Glucose Liqui-UV® (Hexokinase) Procedure No. BD1850
Quantitative Determination of Glucose in Serum, Plasma, CSF or Urine.

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 and cerebrovascular accidents. Hypoglycemia may be the result of an insulinoma, insulin administration, inborn error of carbohydrate metabolism or fasting. Many analytical procedures have been developed to measure blood glucose. Those in common use can be classified as enzymatic (hexokinase, glucose oxidase, and glucose dehydrogenase).

The enzymatic hexokinase (HK) catalyses the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate and adenosine diphosphate (ADP). In the presence of NAD, the enzymeglucohexokinase (G6P-DH), oxidizes glucose-6-phosphato-6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

Glucose + ATP → HK) → glucose-6-phosphate + ADP
Glucose-6-phosphate + NAD → (G6P-DH) → gluconate-6-P + NADH

Reagent
R1: Glucose Buffer Reagent, BD184a
Concentrations in the final mixture:
- TRIS Buffer (pH 7.8) 80 mmol/L
- Mg^2+ 4 mmol/L
- ATP 1.7 mmol/L
- NAD 1.7 mmol/L

R2: Glucose Hexokinase Reagent, BD185b
Glucose Enzyme Reagent (R2), Catalog No. 1062
Mg^2+ 4 mmol/L
Hexokinase > 1.5 KU/L
G6P-DH > 1.5 KU/L

Glucose Standard, 100 mg/dL, BD184b
Contains 5.55 mmol/L Glucose in 0.5 mol/L Benzoic acid.

Precautions:
For In Vitro Diagnostic Use
Reagent Preparation: Glucose Buffer and Enzyme Reagents are supplied ready-to-use. To prepare Working Reagent, combine one part of R2 to five parts of R1. Example: 5 mL of R2 to 25 mL of R1. Working reagent stability is 90 days.

Reagent Storage and Stability: Glucose Reagent and Standard are stable until the expiration dates on their respective labels when stored at 2-8°C. Reagent absorbance > 0.50 at 340 nm (1 cm/cuvette) indicates deterioration.

Materials Required But Not Provided
Spectrophotometer capable of absorbance readings at 334, 340 or 365 nm.
Pipets capable of accurately delivering 0.01 and 1.0 mL.
Heating block or water bath, 37°C (optional)
Cuvettes; Vortex mixer; Interval timer

Specimen Collection and Preparation
Serum: Remove from clot within 30 minutes of collection in order to prevent glycolysis.
Plasma: An anticoagulant containing fluoride is recommended, but any of the common anticoagulants may be used if plasma is separated from cells promptly after centrifugation.
CSF: No special preparation is required.
Sample Stability: 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 -1°C. CSF samples should be analyzed immediately because of possible bacterial contamination. Urine samples are stable for 1 day at 4°C.

Interfering Substances: Ascorbic acid levels up to 30 mg/dL, bilirubin levels up to 30 mg/dL, lipemia levels up to 2000 mg/dL (triglycerides) and hemoglobin levels up to 500 mg/dL show no interference in this test. For a more comprehensive review of factors affecting glucose assays, refer to the published work by Young.  

Automated Procedure
Parameters:
- Wavelength: 340 nm (334 nm, 365 nm)
- Reaction Type: Endpoint
- Reaction Direction: Increasing
- Reaction Temperature: 37°C
- Sample/Reagent Ratio: 1:100
- Equilibration Time: 3 Seconds
- Read Time: 4 Seconds
- Lag Time: 300 Seconds
- Blank Absorbance Limit: 0.200
- Standard: 100 mg/dL
- Low Normal: 70 mg/dL
- High Normal: 105 mg/dL
- Linearity: 500 mg/dL

Above parameters should be employed in programming automated analyzers for Glucose. Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Interchim Customer Service Department.

Test Performance

1. Pipet into cuvets the following volumes (mL) and mix well:

   | Reagent Blank (RB) | Standard (S) | Unknown (U) |
---|---|---|---|
Hexokinase Rgt | 1.0 | 1.0 | 1.0 |
Standard | | 0.01 | — |
Sample | | | 0.01 |

2. Incubate all cuvets at 37°C for 5 minutes.
3. Read S and U vs RB at 340 nm.

Quality Control: Interchim Ser-T-Fy® I, Normal Control Serum, #FT7670 and Ser-T-Fy® II, Abnormal Control Serum, # FT7680 are recommended for verifying accuracy and precision. Other commercially available controls with Glucose values assayed by this method are also suitable.

Calibration
Calibration is required. A suitable aqueous glucose standard such as the Interchim Glucose standard supplied or a serum based calibrator such as the Interchim Ser-T-Cal® Multicalibrator, #FT7640 is recommended.

Results
Values are derived by the following equation:

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

where Au and As are the absorbance values of UNKNOWN and CALIBRATOR, respectively, and 100 the concentration of the CALIBRATOR (mg/dL).

Example: Following readings were obtained using 1 cm cuvets:

\[ \text{Au} = 0.100, \text{As} = 0.126 \]
\[ \text{Glucose (mg/dL)} = \frac{0.100 \times 100}{0.126} = 79 \]

NOTE: Samples having glucose values greater than 500 mg/dL are diluted 1:2 (1 + 1) with distilled water, the assay repeated and results multiplied by the dilution factor 2.

Expected Values
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)
Urine 5 - 15 mg/dL (0.278 - 0.83 mmol/L)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics

Precision: Using controls containing glucose in the normal range and abnormal range, assays were performed over a 20 day period. Coefficients of variation (CV) were within run 2.1 and 2.2and between runs 0.9 and 0.9 respectively.

Correlation: Determination of glucose by the procedure described (y) and by another commercially available glucose hexokinase method (x) on 76 sera, showed a correlation coefficient (r) of 0.989 and a regression equation of y = 1.00x - 0.00 mg/dL.

Linearity: When performed as directed, this method is linear from 0 to 500 mg/dL.

Sensitivity: Based on an instrument resolution of A = 0.001, the method showed a sensitivity of 2 mg/dL.

References

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