Enzymatic Glucose Reagent
Glucose Oxidase Method

PRODUCT SUMMARY

Stability	:	3 Months at 2-8°C
Linear Range	:	Up to 40 mmol/L (720 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Endpoint
Reagent Preparation	:	Add specified volume of distilled or deionised water.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xn - Harmful

INTENDED USE
This reagent is intended for the in vitro quantitative determination of glucose in human serum, plasma or urine.

CLINICAL SIGNIFICANCE
The accurate estimation of glucose is important in the diagnosis and management of hyperglycaemia and hypoglycaemia. Hyperglycaemia may occur as a result of diabetes mellitus, in patients receiving glucose containing fluids intravenously, during severe stress and cerebrovascular accidents. Hypoglycaemia may be the result of an insulinoma, insulin administration, inborn errors of carbohydrate metabolism or fasting.[1] Often in the investigation of these disorders glucose determinations are performed in conjunction with various tolerance tests or stimulation tests. For a more detailed discussion of glucose metabolism the user should refer to a standard text book such as Kaplan.[2]

METHODOLOGY
The glucose oxidase reaction in conjunction with an auxiliary reaction has been widely used for the determination of glucose in biological fluids. Many different auxiliary reactions have been developed in order to improve the overall specificity of the reaction system or retain the inherent specificity of glucose oxidase.[3] The method utilised in this reagent is based on the hydrogen peroxide indicator reaction which couples 4-aminoantipyrine to a phenolic compound as first proposed by Trinder.[4] This method has been validated in an extensive study by Pennock et al.[5] Pennock compared Trinder's method with six other common methods and found it highly reliable with respect to both accuracy and precision. The method was further shown by Pennock[5] and Sharp[6] and Szasz et al[7] to be resistant to well known interfering compounds such as uric acid, glutathione and creatinine.

1. $\text{Glucose} + O_2 + H_2O \xrightarrow{\textit{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$
2. $H_2O_2 + \text{HBA} + \text{4-AAP} \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine dye} + H_2O$

1. Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide.
2. The hydrogen peroxide reacts in the presence of peroxidase with HBA and 4-aminoantipyrine forming a red quinoneimine dye. The intensity of the colour formed is proportional to the glucose concentration and can be measured photometrically between 460 and 560 nm.

Abbreviations

HBA = 4-hydroxybenzoic acid
4-AAP = 4 -aminoantipyrine

REAGENT COMPOSITION

Active Ingredients	Concentration
Glucose oxidase	> 12,000 U/L
Peroxidase	> 60 U/L
4-aminoantipyrine	0.3 mmol/L
4-hydroxybenzoic acid	6 mmol/L
Phosphate buffer	71 mmol/L

Also contains non-reactive fillers and stabilizers.
pH 7.5 ± 0.10 at 20°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Glucose Oxidase Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

R22 Harmful if swallowed.
S28 After contact with skin, wash immediately with plenty of soap and water.

REAGENT PREPARATION
Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label.

STABILITY AND STORAGE
Prior to Use:
When stored at 2-8°C reagent is stable until the expiration date stated on the vial and kit box label.
Reconstituted Reagent:
When stored capped at 2-8°C the reagent is stable for 3 months.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent absorbance >0.60 AU (500 nm, 1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Collection: The stability of glucose specimens is reduced by bacterial contamination and glycolysis. In order to inhibit glycolysis samples should be collected into tubes containing Sodium Fluoride. As soon as possible serum or plasma should be separated from the cells.
Serum: Use non-haemolysed serum.
Plasma: Use heparin or EDTA.
Urine: If a delay in transport to the laboratory is expected the use of a chemical preservative such as merthiolate (0.23 mmol/L) is recommended.[8]
Storage: Serum glucose is stable for 4 hours at 30°C and 24 hours at 4°C For long term storage samples should be placed in sealed containers and frozen at -10°C.[4,5] Urine samples are stable for 1 day at 4°C.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 500 nm (460-560 nm).
- Distilled or deionised water for reagent preparation and related equipment, eg: pipettes.
- Analyser specific consumables, eg: sample cups.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous glucose standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	500 nm (460 - 560 nm)
Secondary Wavelength	600 - 660 nm
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:150
e.g. Sample vol	3 µL
Reagent vol	450 µL
Incubation Time	10 minutes
Reagent Blank Limits	Low 0.00 AU
(500nm, 1cm lightpath)	High 0.60 AU
Linearity	Up to 40 mmol/L (720 mg/dL)
Sensitivity (500nm, 1cm lightpath)	0.035 ΔA per mmol/L (0.002 ΔA per mg/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Glucose} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:
Absorbance of Calibrator = 0.40
Absorbance of unknown = 0.10
Value of Calibrator = 13.2 mmol/L (238 mg/dL)

$$\text{Glucose} = \frac{0.10}{0.40} \times 13.2 = 3.3 \text{ mmol/L}$$

$$\text{Glucose} = \frac{0.10}{0.40} \times 238 = 59.5 \text{ mg/dL}$$

Thermo SCIENTIFIC

For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in litres.

Urine Glucose (mmol/24 hours) = Glucose Result (mmol/L) x Dilution Factor x Volume (L)

Example:

Glucose result	=	0.7 mmol/L (12.6 mg/dL)
Dilution of Urine	=	Neat
24 Hour volume of urine	=	0.95 Litres

Urine Glucose	=	0.7 x 1 x 0.95	=	0.67 mmol/24 hours
Urine Glucose	=	12.6 x 1 x 0.95	=	11.97 mg/24 hours

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with glucose values above 40 mmol/L (720 mg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
3. Unit Conversion: mmol/L x 18 = mg/dL.
4. Avoid direct sunlight.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values for this methodology should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the established limits indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control contact Technical Services or your local distributor

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin, lipaemia and ascorbate were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 1000 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to 975 µmol/L (57 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to 600 µmol/L (35 mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides up to 11.5 mmol/L (1000 mg/dL).
   Ascorbate: No interference from ascorbate up to 0.71 mmol/L (12.5 mg/dL).
2. For a more comprehensive review of factors affecting glucose assays refer to the publication by Young.[9]

EXPECTED VALUES

Serum/Plasma:[10]	3.89 - 5.83 mmol/L (70 - 105 mg/dL)
Urine:[11]	0.28 - 0.83 mmol/L (5 - 15 mg/dL)

For the diagnosis of diabetes or impaired Glucose Tolerance (GT) the W.H.O. recommend the following criteria:[12]

	Plasma Venous	Capillary
Diabetes		
Fasting	≥7.8 mmol/L (≥140 mg/dL)	≥7.8 mmol/L (≥140 mg/dL)
2 hrs after glucose load	≥11.1 mmol/L (≥200 mg/dL)	≥12.2 mmol/L (≥200 mg/dL)
Impaired GT		
Fasting	<7.8 mmol/L (<140 mg/dL)	<7.8 mmol/L (<140 mg/dL)
2 hrs after glucose load	7.8-11.1 mmol/L (140-200 mg/dL)	8.9-12.2 mmol/L (160-220 mg/dL)

PERFORMANCE DATA

The following data was obtained with the Glucose Oxidase Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on the specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.[13]

Within run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.57 / 100.2	18.45 / 332.1
S.D. (mmol/L / mg/dL)	0.08 / 1.39	0.20 / 3.58
C.V. (%)	1.4	1.1

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.57 / 100.2	18.45 / 332.1
S.D. (mmol/L / mg/dL)	0.16 / 2.9	0.44 / 7.9
C.V. (%)	3.0	2.4

ACCURACY

Comparison studies were done using another commercially available glucose oxidase reagent. Normal and abnormal patient serum were assayed in parallel. The results were compared be least squares regression and the following statistics were obtained.

Number of sample pairs	60
Range of sample results	0.2 - 36.2 mmol/L (3.6 - 651.6 mg/dL)
Mean of reference method results	11.8 mmol/L (212.4 mg/dL)
Mean of Glucose results	11.9 mmol/L (214.2 mg/dL)
Slope	1.008
Intercept	0.08 mmol/L (1.44 mg/dL)
Correlation coefficient	0.998

LINEARITY

When run as recommended the assay is linear up to 40 mmol/L (720 mg/dL).

SENSITIVITY

When run as recommended the sensitivity of the assay is 0.035ΔA per mmol/L or 0.002 ΔA per mg/dL (1cm light path, 500nm).

REFERENCES

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REF

Reorder Information

Catalogue No.	Configuration
TR15103/1530-500	10 x 50 mL
TR15104	10 x 200 mL