TECO DIAGNOSTICS
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GLUCOSE (HEXOKINASE) LIQUID REAGENT

INTENDED USE
For the *In Vitro* quantitative determination of glucose in human serum or plasma.

INTRODUCTION
Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated glucose levels are mainly associated with insulinemia or insulin-induced hypoglycemia.[1] A number of secondary factors also can contribute to elevated blood glucose levels. These include pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease.[2]

An enzymatic approach for glucose determination involves hexokinase coupled with glucose-6-phosphate dehydrogenase.[3] A revision of this approach is proposed by the U.S. Center For Disease Control as the reference method for glucose and forms the basis of the reagent for glucose.[4]

PRINCIPLE
Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

G-6-P + NAD $\xrightarrow{G6PDH}$ 6-Phosphogluconate + NADH

The enzymatic hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate and adenosine diphosphate (ADP). In the presence of NAD, the enzyme glucose-6-phosphate dehydrogenase (G6PDH) oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

REAGENT COMPOSITION
After the addition of R2 to Glucose (HK) R1 Reagent as directed, the reagent contains:

Hexokinase	>750 U/L
G6PDH	>1000 U/L
ATP	>1.0 mmol/L
NAD	>1.0 mmol/L
Buffer (pH 7.5)	100 mmol/L
Stabilizers and preservatives	

PRECAUTIONS
1. This reagent is for *In Vitro* diagnostic use only.
2. The reagent contains sodium azide as a preservative. Do not ingest. Avoid skin and eye contact.
3. All specimens and controls should be handled as potentially infectious, use safe laboratory procedures (NCCLS M29-T2).

REAGENT PREPARATION
Add one part of Glucose R2 Reagent to five parts of Glucose (HK) R1 Reagent. Mix gently.

REAGENT STORAGE
1. This reagent and standard should be stored refrigerated at 2-8°C. The reagents are stable until the expiration date appearing on the label when stored as directed.
2. The "working" reagent is stable for 30 days when stored at 2 - 8°C, and protected from direct light.
3. Avoid microbial contamination.

REAGENT DETERIORATION
Do not use if:
1. Reagent has an absorbance greater than 0.30 when measured against water at 340 nm.
2. The reagent fails to recover stated control values or meet stated linearity.
3. The reconstituted reagent develops turbidity, indicating contamination.

SPECIMEN COLLECTION
1. Either serum or plasma may be used.
2. Plasma or serum samples without preservatives should be separated from the cells or clot within a half hour of being drawn.
3. Glucose in separated unhemolyzed serum is generally stable up to eight hours at 25°C and up to 72 hours at 4°C.[5]
4. Glycolysis can be inhibited by collecting the specimen in sodium fluoride. Glucose in a sodium fluoride-oxalate mixture is reported to be stable up to 24 hours at 25°C.[5]

INTERFERING SUBSTANCES
Grossly lipemic or icteric sera may cause falsely elevated glucose values, and may require the use of a serum blank. Young et al. give a complete list of drug and other substances that may affect glucose values.[6]

MATERIALS PROVIDED
1. Glucose (HK) R1 Reagent
2. Glucose (HK) R2 Reagent
3. Glucose standard (100 mg/dl)

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Timer.
3. Test tubes and rack.
4. Spectrophotometer capable of reading at 340 nm.
5. Heating block or water bath (37°C).

AUTOMATED PROCEDURE
Refer to specific instrument application instructions.

MANUAL PROCEDURE
1. Prepare reagent according to the instructions.
2. Appropriately label tubes: reagent blank, standard, patient sample, etc.
3. Pipette 1.0 ml of reagent into all tubes and prewarm to 37°C.
4. Add 0.005 ml (5 µl) of sample to respective tubes. Mix well. Incubate all tubes at 37°C for five minutes.
5. After incubation, zero the spectrophotometer with reagent blank at 340 nm.
6. Read and record the absorbance of all tubes.

* TC-MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

LIMITATIONS

The procedure is linear to 500 mg/dl (27.8 mmol/L). Specimens above this limit must be diluted 1:1 with saline, re-run and the result multiplied by two (2) to compensate for the dilution.

CALIBRATION

An aqueous based standard is provided. The use of a serum-based calibrator is recommended.

QUALITY CONTROL

It is recommended that both normal and abnormal quality control sera be used routinely.

CALCULATIONS

Glucose results are expressed as mg/dl (mmol/L)
Abs. = absorbance at 340 nm

$$\frac{\text{Sample Abs.}}{\text{Standard Abs.}} \times \text{Conc. of standard} = \text{Conc. of glucose (mg/dl)}$$

Example: Sample Abs. = 0.155
Standard Abs. = 0.164

$$\frac{0.155}{0.165} \times 100 = 93 \text{ mg/dl}$$

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0556.

EXPECTED VALUES[5]

Normal range is reported to be 65-100 mg/dl. This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations. In a study of 50 samples, the expected values were found to be 65 - 112 mg/dl.

PERFORMANCE CHARACTERISTICS

1. Linearity: 500 mg/dl.
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, this procedure has a sensitivity of ~~0.30~~0.909 mg/dl.
3. Comparison: Glucose (HK) was compared to a commercially available glucose hexokinase method with the resulting linear regression equation of ~~Y = 0.999x + 0.44~~Y=1.0488X-3.2547. Coefficient of correlation R^2 = 0.997~~9~~. Thirty-two patient sera and controls ranging from 4~~6~~ mg/dl to ~~376~~ 285 mg/dl were assayed by the two methods.
4. Precision:

Within-Run

	Serum 1	Serum 2
Mean (mg/dl)	~~93~~96.7	~~301~~294
Std. Deviation (mg/dl)	~~1.58~~1.73	~~2.59~~1.95
C.V. (%)	~~1.70~~1.79	~~0.90~~0.665

Run-to-Run

	Serum 1	Serum 2
Mean (mg/dl)	~~93~~94	~~300~~290
Std. Deviation (mg/dl)	~~1.12~~3.90	~~2.91~~6.93
C.V. (%)	~~1.20~~4.15	~~1.00~~2.39

REFERENCES

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4. A Proposed Method for Determining Glucose Using Hexokinase and Glucose-6-phosphate Dehydrogenase, Public Health Service, Center for Disease Control, (1976).
5. Tietz, N.W., *Fundamentals of Clinical Chemistry*, 2nd. Ed., W.B. Saunders Co., Philadelphia, PA 243 (1976).
6. Young, D.S. et. al.: *Clin. Chem.* 21:5 (1975).

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