CE

Stanbio Glucose LiquiColor® (Oxidase) Procedure No. 1070

Intended Use: For the Quantitative Determination of Glucose in Serum, Plasma or CSF

Summary and Principle

The accurate estimation of glucose is important in the diagnosis and management of hyperglycemia and hypoglycemia. Hyperglycemia may occur as a result of diabetes mellitus, in patients receiving intravenous glucose fluids, during severe stress or as a result of cerebrovascular accidents. Hypoglycemia may be the result of an insulinoma, insulin administration,
inborn error of carbohydrate metabolism or fasting.[1]

Measurement of blood glucose levels was among the first chemical procedures employed in clinical laboratory medicine.[2] The glucose oxidase methodology was introduced by Keilin and Hartree[3] in 1948. Keston[4] later reported use of the combined glucose oxidase-peroxidase reagent, followed by the Teller[5] addition of a chromogenic reagent to Keston's procedure. The Stanbio single reagent glucose method is based on a technique described by Trinder et al.[6]

Glucose is oxidized in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts, under the influence of peroxidase (POD), with phenol and 4-aminoantipyrine to form a red-violet quinone complex. The intensity of the color is proportional to glucose concentration.

$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{GOD} H_2O_2 + \text{D-Gluconic Acid}$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{POD} \text{Quinone Complex} + H_2O$$

Reagents

Glucose Reagent, Ref. No. 1071

Phosphate Buffer	200 mmol/L
Phenol	4 mmol/L
4-Aminoantipyrine	0.2 mmol/L
Glucose Oxidase	> 15 KU/L
Peroxidase	> 1.2 KU/L

Non reactive ingredients and preservatives

Glucose Standard, 100 mg/dL, Ref. No. 1072

Contains 5.55 mmol/L Glucose in 0.5 mol/L benzoic acid.

Precautions: The reagent is for "*In Vitro Diagnostic Use*". Normal precautions exercised in handling laboratory reagents should be followed. The reagent contains sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Reagent Preparation: The Glucose reagent is supplied ready-to-use.

Reagent Storage and Stability: The reagent is stable up to the end of its labeled expiration date, if properly stored at 2-8°C, protected from light and contamination is avoided. Do not freeze the reagent! Measurement is not influenced by reagent color changes as long as absorbance of the reagent is < 0.80 at 500 nm in a 1 cm lightpath. Discard if it is found to contain particulate matter. The standard is stable up to the end of the labeled expiration date, if properly stored at 2-8°C and contamination is avoided.

Material Required But Not Provided

Spectrophotometer capable of absorbance reading at 500 nm (492-520 nm) • Constant temperature block or bath, 37°C, or temperature controlled cuvette • Accurate pipetting devices • Test tubes • Interval timer

Specimen Collection and Storage

Non-hemolyzed serum is the specimen of choice. Remove from clot within 30 minutes of collection in order to prevent glycolysis. An anticoagulant containing fluoride is recommended, but any of the common anticoagulants may be used if the plasma is separated from the cells promptly after centrifugation. No special preparation is required for CSF specimens. Glucose in serum/plasma processed in the manner described is stable for 48 hours at 2-8°C. For long term storage, samples should be placed in sealed containers and frozen at -20°C.[7,8] CSF samples should be analyzed immediately because of possible bacterial contamination.

Interfering Substances: Excessive levels of ascorbic acid can produce falsely low glucose values. For a more comprehensive review of factors affecting glucose assays, refer to the publication by Young.[9]

Automated Procedure

Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Stanbio's Technical Service Department.

Manual Procedure (Linear to 400 mg/dL)*

1. Remove the amount of reagent to be used for testing and allow to warm to ambient temperature.
2. Glucose reagent is supplied ready-to-use.
3. Zero spectrophotometer at 500 nm with distilled water.
4. For each standard, sample and control, add 1.0 mL reagent to cuvettes/test tubes and warm to 37°C for 5 minutes.
5. Add 10 µL (0.010 mL) of each sample to its respective cuvette/test tube, mix gently and return to 37°C incubation.
6. After 5 minutes of incubation, read and record the absorbance of all samples.

* If linearity is desired to 500 mg/dL, increase reagent volume to 1.5 mL and proceed using 10 µL of sample.

Manual Procedure (Linear to 650 mg/dL)

1. Remove the amount of reagent to be used for testing and allow to warm to ambient temperature.
2. Glucose reagent is supplied ready-to-use.
3. Zero spectrophotometer at 500 nm with distilled water.
4. For each standard, sample and control, add 1.0 mL reagent to cuvettes/test tubes and warm to 37°C for 5 minutes.
5. Add 5 µL (0.005 mL) of each sample to its respective cuvette/test tube, mix gently and return to 37°C incubation.
6. After 5 minutes of incubation, read and record the absorbance of all samples.

Quality Control: Stanbio Ser-T-Fy® 1, Level 1 Control Serum, Ref. No. G427-86, and Ser-T-Fy® 2, Level 2 Control Serum, Ref. No. G428-86, are recommended for each run. Other commercially available controls with glucose values assayed by this method are also suitable. Glucose values determined in these materials, by this procedure, should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

Calibration: Calibration is required. Stanbio Laboratory recommends the use of the standard provided in the kit when using a manual spectrophotometer. For automated analyzers, Stanbio Laboratory recommends the use of the Ser-T-Cal® Multi-Calibrator, Ref. No. 0550-605. For more information about automated analyzer calibration consult the calibration guidelines of your analyzer.

Results

Values are derived by comparing the absorbance of the unknown (u) with that of a standard (s) identically treated.

$$\text{Glucose (mg/dL)} = \frac{Au}{As} \times 100$$

Where Au and As are the absorbances of unknown and standard, respectively, and 100 the concentration of standard (mg/ dL)

Example: Au = 0.370, As = 0.280

$$\text{Glucose (mg/dL)} = \frac{0.370}{0.280} \times 100 = 132$$

Limitations

If the glucose value exceeds the linearity as described in the method run, the specimen should be diluted 2-fold (1+1) with distilled water, the assay repeated and results multiplied by the dilution factor of 2.

Expected Values[10]

Normal Range:

Serum/Plasma: 70 -105 mg/dL, (3.89 – 5.83 mmol/L)
CSF: 40 - 75 mg/dL, (2.22 – 4.17 mmol/L)

These ranges should serve only as a guideline. It is ultimately the responsibility of the laboratory to establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics[11]

Precision: Using a serum containing glucose in the normal range and another with an elevated value, a series of 5 assays were performed on each of 5 days. Coefficients of variation (CV) were within runs of 1.6% and 1.2 % and between runs of 3.0% and 2.0 %, respectively.

Correlation: Determination of glucose by the procedure described (y) and by the powder form method (x) on 64 sera showed a correlation coefficient (r) of 0.995 and the regression equation of y = 0.98x - 1.99 mg/dL.

Linearity: When performed as directed, this method is linear as listed for each test procedure.

References

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2. Folin O., Wu H.: J Biol Chem 41:367, 1920
3. Keilin D., Hartree E.F.: Biochem J 42:230, 1948
4. Keston AS: Abstr 129th Meeting, Am Chem Soc, 1956, p 31c.
5. Teller J.D.: Abstr 130th Meeting, Am Chem Soc, 1956, p 69c.
6. Trinder, P., "Determination of Blood Glucose Using 4-Aminophenazone." J. Clin. Path., 22:246 (1959).
7. Pencock CA, et al. Clin Chem Acta 1973; 49:193.
8. Shepard MDS, Mazzachi RD. The Clin Biochem 1983;4:61-67.
9. Young DS, Effects of Drugs on Clinical Laboratory Tests, 3rd Ed, 1990; 3:168-182.
10. Cooper G.R., McDaniel V: Manual of Methods for the Determination of Glucose, CDC, USPHS, Atlanta.
11. Caraway W.T.: IN Fundamentals of Clinical Chemistry, 2nd ed., N.W. Tietz, Ed. Saunders, Philadelphia, 1976, p 242.
12. Stanbio Laboratory Data

For Technical Service call: 1-800-531-5535 • (830) 249-0772

Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit		Manufactured by
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between temperature indicated	LOT	Lot Number	REF	Reference No.

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