LJ Charts in TTI: Interpretation & Trouble shooting

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Rotary Bangalore & Initiative

Quality Control: Essentials

- Policies & Procedure: Strict adherence
- Training of staff
- Documentation
- Each test run must include one full set of controls that yield results within the limits of standard for acceptability and validity
- Physical parameters (incubation time, temperature range, reagents conc.) should be strictly followed
- Use test kits before expiry date

Controls

Internal controls

- Set of controls (Positive & Negative) provided along with the kit
- To be used only in those batches of kit from which they originate
- Do not detect minor deterioration of kits
- Need for external controls

External controls

- Set of controls included from outside
- Put to continually monitor assay performance (validity)
- Positive (Borderline Reactive) & Negative
- Detect minor error in the assay performance

Why use Third Party Control/IQC/External ?

- Kit controls only calibartors, used to calculate cutoff
- Acceptance range of internal controls wide, PC > 0.800
- Can detect systematic error, equipment malfunction, pipetting errors
- Internal control cannot track changes i.e. trends
  - A problem is about to occur, “near miss “ has occurred
- External control is out for range, indicative that a low positive test sample could be false negative

Monitor changes over a period of time

“Smoke detector” to detect QC problem (fire) by identifying an incorrect result

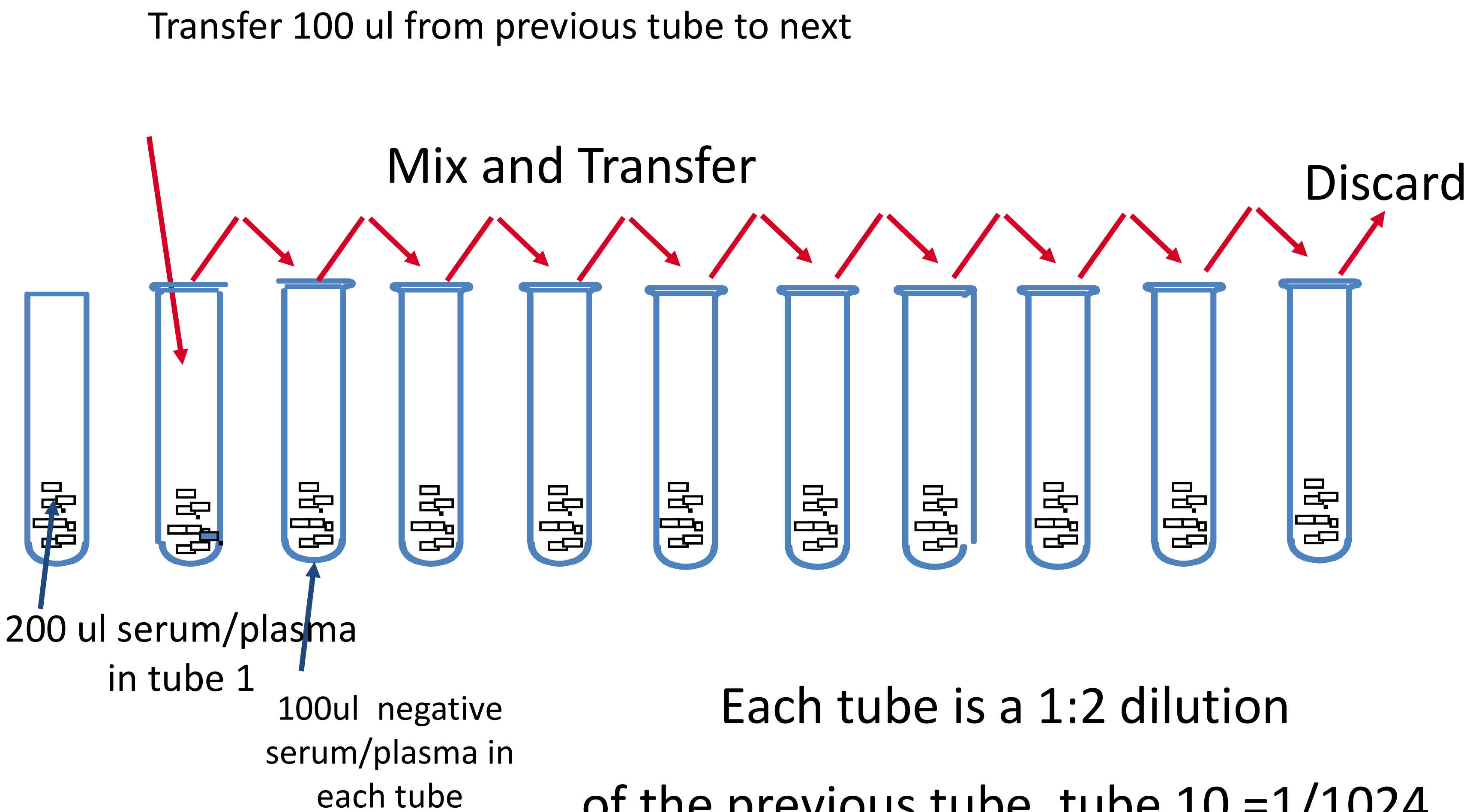
Why use External Control ?

- Compliance to accreditation requirements
- Basic Quality Assurance compliance
- Demonstrate competence of Staff
- Develop confidence on the lab analyzer performance

External Controls for TTI

- Can be prepared in house: Diluted positive specimen
- Aliquots & frozen
- Available commercially: direct use for testing

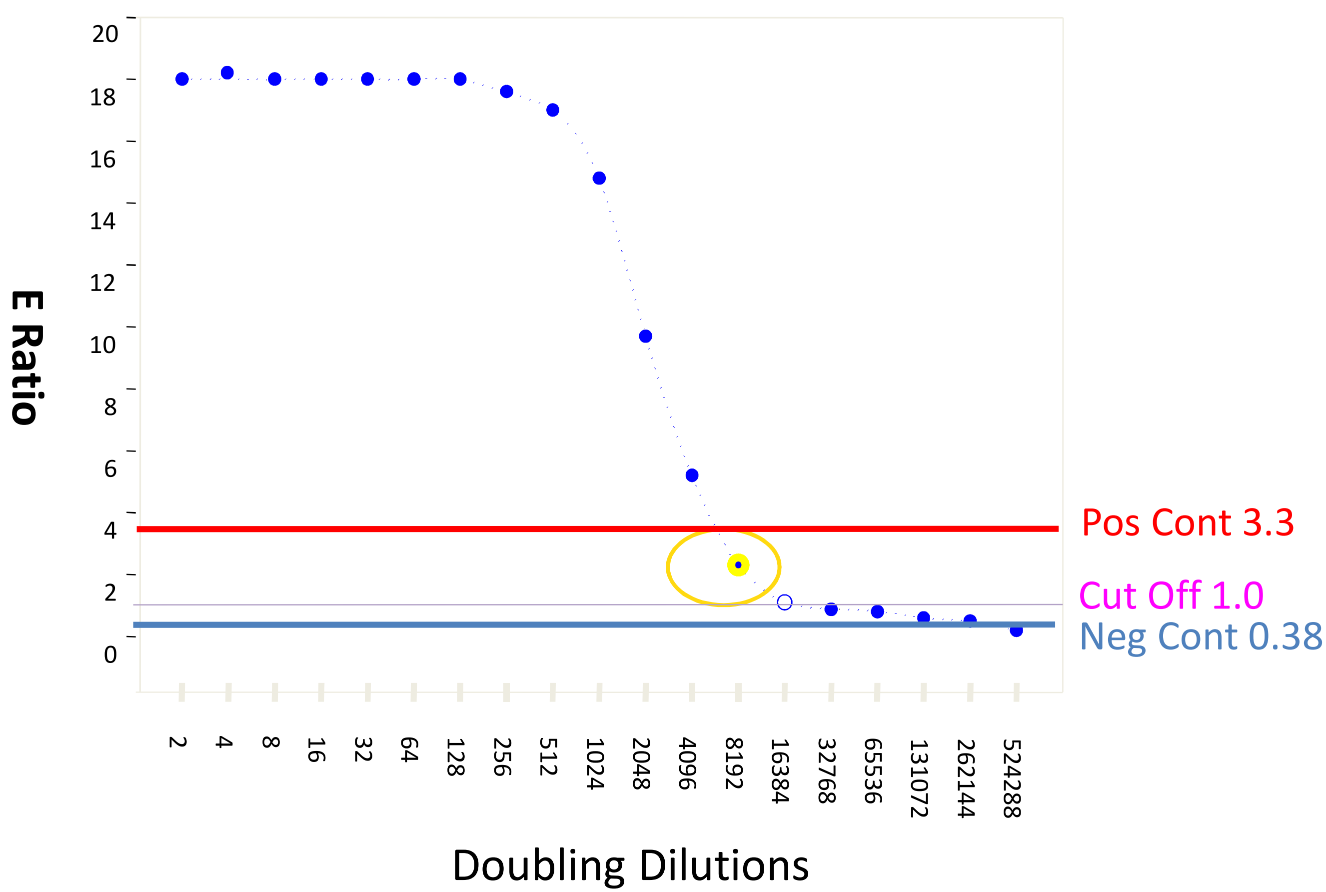
Making Suitable Dilutions



Each tube is a 1:2 dilution

of the previous tube, tube 10 =1/1024

Selecting a Suitable Sample Dilution

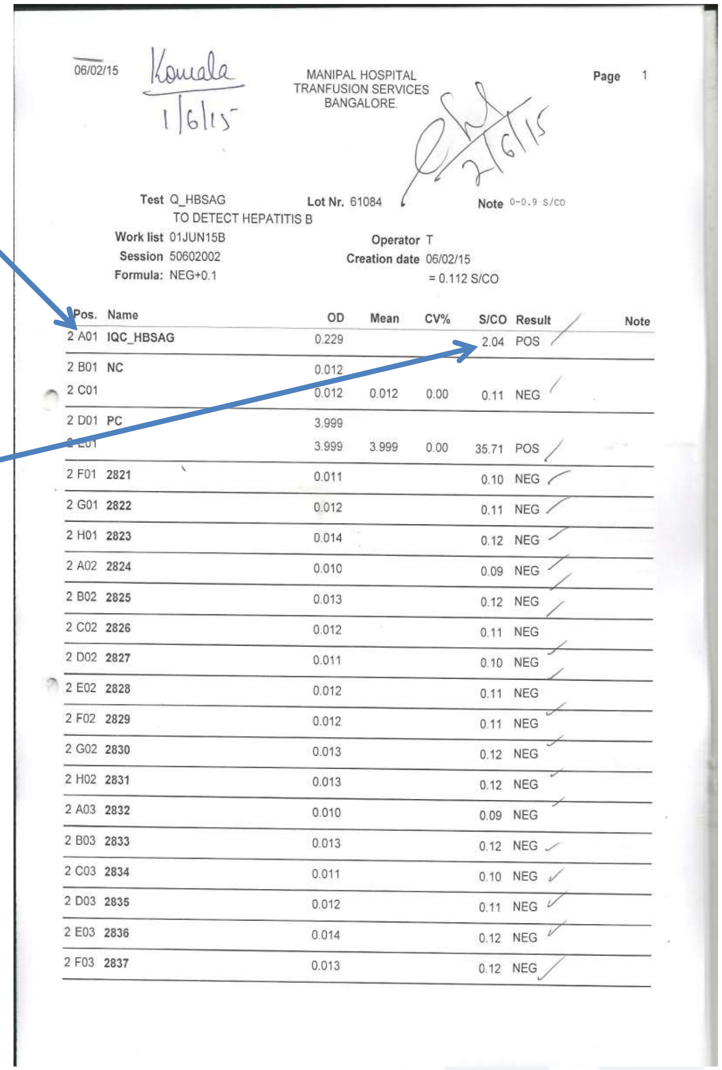


Preparation of Levy Jennings chart

- Run this weak QC sample with each assay
- Calculate E Ratio(ELISA Ratio)

$$= \frac{\text{OD of weak QC sample}}{\text{Cut-off value}}$$

- Statistical analysis of the QC data- by plotting on LJ-graph
- Minimum 20 values required



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Test Q_HBSAG
TO DETECT HEPATITIS B
Lot Nr. 61084
Note 0-0.9 S/CO
Work list 01JUN15B
Session 50602002
Formula: NEG+0.1
Operator T
Creation date 06/02/15
= 0.112 S/CO

Pos.	Name	OD	Mean	CV%	S/CO	Result	Note
2 A01	IQC_HBSAG	0.229			2.04	POS	
2 B01	NC	0.012					
2 C01		0.012	0.012	0.00	0.11	NEG	
2 D01	PC	3.999					
2 E01		3.999	3.999	0.00	35.71	POS	
2 F01	2821	0.011			0.10	NEG	
2 G01	2822	0.012			0.11	NEG	
2 H01	2823	0.014			0.12	NEG	
2 A02	2824	0.010			0.09	NEG	
2 B02	2825	0.013			0.12	NEG	
2 C02	2826	0.012			0.11	NEG	
2 D02	2827	0.011			0.10	NEG	
2 E02	2828	0.012			0.11	NEG	
2 F02	2829	0.012			0.11	NEG	
2 G02	2830	0.013			0.12	NEG	
2 H02	2831	0.013			0.12	NEG	
2 A03	2832	0.010			0.09	NEG	
2 B03	2833	0.013			0.12	NEG	
2 C03	2834	0.011			0.10	NEG	
2 D03	2835	0.012			0.11	NEG	
2 E03	2836	0.014			0.12	NEG	
2 F03	2837	0.013			0.12	NEG	

Why ELISA Ratio?

- Why plot ELISA ratios? Why not plot OD’s directly?
- Plotting E-ratio has these advantages
  - Minimizes kit to kit variation(company to company lot to lot):

    The difference in OD’s and cut-off’s get negated by E-ratio
  - Minimizes technician to technician variation
  - Minimizes instrument to instrument variation

Why L-J charts?

- Easy to plot
- Easy to interpret
- Easy to apply Westgard rules
- Easy availability of online tools

How to create L-J charts?

By using common materials

- Manually - arithmetic graph paper
- MS Excel Software in computer
- Quality software programs
- Inbuilt into Autoanalyzer

How to create L-J chart?

By using simple statistics

- Mean → TARGET
- Standard Deviation (SD)
  - ± 1SD
  - ± 2SD
  - ± 3SD

  → CONTROL LIMITS
- Coefficient of Variation (CV %)

Creating L-J chart …

- The mean and standard deviation of the control being used should be determined based on at least 20 measurements over 20 days.
- Statistical data should be determined based on >10 measurements. *(In case of perishable and less quantity control material).*

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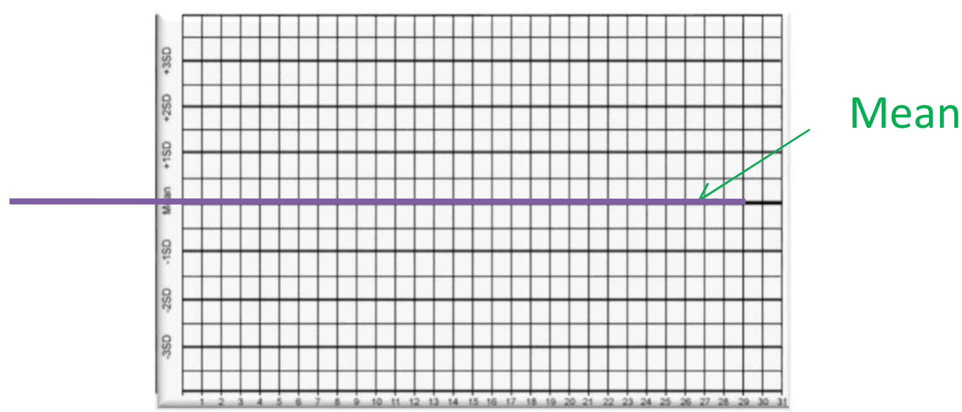
Mean /Target Value

- **Mean** ($\bar{x}$) is the sum of all the measurements (Σ) divided by the number of measurements (n)

$$Mean = \bar{x} = \frac{x_1 + x_2 + x_3 + x_4 + \ldots + x_N}{N}$$

N = the number of data points in the set



QC at Analytical stage

Standard Deviation-Dispersion from target value

- SD quantifies the degree of dispersion of data points about the mean.

$$SD = \sqrt{\frac{(x_1-\bar{x})^2+(x_2-\bar{x})^2+(x_3-\bar{x})^2+\ldots+(x_N-\bar{x})^2}{N}}$$

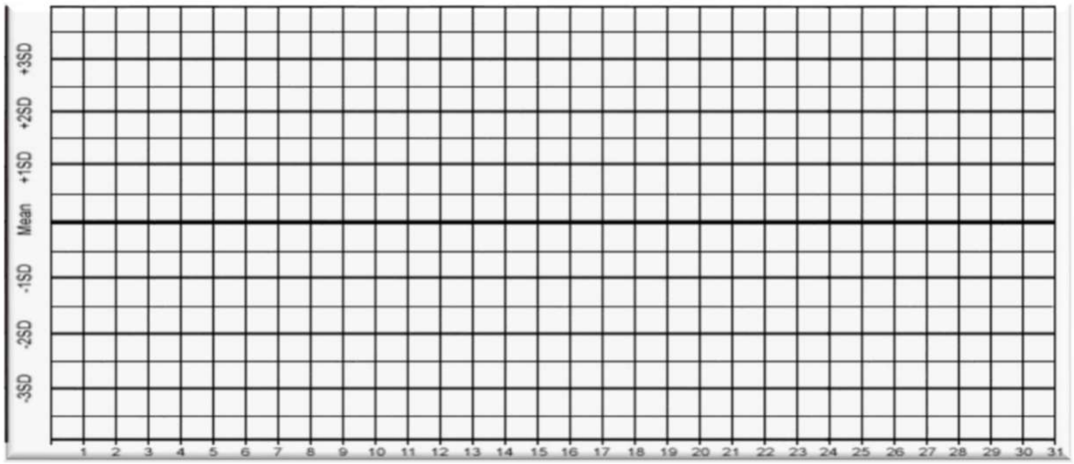
Co-efficient of Variation %

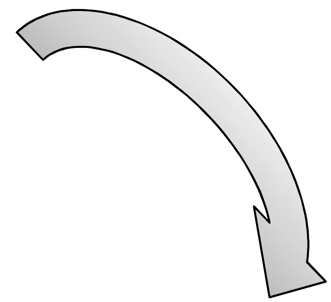
- Coefficient of Variation
- Ratio of the standard deviation to the mean expressed as a percentage.

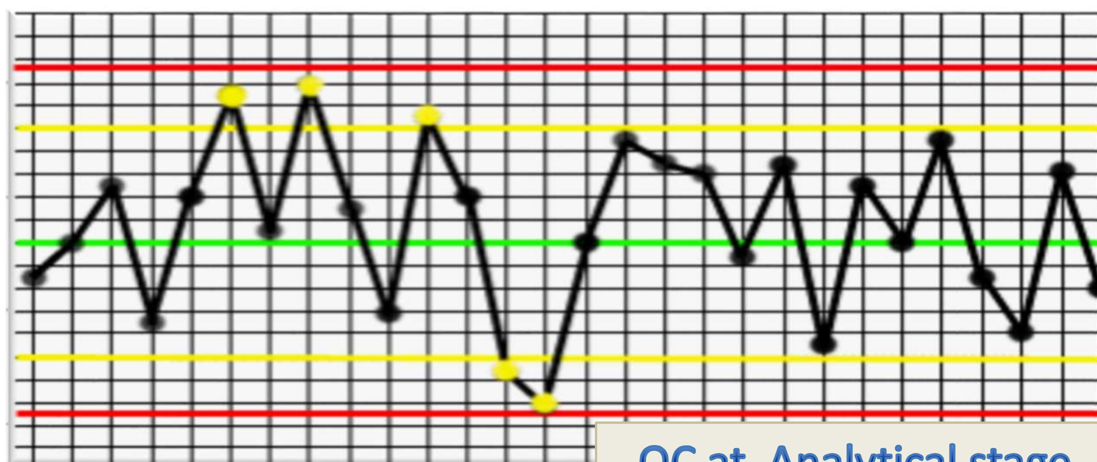
$$CV\ \% = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

- Statistical equalizer
- Performance of kits and reagents can be evaluated

L-J chart is ready for QC monitoring







QC at Analytical stage

Calculate Control Limits

SD is used to set limits upon which control result acceptability is determined.

Mean + (3 x SD) = +3SD

Mean + (2 x SD) = +2SD

Mean + (1 x SD) = 1SD

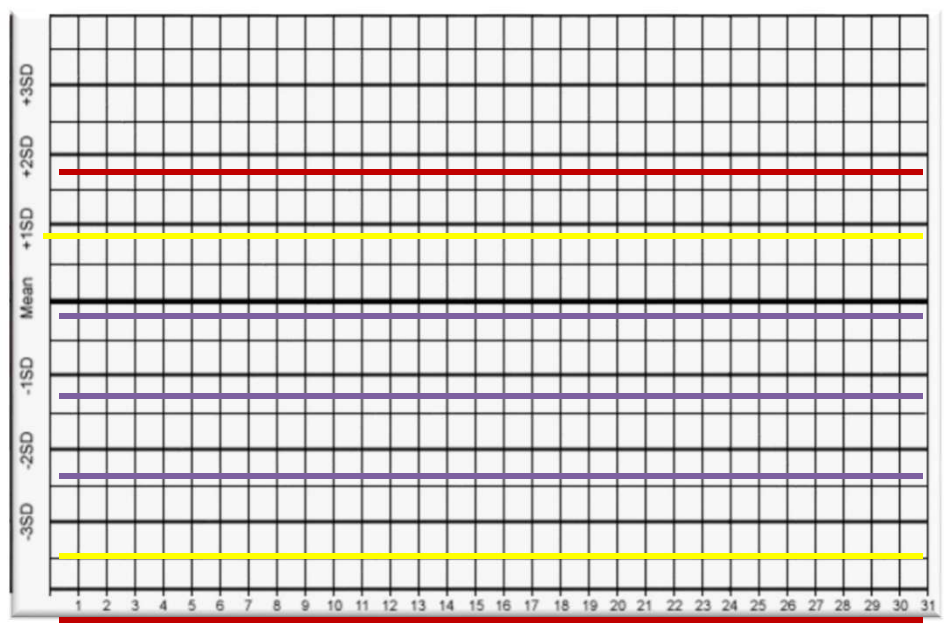
Upper Control Limits

Mean - (1 x SD) = - 1SD

Mean - (2 x SD) = - 2SD

Mean - (3 x SD) = - 3SD

Lower Control Limits



Monitoring QC Data

- Plot Levy-Jennings each day, make decision regarding acceptability
- Meet pre-established limits, usually 95% confidence interval
- Monitoring over time provides information about the ***precision*** and long-term ***accuracy*** of measurement
- Review charts at defined intervals
- Consider using Westgard Control Rules

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QC Results

- Random Error – factors affecting precision
  - Change in volume, temp, poor mixing, sample preparation errors, centrifugation
  - Transcription errors
  - Poor Technical skill: Lack of training, not following SOP
- Systematic error – factors affecting accuracy
  - Change in kit lot, calibrators
  - Reagent stability, degradation, deterioration
  - Inaccurate storage of reagents
  - Incorrect instrument settings

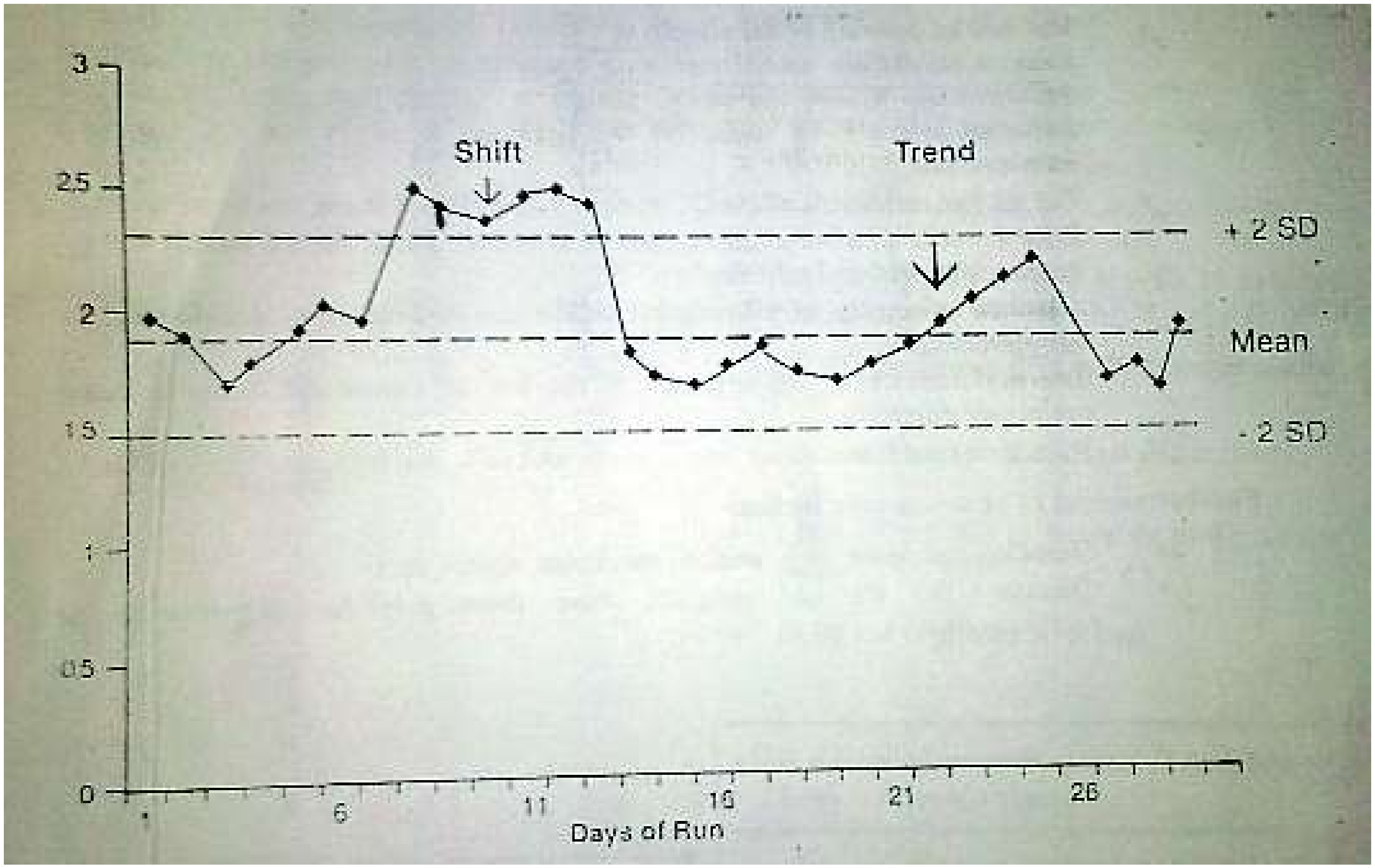
QC Results: Systematic Variation

- Shift – six consecutive control values fall on one side of the mean, values approx equal
  - New kit lots
  - New reagents, buffers
  - Change in incubation temperature
  - New technologist, different pipetting techniques
  - Change in washer, reader

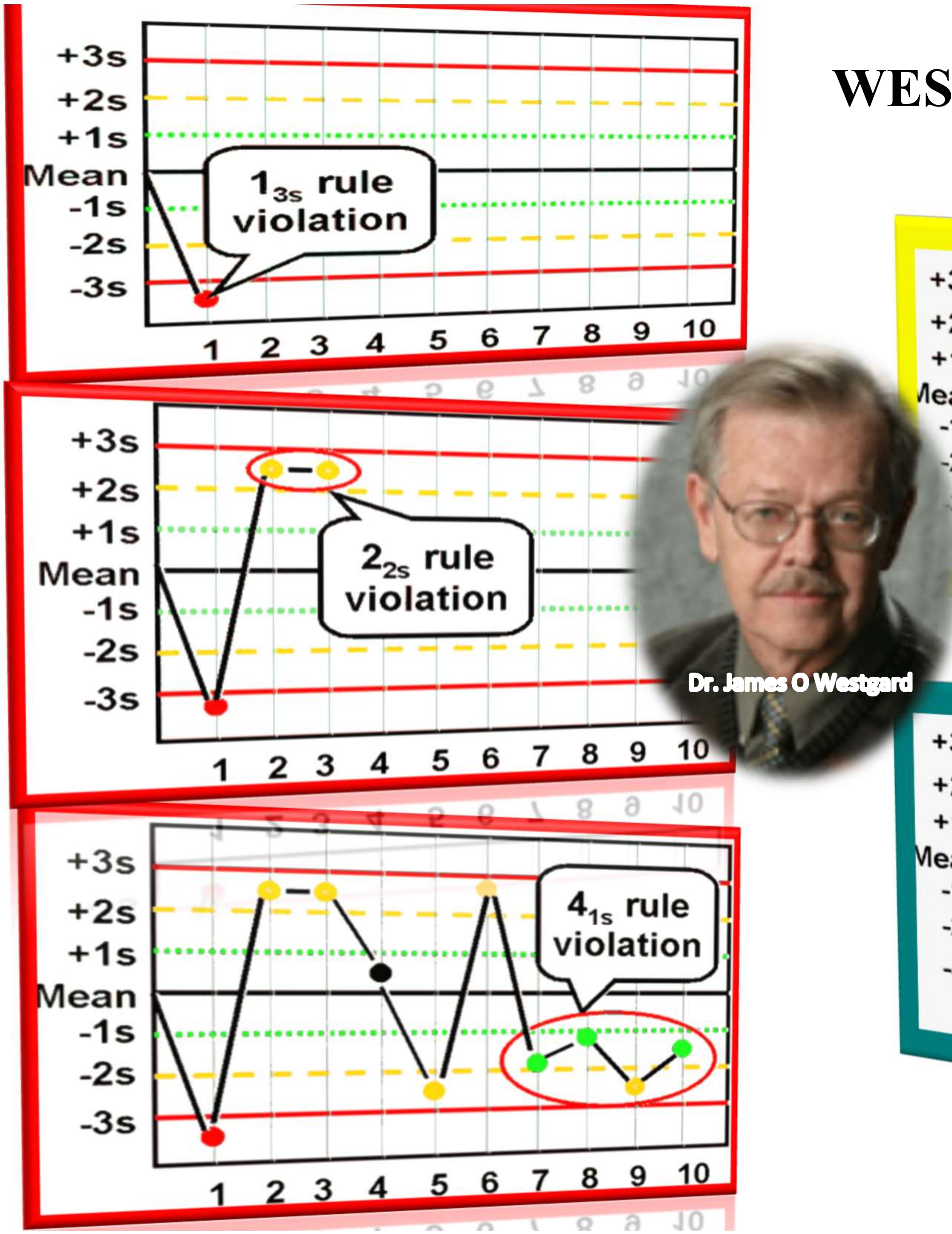
QC Results: Systematic Variation

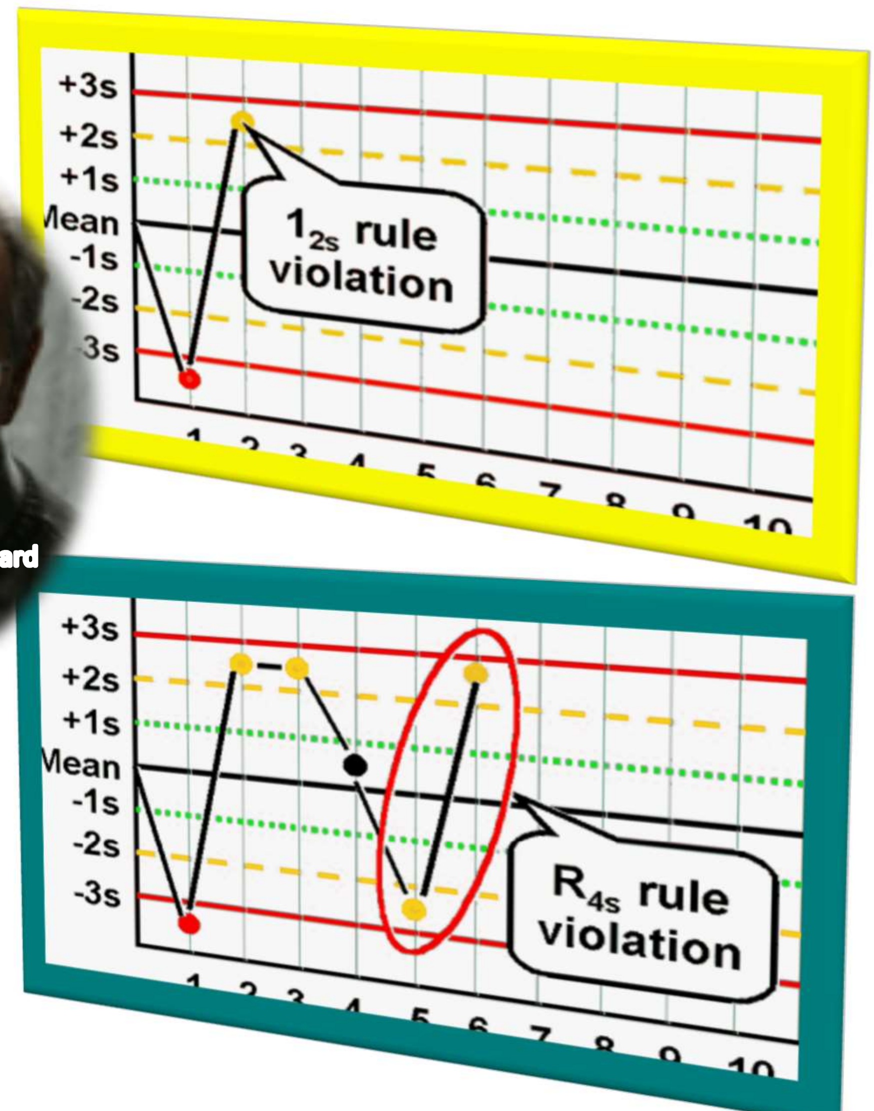
- Trend – six consecutive points fall in one general direction, gradual increase or decrease in value
  - Deterioration of reagents, conjugates, controls
  - Faltering equipment e,g. pipette slowly losing calibration
  - Gradual deterioration of equipment

Shift and Trend



WESTGARD RULES





QC at Analytical stage

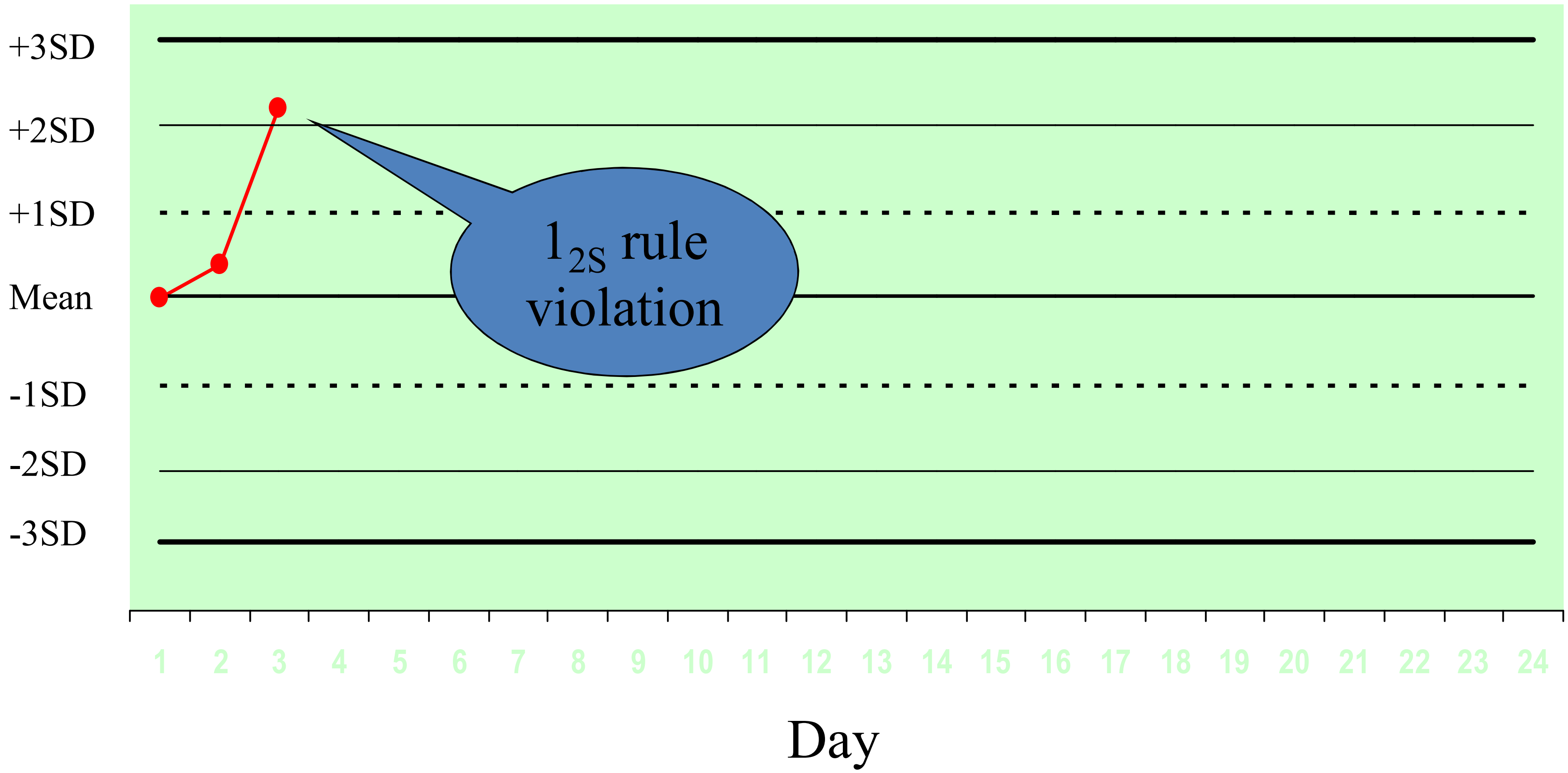
Westgard Rules (James Westgard)

QC Rule	Definition	Type of error
1 - 2s	1 result > ± 2 SD	Random or Systematic
1 - 3s	1 result > ± 3 SD	Random or Systematic
2 - 2s	2 consecutive results > ± 2 SD	Random or Systematic
4 -1s	4 consecutive results > ± 1 SD	Systematic
10x	10 consecutive results on one side of the mean	Systematic

Westgard – 1 2s Rule

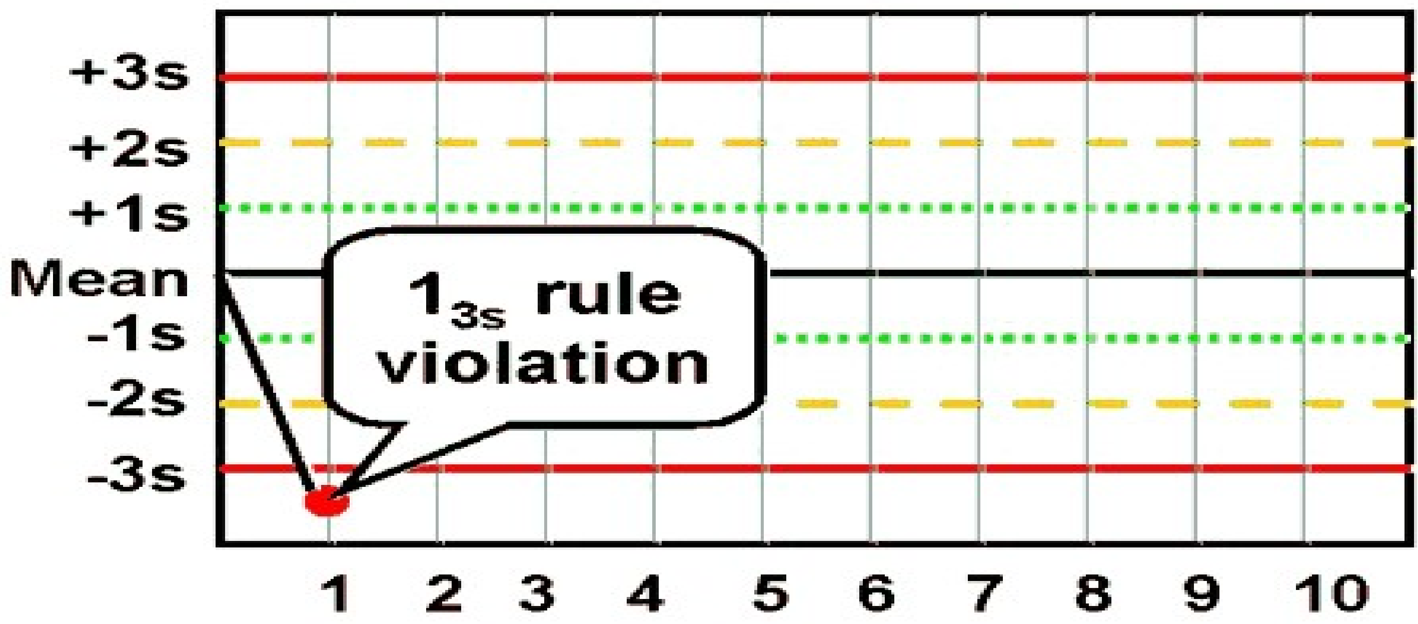
- "warning rule"
- Control result falls outside ±2SD
- Alerts tech to possible problems
- Not cause for rejecting a run

1_{2S} Rule = A warning to trigger careful inspection of the control data



Westgard – 1 3s Rule

- If control result falls outside of ± 3SD, rule is violated
- Run to be rejected, run regarded as Out of Control

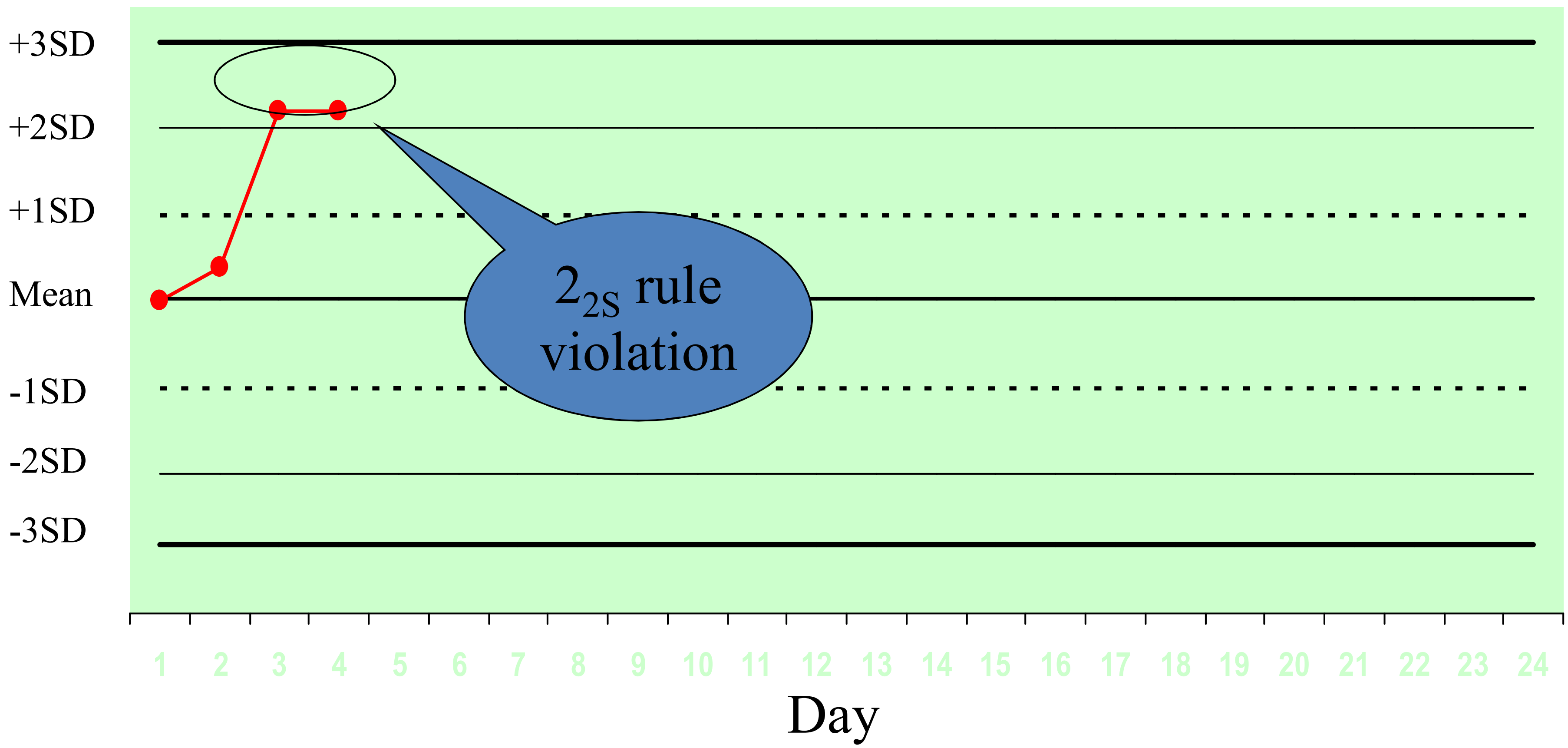


Westgard – 2 2s Rule

- 2 consecutive control values for the same level fall outside of ±2SD in the same direction
- Warning rule, requires corrective action

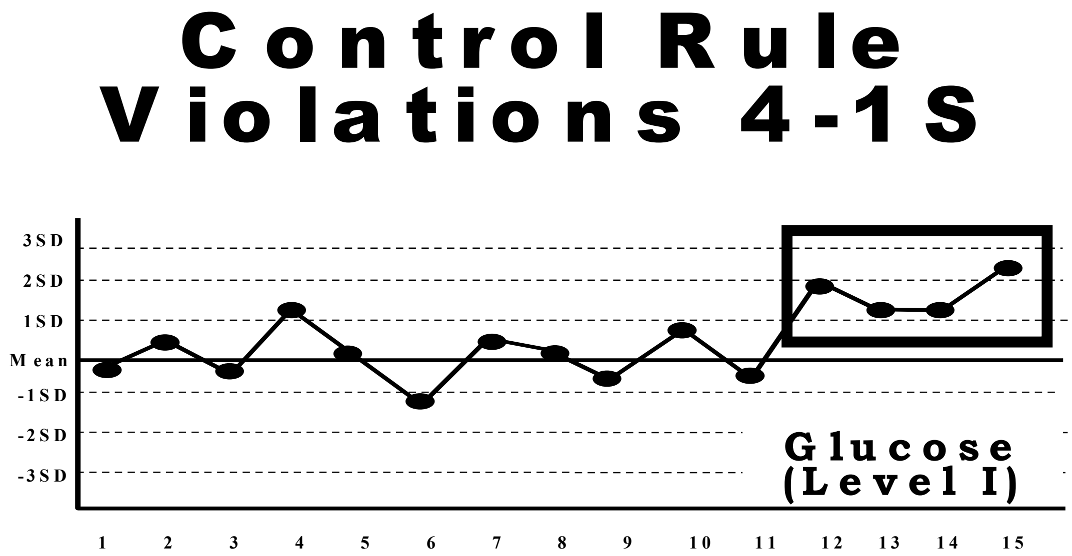
2_{2S} Rule =

+2SD or -2SD control limit



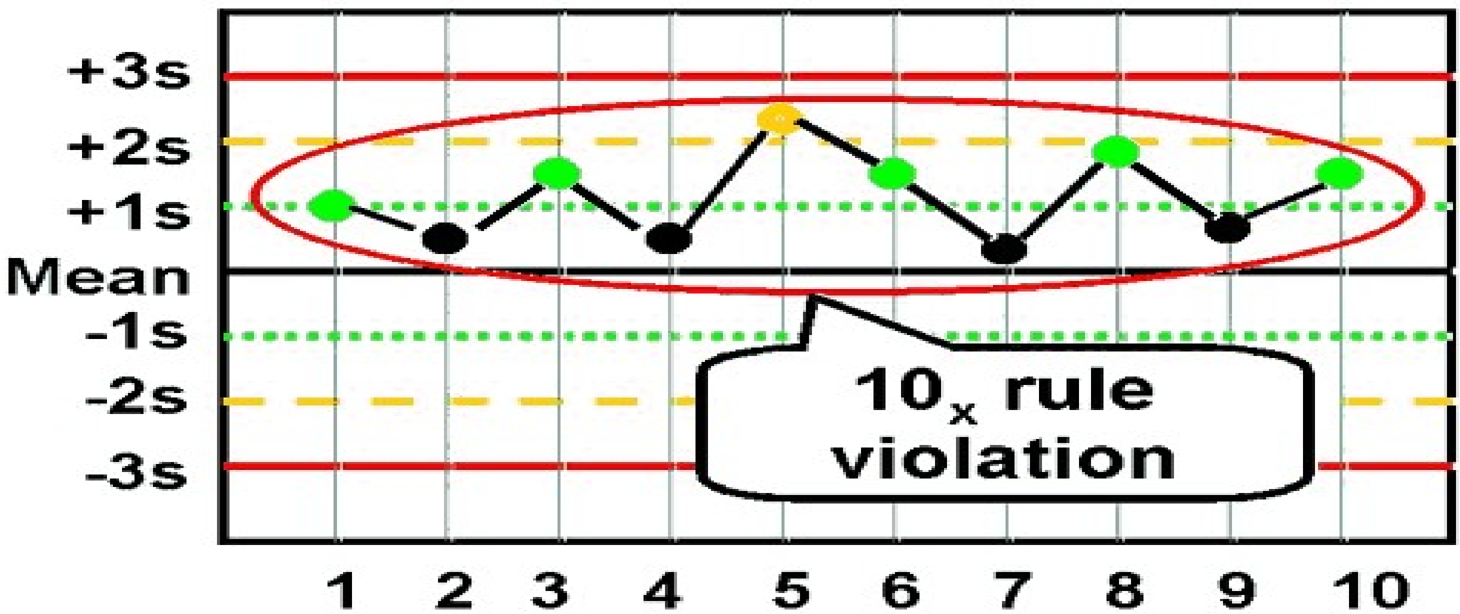
Westgard – 4 1s Rule

- Four consecutive QC results for one level of control are outside ±1SD
- Warning rule



Westgard – 10_X Rule

- Ten consecutive QC results for one level of control are on one side of the mean
- Needs attention, may not be applicable for Qualitative assay & Automation



What to do when Control Value is out of limit?

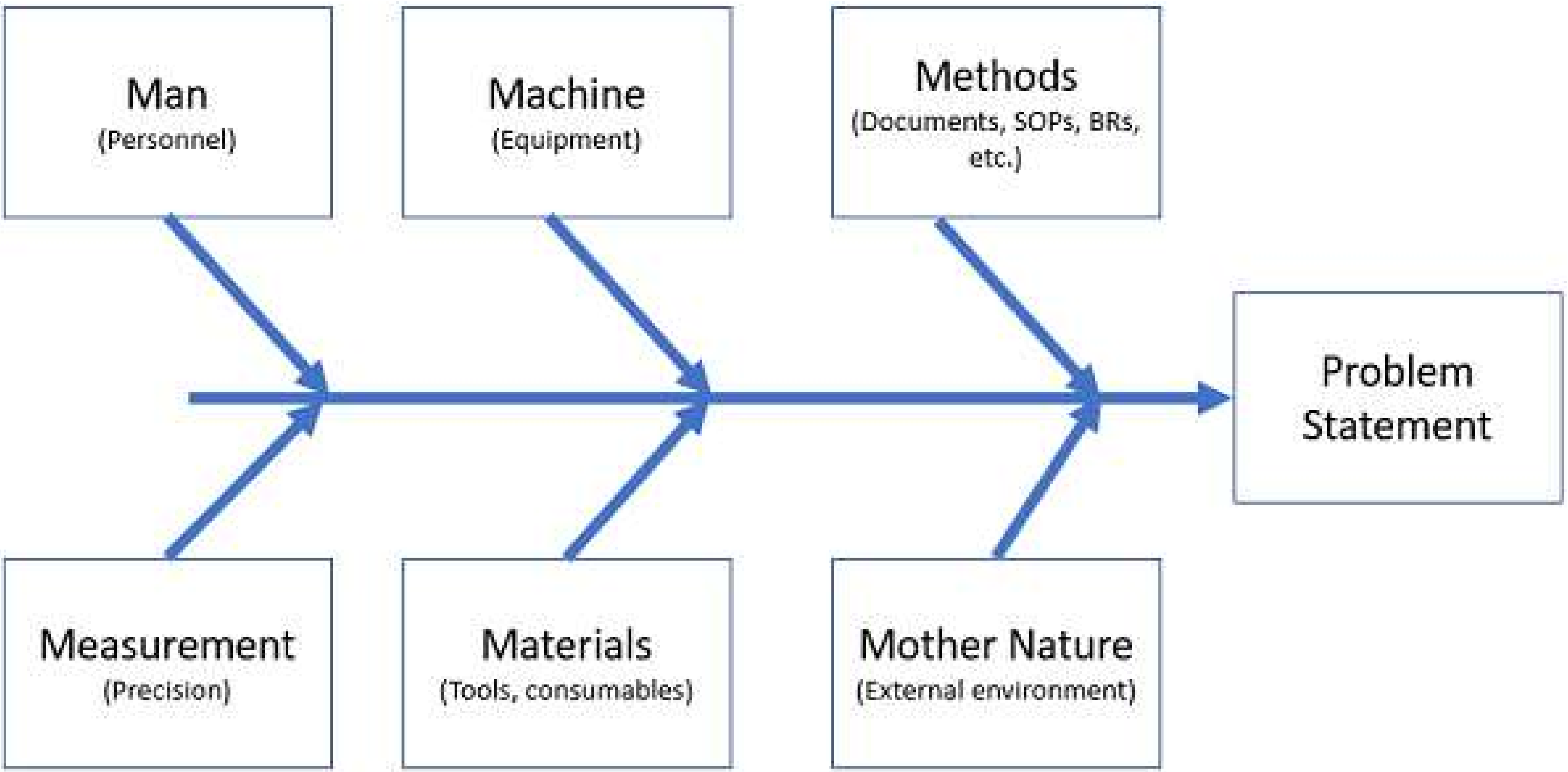
- Rejection Rule
- “out of control”
- Stop testing
- Identify and correct problem
- Repeat testing on the samples and controls
- Do not report results until problem is solved and controls indicate proper performance

Root Cause Analysis & CAPA

- Document all the results
- Repeat the test run when rejection occurs
- Analyze rejections & warning
- Identify the cause of error: Equipment, procedure, personnel, environment, reagents
- Resolve the problem accordingly

Where is the Problem??

Root Cause Analysis



Summary

- QA in TTI: use of External Control
- Prepare LJ chart
- Apply Westgard rules for analysis
- Rejection & warnings
- Root cause analysis
- Corrective & Preventive actions
- Regular monitoring of TTI testing & continual improvement

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Thank you

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