Al-Mustaqbal University College

Pharmacy Department – Fifth Class

Clinical Chemistry

Specimen Collection

First Lec.

Asst. Lec. ZAINAB GHALEB

Out Line

- Introduction of Clinical Chemistry.
- Collection of Blood Samples.
- Types of Blood Samples.
- Anticoagulants.
- Types of Anticoagulants.
- Beer – Lambert Law.

Introduction

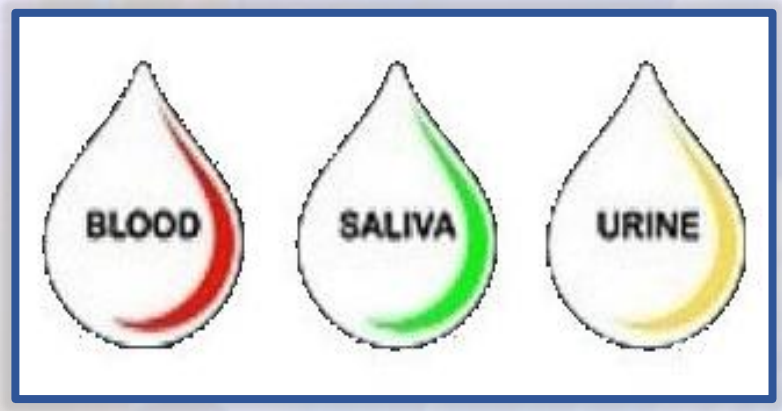
What is Clinical Chemistry?

- **Clinical Chemistry** is the branch of laboratory medicine that focuses primarily on **Molecules**.

- The tests in a Clinical Chemistry lab measure **concentrations** of biologically important **ions** (**salts & minerals**), **small organic molecules** and **large macromolecules** (**primarily proteins**).

The function of Clinical Chemistry Labs is:

- Perform Quantitative and Qualitative tests of Body Fluids such as (Blood, Urine, CSF), as well as Feces, Calculi, Tissues and other materials.



- Help in Diagnosis and Treatment of diseases therefore these tests must be down as accurately as possible.

Combinations of Tests (Panel):

- When an individual test alone is not sufficient to assess a medical condition, a combination of several tests may be used.
- The pattern of results from the combination of tests may provide better insight into the status of the patient than any single test result.
- Such tests, done on the same sample, are often ordered as a group called a panel or profile.

Examples:

LIPID PROFILE

- Total Cholesterol
- LDL Cholesterol
- HDL Cholesterol
- Triglycerides

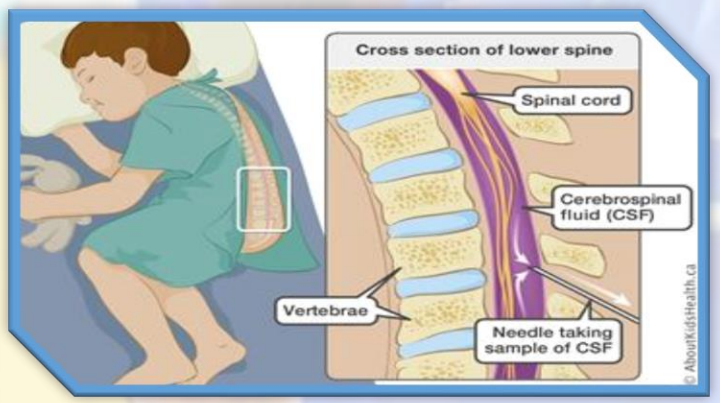
HEPATIC PANEL (LIVER PROFILE)

- Albumin
- Total Protein
- Alkaline Phosphatase
- Alanine Aminotransferase (ALT)
- Aspartate Aminotransferase (AST)
- Total Bilirubin
- Direct Bilirubin

Types of Lab. Samples

- The most common **Sample** that be examined in **Clinical Lab** is **Blood**.

- Other Samples that can be analyzed: Urine, Cerebrospinal Fluid (CSF).



Collection of Blood Samples

- Most Quantitative tests in Clinical Chemistry Labs are carried out on Blood Sample. It can be obtained from:
  - ✓ Venous Blood.
  - ✓ Capillary Blood.
  - ✓ Arterial Blood = Rarely examined.

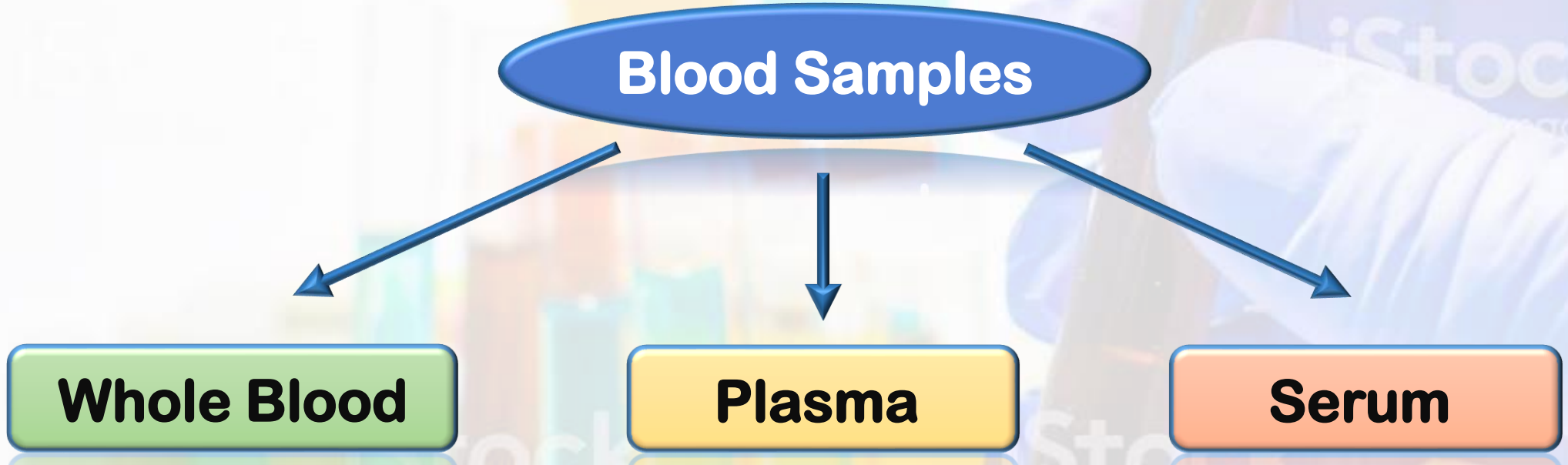
- **Venous & Capillary Blood are most common in the Determinations made on Blood.**

✓ **Capillary Blood = obtained from a Finger or Thumb.**

✓ **Venous Blood = obtained from any Prominent Vein.**

Types of Blood Samples

- **Blood Samples** can be <u>divided</u> according to the type of the test into:

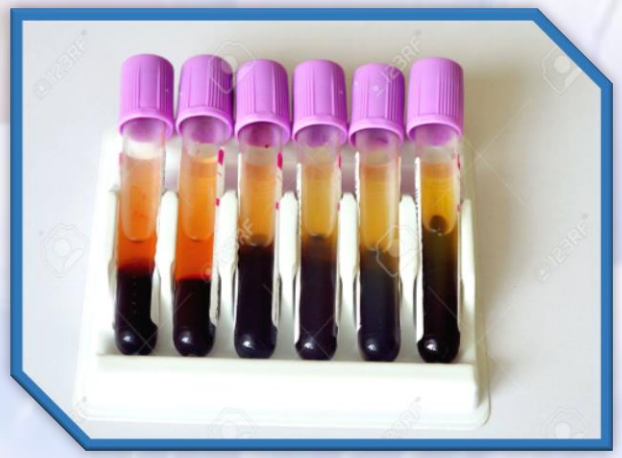


Whole Blood Sample

- ✓ Blood drawn from patient must be directly put in a tube containing an Anticoagulant to prevent the Clotting.
- ✓ The Blood must be mix thoroughly with Anticoagulant inside the tube and is not centrifuged.
- ✓ Used for tests(Example): Hemoglobin (Hb), Ammonia.

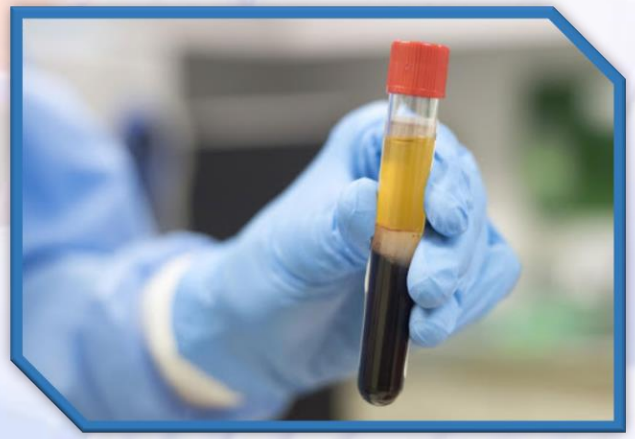
Plasma Sample

- ✓ Blood drawn from patient must be directly put in Anticoagulant tube to Prevent the Clotting.
- ✓ Then tube must be put in Centrifuge to Separate the Red cell from the Supernatant (plasma).
- ✓ Used for tests (Example): Chloride, Ascorbic acid (Vit C), Fibrinogen, Bicarbonate.



Serum Sample

- ✓ Blood drawn from patient put in a tube without anticoagulant and allowed to Clot.
- ✓ Then the tube put in the Centrifuge to Separate the Clot from Serum.



- ✓ Used for most tests (Example): Total Protein, Cho, TG, Creatinine, Uric acid, Bilirubin, Ca, Na, K, Urea.

❑ Plasma differs from Serum in Containing Fibrinogen and Anticoagulant.

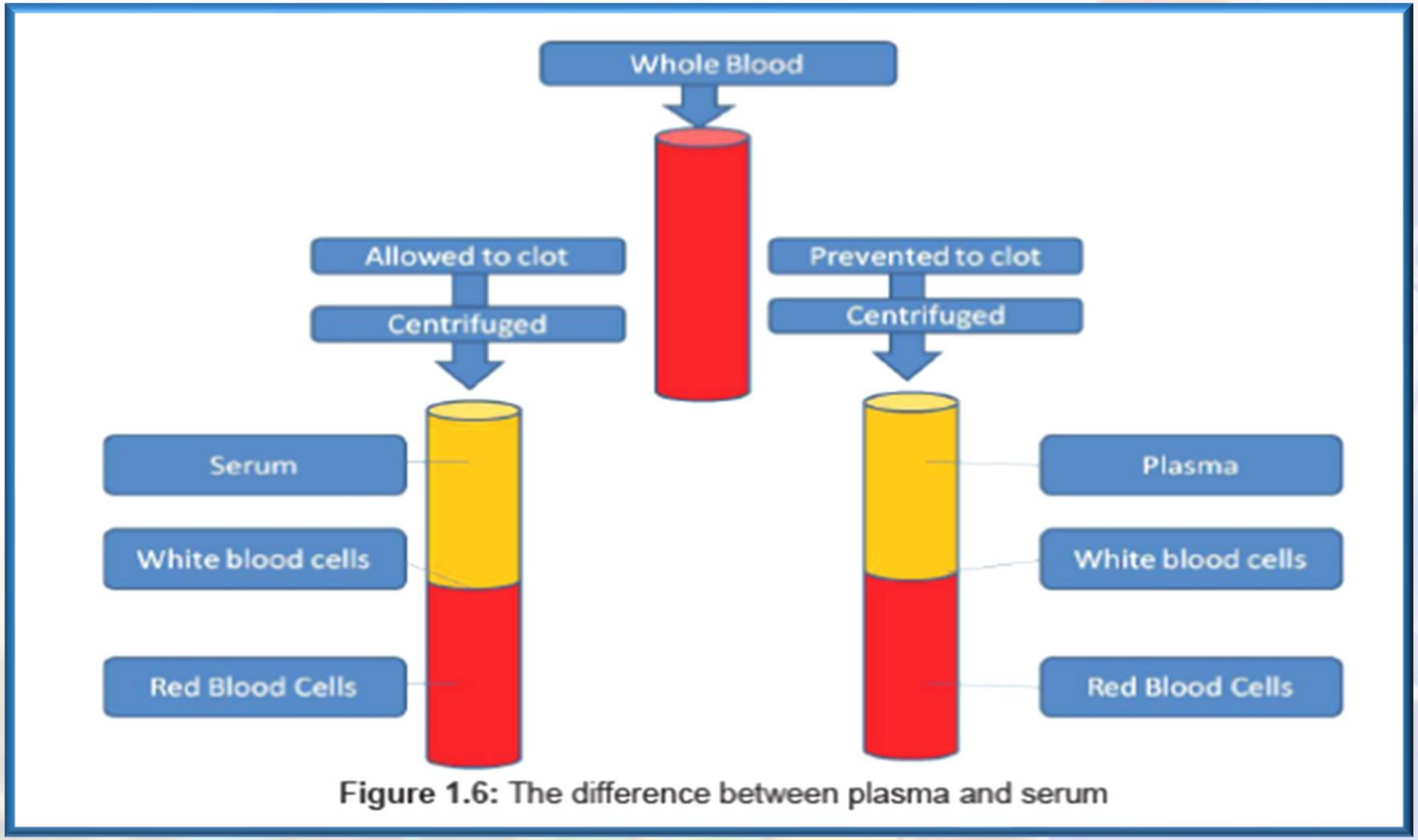
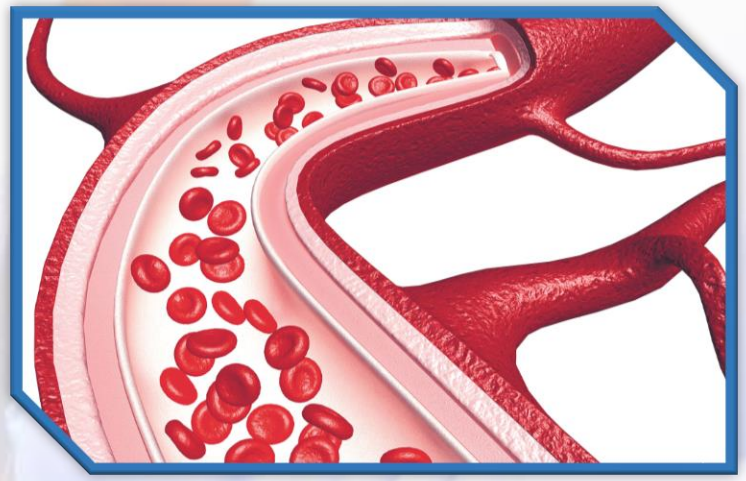


Figure 1.6: The difference between plasma and serum

Anticoagulant

What is Anticoagulant:

- A Chemical substance used to **Prevent** the **Coagulation of Blood**.



- The **Anticoagulant** is **applied** when using **Whole Blood** or **Plasma** is required.

- Used for Preventing Coagulation of Blood without Diluting & without causing a change in the Volume of Red cells.
- Salt causes Precipitation of Ca^{2+} in the form of Calcium Salt and this prevent Coagulation.
- Some of the Commonly used Anticoagulants are:

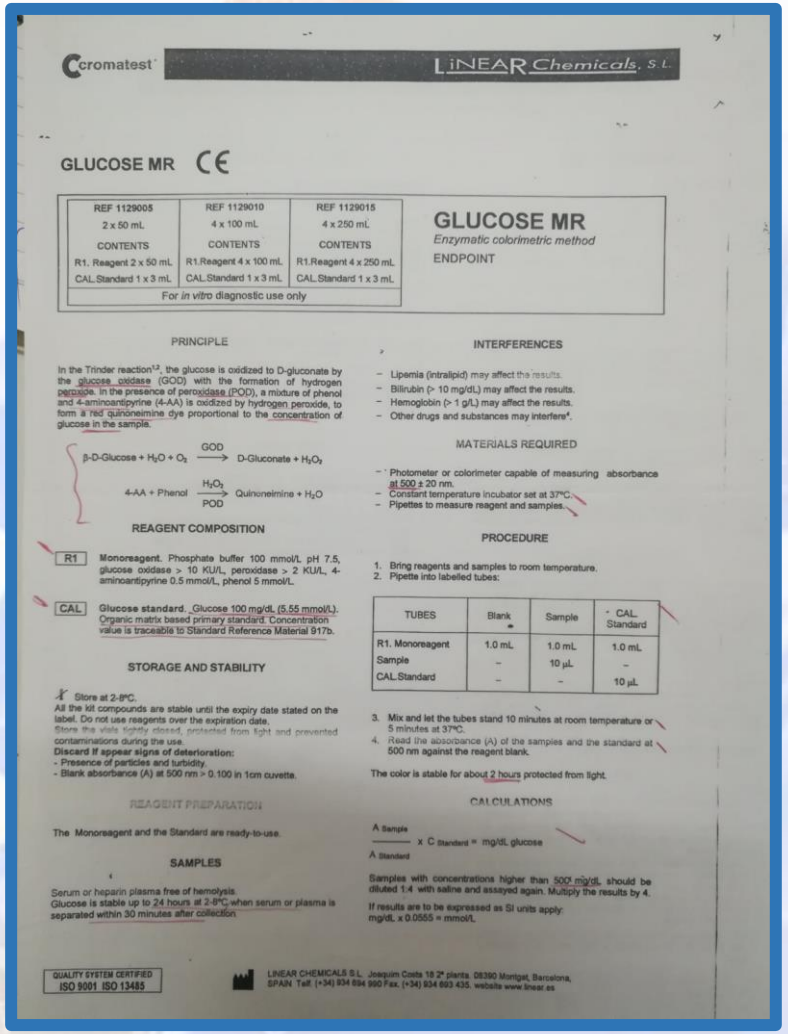
Types of Anticoagulant

- **EDTA** (Ethylene Diamine Tetra-acetic Acid): it may be a Disodium or Dipotassium salt. Act by removing Calcium ions by Chelation.
- **Oxalate** & **Citrate**: act as Anticoagulants by Removing Calcium Ions essential for Blood Coagulation.

- **Sod-fluoride:** it is used when **Blood** is collected for **Glucose** or **Lactate** estimation.
- **Heparin**: Inhibit the formation of **Thrombin** from **Prothrombin** it not produces a change in the compositions of Blood.

Chemicals:

❑ In clinical Lab we used (Kit):



cromatest LINEAR Chemicals, s.l.

GLUCOSE MR CE

REF 1129005	REF 1129010	REF 1129015
2 x 50 mL	4 x 100 mL	4 x 250 mL
CONTENTS	CONTENTS	CONTENTS
R1. Reagent 2 x 50 mL	R1.Reagent 4 x 100 mL	R1.Reagent 4 x 250 mL
CAL.Standard 1 x 3 mL	CAL.Standard 1 x 3 mL	CAL.Standard 1 x 3 mL
For *in vitro* diagnostic use only		

GLUCOSE MR
Enzymatic colorimetric method
ENDPOINT

PRINCIPLE

In the Trinder reaction[1,2], the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide, to form a red quinoneimine dye proportional to the concentration of glucose in the sample.

β-D-Glucose + H_2O + O_2 —GOD→ D-Gluconate + H_2O_2

4-AA + Phenol —H_2O_2, POD→ Quinoneimine + H_2O

REAGENT COMPOSITION

R1 Monoreagent. Phosphate buffer 100 mmol/L pH 7.5, glucose oxidase > 10 KU/L, peroxidase > 2 KU/L, 4-aminoantipyrine 0.5 mmol/L, phenol 5 mmol/L.

CAL Glucose standard. Glucose 100 mg/dL (5.55 mmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 917b.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard if appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 500 nm > 0.100 in 1cm cuvette.

REAGENT PREPARATION

The Monoreagent and the Standard are ready-to-use.

SAMPLES

Serum or heparin plasma free of hemolysis.
Glucose is stable up to 24 hours at 2-8ºC when serum or plasma is separated within 30 minutes after collection.

INTERFERENCES

- Lipemia (intralipid) may affect the results.
- Bilirubin (> 10 mg/dL) may affect the results.
- Hemoglobin (> 1 g/L) may affect the results.
- Other drugs and substances may interfere[4].

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 500 ± 20 nm.
- Constant temperature incubator set at 37ºC.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	Sample	CAL. Standard
R1. Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
CAL.Standard	–	–	10 µL

3. Mix and let the tubes stand 10 minutes at room temperature or 5 minutes at 37ºC.
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for about 2 hours protected from light.

CALCULATIONS

$\frac{A_{Sample}}{A_{Standard}}$ x $C_{Standard}$ = mg/dL glucose

Samples with concentrations higher than 500 mg/dL should be diluted 1:4 with saline and assayed again. Multiply the results by 4.

If results are to be expressed as SI units apply:
mg/dL x 0.0555 = mmol/L

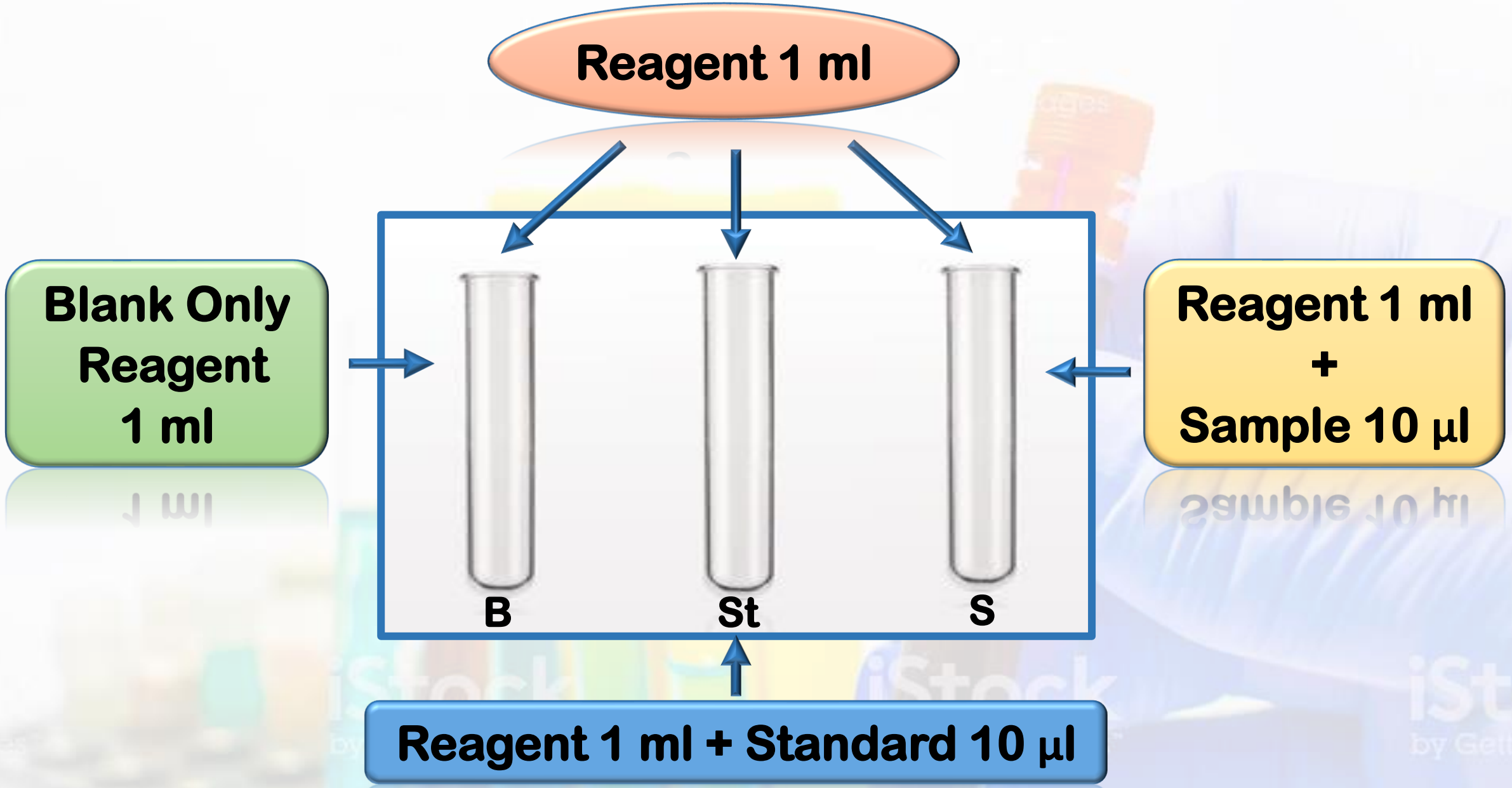
QUALITY SYSTEM CERTIFIED ISO 9001 ISO 13485

LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN. Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es

Procedure:

Solutions	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Micropipette:

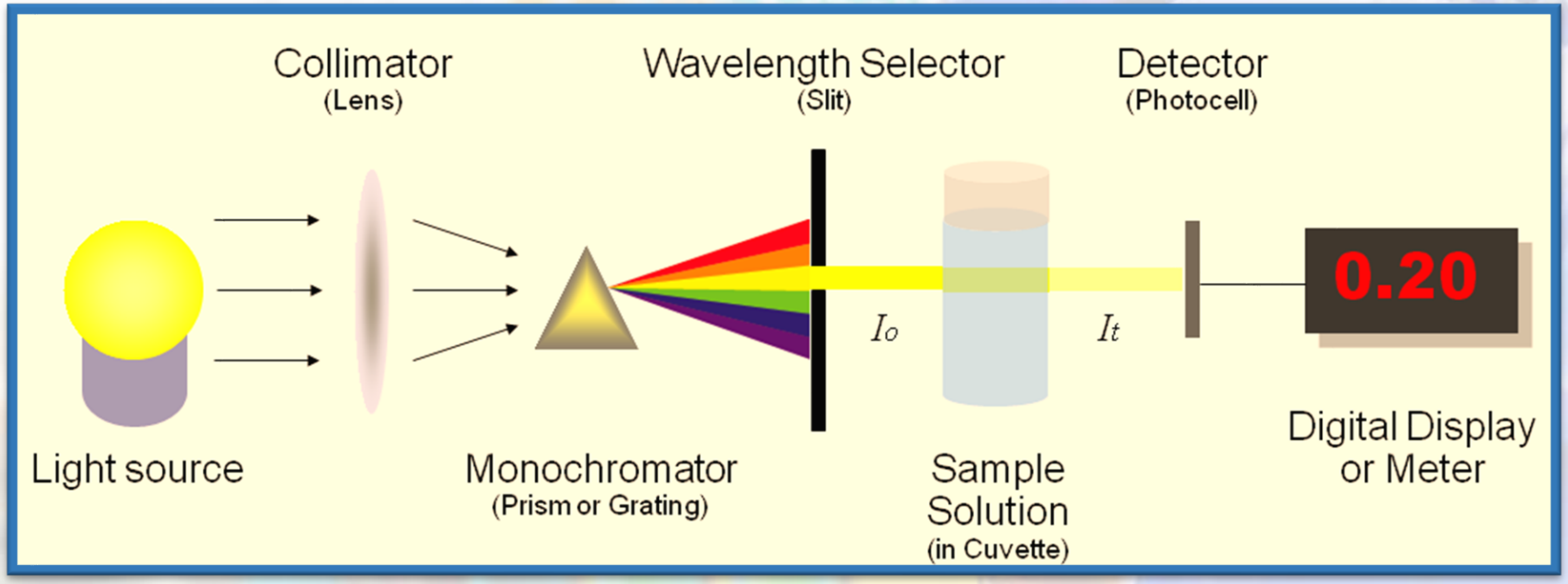

Reagent 1 ml
Blank Only
Reagent
1 ml
Reagent 1 ml
+
Sample 10 µl
B
St
S
Reagent 1 ml + Standard 10 µl

Calculations:

- Measurements in Clinical Lab are based upon (Photometric measurement) which defined as making a measurement of Light Intensity depended of Wavelength.

- Most Instrument used is called Spectrophotometer.

- **Beer – Lambert law**: absorbance is directly proportional to the light path through the cell. **A= abc**
- Where **A** is absorbance **a**= proportionality constant **b**= light bath in cm **c**= concentration.



❑ According to Beer-Lambert Law we can compare the conc. of Sample (test) with conc. of Standard:

a & b are constant

A= abc — For Standard

A= abc — For Sample

So, the Equation is:

$$C\ sample = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Standard conc.}$$

Units:

- **Results in Clinical Biochemistry can be expressed in a variety of units such as:**

- **mmol/l.**
- **mg/100ml or mg/dl.**


Thank You!