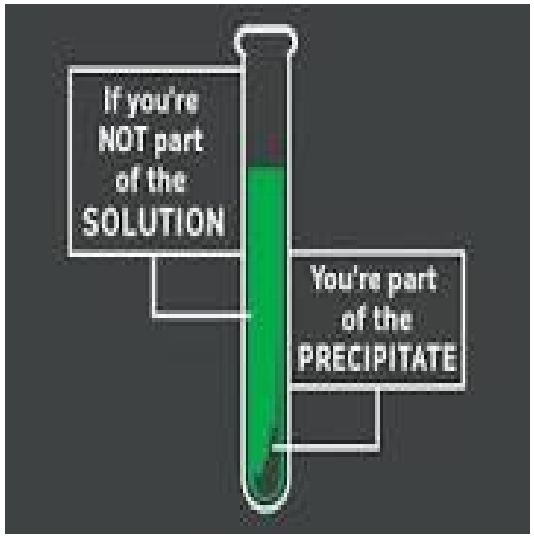
Introduction to Clinical Chemistry



What Is Clinical Chemistry?

Clinical chemistry (or clinical biochemistry) is a branch of laboratory medicine that is generally concerned with analysis of bodily fluids for diagnostic and therapeutic purposes . Clinical chemistry studies chemical and biochemical mechanisms of the body in relation to disease.

Applied techniques include the use and measurement of enzyme activities, spectrophotometry, electrophoresis, and immunoassays.

What Is Clinical Chemistry?

Clinical chemistry (or clinical biochemistry) is a branch of laboratory medicine that is generally concerned with analysis of bodily fluids for diagnostic and therapeutic purposes . Clinical chemistry studies chemical and biochemical mechanisms of the body in relation to disease.

Applied techniques include the use and measurement of enzyme activities, spectrophotometry, electrophoresis, and immunoassays.

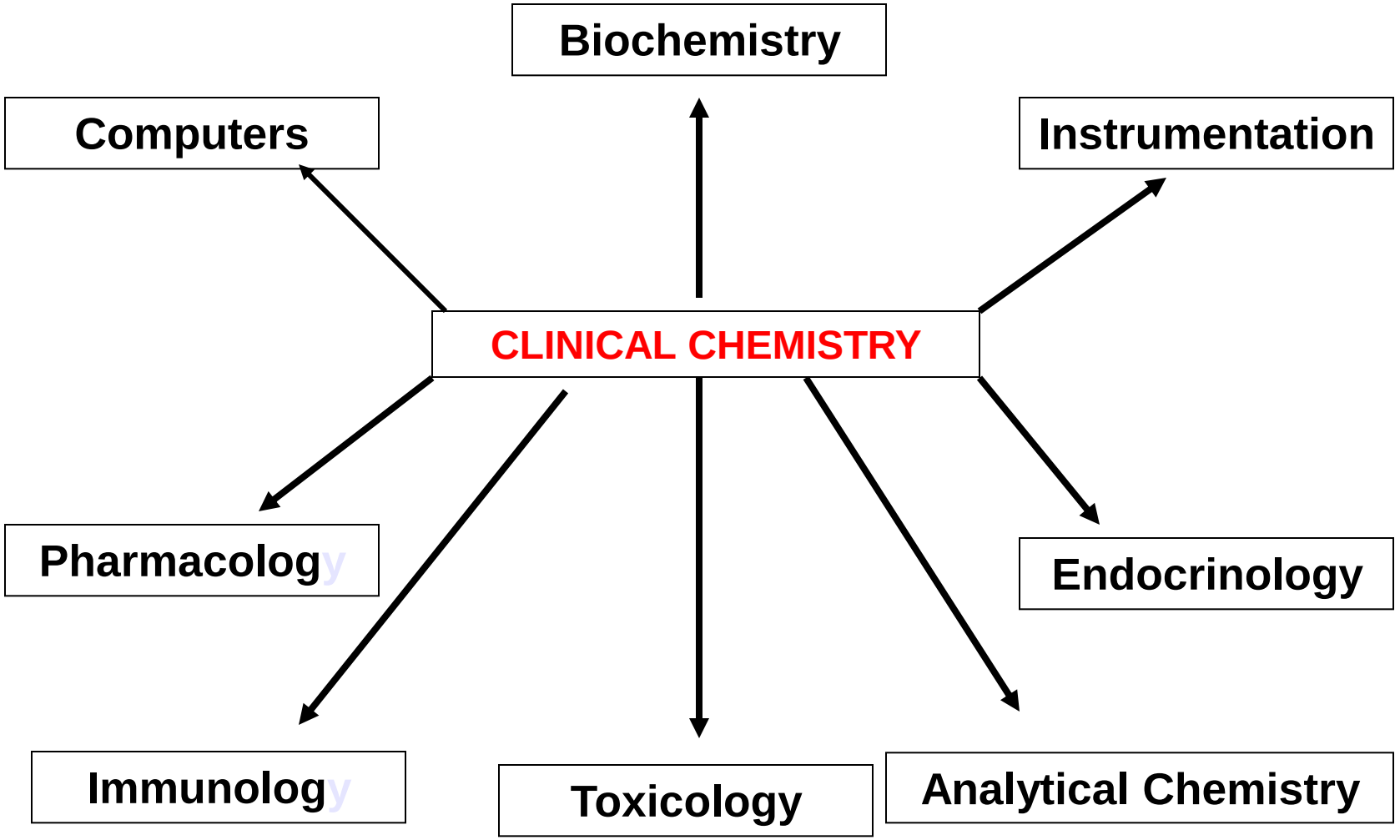
In Conclusion, Clinical Chemistry is

The *systematic study* of *biochemical processes* associated with *health & disease* & the *measurement of constituents* in *body fluids or tissues* to facilitate *diagnosis of disease*.

The purpose & function of clinical laboratories :

1. Confirming or rejecting a diagnosis (CK-MB in MI)
2. Establishing a prognosis (creatinine concentration in progressive renal disease)
1. Detecting disease through case finding or screening (HbS)
2. Monitoring follow-up therapy (Tumor markers)
3. Providing guidelines in patient management (HbA1c in diabetic patients)

SCOPE OF CLINICAL CHEMISTRY



Clinical Laboratory Departments

Clinical Chemistry unit	Clinical Microbiology unit	Clinical Hematology unit
Clinical immunology unit	Cytogenetics unit	Molecular biology unit

Clinical Laboratory Procedures

Laboratory procedures divides into 3 stages:

Pre-analytical stage:

Reading the request form

Recording the patient data and history

Collection of the specimen

Analytical stage :

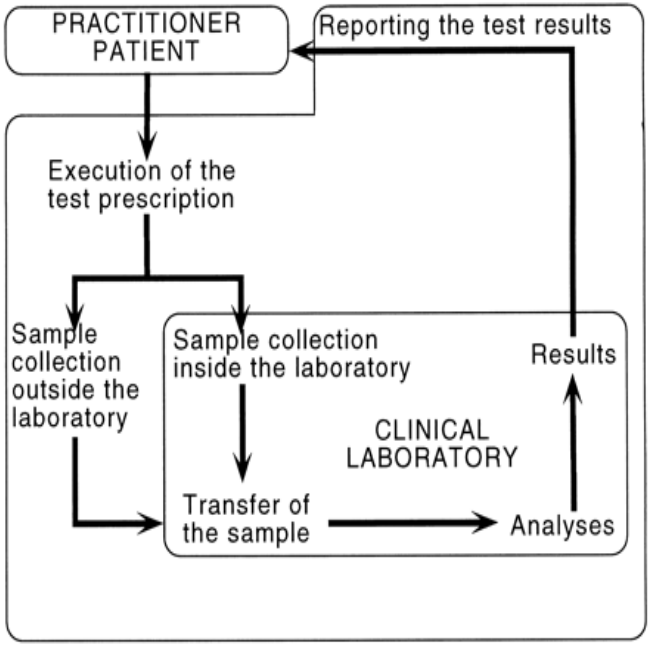
checks the test method, the reagents, standards, and control materials

checks the used instruments. Perform the test and calculate the results

Post-analytical stage:

Reporting the test result which include

1. Test name and type of specimen analyzed
2. Test results clearly
3. The measurement unit and reference range



Types of Specimens for Chemical Analysis

- Blood

- Serum - clear liquid that can be separated from clotted blood.

  (It does not have fibrinogen or the other clotting factors)

- Plasma - liquid portion of the blood, obtained by centrifugation of blood treated with anticoagulant

- Whole blood

- Urine – often 24 hours collection
- Body fluids: Cerebrospinal Spinal Fluid (CSF) and other fluids (Peritoneal Fluid, Amniotic Fluid)

- Synovial fluid

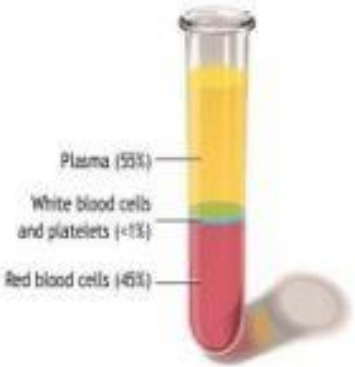
2. Plasma vs. serum

•**Plasma** is the liquid, cell-free part of blood, that has been **treated with anti-coagulants**.

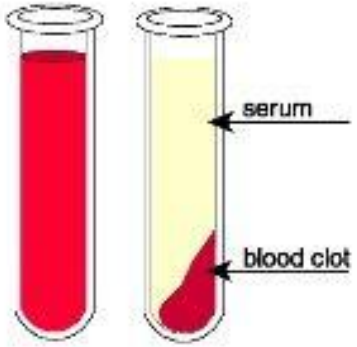
Serum is the liquid part of blood **AFTER coagulation**, therfore devoid of clotting factors as fibrinogen.

Anticoagulated

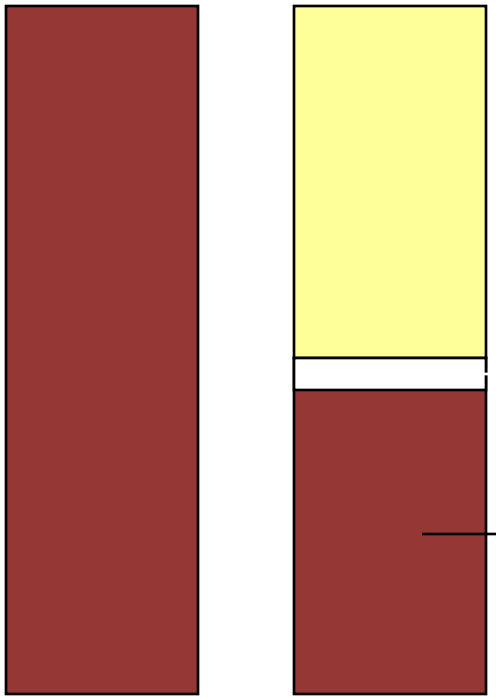
Clotted



•serum= plasma - fibrinogen



Blood Composition



Whole Blood

Plasma

Plasma is fluid component of blood. Comprises ~55% of total volume of whole blood. Contains proteins, sugars, vitamins, minerals, lipids, lipoproteins and clotting factors.

White Blood cells (WBC) & Platelets

Red Blood cells (RBC)

Cellular Components

Arterial puncture

- Arterial blood is ***oxygenated blood***. It is uniform in composition throughout the body.

- Arterial blood is used to measure ***oxygen tension, carbon dioxide tension*** and ***blood pH.***

- Blood gas analyses (BGA) are critical to patients with ***pulmonary problems, oxygen therapy, cardiovascular problems and those undergoing major operations***.

VACUTAINER TUBES

- **These are tubes for blood collection which are color-coded based on the anticoagulant present. They come in various sizes; 2, 5, 7, and 10 ml.**

- **Blood is drawn in this order: Blood culture tubes, red top, blue top, green top, lavender top and gray top**

Collection tubes

- Red-top tubes contain no anticoagulants or preservatives
- Red-top tubes are used for collecting serum
  - 10-15 minutes is required to allow blood to clot before centrifuging
  - Used for blood bank specimens, some chemistries

Collection tubes

- Gold (and “tiger”) top tubes contain a gel that forms a physical barrier between the serum and cells after centrifugation
- No other additives are present
- Gel barrier may affect some lab tests

Collection tubes

- Used for Glucose measurement.
- After blood collection, glucose concentration decreases significantly because of cellular metabolism
- Gray-top tubes contain either:
  - Sodium fluoride and potassium oxalate
  - ➢ preservatives stabilize glucose in plasma by inhibiting enzymes of the glycolytic pathway
  - NaF/oxalate inhibits enolase
  - Iodoacetate inhibits glucose-3-phosphate dehydrogenase

Collection tubes

- Green-top tubes contain either the Na, K, or lithium (Li) salt of heparin. Most widely used anticoagulant for chemistry tests.
  - Should not be used for Na, K or Li measurement
- Heparin accelerates the action of antithrombin III, which inhibits thrombin, so blood does not clot (plasma)

Collection tubes

- Lavender-top tubes contain the K salt of ethylenediaminetetraacetic acid (EDTA), which chelates calcium (essential for clot formation) and inhibits coagulation
- Used for hematology, and some chemistries
- Cannot be used for K or Ca tests

Collection tubes

- Blue-top (or black|) tubes contain sodium citrate, which chelates calcium and inhibits coagulation

- Used for coagulation studies because it is easily reversible.

Collection tubes

- Brown and Royal Blue top tubes are specially cleaned for trace metal studies
  - Brown-top tubes are used for lead (Pb) analysis
  - Royal blue-top tubes are used for other trace element studies.

Factors and Variables Affecting the Laboratory Results

Pre-Collection Variables

- **Diurnal variation**

The cyclical variations include:

-Iron peaks early to late morning; decreases up to 30% during the day

-Cortisol peaks 4–6 a.m.; lowest 8 p.m-12 a.m.; 50% lower at 8 p.m. than at 8 a.m.

-LH and FSH in women during menstrual cycle

- **Time of Collection :** Blood sugar

- **Exercise**: may elevate *creatine phosphokinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)*
- **Stress.**

-Total cholesterol has been reported to increase with stress, and high-density-lipoprotein cholesterol (HDL) to decrease as much as 15%.

-Stress cause high glucose and cortisol due to adrenal stimulation

- **Diet.**

-Glucose and triglycerides, absorbed from food, increase after eating.

-High protein, low carbohydrate diets, greatly increases the serum BUN and uric acid.

- **Tobacco smoking** : Catecholamines, cortisol, Eosinophil, neutrophils, monocytes

Sample Rejection

Various reasons for specimen rejection include:

- Hemolysis / lipemia
- Clots present in an anticoagulated specimen
- Non fasting specimen when test requires fasting
- Improper blood collection tube
- wrong sample volume
- Unlabeled or mislabeled specimen
- Incorrect specimen storage.
- Blood sample is taken from an arm in which an intravenous (IV) infusion is running.

Analytical Results

Clinical Variations

Inter-individual Variation

- Age
- Sex
- Race
- Genetics
- Long term health status

Intra-individual Variation

- Diet
- Exercise
- Drugs
- Sleep pattern
- Posture
- Time of venipucture
- Length of time tourniquet is applied

Analytical Variations

Pre-analytical Variation

- Transport
- Exposure to UV light
- Standing time before separation of cells
- Centrifugation time
- Storage conditions

Analytical Variation

- Random errors
- Systematic errors

Post-analytical

- Transcriptions errors
- Results reported to wrong patient

Prolonged venous stasis

Blocking the flow of blood with the tourniquet leads to a sieving effect. Small molecules, water and ions are forced out blood vessels and larger molecules are concentrated

- Increases total protein, iron (Fe), cholesterol, bilirubin
- Decreases potassium

Specimens requiring special treatment

- Should be placed *immediately* on ice
  - Lactate
  - Ammonia
  - Acid phosphatase
  - Plasma catecholamines

Significantly affected by hemolysis

- Hemolysis-rupture of red blood cell ⟶

  Significant increase in potassium, magnesium, phosphorous, LDH, GOT

Causes of blood hemolysis

1. **Collecting blood from a narrow vein**
3. **Using excess EDTA**
4. **Slow drawing blood from the vein**
5. **Forcing blood through the needle into tubes**
6. **Using wet tube or stopper**
7. **Shaking the tube very hard**
8. **Holding blood in too warm area**

Factors that Determine the Eligibility of a Method (Validation)

1- Specificity النوعية

Refers to the ability of the test to detect one single analyte without detecting other analytes that are also present in the sample.

أي ان النوعية تسمح بمعايرة مادة واحدة بشكل كمي

2- Sensitivity الحساسية

is the ability of the test to detect the smallest amount of the analyte in a solution or sample

أي ان حساسية طريقة تتعلق بأصغر كمية من المادة يمكن كشفها بهذه الطريقة

Accuracy and Precision

3- Accuracy الضبط is the extent to which the *mean measurement is close to the true value.*

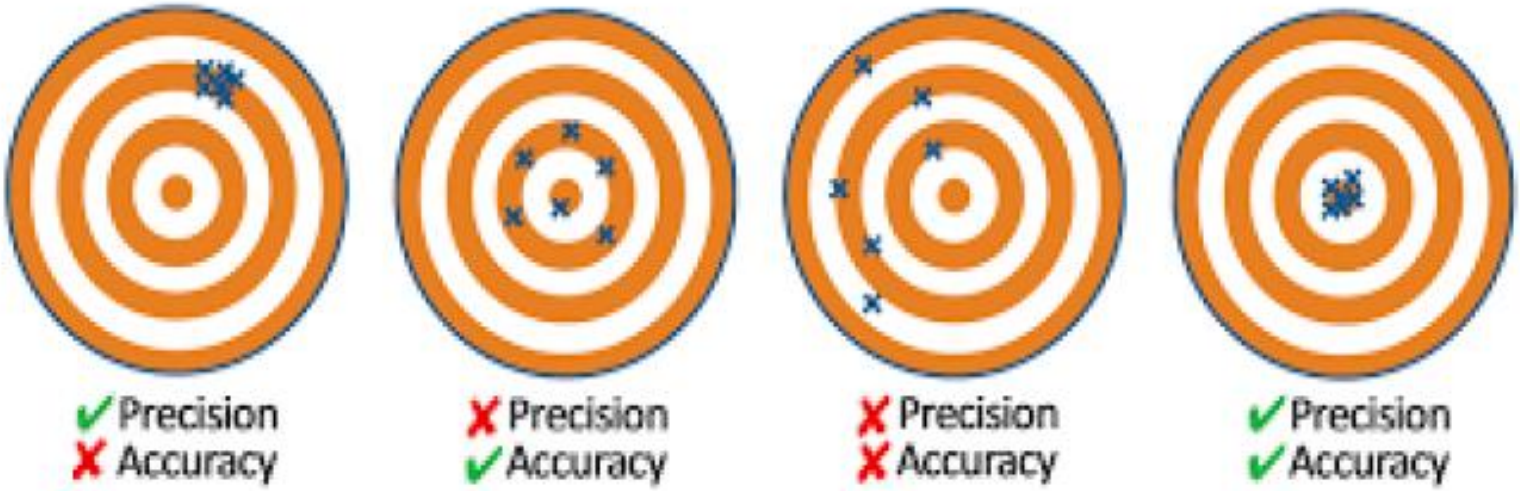
يدل ضبط طريقة على مدى قرب نتيجة معايرة مكون من الكمية الحقيقية له

4- Precision الدقة is the ***reproducibility*** of a laboratory determination when it is run repeatedly under identical conditions

- precision is commonly expressed in terms of *standard deviation (SD), variance* **or** *coefficient of variation (CV)*

وهي مقياس حساس لقابلية تكرارية النتائج

Precision vs Accuracy



Quality Control (QC)

- **is concerned with the *analytic phase***
- monitors the ***over-all reliability of laboratory results*** in terms of ***accuracy and precision***

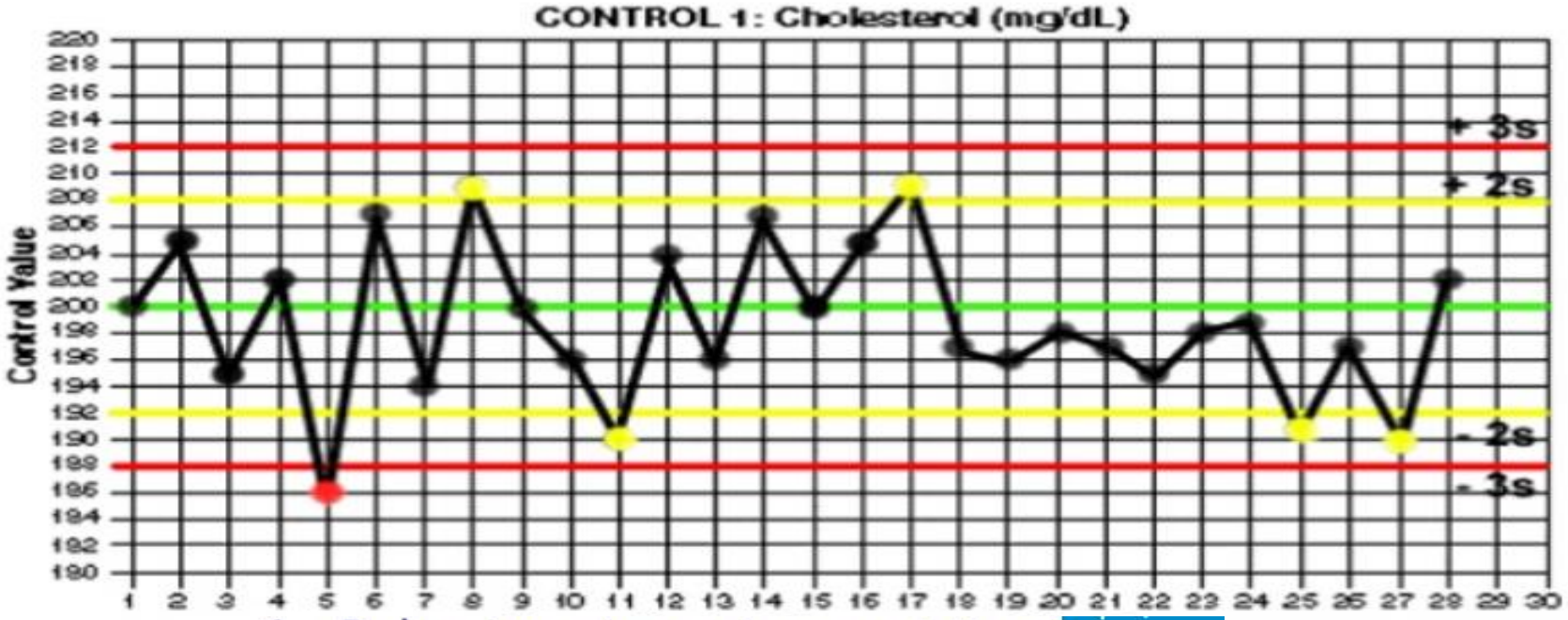
Laboratory quality control is designed to **detect, reduce, and correct** deficiencies in a laboratory's internal analytical process prior to the release of patient results, in order to **improve the quality of the results** reported by the laboratory.

QC in the medical Lab is a statistical process used to monitor and evaluate the analytical process

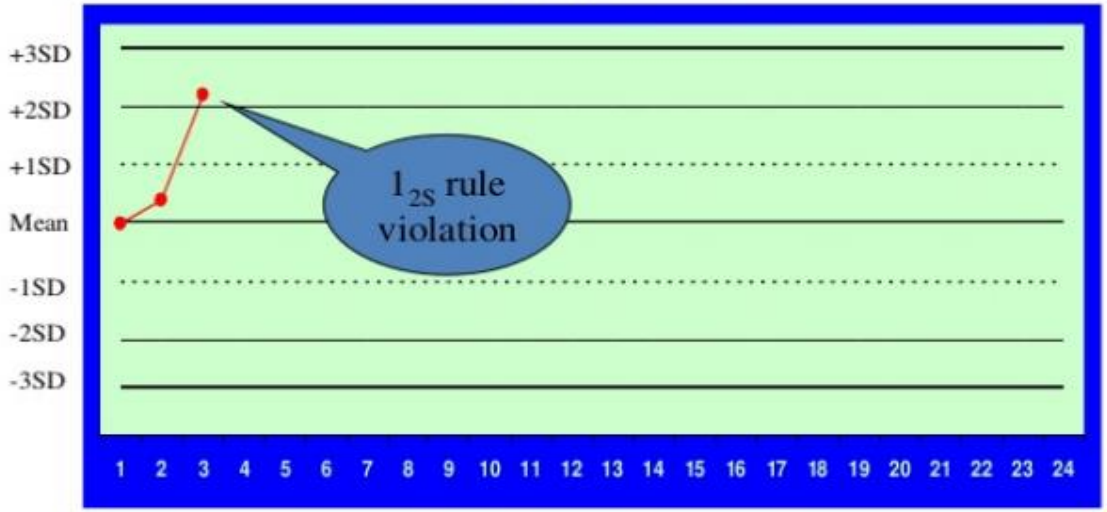
Analysis of Control Materials

- Need data set of at least 20 points obtained over 20-30 days in different times of day
- Calculate mean, standard deviation, Coefficient of variation and determine target ranges
- Develop **Levey-Jenning** charts
- Plot –on the chart- control values each run/ day

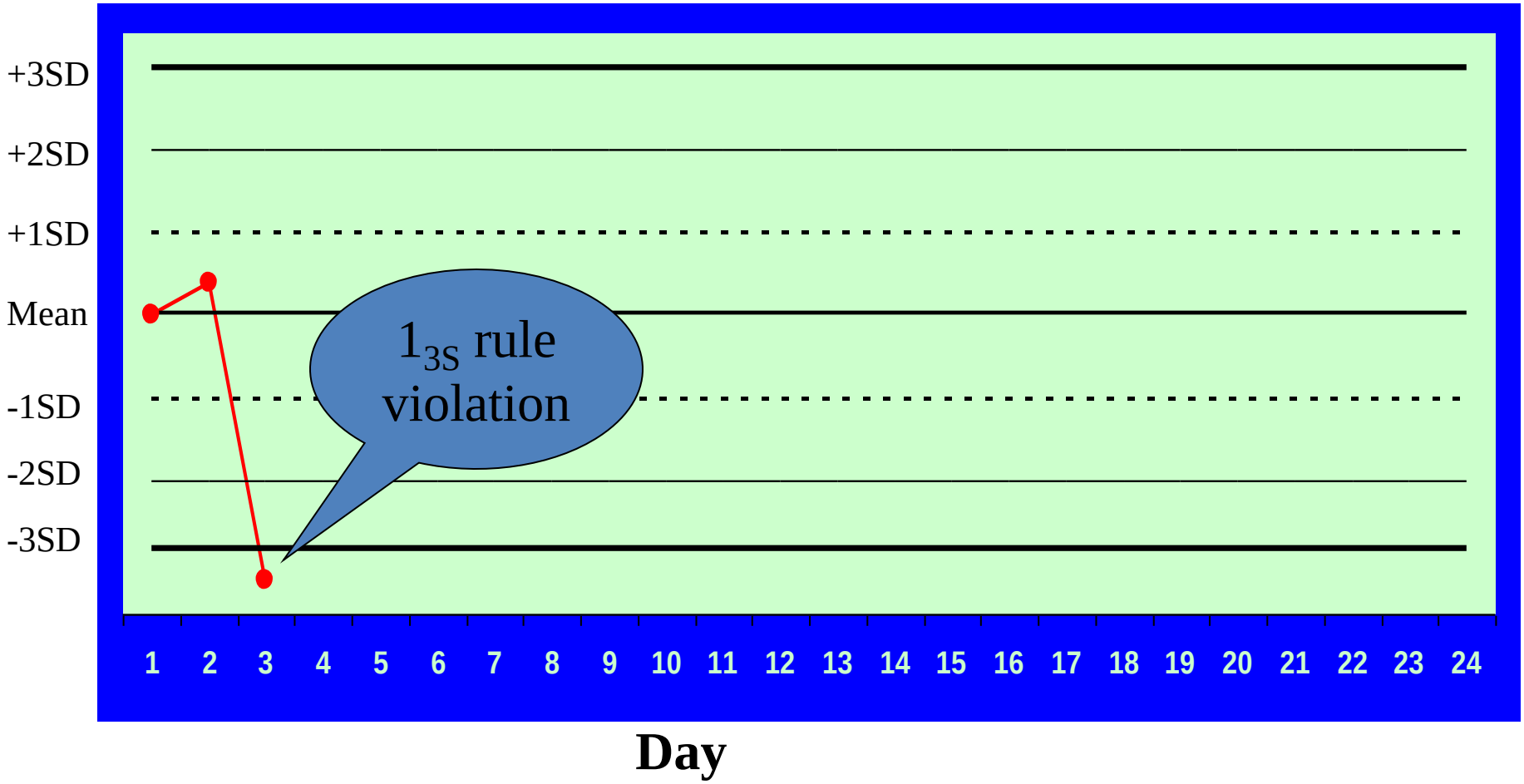
Monitor over time and at defined intervals



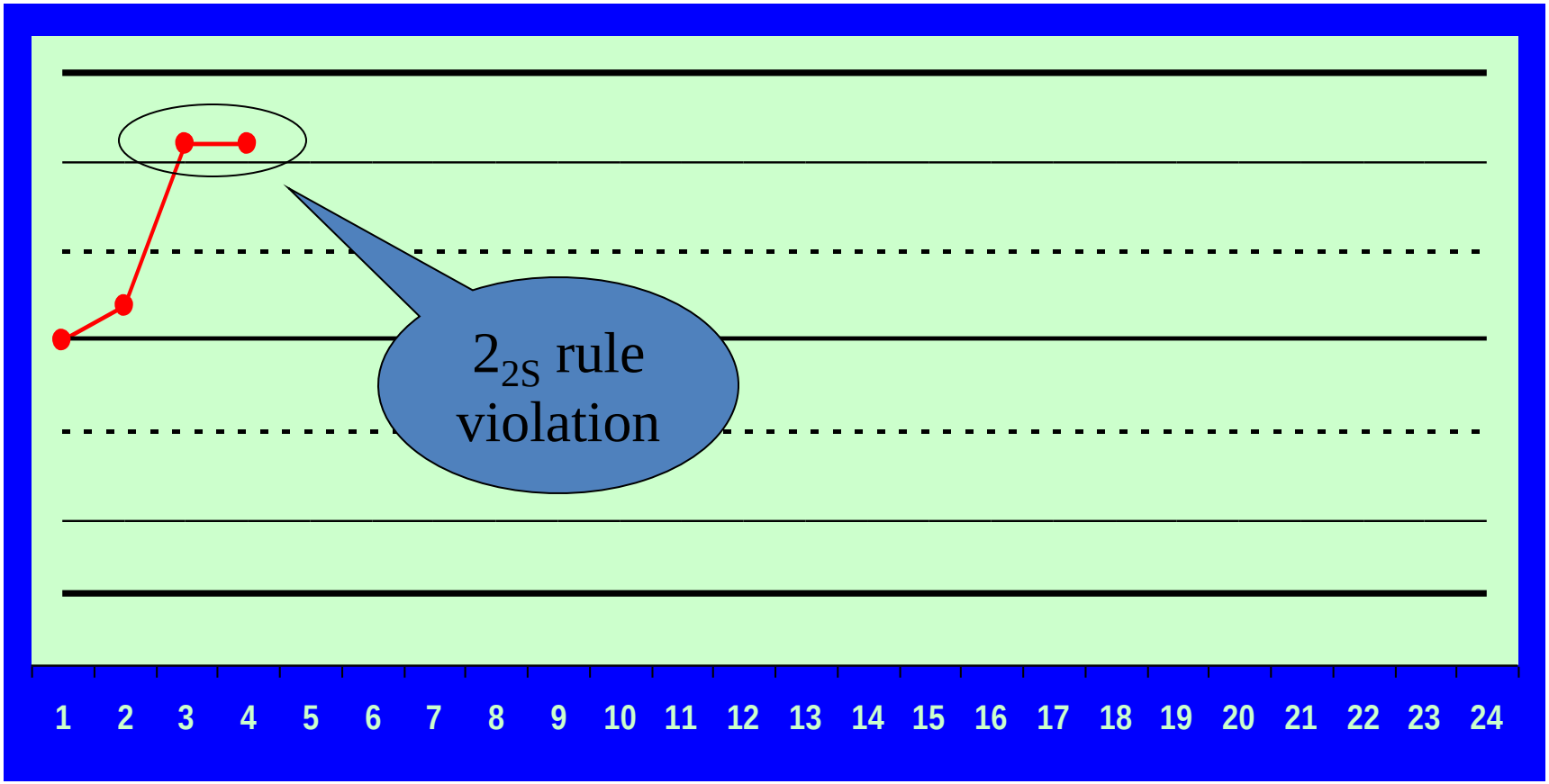
1_{2S} Rule = A warning to trigger careful inspection of the control data



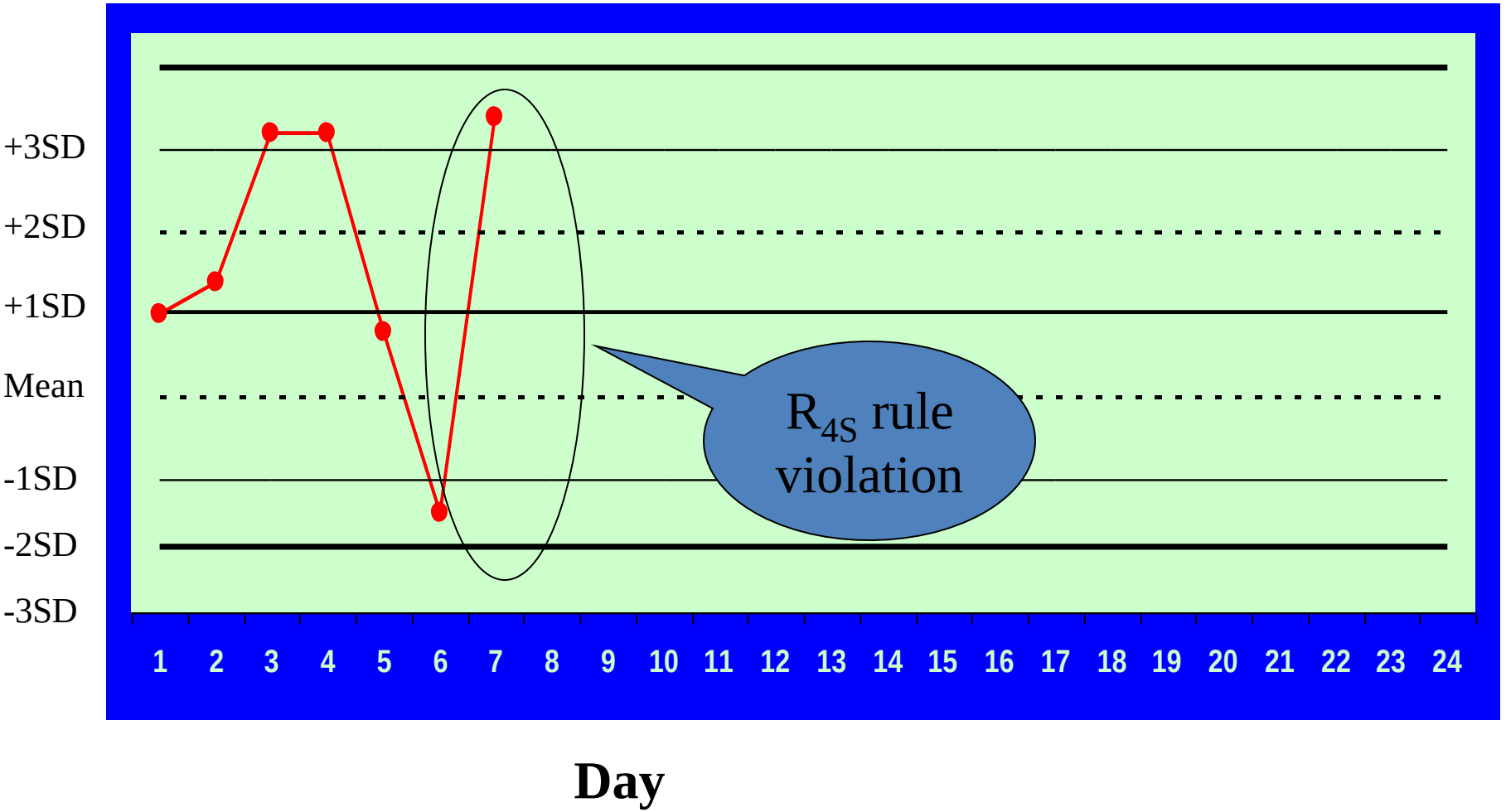
1_{3S} Rule = Reject the run when a single control measurement exceeds the +3SD or -3SD control limit



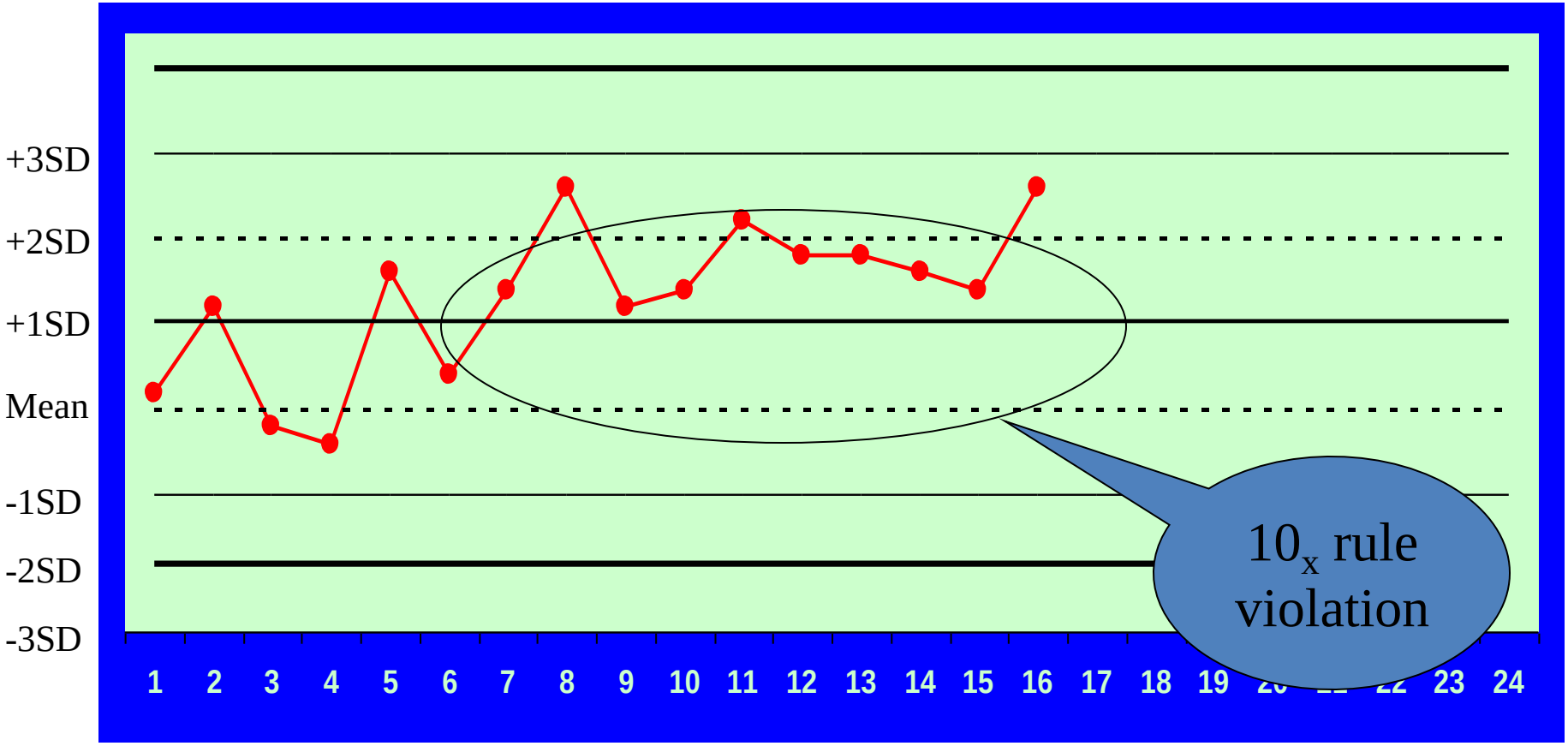
2_{2S} Rule = Reject the run when 2 consecutive control measurements exceed the same +2SD or -2SD control limit



R_{4S} Rule = Reject the run when 1 control measurement exceed the +2SD and the other exceeds the -2SD control limit



10_x Rule = Reject the run when 10 consecutive control measurements fall on one side of the mean



Quality control is a measure of precision, or how well the measurement system **reproduces the same result over time and under varying operating conditions**.

Laboratory quality control material is usually run at the beginning of each shift, after an instrument is serviced, when reagent lots are changed, after calibration, and whenever patient results seem inappropriate.

Control is a solution (usually ***pooled serum samples***) whose constituents are diverse but are known (***a range of values per analyte***). This can be run simultaneously with the **Test** to ***check, verify or validate the accuracy of the results.***

Standard is a solution of ***a particular analyte*** of known characteristics and known value (***exact concentration***). It is used as ***reference for the calculation of the value of the Unknown.***

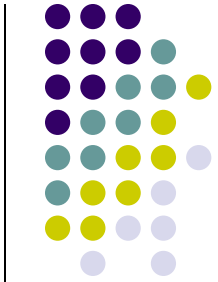
Methods Classification

- The principles that govern the analytic techniques and instrumentations in the lab fall in to four basic areas:
  - 1. Spectrophotometry
  - 2. Luminescence
  - 3. Chromatography
  - 4. Electroanalytic Methods

Spectrophotometric Analysis

- **Spectrophotometric techniques** are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer.
- **The spectrophotometer** can measure the amount of light or electromagnetic radiation (of certain frequency) transmitted or absorbed by the solution.

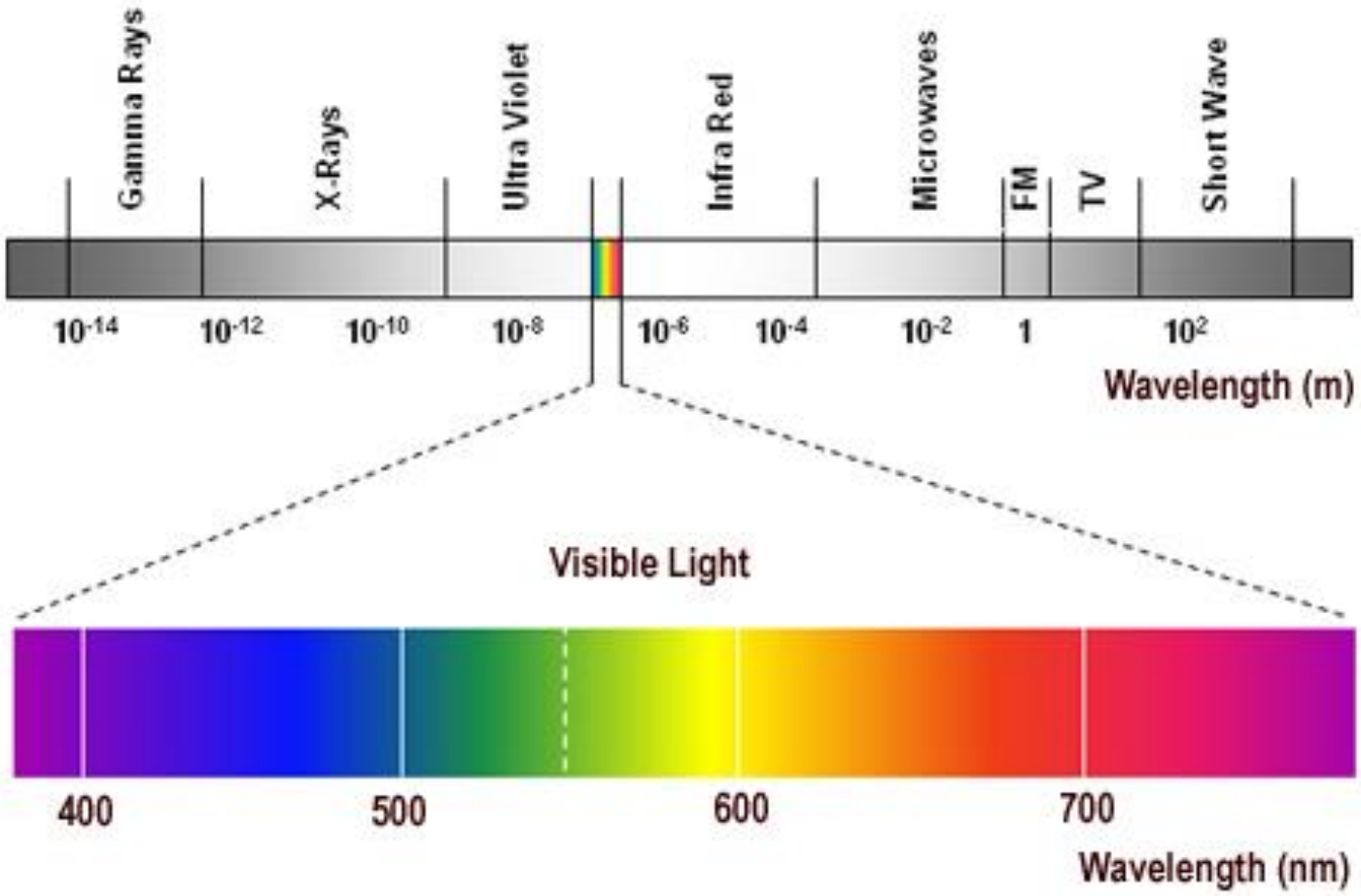
Colors & Wavelengths



Visible Light

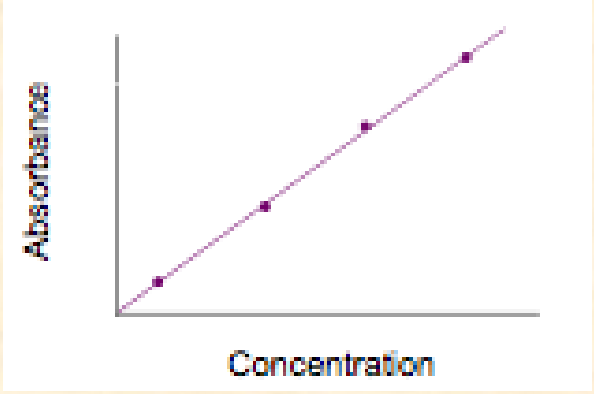
COLOR	WAVELENGTH (λ in nm)
Ultraviolet	< 380
Violet	380 – 435
Blue	436 – 480
Greenish-blue	481 – 490
Bluish-green	491 – 500
Green	501 – 560
Yellowish-green	561 – 580
Yellow	581 – 595
Orange	596 – 650
Red	651 – 780
Near Infrared	> 780

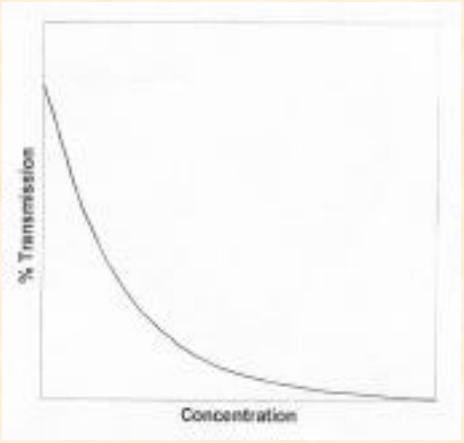
Regions of Electromagnetic Spectrum- the “colour” of light



Spectrophotometer

- compounds absorb light radiation of a specific wavelength.
- the amount of light radiation absorbed by a sample is measured.
- The light absorption is directly related to the concentration of the compound in the sample.
- As concentration increases, light absorption increases, linearly, As concentration increases, light transmission decreases, exponentially





Parts of Spectrophotometer