INTRODUCTION

1. ***Infinite*** Liquid *STAT* Glucose is a reagent set for determination of **True Glucose, based on enzymatic method;** using Glucose, oxidase and Peroxidase.
2. ***Infinite*** Liquid *STAT* Glucose is a **ready-to-use** reagent.
3. ***Infinite*** Liquid *STAT* Glucose estimates glucose in just **one minute** using a initial rate method and in **7 minutes** at 37°C or **15 minutes** at R.T. by end point method.
4. ***Infinite*** Liquid *STAT* Glucose is **linear** upto 700 mg% glucose for kinetic assay procedure and 500 mg% for endpoint procedure.
5. ***Infinite*** Liquid *STAT* Glucose can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
6. **Sodium Fluoride** (as an anticoagulant upto 10 mg/ml blood) does not effect glucose assay.
7. The influence of **Ascorbate, Bilirubin, Antidiabetic drugs** and **Haemoglobin** is negligible.

PRINCIPLE

Glucose oxidase (GOD) converts glucose to gluconic acid. Hydrogen peroxide formed in this reaction, in presence of Peroxidase (POD), oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm. (500-550 nm.). The intensity of the colour complex is directly proportional to the concentration of glucose in specimen.

$$\beta\text{-D Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{POD} \text{Red Dye} + H_2O$$

REAGENT STORAGE & STABILITY & HANDLING

The kit should be stored at 2-8°C and is stable till the expiry date indicated on the label.

The reagent and standard are ready-to-use and are stable till expiry when stored at 2-8°C. **DO NOT FREEZE THE REAGENT.**

The reagent should be stored only in the amber bottle provided to protect it from direct light. This is critical because the reagent is light sensitive (auto oxidation of chromogen system by light and air). Before use swirl in the reagent gently. **DO NOT SHAKE VIGOROUSLY.**

Over time, the reagent may develop a light pink colour, this is expected and does not affect the reagent performance. Discard the reagent if the absorbance of the same exceeds 0.300 O.D. against distilled water at 505 nm.

Contamination of the reagent should be strictly avoided. Should the reagent develop turbidity discard the reagent.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Phosphate Buffer, pH 7.0	170 mmol/l
• Glucose oxidase	15000 IU/l
• Peroxidase	1500 IU/l
• 4 - amino antipyrine	0.28 mmol/l
• Phenol	16 mmol/l
• Stabilizers and inactive ingredients	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Serum or plasma should be separated from the cells at the earliest possible (within 30 minutes), as the rate of glycolysis is approximately 7 mg% per hour at room temperature.

For plasma separation following anticoagulants may be used.

• EDTA	2 mg/ml of blood
• CITRATE	6 mg/ml of blood
• HEPARIN	200 IU/ml of blood
• OXALATE	3 mg/ml of blood
• SODIUM FLUORIDE	10 mg/ml of blood

Sodium Fluoride is preferred as anticoagulant due to the antiglycolytic activity. Higher concentration of Sodium fluoride i.e. more than 10 mg/ml blood should be avoided as it may inhibit the colour development.

Glucose is stable for 24 hours in neatly separated plasma and serum if the estimation is not possible within 24 hours then the specimen should be preserved at -10°C and should be used within 30 days.

PROCEDURE FOR END-POINT

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** ..... **7 mins. at 37°C / 15 mins. at R.T. (25-30°C)**
- ☐ **Wavelength** ..... **505 nm. (500 - 550 nm.)**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Blank absorbance limit** ..... **< 0.300 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **100 mg%**
- ☐ **Linearity** ..... **500 mg/dl**

Manual assay procedure

Prewarm at room temperature (25-30°C) the required amount of reagent before use.

Perform the assay as given below :

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.01 ml	0.01 ml	—
Reagent	1.0 ml	1.0 ml	1.0 ml

Incubation

Incubate the assay mixture for 7 minutes at 37°C or 15 minutes at room temperature (25-30°C). After completion of incubation period measure the absorbance against blank at 505 nm. Final colour is stable for two hours if not exposed to direct light.

Calculation:

❶ **With standard**

$$\text{Conc. (mg\%)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

❷ **With factor for wavelength range : 500 - 510 nm.**

Conc. (mg%) = 269 x Absorbance of Sample

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PROCEDURE FOR KINETIC ASSAY

- ☐ **Reaction type** ..... **2 Point-Kinetic**
- ☐ **Reaction direction** ..... **Upward**
- ☐ **Wavelength** ..... **505 nm. (500 - 550 nm.)**
- ☐ **Flowcell temperature** ..... **37°C**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Delay time** ..... **20 secs.**
- ☐ **No. of readings** **2**
- ☐ **Interval** ..... **40 secs.**
- ☐ **Blank absorbance limit** ..... **< 0.300 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Factor** ..... **100÷(Abs. of std.)**
- ☐ **Linearity** ..... **700 mg/dl**

Manual assay procedure

Prewarm at 37°C the required amount of reagent before use. Perform the assay as given below :

1.0 ml procedure

Standard / Specimen	**0.01ml (10 µl)**
Reagent	**1.0 ml**

First carry out the assay of standard.

Mix and start stop watch simultaneously. Record absorbance at exactly 20th second after standard addition and then again at 60th second.

Subsequently, carry out the assay of the specimen following exactly the same procedure mentioned above.

Calculation:

Calculate the change in absorbance (Δ Abs.) of standard and specimen.

$$\text{Factor} = \frac{\text{Concentration of Std.}}{\Delta \text{ Abs. of Standard}} = \frac{100}{\Delta \text{ Abs. of Standard}}$$

Glucose in mg % = Δ Abs. of Sample x Factor

NOTE :

The specimen to reagent ratio can be altered proportionally without affecting the results.

EXPECTED VALUES

Fasting blood Glucose	:	**60 to 110 mg%**
Postprandial Blood Glucose	:	**< 145 mg%**

NOTE :

Expected range varies from population to population therefore each laboratory should establish its own normal range.

The whole blood glucose value is generally 10 to 15 per cent lower than the serum or plasma glucose values due to the cell stroma.

No significant difference exists between capillary and intravenous blood glucose value, except at the peak of the Glucose Tolerance Test (GTT) where capillary blood glucose value is approximately 20% higher.

PROCEDURE LIMITATIONS

1. Discard the reagent if the absorbance exceeds 0.300 against distilled water at 505 nm.
2. If the glucose value exceeds linearity limit then dilute the specimen suitably with normal saline and repeat the assay. In such case the assay value should be multiplied with the dilution factor to obtain correct glucose value of the specimen.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

1. Trinder P. ***Annals. Clin. Biochem.*** 6, 24 (1969).
2. Young, D.S. et al, ***Clinical Chemistry,*** 21, 1D (1975).
3. Bergmayer, H.V., ***"Methods of Enzymatic Analysis",*** A.P.N.Y. (1974). Page 1196.
4. M. Dashora, et al, ***Proceeding of XI Annual Conf. ACBI.***
5. M. Dashora, ***Proceeding of XIII Annual Conf. ACBI,*** Page 17

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

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