Tehran University of Medical Sciences

Department of Laboratory Hematology and Transfusion Medicine

IQC in Hematology

Presented By:

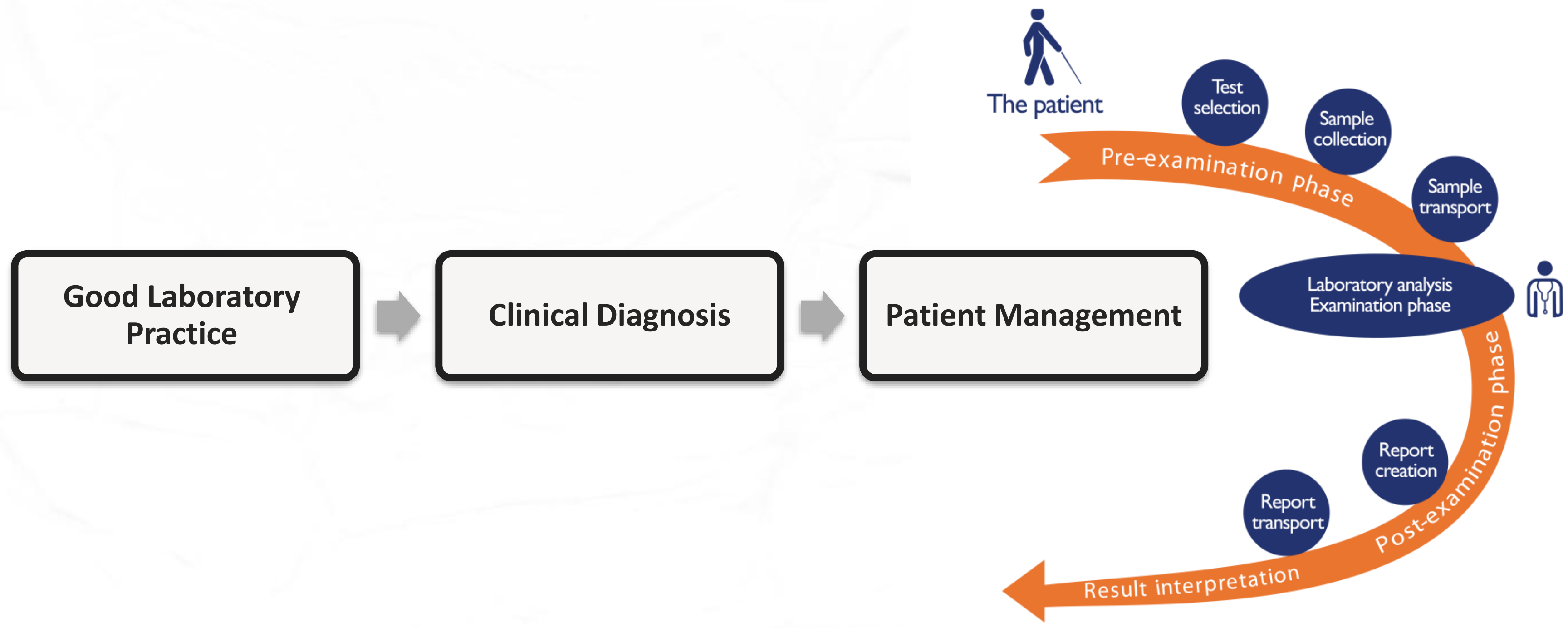
Dr Shaban Alizadeh

Professor of Laboratory Hematology and Transfusion Medicine

Adel Naimi

PhD Student in Laboratory Hematology and Transfusion Medicine

Linking Lab Results to Clinical Decisions



How to Categorize Quality Management?

Based on workflow:

- Pre-analytic
- Analytic
- Post-analytic

Based on level:

- Quality control
- Quality assurance
- Total quality management

QC vs QA

Quality Control	Quality Assurance
Reactive	Proactive
Identifies defects	Prevents defects
Product-based	Process-based
Takes place after production	Takes place during production

QA in Hematology Laboratory

Main Objective

- Ensuring diagnostic results with high accuracy and precision
- Results must be reliable and reproducible

Potential Sources of Errors

- Use of unreliable standards or reagents
- Improper calibration of equipment
- Incorrect execution of laboratory methods
- Errors may be systematic or random

Phases of QA Coverage

- Pre-analytical phase
- Analytical phase → EQA & IQC
- Post-analytical phase

Internal Quality Control (IQC)

Any Time

- Correlation system
- Correlation of PBS with blood counts
- Correlation of blood count changes with clinical events

Daily

- Testing specific control samples and plotting quality control charts (Levey-Jennings, Cusum, Youden)
- Performing replicate and Duplicate & Check testing on all or selected samples
- Delta check: comparing current results with previous patient results

Periodic

- Calibration of cell counters and hemoglobinometers
- Calibration of pipettes and automatic dispensers (initial + scheduled)
- Calibration of photometers and spectrophotometers (initial + scheduled, at least every 6 months)

Correlation system

True Leukocyte Count: True WBC Count = Total Count × 100 / (nRBC + 100)

WBC Count (40X): WBC Count = Count in 40X × 2000

Platelet Count (100X): Platelet Count = Count in 100X × 20,000

Red Blood Cell Indices:

- MCV → Microcytic / Macrocytic
- MCH → Hypochromic / Hyperchromic

Rule	Formula	Notes
1	**Hb × 3 = Hct ± 2**	**Check Hb vs Hct consistency**
2	**RBC × 3.3 = Hb ± 1.5**	**Verify RBC vs Hb**
3	**RBC × 9 = Hct ± 3**	**Verify RBC vs Hct**

Duplicate Tests on Patients' Specimens

Purpose

To check the precision of routine laboratory work by performing duplicate tests

Procedure

Test 10 consecutive specimens in duplicate under controlled conditions

Calculate differences between paired results and determine SD

Differences should not exceed ±2SD within the same batch

Strengths & Limitations

✅ Detects **random errors** effectively

❌ Does not detect **systematic errors**

❌ Insensitive to gradual drift (unless tested later on stable specimens)

❌ If method is poor, wide SD reduces effectiveness

Applicability

Suitable for manual and automated methods

Impractical for routine blood counts in busy labs

Alternative: test 3–4 specimens in duplicate per batch for rough consistency check

Example: Duplicate Test Analysis

First count (WBC)	Second count (WBC)	d	d^2
5.4	5.8	-0.4	0.16
8.3	10.5	-2.2	4.84
17.2	18	-0.8	0.64
5.4	5.4	0	0
12.2	11.8	0.4	0.16
			$\sum$=5.8

$$SD = \sqrt{\frac{\sum d^2}{2n}} = \sqrt{\frac{5.8}{10}} = 0.76$$

2SD = 1.5

d > 2SD → RE

Check Test

Definition

Similar to duplicate test, but **compares previous run results**

Applications

Detects problems between two runs (e.g., reagent failure, equipment malfunction, sample instability).

Ensures instrument stability over time.

Procedure

Select **4–5 specimens** from the previous run

Retest and compare with prior results

Calculate **differences (d) and SD**

Recommendation: Store specimens at **4 °C**

Check Test

Applicability

- ✅ Suitable for **Hb & RBC**
- ❌ Less Suitable for **WBC & Platelets**
- ❌ **Unsuitable for Hct (due to cell swelling and Hct shift)**

Evaluation Criteria

Results should agree within ±2SD.

If SD of check test > ±2SD of duplicate test (with proper storage), possible causes:

- Sample deterioration
- Malfunction of cell counter
- Aperture blockage
- Reagent instability

Delta test

Parameter	Acceptable Δ	Acceptable Δ (Notes)
Hb	±2.0	Changes beyond this require review
Hct	±5.0	Sensitive to plasma volume changes
RBC	>10%	Reduction >10% needs investigation
WBC	20-25%	Normal to abnormal or very abnormal
Plt	>50%	Increase or decrease >50% is a flag
MCV	±6.0	Changes >6 fL are unusual
MCH	±5.0	Changes >5 pg require review

Replicate Test

Definition

11 tests on a single specimen to evaluate **repeatability & precision**

Purpose

Assess **stability & consistency** of results

Compare **instrument/method performance**

Limitations

Same pipette/reagent → cannot detect **systematic or environmental errors**

Applications

Assess **control blood repeatability**

Calculate **CV & SD** for QC

Analysis Example (Hemoglobin CV)

Clinical Lab: 5-10 %

Routine labs: 2–3%

Specialized labs: 1.5%

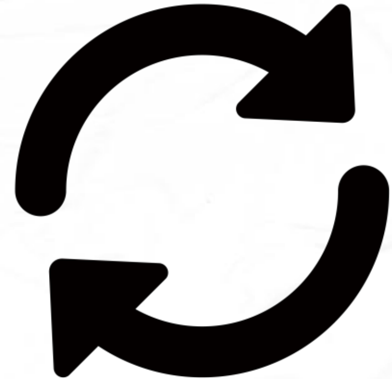
Research/global standards: <1%

Key Points

High CV → minimal clinical requirement only

Low CV/SD → essential for accurate interpretation

Proper **calibration & standard controls** required



Correlation of Test Results with Clinical Condition

Definition

- **Interpret results in clinical context**
- **Report only consistent findings**
- **Physician-lab collaboration is essential**

Applications

1. Detecting Unexpected Results

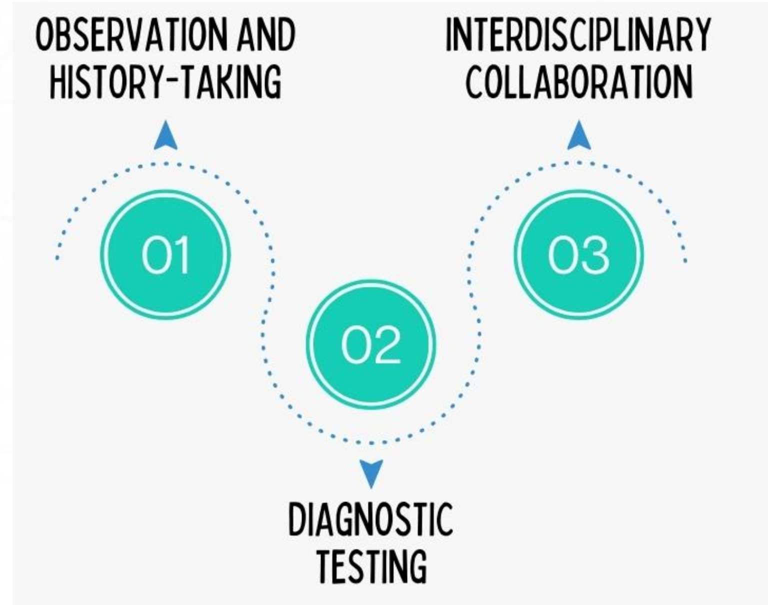
- **High Hematocrit & Hemoglobin** in a pale patient
- **Normal Bilirubin** in a patient with severe jaundice

2. Supporting Clinical Findings

- **Purpura** in a thrombocytopenic patient
- **Periumbilical pain** with neutrophilia
- **Prolonged PT** in a patient on Warfarin
- **Prolonged BT** in a patient on Aspirin
- **High Creatinine** in a patient on dialysis

Key Message

- Lab data must **make sense clinically**
- Ensures **reliable, meaningful reporting**



Correlation of Test Results with Other Laboratory Findings

Key Correlations

- **Hematology:** High MCV → macrocytosis, Low MCV → microcytosis
- **Cell counters** should align with **peripheral blood smear**
- Examples: Hct ≈ 3 × Hb, ESR with CRP, WBC with CRP

Applications / Group Evaluation

- Cost-free method using **related test panels**
- Blood bank: direct vs. indirect grouping
- Thyroid: High T4 suppresses TSH
- Muscle: CK with AST/LDH

Limitations

- Not suitable for **single tests** (e.g., CBC alone)
- Not for **unrelated tests** (e.g., CBC vs. urinalysis)

Error Detection

- Identifies **non-analytical errors**: mislabeling, clots, storage issues

Cell Counter Performance Monitoring Using Hematology Control

Evaluate results for each control level: Low, Medium, High (with expected values from the company brochure)

Use Levy-Jennings chart to monitor:

- Dispersion, shifts, and trends.
- Apply multi-rule assessment for accuracy

Use CUSUM chart or cumulative frequency plots.

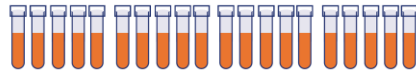
Use Youden plot for cross-laboratory comparison.

Compare results with other labs using the same control sample (external QA).

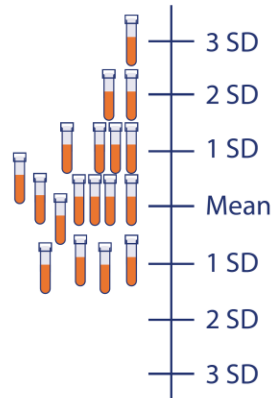
Use of Quality Control Charts

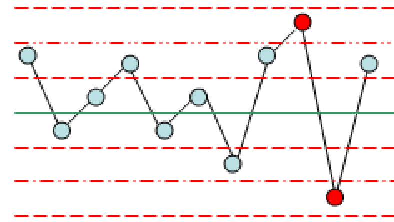
Obtain control material

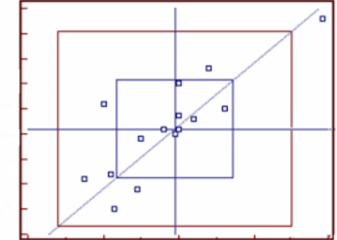
Run each control
20 times over 30 days

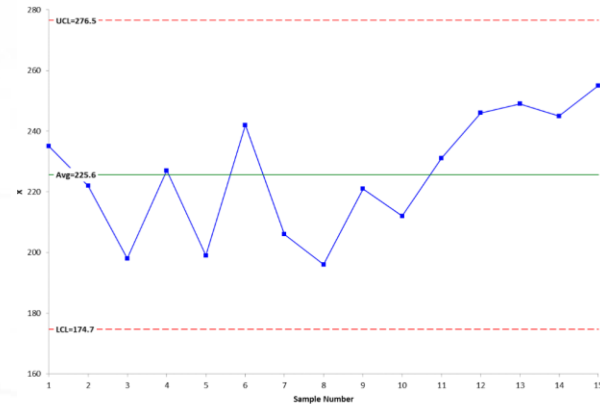


Calculate mean and
± 1, 2, 3 standard deviations

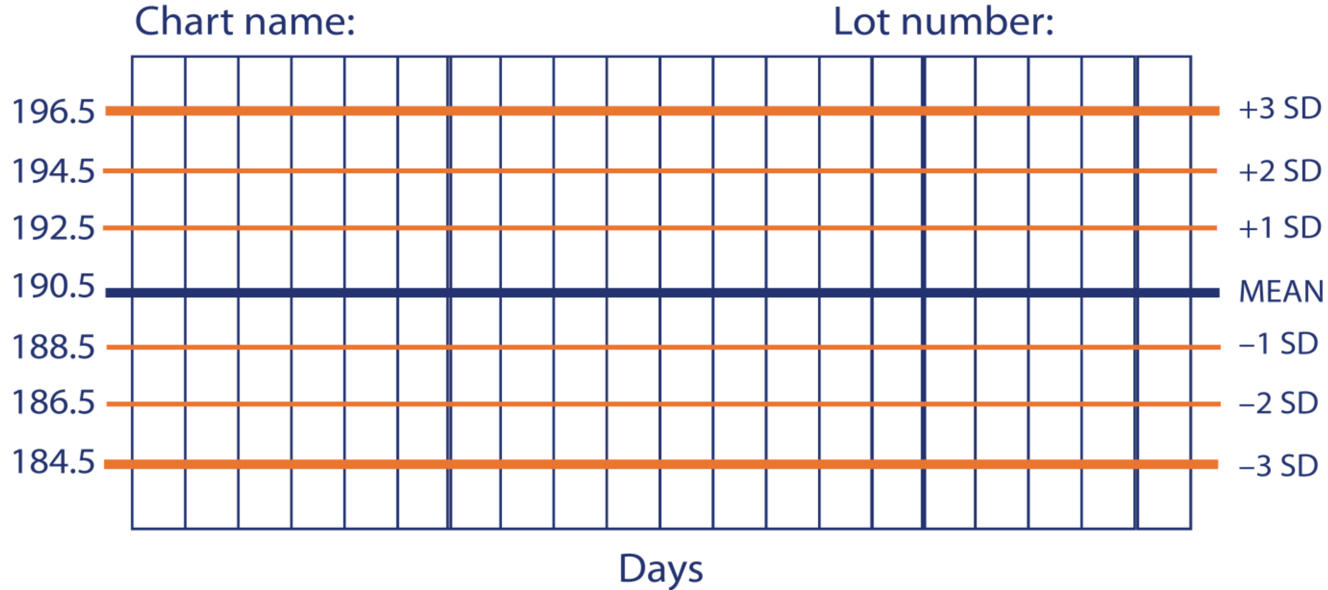




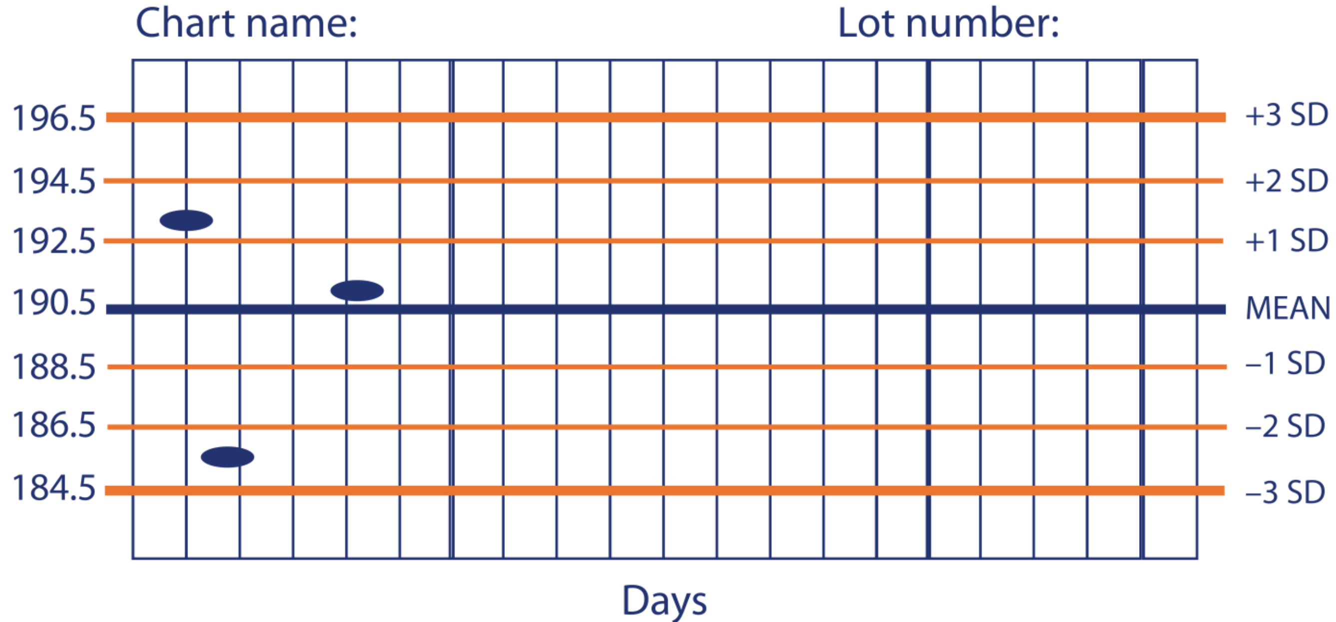




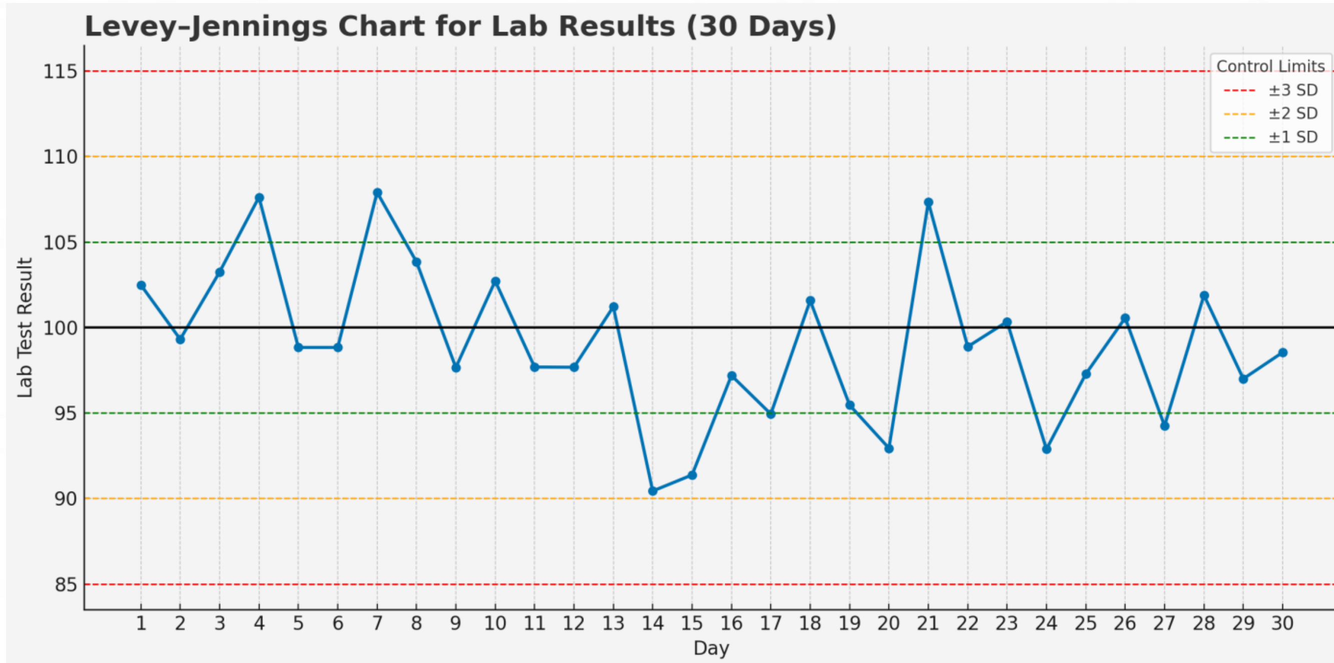
Use of Quality Control Charts



Use of Quality Control Charts



Quality Control Using Westgard Multi-Rules



Use of Quality Control Charts

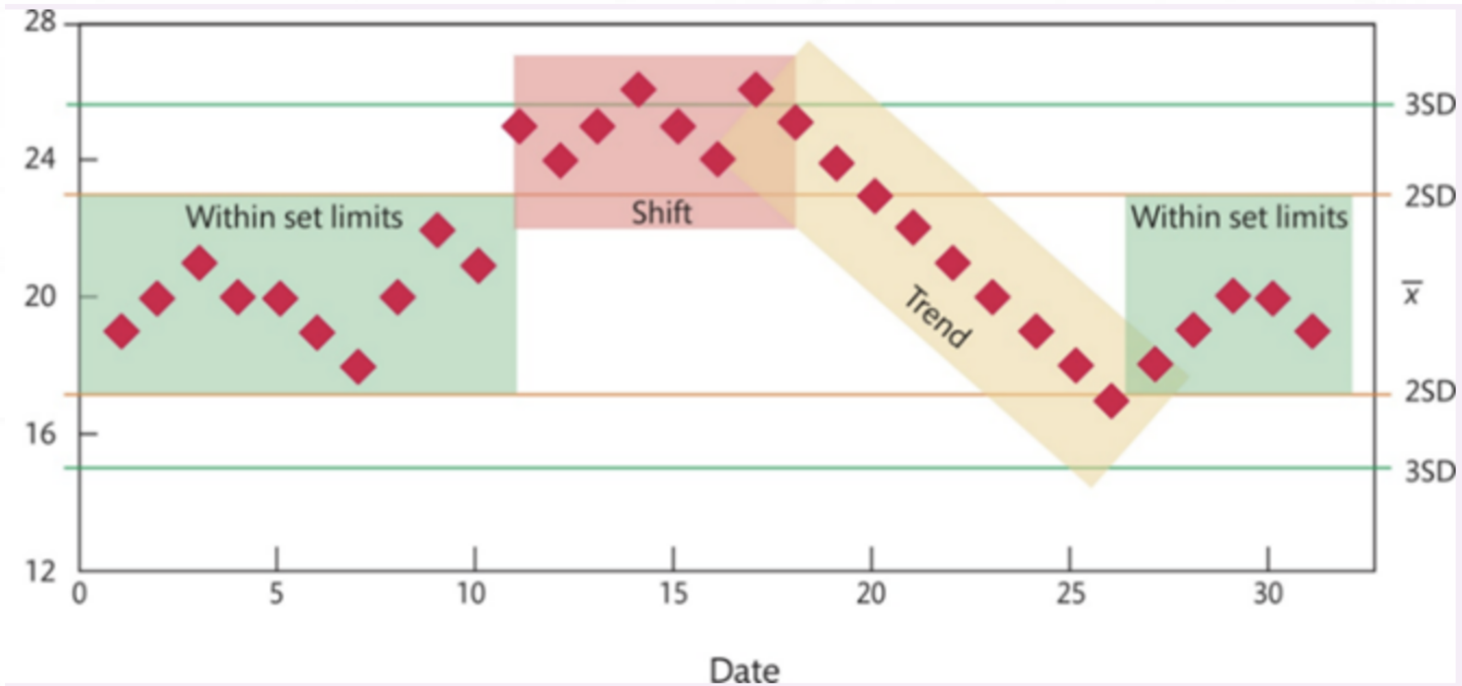
	X	X-X	(X-X)^2
	12.44	0.14	0.02
	12.5	0.2	0.04
	12.2	-0.1	0.01
	11.7	-0.6	0.36
	12	-0.3	0.09
	12.6	0.3	0.09
	12.6	0.3	0.09
	12.7	0.4	0.16
	12.3	0	0
	12	-0.3	0.09
Sum=	123		0.95
Mean=	12.3		

$$SD = \sqrt{\frac{\sum(X-X)^2}{n-1}}$$

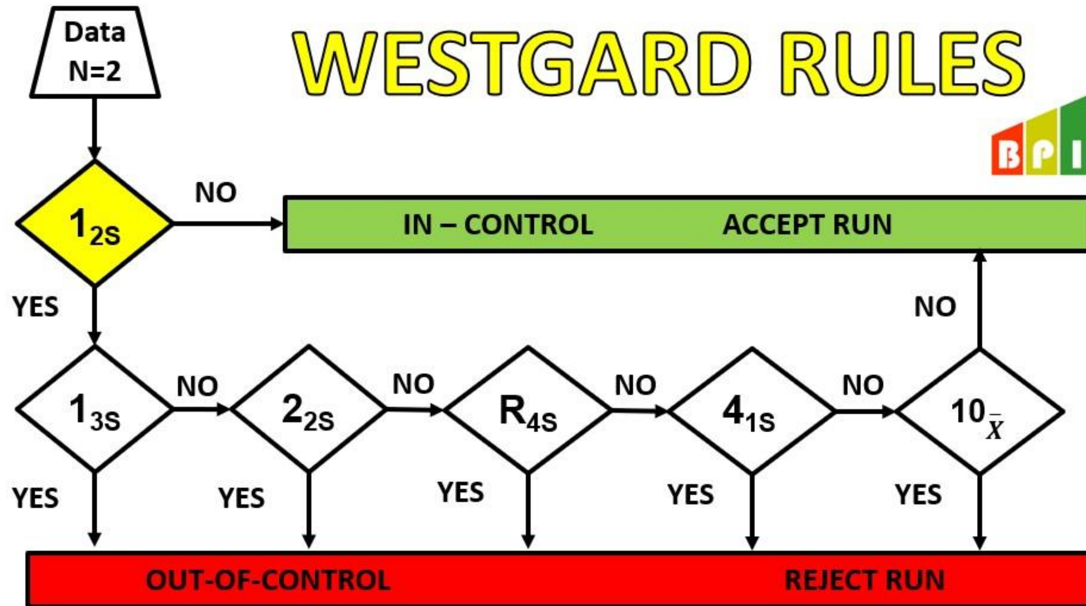
$$SD = \sqrt{\frac{0.95}{9}} = \sqrt{0.106} = 0.33$$

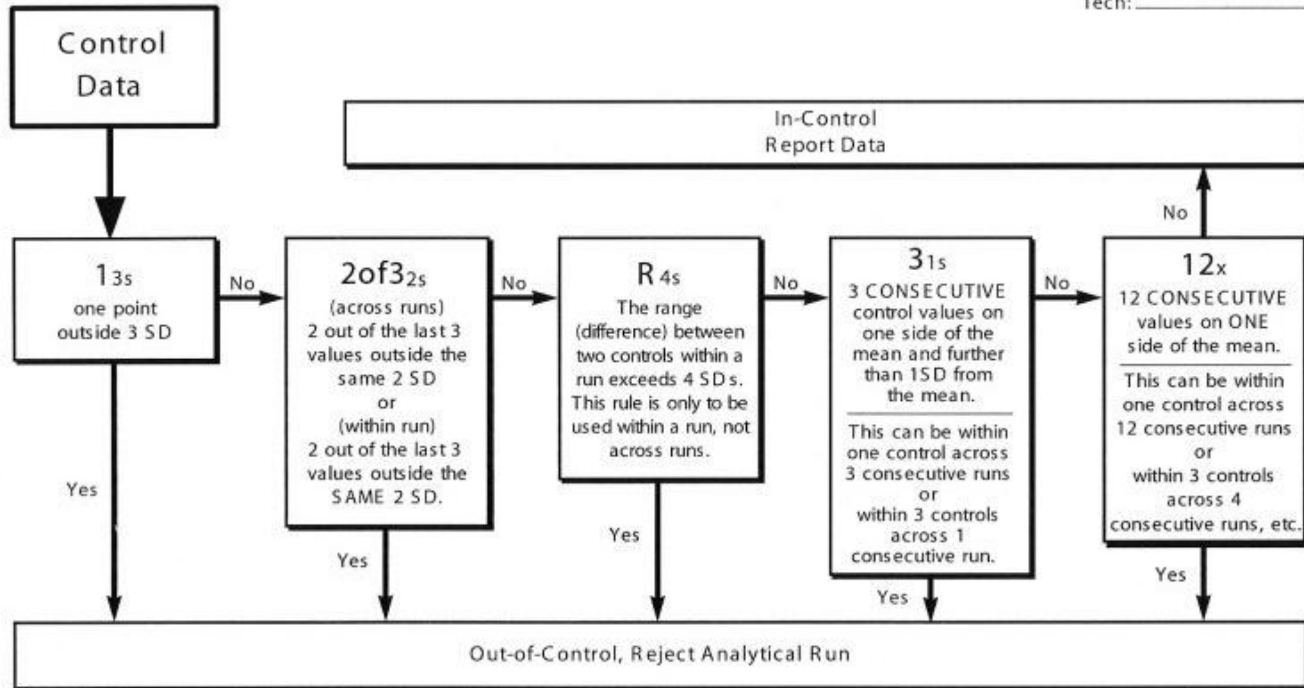
2SD=0.66

Evaluation of Levey-Jennings Chart for Dispersion, Shift, and Trend Patterns

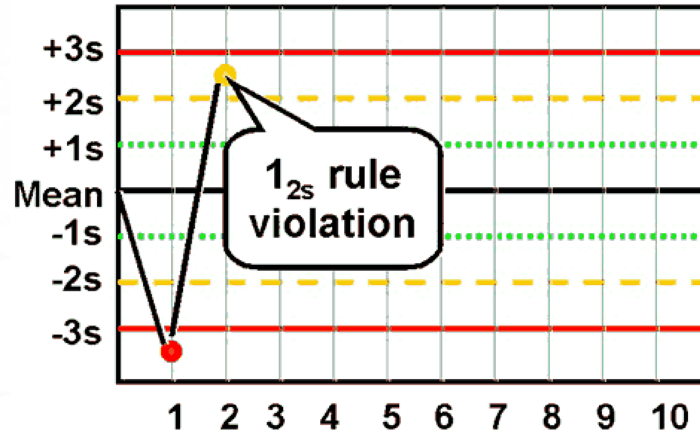


Quality Control Using Westgard Multi-Rules

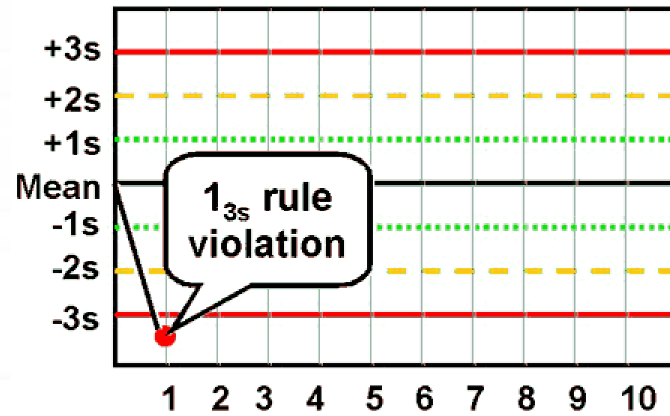



Tech: ____________
Control Data
In-Control Report Data
1 3s
one point outside 3 SD
No
2of3 2s
(across runs)
2 out of the last 3 values outside the same 2 SD
or
(within run)
2 out of the last 3 values outside the SAME 2 SD.
No
R 4s
The range (difference) between two controls within a run exceeds 4 SDs. This rule is only to be used within a run, not across runs.
No
3 1s
3 CONSECUTIVE control values on one side of the mean and further than 1SD from the mean.
This can be within one control across 3 consecutive runs
or
within 3 controls across 1 consecutive run.
No
12x
12 CONSECUTIVE values on ONE side of the mean.
This can be within one control across 12 consecutive runs
or
within 3 controls across 4 consecutive runs, etc.
No
Yes
Yes
Yes
Yes
Yes
Out-of-Control, Reject Analytical Run

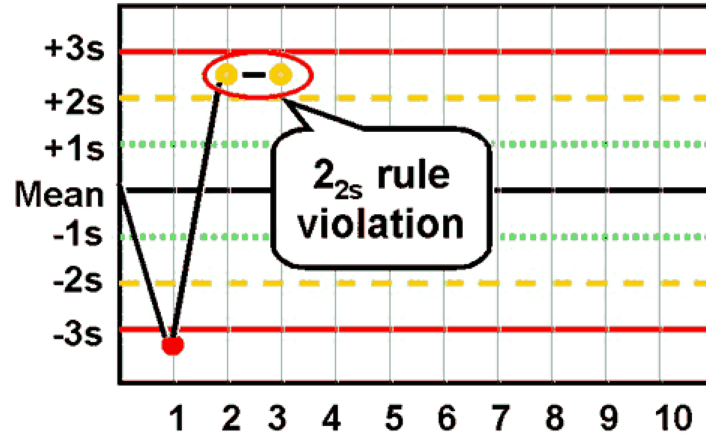
1_{2s}



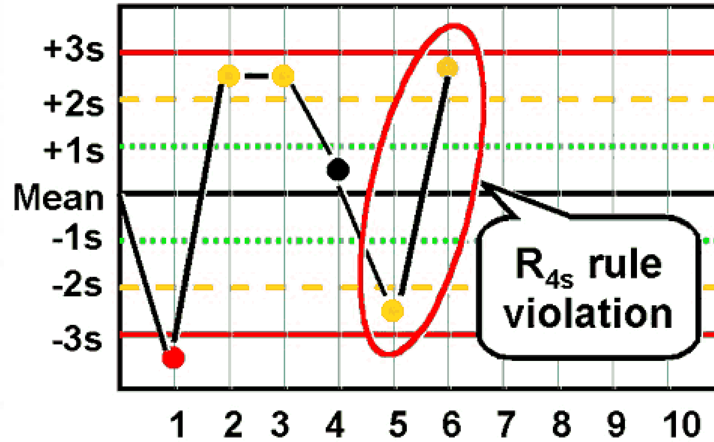
1_{3s}



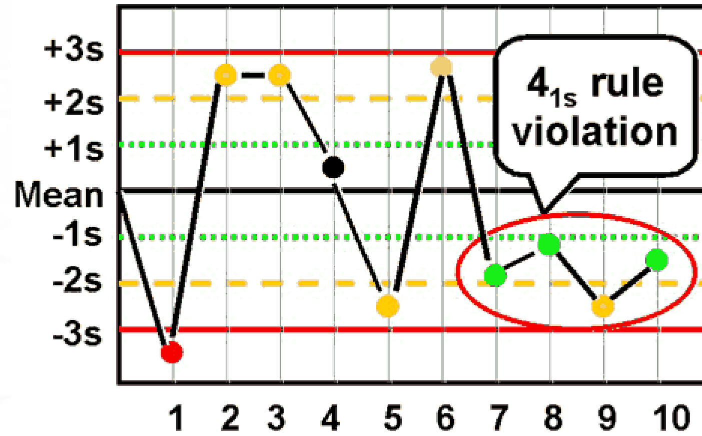
2_{2s}



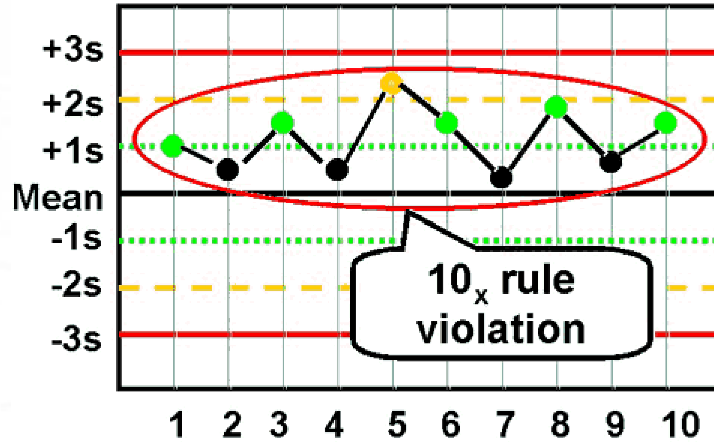
R_{4s}



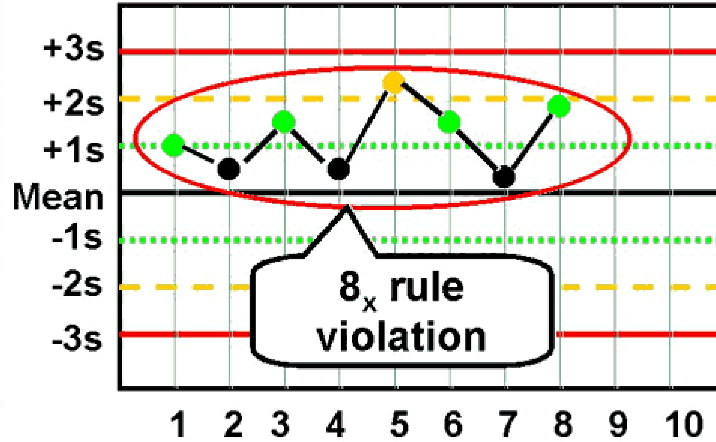
4_{1s}



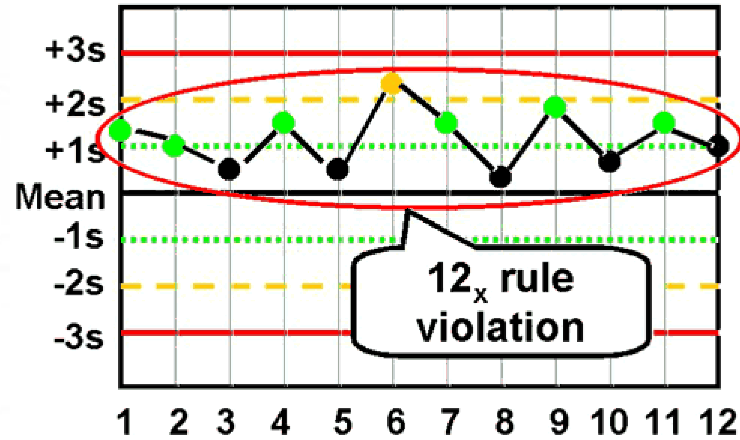
10_x



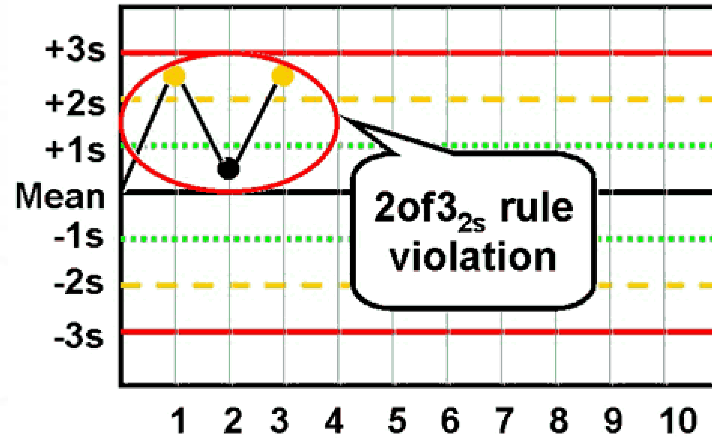
8_x



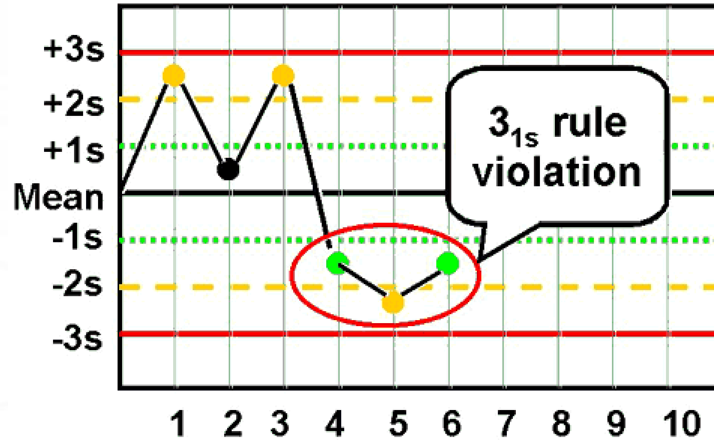
1_{2x}



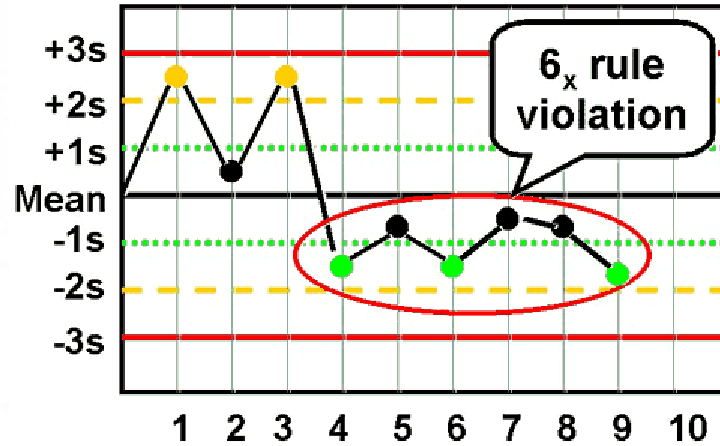
$2of3_{2s}$



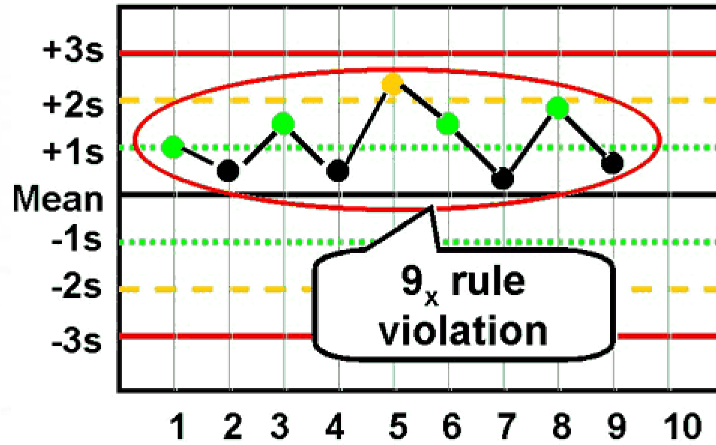
3_{1s}



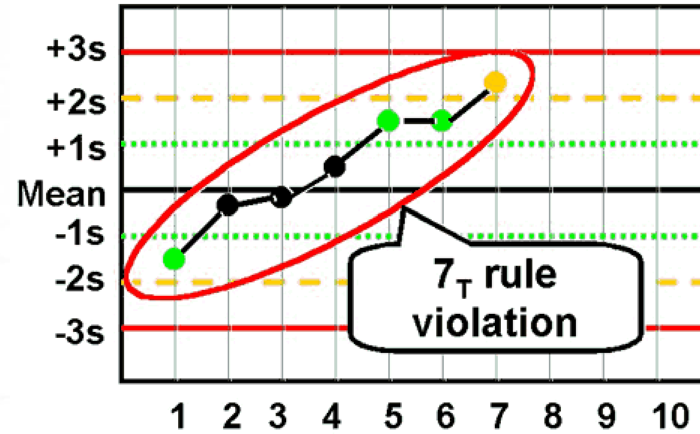
6_x



9_x



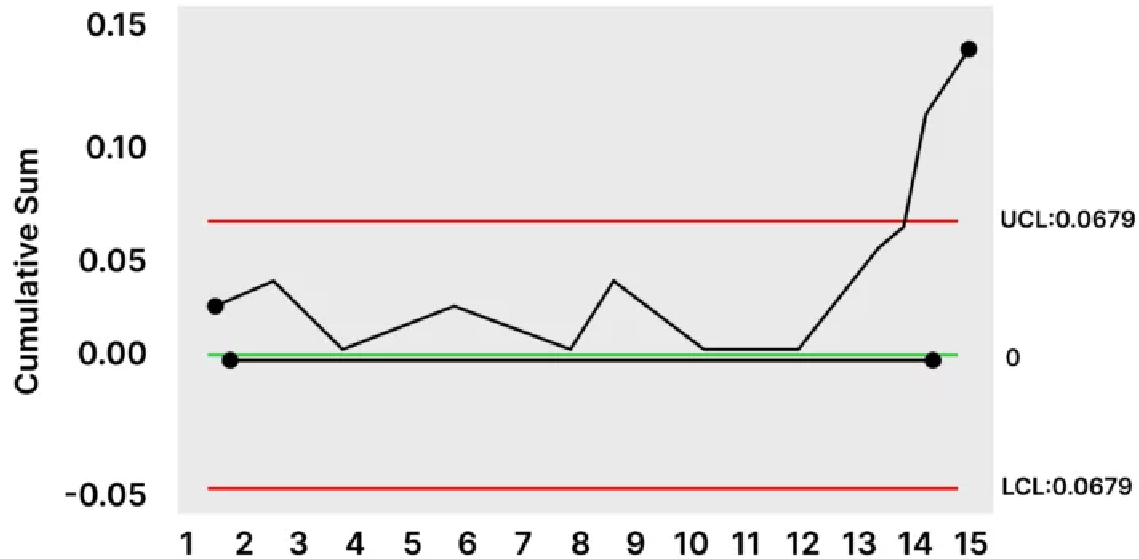
7_T



Westgard QC Rules at a Glance

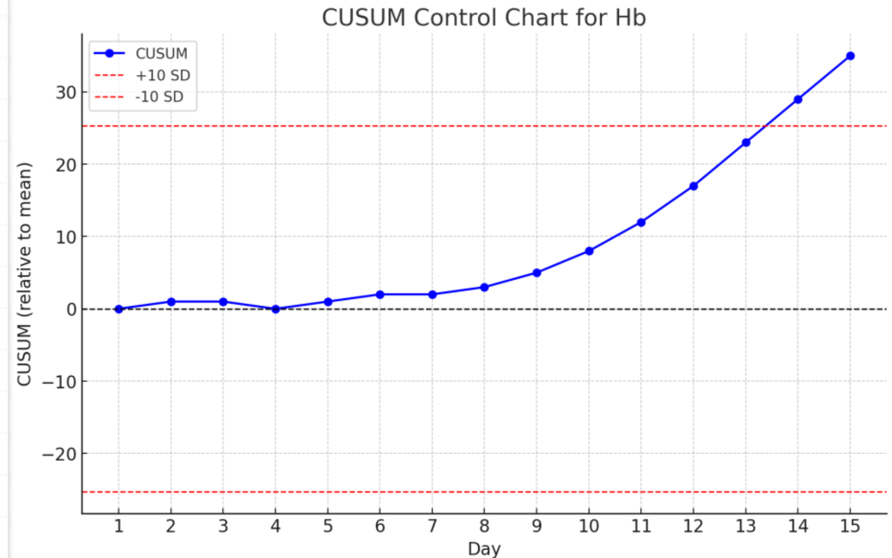
12S	±3 SD > One control > ±2 SD	⚠️ Warning only	Accept if isolated; investigate if repeated
13S	One control > ±3 SD	❌ Random/Systematic error	Reject run, troubleshoot
22S	Two consecutive > ±2 SD (same side)	❌ Systematic error	Reject run, recalibrate
R4S	Difference ≥ 4 SD between two results	❌ Random error	Reject run, repeat test
41S	Four consecutive > ±1 SD (same side)	❌ Systematic shift	Reject run, investigate bias
8X / 10X / 12X	8–12 consecutive results on same side of mean	❌ Systematic error	Reject run, check calibration
2 of 3 (2S)	2 of 3 consecutive > ±2 SD (same side)	❌ Systematic error	Reject run, troubleshoot
3 1S	3 consecutive > ±1 SD (same side)	❌ Systematic error	Reject run
6X / 9X	6 or 9 consecutive results on same side of mean	❌ Systematic drift/shift	Reject run
7T (Trend)	7 consecutive ↑ or ↓ values	❌ Trend (drift)	Reject run, check instrument

CUSUM Chart in Cell Counter Performance Evaluation

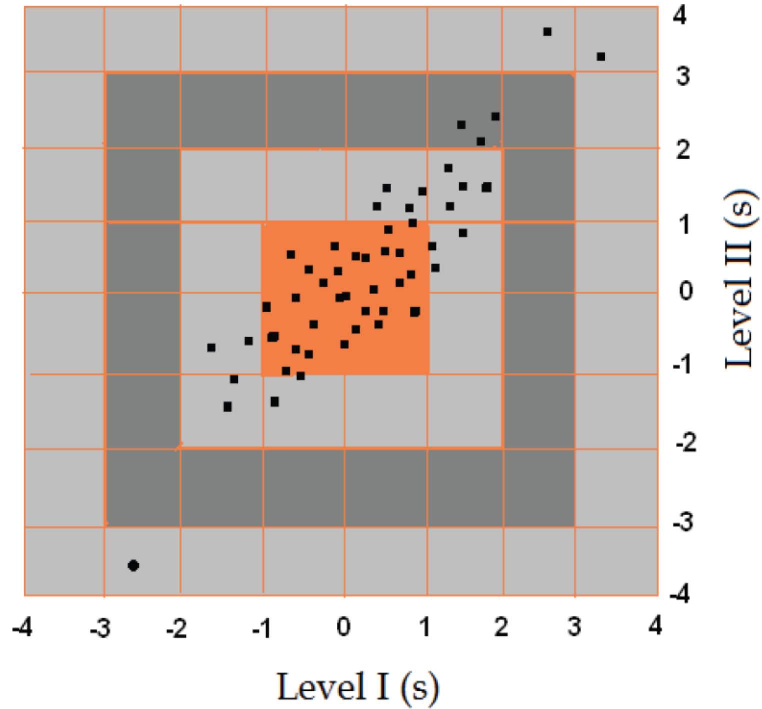


CUSUM Data Example for Hemoglobin QC

Day	Hb g/L	Hb – 144	Difference from Target	CUSUM
1	144	144–144=0	0	0
2	145	145–144=1	+1	0+1=+1
3	144	144–144=0	0	+1+0=+1
4	143	143–144=–1	–1	+1–1=0
5	145	145–144=1	+1	0+1=+1
6	145	145–144=1	+1	+1+1=+2
7	144	144–144=0	0	+2+0=+2
8	145	145–144=1	+1	+2+1=+3
9	146	146–144=2	+2	+3+2=+5
10	147	147-144=	+3	+5+3=+8
11	148	148-144=4	+4	+9+4=+12
12	149	149-144=5	+5	+12+5=+17
13	150	150-144=6	+6	+17+6=+23
14	150	150-144=6	+6	+23+6=+29
15	150	150-144=6	+6	+29+6=+35



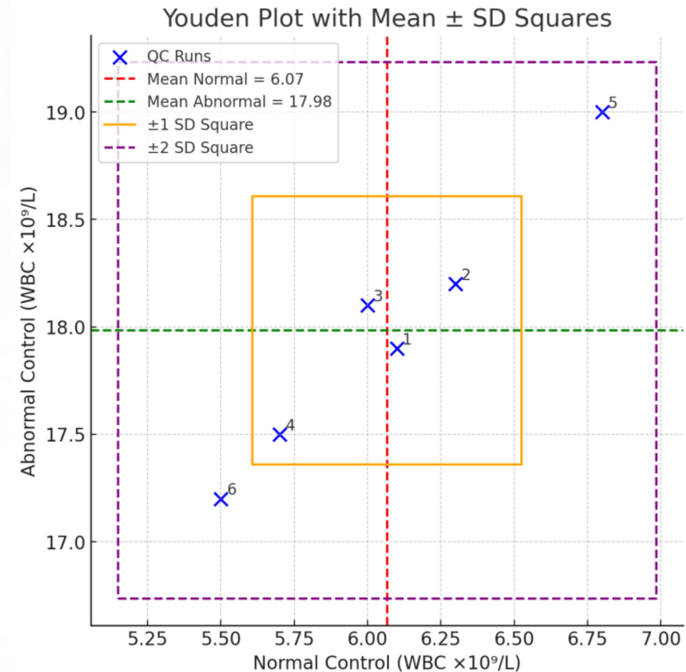
Youden (XY) Plot in Cell Counter Performance Evaluation



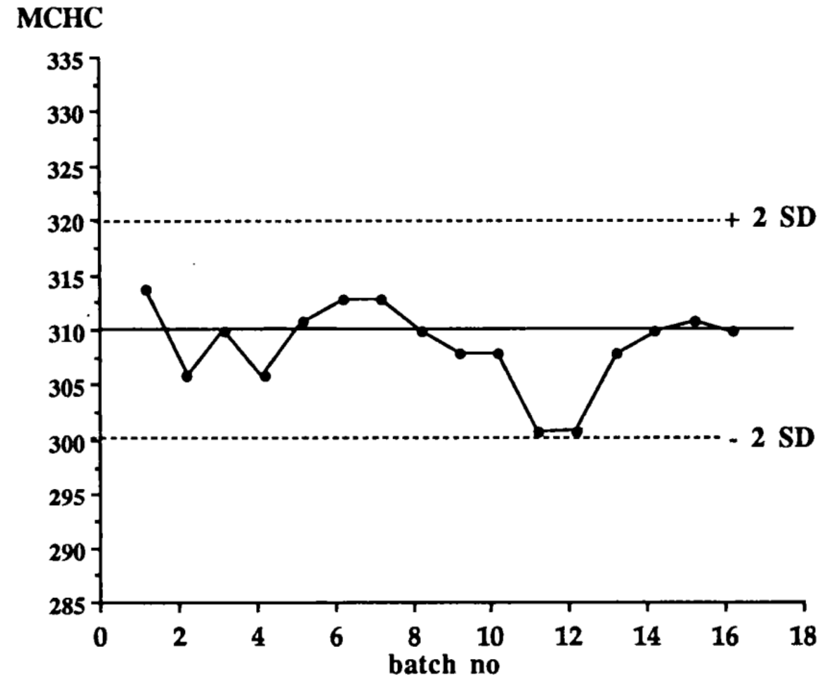
Example of Youden Plot in Hematology QC

	Normal Control	Abnormal Control
Mean	6.1	18.0
SD	0.5	0.6
2SD	0.9	1.2

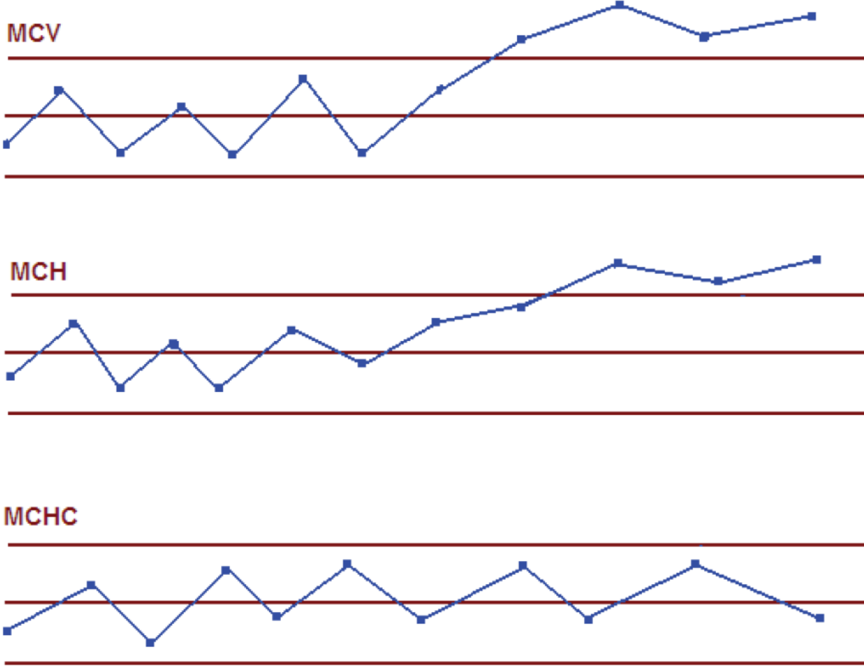
Run	Normal Control	Abnormal Control
1	6.1	17.9
2	6.3	18.2
3	6.0	18.1
4	5.7	17.5
5	6.8	19.0
6	5.5	17.2



Use of MCHC in Quality Control

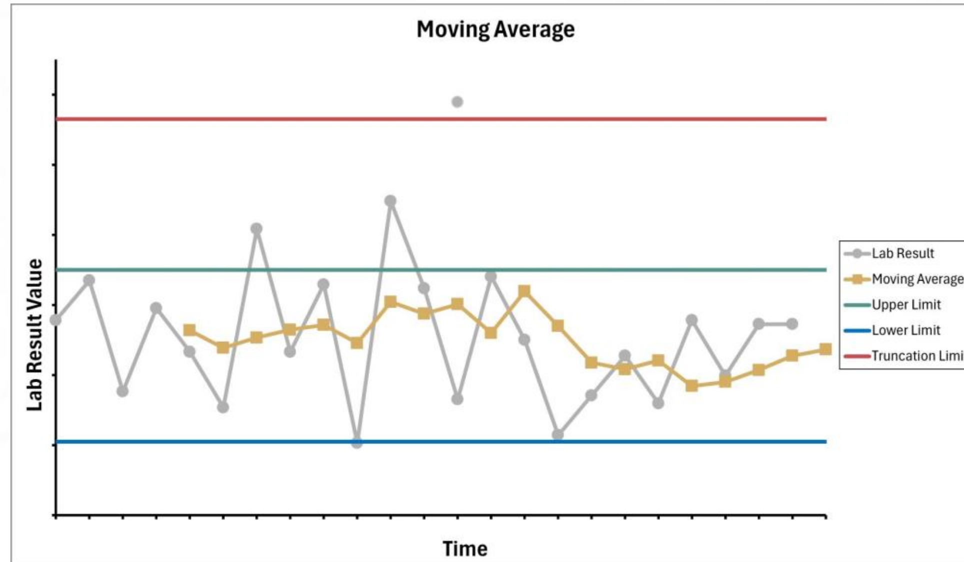


Monitoring Hematology Analyzers with the Moving Average Test



Moving Average = complementary QC tool

a real-time, patient-based quality control method



$$\%Deviation = \frac{MCHC_{observed} - MCHC_{baseline}}{MCHC_{baseline}} \times 100$$

Accuracy Evaluation of Hematology Analyzers

Limit of Previous QC: Focused on precision, not accuracy

Control vs. Calibrator:

- Calibrator → Narrow SD, true accuracy
- Control → Wide SD, less reliable

When to (Re)Calibrate:

- Visible errors in results
- Systematic trends in QC charts
- Sudden shifts indicating bias change

Trueness Evaluation Methods

- Against Calibrator: run calibrator → compare measured vs brochure value → report bias

  Between Two Instruments: run 10 concurrent patient samples; comparator must be calibrated & reference-verified

  Statistical: t-test (means), F-ratio (variances), Chi-squared (deviation/homogeneity)

Comparing Means with t-test

Definition & Aim

- Compare means ± SD of two datasets
- No overlap → significant difference → not calibrated

Probability Ranges

- ±1 SD → 68%
- ±2 SD → 95% (QC hematology)
- ±3 SD → 99.7%

Accurate Method

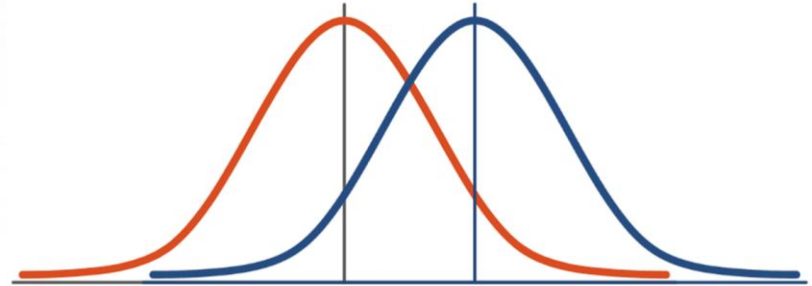
- Use Standard Error of Difference (SE diff)
- Significant if $|Mean_1 - Mean_2| > SE\ diff$

Formula

$SE\ diff = \sqrt{(SD_1^2 / n_1) + (SD_2^2 / n_2)}$

Applications

- New method vs. reference method
- Cell counter calibration (2 days)
- Compare two counters



How to Perform a t-test (Difference Between Means)

Sample	Cell Counter I	Cell Counter II
1	12.0	11.5
2	12.5	12.0
3	13.0	12.0
4	13.0	11.8
5	11.5	11.0
6	12.0	11.4
7	12.8	12.0
8	11.6	10.6
9	10.0	11.5
10	12.0	11.0

$$SE_{diff} = \sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}} \qquad t = \frac{Mean_1 - Mean_2}{SE_{diff}}$$

If $t_{calculated} > t_{critical}$ → the difference is significant.

If $t_{calculated} \leq t_{critical}$ → the difference is not significant.

$$Mean_1 = 12.04, \quad Mean_2 = 11.48 \qquad \Delta Mean = 0.56$$

$$SE_{diff} = \sqrt{\frac{0.809}{10} + \frac{0.240}{10}} = \sqrt{0.081 + 0.024} = \sqrt{0.105} = 0.324$$

$$t = \frac{0.56}{0.324} = 1.728$$

$$t_{critical} = 2.262$$

$$1.728 < 2.262 \Rightarrow NotSignificant$$

Evaluating Cell Counter Calibration with F-Test

Core Idea:

Use a calibrated cell counter to assess others via variance comparison (F-test for precision).

Steps:

- Calculate F-ratio.
- Compare with critical F-value (95% confidence, P=0.05).
- Degrees of freedom: (n_1-1, n_2-1).

Results:

- F < Critical: Analyzer calibrated.
- F > Critical: Recalibration needed.

Note:

- F-test: Precision (random error).
- t-test: Accuracy (mean differences).

$$\textbf{F test} = \frac{\textbf{Larger Sample Variance}}{\textbf{Smaller Sample Variance}}$$

How to Perform a F-test

Measurement	Group A	Group B
1	12.0	11.5
2	12.3	12.8
3	11.8	10.9
4	12.2	13.2
5	12.1	11.0
6	11.9	12.5
7	12.4	13.1
8	12.2	11.2
9	11.7	12.9
10	12.0	10.8
Mean	12.06	11.89
Variance	0.30	1.01

$$\text{F test} = \frac{\text{Larger Sample Variance}}{\text{Smaller Sample Variance}}$$

$$F = \frac{1.01}{0.30} = 3.37$$

$$F_{\text{critical}} = 3.18$$

If $F > F_{\text{critical}}$ → Significant difference in variances

If $F \leq F_{\text{critical}}$ → No significant difference

$$3.37 > 3.18 \quad \Rightarrow \quad \text{Significant difference} \rightarrow \text{Recalibrate analyzer}$$

Validating Hematology Analyzer Accuracy with Chi-Square Test

Objective:

- Compare observed (O) cell counter results with expected (E) values to assess calibration needs.

Methodology:

- Calculate O - E for each sample.
- Compute $\chi^2 = \Sigma [(O - E)^2 / E]$.
- Compare χ^2 with critical value (P=0.05, df):
  - $\chi^2 \geq$ Critical: Recalibrate.
  - $\chi^2 <$ Critical: No calibration needed.

$$x_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Observed Value *(aka from your sample)*

Expected Value *(aka what you already know or thought to be true before your study)*

Square the result to get positive values only

Chi-squared symbol

Sigma *(sum/add everything up)*

Expected Value

Evaluating Cell Counter Calibration with Chi-Square Test

Sample	Observed Value (x)	Expected Value (μ)	$(x - \mu)^2$	$(x - \mu)^2 / \mu$
1	10.2	10.0	0.04	0.004
2	9.8	10.0	0.04	0.004
3	10.5	10.0	0.25	0.025
4	9.7	10.0	0.09	0.009
5	10.1	10.0	0.01	0.001
6	10.3	10.0	0.09	0.009

$$\chi^2 = \sum \frac{(x_i - \mu_i)^2}{\mu_i}$$

$$\chi^2 = 0.004 + 0.004 + 0.025 + 0.009 + 0.001 + 0.009 = 0.052$$

$$\chi^2 = 0.052$$

For $\alpha = 0.05$: The critical value is 11.07

Verifying Cell Counter Stability with t Britin Method

Objective:

Check stability of 7 CBC parameters in EDTA blood over 2 days.

MCH ،MCV ،HCT ،HGB ،WBC ،RBC ،PLT

Methodology:

- Test 5-10 normal samples (day 1).
- Refrigerate 24h, retest (day 2).
- Calculate t-statistic (differences, mean, SD, t-test).
- Compare t with critical value (2.776, P=0.05, df=4):
  - t > 2.776: Recalibrate.
  - t < 2.776: Calibration valid.

Example (HGB):

- 5 samples: t = 2.67 < 2.776 → Calibration valid.

Application:

Ensures reliable CBC results for routine testing.

Calibration Methods of Hematology Analyzers

Calibration Procedure

Evaluate **accuracy** and **repeatability** of the instrument.
Perform calibration using **fresh whole blood**.

Need for Calibration

Initial setup of the device
Repair or replacement of measurement-related components
Use of new reagents with a different lot number
Observation of shifts or deviations in control charts
Recommendation by manufacturer or technical support
Poor performance in external quality control results

Accurate and precise

Precise but biased

Imprecise

Evaluation of Analyzer Precision (Repeatability)

Three fresh EDTA blood samples are selected.

Each sample is analyzed **three** times by the device.

For each parameter of interest, **SD** and **CV** are calculated using standard formulas.

Acceptance criteria:

- If CV values fall within the acceptable range → **Repeatability is confirmed.**
- If CV values exceed the defined limits → **Repeatability issues must be addressed prior to calibration.**

Parameter	Acceptable CV Range
RBC	< 2%
MCV	< 2%
HCT	< 3%
Hb	< 2%
WBC	< 3%
PLT	< 5%

Corrective actions may include:

- Repeated washing of the analyzer
- Technical inspection of the device
- Additional troubleshooting as needed

Calibration Using Commercial Calibrators

Commercial cellular calibrators are available for specific analyzer models

- *Examples:* Coulter, Sysmex, CDS CAL, Para Tech, etc.

Target values are assigned using reference methods and pre-calibrated analyzers

Effective only if **manufacturer's instructions** are strictly followed

Compared to calibration with fresh blood and reference methods, commercial calibrators are:

- Easier and faster to use
- Likely to provide better results in most laboratories

Alternative when commercial calibrators are not available:

- **CPD blood** (citrate-phosphate-dextrose) stabilized with mild **formalin** or **glutaraldehyde**
- Can be used as a **daily control** (stable for several months)
- Each parameter must first be measured by a **previously calibrated analyzer**

Comparison: Commercial vs. In-lab Calibration

Aspect	Commercial Calibrators	In-lab Prepared (CPD + Fixatives)
Source	Commercially produced (Coulter, Sysmex, CDS CAL, Para Tech, etc.)	CPD blood fixed with formalin or glutaraldehyde
Target Values	Pre-assigned using reference methods & calibrated analyzers	Must be measured by a pre-calibrated analyzer
Ease of Use	Easy, ready-to-use, faster	Requires preparation & handling in lab
Reliability	High, if manufacturer’s instructions are followed	Dependent on stability & preparation quality
Stability	Shelf-stable (as specified by manufacturer)	Stable for several months with fixatives
Suitability	Recommended for routine calibration in most laboratories	Alternative when commercial calibrators are unavailable

Calibration Using Fresh Whole Blood

Procedure

- Follow recommendations of **Brittain (1969)** and **Gilmer (1977)**
- Collect **10–20 fresh EDTA blood samples** within normal ranges (Hb ≈ 10 g/dL, HCT ≈ 35%)
- Measure each parameter **three times** using **reference methods**
- Calculate **mean values** for calibration

Reference Methods

- **Hemoglobin (Hb):** HiCN method using certified standard & photometer
- **Hematocrit (HCT):** Microhematocrit centrifugation
  - Plasma trapping correction (1.5–3%) optional; ICSH does not require it
- **RBC Count:** Dilution with isotonic solution, counted by single-channel analyzers
- **WBC Count:** Dilution with lysing reagent, then count
- **Erythrocyte Indices (MCV, MCH, MCHC):** Calculated from Hb, RBC, and HCT

Calibration Approaches

No.	Reference Method	Analyzer Result	CF (%)
1	47.0	46.0	2.13
2	50.0	49.0	2
3	36.6	33.0	9.84
4	41.6	39.0	6.25
5	32.3	33.3	-3.1
6	38.6	36.3	5.96
7	45.3	42.3	6.62
8	53.0	49.6	6.42
9	36.0	33.6	6.67

$$\text{CF (\%)} = \frac{\text{Reference Mean} - \text{Analyzer Mean}}{\text{Analyzer Mean}} \times 100$$

Mean of CF = +4.75%

Interpretation of CF:

- **Positive CF** → Analyzer underestimates results compared to the reference.
- **Negative CF** → Analyzer overestimates results.
- **CF = 0** → No calibration required.

Calibration Approaches

No.	Reference Mean	Analyzer Mean	CF (%)
1	47.0	46.0	2.13
2	50.0	49.0	2
3	36.6	33.0	9.84
4	41.6	39.0	6.25
5	32.3	33.3	-3.1
6	38.6	36.3	5.96
7	45.3	42.3	6.62
8	53.0	49.6	6.42
9	36.0	33.6	6.67
10	45.6	41.6	+8.77

$$C = B \times \left(1 - \frac{A}{100}\right)$$

A = mean CF (excluding extremes)
B = previous calibration factor
C = new calibration factor

Highest CF = **+9.84%**
Lowest CF = **–3.1%**
Remaining 8 CFs average (**A**) = **+5.6%**
Previous calibration factor (**B**) = **95%**
New calibration factor (**C**) = **89.68%**

Formula for New Calibration Factor

$$\text{New CF} = \text{Previous CF} \times \frac{\text{Reference Mean}}{\text{Analyzer Mean}}$$

Example (Hemoglobin Calibration)

Reference Mean = **15.6 g/dL**
Analyzer Mean = **15.5 g/dL**
Previous Calibration Factor = **100**

New Factor = 100 × (15.6 / 15.5) ≈ 100.65

Calibration Methods in Hematology Analyzers

Manual Calibration	Automatic Calibration
• Run calibrator ≥5 times and calculate the mean for each parameter. • Select Manual Calibration in the menu. • Previous factors are displayed; calculate and enter new factors for parameters outside the acceptable range (typically 75–125). • Formula: • New Factor = Previous Factor × (Reference Mean / Analyzer Mean) • Example: • WBC: Reference = 8.4, Analyzer Mean = 8.12, Previous Factor = 98.9 • New Factor ≈ 102.3	• Select Auto Calibration from the menu. • Enter calibrator reference values (date, serial, parameters). • Run calibrator ≥5 times (depending on analyzer). • Analyzer calculates and applies the new factor automatically. • After calibration, a shift in control sample results may occur (normal effect of calibration).

Causes of Unacceptable Results

Evaluation of Non-analytical errors

QC Sample

Improper preparation
Poor storage conditions
Analyte instability

Control Range

Wrong target value
Wrong standard deviation

Interpretation of Results

Wrong number of control samples
Wrong control rules

Causes of Unacceptable Results

Evaluation of analytical errors

RE

- Fluctuation in light source
- Fluctuation in electrical current
- Fluctuation in sample volume
- Air bubbles in the system
- Temperature fluctuations
- Improper mixing of sample with reagent

SE (Progressive)

- Gradual change in light source
- Gradual change in wavelength
- Buildup of material in tubing
- Clogging of apertures
- Change in electronic circuits
- Calibration drift

SE (Abrupt)

- Not following procedures
- Sudden change in light source
- Sudden change in wavelength
- Sudden change in reagent wave
- Sudden change in sample properties
- Sudden change in ambient humidity or temperature
- Calibration failure

Thank you

for your attention