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Liquid Glucose (Hexokinase) Reagent Set

Intended Use
For the *in vitro* quantitative measurement of glucose in serum.

Test Summary
The measurement of glucose concentrations in biological fluids has been well documented. Glucose testing can be diagnostically significant in diabetes, hypoglycemia, and various adrenal and pituitary disorders.

Enzymatic methods for the measurement of glucose were first described by Keilin and Hartree.[1] The U.S. Food and Drug Administration has proposed as the reference method for glucose a totally enzymatic procedure using hexokinase and glucose-6-phosphate dehydrogenase.[2] Passey, et.al.[3] have critically reviewed ten glucose methods and have used the hexokinase procedure as the reference method.

Principle

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{HK} G_6P + \text{ADP}$$

$$G_6P + NAD^+ \xrightarrow{G_6PDH} \text{6-Phosphogluconate} + NADH + H^+$$

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) producing 6-phosphogluconate and NADH.
The formation of NADH causes increase in absorbance at 340 nm which is directly proportional the concentration of glucose in the sample.

Reagents
Glucose Reagent: A buffered solution containing 2 mmol/L nicotinamide adenine dinucleotide, 4 mmol/L adenosine triphosphate, 2 mmol/L magnesium, > 2000 U/L hexokinase (yeast), > 4000 U/L glucose-6-phosphate dehydrogenase (microbial), stabilizers, and preservatives.

Warnings and Precautions for Use
S24/25: Avoid contact with skin and eyes.
See Material Safety Data Sheet for additional information.

Reagent Preparation, Storage and Stability
Reagents are ready for use.
Supplied reagent is stable at 2-8°C until expiry date. Stability claims are based on real time studies

Reagent Deterioration
The reagent solution should be clear. Turbidity would indicate deterioration.

Disposal
Reagents must be disposed of in accordance with all Federal, State, and local regulations.

Specimen Collection and Storage
1. Fresh, clear, unhemolyzed serum. Serum should be separated from cells as soon as possible to minimize glucose decomposition by glycolysis.
2. In properly handled samples, glucose concentrations are stable for up to 3 days at 4°C.[4]

Analytical Specificity (CLSI EP7)[5]
Cross contamination studies have not been performed on automated instruments. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.
Interferences from icterus, lipemia, and hemolysis were evaluated for this method on a Roche/Hitachi 704® analyzer.

Concentration of Analyte		Substance Tested	Concentration of interferent where interference is insignificant	
Conventional Units	SI Units			
96 mg/dL	5.3 mmol/L	Hemoglobin	1000 mg/dL	155 µmol/L
99 mg/dL	5.5 mmol/L	Bilirubin	20 mg/dL	342 µmol/L
91 mg/dL	5.0 mmol/L	Intralipid	100 mg/dL	300 mg/dL (3.4 mmol/L) Simulated triglycerides

When assaying turbid or lipemic samples, it is recommended that a serum blank correction be performed. The blank can be prepared using 25 µL of sample and 2.5mL of deionized water. The absorbance of this solution is determined at 340 nm and subtracted from the absorbance of that sample with reagent.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S.[6]

The information presented above is based on results from the manufacturer's studies and is current at the date of publication.

Materials Provided
Glucose (Hexokinase) Reagent.

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry Control, catalog number C7592-100

Test Conditions
For the data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:100 and a wavelength reading of 340 nm.

Mindray BS-200 Test Parameters

Test :	GLU	R1 :	300
No.:	018	R2 :	0
Full Name:	Glucose	Sample Volume:	3
Standard No.:		R1 Blank:	
Reac.Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	340nm	Linearity Range:	0 - 600
Sec. Wave:	405nm	Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 12	Factor: Compensate: Slope 1.0	Intercept: 0
Incuba.Time:		□ Prozone check	
Unit:	mg/dl	q1: q2: q3: q4:	
Precision:	Integer	PC: Abs:	

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Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Correlation Coefficient:	0		

Limitations
A sample with a glucose concentration exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

Calibration
Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used.

Quality Control
A normal and abnormal concentration control should be analyzed as required in accordance with local, state and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

Calculations
The analyzer automatically calculates the glucose concentration of each sample.

Reference Intervals[4]
70-105 mg/dL (3.9-5.8 mmol/L)
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Performance Characteristics
Data presented was collected on a Roche/Hitachi® 704 analyzer unless otherwise stated.

RESULTS
Glucose concentration is reported as mg/dL (mmol/L).

Reportable Range (CLSI EP6) [5]
The linearity of the procedure described is 600 mg/dL (33.3 mmol/L). The lower limit of detection of the procedure described is 0.6 mg/dL (0.03 mmol/L). This data results in a reportable range of 0.6 to 600 mg/dL (0.03 to 33.3 mmol/L).

Accuracy (CLSI EP9)[5]
The performance of this method (y) was compared with the performance of asimilar glucose method (x) on a Hitachi® 704. Fifty patient serum samples ranging from 38 to 295 mg/dL (2.1 to 16.4 mmol/L) were tested and gave a correlation coefficient of 0.9992. Linear regression analysis gave the following equation:

This method = 0.9849 (reference method) + 2.3 mg/dL (0.13 mmol/L).

Precision (CLSI EP5) [5]
Data was collected on two concentrations of a control sera using a single lot of reagent in forty runs conducted over twenty days.

Concentration		Total SD		Total CV%	Within Run SD		Within run CV%
mg/dL	mmol/L	mg/dL	mmol/L		mg/dL	mmol/L	
89	4.9	1.1	0.06	1.3	0.4	0.02	0.4
257	14.3	3.1	0.17	1.2	1.1	0.06	0.4

References

1. Keilin, D. Hartree, E.F., Biochem. J. 42, 250 (1948).
2. United States Department of Health, Education and Welfare, Food and Administration. *In Vitro Diagnostic Products for Human Use, Proposed Establishment of Glucose*, Fed. Regist. 39, No. 126, 24136-24147 (1974).
3. Passey, R.B., Gillum, R.L., Fuller, J.B., Urry, F.M., Giles, M.L., *Evaluation and Comparison of Ten Glucose Methods and the Reference Method Recommended inthe Proposed Product Class Standard* (1974), Clin. Chem. 23, 131-139 (1977).
4. Burtis, C.A., Ashwood, E.R., Editors, *Tietz Textbook of Clinical Chemistry,Second Edition*, W.B. Saunders Company, Philadelphia, PA (1994).
5. *CLSI Method Evaluation Protocols*, Clinical and Laboratory Standards Institute, Wayne, PA.
6. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests*, 3rd ed., AACC Press, Washington (1990).

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Use by (YYYY-MM) | LOT Lot and batch code | REF Catalog number | Manufacturer | IVD *In vitro* diagnostic medical device
Temperature limitation | Consult instructions for use | CE mark | EC REP Authorized representative in the European Community