

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. $\frac{1}{2}$ 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

$$\frac{C_1}{C_2} = \frac{D_1}{D_2}$$

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

NORMAL RANGE:

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

EXPERIMENT No: 7

Date: 27-11-2018

TO ESTIMATE THE CONCENTRATION OF GLUCOSE IN THE GIVEN BLOOD

CALCULATIONS:

conc: of standard glucose solution, $C_1 = \frac{0.2 \text{ mg}}{2 \text{ ml}}$

conc: of unknown solution = ?

optical density of standard solution, $D_1 = 30$

optical density of unknown solution, $D_2 = 35$

$$\frac{C_1}{C_2} = \frac{D_1}{D_2}$$

$$C_2 = \frac{C_1 \times D_2}{D_1} \times D.F$$
$$= \frac{0.2 \times 35}{30} \times 10$$

$$C_2 = 2.3 \text{ mg}$$

\Rightarrow 2 ml of protein free filtrate contain 2.3 mg of glucose

\Rightarrow 1 ml of PFF solution contain = 1.15

\Rightarrow 100 ml " " " " = 1.15×100
= 115 mg

CamScanner
18/11/19

RESULT: 100 ml of PFF solution have glucose = 115 mg