DETERMINATION OF BLOOD GLUCOSE

Fasting blood sample is preferred for the determination of glucose as it eliminates the chances of variation due to the absorption of glucose from the gastro-intestinal tract.

METHOD:

Folin & Wu Method.

PRINCIPLE:

The protein free filtrate (PFF) is heated with alkaline copper sulphate solution in a special tube (Folin-Wu Tube) to prevent reoxidation. Glucose and other reducing substances reduce cupric to cuprous oxide, which on reaction with phosphomolybdic acid produces a blue colour. The blue colour is produced due to the reduction of Mo^{6+} to Mo^{3+} or Mo^{4+}.

The density of the colour is proportional to the amount of reducing substances present in the PFF.

REAGENTS:

1. 10% Sodium Tungstate,
2. ⅔ N H_2SO_4,
3. Alkaline copper sulphate solution,
4. Phosphomolybdic Acid, and
5. Standard Glucose Solution (0.2 mg / 2ml).

PROCEDURE:

Label three (3) Folin-Wu Tubes as Unknown (U), Standard (S) and Blank (B).

Transfer 2ml PFF to tube 'U', 2ml standard glucose solution to tube 'S' and 2ml distilled water to tube 'B' respectively.

Add 2ml alkaline copper sulphate solution to each tube.

Mix and keep them in boiling water bath for 8 minutes Remove and cool the tubes under running tap water.

Add 2ml Phosphomolybdic acid and mix. Let the tubes stand for 2 minutes.

Dilute the contents of each tube up to 25ml mark with distilled water and mix by inversion.

Read the optical density in photoelectric colorimeter at 420nm.

No.	REAGENTS.	TUBES.		
		U	S	B
1.	P.F.F.	2ml	-	-
2.	Standard Glucose Solution.	-	2ml	-
3.	Distilled Water.	-	-	2ml
4.	Alkaline Copper Sulphate Solution.	2ml	2ml	2ml
5.	Keep in BOILING water bath. Remove after 8 minutes and cool under running tap water.			
6.	Phosphomolybdic Acid.	2ml	2ml	2ml
7.	After 2 minutes, dilute with distilled water up to 25ml mark.			
8.	Record the Optical Density at 420nm.			

CALCULATIONS:

Optical Density of Unknown (U) = OD_U
Optical Density of Standard (S) = OD_S
Concentration of Standard Solution = C_S
PFF Dilution Factor = D
Volume of PFF Used. = V

$$\text{BLOOD GLUCOSE (mg/dl)} = \frac{OD_U \times C_S}{OD_S} \times \frac{D}{V} \times 100$$

NORMAL RANGE:

Fasting Blood Glucose = 65-110 mg/dl
Random Blood Glucose = Up to 140 mg/dl

This method does not give true blood glucose values as the other non-glucose reducing substances present in the blood such as glutathione, ascorbic acid etc. also interfere and is a relatively constant fraction showing 20–30 mg/dl higher values. Therefore the values for blood glucose obtained by this method are quite reliable and can be used for the purpose of comparison and diagnosis.

HYPERGLYCAEMIA.

- Diabetes mellitus,
- Pancreatic disease.
- Hyperthyroidism,
- Epinephrine administration,
- Adrenal Diabetes,
- Pituitary Diabetes,
- Certain Hepatic Disorders,
- Ether Anaesthesia.

HYPOGLYCAEMIA.

- Reactive hypoglycaemia,
- Insulin administration,
- Hyper-insulinaemia,
- Insulinoma,
- Hypo-endocrino-pathies, e.g.,
  Hypopituitarism,
  Cretinism,
  Myxoedema and
  Addison's Disease.

EXPERIMENT No: 8 Date: _____

TO ESTIMATE THE CONCENTRATION OF GLUCOSE IN THE GIVEN BLOOD

CALCULATIONS:

Optical Density of unknow, ODU = 52

Optical Density of standard, S = 50

Concentration of Standard solution = 0

PFF dilution factor = D = 10

Volume of unknown = CU = ?

Volume of PFF used = U = 2ml

$$\frac{Cs}{CU} = \frac{ODs}{ODU} \times 10$$

$$CU = \frac{ODU}{ODs} \times Cs \times 10 = \frac{52}{50} \times 0.2 \times 10 = 2.08ml$$

2ml PFF has glucose = 2.08mg

1ml PFF has glucose = $\frac{2.08}{2}$ = 1.04mg/ml

$$= 1.04\frac{mg}{ml} \times 100 = 104mg/dl$$

RESULT: 104mg/dl