Quantitative Determination of Blood Glucose

One of the most commonly performed procedures in clinical chemistry laboratory is assay of blood glucose. Used for the diagnosis and treatment or management of diabetes mellitus and other diseases.

Specimen:

Whole blood, or free of hemolysis plasma or serum, taken from patients in a:

fasting state (no food or drink other than water for 8-12 hrs). Or:

postprandial taken after 2 hrs the patient has had a meal.

Reference Values: (Normal Values)

Glucose, fasting, serum: Child 70-105 mg/dL (3.89-5.83 mmol/L)

Adult 70-105 mg/dL (3.89-5.83 mmol/L)

Whole blood: Adult 60-95 mg/dL (3.33-5.27 mmol/L)

Hyperglycemia: increase in blood glucose.

1- In diabetes mellitus.

2- Traumatic injury.

3- Gestational diabetes in some pregnancies.

Hypoglycemia: decrease of blood glucose. It is a life-threatening.

1- In liver disease with impaired glycogen metabolism.

2- Over dose of insulin, insulinoma.

3- Galactosemia.

4- Adrenal failure.

5- Pitutary failure.

6- ACTH or GH deficiency.

Methods:

1- Enzymatic methods like hexokinase method and glucose oxidase method.

2- Oxidation-Reduction Methods.

3- Aromatic Amine Methods.

Glucose + Glucose oxidase --------- gluconic acid + H_2O_2 + phenol + 4-aminoantipyrine

4-aminoantipyrine + peroxidase --------- Qunoneimine dye + $4H_2O$

Sample Handling:

Samples for glucose analysis should be delivered to the laboratory as soon as possible after being drawn from the patient. Effect of glycolysis by enzymes of RBCs will affect glucose. It must be separated within 30 min. Use sodium fluoride tubes to avoid glycolysis, and mix well. Sodium fluoride is good to preserve glucose.

Procedure:

1- Collect a blood specimens in a serum sample tube.

2- Leave it to coagulate for 20min.

3- Separate serum sample from the blood by centrifuga on 4000 rpm for 5min.

4- Separate serum in labeled (T) tube.

5- Prepare the following set of test tubes:

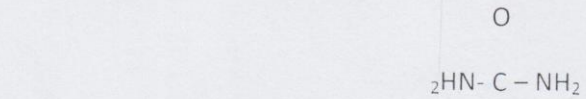
	T (Test)	S (Standard)	B (Blank)
Serum	10 microleter	---	---
Standard Solution	---	10 microleter	---
Distilled Water	---	---	10 microleter
Working Reagent	100 microleter	100 microleter	100 microleter

Mix well. Put it in 37 Centigrade for 5 min.

6- Measure the Absorbance (Optical Density, OD) readings by spectrophotometer at 520 nm.

Quantitative Determination of Blood Urea

Urea is the major non-protein nitrogen compound in the blood; others are amino acids, uric acid, creatinine, creatine and ammonia.



Urea is synthesized in the liver. As the proteins break downs into amino acids, urea will produce as a by-product of the deamination reactions of amino acids.

Urea elimination in the urine is the major route for nitrogen excreation. It is filtered from the blood at the glomerulus, but passive tubular reabsrorption occur in low rate of urine flow.

Clinical Significance:

The assay of urea is estimate of renal function. Blood urea concentration is used as an index of glomerular function, but blood creatinine is more accurate.

Because the concentration of urea is directly related to protein metabolism, the protein content of the diet will affect the amount of the urea in the blood.

Urea production increased by a high protein intake, high catabolic states, during dehydration and by absorption of amino acids and peptide after gastrointestinal hemorrhage.

Urea production decreased in patients with low protein intake, liver disease.

Reference Values: (Normal Values, Expected Values)

Serum Urea Nitrogen	mg/dL	mmol/L
adult	7 - 18	2.5 – 6.4
> 60 yr	8 - 21	2.9 – 7.5
Infant/child	5 - 18	1.8 – 6.4
Serum Urea, adult	5 - 39	2.5 – 6.4
Urine Urea Nitrogen	g/24 hr	mmol/24 hr
adult	12 - 20	428 - 714

Uremia: the condition of abnormally high urea nitrogen in the blood.

Azotemia: a significant increase of urea and creatinine in the blood.

Method: Enzymatic method reactions:

Urea + H_2O + urease ---------- $(NH_4)_2CO_3$

NH_4^+ + a-ketoglutaric acid + NADH + Glutamate dehydrogenase --------- ADP + H^+ + NAD^+ + Glutamic acid

Specimen:

Serum, plasma (Na or Li Heparin), urine or other biological fluids.

Sample Handling:

Avoid Fluoride containing tube because it interfere with urease method by inhibit urease reaction.

Since urea can be lost through bacterial action, the specimen should be analyzed within a few hours a er collec on or should be preserved by refrigera on at 4-8 C up to 72 hrs.

Procedure:

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2- Leave it to coagulate for 20min.

3- Separate serum sample from the blood by centrifuga on 4000 rpm for 5min.

4- Separate serum in labeled (T) tube.

5- Prepare the following set of test tubes:

	T (Test)	S (Standard)	B (Blank)
Serum	10 microleter	---	---
Standard Solution	---	10 microleter	---
Distilled Water	---	---	10 microleter
Working Reagent	1 mL	1 mL	1 mL
Leave it for 5 min in 37 C			
R2	200 microleter	200 microleter	200 microleter

Quantitative Determination of Blood Total Cholesterol

Total cholesterol assay, associated to assay of other lipids in serum is used in the diagnosis of hyperlipidemia. Increased levels are also seen in hepatic and thyroid disorders. Total cholesterol, triglycerides, HDL-Cholesterol, and LDL-Cholesterol determination is useful in prediction of CHD coronary heart diseases. So this assay is used in diagnosis and treatment of atherosclerotic diseases. Hypercholesterolemia observed in diabetic.

The major lipids present in plasma are:

Fatty acids, triglycerides, cholesterol, and phospholipids. In much smaller amounts are: steroid hormones and fat-soluble vitamins.

Cholesterol is the precursor of steroid hormones and bile salts.

Cholesterol is present in dietary fat (animal source) and synthesized in many tissues including the liver.

Cholesterol and lipids except fatty acids are circulates in blood's lipoproteins: chylomicrones, VLDL, IDL, LDL, and HDL (total cholesterol).

LDL : the principle carrier of cholesterol. 50%of total cholesterol is LDL-Cholesterol (bad cholesterol).

LDL CHOL = total CHOL – (HDL CHOL + TG / 2.2) mmol/L

HDL: carrier of cholesterol from peripheral tissues to liver, lowering total cholesterol in blood, (HDL-Cholesterol, good cholesterol).

Reference Values: (Normal Values, Expected Values)

Plasma cholesterol concentration varies for different populations.

Generally, in adults:

Total Cholesterol	mg/dL	mmol/L
Normal	< 200	< 5.2
Low risk of atherosclerosis	200 -240	5.2 – 6.2
High risk of atherosclerosis	> 240	> 6.2

Hypercholesterolemia: increase of cholesterol in blood.

Hypocholesterolemia: decrease of cholesterol in blood.

Method: Enzymatic method reactions:

Cholesterol esters + cholesterol esterase --------- cholesterol + free fatty acids

Cholesterol + O2 + cholesterol oxidase --------- cholest-4-ene-3one + H2O2

2 H2O2 + phenol + 4-aminoantipyrine + peroxidase --------- Quinoneimine (pink) + 4 H2O

Specimen:

Serum or plasma (Heparin or EDTA) , taken from patients in a:

fasting state (no food or drink other than water for 8-12 hrs).

Sample Handling:

Do not use oxalate, fluoride, or citrate tubes. Separate serum from cells within 2 hours. Cholesterol is stable in the specimen for 5-7 days at 2-8 C. Avoid repeated freezing.

Procedure:

1- Collect a blood specimens in a serum sample tube.

2- Leave it to coagulate for 20min.

3- Separate serum sample from the blood by centrifuga on 4000 rpm for 5min.

4- Separate serum in labeled (T) tube.

5- Prepare the following set of test tubes:

	T (Test)	S (Standard)	B (Blank)
Serum	10 microleter	---	---
Standard Solution	---	10 microleter	---
Distilled Water	---	---	10 microleter
Working Reagent	1 mL	1 mL	1 mL

Mix well. Put it in 37 Centigrade for 5 min or 10 min at room temperature.

6- Measure the Absorbance (Optical Density, OD) readings by spectrophotometer at 500 nm.