Methodology

The Hexokinase / glucose-6-phosphate dehydrogenase method developed by the American Association of Clinical Chemistry and Centres for Disease Control has been accepted as the reference method for glucose determination. In this procedure, free fructose prepared by the Somogyi technique using ZnSO4 / I2/S2O3 precipitation is used. For routine laboratory use however serum or plasma without protein removal is the preferred method. The Glucose Hexokinase reagent is based on this reference method.

The series of reactions involved in the assay system is as follows:

1. Hexokinase catalyses the phosphorylation of glucose by ATP producing ADP and glucose-6-phosphate.

   Glucose + ATP \[ \rightarrow \] Hexokinase \[ \rightarrow \] G-6-P + ADP

2. Glucose-6-phosphate is oxidised to 6-phosphogluconate with the reduction of NAD+ to NADH by G-6-PDH. The amount of NADH formed is proportional to the concentration of glucose in the sample and can be measured by the increase in absorbance at 340 nm.

   G-6-P + NAD+ \[ \rightarrow \] G-6-PDH \[ \rightarrow \] 6-PG + NADH + H+

**Indications of Reagent Deterioration:**

- Turbidity;
- Reagent absorbance >0.5 (340 nm, 1 cm lightpath); and/or
- Failure to recover control values within the assigned range.

**Specimen Collection and Handling**

- **Collection:** The stability of glucose specimens is reduced by bacterial contamination and glycolysis. In order to inhibit glycolysis samples should be collected into tubes containing Sodium Fluoride. As soon as possible serum or plasma should be separated from the cells.
- **Serum:** Use non-haemolysed serum.
- **Plasma:** Use heparin.
- **Urine:** If a delay in transport to the laboratory is expected the use of a chemical preservative such as merthiolate (0.23 mmol/L) is recommended.
- **Storage:** In separated, non-haemolysed serum or plasma, glucose is stable for up to 72 hours at 4°C or as long as 8 hours at 25°C.5,6 In the presence of sodium fluoride, glucose is stabilized for as long as 3 days at room temperature.6 For long term storage samples should be placed in sealed containers and frozen at -10°C.7 Urine samples are stable for 1 day at 4°C.8

**Additional Equipment Required But Not Provided:**

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm (334-365 nm).
- Analyzer specific consumables, e.g.: sample cups.
- Normal and abnormal asayed control material.
- Calibrator or a suitable aqueous glucose standard.

**Assay Procedure**

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

**System Parameters**

- **Temperature:** 37°C
- **Primary Wavelength:** 340 nm (334 - 365 nm)
- **Secondary Wavelength:** 380 nm (380 - 410 nm)
- **Assay Type:** End Point
- **Direction:** Increase
- **Sample:Reagent ratio:** 1:150
- **Incubation Time:** 3 minutes
- **Reagent Blank Limits:** Low 0.00 AU (340 nm, 1 cm lightpath) High 0.50 AU (340 nm, 1 cm lightpath)
- **Linearity:** 0-45 mmol/L (0-810 mg/dL)
- **Analytical Sensitivity:** 0.038 \( \Delta \text{Abs per mmol/L} \) (340 nm, 1 cm lightpath)

**Calculations**

Results are calculated, usually automatically by the instrument, as follows:

\[
\text{Glucose} = - \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value} - \text{Value of Calibrator}
\]

**Example:**

- Absorbance of Calibrator = 0.30
- Absorbance of unknown = 0.10
- Value of Calibrator = 13.2 mmol/L (238 mg/dL)

\[
\text{Glucose} = \frac{0.10}{0.30} \times 13.2 = 4.4 \text{ mmol/L (238 mg/dL)}
\]

- Glucose = 0.10 x 238 = 79 mg/dL
For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in liters. 

Urine Glucose = Glucose Result x Dilution x Volume (L) (mmol/24 hours) (mmol/L) Factor

Example:
- Glucose result = 0.7 mmol/L (12.6 mg/dL)
- Dilution of Urine = Neat
- 24 Hour volume of urine = 0.35 Liters

Urine Glucose = 0.7 x 1 x 0.95 = 0.67 mmol/24 hours
Urine Glucose = 12.6 x 1 x 0.95 = 11.97 mg/24 hours

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. May also be run at 334 or 365 nm.
3. Specimens with glucose values above 45 mmol/L (810 mg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
4. Unit Conversion: mmol/L x 18 = mg/dL

CALIBRATION
Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (e.g. NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturer's specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:
- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values for this methodology should be run as unknown samples:
- At least once per day as established by the laboratory.
- When a new batch of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the established limits indicate the assay may be out of control. The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control contact Technical Services or your local distributor

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin (free and conjugated) and lipaemia were carried out. The following results were obtained:
   - Haemoglobin: No interference from haemoglobin up to 470 mg/dL.
   - Free Bilirubin: No interference from free bilirubin up to 281 µmol/L (16.4 mg/dL).
   - Conjugated Bilirubin: No interference from conjugated bilirubin up to 298 µmol/L (17.4 mg/dL).
   - Lipaemia: No interference from lipaemia, measured as triglycerides, up to 23 mmol/L (2000 mg/dL).
2. Young DS1 has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES
- Fasting serum: 4.11 - 5.56 mmol/L (74 - 100 mg/dL)
- Urine: 0.06 - 0.83 mmol/L (1 - 15 mg/dL)

For the diagnosis of diabetes, Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT) the W.H.O. recommend the following criteria:

**Diabetes**
- Fasting plasma glucose ≥7.0 mmol/L (≥126 mg/dL)
- 2 hours after glucose load ≥11.1 mmol/L (≥200 mg/dL)

**IFG**
- Fasting plasma glucose 6.1 - 6.9 mmol/L (110 - 125 mg/dL)
- ≥ 7.0 mmol/L (≥126 mg/dL)
- 2 hours after glucose load 7.8 - 11.0 mmol/L (140 - 199 mg/dL)

**PERFORMANCE DATA**
The following data was obtained with the Infinity Glucose Hexokinase Liquid Stable Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on the specific analyzer used.

**IMPRECISION**
Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EPS-T procedure.

**Within run:**
- LEVEL I
  - Number of data points: 80
  - Mean (mmol/L / mg/dL): 5.09 / 91.6
  - SD (mmol/L / mg/dL): 0.08 / 1.44
  - C.V. (%): 1.6
  - Total: 80
- LEVEL II
  - Number of data points: 80
  - Mean (mmol/L / mg/dL): 5.09 / 91.6
  - SD (mmol/L / mg/dL): 0.36 / 6.8
  - C.V. (%): 3.6

**Total:**
- LEVEL I
  - Number of data points: 80
  - Mean (mmol/L / mg/dL): 19.27 / 346.9
  - C.V. (%): 1.4
- LEVEL II
  - Number of data points: 80
  - Mean (mmol/L / mg/dL): 19.27 / 346.9
  - C.V. (%): 4.4

**METHOD COMPARISON**
Comparison studies were done using another commercially available glucose hexokinase reagent as a reference. Normal and abnormal patient serum and urine samples were assayed in parallel. The results were compared by least squares regression and the following statistics were obtained.

**Serum/plasma:**
- Number of sample pairs: 60
- Range of sample results: 2.3 - 26.7 mmol/L (41.4 - 480.6 mg/dL)
- Mean of reference method results: 6.25 mmol/L (112.5 mg/dL)
- Mean of Infinity Glucose HK results: 6.27 mmol/L (112.9 mg/dL)
- Slope: 1.021
- Intercept: -0.13 mmol/L (-2.34 mg/dL)
- Correlation coefficient: 0.9993

**Urine:**
- Number of sample pairs: 60
- Range of sample results: 0.0 - 44.0 mmol/L (0.0 - 792.0 mg/dL)
- Mean of reference method results: 9.8 mmol/L (176 mg/dL)
- Mean of Infinity Glucose HK results: 10.4 mmol/L (187 mg/dL)
- Slope: 1.086
- Intercept: -0.29 mmol/L (-5.22 mg/dL)
- Correlation coefficient: 0.9962

**LINEARITY**
When run as recommended the assay is linear between 0 and 45 mmol/L (0 - 810 mg/dL).

**ANALYTICAL SENSITIVITY**
When run as recommended the sensitivity of the assay is 0.038 µAbs per mmol/L / 0.002 µAbs per mg/dL (1 cm light path, 4cm nm).

**REFERENCES**

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