GLUCOSE - GOD – PAP

Diagnostic Single reagent for the in-vitro quantitative determination of glucose in human serum, plasma, urine and CSF on both manual and automated systems.

REF: V/GL02.050	100 test	REF: V/GL02.125	250 test	REF: V/GL04.125	500 test
REF: V/GL01.100	100 test	REF: V/GL01.250	250 test	REF: V/GL02.250	500 test
REF: V/GL02.100	200 test	REF: V/GL03.100	300 test	REF: V/GL04.250	1000 test

CLINICAL SIGNIFICANCE [1]

Glucose is the major carbohydrate present in blood. Its oxidation represents the major source of cellular energy in the body. The glucose level in blood is maintained with a narrow level under diverse conditions (feeding, fasting, or exercise) by regulatory hormones such as insulin, glucagon or epinephrine.
Accurate measurement of glucose in body fluid is important in diagnosis and management of diabetes, hypoglycemia, adrenal dysfunction and various other conditions.
High levels of serum glucose (hyperglycemia) may be seen in case of Diabetes mellitus, in patients receiving glucose containing fluids intravenously, in cerebrovascular accidents, during severe stress, hyperparathyroidism, pancreatitis, and renal failure.
Decreased levels of glucose (hypoglycemia) can be due to an insulinoma, inborn errors of carbohydrate metabolism, extensive liver disease, hypothyroidism, hypopituitarism, or fasting.

METHOD PRINCIPLE [2]

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye as indicator with absorbance proportional to the concentration of glucose in sample.

$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Violet Quinone Dye} + 2H_2O$$

REAGENT COMPOSITION

R1: Glucose standard	100 mg/dl (5.55mmol/L)
R2: Reagent	
Phosphate Buffer	100 mmol/L
Phenol	4.0 mmol/L
4-amino-antipyrine	1.0 mmol/L
Glucose oxidase	> 20 KU /L
Peroxidase	> 2.0 KU/L
Sodium Azide	8 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** Glucose reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie Glucose reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8˚C. Once opened, the opened vial is stable till its expiry date at the specified temperature.

Deterioration

The **Lab.Vie** glucose reagent is normally clear or pale pink. Do not use Glucose reagent if it is turbid or if the absorbance is greater than 0.15 at 546 nm.

SPECIMEN COLLECTION AND PRESERVATION [5,6]

Serum or plasma

Individuals should be fasting before sample collection. Serum or plasma should be separated within 30 minutes to prevent glycolysis. If anticoagulants (heparin, EDTA, or fluoride) are used an average decrease of 7% in 1 hour (10 mg/dl) is seen then glucose stabilizes. Glucose is stable in serum up to 8 hours at 25˚C or up to 72 hours at 4˚C, and in plasma (fluoride) up to 24 hours at 25˚C.

Urine

Urine samples are stable 1 day at 4˚C, in case of delay due to transportation or for 24 hour urine collection, it is recommended to add either merthiolate (0.23 mmol/L) or 5 ml glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40% of their glucose after 24 hour storage at room temperature; therefore, keep samples on ice during collection.

CSF

Sample should be analyzed for glucose immediately to avoid contamination with bacteria. If a delay in measurement is unavoidable, the sample should be centrifuged and stored at 4˚C.

SYSTEM PARAMETERS

Wavelength	546 nm (492 – 550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	37 ˚C or 20– 25˚C
Incubation time	20 min. at 20–25˚C or10 min. at 37˚C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU High 0.15 AU
Sensitivity	5 mg/dL (0.27 mmol/L)
Linearity	500 mg/dL (27.7 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotomete

ASSAY PROCEDURE

	Blank	*Standard*	*Specimen*
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl

Mix and incubate for 10 minutes at 37°C or 20 minutes at 15-25°C. Measure absorbance of specimen "A" and standard "A" against reagent blank within 30 minutes.

CALCULATION

$$\text{Glucose concentration (mg/dl)} = \frac{\text{(A specimen)} \times 100}{\text{(A standard)}}$$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range, please contact technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	103	228	109	235
SD. mg/dl	1.12	1.19	1.23	1.27
CV. %	1.09	0.83	1.17	0.98

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie** Glucose reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.991.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 5 mg/dL (0.27 mmol/L).

Linearity
The reaction is linear up to glucose concentration of 500 mg/dl; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

INTERFERING SUBSTANCES [3]

Haemolysis
No significant interference from haemoglobin up to 500 mg/dl.

Icterus
No significant interference from free and conjugated bilirubin up to levels of 15 mg/dl (257 mmol/L).

lipemia
Lipid disturb measurements if present in high concentration (More than 500 mg/dl).

Others
Turbidity caused by insoluble uranyl phosphate may result in false high levels.

Reducing Substances
Large amounts of reducing substances as ascorbic acid, creatinine, glutathione and uric acid react with hydrogen peroxide and stimulate low glucose concentration.

EXPECTED VALUES [4]

Serum and plasma	***mg/dl***	***[mmol/L]***
Glucose fasting:		
Newborns ≤ 1day	40-60	[2.2-3.3]
Newborns > 1day	50-80	[2.8-4.4]
Children	60-100	[3.3-5.6]
Adults	70 -110	[4.1-5.9]
60-90 years	82-115	[4.6-6.4]
> 90 years	75-121	[4.2-6.7]
Glucose 2h postprandial	< 140	[< 6.6]
Urine	***g/24hrs***	***[mmol/24hrs]***
Random	5.0 – 15	[0.28-0.83]
24 hours	< 0.5	[< 0.28]
CSF	***mg/dl***	***[mmol/L]***
Adults	40-45	[2.2-4.2]

DYNAMIC RANGE

5 - 500 mg/dl (0.27 - 27.7 mmol/L).

REFERENCES

1. Caraway WT, Watts NB. Carbohydrates In: Tietz NW, ed.Fundamentals of Clinical Chemistry. 3ry ed. Philadephia WB saunde-rs 1987:422-447.
2. Trinder, P., Ann. Clin. Biochem. (1969), 6:24.
3. Young D.S., Effects of drugs on clinical laboratory tests. 4th Ed. (1995), p.3-274 to 3-294.
4. Tietz N. W., (Ed.), Textbook of Clinical Chemistry. Burtis CA and Ashwood ER, Fifth Edition, 2012.
5. Howanitz PJ, Howanitz JH. Carbohydrates. In: Henry JB,ed. Clinical diagnosis and mana-Gement by laboratory methods. 17th ed Philadelphia: WB Saunders 1984:165-179
6. Weissman M, klein B. Evaluation, of glucose determination In untreated serum samples. Clin Chem.1958; 4:420-422.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		