INTRODUCTION

1. AutoZyme ***STAT*** Glucose is a reagent set for determination of **True Glucose based on enzymatic method** using Glucose oxidase and Peroxidase.
2. AutoZyme ***STAT*** Glucose estimates glucose in just **one minute** using an initial rate method and in 7 minutes by end-point method.
3. AutoZyme ***STAT*** Glucose pack sizes 10 x 100 ml and 4 x 500 ml are provided with **2 standards** of 100 mg% and 300 mg%.
4. AutoZyme ***STAT*** Glucose is **Linear** upto 700 mg% glucose for kinetic assay procedure and 500 mg% using end-point procedure.
5. AutoZyme ***STAT*** Glucose is a High Stability Reagent.
6. AutoZyme ***STAT*** Glucose can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
7. AutoZyme ***STAT*** Glucose has **one step reconstitution.** It involves the mixing of ENZYME and DILUENT.
8. **Sodium Fluoride** (as an anticoagulant upto 10 mg/ml blood) does not effect glucose assay.
9. The influence of **Ascorbate, Bilirubin, Antidiabetic drugs** and **Haemoglobin** is negligible.
10. **Very small volume** of serum or plasma is required for assay.
11. The **shelf-life** of AutoZyme ***STAT*** Glucose is 18 months.

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 / 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 Glucose in specimen.

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

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

REAGENT AVAILABILITY

	10 x 100 ml (Code : GU-2)	4 x 500 ml (Code : GU-3)
• **ENZYME** Vials	10	4
• **STANDARD** bottle - 100 mg%	1	1
• **STANDARD** bottle - 300 mg%	1	1
• **WORKING** Solution Container*	1	—
• **DILUENT** bottles	2	4

*(Empty bottle for preparation and storage of working solution)

***GLUCOSE standard 100 mg% and 300 mg% are assayed against National Bureau of Standards (NBS) reference material from Washington.**

ENZYME and STANDARD are to be stored at 2-8°C.

Diluent Reagent may be stored below 30°C and away from direct light.

The reagents are for ***in vitro*** diagnostic use.

PREPARATION OF WORKING SOLUTION

4 x 500 ml (Code GU-3)

Transfer the content of one vial of **Enzyme** into one bottle of **Diluent** and mix by gentle swirling or inversion. Do not shake vigorously.

10 x 100 ml (Code GU-2)

Transfer 100 mL. **Diluent** into working solution bottle. Now add contents of one vial of **Enzyme** into it. Mix by gentle swirling or inversion. Do not shake vigorously.

REAGENT STORAGE & STABILITY

Stability of reconstituted working reagent is printed on the bottle label. **(DO NOT FREEZE).** The working solution should be stored in the dark bottle (diluent bottle or the working solution bottle) provided. This is critical because the reagent is light sensitive (auto oxidation of chromogen system by light and air), it should therefore be kept away from direct light.

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-aminoantipyrine	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 its antiglycolytic activity. The 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 (10µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **100 mg%**
- ☐ **Linearity** ..... **500 mg/dl**

Manual assay procedure

Prewarm at room temperature the required amount of working solution before use.

Perform the assay as given below :

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.01 ml	0.01 ml	—
Working solution	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 at least two hours if not exposed to direct light.

Calculation :

$$\text{GLUCOSE in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

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PROCEDURE FOR KINETIC ASSAY

- ☐ Reaction type.....2 Point-Kinetic
- ☐ Reaction direction.....Upward
- ☐ Wavelength .....505 nm. (500 - 550 nm.)
- ☐ Flow cell 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 ÷ (Δ Abs. of std.)
- ☐ Linearity .....700 mg/dl

Manual assay procedure

Prewarm at 37°C the required amount of working solution before use.

Perform the assay as given below :

1.0 ml procedure

Standard/Specimen	0.01 ml (10 µl)
Working solution	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 Std. 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 Specimen x Factor.

NOTE : The specimen to working solution ratio can be altered proportionally without affecting the results.

EXPECTED VALUES

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

PROCEDURE LIMITATIONS

1. Discard the working solution if the absorbance is more than 0.300 against distilled water at 505 nm.
2. If the glucose value exceeds linearity limit then dilute 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 realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of the 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,*** Page..
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

AR. No.: I 19 *GU-2009-05-00I*

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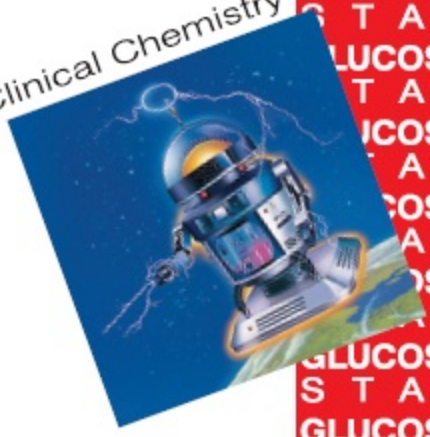

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