POINTE SCIENTIFIC, INC.
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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 ℃.[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.

Interference
The method is not influenced (< 10%) by hemoglobin values up to 500mg/dl, bilirubin levels up to 15mg/dl and lipemia / Triglycerides (Intralipid used to simulate) to 100mg/dl intralip or 300mg/dl simulated triglyceride. The studies were performed on the Beckman Coulter AU™400 analyzer following a modification of the guidelines contained in NCCLS document EP7-P

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. Controls
2. Calibrator
3. Beckman Coulter AU™ analyzer
4. Application and Instrument manuals

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **Glucose** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **2** DIL. VOL.:**0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **175** DIL. VOL.:**0** MIN. OD MAX. OD
R2 VOLUME: DIL. VOL.:**0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **340** ∇ SEC. **380** ∇ FIRST L: **-2.000** FIRST H:**2.500**
METHOD: **END** ∇ LAST L: **-2.000** LAST H:**2.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **18** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**
NORMAL RANGES: AGE L AGE H

	SEX		YEAR	MONTH	YEAR	MONTH	L	H
○	1. #	∇	#	#	#	#	#	#
○	2. #	∇	#	#	#	#	#	#
○	3. #	∇	#	#	#	#	#	#
○	4. #	∇	#	#	#	#	#	#

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○	5. #	∇ #	#	#	#	#	#	#
○	6. #	∇ #	#	#	#	#	#	#
	7. NONE SELECTED						#	#
	8. OUT OF RANGE	L	H				#	#

PANIC VALUE: **#** **#** UNIT:**mg/dl** DECIMAL PLACES: **0**

CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

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 Beckman AU400 analyzer unless otherwise stated.

RESULTS

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

Linearity: 500 mg/dl

Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.997 and a regression equation of y = 0.985x – 0.14. (n = 37, range 54 – 306 mg/dl)

Precision:

Within Day (N=20)			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
28	0.5	1.8	95	2.0	2.1
97	1.2	1.2	305	2.6	0.8
214	3.4	1.6			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6 using a Beckman Coulter AU™400 analyzer [7]

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).
7. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)

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