Demonstrated by

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CLS333

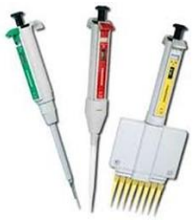
Introduction

Part#1

- **Course Description:**
- This is a required course in biochemistry for preparing a generalist in medical technology program. The course will provide a useful application of basic biochemistry along with physiological chemistry in developing an appreciation of the clinical significance of the tests, test methodology and interpretation of test results
- Carbohydrates
- Proteins
- Lipids
- Liver function
- Renal function

***lab tools:**

- Mostly we will use:
- **Micropipette.**



*Pipettes are used to accurately measure and dispense small vo liquid

How to Use a Micropipette:
1. Select the correct size micropipette and tips.
2. Dial the volume adjustment knob to set the proper volume in the digital volume indicator.
3. Place the tip securely on the micropipette.
4. Hold the micropipette vertically over the solution and push the plunger down to the first stop.
5. Insert the tip into the solution.

6. Slowly release the plunger and note that the solution is drawn into the tip.

7. Look at the tip to be sure that you do not have bubbles in the tip.

8. Dispense the solution touching the tip to the side of the target container. Slowly depress the plunger to the second stop. Before releasing the plunger, remove tip from target container.

9. Be sure the tip is empty, then use the tip ejector to dispose of the tip into an appropriate disposal container.

- **Spectrophotometer**
- **Test tubes**

<u>*Samples:</u>

Serum is plasma without clotting factors; whereas plasma is the liquid component of blood.

- Test tubes usually have additives to make blood behave in specific ways until it can be tested. Some additives cause blood to clot, others prevent it from clotting. Different chemicals are used for different lab tests.
- Tests requiring **serum** are drawn into a test tube containing a clot activator. The tube will then be centrifuged.

✓ Light Blue – for coagulation tests (i.e. when a person is taking blood thinners)
✓ Gold (or Red plastic tubes*) - for tests needing serum instead of whole blood or plasma**
✓ Green - for some of the chemistry tests
✓ Purple/Violet- hematology tests (CBC, ESR…)
✓ Grey- for blood sugar tests

***student should know the tubes e.g.**

Grey-red-bright blue-green-yellow-lavender tops

Lab 1

Glucose

Quantitative determination of glucose in serum or plasma

-Glucose is a major carbohydrate present in the peripheral blood . The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations help to diagnose and treat the diabetes mellitus(D.M).

-The blood glucose level is normally maintained within a narrow range under various conditions by hormones ,such as insulin, glucagon, or epinephrine.

-Patients with diabetes demonstrate an inability to produce insulin.

-Clinical diagnosis should not be made on a single test result.

***clinical significance:**

-Elevated glucose associated with:

pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease

-Low glucose associated with:

insulinoma, hypopituitarism , neoplasms, or insulin hypoglycemia

***principle:**

$$Glucose + O_2 + H_2O \xrightarrow{GOD} Gluconic\ acid + H_2O_2$$

$$H_2O_2 + 4\ aminoantipyrine + PHBS \xrightarrow{POD} quinoneimine\ dye(red\ color) + 4H_2O$$

The quinoneimine dye has an absorption maximum at 510 nm. The amount of color produced is directly proportional to the glucose content of the sample.

***specimen:**

Serum or plasma or CSF

Free of hemolysis or clot(glycolysis)

Stability: stable at 2-8 ºC for one day.

***procedure:**

1. Assays conditions:

Wavelength……..510 nm (490-550)

Cuvette……1 cm light path

Temperature……37 ºc

2 .Adjust the instrument to zero with distilled water

3. Pipette into a cuvette

	Blank	Standard	sample
WR (mL)	1.0	1.0	1.0
Standard µL		10	
Sample µL			10

4. Mix and incubate for 10 min at 37ºc
5. Read the absorbance (A).

* The color is stable for at least 30 min

Calculation

(A)Sample x 100(std conc)= mg/dL
(A)standard

Conversion factor: mg/dL x 0.0555=mmol/L

Normal Range:
Fasting……70-105 mg/dL
Or ……3.9-5.8 mmol/L

***limitation:**

The following substances reportedly cause decreased glucose values :

Ascorbic Acid when greater than 5 mg/dL

Hemoglobin when greater than 0.7 g/dL

Bilirubin when greater than 15 mg/dL

Uric Acid when greater than 2.0 mg/dL

Cysteine when greater than 10 mg/dL

***Fasting plasma glucose (FPG) versus oral glucose tolerance test (OGTT):**

OGTT is performed by serial measurement of plasma glucose before and after a specific amount of glucose is given orally.

Although OGTT is more sensitive than FPG, it is affected by a large number of factors that result in poor reproducibility.

Therefore, an OGTT is rarely necessary for the diagnosis of DM.

***Whole blood versus plasma glucose:**

Most lab instruments measures plasma glucose, the results is more reliable.

It is 10-15% higher than whole blood glucose, since RBC contain less water per unit volume than plasma.

***Capillary versus venous plasma glucose:**

There is little difference between them.

in hyperglycemia, capillary plasma glucose may be significantly higher than venous plasma glucose.

***Hemoglobin A1C (glycosylated Hemoglobin):**

Whole blood sample used to monitor the degree of the control of blood glucose level during the past 2 months

Lab 2

Total protein

Quantitative determination of total protein in serum using a biuret reaction

***Definition:**

A large group of nitrogenous organic compounds that are essential constituents of living cells; consist of polymers of amino acids (are joined together by the peptide bonds); essential in the diet of animals for growth and for repair of tissues; can be obtained from meat , eggs and milk.

***Total Serum Protein:**

A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin.

Types:-

- ✓ Albumin.
- ✓ α1globulin (α1 Antitrypsin)
- ✓ α2globulin (haptoglobulin)
- ✓ β globulin (transferrin, fibrinogen)
- ✓ γ globulin (Immunoglobulins).

***Function of T.P:**

1. Structural protein: e.g. keratin
2. Enzyme and catalytic protein: e.g. pepsin
3. Transport protein: Hb, serum albumin

4. Hormonal protein: e.g. hormones as insulin, adrenalin

5. Contractile protein: e.g. actin and myosin

6. Storage protein: e.g. oval albumin, glutamine

7. Genetic protein: e.g. nucleic acid (DNA & RNA)

8. Defense protein: e.g. IG (immuno-globulins).

9. Receptor protein: hormones .

***sources:**

Albumin and most of α and β globulins are formed in liver.

IGs are synthesized by the plasma cells in lymph nodes, bone marrow and spleen.

TP= Globulin+ Alb.

Serum globulin can be separated into several subgroups by serum protein electrophoresis.

Albumin is tested for liver and kidney diseases

Globulin is tested for multiple myeloma.

***Methods for determination of total protein in serum:**

-Kjeldahl method.

-Refractometry.

-Biuret**

-Dye Binding.

-Ultraviolet Absorption

***clinical significance:**

Through osmotic pressure, serum protein is involved in the maintenance of normal distribution of water between blood and tissues . The several fractions of serum protein vary independently and widely in disease.

-Low protein is primarily caused by malnutrition, impaired synthesis, loss (as by haemorrhage) or excessive protein catabolism.

-Elevated protein levels are caused mainly by dehydration

*Hypoproteinemia or Hemodilution:

seen in : starvation, mal-absorption and burns.

*Hyperproteinmia or Hemoconcentration:

Occurs due to:

-dehydration (diarrhea, vomiting)

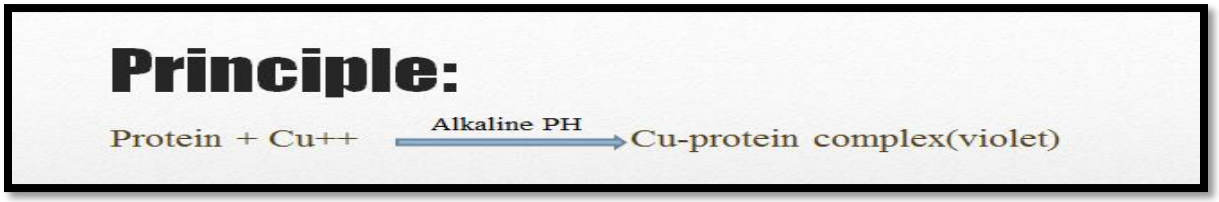
-excess synthesis of plasma proteins (multiple myeloma)

***protein in other body fluid:**

A: Urine protein:

proteinuria: due to increase concentration of total protein in urine > 12mg/dl.

B: CSF protein.



The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

***Effective Reagents:**

- ✓ Sodium potassium tartrate combined with Cu++ to prevent precipitation in the alkaline solution.
- ✓ Potassium iodide which acts as an antioxidant

Procedure:

	Blank	Std	Test
Biuret Rgt ml	1	1	1
Std µl	-	20	-
Sample µl	-	-	20

Mix and let stand at RT for 10 min.

Read the absorbance of standard and test at 540 ± 5 nm against blank.

The final color developed in the reaction is stable for at least 60 minutes

Calculation

$$\frac{\text{A test}}{\text{A Std}} \times \text{Conc.of Std (5g/dl)} = \text{T.P in test (g/dl)}$$

***Normal Range:**

Healthy young and middle aged adults

6.0-8.0 g/dl (60-80 g/I) Recumbent

6.5-8.5 g/dl (65-85 g/I) Ambulatory

Lab 3

Albumin

Quantitative determination of albumin in serum using the bromocresol green (BCG) dye binding method.

One of the most important serum proteins produced in the liver and It is protein that is water soluble

***Functions:**

-Nutrition

-Regulation of osmotic pressure

-Bind and transport of bilirubin, steroids, fatty acids & Ca

***Clinical significance:**

*Elevated serum albumin

-Associated with dehydration

-Skin lesions (ex. Dermatitis, burns, dehydration)

*Low serum albumin

-Malnutrition

-liver disease (Cirrhosis)

-Renal disease

-rheumatoid arthritis

The result is converting the color to Green-blue complex.

The absorbance of the solution increases in direct proportion to the albumin concentration

Procedure

	Blank	Std	Test
Alb Rgt -ml	2.5	2.5	2.5
Std-µl	---	10	---
Sample-µl	---	---	10

Mix and allow to stand at RT for 15 min.
Adjust the wavelength at 630nm and Read absorbance of all tubes within 60 min.

Calculation

$$\frac{A\ Test}{A\ Std} \times Conc.of\ Std(5\ g/dl) = Alb\ in\ test(g/dl)$$

***Normal Range:**

3.8-5.0 g/dl = 38-50 g/l

***Limitation:**

-Excessive hemolysis lead to increase Albumin

-Color Reagent has a reduced sensitivity to albumin in the presence of detergents and dioxane

-Ampicillin interferes with BCG methods.

-At a neutral pH and low albumin concentration, BCG will bind with some of the alpha and beta-globulins present in human serum.

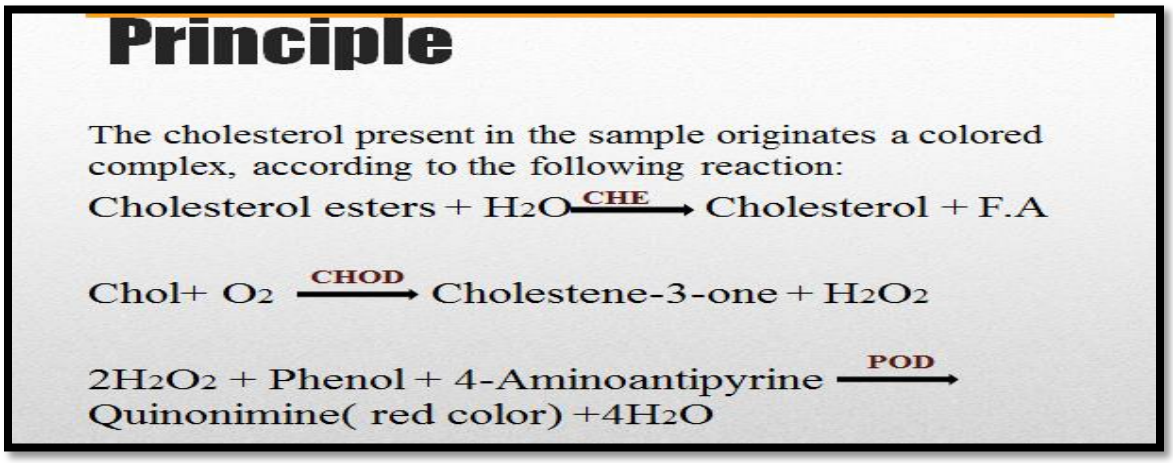
Lab 4

Cholesterol

Quantitative determination of total cholesterol in serum /plasma by enzymatic color/endpoint method

- Cholesterol is a fatty substance found in blood, bile and brain tissues.
- It serves as a precursor to bile acids, steroids and vitamin D. The determination of serum cholesterol is a major aid in the diagnosis and classification of lipemias,hepatic and thyroid diseases

- -High blood cholesterol is one of the major risk factors for heart disease.
- -Major dietary sources of it cheese, egg yolks ,beef, fish, and shrimp



CHE= cholesterol esterase

CHOD= cholesterol oxidase

POD= peroxidase

This dye is proportional to the cholesterol concentration of Cholesterol in the sample

***Specimen:**

Serum or heparinized sample

Procedure

	Blank	Std	Test
Chol Rgt-ml	1	1	1
Pre-warm at 37 C for 3min then:			
Std-µl	---	10	---
Sample-µl	---	---	10
Mix and incubate at 37C for 10 min, then read A at 505 nm			

Calculation

$$\frac{A\ test}{A\ Std} \times \text{Conc. Of Std(200 mg/dl)} = \text{Chol (mg/dl)}$$

***Normal Range:**

Desirable.....<200 mg/dl

Borderline high.....200-239

High....≥ 240

***Interfering Substances:**

- Anticoagulants, such as fluoride and oxalate will result in false low values.
- The test is not influenced by hemoglobin values up to 200 mg/dl or by bilirubin levels up to 10 mg/dl.
- Interference from grossly icteric and heavily hemolysed specimens is correctable by use of a serum/ plasma blank.

***Limitation:**

This reagent is linear up to 500 mg/dl.

- Samples with value of above 500 mg/dl should be diluted 1:1 with isotonic saline and re-run. Multiply the final results by two (2).
- Grossly lipemic serums require a "sample blank". Add (10 µl) of sample to 1.0 ml saline, mix and read the absorbance against water