Quality Control/Assurance

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LEARNING OBJECTIVES

After the end of the whole series of lectures, you should be able to:

- Know what quality assurance/control is & its importance in the clinical laboratory
- Know the different phases of clinical laboratory testing process
- Know the types of laboratory errors
- Know quality control at the analytical phase and its types
- Know the use of Levey-Jennings control chart in clinical laboratory
- Know method evaluation in clinical laboratory

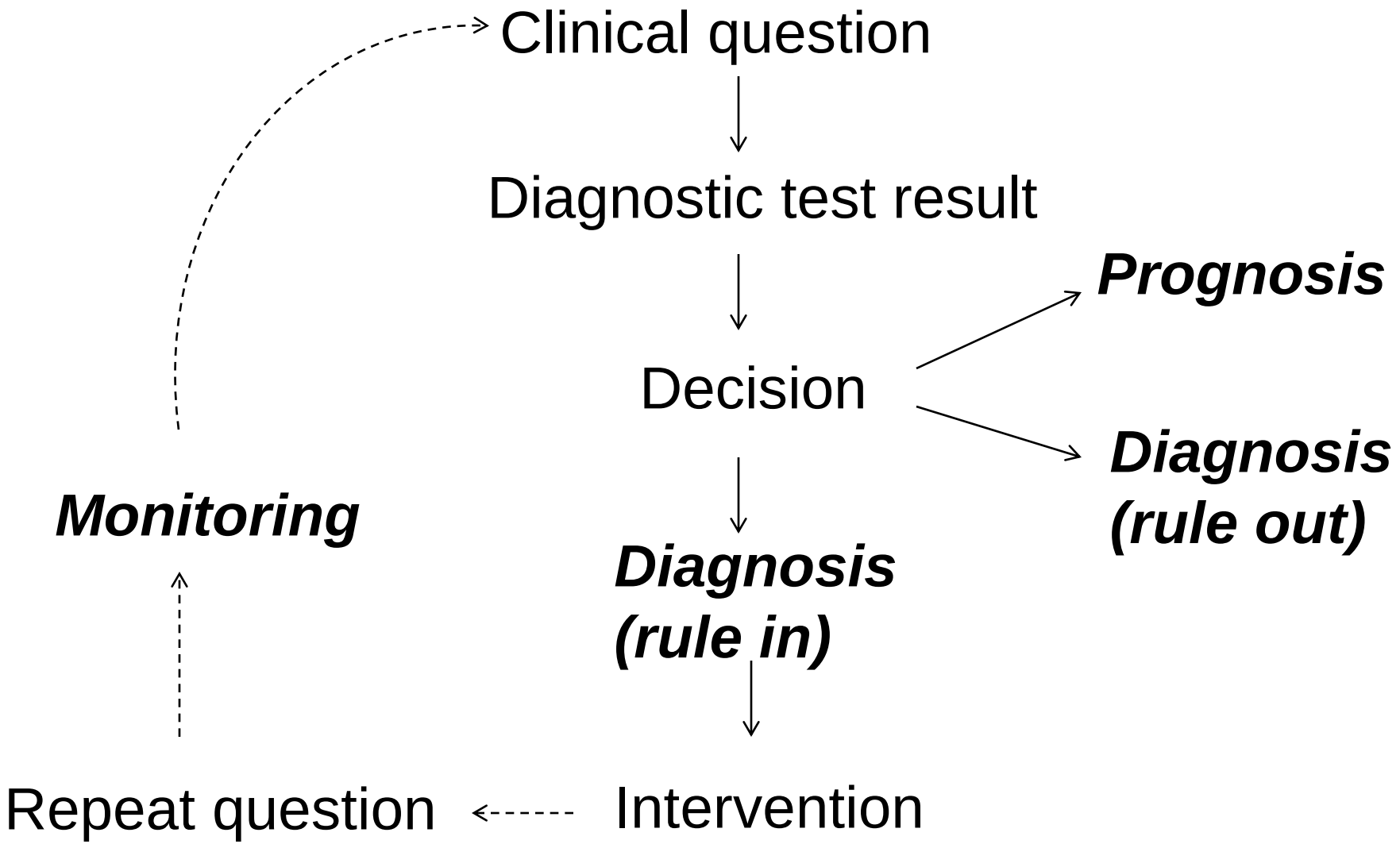
- **What is quality?**

  Conformance to the requirements of users or customers and the satisfaction of their needs and expectations

- In laboratory, who are the users?

- What are they requiring from the laboratory?

Test Result vrs Decision



Roles of Clinical Laboratory Tests

Laboratory tests are important for:

- Diagnosis of diseases (rule in or rule out)
- Monitoring of diseases
- Prognosis of diseases
- Screening of diseases
- Confirmation of previous normal/abnormal test
- Research purposes/education of medical scientists
- Medico-legal purposes

Clinical Laboratory Tests

- **Types of Tests**
  - Core or routine tests
  - Specialized tests
  - Emergency tests

- Laboratory patients' results are essential: WHY?

  60-70% of **<u>all important medical decisions</u>** on:

A. Admissions;
B. Medications; and
C. Discharge

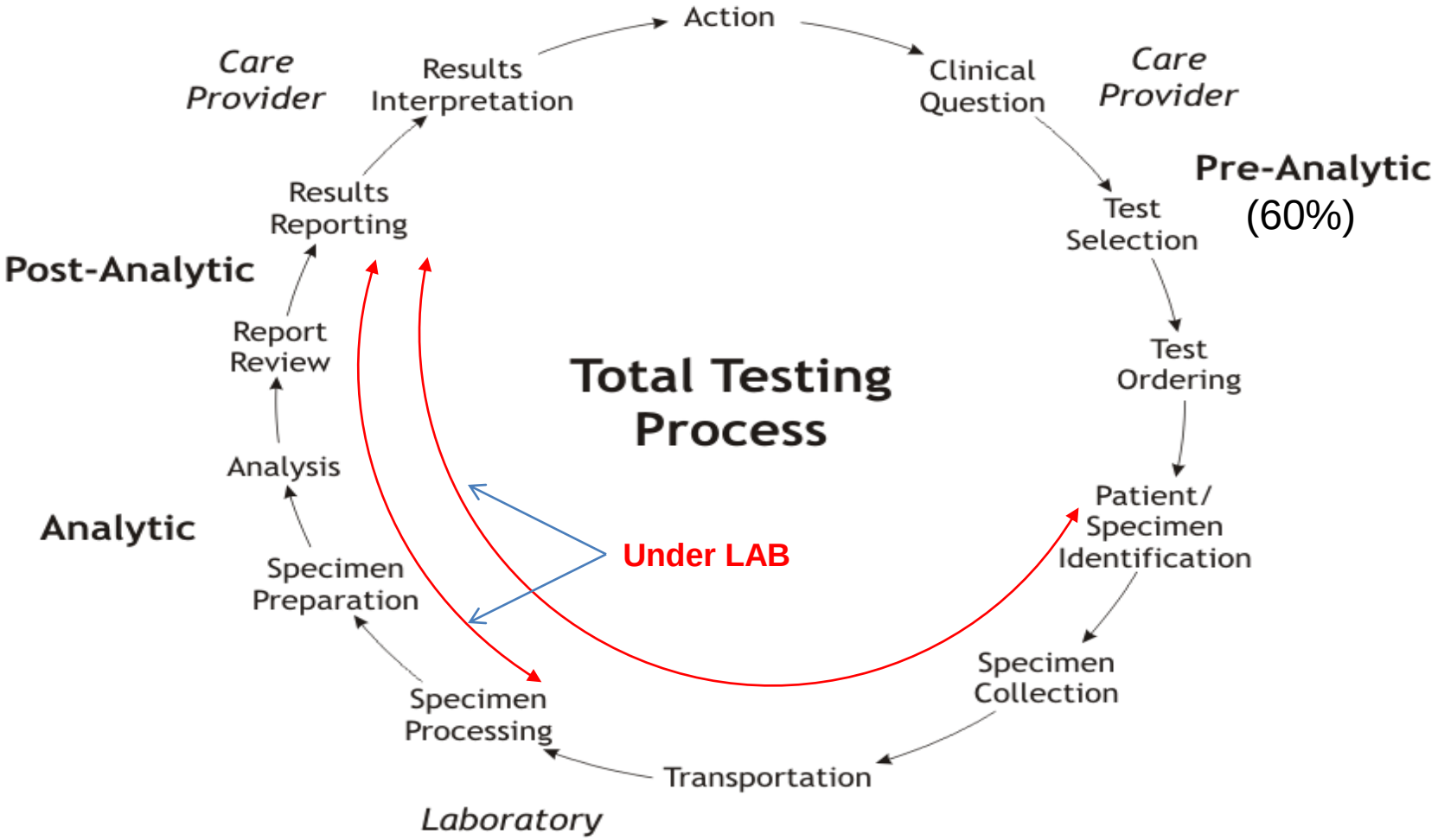
} Are based on **evidence from laboratory results**

Total Testing Process

The total laboratory testing process consists of 3 phases:

1. **Pre-analytical phase:** specimen collection, transport, and processing *(and storage)*
2. **Analytical phase:** testing/analysis of sample
3. **Post-analytical phase:** test results transmission, interpretation, follow-up, retesting

Total Testing Process



Reliability of Laboratory Results

- To ensure reliability of a laboratory's results, an effective **quality assurance** system should be implemented and monitored throughout the entire testing process

- What is **quality assurance?**

- And what is **quality control?**

Quality Assurance & Quality Control

- **Quality Assurance (QA):** is all of the procedures, actions and activities that take place to ensure that the final results reported by the laboratory are correct/accurate

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

- "The aim of quality control is simply to ensure that the results generated by the test are correct. However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time"

Quality Control

- Quality control: there are two types - internal quality control and external quality control (e.g. IEQAS - UK)

- What is internal quality control?

- What is external quality control?
  - Proficiency testing/interlaboratory comparison???

Quality Control: Terms

Some terms to understand:

- **True value** – an ideal concept, which cannot be achieved

- **Accepted True value** – The value approximating the ‘True Value’; the difference between the two values is negligible

- **Error**
  - Error is the discrepancy between the result obtained in the testing process and its ‘True Value’/‘Accepted True Value’

Types of Error

1. Pre-analytical error
2. Analytical error
3. Post-analytical error

Pre-Analytical Errors

- Errors which occur in the pre-analytical phase or before the analysis of the samples
- Contribute to about **75%** of total laboratory errors
- Most difficult to monitor and control since most of them occur **beyond** the laboratory
- However, through **Quality Assurance** measures, the laboratory should try to maintain control over these factors

Pre-Analytical Errors

- Errors could come from all the following variables:
  - Patient preparation
  - Patient identification
  - Site selection/preparation
  - Tube/needle selection
  - Tourniquet placement/time
  - Order of draw
  - Specimen labelling/identification
  - Specimen handling/processing
  - Specimen transport

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THE INFORMED PATIENT

By LAURA LANDRO

Hospitals Move to Cut Dangerous Lab Errors

Improved Specimen Collection And Efficiency Help Increase Accuracy of Medical Testing

June 14, 2006; Page D1

Pre-Analytical Errors

Patient preparation

- Laboratory tests are affected by many factors (diet, alcohol, drugs, smoking, exercise, stress, and posture);
- Example - Some tests:
  - Require fasting for at least 12 hours prior to venipuncture, e.g glucose (FBS) and Lipid profile
  - have diurnal variations, e.g. cortisol and adrenocorticotropin (ACTH), where the analyte is at its highest level in the morning, and the levels gradually decrease during the course of the day.
- The laboratory must define the instructions and procedures for patient preparation and specimen acquisition
- These should be included in hospital procedure manuals

Pre-Analytical Errors

Specimen Identification/Labelling

- Tubes must be labelled in front of the patient
    - At bedside
    - At phlebotomist chair
- Label must be permanently attached & contain:
    - full name
    - Age
    - Date, time as required e.g. TDM, OGTT
    - Blood collector's initials

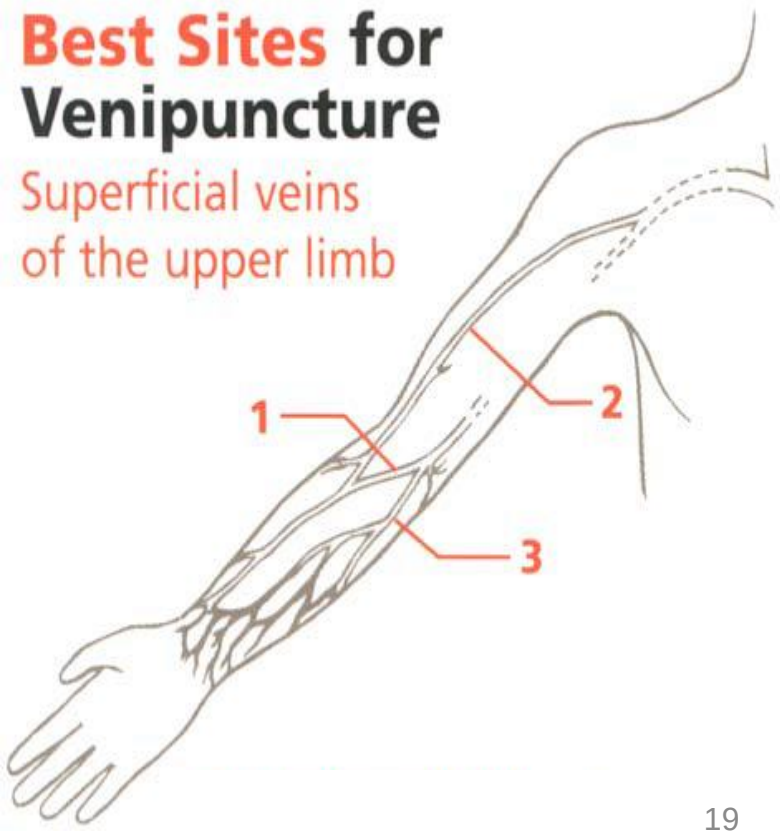
Impact

- Recollection, Reanalysis, Misdiagnosis, Labour costs

Site Selection

Common veins used for venipuncture:

1. Median Cubital (preferred site)
2. Cephalic
3. Basilic



Site preparation

- Cleanse with:
    - 70% Alcohol.....why???
- Cleansing should be air-dry
    - Haemolysis
    - Patient discomfort
- Cleansing should be done in circular motion & outward from site

Avoid These Sites

Venipuncture should be avoided from:

- An arm with IV infusion
- Side where mastectomy was performed; lymphostasis affects blood composition
- Area where edema is present
- Scarred or sclerosed veins
- Extremity that appears bruised, reddened, swollen or infected
- Extremity that has a dialysis shunt or fistula
- Above a cannula

Needle Selection

- 19-23 gauge most commonly used*
- 22-23 gauge in children*
- Right selection of needle avoids haemolysis

*Clinical and Laboratory Standards Institute (CLSI)

Tourniquet placement/time

- Place 3 to 4 inches above the venipuncture site
- Maximum time for tourniquet on arm: **1 minute**
- Release tourniquet as soon as blood starts to flow into first tube
- Haemoconcentration & venous stasis after 1 min and up to 3 mins
  - Can increase
    - serum protein & protein-bound analytes (5-15%)
    - lactic acid
- Avoid pumping the fist
  - ↑K, Phosphate, lactate, ionized calcium
- Reverts to normal within 10 mins after tourniquet removal

Order of Draw

Order of draw (CLSI recommendation):

- Blood culture tube
- Coagulation tube (e.g. citrate, blue stopper)
- Serum tube with or without clot activator, with or without gel (e.g. red stopper)
- Heparin tube with or without gel plasma separator (e.g. green stopper)
- EDTA (e.g. lavender stopper)
- Glycolytic inhibitor tube (e.g. gray stopper)

Mixing of specimens

- Invert tubes a number of times & gently as recommended by the manufacturer immediately after sample collection, examples:
  - Invert SST 5 times
  - Invert sodium citrate tubes 3 to 4 times
  - Other additive tubes invert 8-10 times

Consequences if not mixed

- Tubes with anticoagulants will clot
- SST tubes will not clot completely
- Specimen will often need to be redrawn
- Improper mixing

  Microclots, clotted specimens

  Haemolysis

Handling of Specimens

Some samples need to be handled with special care

- Light-sensitive analytes include:
    - Bilirubin
    - Carotene
    - Vitamin A
    - Vitamin B6
- Transport light-sensitive specimen wrapped in Al foil

Transportation of Samples

Mode of transportation and time

- **By Hand or Courier service**
  - Adequate packaging/handling to ensure constituent stability for the tests requested
  - Transport conditions too hot/too cold (E.g. In hands, inside pockets, etc)

Time is important

  - Rapid transport and short storage times improve the reliability of laboratory results

Sample Processing

Allow clot formation: Blood with no coagulation abnormalities clots in 45 min.+/- 15 min

- Blood from patients on anticoagulant therapy or with coagulopathies takes longer to clot

Centrifugation

- After complete clotting of sample or maintenance of anticoagulation
- Centrifugation and separation of cells from serum/plasma
- Centrifuge between 1100 and 1300 g for 10 mins for swing head units or 15 mins for fixed angle rotor unit
- Impact: Recentrifugation (delay):
  - ↑K ; ↓Glucose

Maintenance of Samples

Maintenance of serum/plasma samples

- Separated serum/plasma should remain at room temperature for no longer than 8 hours
- If assays will not be completed within 8 hours, refrigerate at 2 – 8ºC
- If assays are not completed within 48 hours, freeze at -20ºC

Whole Blood

- Plasma/serum should be separated from contact with cells within recommended time limits*

*CLSI recommends a maximum limit of 2 hours from time of collection

Tube Orientation & Closure

Tube Orientation

- It is recommended to place tubes of blood in a vertical closure-up position for delivery to laboratory

Tube Closure

- Tubes of blood are to be kept closed at all times
  - ↑ in pH due to loss of CO_2
  - Increase in pH, ↓ionized calcium, acid phosphastase
- Eliminates exogenous contamination of specimen
- Prevents evaporation, spills, aerosols

Pre-Analytical Error

Quality assurance measures such as:

- Well developed standard operating procedures (SOP) for sample collection
- Well trained phlebotomy staff/employing qualified professionals (e.g. BMS) or in-service training of staff
- Use of easy patient & specimen identification methods (such as bar code identification?)
- Willingness to be information resource and/or trainers for physicians and floor personnel often involved with specimen collection

Post-Analytical error

- Errors that occur after the analysis of the specimen
  - Incorrect data entry of lab results
  - Physician not notified of a panic or critical value
  - Incorrect interpretation of lab results by physician
  - Incorrect reference values

- **Quality Assurance** measures must be implemented if problems identified

- **NB: Reading assignment:** How is reference/normal range established?

Analytical Errors

- Errors which occur during the analysis of samples
- It could be classified into two:
  1. Random errors/imprecision/scatter
  2. Systematic errors/inaccuracy (bias)
- Huge improvement in management of these errors because of technological advancement in laboratory instrumentation and quality control measures

Analytical Error

Random Error

- An error which varies in an unpredictable manner and causes variation in repeated estimates or measurements

- Random errors cause imprecision/scatter in results
- Examples:
  - Faulty technique (incorrect/ variable pipetting, inadequate mixing of sample with reagents, inconsistent incubation)
  - Fluctuating/erratic instruments due to unreliable electricity supply
  - Presence of interfering substances e.g. RBC
  - Dirty glassware & equipment

Analytical Error

Random Errors (Cont)

Examples:

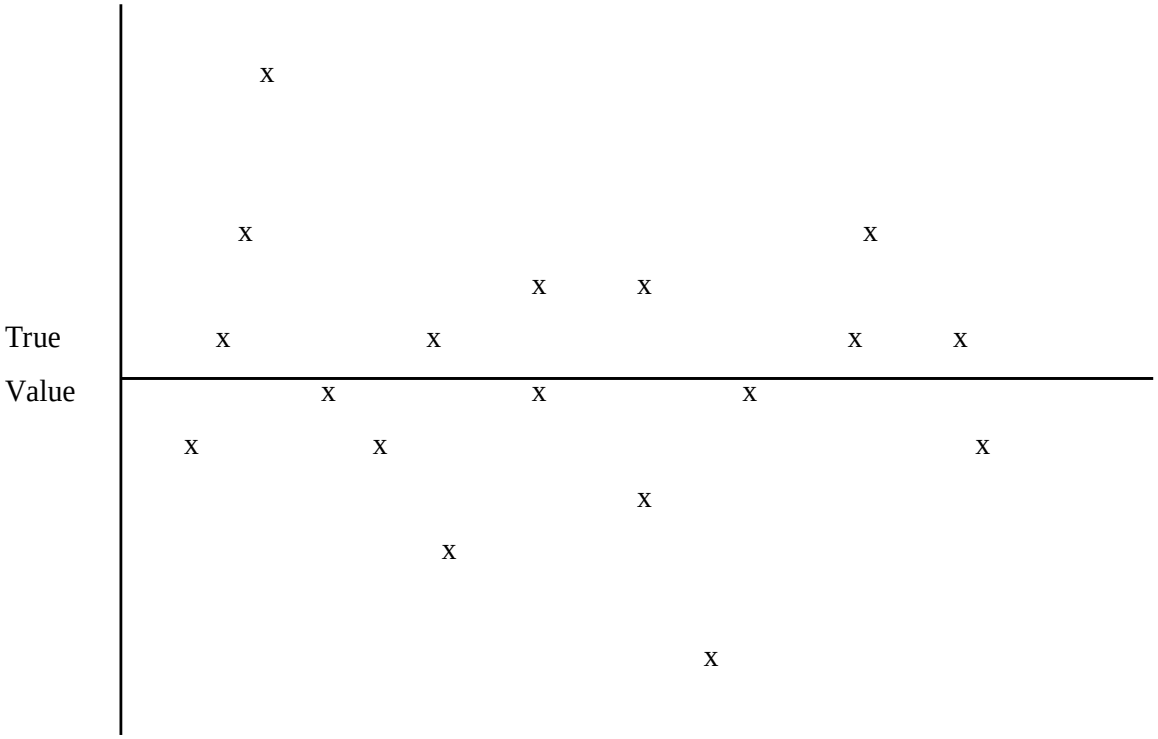
- Dirty or finger marked cuvettes or reading absorbance when there are air bubbles

- Heavy work schedules resulting in short cuts or mistakes

- Low workload resulting in loss of concentration

Analytical Errors

Random Errors



Analytical Errors

Systematic Error (SE)

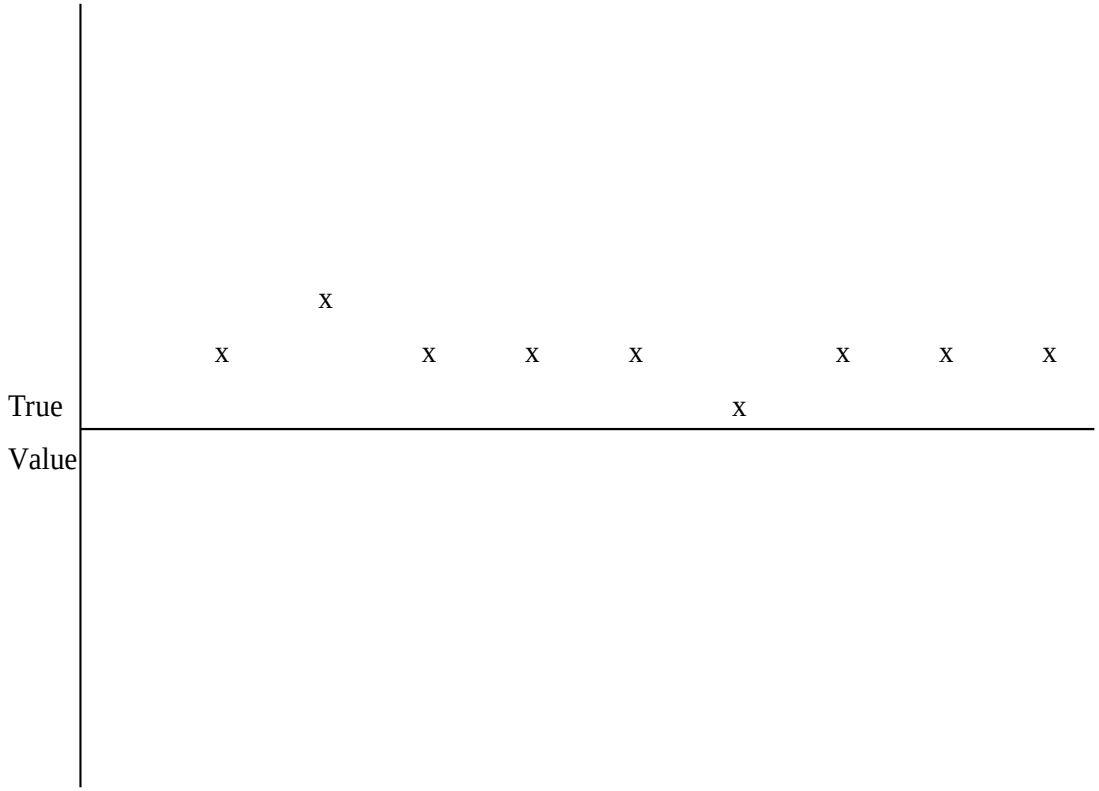
- An error, which in the course of a number of measurements of the same value of a given sample, remains constant **(i.e. Constant SE)** or varies in a predictable way **(i.e. Proportional SE)**
- Systematic errors create a characteristic bias in the test results and can be accounted for by applying a correction
- Systematic errors cause inaccuracies in test results

Examples:

- Use of unsatisfactory reagents
- Incorrect or infrequent calibration of a test method
- Use of inappropriately prepared, stored or expired control sera/calibrator
- Reading of tests at incorrect wavelength

Analytical Errors

Systematic Errors



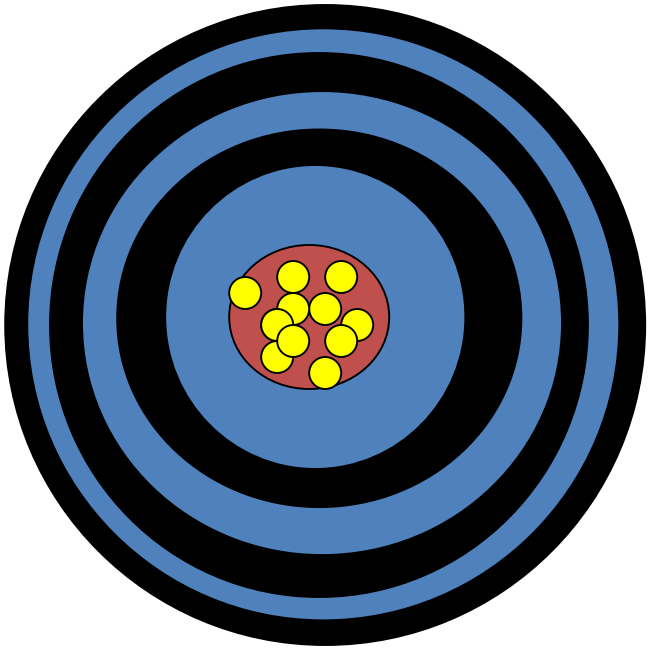
Analytical Error

- Random errors affect precision
  - Precision: Closeness of agreement between quantity values obtained by replicate measurements of a quantity, under specified conditions; thus, how well a series of replicate measurements agree with each other

- Systematic errors affect accuracy
  - Accuracy is the closeness of the agreement between the measured value of an analyte and its ‘true’ value

Analytical Error

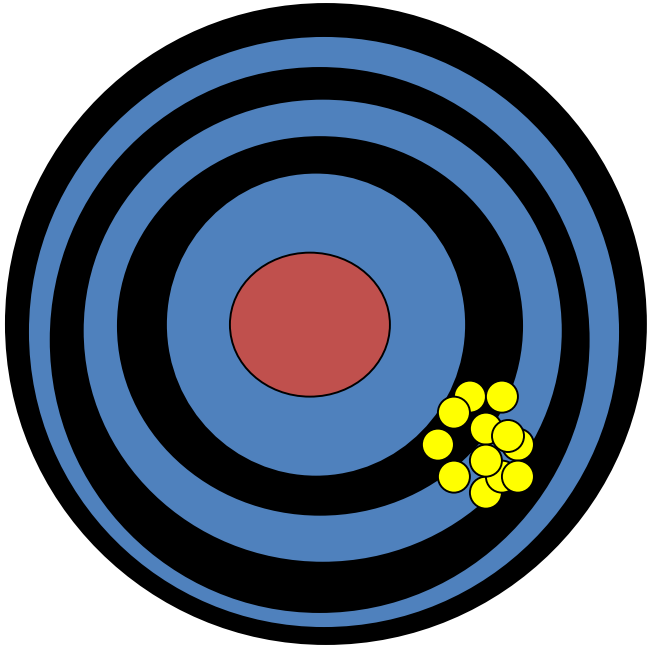
Precise and Accurate



Analytical Error

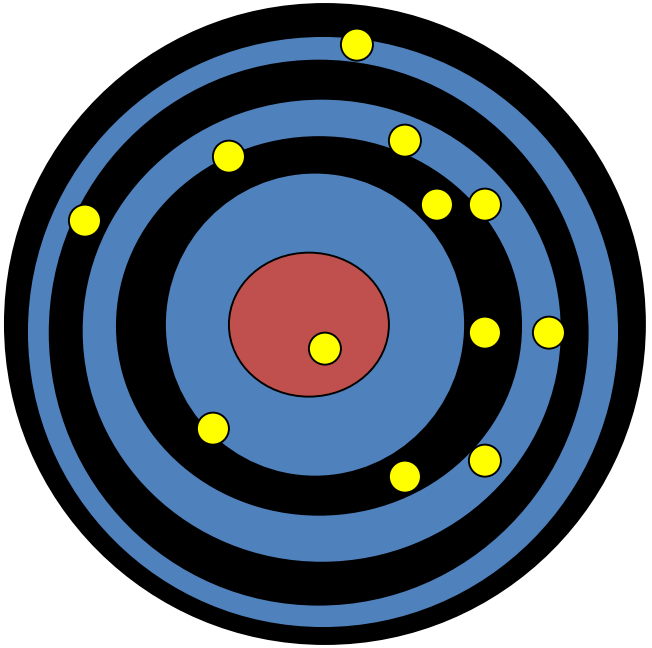
Precision and Accuracy

- **Precise and inaccurate**



Systematic Error (SE)

- **Imprecise and inaccurate**



Random Error & SE

Statistical Control of Analytical Errors/Methods

- Performance of analytical methods is typically monitored through analysis of ***specimens with known concentrations*** **(i.e. controls)** and followed by ***comparison of observed values with the known values***

- The known values are usually represented by **interval of acceptable values** or **lower and upper control limits**

- These are **INTERNAL quality control (QC) measures**

Statistical Control of Analytical Errors/Methods

Purposes of IQC

- "The main objective of internal quality control (IQC) is to ensure day-to-day consistency"(WHO 1981)

There are three purposes of IQC:

1. To monitor the accuracy and precision of the complete analytical process;
2. To detect immediate errors that occur due to test-system failure, adverse environmental conditions, and operator performance; and
3. **To monitor over time** the accuracy and precision of test performance that **may be influenced by changes** in test system performance and environmental conditions, and variance in operator performance

Statistical Control of Analytical Errors/Methods

- **Control Materials**
  - Are specimens analysed for QC purposes
  - Normally resemble the patient sample
    - Have same characteristics as patient sample such as matrix
  - Can be purchased as:
    - **'assayed'** – come with range of established values; more expensive
    - '**un-assayed**' - the lab must use statistical measures to establish their range of values
  - Generally supplied in lyophilized or freeze-dried forms; then reconstitute by adding distilled water/diluent solution
  - Can be internally prepared: But???
  - The control results of any run/analysis must be compared to the 'range of expected' results (established by ……who?) to determine acceptability of the analysis

Statistical Control of Analytical Errors/Methods

Quality Control (QC) Data:

- Collection of QC data
  - For some tests/assays, control results are positive or negative (yes it worked, or no it did not)
  - **For other tests, such as those that produce a digital data result which must be tabulated over a period of time and statistical analysis performed**
    - **Our focus is on this**

Statistical Control of Analytical Errors/Methods

- **Common statistical tools used in QC:**
  - Mean/average
  - Standard deviation (SD)

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

  - Coefficient of variation (CV)
    - **CV% = Standard deviation X 100%**

      mean

Statistical Control of Analytical Errors/Methods

- **Common statistical tools used in QC:**
  - Target/expected values of IQC
    - Find the mean of the QC data; and
    - Find the SD
    - Set the target or expected values
      - Mean±1SD, **2SD**, 3SD

So, how do we determine the range of acceptable QC results for the following QC data?

- Scenario: cholesterol assay
  - Mean of group of control values = 104 mg/dL
  - Standard Deviation = 5 mg/dL
  - Determine the target/acceptable/expected Range or control limits (lower and upper limits) using ± 2SD; (which will allow you to evaluate acceptability of performance of the control on subsequent days)
- Is a control value of 100 mg/dL acceptable? What about 120 mg/dL???
- The target/acceptable range is 94-114 mg/dl

Control Charts

• Control charts are used to compare the observed control values with the control limits/expected control range

•Such charts provide visual display for a quick inspection and review

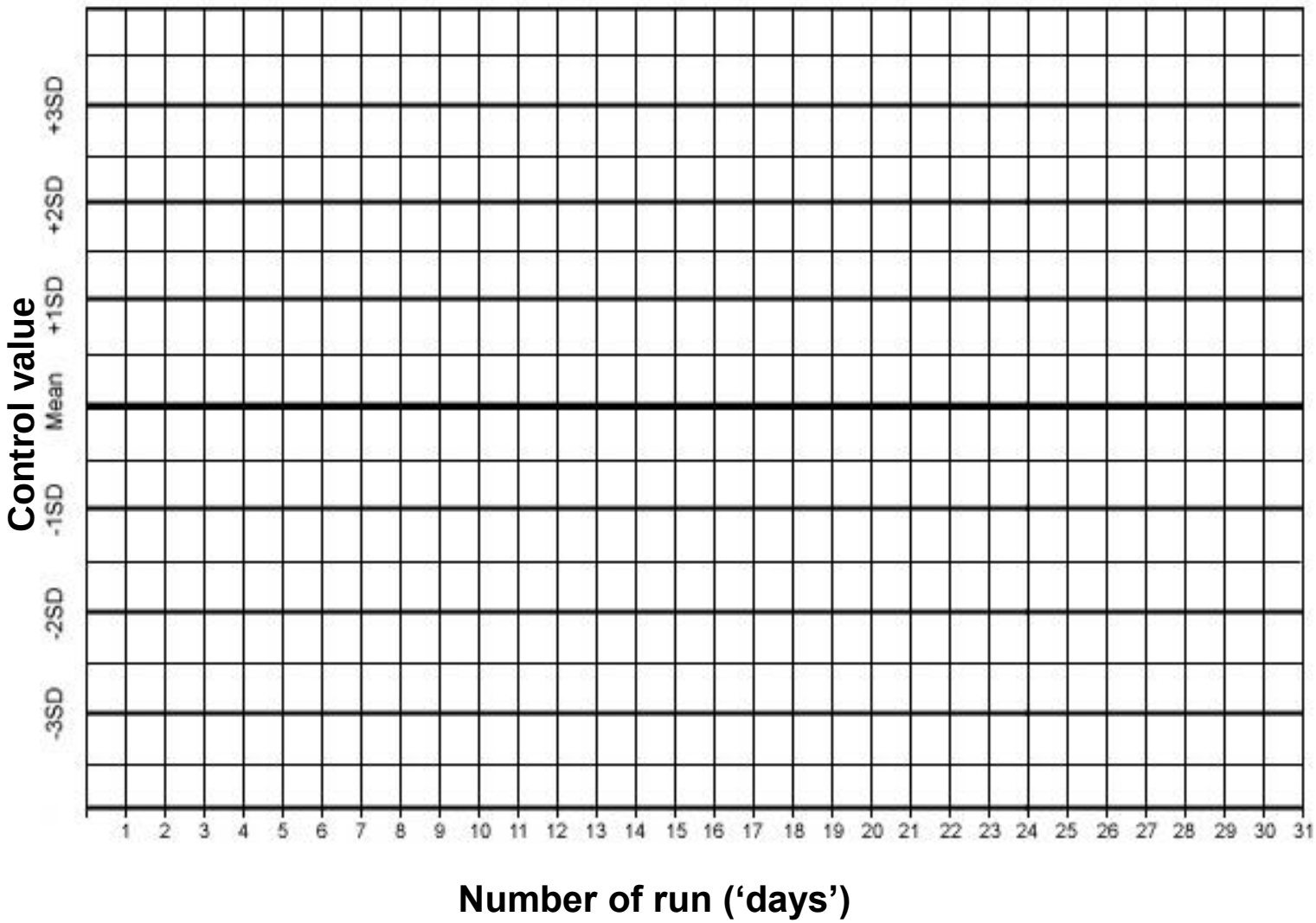
A common one is **Levey-Jennings** Control Chart

Levey-Jennings Control Chart

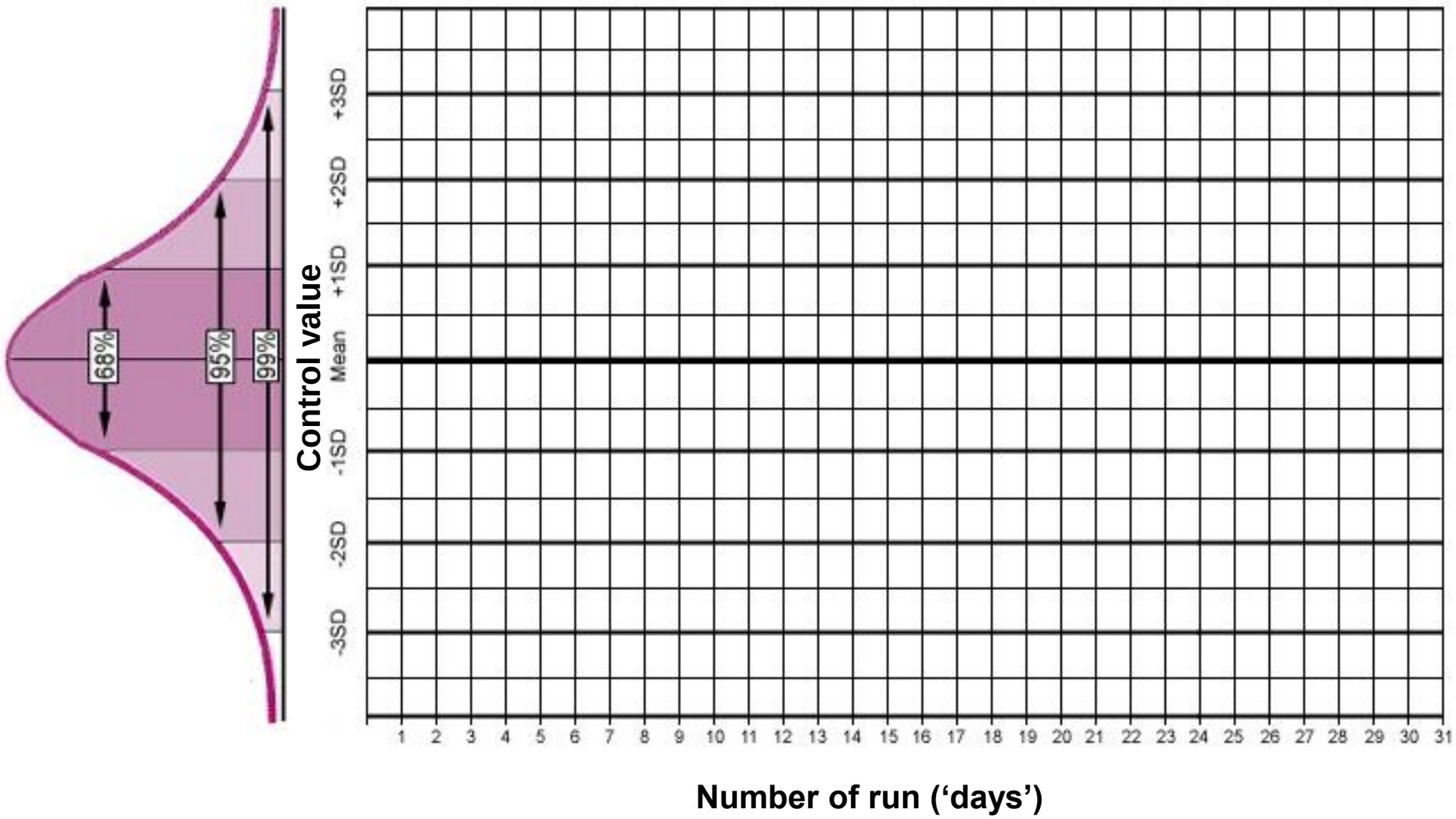
A **Levey-Jennings** control chart makes use of QC specimens/data and is developed in the following manner:-

- Run the IQC specimen for **at least 20 or more** and record down the values
- Calculate the **mean** and **standard deviations (SD)**
- Make a plot with the assay run on the x-axis, and control values on the y axis
- Draw the following lines across the y-axis: **mean; -3, -2, -1, 1, 2, and 3 SD**
- Plot the control results obtained for the IQC specimen for subsequent assay runs

Levey Jennings Control Chart



Levey-Jennings Control Chart



Example: Serum cholesterol (mg/dl)

Mean = 150 mg/dl
SD = 5 mg/dl

Mean = 100 mg/dl
SD = 4 mg/dl

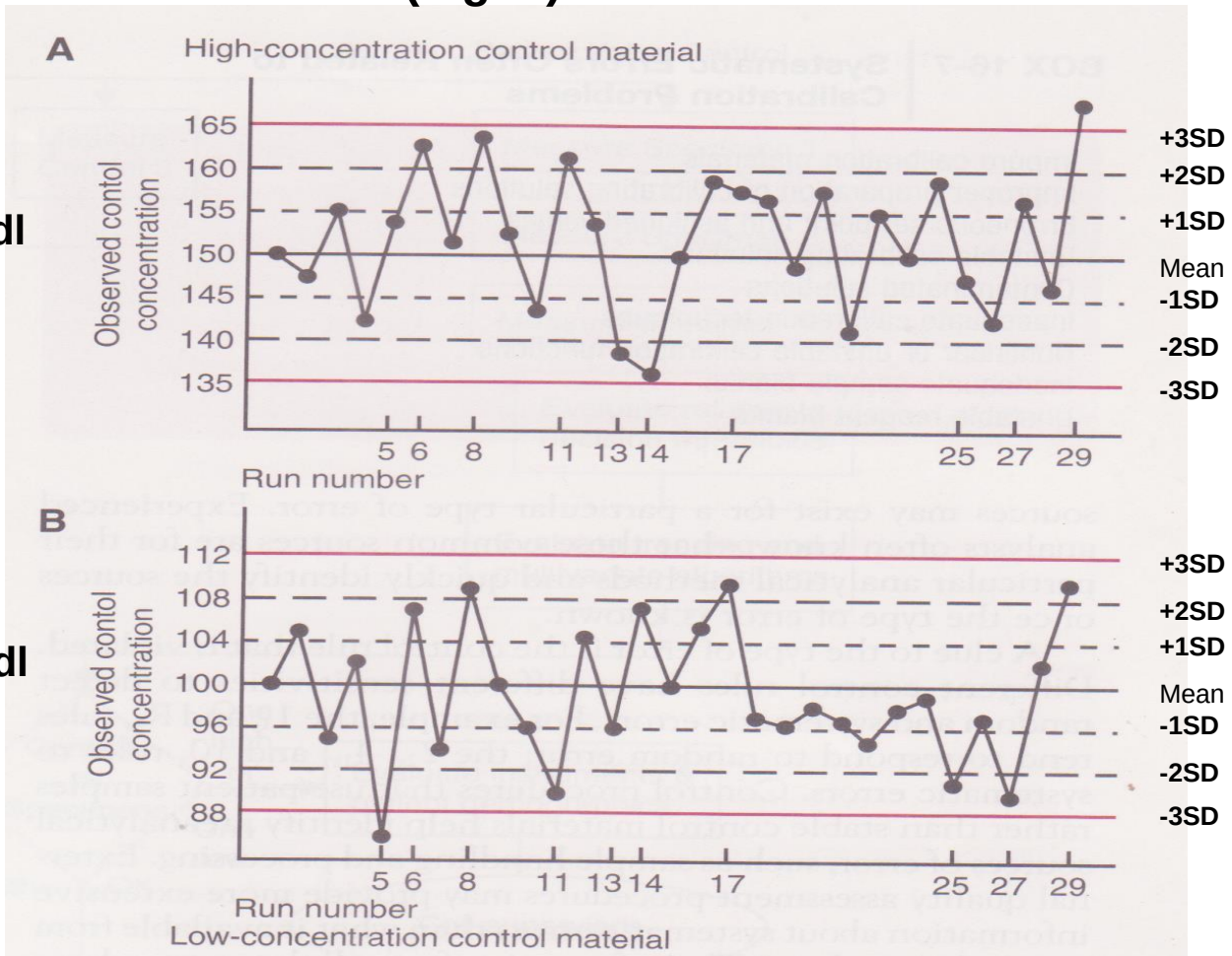
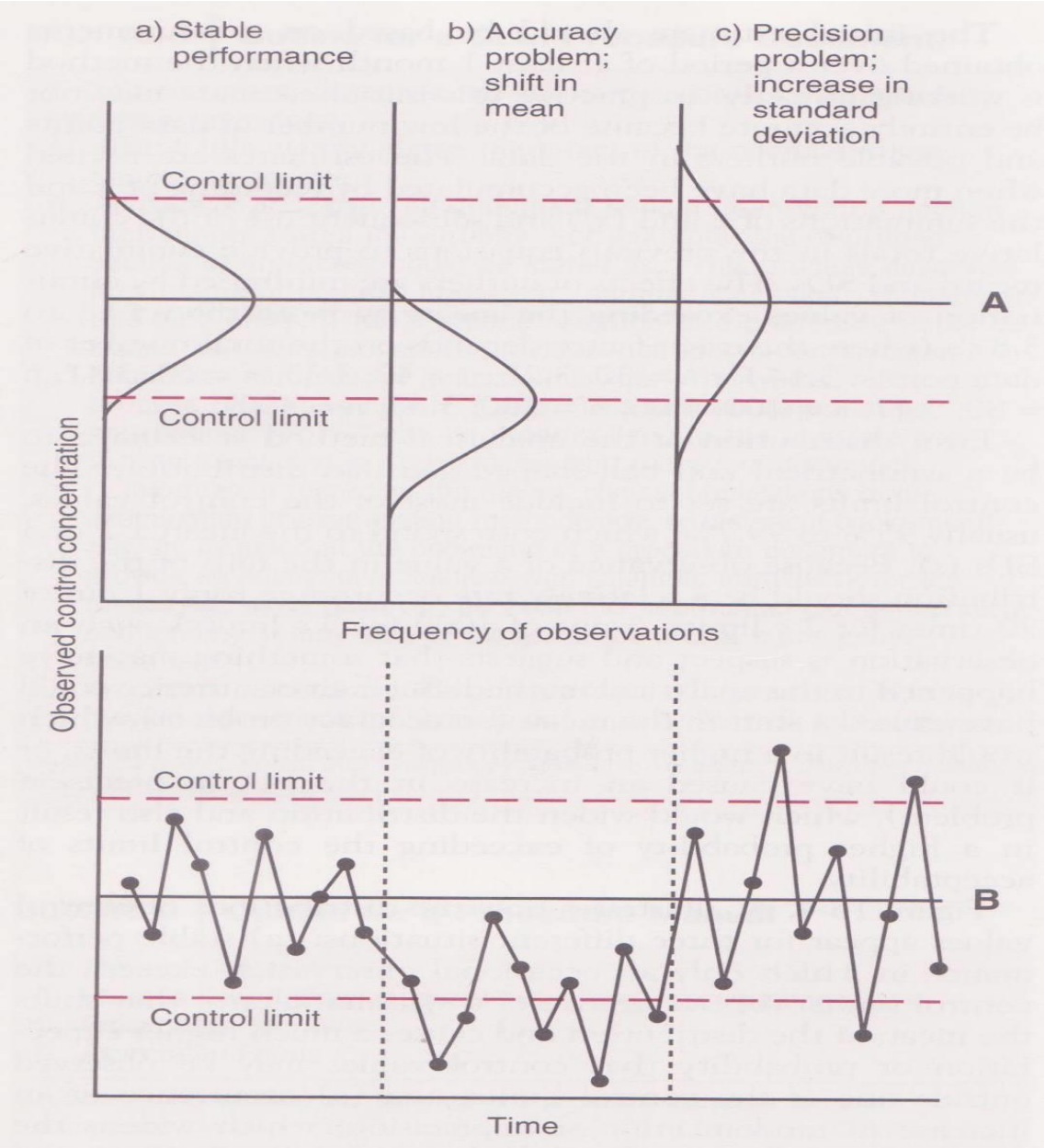


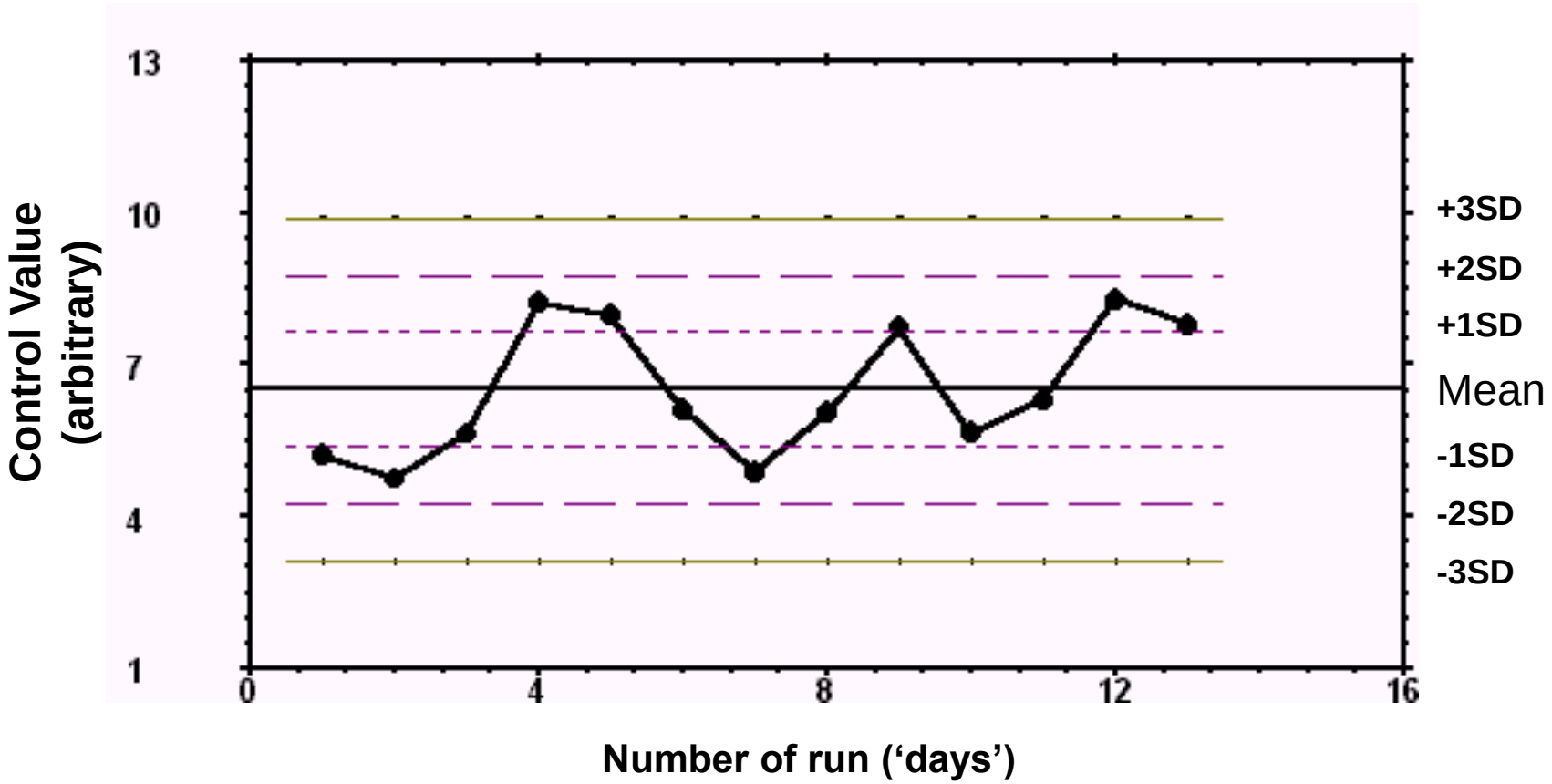
Figure 16-5 Westgard multirule control chart with control limits drawn at the mean ± 1*s*, 2*s*, and 3*s*. Concentration is plotted on the *y*-axis versus time (run number) on the *x*-axis. **A,** Chart for high-concentration control material. **B,** Chart for low-concentration control material. *s*, Standard deviation. (From Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem 1981;27:493–501.)

Example


a) Stable performance
b) Accuracy problem; shift in mean
c) Precision problem; increase in standard deviation
Control limit
Control limit
A
Observed control concentration
Frequency of observations
Control limit
Control limit
B
Time

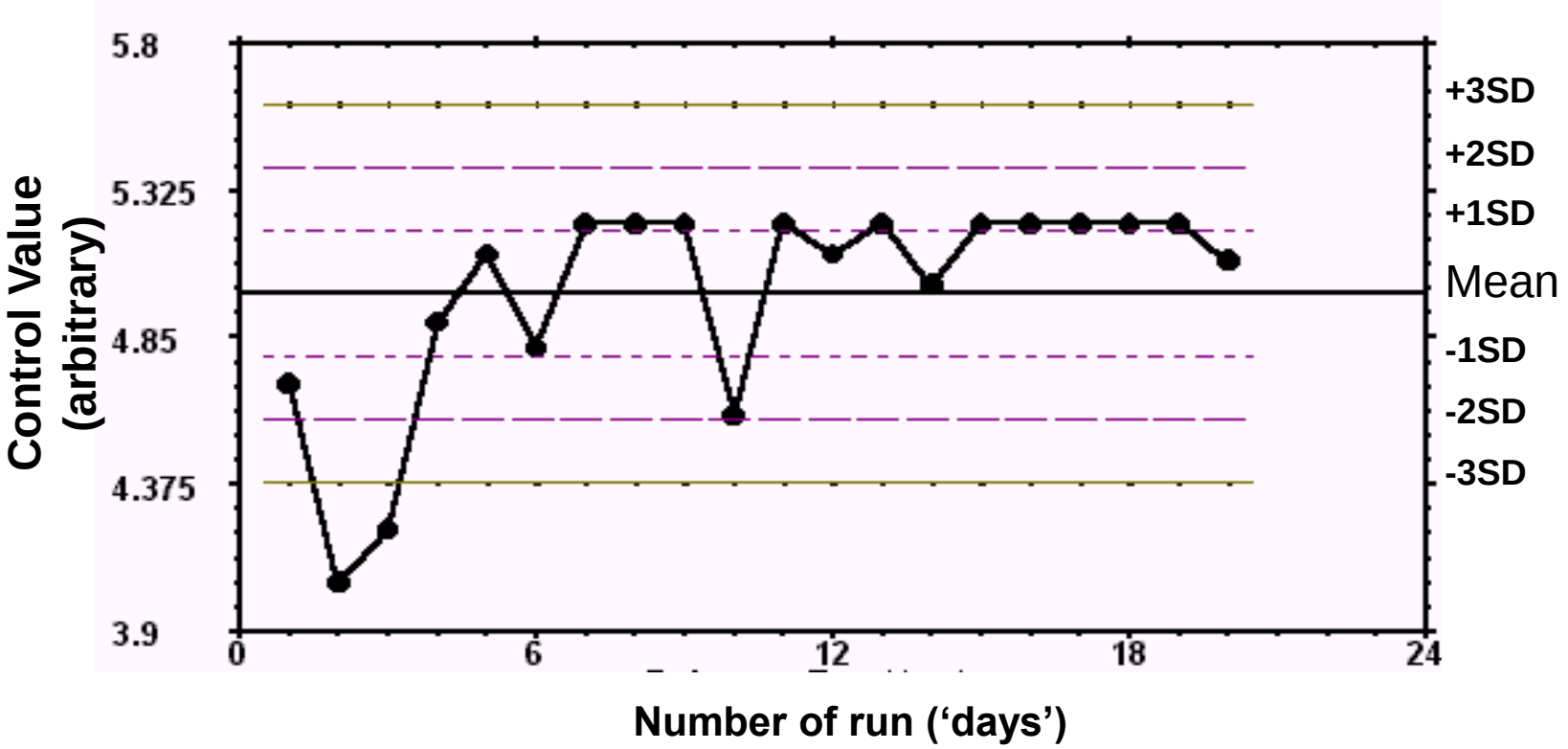
Levey-Jennings Control Chart

What does the normal pattern look like?
(random dispersion)



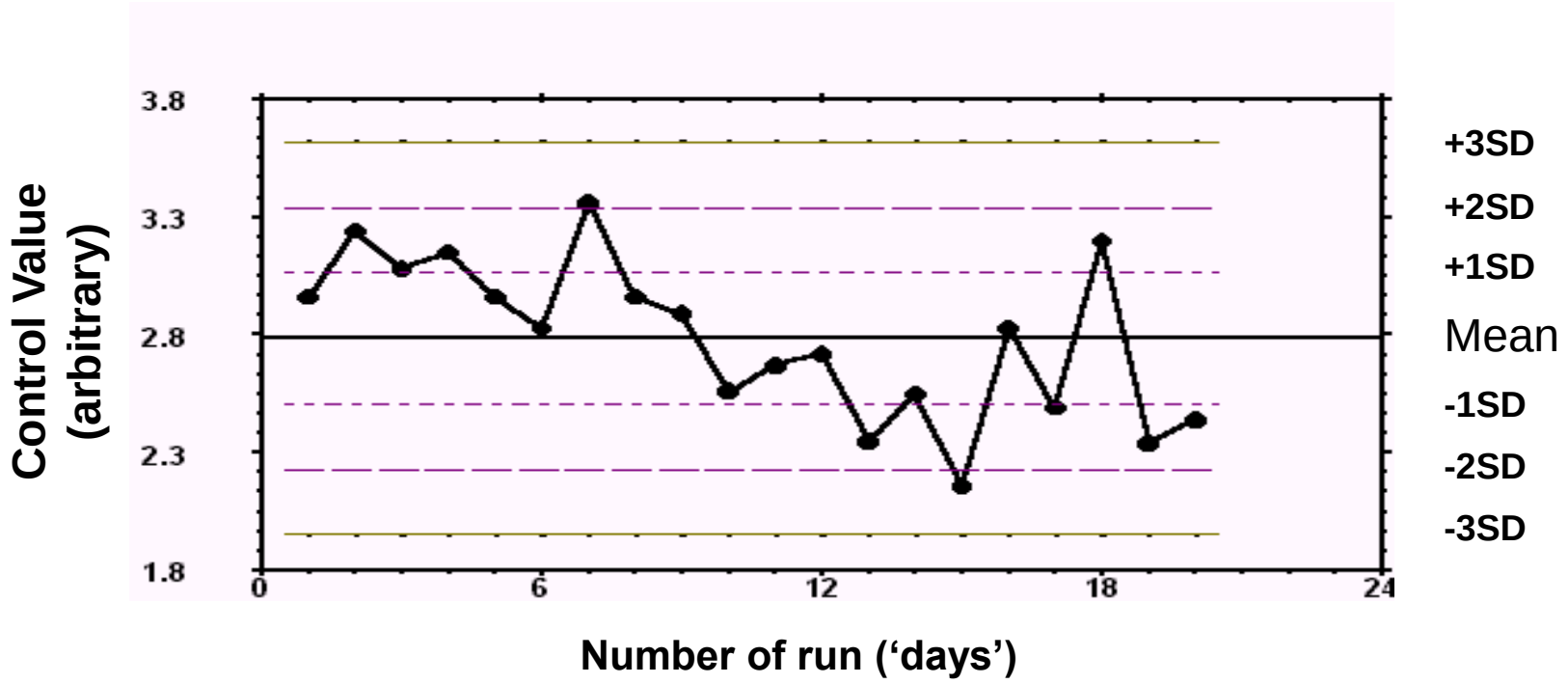
Levey-Jennings Control Chart

- **Shift** – when there are 6 or more consecutive data results on the same side of the mean



Levey-Jennings Control Chart

- **Trend** – when there is a consistent increase or decrease in the data points over a period of 6 or more days



Westgard Rules

- Statistical rules which guide in the **detection of both random and systematic errors** in QC data

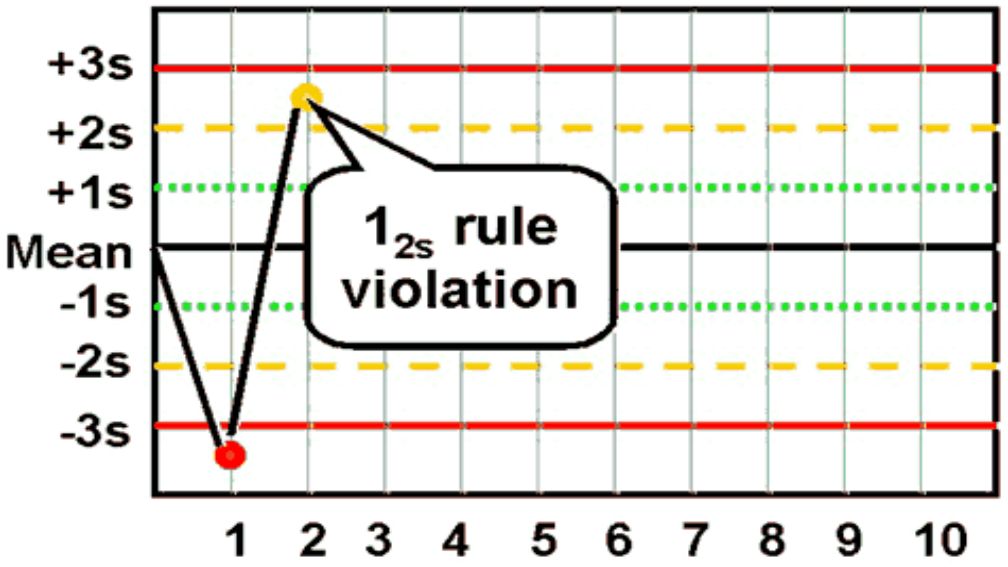
- Established by James O. Westgard, PhD
  - http://www.westgard.com/mltirule.htm

- The rules are applied **when two** (or more??) con**trol materials are used**

- The control rules are given symbols as A_L or n_L; where A is abbreviation for a statistic, n is the number of control observations, L refers to the control limits

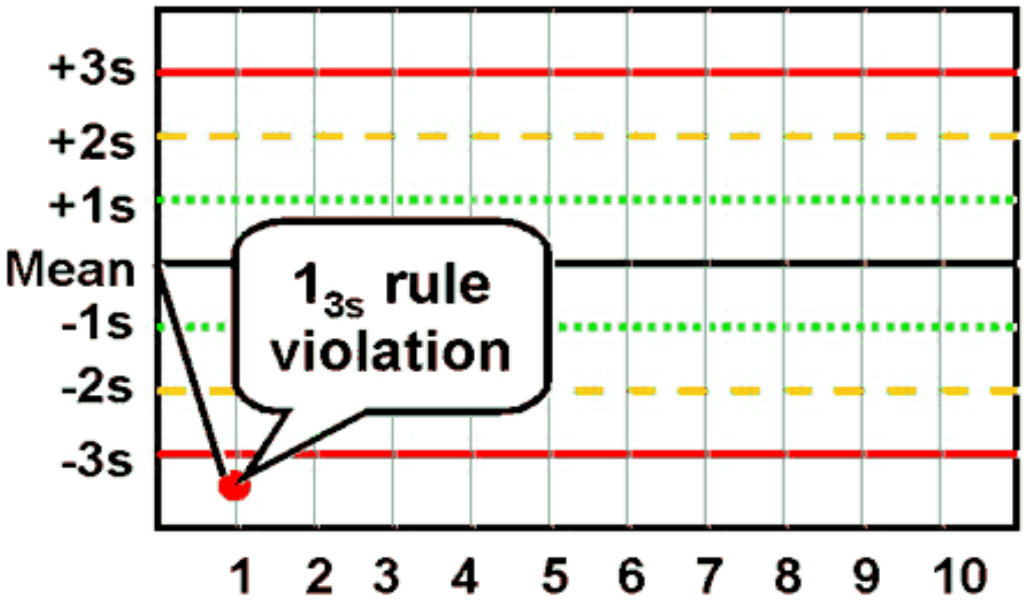
Quality Control

- Common Westgard rules
  - 1_{2s}
    - A single control measurement exceeds two standard deviations from the target mean
  - Action – must consider other rule violations
    - This is a warning



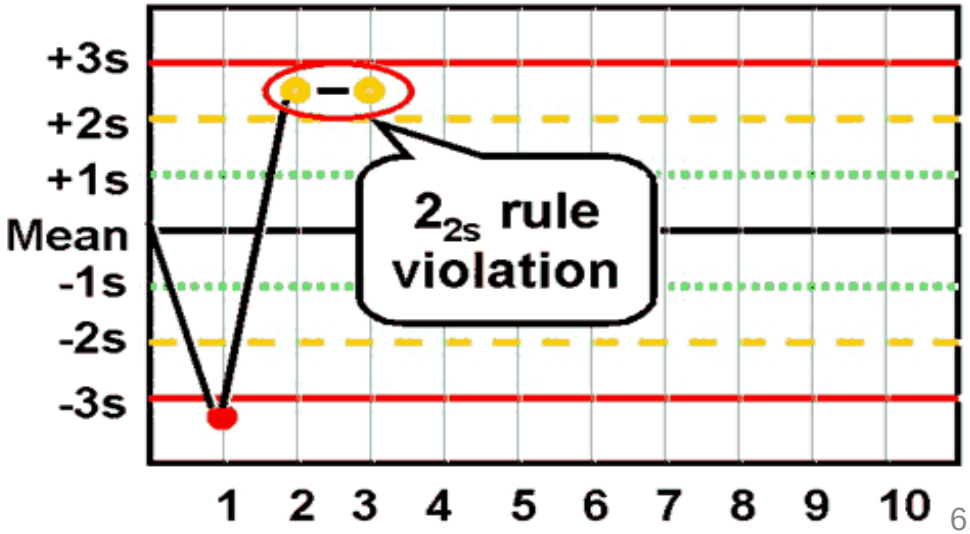
Quality Control

- Common Westgard rules
  - 1_{3s}
    - A single control measurement exceeds three standard deviations from the target mean
    - Primarily sensitive to random errors
  - Action - Reject



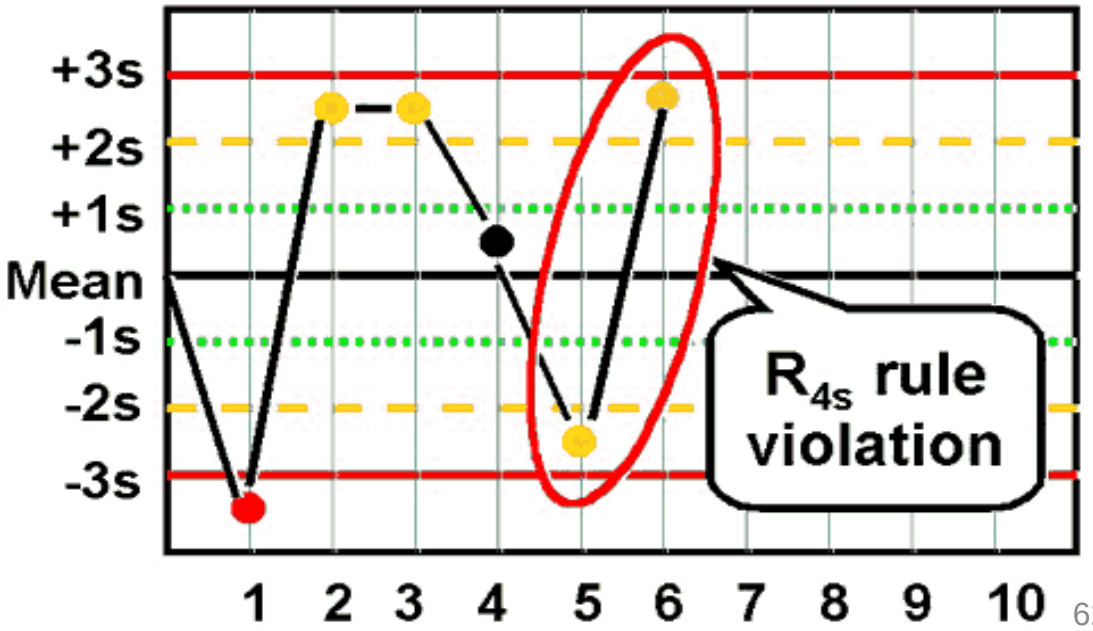
Quality Control

- Common Westgard rules
  - 2_{2s}
    - Two consecutive control measurements exceed the same mean plus 2S or the same mean minus 2S control limit
    - Primarily sensitive to systematic errors
  - Action – Reject



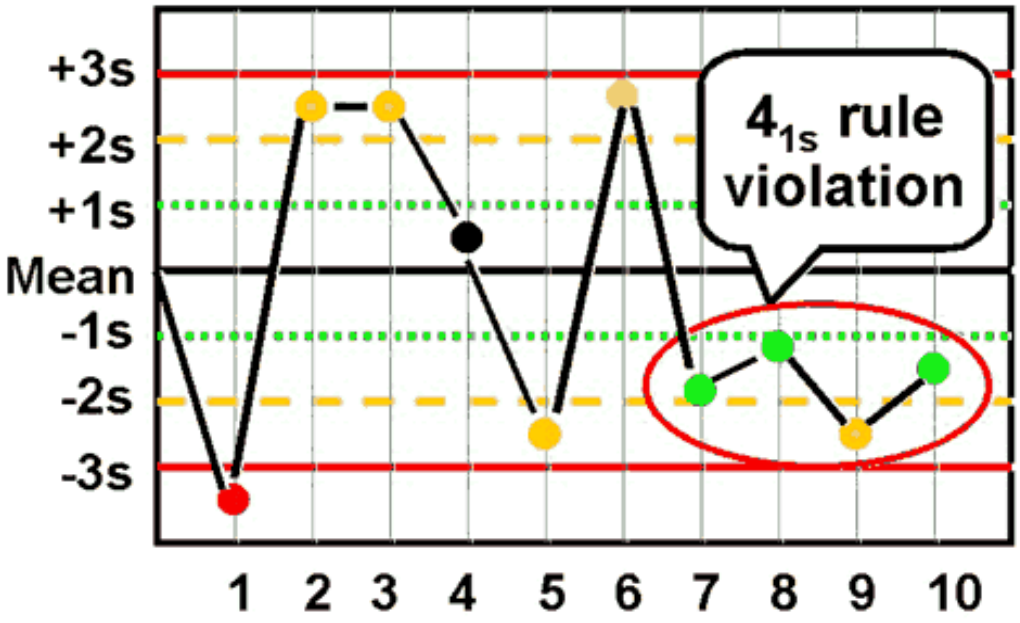
Quality Control

- Common Westgard rules
  - R_{4s}
    - One control measurement in a group exceeds the mean plus 2S and another exceeds the mean minus 2S
    - Primarily sensitive to random errors
  - Action – Reject



Quality Control

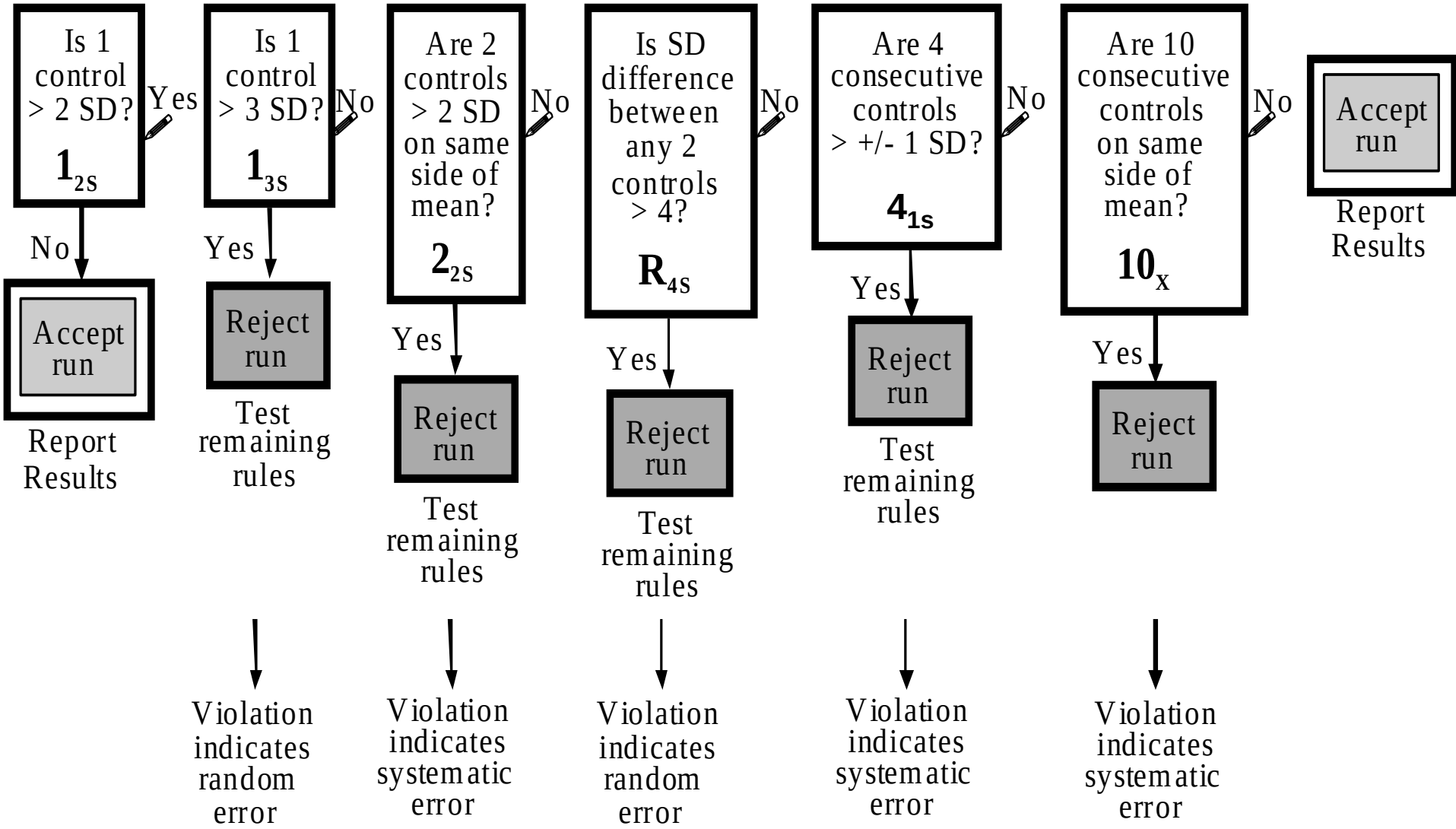
- Common Westgard rules
  - 4_{1s}
    - Four consecutive control measurements exceed the same mean plus 1S or the same mean minus 1S control limit
    - Sensitive to systematic errors
  - Action – Reject

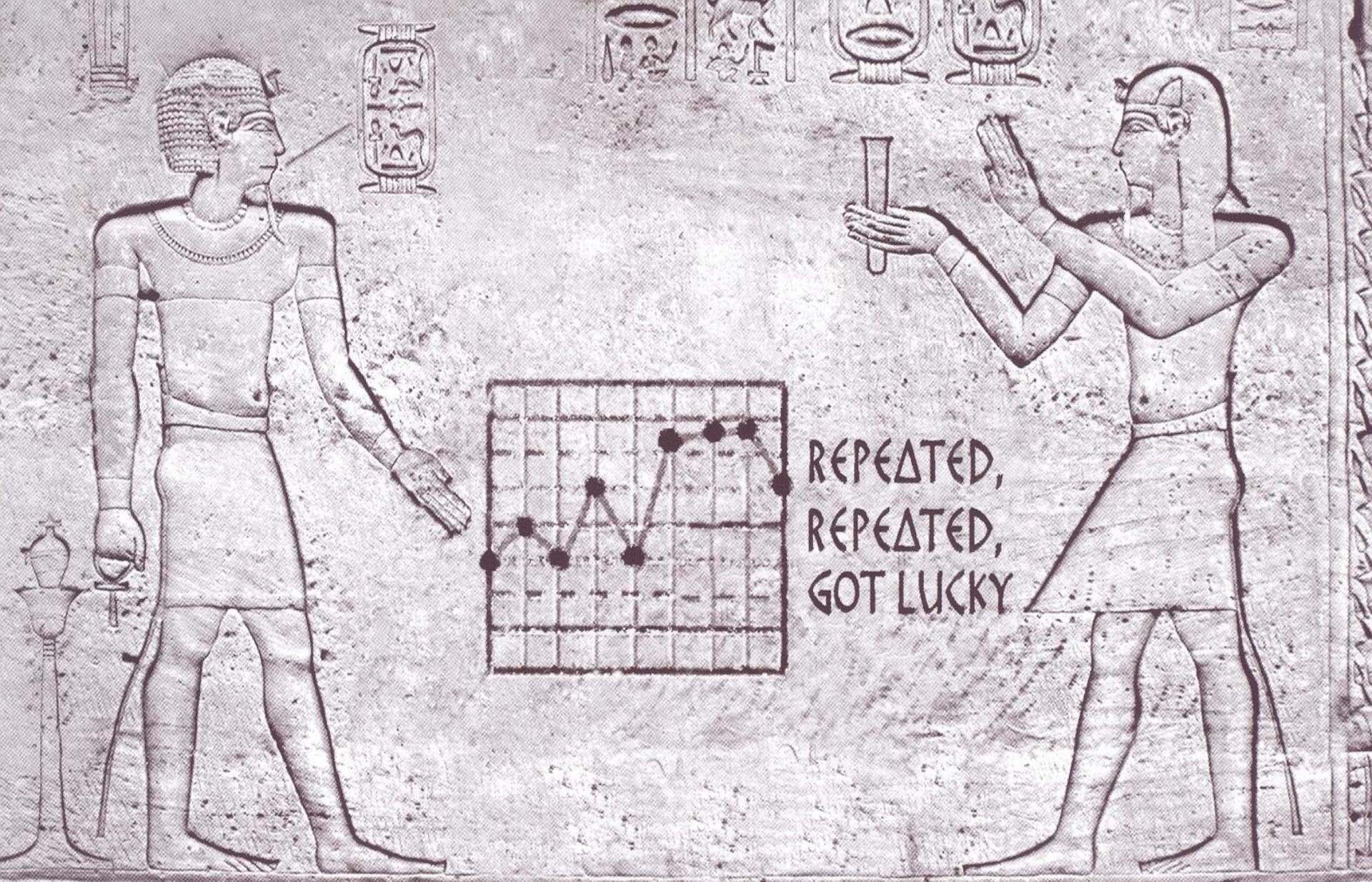


Quality Control

- Common Westgard rules
  - 10_x
    - Ten consecutive control observations falling on one side of the mean
    - Sensitive to systematic errors
  - Action – Reject

Quality Control





HOW OLD ARE YOUR QC PRACTICES?

But what if your control specimen is “out of control?”

- “Out of control” means that there is too much dispersion in the control results (current) compared with the expected control range/limit

- This suggests that something is wrong with the process that generated that observation/result

- *Patient test results cannot be reported to physicians when there is something wrong with the testing process that is generating results*

- *Remember … No information is better than wrong information!!!!!*

But what if your control specimen is “out of control?”

- Some Corrective Measures

Things that can go Wrong	Corrective Action
Instrument malfunction	Identify malfunction and fix
Reagents: preparation, contamination, volume	New reagents
Tech error	Identify error and repeat test
Control sample/specimen is old or prepared improperly	Use new control

How to implement an IQC programme

1. Establish written policies and procedures
2. Assign responsibility for monitoring and reviewing
3. Train staff
4. Obtain control materials
5. Collect data
6. Set target values (mean, SD)
7. Establish Levey-Jennings charts
8. Routinely plot control data
9. Establish and implement troubleshooting and corrective action protocols
10. Establish and maintain system for documentation

METHOD EVALUATION

Method Selection

- Before any new method is introduced into a lab both **managerial** and **technical information** must be compiled and carefully considered

- The information should be collected from different sources e.g. manufacturers, sales representatives, scientific presentation and scientific literature

Managerial information to be considered

- Instrument cost
- Personnel requirement
- Instrument size (versus available space)
- Cost per test
- Sample volume
- Specimen types
- Environmental requirements

Technical information to be considered

- Analytical sensitivity or detection limit of the test (smallest concentration that can be accurately measured)
- Analytical specificity of the test (the ability to measure only the analyte of interest)
- Linear range or AMR (analytical measurement range) is an assessment of the lowest and highest levels at which an analyte can be accurately measured
- Interfering substances
- Estimation of imprecision and inaccuracy
- Reference Intervals (Normal values)

NB: Reading assignment: How is reference/normal range established?

Summary: Selecting an Analytical Method

1. The principle of the assay
2. The composition of reagents & reference materials, the quantity provided, & their storage
3. The stability of reagents & reference materials
4. Possible hazards, appropriate safety precautions
5. The type, quantity & disposal of waste
6. Specimen requirements – collection, volume, storage
7. Anticipated analytical performance – accuracy, precision, range
8. The reference interval – derivation, values in health & disease
9. The detailed protocol for performing the test
10. The availability of technical support, supplies

Method Evaluation

- Before any new method is introduced, an initial evaluation should be carried out

- The imprecision and inaccuracy are estimated and compared with the maximum allowable error for the test based on medical criteria or significance

- If the imprecision and inaccuracy exceed the maximum allowable error, the method is either rejected or modified and re-evaluated

Estimation of imprecision

- It involves the repeated estimation of the concentration of aliquots of a given sample over a period of at least 20 days (**replication experiment**)

- The control material should span a clinically meaningful range of concentrations (normal or abnormal)

- When the imprecision data are generated, the mean, standard deviation and coefficient of variation are calculated

Estimation of imprecision (cont.)

- Two main types:
  - within run: imprecision within one run - is indicated by the standard deviation of the controls analyzed within one run
  - Between run: imprecision between runs

- The random error or imprecision of a test procedure is measured in terms of coefficient of variation (CV) and standard deviation (SD). The smaller the CV and SD, the better the precision

Estimation of Inaccuracy (Bias)

- One way of estimating inaccuracy is by the **comparison of methods technique/ experiment**

- This involves the estimation of a given analyte using both the test method and a comparative method

- It is recommended that at least 40 and preferably 100 samples should be estimated by both methods

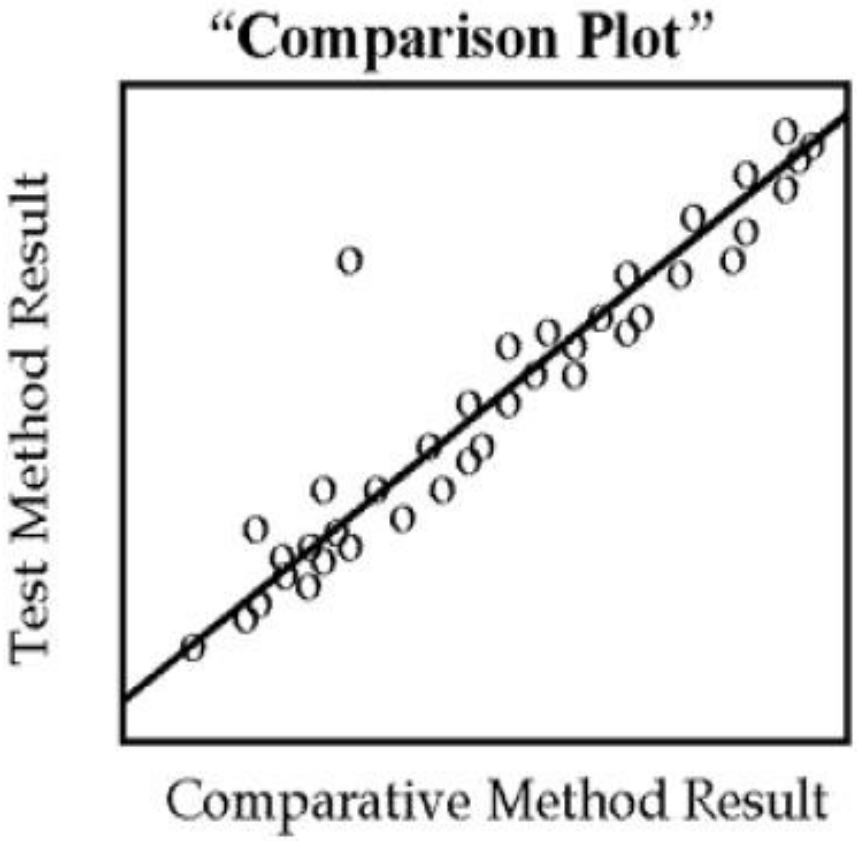
Estimation of Inaccuracy (Bias)

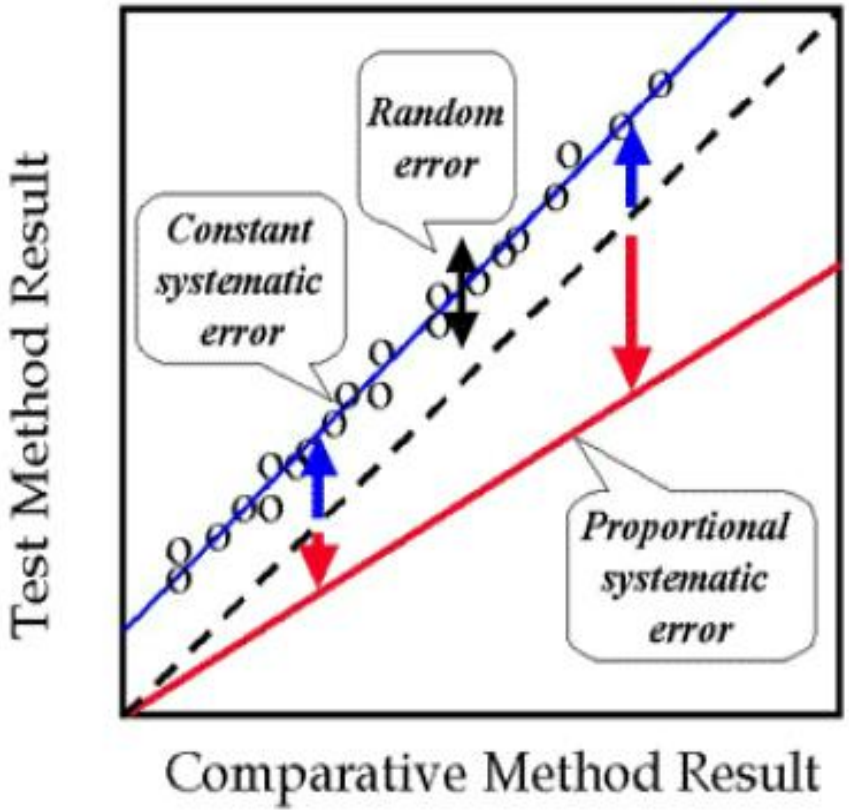
- Duplicates of each sample should be analyzed by both methods

- The analyses by both methods should be performed on the same day, preferably within 4 hours

- A graph of the test method data (y-axis) versus the comparative method (x-axis) allows visualization of method comparison

Correlation/Comparison Plot





Estimation of Inaccuracy (Bias) (cont.)

- Find the bias: it is the difference between the averages by the two methods, which is also the same as the average difference for all the specimens analyzed by the two methods

- It provides an estimate of the systematic error or average difference that is expected between the methods

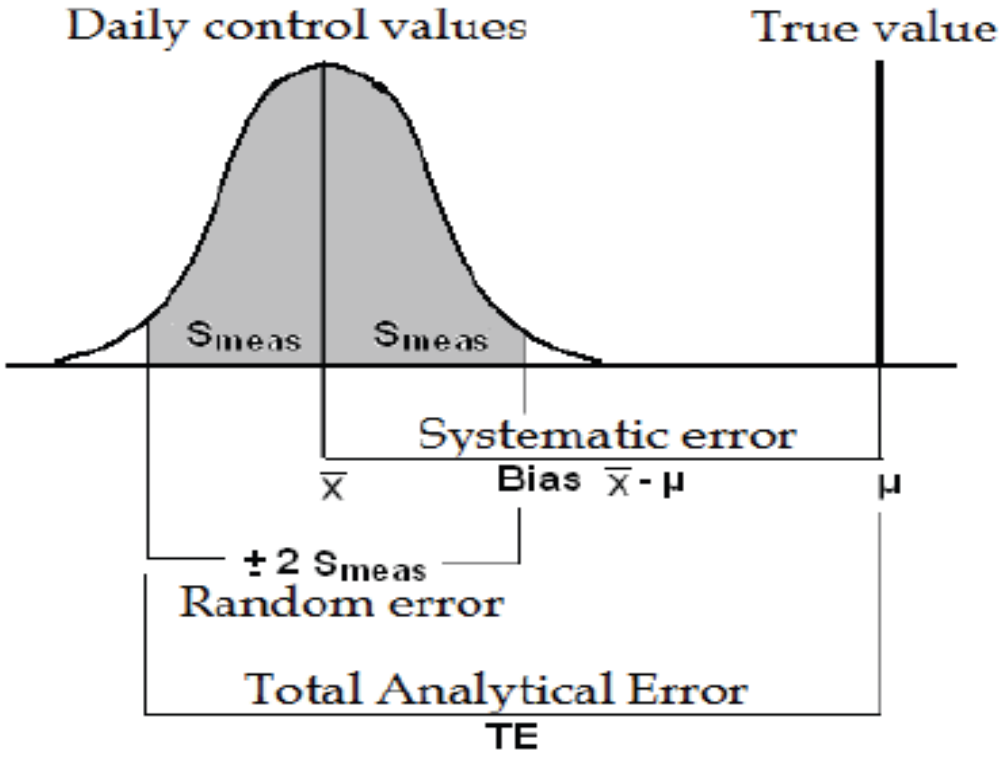
- Thus, the smaller the bias, the smaller the systematic error, and the better the agreement/accuracy

Total Analytical Error

- Total error:
  - Sum of random plus systematic errors

- If the total error exceeds the maximum allowable error, the method is either rejected or modified and re-evaluated

Total Analytical Error



References

- Burtis CA, Ashwood ER and Bruns DE (2008). Tietz Fundamentals of clinical chemistry. Sixth edition. Saunders & Elsevier: USA. Pages 201-262.
- Bishop, M., Fody, E., & Schoeff, l. (2010). *Clinical Chemistry: Techniques, principles, Correlations.* Baltimore: Wolters Kluwer Lippincott Williams & Wilkins.