

Glucose (Trinder)

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

The glucose oxidase and peroxidase method was first reported in 1956 by Keston (10.1) and Teller (10.2). In 1958, Beach and Turner reported on the specificity of the reaction for beta-d-glucose, and determined its accuracy by comparative tests with other glucose determination methods. The Biotron Diagnostics Glucose Test is a modification of the Meites and Gochman (10.3, 10.4) glucose determination.

2.2 TEST PRINCIPLE

D-glucose in the sample is oxidised by the enzyme glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase, reacts with hydroxybenzoate and 4-aminophenazone to form a quinone complex. The intensity of the color formed is proportional to the glucose concentration in the sample.

Glucose oxidase

d-Glucose -----> gluconic acid + H₂O₂

Peroxidase

H₂O₂ + 4-aminophenazone -----> quinone complex

+ hydroxybenzoate

2.3 CLINICAL SIGNIFICANCE (10.8)

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. The specimen should be collected following a 12 hour fast. Plasma prepared from blood collected with an anticoagulant containing fluoride may be used.

Samples must be separated within 30 minutes of collection as glycolysis occurs in whole blood at a rate of 7% per hour.(10.6)

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 µl. 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

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. (10.6)

Fluoridated plasma for glucose assay is stable at 2°-8° C (refrigerated) for 5 days (10.5.)

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

(5 X 50 ml)

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

4.1 GLUCOSE ENZYME REAGENT

Each vial contains, after reconstitution with deionized water:

glucose oxidase ≥ 20,000 U/L

peroxidase ≥ 5000 U/L

4-aminophenazone ≥ 0.2 mM

p-hydroxybenzoate ≥ 10 mM

stabilizer, and a preservative

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 working reagent is prepared by adding 50 ml of deionized water to each glucose reagent vial. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

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4.4 STORAGE AND STABILITY

When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label. The working reagent is stable for 30 days if kept at 2-8° C (refrigerated) and protected from light.

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 500-540 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 500-540 nm.
- 4.5.3 Test tubes capable of holding 3 ml.
- 4.5.4 Pipettes capable of delivering 2.5 ml and 20 µl.
- 4.5.5 Deionized or distilled water for preparing the reagent blank.
- 4.5.6 Timer for a 3 minute incubation.
- 4.5.7 Constant temperature source for 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	500-540 nm
Temperature	37° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	3 min at 37° C
Sample volume	20 µl
Reagent volume	2.5 ml
Total volume	2.52 ml
Sample to reagent ratio	1/125

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 500-540 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 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 working reagent to room temperature. (See 4.3 Reagent Preparation Section.)
- 5.4.2 Into separate test tubes pipette 20 µl of distilled water, calibrator, or serum to be assayed.
- 5.4.3 Add 2.5 ml of working reagent to each test tube and mix.
- 5.4.4 Following incubation for 3 minutes at 37° C determine the absorbance of the calibrator (As) and of each serum (A) at 500-540 nm using the distilled water sample as the reagent blank.

5.5 CALCULATION AND RESULTS

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

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 (10.5)

The range of expected values is:

$$60 - 110 \text{ mg/dl}$$

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 (10.8)

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.

6.3 LIMITATIONS OF PROCEDURE

- 6.3.1 A comprehensive list of drugs and other substances which can affect the glucose concentration in serum is given by Young. (10.7)
- 6.3.2 Bilirubin has no effect if present in concentrations of less than 8 mg/dl.
- 6.3.3 Uric acid has no effect if present in concentrations of less than 12 mg/dl.

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 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, 20 replicates of 2 control sera were run.

<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
89	± 2.8	3.1
298	± 8.5	2.8

Between-Run

In this study, 8 runs were made with a pooled serum sample.

<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
97	± 3.3	3.4

9.2 CORRELATION

A correlation study was done on the SMA 12/60 (registered trademark of Technicon Corp.) comparing this method and a similar glucose method.

<u>Number of Samples</u>	<u>Regression Equation</u> <u>y=Biotron, x=Comparative</u>	<u>Correlation Coefficient</u>
20	$y = 1.014 x - 2.39$	0.98

9.3 RECOVERY STUDY

In this study, known aqueous glucose standards of varying concentration were added to a pooled serum sample. With this method, recovery was in the range of 90-99%.

9.4 LINEARITY

This method is linear through 400 mg/dl, beyond which the specimen should be diluted 1 to 1 with deionized water. Reassay the specimen and multiply the result by 2.

10.0 REFERENCES

- 10.1 Keston, A., Abstracts of Paper Presented at the 129th Meeting of the Am. Chem. Soc., Section 69C, 1956.
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- 10.4 Gochman, N., and Schmitz, J.M., Clin. Chem. 18, 943(1972).
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- 10.7 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
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