

CLINICAL LABORATORY

BASIC OVERVIEW

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Laboratory Roles and Responsibility

The responsibilities of the clinical lab include :

- ❖ Correct Identification, collection and processing of patient specimens
- ❖ Accurate performance of testing
- ❖ Timely reporting of result
- ❖ Communication with physicians and other healthcare professionals

Analyst testing is used to help diagnose, monitor and treat disease



Laboratory Workflow

There are 6 main steps in how a sample flows through the lab from other creation to final test result

1. Test ordered
2. Sample is collected
3. Sample is delivered to the lab
4. Sample is processed
5. Sample is analysed
6. Result are reported



Laboratory Speciment

Most common laboratory specimens types :

- ❖ Blood
- ❖ Urine

Additional Laboratory specimens :

- ❖ Body Fluid
- ❖ Sputum
- ❖ Stool
- ❖ Tissue samples
- ❖ Culture swabs

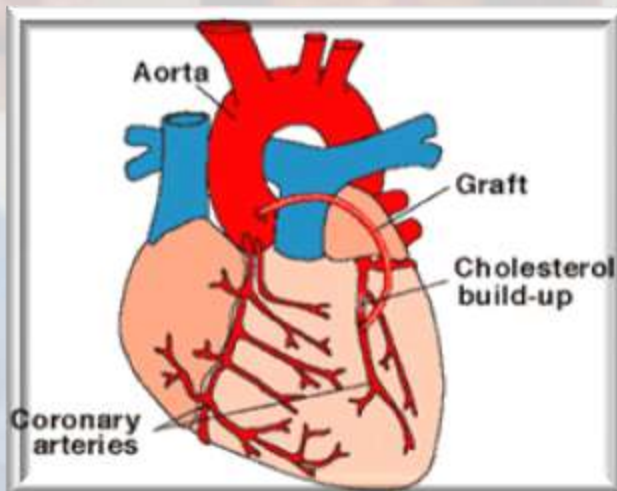


Sampling / Phlebotomy

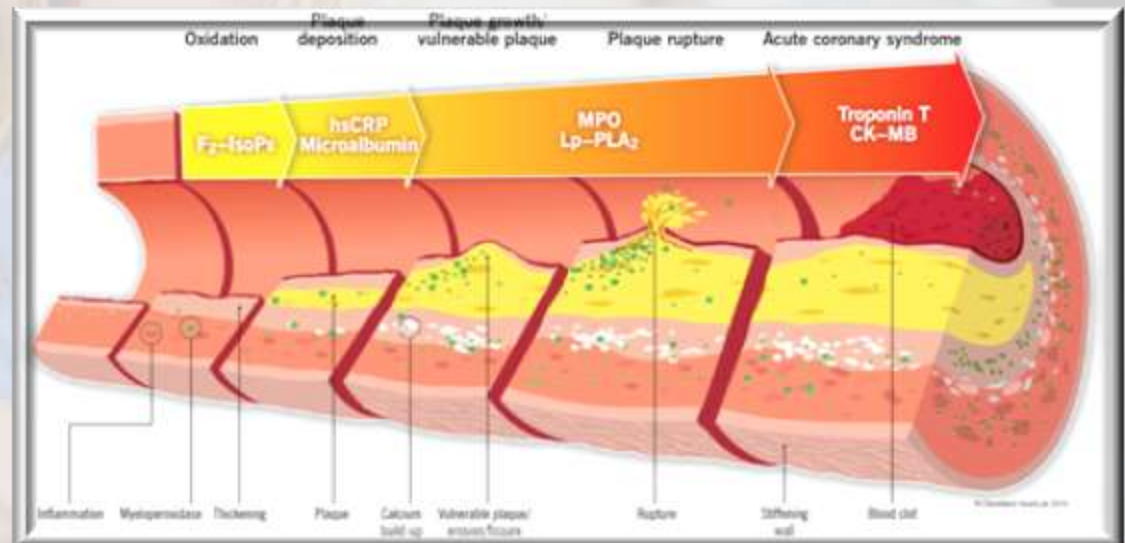


Types of Samples : Serum, Plasma, Blood, Urine, CSF

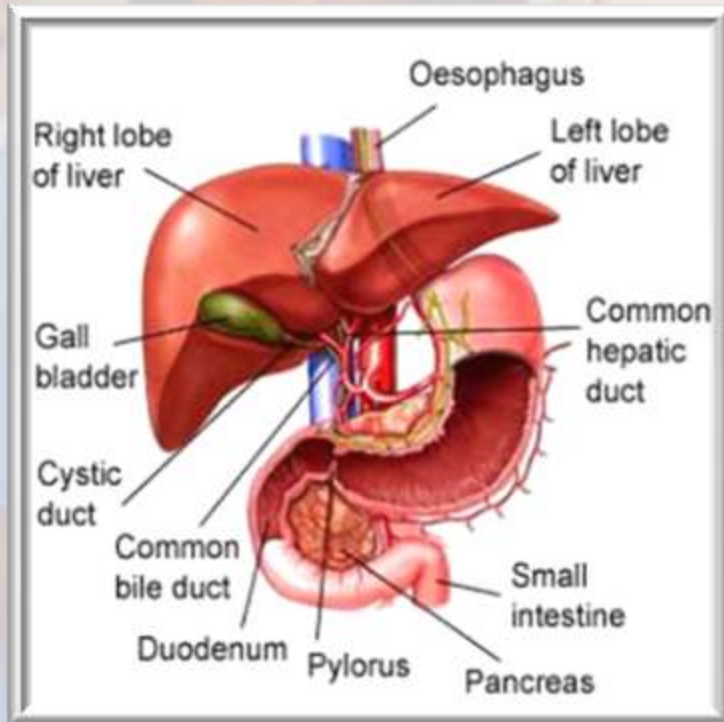
Why do we analyze our blood



Corronary disease :
CK, LDH, Troponin T

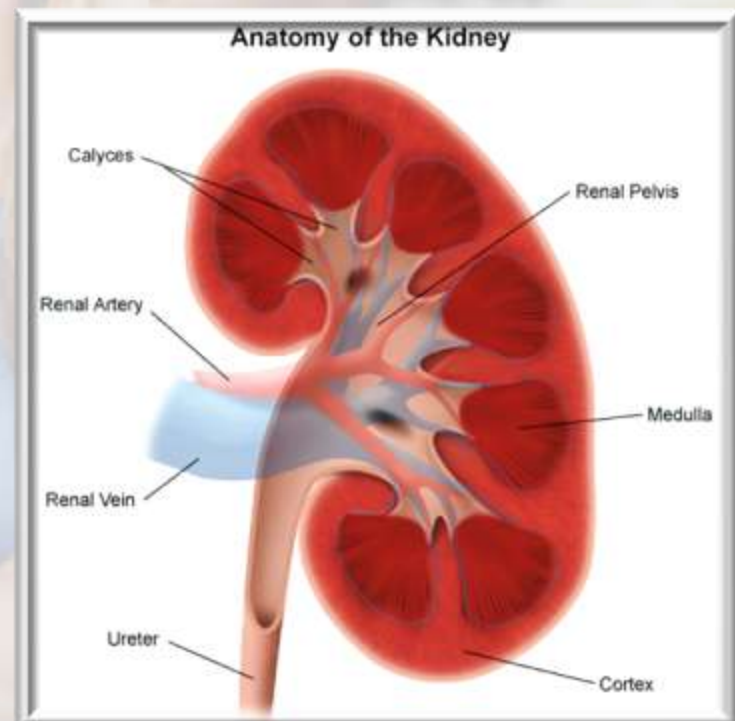


Why do we analyze our blood

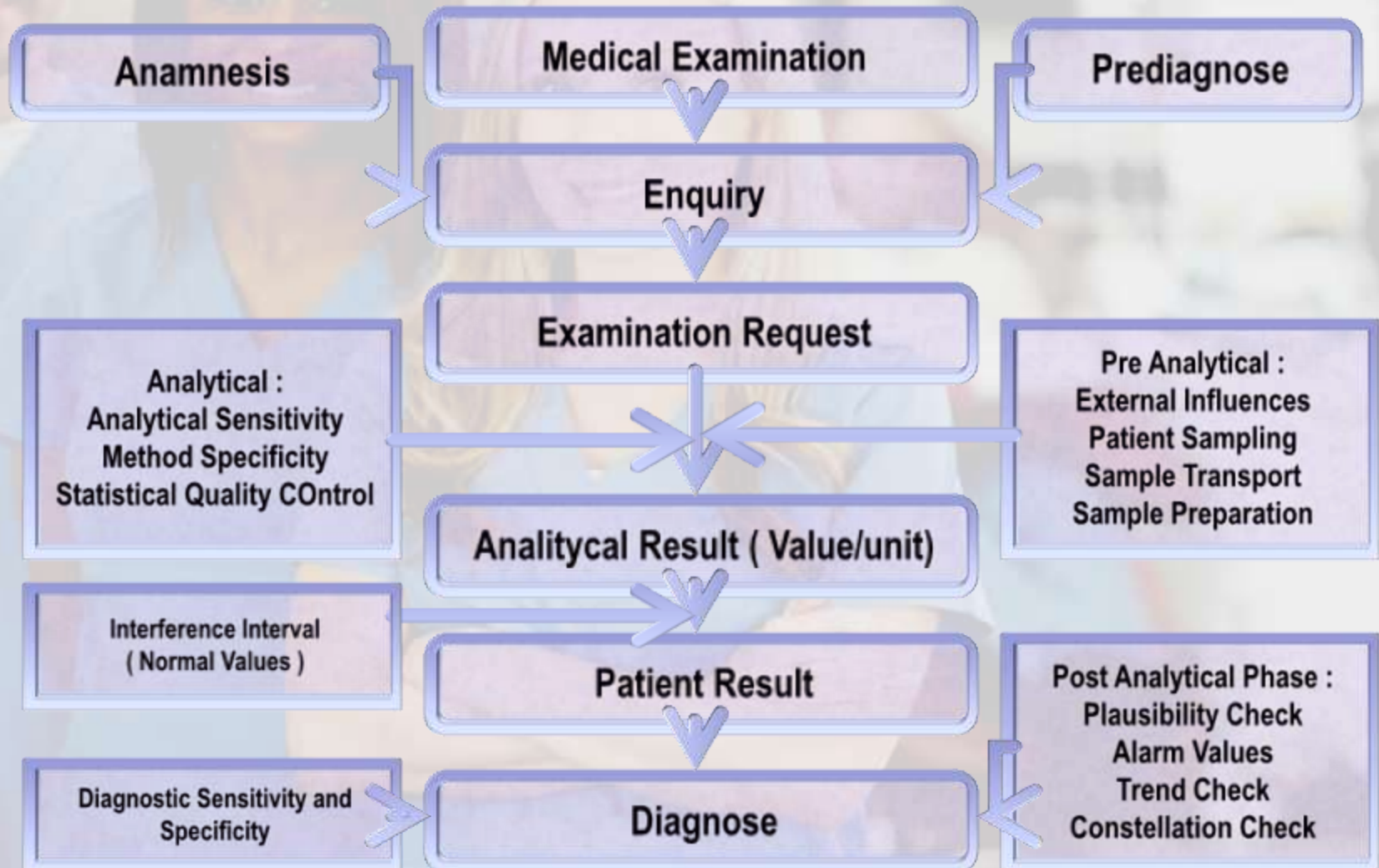


Liver Disease
Bilirubin

Kidney Disease
BUN, Creatinine



Medical Examination Overview



Medical Examination

- ❖ Objective is to get an answer about the health status of patient
- ❖ The physician determines on the basis of the anamneses, his clinical examination and on the basis of additional known information an enquiry → Examination Request
- ❖ His is Followed by the necessary preparation of the patient and blood sampling

What is Blood composition ?

Blood Composition :

- Plasma
- Cells



55 % Plasma

- Yellow, Sticky liquid
- Transport of nutrients (protein, Fats, Carbon Hydrates)
- Hormones

44 % Erythrocyte

- Red Blood cells
- Contain haemoglobin
- O₂ & CO₂ transport

Sample Container What do we use ?


Different type of sample collection in commercially available blood collection system (Beckton Dickinson Vacutainer, Sarstedt Monovetten,...)




Anti Coagulant agent	Ext	Application	Colour
Non coagulant	Serum	Clin chem, sero, immun	Red
heparin	plasma	Clin chem	Green
EDTA	Plasma	Hema, special chem, immun	Lilac
Citrate	Plasma	Coagulation test PT/APTT	Bleu
Na-Fluoride/K-Oxalat	Plasma	Glucose, Lactat	Grey
			Pink
			Yellow

What samples do we analyze ?


Invert
5
Times



Clot
30
Minutes



Spin
10
Minutes



• Gently invert 5 times to mix clot activator with blood.

• Allow blood to clot for a minimum of 30 minutes in a vertical position.

• Observe a dense clot.

• Centrifuge at FULL SPEED (between 1100 and 1300g) for 10 minutes for swing-head units or 15 minutes for fixed angle units (balance tube in centrifuge).

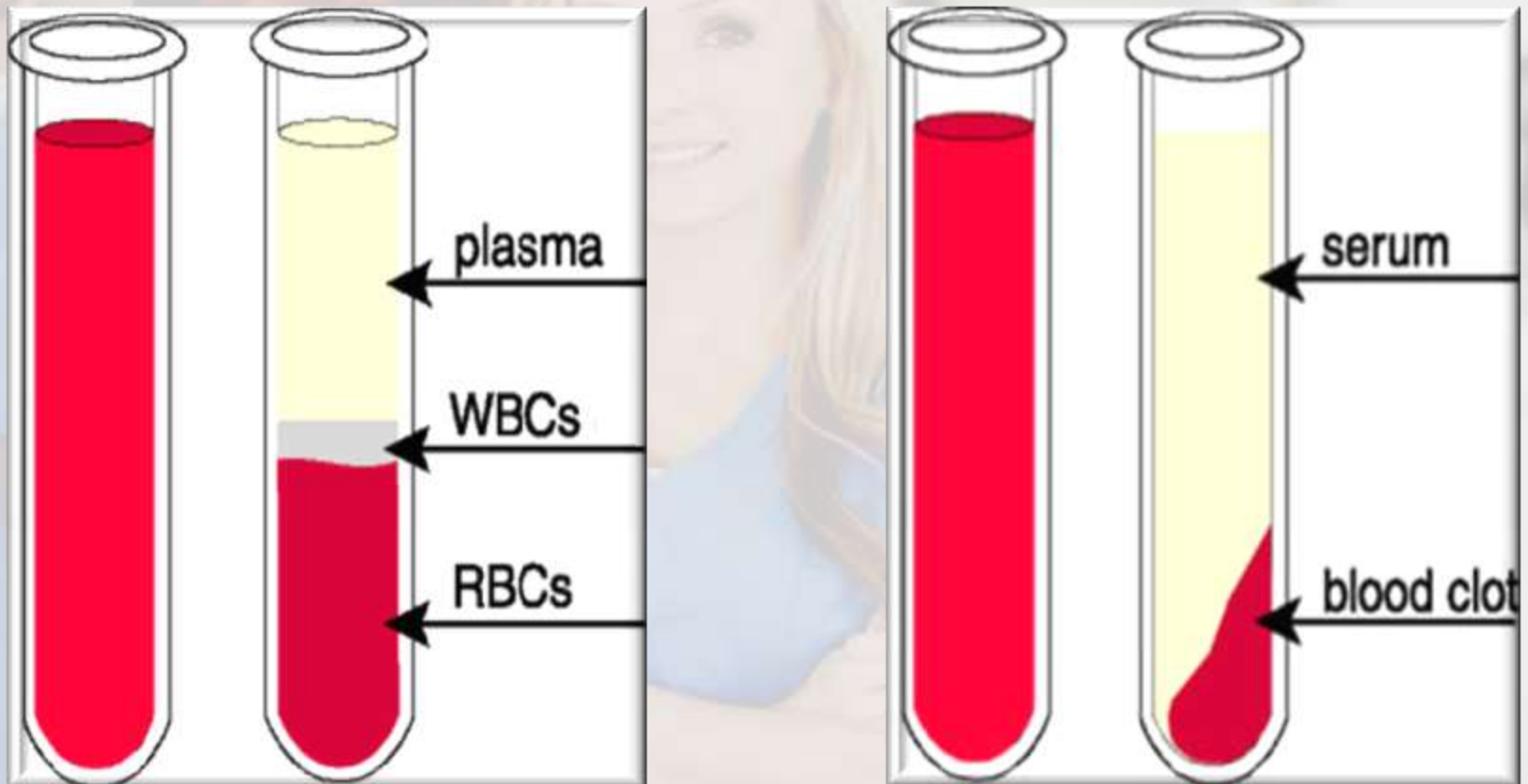
• Barrier will form, separating serum specimen from clot.

• Transport spun tube to laboratory.

BD Vacutainer Technical Services
1.800.631.0174



Plasma Versus Serum



Pre-Analytical



Possible Influences

- ❖ Age
- ❖ gender
- ❖ Genetic Influences
- ❖ Nutritional Influences
- ❖ Pregnancy
- ❖ Biorhythm (diurnal rhythm causing analytical fluctuations)
- ❖ Muscular mass, body weight
- ❖ Physical activity or inactivity
- ❖ Psychological stress (fear for blood collection, surgery)
- ❖ Use of medicines

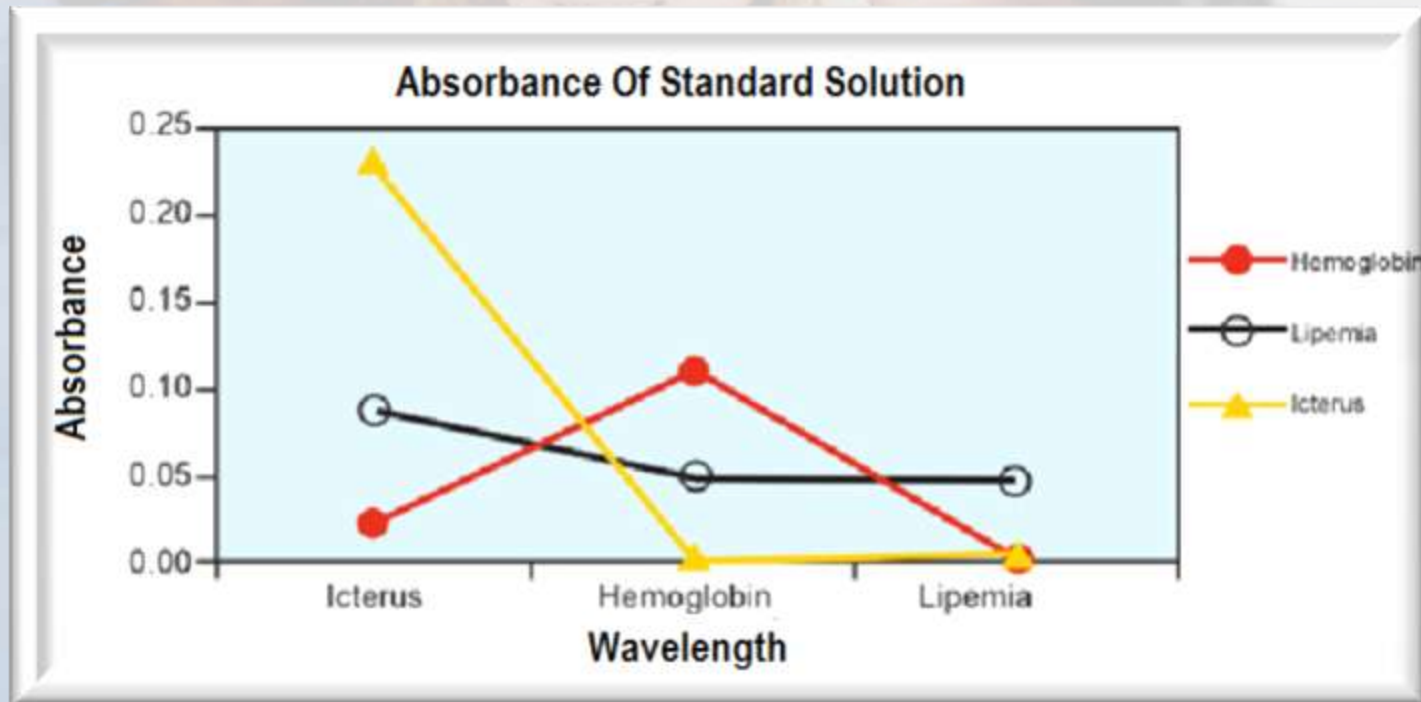
Pre-Analytical

Disturbing Influences

- ❖ Sample collection (body position, venous congestion, ...)
- ❖ Sample condition (haemolytic, Lipemic, Icteric)
- ❖ Normal serum obtained from an individual in good health is usually clear, pale yellow in color. However, the color of the patient's serum may appear different for various reasons such as disease or improper handling of the blood specimen.
- ❖ Lipemia (lipo) results from increased levels of lipoproteins associated with triglycerides, and it can cause the serum to appear white.
- ❖ Hemolysis (hemo) is caused usually by the release of hemoglobin from ruptured red blood cells during sample collection and/or sample handling; this interference can cause the serum to appear red.

Pre-Analytical

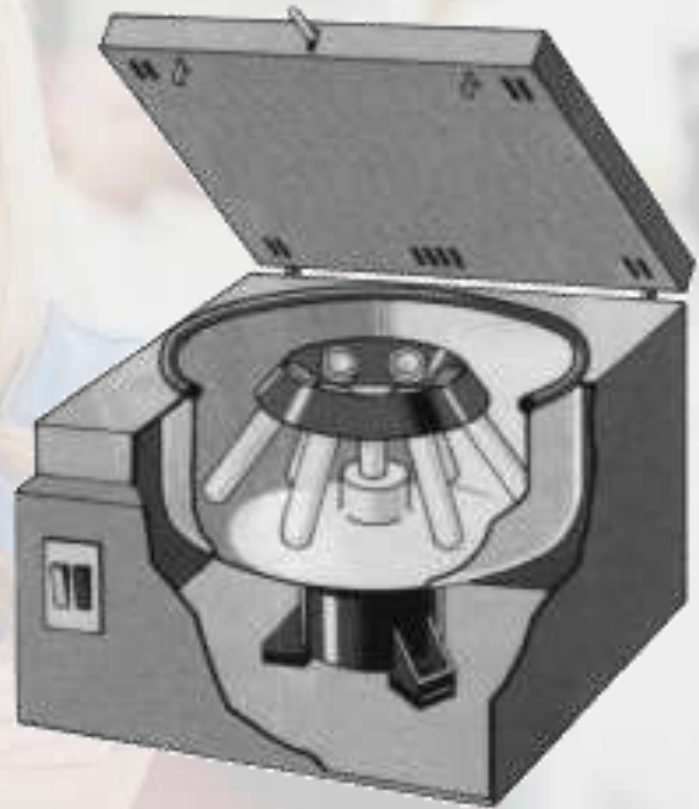
Icterus (Icte) is the result of increasing levels of bilirubin, and it can cause the serum to appear yellow.



Pre-Analytical

Separation Of Samples

- ❖ Centrifugation
- ❖ Deproteinization
- ❖ Chromatography
- ❖ Electrophoresis



Pre-Analytical

After the centrifugation if the sample was without anticoagulant
The supernatant fluid is SERUM
otherwise is plasma.

As anticoagulants they use EDTA K3, EDTA K2, Heparin, Citric Acid (:1, Citric Acid 4:1 NaF and others.

If we use plasma we must know the type of the anti-coagulant due to different interferences
Ca, Na, Fe, ALP



Pre-Analytical



Pre-Analytical

- ❖ Some photometric assays may be influenced by the presence of these abnormal serum colors and the reliability of the test result may be decreased.
- ❖ Haemolysis can cause analytical interferences such as high K⁺ caused by release from erythrocytes, or can interfere with the measuring technique (photometry)
- ❖ Inadequate sample transport
- ❖ Wrong centrifugation
- ❖ Inadequate sample storage (bilirubin)

Pre-Analytical

Serum

- 30-45 Minutes clotting (preferably in the dark)
- 10-15 minutes centrifugation @ 1000-1500 g

Plasma

- Immediate 10-15 minutes centrifugation @ 1000-1500 g

Pre-Analytical

Sample transport and storage

- ❖ Properly packed
- ❖ Transport must be save biohazardous material
- ❖ 4 hours stable @ 15-25⁰C closed to avoid evaporation
- ❖ 24 hours stable @ 4-8⁰C Dry Ice, cool packs, refrigerator, etc

Pre-Analytical

Example : Potasium

- ❖ Plasma is recommended for rapid centrifugation, use only serum or plasma for single patients
- ❖ Sample preparation (heparin plasma), centrifuge within 30-45 minutes after collection, erythrocytes produce hemocysteine, which continues after sampling
- ❖ Store on ice if centrifugation within 30-45 minutes is not possible
- ❖ Store plasma at 20°C if sample can be measured within 48 hours

Analytical

Adequate test methodology

- ❖ Standard operating procedure
- ❖ Understandable
- ❖ Traceable

Routine test must be

- ❖ Easy to be executed
- ❖ Reliable
- ❖ Low risk failure



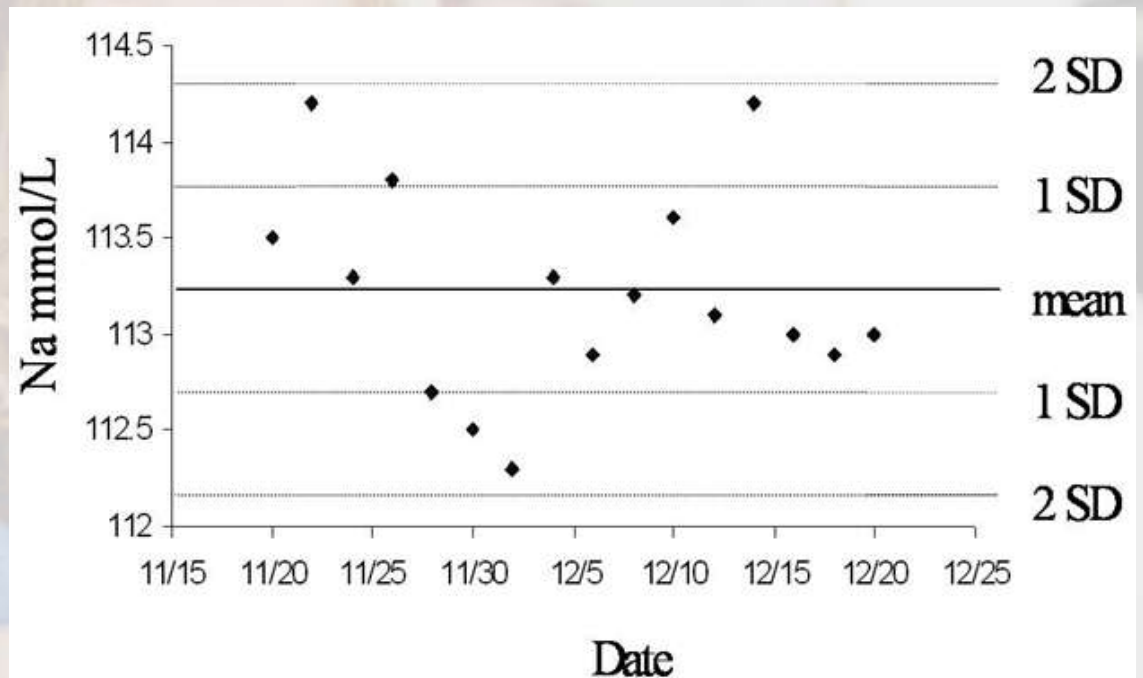
Statistical Quality Control

Samples with known concentration

- ❖ Low
- ❖ Medium
- ❖ High

As part of daily routine

- ❖ Begin of the run
- ❖ Middle in the Run
- ❖ End of the day
- ❖ Random



Pre-Analytical

Test Report

Demographic Information

- ❖ Patient name, Patient ID, Lab number
- ❖ Sample matrix, visual distortions
- ❖ Date, Time sample collection, arrival in the lab, time of analyses

Analytical results

- ❖ Test name, Unit Reference values, comments (High/Low, diluted, duplicates,...)

Analytical Result

Expected Values

Reference range

- ❖ Normal Values
- ❖ Based on a large pool of healthy persons

Differences between

- ❖ Children vs adult
- ❖ Male vs female
- ❖ Serum vs plasma
- ❖ Population
- ❖ biorhytem



Diagnose

After checking the reliability of the analysis

- ❖ Analytical range
- ❖ Statistical Quality Control
- ❖ Pre-analytical and analytical disturbances
- ❖ Plausibility of the result
 - ✓ Compared with previous result
 - ✓ Fit with the situation of the patient

Method Of Clinical Chemistry

Photometry

chemiluminescence

Potentiometry (ISE)

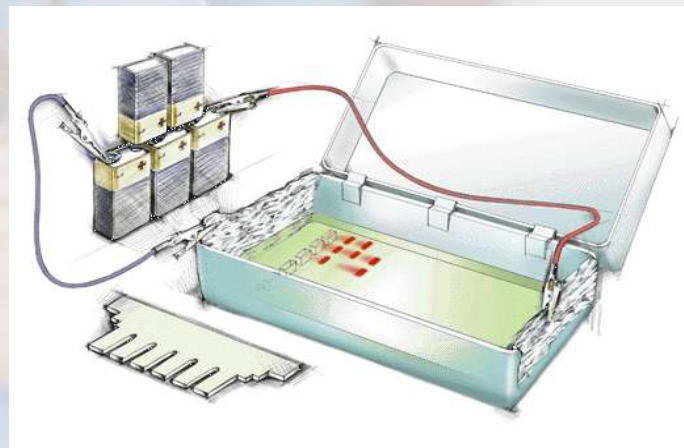
Electrophoresis

Nephelometry

Y-Counter

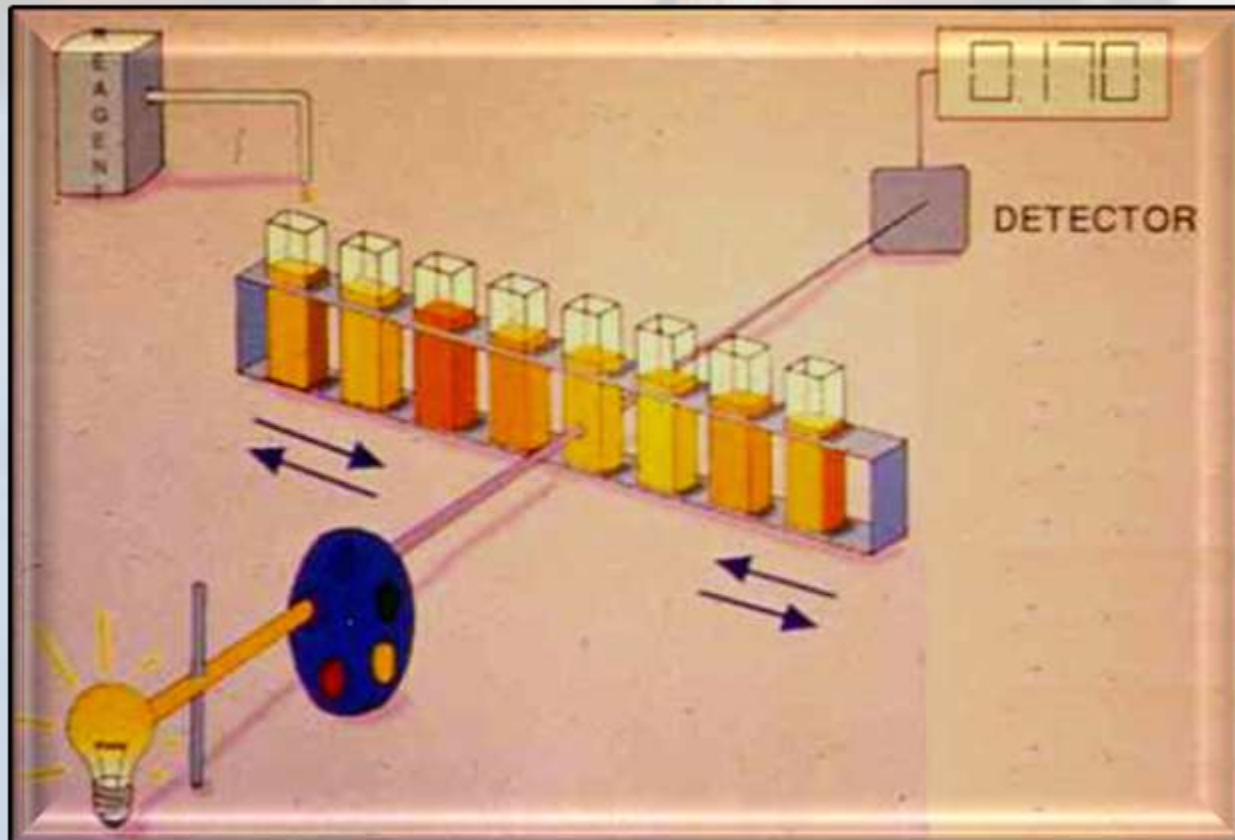
Mass Absorption

Osmometry

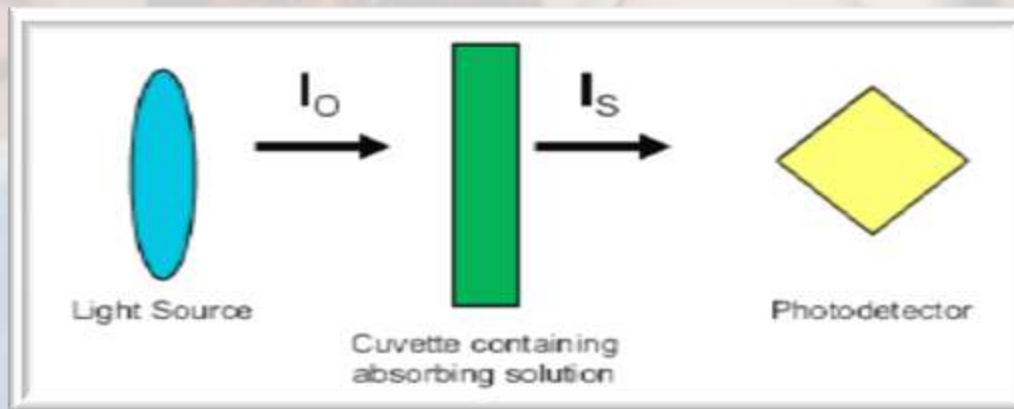


Photometry

In photometry, an aliquote of sample containing analyte is mixed in a cuvette with a liquid reagent. The reagent react with analyte producing a change in absorbance (color) within the reaction solution. The absorbance is measured using a photometry system



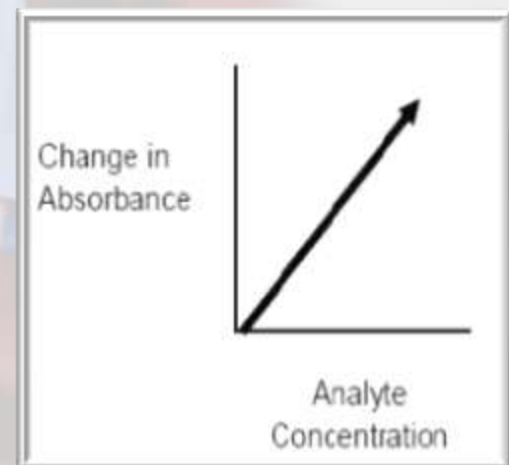
Photometry



This is achieved by comparing the amount of transmitted (I_s) light to the amount of light entering (I_0).

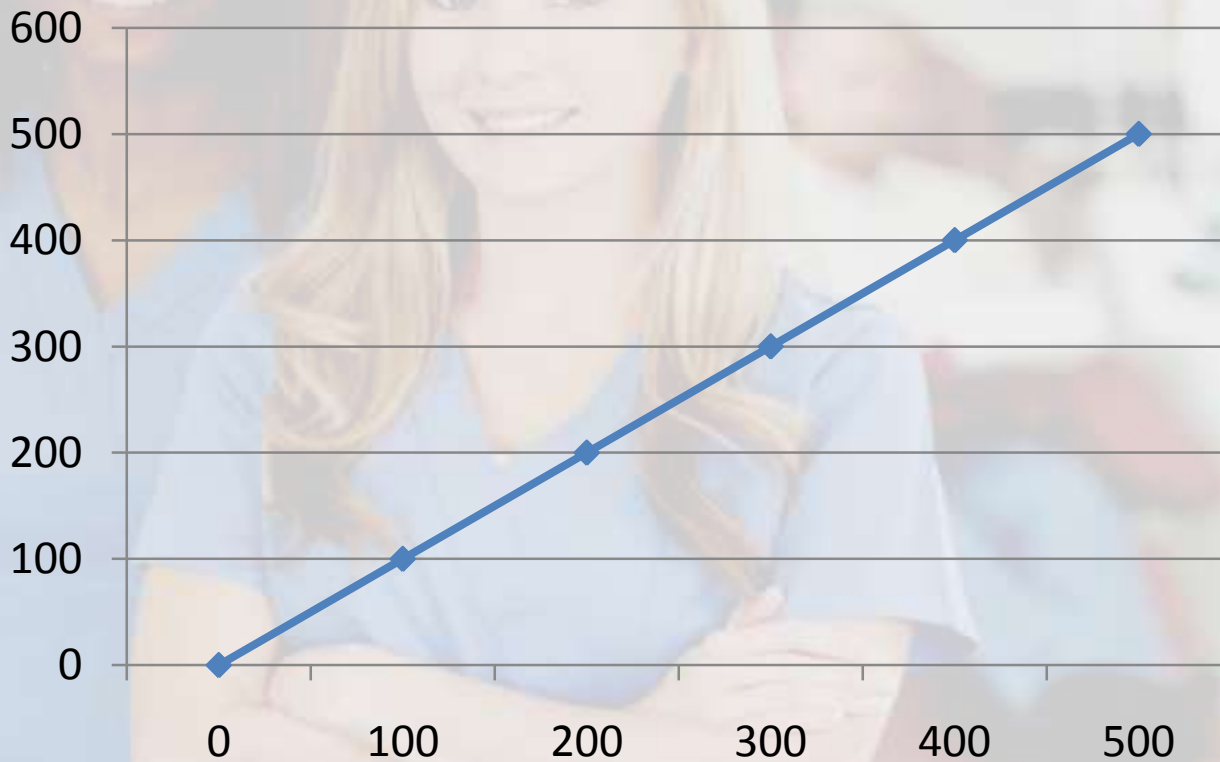
The change in absorbance is proportional to the concentration of analyte in the sample.

Typically, more analyte in the sample generates a darker colored solution in the cuvette, thus, less light gets through to the detector.



Linear Calibration Curve

(Linear Reaction)



Slope = angle of line

Calculation

If a blank and only one calibrator are run, the factor is determined as :

$$F \text{ Conc std} = \frac{\text{Concstd}}{R_{\text{std}} - R_{\text{blk}}}$$

Where :

Conc std = concentration of the calibrator

R std = response of the calibrator

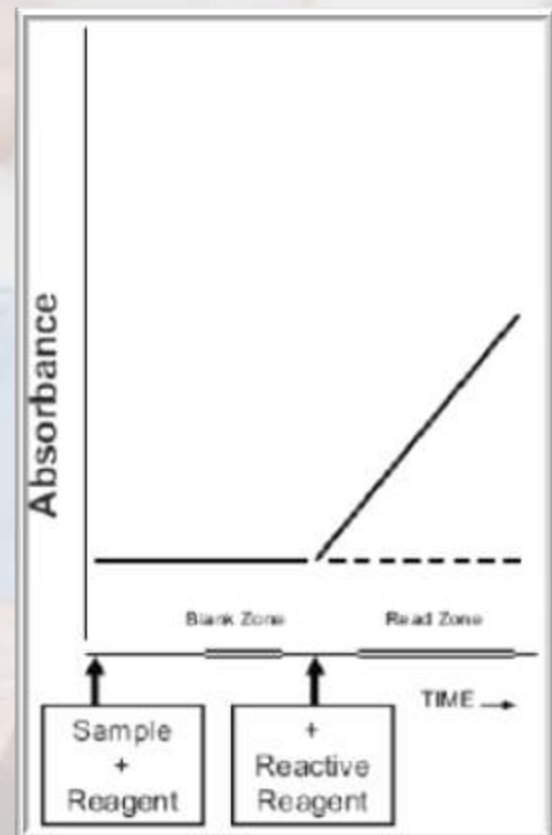
R blk = response of the blank

Type of Calibration	Number and type of Calibration	Conversion into concentration
Absolute calculation (ABS calc)	Reagent Blank	Reaction ABS X FV
One point calibration curve (STD calc)	Reagent blank one standard	Reaction ABS X FV/(STDABS)

Rate Method

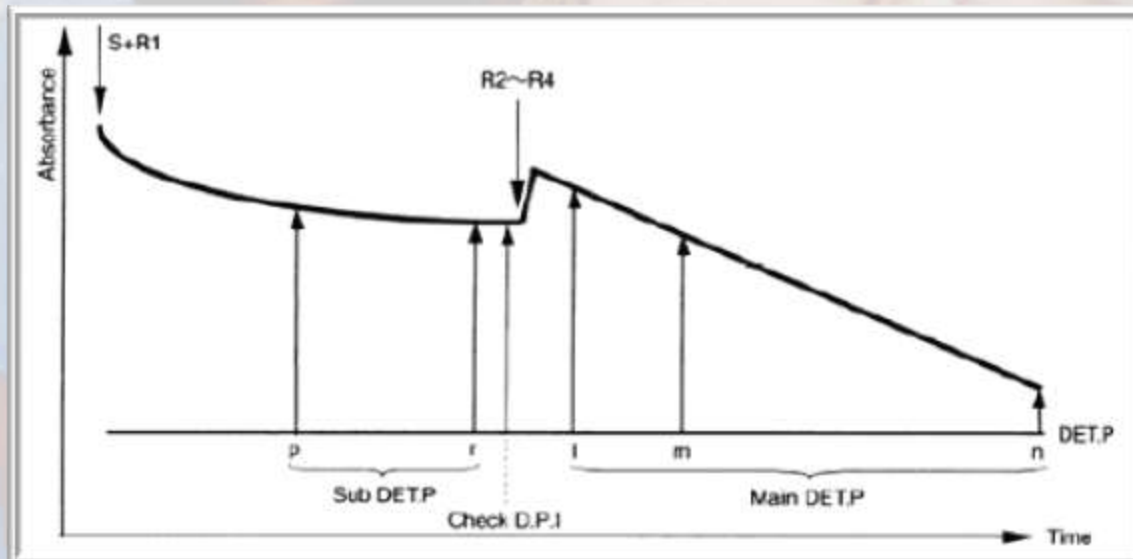
Rate reaction (Reaction change as a function of time): using this principle, a result is calculated from the change in signal per unit of time. The rate of the signal change is measured. These reactions can also be described as either **up** and **down**. Enzymes are measured using the rate reaction. Examples of rate-up are CK and LDH. Examples of rate ALT and AST.

Rate-Up Reaction



Rate or Zero Order Kinetics

RRA is the rate method of obtaining concentration or activation value from absorbance change per minute between two points using the least – squares method



L, m, n, p, r	Measurement points
S	sample volume
V, V_p, m	Reagent Volume
A_{mn}	Mean Absorbance, exclude min&max
Δa_{mn}	The change in absorbance per minute between measurement
T_{mn}	Time (min) between measurement points m & n
$A(tp)$	Absorbance obtained by substituting the time at measurement point into the approximation curve
$\Delta A(tp)$	The slope of the reagent to the approximation curve (the change of absorbance
per	minute)
K_{pm}	Liquid – volume correction coefficient

Example of setting general reaction process (decrease reaction) and measurement points using the RRA method

$$K_{pm} = (S+V_p)/(S+V_m)$$

Rate, Zero Order

Decrease :

340 nm AST/GOT-ALT/GPT, LDH P—L, Aldolase

FACTOR or FV = $(V_{\text{total}} \times 1000) / (V_{\text{sample}} \times \text{Light Path} \times \text{MEC})$

Increase :

340 nm : LDH L- P, CK, CKMB, HBDH, ELASTASE, LAP

405 nm : ALP, ACP, NP

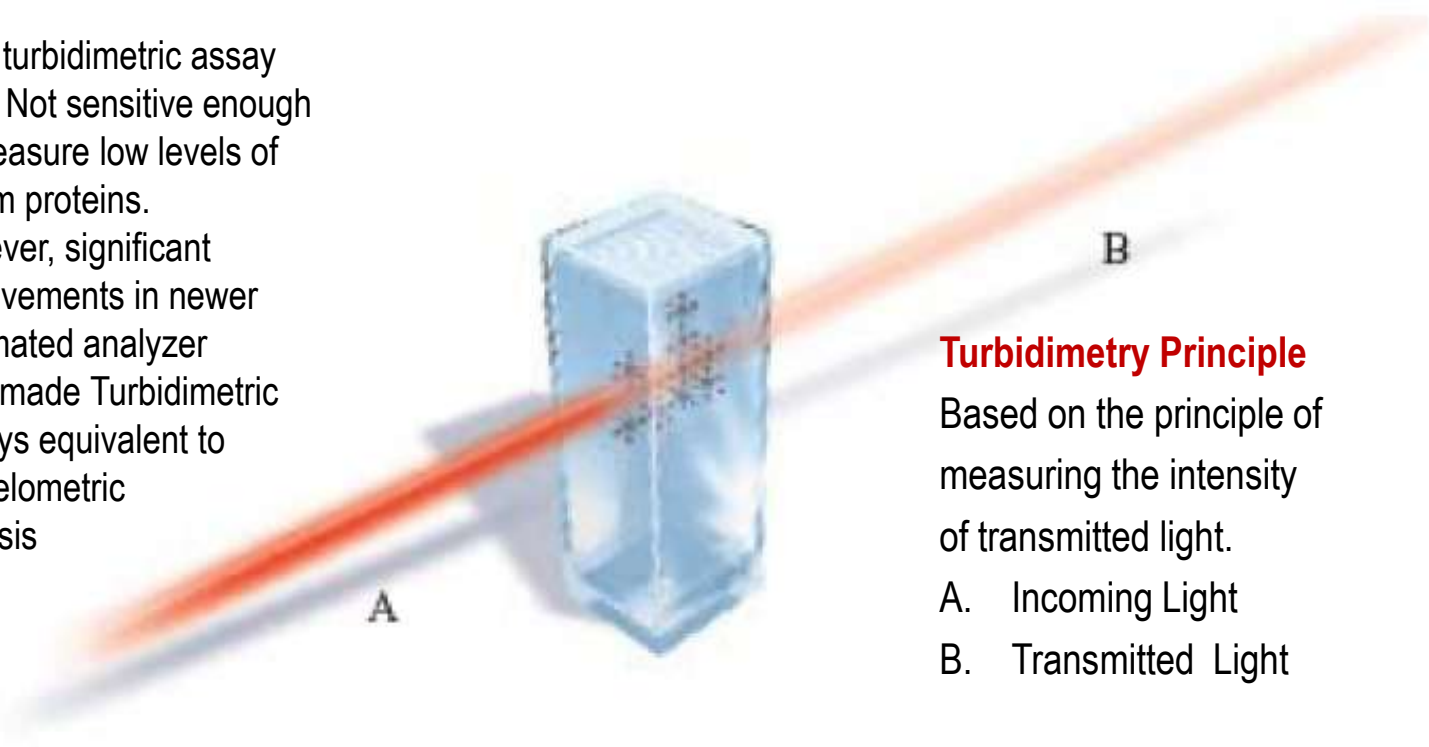
Factor or FV = $((V_{\text{total}} \times 1000) / (V_{\text{sample}} \times \text{Light Path} \times \text{MEC}))$

Turbidimetric Assay

Turbidimetric Assay

Turbidimetric assays measure the intensity of the transmitted light as shown below.

Early turbidimetric assay
Were Not sensitive enough
To Measure low levels of
Serum proteins.
However, significant
improvements in newer
automated analyzer
have made Turbidimetric
assays equivalent to
nephelometric
analysis



Turbidimetry Principle

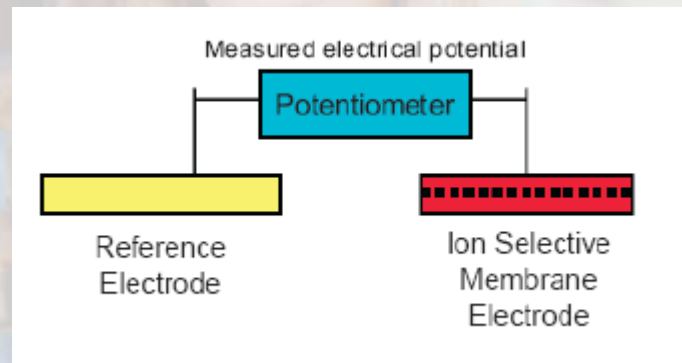
Based on the principle of measuring the intensity of transmitted light.

A. Incoming Light

B. Transmitted Light

Potentiometry

Potentiometry is based on electronical reaction and is the measurement of the electrical potential between two electrodes in an electronial cell. Examples of analytes that typically utilize potometry for their measurement are the electrolytes sodium (Na^+), potassium (K^+) and chloride (Cl^-).



Ion selective membrane electrodes (ISE) are utilized with spesific permeability to selected anions and cations (e.g, Valinomycin membrane to measure (K^+) . Sample containing analyte is brought into contact with the ion specific membrane. Concentration are calculated from the measured potential through the Nernst equation.

Potentiometry

Direct potentiometry : this is the simplest method of making ion-selective electrode measurements. The electrodes are immersed in test solution and the electrode potential is measured directly to this measurement by reading the answer from a calibration graph of concentration versus millivolts.

Indirect potentiometry : dilution of the sample (less volume, less problems, less interventions)

Case Example



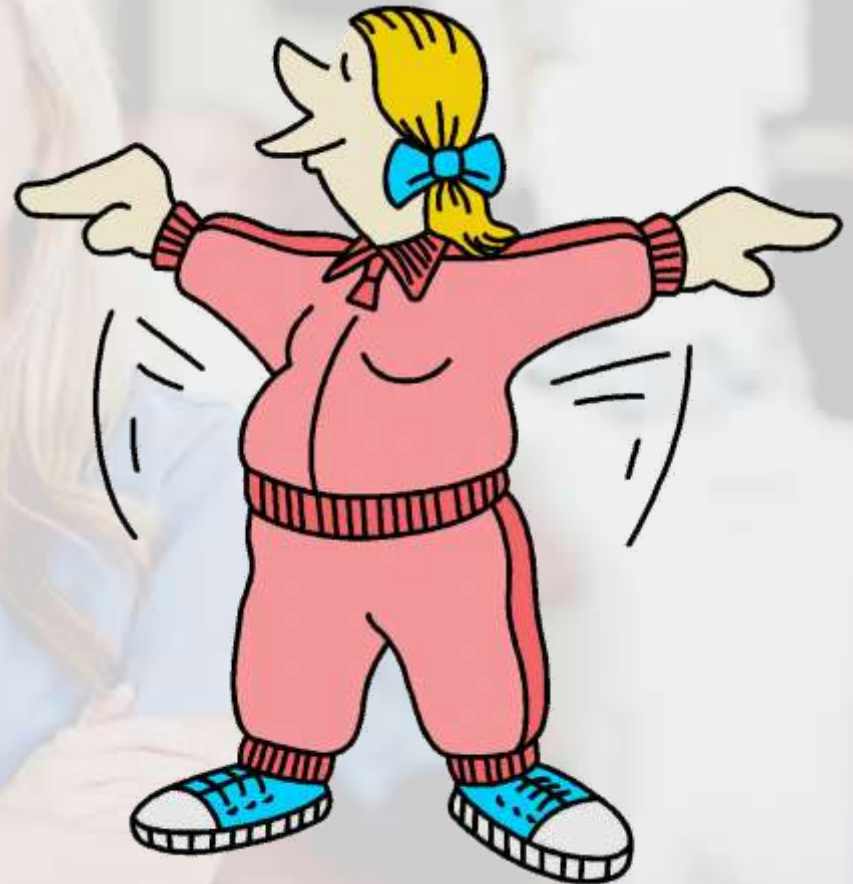
Where do
you find
high levels
of proteins

Proteins are present in all body fluids. Their concentration is normally high only in blood, serum, plasma, lymph fluid, and some exudates. There is a small amount of protein in spinal fluid and trace of protein in urine.

Proteins have many purposes. They function as antibodies, form part of the endocrine system, and provide a complex blood-clotting system. Additionally, they are carriers for other compounds, provide tissue nutrients, and function as enzymes. To determine disease processes it is important to compare levels for each fraction of the proteins to normal values

Pre-analytical factors that affect serum proteins concentration

- 1) Time of the day
- 2) Position
- 3) Exercise
- 4) Fasting vs non fasting
- 5) Medications
- 6) Time of year (season)
- 7) Age and gender
- 8) Geographic location
- 9) Venipuncture technique
- 10) Sample handling and storage



Patient Result

Test Report

Demographic Information

- Patient name, Patient ID, Lab Number
- Sample Mtrix, Visual distortions
- Date, time sample collection, arrival in the lab, time of analyses

Ana

Auto Validation



Automatic

Limits defined by the lab

Delta Check

Quality Control with westgard rules

Messages from the system

Diagnose

