GLUCOSE

(GOD-PAP)

Single Reagent

INTENDED USE

Vitro glucose reagent is intended for the in vitro quantitative determination of glucose in serum, plasma, cerebrospinal fluid (CSF), or urine on both automated and manual systems

METHOD

Enzymatic, colorimetric method (GOD/PAP) with glucose oxidase, and 4-aminoantipyrine.

Liquid stable single reagent

CLINICAL SIGNIFICANCE

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas[1]. The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure, and liver disease. Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism, or insulin induced hypoglycemia[2]. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glucosuria, to detect renal tubular defects, and in the management of diabetes mellitus. Glucose measurement in cerebrospinal fluid is used for evaluation of meningitis, neoplastic involvement of meninges, and other neurological disorders[3].

ASSAY PRINCIPLE

A large number of methods exist for determination of glucose in body fluids. Numerous chemical methods depend on the reduction of heavy metals or nitroaromatic acids by the aldehyde group of glucose. All these methods suffer from one or more problems including lack of specificity, requirement for prolonged incubation, and the use of noxious chemicals. Enzymatic methods for glucose determination were first described in 1948 by Keilin and Hartee[4] using glucose oxidase in a manometric technique. Keston[5] modified this method in the early 1950's using glucose oxidase/peroxidase enzyme system and o-dianisidine chromogen systems. Since then, various alternative chromogen systems have been proposed. The Trinder method replaces carcinogenic o-dianisidine with phenol plus 4-aminoantipyrine[6]. This method is less influenced by interfering substances and does not suffer from the many drawbacks of earlier methods. Vitro glucose reagent combines the use of glucose oxidase enzymes with the peroxidase/phenol/4-aminoantipyrine system of Trinder for the measurement of glucose in human serum, urine, or CSF.

The series of reactions involved in the assay system is as follows:

1. Glucose oxidized by glucose oxidase (GOD) to gluconic acid.
2. In presence of peroxidase (POD), the formed hydrogen peroxide formed affects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

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

$$2\,H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{POD} \text{Quinoneimine dye} + 4\,H_2O$$

The intensity of the color produced is directly proportional to glucose concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Serum or plasma[1]		
Adults (fasting)	70 – 110 mg/dl	(3.9 – 6.1 mmol/l)
Full-term infants	30 – 90 mg/dl	(1.7 – 5.0 mmol/l)

For diagnosis of diabetes mellitus, the WHO recommends the following limits[7] (venous):

Fasting	≥140 mg/dl	(7.8 mmol/l)
2 hrs after glucose load	≥200 mg/dl	(11.1 mmol/l)
Urine[8, 9]		
Random	5.0 - 15 mg/dl	(0.28 – 0.83 mmol/l)
24 hours	<0.5 g/day	(2.8 mmol/day)
***CSF[3]**		
Adults	40 - 75 mg/dl	(2.2 – 4.2 mmol/l)

*CSF glucose values should be approximately 60% of the plasma values and must always be compared with concurrently measured plasma values for adequate clinical interpretation.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range. For diagnostic purposes, the glucose results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Glucose standard	100	mg/dl
R_2	Phosphate buffer, pH 7.0	100	mmol/l
	Phenol	11	mmol/l
	Glucose oxidase	15	KU/l
	Peroxidase	2	KU/l
	4-Aminoantipyrine	0.4	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

Serum, plasma*, urine, and cerebrospinal fluid.

* The only accepted anticoagulants are heparin, EDTA, and fluoride.

Specimen Preparation & Stability For serum specimen

- **For serum specimen**

Patients should be following their usual diet and be in their usual state of health. Patients should refrain from eating for 6 to 8 hours before fasting blood sample. Serum must be separated from the clot promptly since the rate of glucose decrease is approximately 7% in one hour in whole blood[10]. This decrease is the result of glycolysis. Glucose in separated non-hemolyzed serum is generally stable up to 8 hours at 25^{0}C or up to 72 hours at 4^{0}C. Glycolysis can be inhibited by collecting the specimen in sodium fluoride tubes[1].

- **For urine specimen**

Collect urine in a dark bottle. For 24 hours collections add 5 ml glacial acetic acid to the container before collection for preservation. Unpreserved urine samples may lose up to 40% of their glucose after 24–hours storage at room temperature[3]. It is recommended to keep specimens on ice during collection.

- **For CSF specimen**

CSF may be contaminated with bacteria and often contains other cellular constituents. CSF samples should therefore be analyzed for glucose immediately[3].

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum, plasma, CSF or diluted urine

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	10 µl	……
Specimen	……	……	10 µl

Mix, incubate for 10 minutes at 37^{0}C or 15 minutes at 20-25^{0}C.
Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

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EC REP | **Medical Device Safety Services**
MDSS GmbH, Burckhardtstr. 1
30163 Hannover, Germany.

Calculate the glucose concentration by using the following formulae:

$$\text{Glucose Concentration} = \frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

For urine specimen the results must be multiplied by the dilution factor and 24 hours collections by the volume in liters.

- **Unit conversion**

mg/dl x 0.055 = mmol/l

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established glucose values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

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 on fresh control material still remain outside the limits, then repeat the test with fresh reagent.

If results are still out of control, contact Vitro Technical Services

INTERFERING SUBSTANCES

- **Anticoagulants:**

Heparin, EDTA, and fluoride are only accepted anticoagulant.

- **Bilirubin:**

No interference from free or conjugated bilirubin up to a level of 15 mg/dl.

- **Drugs:**

Young[11] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

- **Haemoglobin:**

No interference from haemoglobin up to a level of 200 mg/dl.

- **Lipemia:**

Grossly lipemic or iceteric sera will cause false glucose values and required the use of a serum blank.

WARNING & PRECAUTION

- Vitro glucose reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The reagent blank will not exceed an absorbance of 0.1 but don't use the reagent if it is turbid or if the absorbance is greater than 0.2 at 500 nm.
- If specimen is grossly lipemic or iceteric use serum blank by adding 10 µl of patient serum to 1.0 ml distilled water and read absorbance against water blank. Subtract this absorbance from the test absorbance to correct for the lipemia or icterus.

PERFORMANCE EVALUATION

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (mg/dl)	90	314
SD ((mg/dl)	2.2	3.1
CV (%)	2.4	1.0

	Between Day	
Control	Level I	Level II
Number of samples	40	40
Mean (mg/dl)	90	313
SD (mg/dl)	4.8	17.6
CV (%)	5.2	5.7

Method Comparison

Number of sample pairs 45
Range of sample results 56-306 mg/dl
Mean of reference method results125 mg/dl
Mean of glucose results120 mg/dl
Slope 1.002
Intercept 2.8 mg/dl
Correlation coefficient 0.998

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.
When run as recommended the sensitivity of this assay is 5.0 mg/dl (0.28 mmol/l).

Linearity

When run as recommended, the assay is linear up to 500 mg/dl (27.5 mmol/l).
If result exceeds 500 mg/dl (27.5 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

BIBLIOGRAPHY

1. **Caraway, WT & Watts, MB (1987):** Carbohydrates. In: Tietz NW, ed. Fundamentals of clinical Chemistry. 3rd ed. Philadelphia: WB Saunders; 422-447.
2. **Howanitz, PJ& Howanitz, JH.(1984):** Carbohydrates. In: Henry JB, ed. Clinical Diagnosis and Management by laboratory Methods. 17th ed. Philadelphia: WB Saunders; 165-179.
3. **Sacks, DB (1994):** Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia: WB Saunders; 928-1001.
4. **Keilin, D & Hartree, EF (1948):** Biochem. J. 42:250.
5. **Keston, AS, (1956):** Abstract of Papers, 129th Meeting, Am. Chem, Soc., Dallas, TX, April 1956.
6. **Trinder, P (1969):** Ann. Clin. Biochem. 6: 24.
7. **WHO (1985):** Technical Report Series, No. 727 Diabetes mellitus: Report of a WHO study Group. Geneva: WHO; 1985.
8. **Richterich, R & Colombo, JP (1978):** Klinische Chemie. 4th ed. Basel: S. Karger; 570.
9. **Tietz, NW, ed (1995):** Clinical Guide to laboratory Tests. 3rd ed. Philadelphia: WB Saunders; 268-273.
10. **Weissman, M & Klein, B (1958):** Evaluation of glucose determination in untreated serum samples. Clin Chem. 4: 420-422.
11. **Young, DS (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE	REF	SIZE
11801	2 X 100 ml	11805	4 x 250 ml
11802	2 X 125 ml	11806	1 x 500 ml
11803	4 X 100 ml	11807	2 x 500 ml
11804	4 X 125 ml	11808	1 x 1000 ml

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