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Liquid Glucose (Hexokinase) Reagent Set

Intended Use
For the quantitative determination of glucose in serum. For *in vitro* diagnostic use only.

Clinical Significance
Determination of glucose in serum is most commonly performed for the diagnosis and treatment of diabetes mellitus.

Test Summary
There are a large number of methods in existence for the measurement of glucose in biological fluids. Early methods such as the Folin-Wu[1] and Somogyi-Nelson[2] depended on the reduction of heavy metals by the aldehyde group of glucose. These methods are subject to interference by carbohydrates other than glucose. The ortho-toluidine method, introduced in 1959[3] and later modified [4,5] to react directly with serum, is specific for aldoses but uses a strong, noxious, corrosive acid requiring incubation at elevated temperatures.
Enzymatic methods were first described in the 1940's[6] with varied modifications described to date.[7,8]
The present hexokinase method is based on a modification of Slein[9], using hexokinase and glucose-6-phosphate-dehydrogenase to catalyze the reaction. The method is also based on the reference method proposed by the FDA for measuring glucose.

Principle
$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} G_6P + \text{ADP}$$

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

Glucose is phosphorylated with adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). The product, glucose–6-phosphate (G_6P) is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glucose-6-phosphate-dehydrogenase (G_6PDH). The formation of NADH causes and increase in absorbance at 340nm. The increase is directly proportional to the amount of glucose in the sample.

Reagent Composition
Liquid Glucose (Hexokinase) Reagent: Hexokinase (yeast) ≥ 2000U/L, G_6PDH (Leuconostoc mesenteroides) ≥ 1500U/L, ATP ≥ 1.5mM, NAD ≥ 2.0mM, Buffer pH 7.5 ± 0.1. Nonreactive stabilizers and sodium azide (0.095%) as preservative.

Reagent Preparation
The reagent is ready to use.

Reagent Storage and Stability
The reagent set is stored at 2-8°C. Once opened the reagent will remain stable at least 30 days when properly stored and handled.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagent contains Sodium Azide as a preservative. Sodium Azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to remove Azide Salts," in the Manual Guide-Safety Management No. CSC-22 issued by the Centers for Disease Control, Atlanta, Georgia.
3. Do not use the reagent if the reagent blank has an absorbance greater than 0.500 against water at 340 nm, or contains obvious microbial growth.
4. Reagent should not be used if it fails to recover stated values in control sera.
5. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. Serum: Use fresh, unhemolyzed serum.
2. Plasma: Unhemolyzed samples from tubes containing oxalate, citrate, EDTA, fluoride or heparin may be used.
3. Specimen collection should be carried out in accordance with NCCLS M29-T2.[10] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.
4. Serum and plasma must be separated from the red cells promptly to prevent glycolysis. Glucose will decrease approximately 7% per hour when left in contact with red cells.[11] The addition of sodium fluoride to the specimen may prevent glycolysis.
5. Glucose in serum or plasma is stable for 8 hours at room temperature and 24 hours refrigerated at 2-8°C.
6. Specimen collection should be carried out in accordance with NCCLS M29-T2.[10] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
1. Young, et al[12] has published a comprehensive list of drugs and substances that may affect glucose values.
2. Bilirubin to the level of 20 mg/dl has been found to exhibit negligible interference (≤5%) in this assay.
3. Hemoglobin to the level of 400 mg/dl has been found to exhibit negligible interference (≤5%) in this assay.

NOTE: Glucose level was 177 mg/dl for the Bilirubin study and 181 mg/dl for the Hemoglobin study.

Materials Provided
Glucose (Hexokinase) Reagent.

Materials Required but not Provided
1. Accurate pipetting devices. (10ul and 1.0ml)
2. Timer. (For five minutes)
3. Test tubes/rack
4. Spectrophotometer with ability to read 340 nm.
5. Heating block or water bath (37°C).

Procedure (Automated-General)

Wavelength:	340 nm
Assay Type:	Endpoint
Sample/Reagent Ratio:	1:101
Reaction Direction:	Increasing
Temperature:	37°C
Incubation Time:	180 seconds
Low Normal:	70 mg/dl
High Normal:	110 mg/dl

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Procedure (Manual)

1. Label test tubes: "Blank", "Standard", "Control", "Sample", etc.
2. Add 1.0ml of reagent to all tubes.
3. Add 0.01ml (10 ul) of sample to respective tubes. Mix and incubate at 37°C for three minutes.
4. Zero spectrophotometer with water at 340nm
5. Read and record the absorbance of all tubes.

Limitations

1. Extremely lipemic samples may give falsely elevated glucose values. Prepare a sample blank by adding 10ul of sample to 1.0 ml isotonic saline, reading against water and subtracting the absorbance reading from the test absorbance.
2. Final reading is stable for 15 minutes after incubation period.
3. If spectrophotometer requires greater than 1.0 ml for accurate reading, 3.0 ml of reagent and 0.03 ml (30 ul) of sample may be used.
4. Samples with values exceeding 500 mg/dl should be diluted 1:1 with saline, re-run and the final answer multiplied by two.

Calibration

Use an NIST-traceable Glucose Standard (100mg/dl) or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Calculation

Abs. = Absorbance

$$\frac{\text{Sample Abs.} - \text{Blank Abs.}}{\text{Standard Abs.} - \text{Blank Abs.}} \times \text{Concentration of standard} = \text{Glucose (mg/dl)}$$

Sample: Blank Abs = 0.08
Sample Abs. = 0.150
Std. Abs. = 0.200
Std. Conc. = 100 mg/dl

Calculation: $\frac{.150-.08}{.200-.08} \times 100 = 58$ mg/dl

Quality Control

The validity of the reaction should be monitored by use of control serums with known normal and abnormal Glucose values. These controls should be run at least with every working shift in which Glucose assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values

Normal range is reported to be 70-110 mg/dl. [13]

Performance

1. Assay Range: 0-500 mg/dl. Samples that exceed 500 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: Results obtained with this reagent (y) in 110 samples ranging in Glucose from 36 to 294 mg/dl were compared with those obtained in the same samples using a dry-powder reagent (x) from the manufacturer based on the same methodology. The correlation coefficient was 0.999 and the regression equation was y=0.97x + 1.8. (Sy-x=14.45).
3. Precision: Precision studies were performed following a modification of the guidelines contained in NCCLS document EP5-T2.[14]

Within Day				Run to Run			
Mean	S.D.	C.V.%	N	Mean	S.D.	C.V.%	N
81	0.99	1.22	20	84	1.48	1.76	20
264	3.45	1.31	20	270	3.53	1.31	20
469	3.92	0.84	20	484	7.69	1.59	20

4. Sensitivity: The sensitivity for the Liquid Glucose (Hexokinase) reagent was investigated by reading the change in absorbance at 340 nm for a saline sample, and serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Liquid Glucose (Hexokinase) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 mg/dl of Glucose gives an absorbance of 0.0057.

References

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