

Intended Use

For **IN VITRO quantitative** determination of Glucose in serum.

Clinical Significance

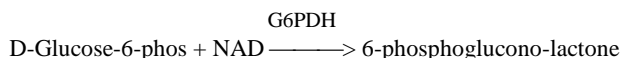
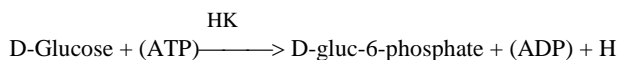
Measurements of Glucose are primarily used for diagnosing diabetes, nephritis, pancreatic disease, hyperthyroidism, hypopituitarism, hyperinsulinism, cretinism, and myxedema, as well as for monitoring the causes and treatments. (1)

Method History

The specificity of the hexokinase (HK) (EC 2.7.11) and the glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) enzyme reactions for the determination of serum Glucose was first reported by Slein in 1950. (2) The Food and Drug Administration has proposed as the reference method for measurement of Glucose, a totally enzymatic procedure using HK and G6PDH. (3) Catachem Glucose Hexokinase method is based upon the work of L.P. Leon, et al.

Method Principle

The enzyme hexokinase catalyzes the phosphorylation of Glucose in the presence of ATP and magnesium ions. The resultant glucose-6-phosphate is then oxidized to 6-phosphoglucono lactone with concomitant reduction of nicotinamide adenine dinucleotide (NAD). The amount of NADH produced is proportional to the Glucose present in the serum sample and it is quantified at 340nm. The reaction scheme illustrates the reactions that occur in this method.

**Reagent Content**

Active ingredients in the reagents will be approximately as follows:

Hexokinase Reagent

Each vial contains:

Buffer	
Hexokinase	≥ 800 U/L
G-6-PDH	≥ 800 U/L
Adenosine-5' - triphosphate	0.83 mM
Nicotinamide-Adenine Dinucleotide	1.21 mM
Non-reactive ingredients and stabilizers	

Precautions

Avoid contact with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent

Catachem Glucose Reagent is packaged ready-for-use. No preparation is required.

Reagent Storage And Stability

Store the reagent at 2-8°C. When stored as directed, this reagent is stable until the expiration date stated on the label. Upon opening and when stored as directed, the Working Glucose Reagent is stable for 60 days at 2-8°C.

Specimen Collection And Preparation

Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In serum the glucose concentration is stable for eight hours at 25°C and 72 hours at 4°C. (1)

Quality Control

To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances

A comprehensive discussion has been reported on the effect of interfering substances on various Glucose methods including the Hexokinase/G6PDH method. (4) A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et. al. (5)

Expected Values

The range of expected values determined for this method is 70-105 mg/dL for individuals less than 50 years of age and 85-125 mg/dL for individuals over 50 years of age. (1) These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Analytical Parameters

Wavelength	340 nm
Temperature	37°C
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	5 min
Reagent Volume	1.0 ml
Sample Volume	0.01 ml (10uL)
Total Volume	1.01 ml
Sample-to-reagent Ratio	1:101

Assay Procedure

1. Pipette 1.0 ml of Glucose Reagent into each of three cuvettes marked "calibrator", sample" and "blank".
2. Pipette 0.01 ml (10 uL) of calibrator or sample into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at 37°C.
4. Set spectrophotometer wavelength at 340 nm and zero the instrument with the cuvette marked "blank".
5. Read the "calibrator" and "sample" absorbencies.
6. Calculate the glucose concentration (mg/dL) in the sample(s), as shown in results and calculations.

Results And Calculations

$$\text{Gluc (mg/dL)} = \frac{\text{sample abs}}{\text{calibrator abs}} \times \text{calibrator (mg/dL)}$$

Example:

$$\text{Sample absorbance} = 0.300$$

$$\text{Calibrator absorbance} = 0.250$$

$$\text{Calibrator (mg/dL)} = 200$$

$$\text{Glucose (mg/dL)} = \frac{0.300}{0.250} \times 200 = 240 \text{ mg/dL}$$

Method Performance Characteristics

Sensitivity: 0.0008 - 0.0012 absorbance units per mg/dL.

Linear Range: 0-600 mg/dL.

Precision: Within-run and day-to-day precision is summarized as follows:

Precision Study

Glucose Mean mg/dL	Within-Run Precision		Total Precision	
	SD mg/dL	CV %	SD mg/dL	CV %
58	1.30	2.40	3.10	5.20
289	1.80	0.70	7.40	2.50
475	3.50	0.70	5.00	1.00

Correlation

A comparison of this method using an automated analyzer and a reference method based upon the Hexokinase/G6PDH reaction resulted in the following regression statistics.

Range	=	60 - 472 mg/dL
N	=	160
Y	=	0.993 x +2.0
r	=	0.999
Sy.x	=	2.9

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. WB Saunders Company, Philadelphia.
2. Slein MW, Cori GT, Cor, CF. J Biol Chem 186:763-780 (1950).
3. United States Department of Health, Education and Welfare, Food and Drug Administration. In Vitro Diagnostic Products for Human Use, Proposed Establishment of Product Class Standard for Detection of Measurement of Glucose. Fed Regist. 39,126, 24136-24147 (1974).
4. Leon LP, Sansur M, Snyder RL and Horvath C. Clin Chem 23, No. 9 (1977).
5. Young DS, Pestaner LD, Gibberman V. Clin Chem 21, No 5 (1975).

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