

Intended Use

For the quantitative determination of Glucose in serum.

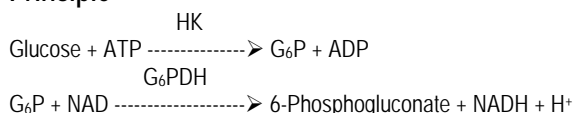
Method History

There are a large number of methods in existence for the measurement of glucose in biological fluids. Early methods such as the Folin-Wu¹ and Somogyi-Nelson² 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 1959³ 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's⁶ 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.

Principle



Glucose is phosphorylated with adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). The product, glucose-6-phosphate (G₆P) is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glucose-6-phosphate-dehydrogenase (G₆PDH). The formation of NADH causes an increase in absorbance at 340 nm. The increase is directly proportional to the amount of glucose in the sample.

Reagents

(Concentrations refer to reconstituted reagent)

Glucose Reagent:

Hexokinase	1000U/L
G ₆ PDH	1000U/L
ATP	1.0mM
NAD	1.0mM
Buffer pH	7.5 ± 0.1
Sodium Azide	0.01 % w/v
Nonreactive stabilizers and fillers.	

Reagent Preparation

Reconstitute reagent vials with volume of distilled water stated on vial label. Swirl gently to dissolve. Do not shake.

Reagent Storage

1. The reagent should be stored refrigerated at 2-8°C.
2. Reconstituted reagent is stable for five days at room temperature and for 60 days refrigerated at 2-8°C.

Reagent Deterioration

Do not use if:

1. Reagent has an absorbance greater than 0.20 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.

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. This reagent contains sodium azide at 0.01%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

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. 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.¹⁰ The addition of sodium fluoride to the specimen may prevent glycolysis.
4. Glucose in serum of plasma is stable for 8 hours at room temperature and 24 hours refrigerated at 2-8°C.

Interferences

Young, et al¹¹ has published a comprehensive list of drugs and substances which may affect glucose values.

Materials Provided

Glucose Hexokinase reagent.

Materials Required but not Provided

1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 340 nm

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Reconstitute reagent vial according to label instructions. (See "Reagent Preparation")
2. Label test tubes labeled "blank", "control", "standard", "sample", etc.
3. Add 1.0ml of reagent to all tubes
4. Add 0.005ml (5 ul) of sample to respective tubes. Mix and let stand at room temperature for three minutes.
5. Zero spectrophotometer with water at 340nm.
6. Read and record absorbance values of all tubes.
7. To determine results, see "Calculations" section.

Procedure Notes

1. Extremely lipemic samples may give falsely elevated glucose values. Prepare a sample blank by adding 5 ul of sample to 1.0ml isotonic saline, reading against water and subtracting the absorbance reading from the test absorbance.
2. Final reading is stable for fifteen minutes after incubation period.
3. If spectrophotometer requires greater than 1.0ml for accurate reading, 3.0ml of reagent and 0.02ml (20 ul) of sample may be used.

Glucose Hexokinase Reagent Set

Limitations

1. Samples with a glucose value exceeding 600mg/dl should be diluted 1:1 with saline, re-run and the final answer multiplied by two.
2. Extremely hemolyzed samples should not be used for the glucose assay.
3. Extremely lipemic samples require a sample blank. See "Procedure Notes".

Calibration

Use an aqueous glucose standard (100 mg/dl) or an appropriate serum based calibrator.

Calculations

Abs. = Absorbance

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

Example: If: Blank Abs. = 0.080
Sample Abs = 0.150
Std. Abs = 0.200
Std. Conc. = 100 mg/dl

Then: $\frac{.150 - .080}{.200 - .080} \times 100 = 58\text{mg/dl}$

Quality Control

The integrity of the reaction should be monitored by use of normal and abnormal control sera with known glucose values.

Expected Values

Normal range is reported to be: 65-110 mg/dl.

It is strongly recommended that each laboratory establish its own normal range.

Performance

1. Linearity: 600 mg/dl
2. Comparison: A study performed between this procedure and another similar hexokinase procedure yielded a correlation coefficient of 0.999 with a regression equation of $y = 0.99x + 0.68$. The study included samples with values ranging from 51 to 506 mg/dl.
3. Precision:

Within Run			Run To Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
104	1.3	1.3	104	1.9	1.8
299	3.8	1.3	312	6.3	2.0

References

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