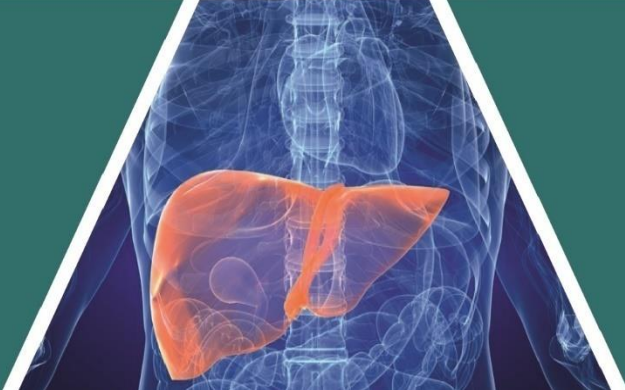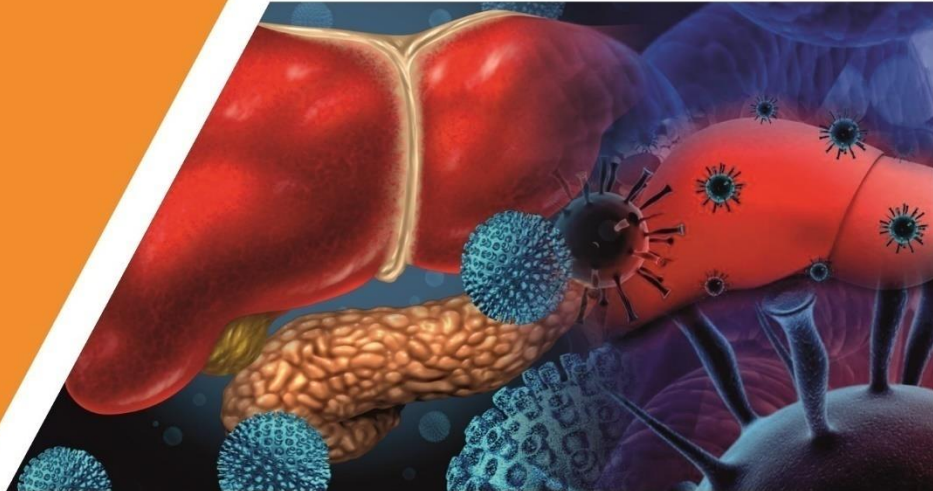**PROJECT PRAKASH**

Pogrammed Approach to Knowledge and Sensitization on Hepatitis

HEPATITIS INDUCTION PROGRAM
FOR LAB TECNICIANS

SEROLOGICAL METHODS IN THE DIAGNOSIS OF VIRAL HEPATITIS & QUALITY CONTROL

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Laboratory diagnosis in Virology

Direct methods

- Electron microscopy
  - Viral Culture
- Nucleic acid detection

Indirect methods

- Serology (antigen & antibody detection)

What are Serological assays?

- Detection of either the antigen or antibody in a patient's sample

- Basic Principle – in vitro antigen-antibody reactions

Different principles of Serological tests:

1. Rapid Diagnostic card Tests

2. Enzyme linked Immunosorbent Assay (ELISA)

3. Chemiluminescent microparticle immunoassay (CLIA)

Serological markers for Viral Hepatitis

Antigens
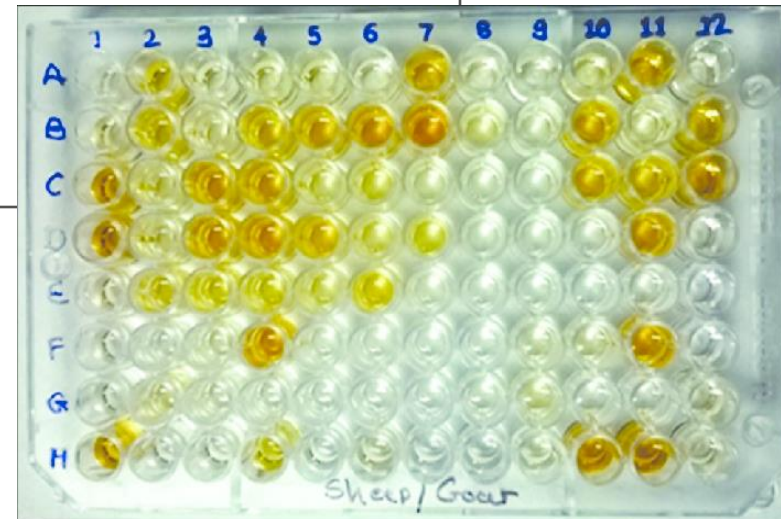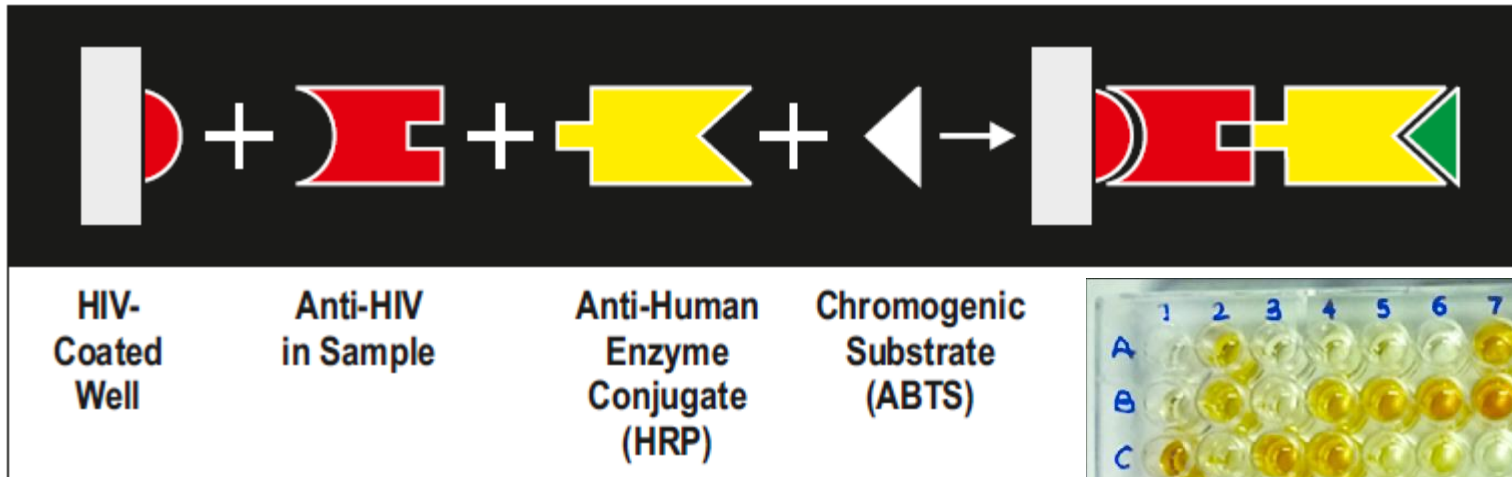
- HBsAg
- HBeAg

- HCVcAg

Antibodies

- Anti-HB core total
- Anti-HB core IgM
- Anti-HBe
- Anti-HBs
- Anti-HCV
- Anti-HDV IgM/IgG
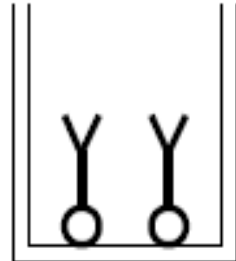- Anti-HAV IgM/IgG
- Anti-HEV IgM/IgG

ELISA

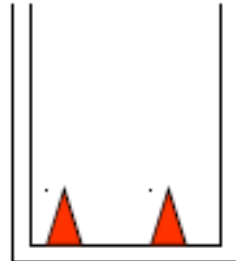## ELISA uses an enzyme to detect the antigen-antibody binding

Principle

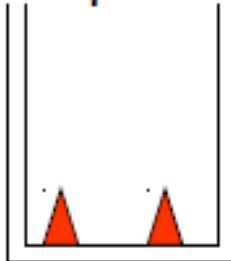| HIV-Coated Well | Anti-HIV in Sample | Anti-Human Enzyme Conjugate (HRP) | Chromogenic Substrate (ABTS) |

Sandwich ELISA

Indirect ELISA

Competitive EILSA

Color product

Antigen

Antibody

Conjugate (Ab to Ab)

Conjugate (Ab to Ag)
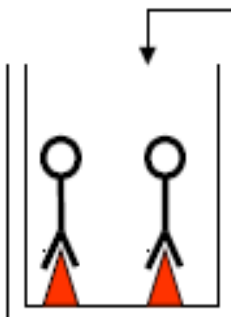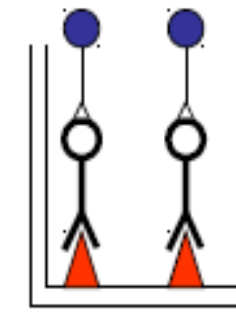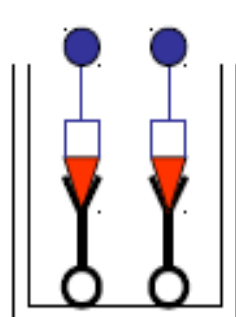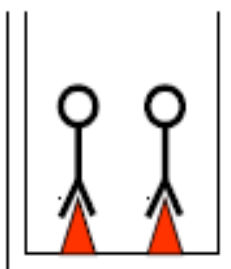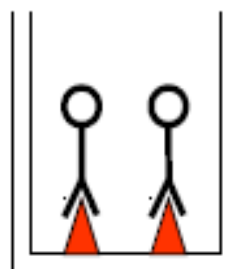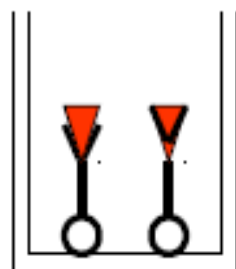
Conjugate is washed out as Ag is not free to bind the conjugate
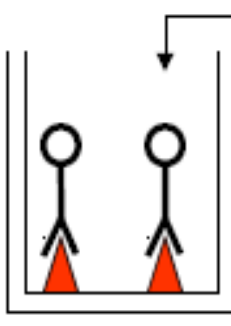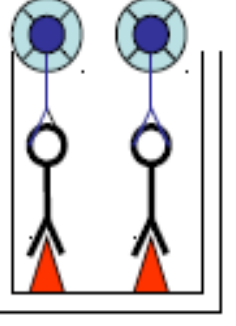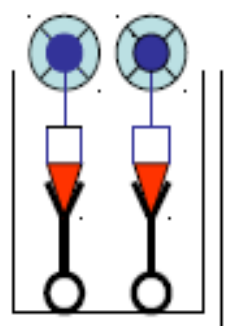
Substrate

Enzyme (tagged to conjugate is not there to act on substrate

ADVANTAGES

- Large number of samples can be tested.

- Takes only 2-3 hours

- Main Screening test

- Very sensitive

- Has become more specific with the use of purified recombinant and synthetic antigens and monoclonal antibodies

DISADVANTAGES

- Expertise needed

- Extensive equipments needed

Automated ELISA systems

RDT - Immunochromatography

- Visual point of care tests

- Useful in resource limited settings

- Technically simple

- Kits can be stored at ambient temperature

- Results can be read within 15 to 20 minutes

- Good sensitivity and specificity comparable to ELISA
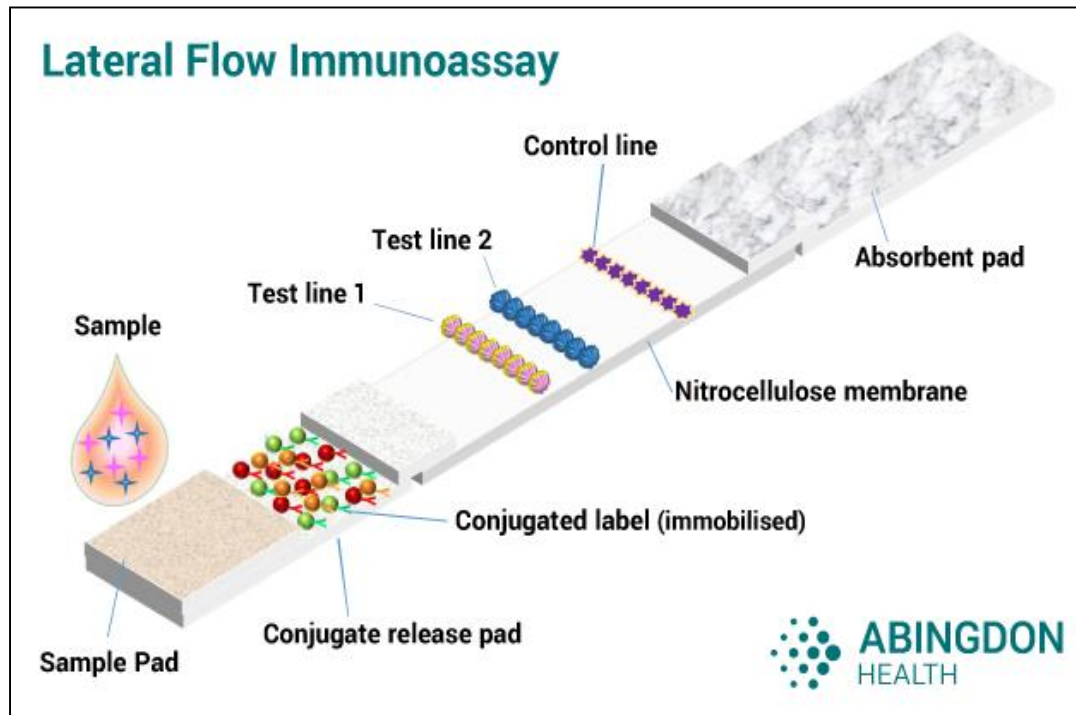
RDT – Immunoconcentration Dot Blot Immunoassay

- Antibody specific to the analyte of interest is immobilized onto a flow-through support contained within a single-use device.

- The test sample is applied to the support material and allowed to pass vertically through the membrane.

Internal Control

HIV-1 peptide

HIV-2 peptide

- After a wash step, a second antibody, conjugated to a label (such as an enzyme or colloidal gold) is applied.

- The flow of sample through the capture field facilitates the kinetics of the reaction and therefore shortens the time to result.
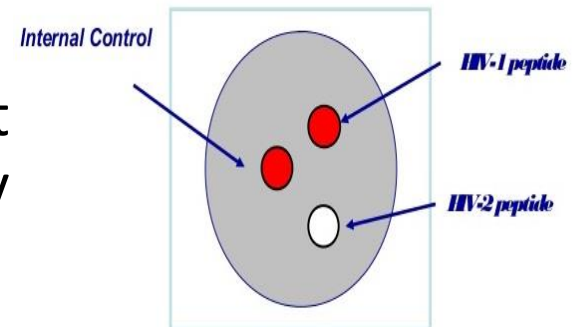
Chemiluminescent Microparticle Immunoassay

- Two chemicals react to form an excited high-energy intermediate, which breaks down releasing some of its energy as light.

- variation of the standard ELISA in which conjugate solution containing pyrogallol, Isoluminol, Acridinium ester or oxalate ester are oxidized by a substrate (trigger) to produce a burst of light (luminescence).This burst can be measured by a luminometer.

Sample + Paramagnetic microparticles (capture molecules)

Immune complex formed

The paramagnetic particles bound to the specific analyte get attached to the wall of the reaction vessel

Washing

Acridinium labelled conjugate is added

Washing

Pre-trigger hydrogen peroxide is added

Trigger sodium hydroxide added and acridinium undergoes an oxidation to form N-Methyl acridone and releases light

The resultant chemiluminescent reaction is measured as Relative Light Units (RLUs)

ELISA	Chemiluminescence
Conjugate is linked to an enzyme	Conjugate is linked to acridinium dye
Chromogenic substrates are used which produce visible colour	Substrates are trigger solutions which oxidize acridinium labelled conjugate into a fluorogenic compound which produces photon of light
Less sensitive (Because Ag or Ab in the sample gets attached to the Ag or Ab on the bottom of the well only. Lesser the surface area, lesser is the amount of Ag or Ab available for detection)	More sensitive (Because paramagnetic particles in the microparticle solution allows the immune complex to get attached to the walls of reaction vessels. More the surface area, more is the amount of Ag or Ab available for detection)
Batch testing required	Even one sample can be run without wastage of reagents
Only one test can be tested for a patient in one run	Multiple tests/markers can be tested for a patient in one run
Long processing time	Short processing time
Technical expertise required	less technical expertise required

QUALITY CONTROL

What is Quality?

Quality Control - QC refers to the measures that must be included during each assay run to verify that the test is working properly

Quality Assurance - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct

"the right test is carried out on the right specimen,

the right result and right interpretation is delivered to the right person at the right time"

Quality Assessment - quality assessment (also known as proficiency testing) is a means to determine the quality of the results generated by the laboratory. Quality assessment is a challenge to the effectiveness of the QA and QC programs

Functional Phases in a laboratory

- Pre-analytical – requisition, specimen acception & rejection criteria, specimen segregation & archiving

- Analytical – equipment, kits, assaying controls & samples

- Post-analytical – validation, interpretation, verification & dispatch of final reports

SPECIMEN
KITS
EQUIPMENTS
PERSONNEL

- SOPs

- Control materials

- Levey Jenning Charts

- Documentation

- CAPA

Defining control materials

- Contain an established amount of the substance being tested - the analyte

- Tested at the same time and in the same way as patient samples

- To validate the reliability of the equipment , evaluate the operator's performance and environmental conditions that might impact results

- Controls should check both low values and high values

- Controls are serum based, but they may also be based on plasma, urine

Difference b/w Calibrators & Controls

Calibrators

A substance with a specific concentration.
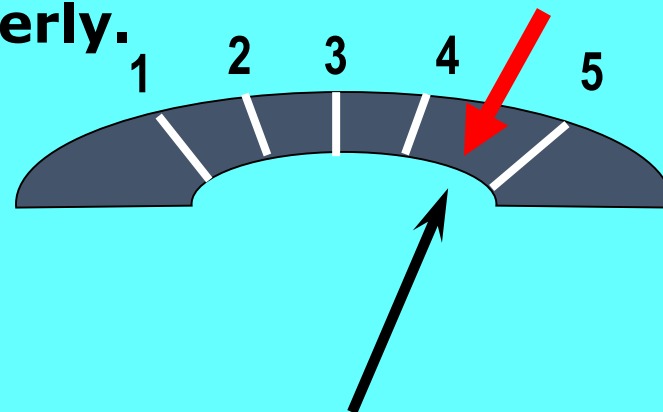
Calibrators are used to set (calibrate) the measuring points on a scale.
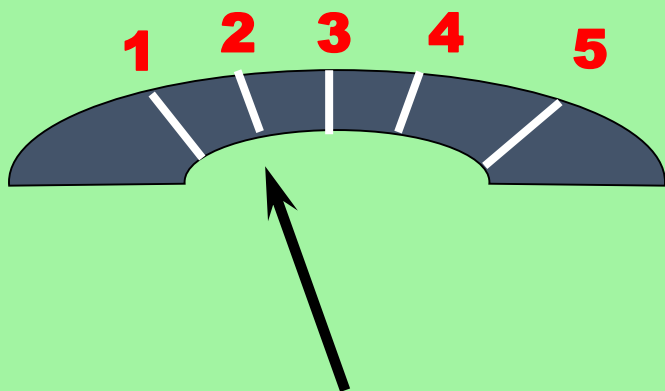
Controls

A substance similar to patients' samples that has an established concentration.

Controls are used to ensure the procedure is working properly.

Controls

- **Internal controls**

  - Set of controls (Positive & Negative) provided along with the kit

  - To be used only in those batches of kit
  - The internal controls do not detect minor deterioration of kits
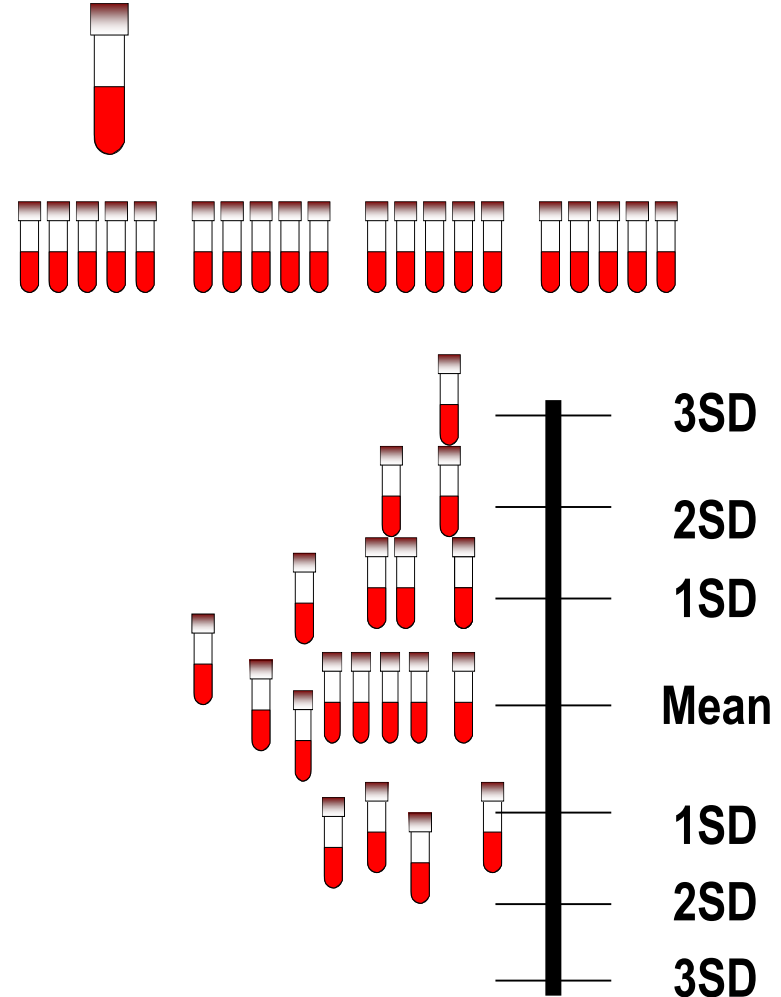
- **External controls**

  - Set of controls included from outside

  - Positive (Borderline Reactive) & Negative

  - Detect minor error in the assay performance

  (Control charts – to monitor the assay of controls)

Steps in Implementing QC chart

- obtain control material

- run each control 20 times over 30 days

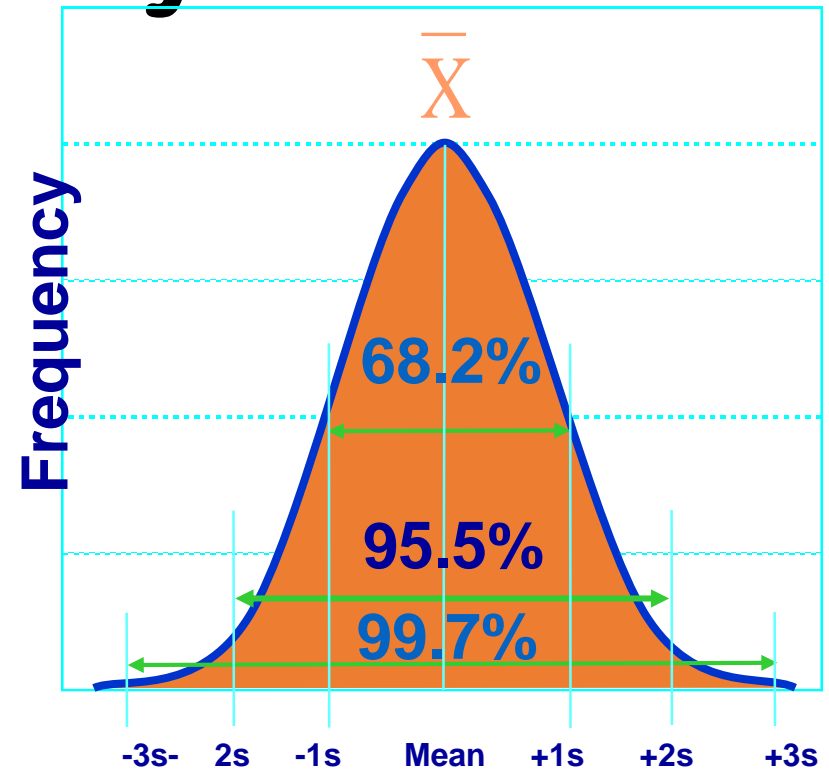- calculate mean and +/-1,2,3 Standard Deviations

3SD

2SD

1SD

Mean

1SD

2SD

3SD

Standard Deviation and Probability

For a set of data with a **normal distribution**, a random measurement will fall within:

± 1 SD 68.3% of the time

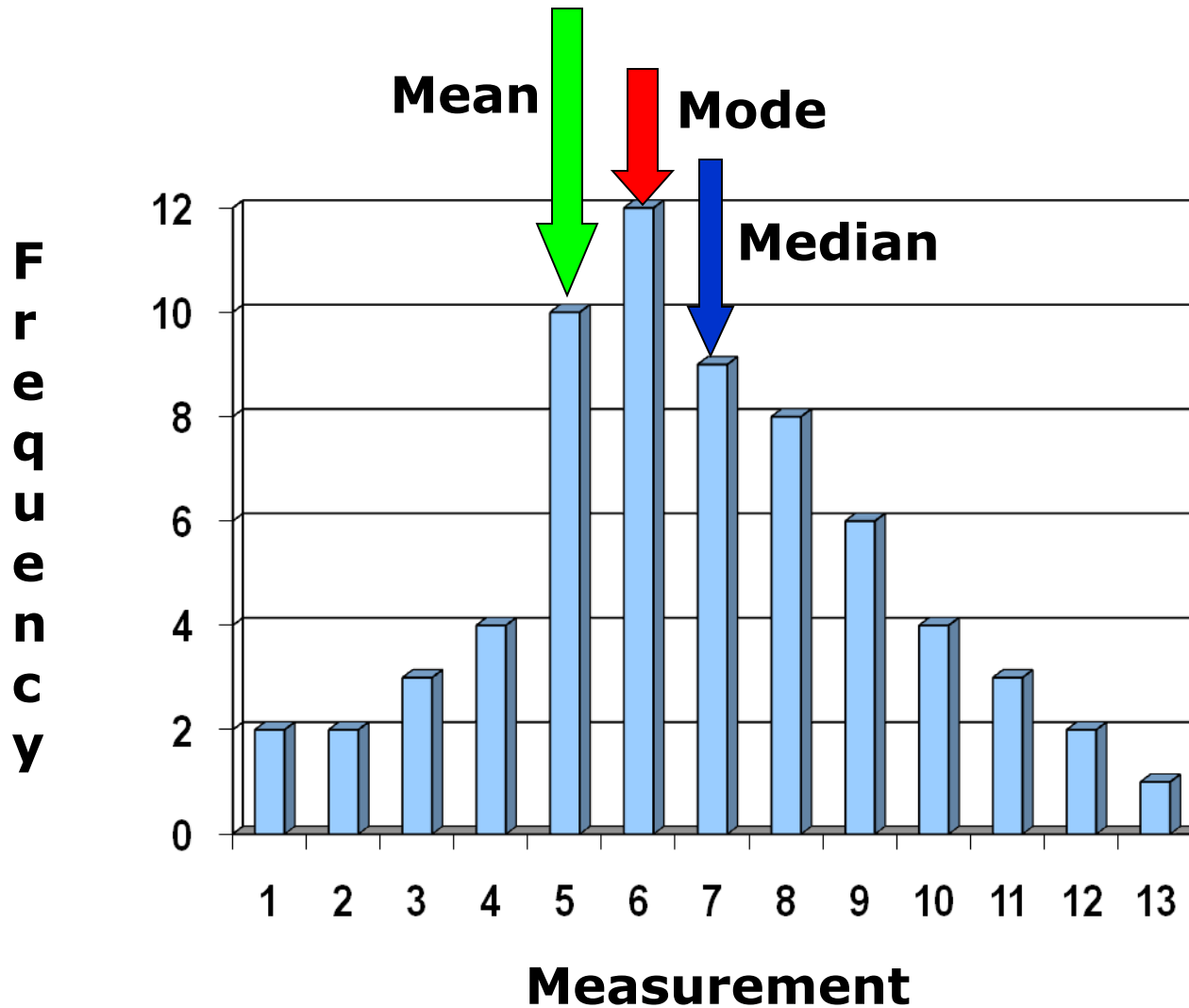**± 2 SD 95.5% of the time**

± 3 SD 99.7% of the time

$\overline{X}$

Frequency

68.2%

95.5%

99.7%

-3s- 2s -1s Mean +1s +2s +3s
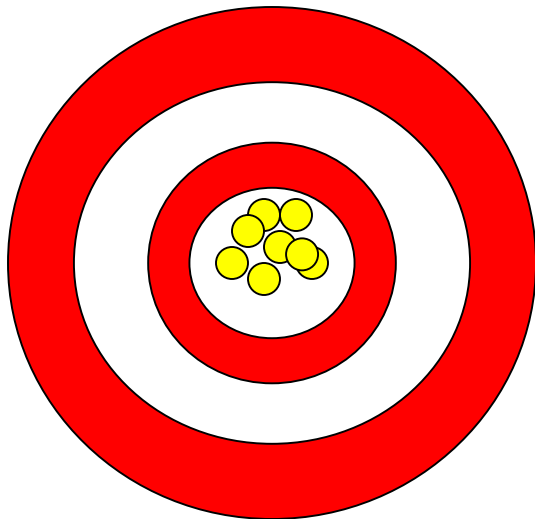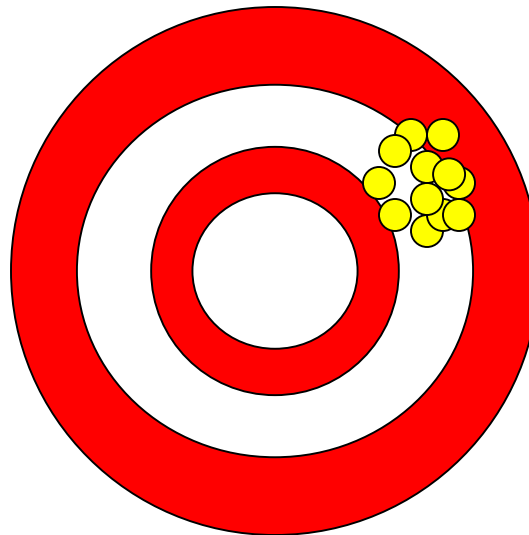
Not all central values are the same

Accuracy and Precision

Accurate and Precise

Precise but Biased

Imprecise
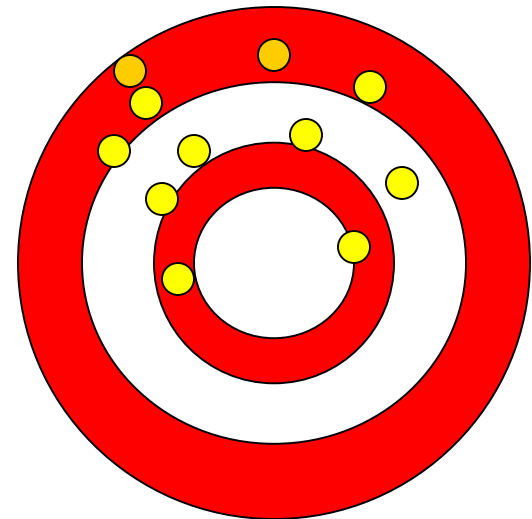
Accurate = Precise but not Biased

Calculation of Mean

$$\overline{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$\overline{X}$ = Mean

X_1 = First measurement

X_2 = Second measurement

X_n = Last measurement in series

n = Total number of measurements

n = number of data points

$\overline{X}$ represents the mean

$x_1 - x_n$ = all of the measurements (1 through n)

$\sum$ is the sum of (add data points)

Standard Deviation (SD)

SD is the principle measure of variability used in the laboratory

$$SD = \sqrt{\frac{\sum (x_1 - \bar{x})^2}{n - 1}}$$

Standard Deviation – Statistical Formula

Coefficient of Variation

The coefficient of variation (CV) is the SD expressed as a percentage of the mean.

$$CV = \frac{SD}{mean} \times 100\%$$

- CV is used to monitor precision
- CV is used to compare methods
- CV ideally should be less than 5%

# Calculation of Mean: ELISA Tests

- Run controls 20 times in 30 days. Record both OD and cut off (CO) values for each measurement.

- Divide the OD by the CO (OD/CO) for each data point or observation. This standardizes the data.

- Add the ratios and divide by the number of measurements to get the mean.

Draw lines for Mean and SDs

(calculated from 20 controls)

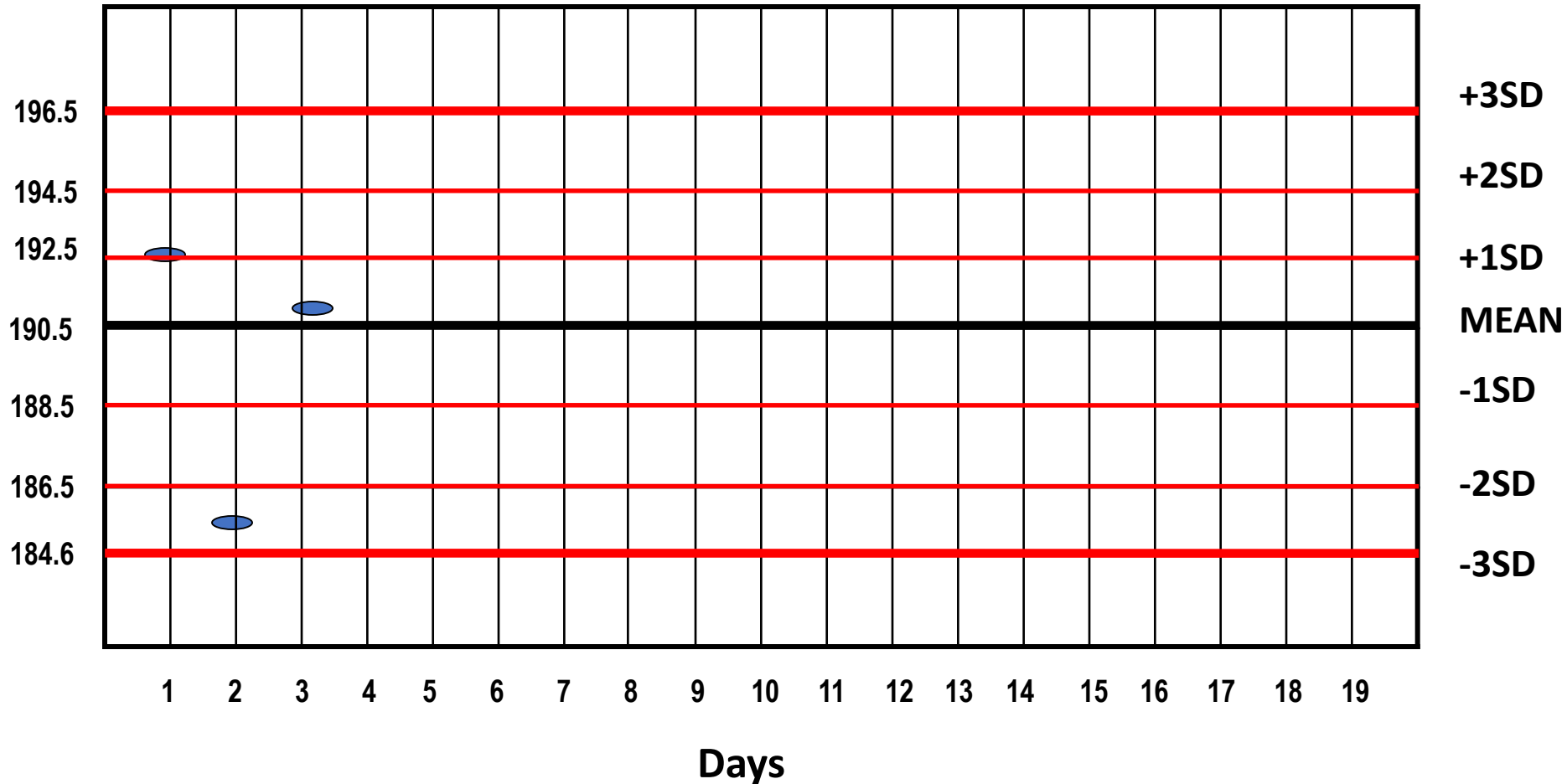**Chart name:**   **Lot number:**

196.5	+3SD
194.5	+2SD
192.5	+1SD
190.5	MEAN
188.5	-1SD
186.5	-2SD
184.6	-3SD
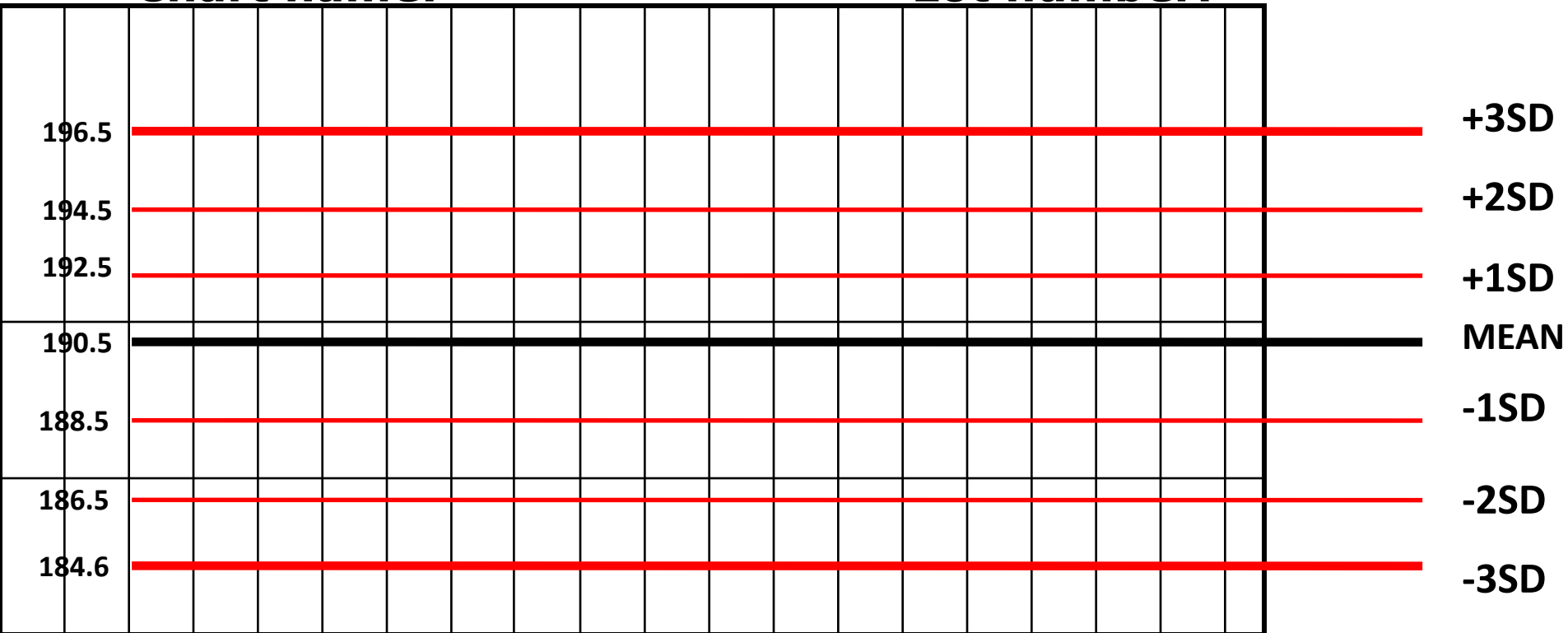
Days

Levey-Jennings Chart

Plot daily control measurements

Number of Controls

Interpretation depends on number of controls run with patients' samples.

- **Good**: If one control:
  - accept results if control is within ± 2SD
    unless shift or trend

- **Better**: If 2 levels of controls
  - apply Westgard multirule system

Frequency of running QC in a lab

- Less than 40 per day - apply at least one level QC once a day.

- Between 40-80 per day - apply two level QC at least once a day.

- More than 80 per day - apply two level QC at least twice a day for such analytes

Detecting error

- **Random error**: variation in QC results with no pattern- only a cause for rejection if outside 2SDs.

- **Systematic error**: not acceptable, correct the source of error
  Examples:
  - **shift**–control on one side of the mean 6 consecutive days
  - **trend**–control moving in one direction– heading toward an "out of control" value
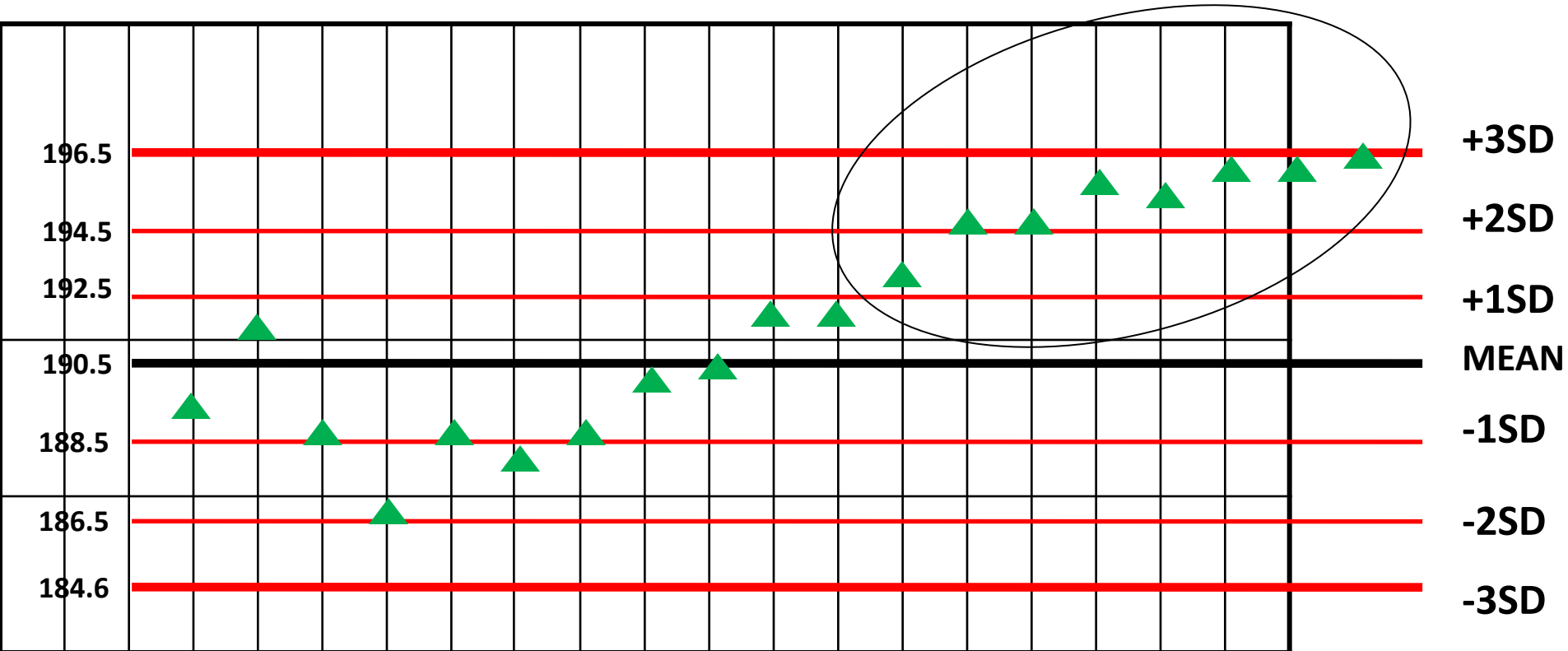
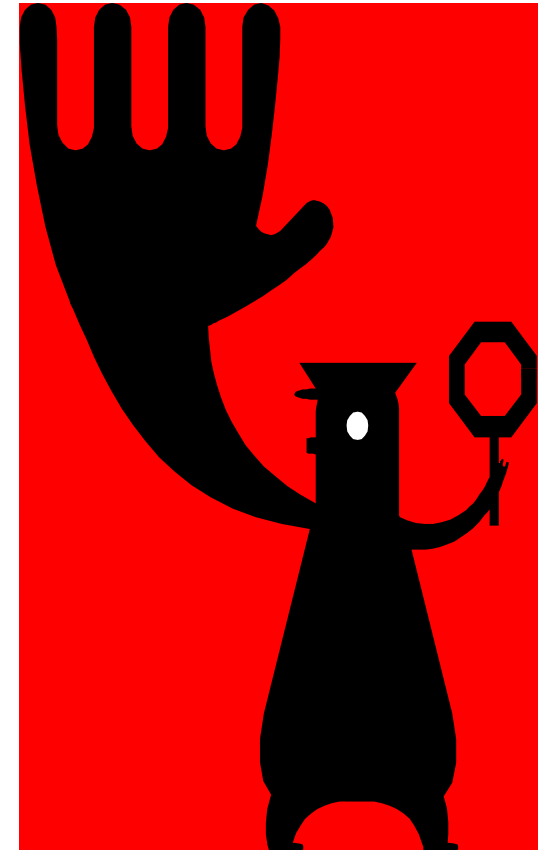# Levey-Jennings Chart
Shift
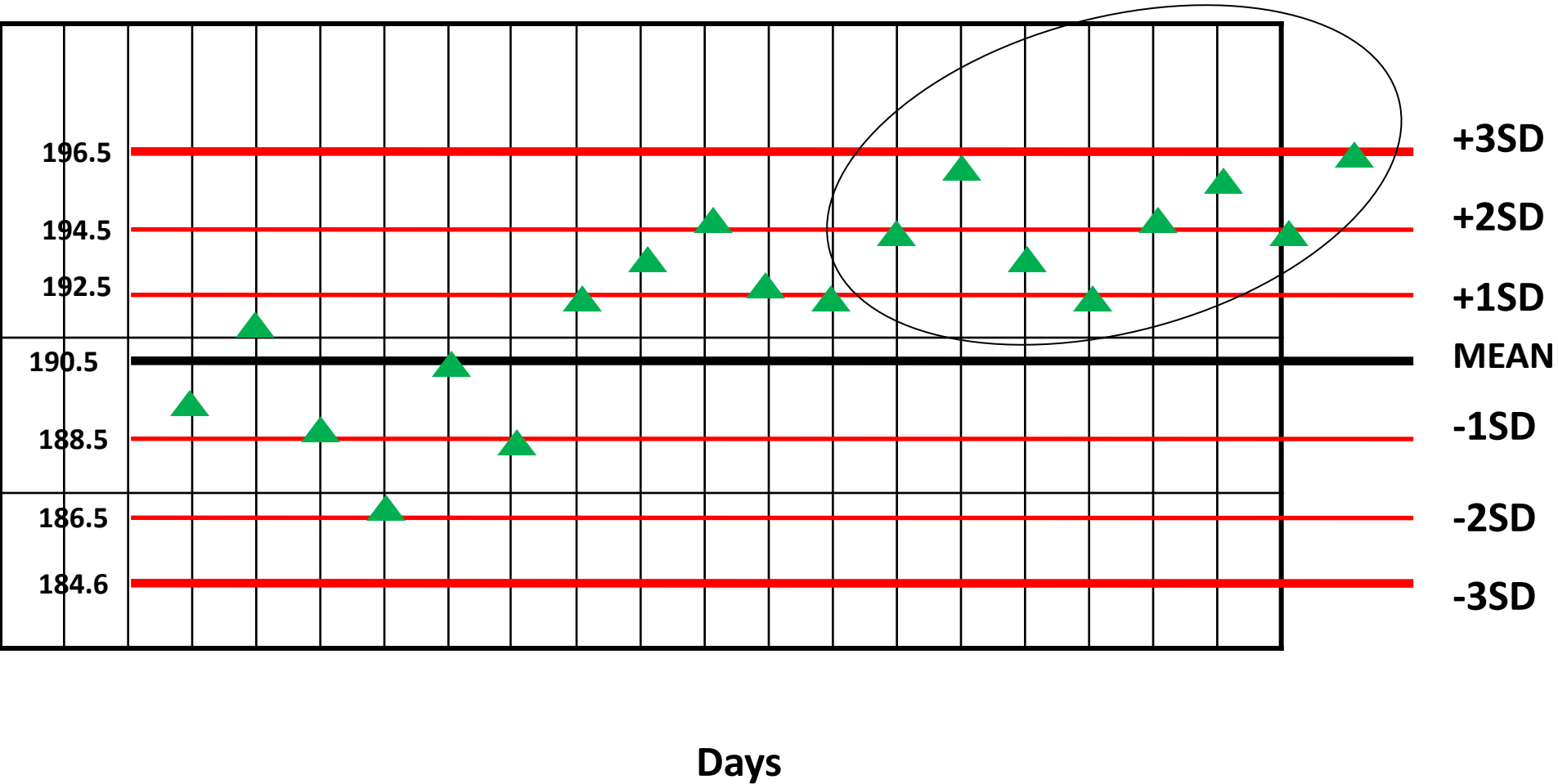
Levey-Jennings Chart

Trend

Days

If QC is out of control

- **STOP testing**

- identify and correct problem

- repeat testing on patient samples and controls after correction

- **Do not report patient results** until problem is solved and controls indicate proper performance

Possible Problems

- degradation of reagents or kits

- degradation of control material

- operator error

- failure to follow manufacturer's instructions

- an outdated procedure manual

- equipment failure

- calibration error

Westgard Multirule System

- a "multi-rule" system developed by

  Dr. James O. Westgard based on statistical concepts

- a combination of decision criteria or

  rules to assess if a system is in control

- use when at least 2 levels of control

  are run with the examination run

- cannot use with only one control

Westgard Multi-rule System Titles

- 1_{2S} rule
- 1_{3S} rule
- 2_{2S} rule
- R_{4S} rule
- 4_{1S} rule
- 10_X rule

Used when 2 levels of control material are analyzed per run.

Westgard – 1$_{2S}$ Rule

- **WARNING RULE** – not cause for rejecting a run
- One of two control results falls outside ±2SD
- Alerts technologist to possible problems
- Must then evaluate the 1$_{3S}$ rule

Westgard – 2_{2S} Rule

- Two consecutive control values for the same level fall outside of ±2SD in the same direction

or

- Both controls in the same run exceed ±2SD

- Patient results cannot be reported

- Requires corrective action

Westgard – R$_{4S}$ Rule

- One control exceeds the mean by –2SD, and the other control exceeds the mean by +2SD

- The range between the two results will therefore exceed 4 SD
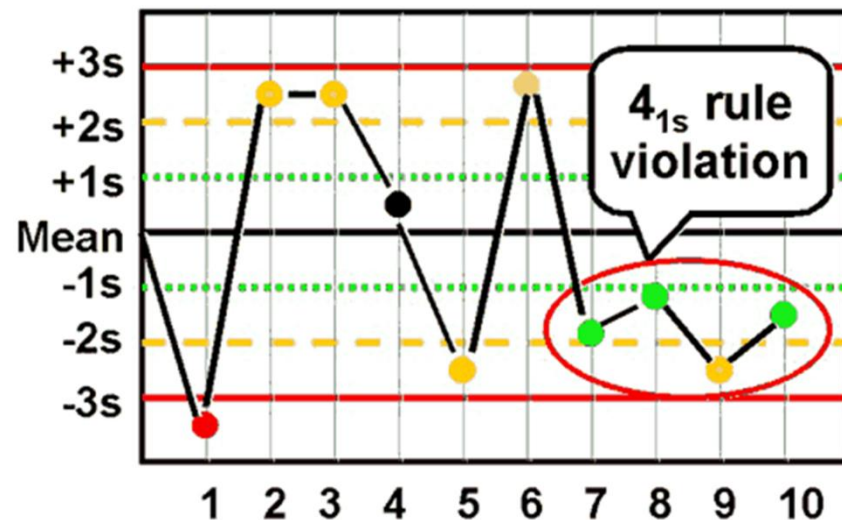
- test run must be rejected
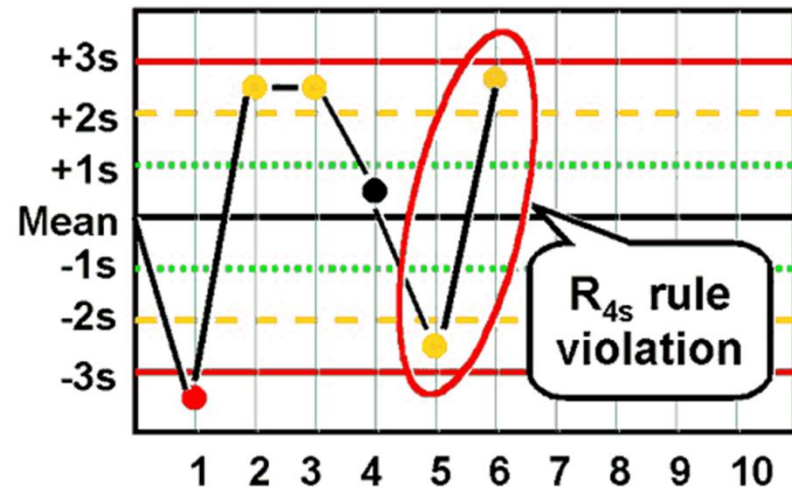
Westgard – 4_{1s} Rule

- requires control data from previous runs

- four consecutive QC results for one level of control are outside ±1SD

                                         or

- both levels of control have consecutive results that are outside ±1SD
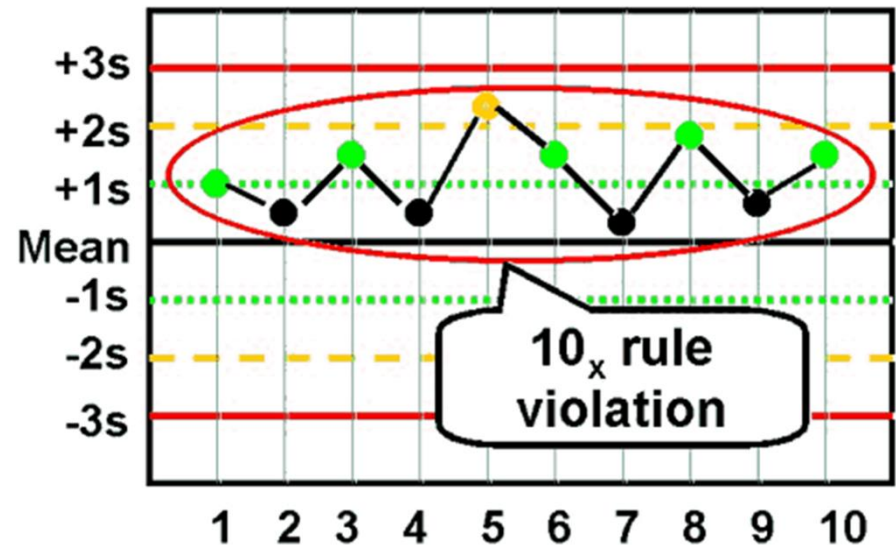
Westgard – $\overline{10}_X$ Rule
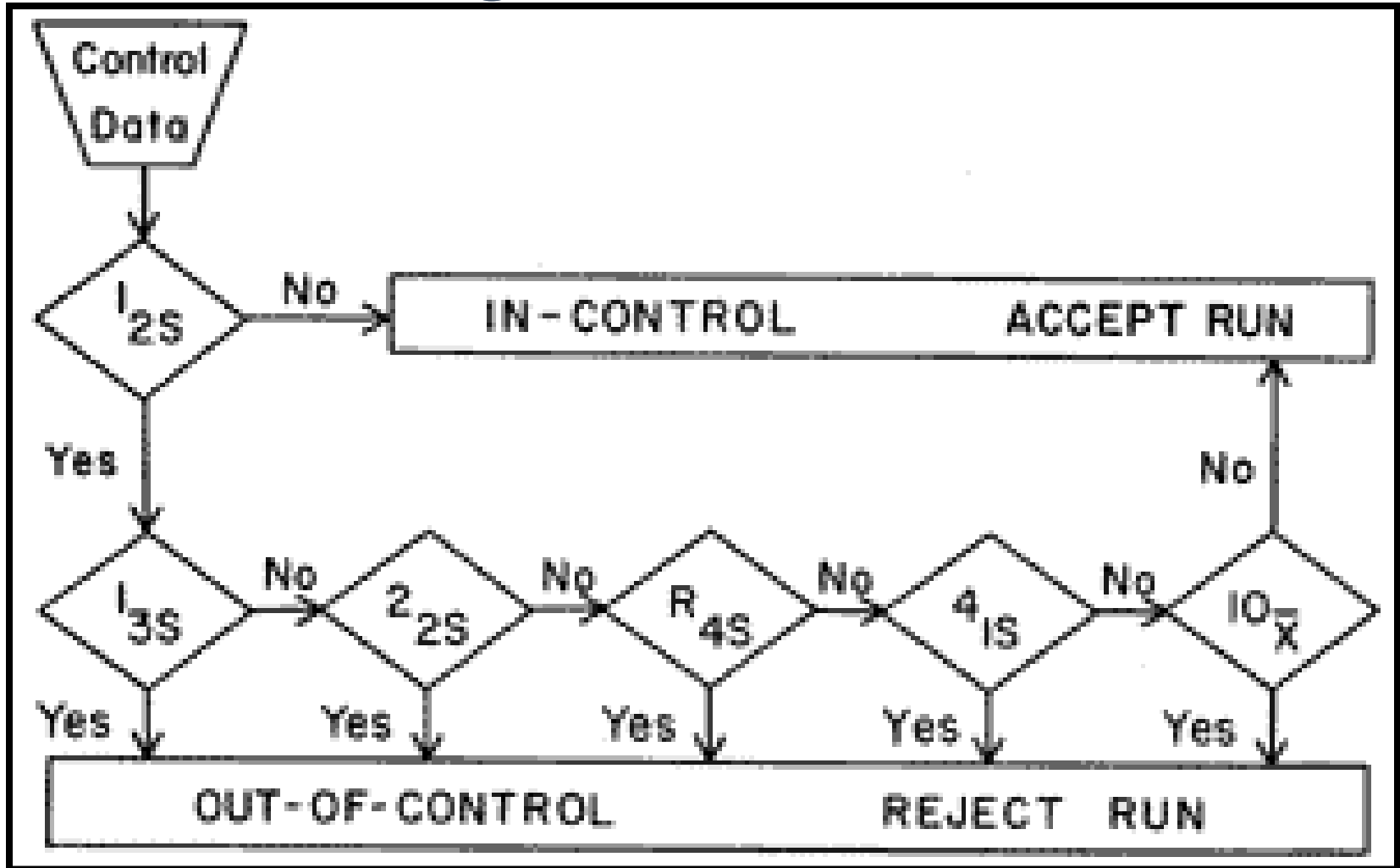
- requires control data from previous runs

- ten consecutive QC results for one level of control are on one side of the mean

                                    or

- both levels of control have five consecutive results that are on the same side of the mean

Westgard Multirule QC

QAP Summary

- Establish QC Policy & QC Procedure
- Secure QC material supply for several months, preferably for one year with
- Same lot number for both normal & abnormal QC
- Construct LJ charts and plot daily QC values
- Scan LJ charts for trends and shifts
- Define "out-of control limits" and corrective actions
- Participate in EQA programmes
- Evaluate IQC & EQA reports once a month towards method / analyzer modifications
- Evaluate the whole QC programme once a year for its effectiveness, cost and areas needing attention

THANK YOU!