and applicable to real laboratory conditions.

Disadvantage:
May not reflect true clinical needs and is often based only on current technological capabilities.

Example:
In an inter-laboratory quality control program, most laboratories show an error of about ±7% in measuring **urea**; therefore, the TEa for urea can reasonably be set at around **±10%**.

Regulatory and Professional Guideline–Based Theory

Concept:
The Total Allowable Error (TEa) is determined by official or professional organizations such as **CLIA (USA)**, **CAP**, **RiliBÄK (Germany)**, or **IFCC**.

Example:
According to CLIA, the TEa for **glucose** is ±10%, and for **sodium** it is ±4 mmol/L.

Advantage:
This approach has legal authority and official reference status.
Standardized and comparable across all laboratories.

Disadvantage:
It may not always align with specific biological or clinical needs.
In this method, the TEa values are pre-defined by official organizations (e.g., CLIA, CAP, or IFCC), and laboratories are required to adhere to these limits.

TEa Hematology Parameter

Hematology Parameter	Total Allowable Error (TEa)
Hemoglobin (Hgb)	±7%
Hematocrit (Hct)	±6%
Red Blood Cell Count (RBC)	±4%
White Blood Cell Count (WBC)	±15%
Platelet Count (PLT)	±25%
Mean Corpuscular Volume (MCV)	±2 fL
Mean Corpuscular Hemoglobin (MCH)	±3%
Mean Corpuscular Hemoglobin Concentration (MCHC)	±3%
Partial thromboplastin time (PTT) & Prothrombin time (PT)	±15%

Verification

Simple definition:

proving that the same method also works correctly in your laboratory with your equipment, staff, and environment.

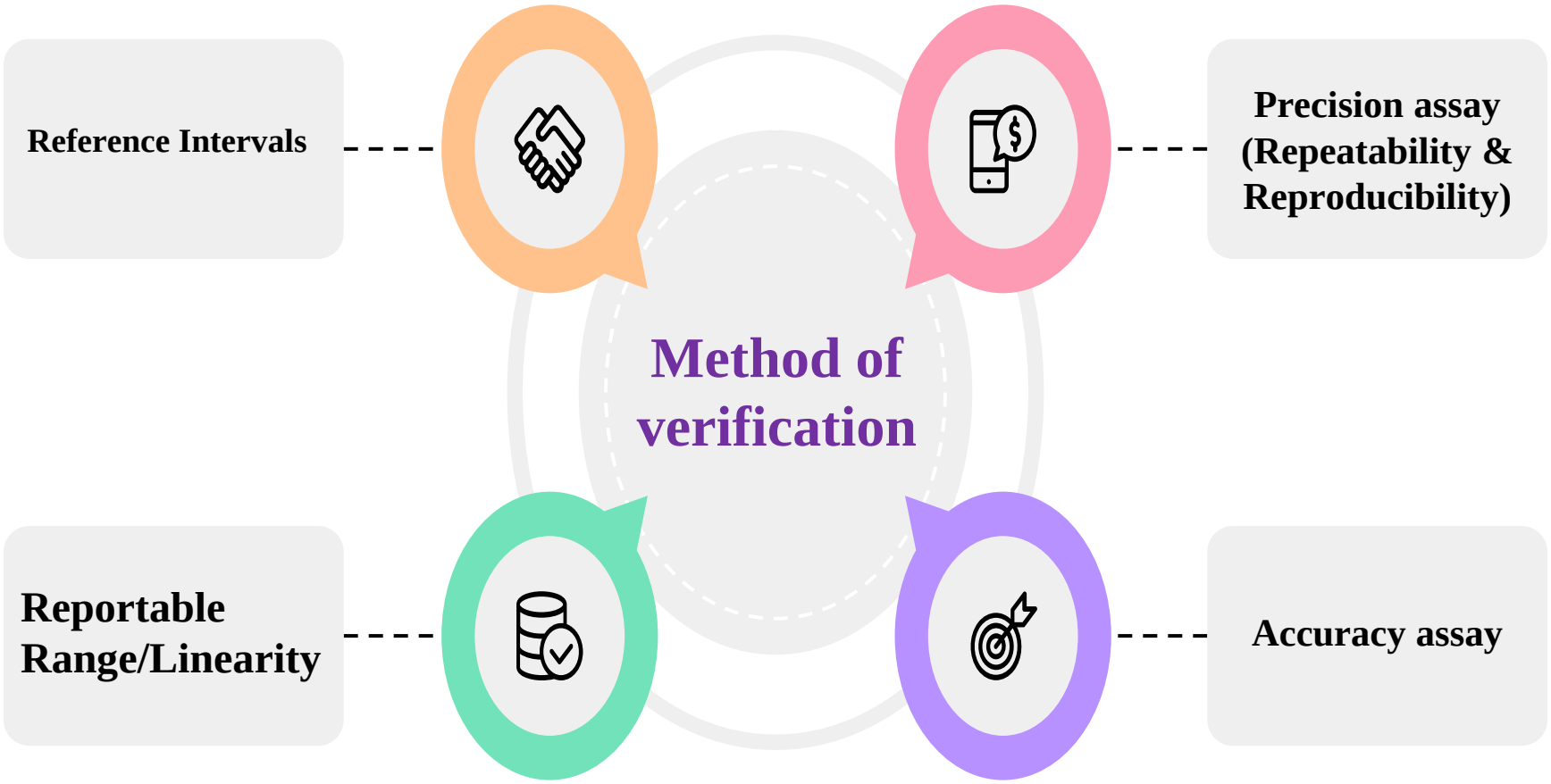
When to perform:

Upon device purchase

Relocation or changes in environmental conditions

Change in kits and reagent

Change in Lot number kits


Reference Intervals
Precision assay (Repeatability & Reproducibility)
Method of verification
Reportable Range/Linearity
Accuracy assay

Precision assay (Repeatability & Reproducibility)

- Measure one or two control levels multiple times within a day (repeatability) and across several days (reproducibility).
- Calculate CV% and ensure it falls within the limits claimed by the manufacturer or CLSI guidelines.
- **Intra-assay (within run):** Same samples run multiple times on the same run and day
- **Inter-assay (between run):** Same samples run in different runs on the same day or different days, and preferably by a different laboratorian.
- Calculating the Coefficient of Variation $$CV\ \% = 100 \times \frac{\text{Standard Deviation}}{\text{Mean}}$$

Repeatability (Within-run Precision)

- Same operator
- Same instrument
- Same reagents
- Short time interval

Example

- Suppose you are measuring **serum glucose** using a biochemistry analyzer.
- You have a control sample with a true value of 100 mg/dL.
- You test this sample **10 times consecutively** in the same run by the same operator

Replicate	Result (mg/dL)
1	100
2	101
3	99
4	100
5	100
6	101
7	99
8	100
9	100
10	101

Mean = 100 mg/dL
Standard Deviation (SD) = 0.8
Coefficient of Variation (CV%) = 0.8%

A low CV indicates **good repeatability** of the assay.

Reproducibility (Between-run Precision)

- Different operators
- Different days

Example

- The same glucose control (100 mg/dL) is tested over three different days by two different operators, each day with three replicates

Day	Operator	Results (mg/dL)
1	A	99, 100, 101
2	B	102, 100, 101
3	A	99, 98, 100

Overall Mean = 100 mg/dL
SD = 1.2
CV% = 1.2%

This shows that the method provides **reliable and consistent results across varying conditions**, indicating good reproducibility.

Repeatability vs Reproducibility

Feature	Repeatability	Reproducibility
Experimental conditions	Constant (same day, operator, and instrument)	Variable (different days, operators, or instruments)
Purpose	Evaluates short-term precision	Evaluates long-term stability and reliability
Example	Ten consecutive runs of the same sample in one day	Testing the same sample over multiple days and operators

General Procedure for Precision Verification

Step	Description
1 **Select control materials**	Choose **two levels of control (normal and pathological)** for each parameter (e.g., normal and low Hb).
2 **Repeatability test**	Analyze each control **10 consecutive times in a single day**.
3 **Reproducibility test**	Analyze the same controls **twice per day for 5 consecutive days**.
4 **Calculate Mean, SD, and CV%**	Formula: **CV% = (SD / Mean) × 100**
5 **Compare results with manufacturer or CLSI criteria (EP15 / EP05)**	If CV% ≤ allowable limit → **method accepted**.

Complete Blood Count (CBC)

Parameter	Type	Repetitions	Example Results (Hb, g/dL)	Mean	SD	CV%	Acceptance Limit	Result
Hb (normal)	Repeatability	10 in one day	13.4, 13.5, 13.6, 13.4, 13.5, 13.6, 13.4, 13.5, 13.5, 13.4	13.5	0.07	0.52%	≤2%	Accepted
Hb (low)	Reproducibility	5 days	Daily means: 8.1, 8.0, 8.2, 8.1, 8.1	8.1	0.07	0.86%	≤2%	Accepted

Interpretation:
CV values below 2% are acceptable for Hb measurement. Higher variability suggests calibration or instrument issues.

Platelet Count

Type	Results ($\times 10^3/\mu L$)	Mean	SD	CV%	Acceptance Limit	Result
Repeatability	240, 243, 239, 241, 242, 241, 240, 242, 243, 241	241.2	1.3	0.54%	≤5%	✔
Reproducibility	Daily means: 240, 238, 243, 242, 241	240.8	1.9	0.79%	≤5%	✔

White Blood Cell Count (WBC)

Type	Results ($\times 10^3/\mu L$)	Mean	SD	CV%	Limit	Result
Repeatability	6.1, 6.0, 6.2, 6.1, 6.1, 6.2, 6.0, 6.1, 6.1, 6.2	6.11	0.07	1.1%	≤3%	✔
Reproducibility	Daily means: 6.0, 6.2, 6.1, 6.0, 6.1	6.08	0.08	1.3%	≤3%	✔

ESR (Erythrocyte Sedimentation Rate – Automated Westergren)

Type	Results (mm/h)	Mean	SD	CV%	Acceptance Limit	Result
Repeatability	25, 26, 25, 24, 26, 25, 25, 26, 24, 25	25.1	0.7	2.8%	≤10%	✔
Reproducibility	Daily means: 25, 24, 26, 25, 25	25.0	0.7	2.8%	≤10%	✔

Reticulocyte Count (%)

Type	Results	Mean	SD	CV%	Acceptance Limit (CLSI)	Result
Repeatability	1.4, 1.5, 1.6, 1.5, 1.4, 1.6, 1.5, 1.5, 1.4, 1.6	1.5	0.07	4.7%	≤10%	✔
Reproducibility	Daily means: 1.5, 1.6, 1.4, 1.5, 1.5	1.5	0.07	4.7%	≤10%	✔

Coagulation Tests (PT / aPTT)

Test	Type	Mean (sec)	SD	CV%	Acceptance Limit	Result
PT (Normal)	Repeatability	13.2	0.15	1.1%	≤3%	✔
PT (Pathologic)	Reproducibility	23.8	0.4	1.7%	≤5%	✔
aPTT (Normal)	Repeatability	31.0	0.5	1.6%	≤5%	✔
aPTT (Pathologic)	Reproducibility	48.2	1.1	2.3%	≤7%	✔

Interpretation and Acceptance Criteria

Outcome	Interpretation
CV% ≤ allowable limit	Precision accepted □
CV% > allowable limit	Review calibration, environmental conditions, or operator error □

Accuracy assay

- Analyze ~20 patient samples or control materials and compare results with those from a reference method or known target values.

- Check whether the deviation is within allowable total error (e.g., ±10 mg/dL for glucose).

Example

Sample	Reference Method (mg/dL)	Test Method (mg/dL)	Difference (A–B)
1	90	92	+2
2	110	108	-2
3	140	145	+5
4	180	175	-5
5	95	97	+2
6	130	137	+7
7	160	153	-7
8	120	124	+4
9	100	103	+3
10	85	83	-2
11	150	156	+6
12	200	192	-8
13	175	170	-5
14	115	118	+3
15	135	140	+5
16	145	144	-1
17	155	160	+5
18	165	163	-2
19	125	130	+5
20	105	99	-6

- Average deviation = ±4.3 mg/dL
- Maximum deviation = ±8 mg/dL
- All differences are within the allowable total error (±10 mg/dL).

Verification of Accuracy in Hematology Tests

Step	Description
Select patient samples	At least **20 fresh patient samples** covering the full analytical range (low, normal, high).
Test using two methods	Analyze each sample using the **new method** and the **reference or existing method**.
Record and compare results	Create a comparison table (New vs Reference).
Calculate bias (mean difference)	$Bias$ = (Mean of new method − Mean of reference method).
Plot data	Use **Passing–Bablok regression** or **Bland–Altman plot** to assess agreement.
Evaluate acceptance criteria	If bias ≤ allowable total error (TEa) → accuracy verified.

Acceptance Criteria (CLSI / CLIA Examples)

Parameter	Allowable Bias (TEa limits)
Hemoglobin (Hb)	±7% or ±0.5 g/dL
Hematocrit (Hct)	±6%
WBC count	±15%
RBC count	±10%
Platelet count	±15%
PT / aPTT	±10%
ESR	±10%

Hemoglobin (Hb)

Procedure:

•20 samples analyzed using both **new analyzer** (e.g., Mindray BC-6200) and **reference analyzer** (e.g., Sysmex XN-1000).

•Results compared by regression and bias calculation.

Sample	Reference (g/dL)	New Method (g/dL)	Difference
1	8.0	8.1	+0.1
2	10.5	10.4	–0.1
3	13.2	13.1	–0.1
4	16.0	16.1	+0.1
...	...	...	...
Mean Bias	—	—	**+0.05 g/dL (0.4%)**

 Interpretation: Bias = 0.4% → within allowable ±7% → accuracy accepted.

$$\text{Mean Bias} = \frac{\sum \text{Differences}}{n}$$

$$\text{Percentage Bias} = \left(\frac{\text{Mean Bias}}{\text{Mean Reference Value}}\right) \times 100$$

WBC Count

Sample	Reference (×10³/µL)	New Method	Difference
1	4.8	4.9	+0.1
2	6.5	6.4	–0.1
3	10.0	10.2	+0.2
4	15.5	15.3	–0.2
Mean Bias	—	—	**0.3%**

- **Interpretation:** Bias < 15% → accuracy confirmed.

🔬 Platelet Count

Sample	Reference (×10³/µL)	New Method	Difference
1	120	118	–2
2	250	247	–3
3	420	425	+5
Mean Bias	—	—	**+0.8%**

- Within ±15% → acceptable accuracy.

Coagulation Tests (PT / aPTT)

Test	Reference (sec)	New Method (sec)	Difference	Bias (%)	Limit	Result
PT	12.8	12.9	+0.1	0.8%	≤10%	✔
aPTT	32.0	31.8	–0.2	0.6%	≤10%	✔

- Both tests show minimal deviation → accuracy verified.

ESR (Automated vs Westergren)

Sample	Reference (mm/h)	New Method	Difference
1	10	11	+10%
2	40	38	–5%
3	80	78	–2.5%
Mean Bias	—	—	**+0.8%**

- Within ±10% → acceptable.

Interpretation of Results

Observation	Interpretation
Bias ≤ allowable total error	Accuracy verified
Bias > allowable limit	Method requires recalibration or troubleshooting
Significant proportional bias	Possible reagent or optical system issue
High random error	Check precision (instrument stability)

Reportable Range/Linearity

- Test samples across the low, medium, and high ranges claimed by the manufacturer (e.g., glucose: 50–400 mg/dL).
- Confirm that results fall within the expected analytical range.
- Manufacturer claim: analytical measurement range 50–400 mg/dL (linear and reportable).
- Test plan: run at least three samples in each range: low (≈50–80), medium (≈120–200), high (≈300–400).
- Compare measured values to the claimed analytical range and note any out-of-range results.

Test each level in duplicate or triplicate	Perform under standard operating conditions.
Plot results	Measured value (y-axis) vs. expected value (x-axis).
Evaluate linearity	Use regression analysis (e.g., linear regression or Passing–Bablok) and calculate the correlation coefficient (r).

Acceptability criteria

- ✓ Correlation coefficient $r \geq 0.99$
- ✓ Slope between 0.95–1.05
- ✓ Intercept near zero
- ✓ Deviations within allowable total error (TEa).

Hemoglobin (Hb) Linearity Verification

Claimed range by manufacturer: 3 – 20 g/dl

Procedure:

•Prepare 5 levels by diluting a high-concentration sample (e.g., 20 g/dL) with isotonic saline.

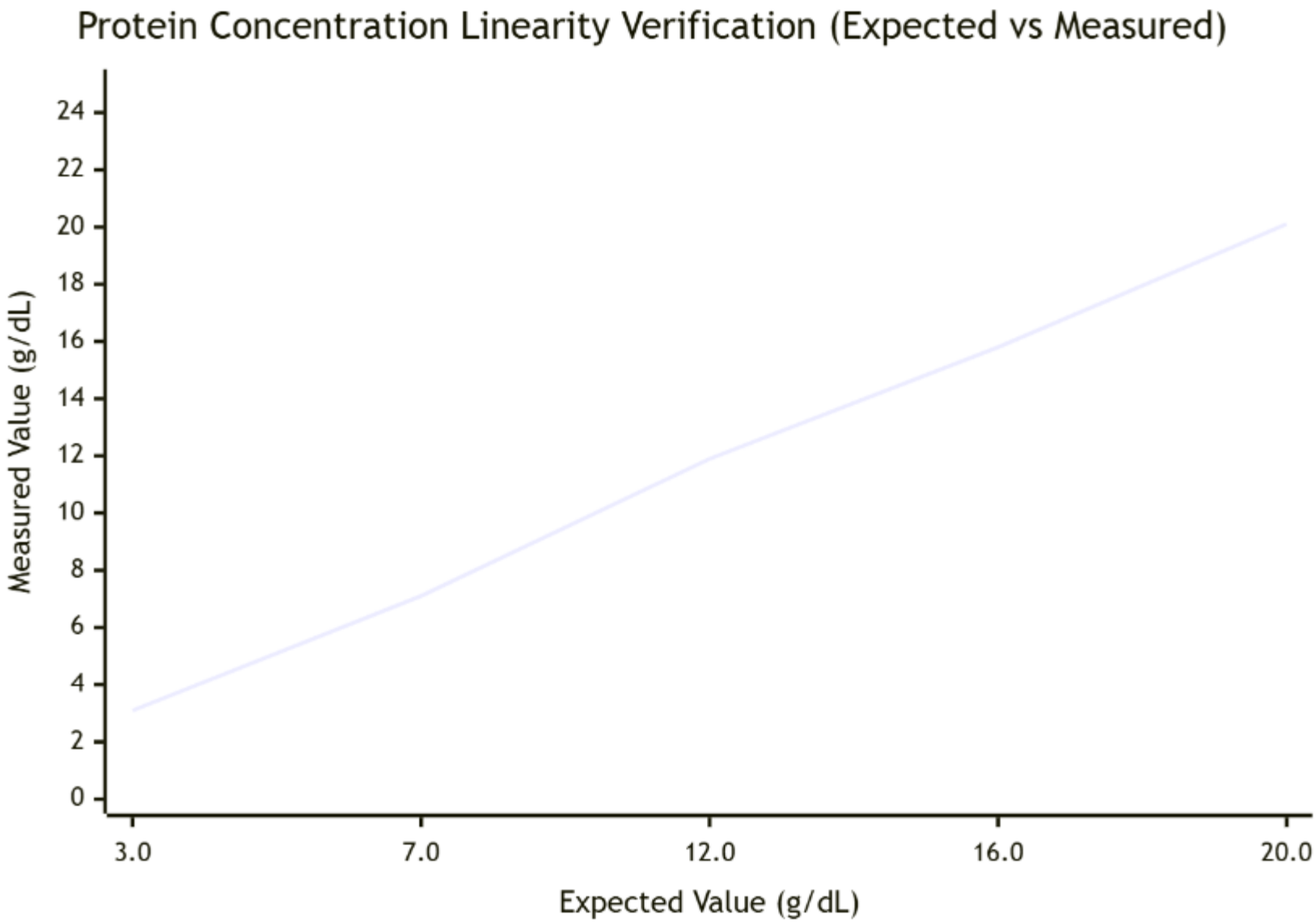
•Measure each level twice.

Expected (g/dL)	Measured (g/dL)	Deviation (%)
3.0	3.1	+3.3
7.0	7.1	+1.4
12.0	11.9	−0.8
16.0	15.8	−1.3
20.0	20.1	+0.5

Result:

$$\text{Deviation } \% = \frac{\text{Measured} - \text{Expected}}{\text{Expected}} \times 100$$

r = 0.999, slope = 0.998, intercept = 0.02 → **Linearity accepted**

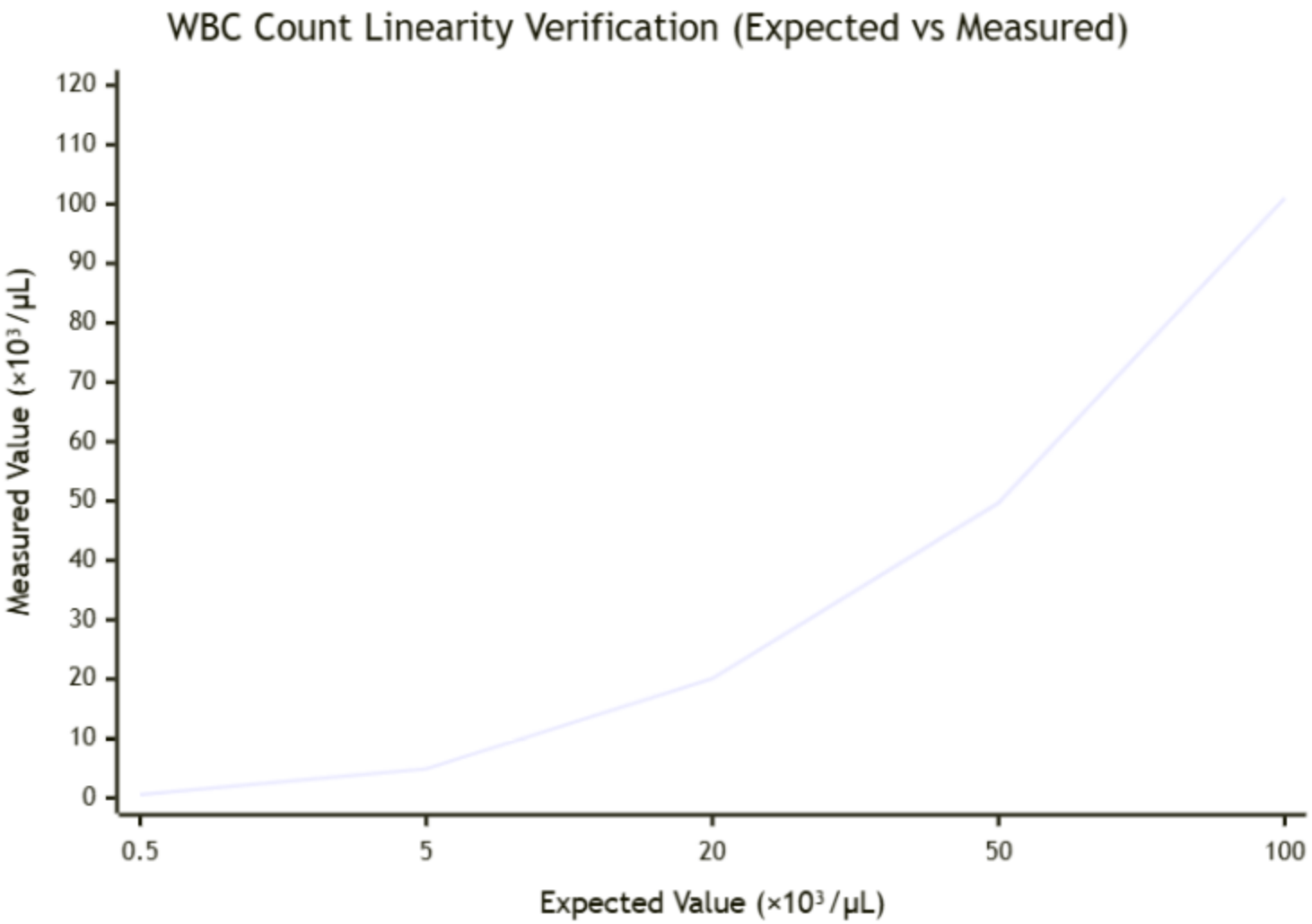

Protein Concentration Linearity Verification (Expected vs Measured)
Measured Value (g/dL)
24
22
20
18
16
14
12
10
8
6
4
2
0
3.0
7.0
12.0
16.0
20.0
Expected Value (g/dL)

White Blood Cell Count (WBC)

Claimed range: 0.1 – 100 $\times 10^3/\mu L$

Expected ($\times 10^3/\mu L$)	Measured	Deviation (%)
0.5	0.52	+4.0
5	4.9	−2.0
20	20.1	+0.5
50	49.7	−0.6
100	101	+1.0

Result:
r = 0.9995, slope = 1.002, intercept = −0.05 → **Acceptable** □

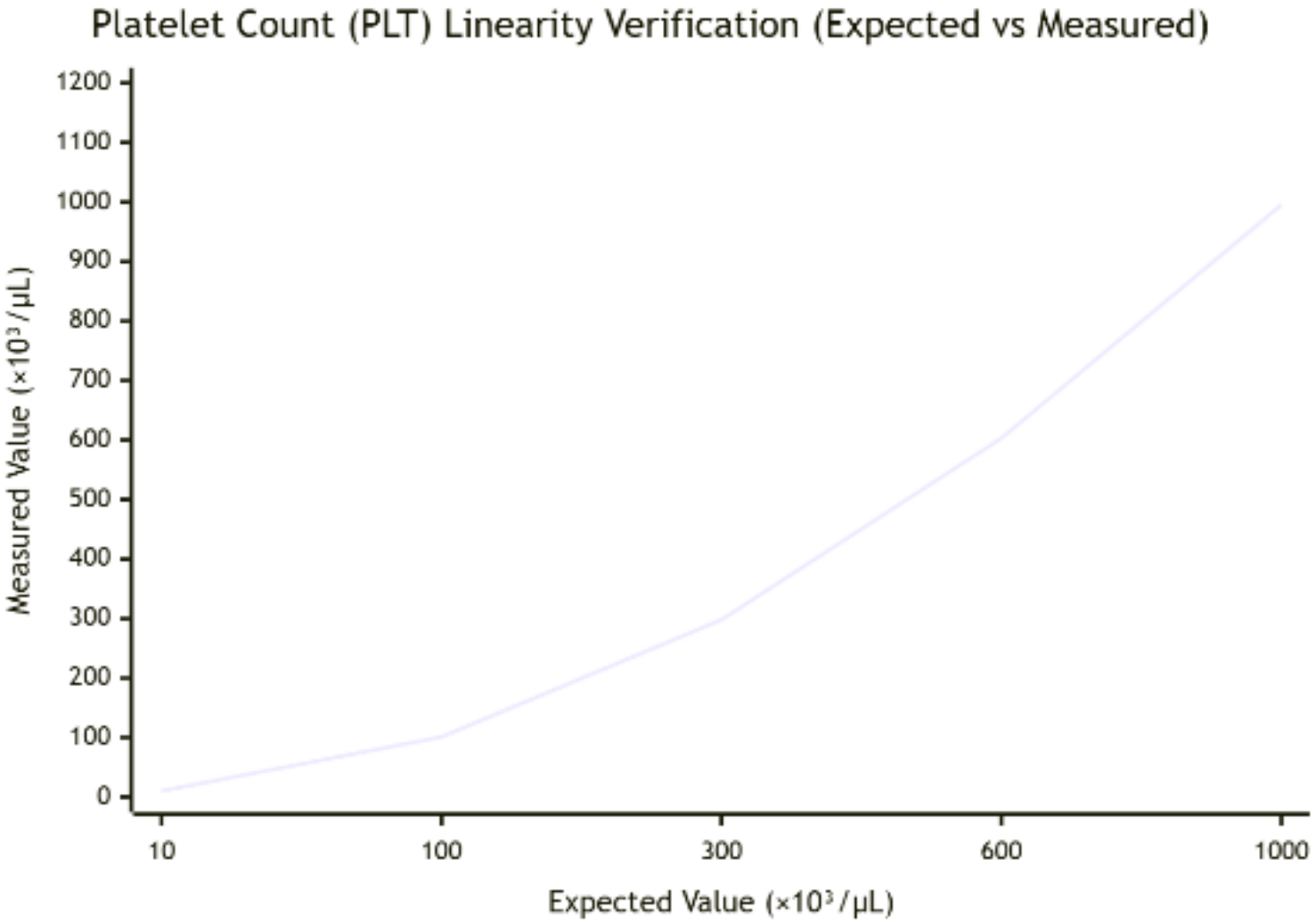

WBC Count Linearity Verification (Expected vs Measured)
Measured Value (×10³/μL)
120
110
100
90
80
70
60
50
40
30
20
10
0
0.5
5
20
50
100
Expected Value (×10³/μL)

Platelet Count (PLT)

Claimed range: 10 – 1000 $\times 10^3/\mu L$

Expected ($\times 10^3/\mu L$)	Measured	Deviation (%)
10	10.2	+2.0
100	101	+1.0
300	298	−0.7
600	603	+0.5
1000	995	−0.5

Result:
r = 0.999, slope = 1.001, intercept = −1.2 → **Linearity confirmed** □


Platelet Count (PLT) Linearity Verification (Expected vs Measured)
Measured Value (×10³/μL)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10
100
300
600
1000
Expected Value (×10³/μL)

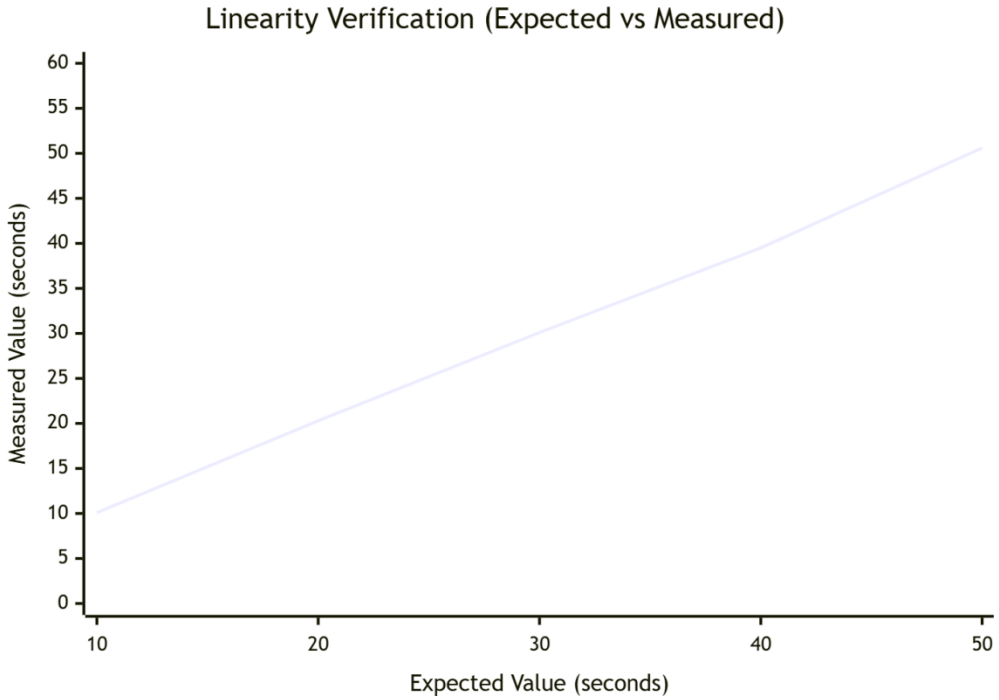
PT and aPTT

Claimed range: PT: 10 – 50 seconds, aPTT: 25 – 120 seconds

Procedure:
Prepare dilutions of abnormal plasma using normal plasma.

Expected (sec)	Measured	Deviation (%)
10	10.1	+1.0
20	20.3	+1.5
30	30.1	+0.3
40	39.5	−1.3
50	50.6	+1.2

Result:
r = 0.998, slope = 1.01, intercept = 0.2 → **Linearity acceptable**


Linearity Verification (Expected vs Measured)
Measured Value (seconds)
60
55
50
45
40
35
30
25
20
15
10
5
0
10
20
30
40
50
Expected Value (seconds)

ESR

Claimed range: 2 – 120 mm/h

Procedure:

Use patient samples of different ESR levels or linearity standards from the manufacturer.

Expected (mm/h)	Measured	Deviation (%)
5	5.2	+4.0
30	29.8	−0.7
60	61.0	+1.7
90	89.5	−0.6
120	121	+0.8

Result:

r = 0.999, slope = 0.998, intercept = 0.4 → **Linearity verified** □

Flags assay

Reference Intervals

- Collect at least 20 samples from healthy individuals.
- At least 95% of the results should fall within the manufacturer's proposed reference interval.

Procedure (Based on CLSI EP28-A3c)

- Select ≥20 healthy volunteers (10 male, 10 female).
- Collect EDTA blood samples and test within 2 hours using the routine analyzer.
- Compare results with the manufacturer’s reference range.
- If ≤2 of 20 results are outside → interval verified; if ≥3 → new interval needed (≥120 individuals).

Example

Test	Manufacturer's Reference Interval	No. of Samples Tested	No. Within Range	No. Outside Range	Verification Result
Hemoglobin (Hb)	12.0–16.0 g/dL (F), 13.0–17.0 g/dL (M)	20	19	1	☐ Verified
WBC	4.0–10.0 ×10³/µL	20	18	2	☐ Verified
Platelets (PLT)	150–400 ×10³/µL	20	19	1	☐ Verified
RBC	4.2–5.4 ×10⁶/µL (F), 4.7–6.1 ×10⁶/µL (M)	20	18	2	☐ Verified
MCV	80–96 fL	20	20	0	☐ Verified
HCT	36–46% (F), 40–50% (M)	20	17	3	☐ Not verified

Verification vs Validation

Feature	Verification	Validation
Purpose	Confirm manufacturer claims	Prove clinical suitability
Timeline	Few days	Several weeks
Scope	Accuracy, precision, linearity	Comprehensive evaluation
Application	FDA-approved devices	Modified or home made methods