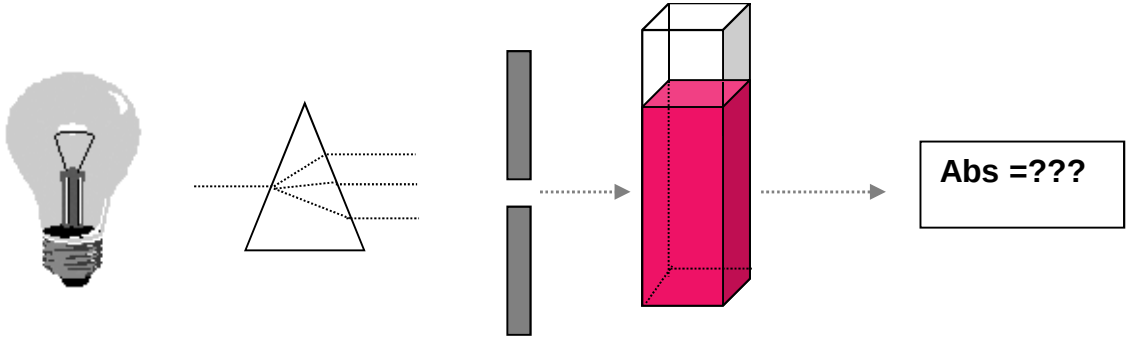

GLUCOSE ASSAY

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Biochemistry II Lab

Rationale

- Glucose is a ubiquitous fuel molecule in biology. It is oxidized through a series of reactions, yielding ATP.
- Ordinarily the concentration of glucose in the blood is maintained at a relatively stable concentration.
- Due to its importance in metabolism, glucose level is a key diagnostic parameter for many metabolic disorders.
- The accurate estimation of glucose is important in the diagnosis & management of hyperglycaemia/ hypoglycaemia.

Matrix for Glucose Assays

- Serum/plasma are the most common specimens.
- Glucose can also measured in other body fluids.
- Serum/plasma should be separated from cells with in 30 minutes of blood collection.
- Prolonged exposure of serum/ plasma to leukocytes, platelets, and erythrocytes allows the cells to consume glucose and lower the glucose concentration.
- Sodium fluoride (NaF) inhibits glycolysis (Enolase)
  - NaF = 2mg/ml of blood is effective.
- The decline of plasma or serum glucose concentration is generally 5% to 10% per hour.

Specimen collection time options

- Random blood sugar (RBS)
- Fasting blood sugar (FBS)
- Two- hours post prandial (2-HPP)

Measuring Glucose in Plasma/Serum

- In past, glucose analysis were often performed with relatively non-specific methods (Oxidation-reduction & Condensation) that resulted in falsely increased values.

Drawbacks:

- Lacks specificity $\longrightarrow$ Non glucose reducing substances
- Requires protein precipitation $\longrightarrow$ Proteins react with Cu
- Corrosive & toxic reagents
- It is laborious

•Clinical chemists turned to enzymatic methods in an attempt to obtain a “true” glucose determination because of the high specificity of an enzyme.

•Commonly used enzymatic methods include:

- Glucose Oxidase
- Glucose Hexokinase

- These methods will not measure other mono or disaccharides. i.e., fructose, galactose, sucrose or lactose

Principle of glucose oxidase method

D-Glucose + O_2 $\xrightarrow{\text{Glucose Oxidase}}$ Gluconic Acid + H_2O_2

$2H_2O_2$+ 4-aminantipyril + Phenol $\xrightarrow{\text{Peroxidase}}$ $2H_2O$ + Quinoneamine

(colorless) (Red colored)

- Glucose oxidase is highly specific for β-D-glucose. Glucose in solution is 36% in α-form & 64% in β-form. Addition of the enzyme mutarotase to the reaction facilitate the conversion of α-D-glucose to β-D-glucose.
- Intensity of the color formed from oxidation of chromogen is proportional to the amount of glucose and absorbance is read at 546nm.

Materials

- Spectrophotometer
- Micropipettes
- Pipette tips (Small & Large)
- Test tubes with rack
- Glucose Monoreagent
- Glucose Standard Solution
- Test Sample
- Control Solutions

Procedure

	Blank	Standard	Sample
Working reagent	1000ul	1000ul	1000ul
Standard	-----	10ul	-----
Sample	-----	-----	10ul
Mix and measure after incubating at +37 ^{0}C for 5 min. or 10 min. at +25 ^{0}C .			

Calculation

$$C_{test} = \frac{A\ test\ X\ C\ std}{A\ std}$$

Normal values (mg/dl)	Dog	Cow	Horse	Sheep	Goat	Man
	61.9-108.3	42.1-75.5	62.2-114.0	44.0-81.2	48.2-76.0	75-115

Conversion factor: mg/dL x 0.0555= mmol/L

Principles of Hexokinase Method

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ G-6-P + ADP

G-6-P + NAD^+ $\xrightarrow{\text{G6- PDH}}$ 6-phosphogluconate + $NADH^+$

- The amount $NADH^+$ produced is directly proportional to glucose amount and absorbance measured at 340nm.

G-6-P= Glucose -6-phosphate

G6-PDH = Glucose -6-phosphate dehydrogenase

- The hexokinase method is considered more accurate than the glucose oxidase methods because the coupling reaction using G-6-PDH is highly specific; therefore, it has less interference than the coupled glucose oxidase procedure.
  - In glucose oxidase procedure uric acid, bilrubin, ascorbic acid falsely decrease values b/c these substances are oxidized by peroxidase which then prevents oxidation of chromogen.

Interferences:

- Hemolyzed samples, Bilirubin affects the test as they absorb near UV wavelength (340 nm):
- Lipemia cause falsely elevated glucose values.

Quality Control

- A normal & abnormal quality control sample should be analyzed along with samples for acceptance or rejection of the analytical run.