

Glucose HK (Liquid)

1.0 INTENDED USE

The reagent is intended for the quantitative determination of Glucose in serum or plasma.

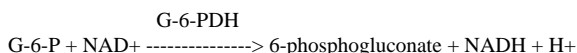
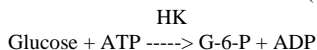
2.0 BACKGROUND

2.1 METHOD AND HISTORY

Enzymatic methods for the determination of glucose were described by Keilin and Hartree (10.1). Passey, et. al. (10.3) have reviewed 10 glucose methods and have used the hexokinase method as the reference procedure.

2.2 TEST PRINCIPLE

Glucose is converted to glucose-6-phosphate (G-6-P) by hexokinase (HK) in the presence of adenosine triphosphate (ATP). G-6-P is converted to 6-phosphogluconate and NADH in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH) and nicotinamide adenine dinucleotide (NAD) and the increase in optical density at 340 nm is measured.



NADH causes an increase in absorbance at 340 nm which is directly proportional to the concentration of glucose in the sample.

2.3 CLINICAL SIGNIFICANCE (10.4)

Elevated levels of glucose (hyperglycemia) are found in uncontrolled diabetes mellitus, hyperthyroidism, hyperadrenalism, uremia and bacterial or viral infections.

Low levels of glucose (hypoglycemia) are found in certain adrenal and pituitary disorders, and in cases of high levels of insulin or other antidiabetic drugs.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

The patient should be fasting for 12 hours prior to specimen collection.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Plasma may be used if citrate, oxalate, heparin or EDTA are employed as the anticoagulant. Hemolyzed samples must not be used since erythrocytes contain glucose-6-phosphate which is an intermediate product in this reaction.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 μ l. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Samples must be separated within 30 minutes of collection as glycolysis occurs in whole blood at a rate of 7% per hour.(10.4) Serum samples may be stored refrigerated (2°-8° C) for 1 week , or frozen (-20° C) for 1 month prior to analysis. Frozen samples should be thawed at room temperature and mixed thoroughly before analysis. Thawed samples should not be refrozen. (10.4)

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS

(2 X 125 ml)

Reagents necessary for the determination of glucose are included in the kit.

4.1 GLUCOSE (HK) REAGENT

The glucose reagent contains:

phosphate buffer	100 mM
magnesium chloride	2mM
ATP	4mM
NAD	2mM
HK (L.mesenteroides)	2000 U/L
G-6-PDH (yeast)	4000 U/L

4.1.1 Standard/Control/Calibrator

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Never pipette by mouth. Exercise the normal precautions required for handling all laboratory reagents.

4.3 REAGENT PREPARATION

The reagent is ready to use as is.

4.4 STORAGE AND STABILITY

When stored at 2°-8°C reagents are stable until the expiration date printed on the label.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 340 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.

- 4.5.3 Test tubes capable of holding 3 ml.
- 4.5.4 Pipettes capable of delivering 2.5 ml and 25 µl.
- 4.5.5 Deionized or distilled water for preparing the reagent blank.
- 4.5.6 Timer for a 5 minute or 10 minute incubation.
- 4.5.7 Constant temperature source which can be adjusted to 37° C.
- 4.5.8 Calibrator .
- 4.5.9 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C, or 18-26° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	5 min at 37° C 10 min at 18-26° C
Sample volume	25 µl
Reagent volume	2.5 ml
Total volume	2.525 ml
Sample to reagent ratio	1/100

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The Glucose (HK) assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

- 5.4.1 Warm the required volume of reagent to room temperature.
- 5.4.2 Into separate test tubes pipette 25 µl of distilled water, calibrator, or serum to be assayed.
- 5.4.3 Add 2.5 ml of reagent to each test tube and mix.
- 5.4.4 Following incubation for 5 minutes at 37° C in a heat block or 10 minutes at 18-26° C (room temperature), determine the absorbance of the calibrator (As) and of each serum (A) at 340 nm using the distilled water sample as the reagent blank.

5.5 CALCULATION AND RESULTS

$$\text{Glucose (mg/dl)} = \frac{A}{A_s} \times \text{X concentration of calibrator}$$

A = absorbance of sample, As = absorbance of calibrator

Example:

$$\text{Glucose (mg/dl)} = \frac{.295}{.355} \times 100 \text{ mg/dl} = 83 \text{ mg/dl}$$

with A = .295 and As = .355, concentration of calibrator = 100 mg/dl

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.4)

The range of expected values in adults for fasting glucose using the glucose (HK) method is: 70-105 mg/dl
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

Reference 10.6 provides a discussion of some critical values.

6.3 LIMITATIONS OF PROCEDURE

- 6.3.1 High levels of fructose in serum, as may occur in essential fructosuria or after excessive ingestion of fruits, may produce falsely elevated glucose levels, i.e., 200mg of fructose will react as 1mg of glucose. Greater than 500mg of fructose are inhibitory. (10.4)
- 6.3.2 Hemoglobin itself is not an interfering substance, however, hemolyzed cells can release enzymes which catabolize glucose as well as glucose-6-phosphate, the latter of which is a reaction intermediate. This effect is minimized when samples are stored in a refrigerator at 2°-8°C especially in the presence of fluoride ion. (10.4)
- 6.3.3 A comprehensive list of drugs and other substances which can affect the glucose concentration in serum is given by Young. (10.5)
- 6.3.4 As with any chemical reaction, users should be alert to the possible effect on results caused by unknown interferences from medications or endogenous substances. All patient results should be evaluated in light of the total clinical status of the patient.
- 6.3.5 Use of this test has not been evaluated in neonatal hypoglycemia. Thus this test is not recommended for use with neonatal (birth to 4 weeks) specimens.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The Glucose (HK) assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details. Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 15 replicates of 2 control sera were run.

<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
79	± 0.57	0.73
309	± 4.08	1.33

Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 2 control sera.

<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
96	± 1.42	1.49
332	± 4.73	1.43

9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing King Glucose (HK) method and a similar glucose (HK) method. The samples range between 35 mg/dl and 559 mg/dl.

<u>Number of Samples</u>	<u>Regression Equation</u>	<u>Correlation Coefficient</u>
44	$y = .987x + .470$	0.999

9.3 LINEARITY

This method is linear to 500 mg/dl. A sample with glucose beyond the linearity limit should be diluted 1 to 1 with deionized water. Reassay the specimen and multiply the results by 2.

10.0 REFERENCES

- Keilin, D., Hartree, E.F., Biochem. J. 42,250 (1948).
- United States Department of Health, Education and Welfare, Food and Drug Administration. In vitro Diagnostic Products for Human Use, Proposed Establishment of Product Class Standard for Detection or Measurement of Glucose, Fed. Regist. 39, No. 126, 24136-24147 (1974).
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- Tietz, N.W., (ed.), Fundamentals of Clinical Chemistry, 2nd ed., W.B. Saunders Co., Toronto, 1213 (1982).
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