

Clinical Biochemistry Protocols

Estimation of Blood Glucose level

by

Folin-Wu method

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Clinical Biochemistry Practical Protocols

Estimation of Blood Glucose level by Folin-Wu method

Aim:

To estimate Glucose level in Blood by Folin-Wu method

Principle:

When glucose or other reducing agents are treated with alkaline copper solution they reduce the copper with the result insoluble cuprous oxide is formed. The reaction depends on temperature, duration of heating, degree of alkalinity. The ratio of glucose to cuprous oxide form may be varied after heating for a period. The cuprous oxide form is allowed to react with phosphomolybdate to form molybdenum blue colored complex which can be read colorimetrically using red filter on at 680nm.

Reagents:

1. 1% Sugar solution: 1 gram of sugar is added in 100ml saturated benzoic acid.
2. Working standard sugar solution: 0.2 mg per 2 ml of the above solution.
3. Alkaline copper solution:
 - a. Solution A: Dissolve 2% Na_2CO_3 in 0.1N NaOH.
 - b. Solution B: 0.5% CuSO_4 in 1% sodium potassium tartarate or Rochelle salt

Mix 50ml of solution A with 1 ml of solution B.

4. Phosphomolybdic acid: To 35grms of molybdic acid add 5 grams of sodium tungstate, 200 ml of 10% NaOH and 200ml of water is added. It is boiled vigorously for 20-30 minutes so as to remove whole of ammonium present in molybdic acid. The solution is cooled and diluted to about 350ml and 125 ml of 85% phosphoric acid (ortho) is added and make up to 500ml with distilled water.
5. 10% Sodium tungstate
6. 2/3 N H_2SO_4

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Procedure:

Deproteination of Blood: 1 ml of Blood is transferred to boiling tube containing 7 ml of water then 1 ml of 10% sodium tungstate is added mixed well followed by 1 ml of 2/3 N H₂SO₄ with shaking. It is allowed to stand for 10 minutes, it is then filtered. This filtrate is called tungstic acid blood filtrate and is taken as Test sample.

2ml of tungstic acid blood filtrate is transferred to folin-wu tube graduated at 25 ml mark and to other similar tubes 2 ml of standard glucose solution and 2ml of water as Blank is added. To each of the three tubes 2 ml of alkaline CuSO₄ is added. Now the surface of the mixture is in line with 4ml mark of the Folin-Wu tube. The tubes are placed in boiling water bath exactly for 8 minutes. It is cooled under running water. To each of the tube 2 ml of phosphomolybdic acid solution is added. After 1 minute it is diluted with water up to the mark. The solution is transferred to a suitable tube and OD is read at 680nm.

Normal values:

- The normal blood sugar level ranges from 8 to 120 mg / 100ml of blood
- In mild diabetic conditions value of blood glucose from 140-300 mg / 100ml of blood and in severe diabetic conditions value is upto 1200mg / 100ml of blood have been noted.
- Low blood sugar level values are formed in insulin administration, Addison's disease, hypoglycemia and hypopituitarism.

Report:

_____ mg of glucose is present in 100 ml of given blood sample.

Calculation:

Mg of glucose / 100ml of blood =

$$\text{mg of glucose in standard X} \times \frac{\text{OD of test} \times 100}{\text{OD of Standard} \times 0.2}$$