Glucose

* Sample collection & storage :-

↓

→ Individual ↓ ① hematocrit, fasting whole Blood glucose conc. 10 to 12 mg/l. lowes than plasma.

→ Both serum & plasma can be used for glucose estimation.

→ POCT device uses to measure glucose conc in the plasma phase.

→ Glycolysis ↓es s. glucose By 5 to 10 mg/dl in 1 hr. in ① uncentrifuged coagulated Blood at room temperature.

→ Rate is highes if ① leucocytosis
② Bact. contamination.

leucocytosis may lead to ↓ of 65 mg/dl of glucose after 1 to 2 hrs in -nce of glycolytic inhibitors

→ Seperated non hemolyzed sterile serum stable glucose conc. as long as 8 hrs at 25°C.

→ Even the plasma free from cell

↓

may contain leukocyte after mod. centrifugation

↓

that also metabolize glucose

* Glycolytic Inhibitors :-

→ ① NaF :
↳ Inhibits glycolysis
↓
glucose is stabilized for 3 days at room temp.
↓
It inhibits activity of enolase
↓
By forming complex ~ Mg^{+2} & P_i
↓
c interferes interaction enzyme & substrate.

→ Weak anticoagulant → binds ~ Ca^{+2}
↓
So used in combinⁿ ~ Oxalate.

→ Flouides inhibits urease activity
↓
So not useful for determination of urea

→ ~~Flouide~~
→ Glycolytic activity of Flouride has late onset
↓
So glycolysis may occur in 1st hr after collection of sample even in Flouride vacuttee.

sod.

(b) Iodoacetate

↓

also inhibit glycolysis. → when want to measure Urea & glucose → Iodoacetate can be use as preservative.

→ In 24 hrs collection of urine

↓

glucose may be preserve. By adding 5 ml of glacial acetic acid to the containes

↓

So pH B/w 4 to 5

↓

Inhibits Bact. activity.

Others preservative for urine :

↓

Sod. Benzoate

Sod. Nitrate.

→ Urine should be stored at 4°C during collection.

* Methods :—

(1) Hexokinase methods :—

$$\text{Glucose} + \text{ATP} \xrightleftharpoons[Mg^{+2}]{\text{Hexokinase}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} \xrightleftharpoons{\text{G-6-P D}} \text{6-phosphogluconate}$$

NADP (NADPH + H^+) → measured at 340nm

* Reb.

Interference :-

① Hb: >0.5mg/dl : interferes.
↓
B'cz phosphate esters & enzymes released from RBC
↓
interfere c the assay.

② Drugs

③ Bilirubin

④ Lipaemia :
↳ TG >500mg/dl
↓
+ve interference.
↓
Sample Blanking
↓
to eliminate lipemic & icteric interference.

↳ prepared By 10 µl sample + Isotonic saline
↓
absorbance taken against water at 340 nm.

⑤ Fructose :
→ ⓗ Fasting has low conc. of fructose

↓

Ingestion of sucrose 1 gm/kg

↓

↑ S. Fructose & to 10 mg/dl ē in 1 hrs, remain high for 2 hrs.

↓

So during GTT, should not administer any fructose.

* Mode of reaction :-

→ End point / equilibrium assay

* Reagent may also contain substances that reacts ē coenzyme tnt in the reagent

↓

they can be detected by taking reagent Blank

↓

If reagent blank at 340nm is > 0.35

↓

they are unsuitable for use.

② Reference method :

→ Serum / plasma deproteinated By adding solution Barium hydroxide & zinc sulfate

↓

Supernatant mixed ~ reagent

↓

method is Based on Hexokinase, G-6-PD & NADH (Not NADPH)

→ Calibrators & Blank carried through entire process.

② Glucose Oxidase Methods :—