Glucose-6-phosphate dehydrogenase (G-6-P-DH) reactions was described in PRINCIPLE OF PROCEDURE

1. Intended for in vitro diagnostic use only.

SUMMARY AND EXPLANATION OF TEST

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue.

The most frequent cause of hyperglycemia is diabetes mellitus. A number of secondary factors also can contribute to elevated blood glucose levels. These include pancreatitis, pituitary or thyroid dysfunction, renal failure, and liver disease. Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels, such as insulinoma, hypoglycemia, neoplasms, or insulin-induced hypoglycemia.1

PRINCIPLE OF PROCEDURE

The method for determination of glucose employing the Hexokinase (HK) Glucose-6-phosphate dehydrogenase (G-6-P-DH) reactions was described in 1962,2 with modifications in 1972.3

The method is highly specific for glucose; the reagent can be used for the determination of glucose in serum and plasma, without prior treatment of these specimens. Common anticoagulants, including fluoride, at the recommended levels, do not interfere.

The assay is based on the following reactions:

\[ HK \]
\[ Glucose + ATP \rightarrow G-6-P + ADP \]
\[ G-6-P + NAD \rightarrow 6-PG + NADH \]

Glucose is phosphorylated by adenosine-5'-triphosphate (ATP), in a reaction catalyzed by HK. The glucose-6-phosphate (G-6-P) formed is oxidized to 6-phosphogluconate (6-PG) by G-6-P-DH. In this same reaction an equimolar amount of Nicotinamide Adenine Dinucleotide (NAD) is reduced to NADH, with a resulting increase in absorbance at 340 nm. The concentration of glucose is proportional to this increase in absorbance.

REAGENTS

Reagent 1 and Reagent 2 are aqueous solutions having the following approximate concentration:

Reactive ingredients:

Reagent 1
- Nicotinamide adenine dinucleotide 5 mmol/L
- Adenosine-5'-triphosphate 24 mmol/L

Reagent 2
- Hexokinase 16,000 U/L
- Glucose-6-phosphate dehydrogenase 20,000 U/L

Non-reactive ingredients:
- Buffers and stabilizers

WARNINGS AND PRECAUTIONS

1. Intended for in vitro diagnostic use only.
2. Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See Clinical Laboratory Safety; Approved Guideline, NCCLS Publication GP17-A.)
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.

INSTRUCTIONS FOR REAGENT HANDLING

The reagents are ready to use and need no further preparation.

STORAGE AND STABILITY

These glucose reagents should be stored in the refrigerator (2 - 8°C). Keep reagent bottles tightly closed when not in use. The reagents are stable until the expiration date on the label. On-board stability of the Glucose UV Reagent is dependent upon instrument application and laboratory conditions. On-board stability is normally defined as leaving the open reagent container on the instrument for 8 hours and tightly capped/sealed for 16 hours at 2 - 8°C. When the reagent is used in an uncooled reagent rack position, typical on-board stability is 9 days. When the reagent is used in a cooled rack, typical on-board stability is 11 days. Reagent 1 should be colorless to light yellow, and Reagent 2 should be colorless. Turbidity may be a sign of contamination; any turbid reagent should be discarded.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Glucose is metabolized by erythrocytes at a rate varying, according to temperature and other conditions. This consumption can be as high as 10 mg/dL per hour.

It is, therefore, very important that serum and plasma be separated from the formed elements of blood as soon as possible after collection.

Either serum or plasma may be used. The acceptable anticoagulants for plasma are EDTA, Sodium Fluoride-Oxalate or heparin. The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without a preservative should be separated from the cells or clot within a half hour of being drawn.

Glucose in separated non-hemolized serum is generally stable for up to 8 hours at 25°C and up to 72 hours at 4°C. Glucose in separated non-hemolized serum is generally stable for up to 24 hours at 25°C.

INTERFERING SUBSTANCES

The hexokinase method has been found to be free of interference from a large number of contaminants. Interference of <10% has been demonstrated from 80 mg/dL bilirubin (conjugated or unconjugated), 1000 mg/dL hemoglobin, or 700 mg/dL triglycerides (as Intralipid).

Young has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including glucose.

PROCEDURE

MATERIALS PROVIDED

One 125 mL bottle and one 40 mL bottle (Order No. R84682).

ADDITIONAL MATERIALS REQUIRED

1. Automated Analyzer, suitable for two reagents, that can measure absorbance at 340 nm.
2. Commercially available calibrator or standard with established values for this method are listed below in the calibration section.

INSTRUCTIONS FOR USE

The reagents are ready to use as supplied. Pour the necessary amount of Reagent 1 into the primary reagent position. Add sufficient Reagent 2 to the Start Reagent position. Place the calibrator in the appropriate position on the instrument if calibration is required. See suggested parameters listings at the end of this package insert. See the appropriate operator’s manual for further details on system programming and operation.

CALIBRATION

Use a commercially available calibrator or standard with established values for this Cliniqa method, e.g. Multi-Analyte Serum Calibrator R65010. Calibration is required every 2 months. Recalibration is required during this time period with a reagent lot number change or if there is a shift in control values.

QUALITY CONTROL

It is recommended that two levels of controls be run with the assay at least once every 8 hours and with each reagent lot change. Commercially available control material with established glucose values may be used for quality control. The assigned value of the control material must be confirmed by this methodology. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or
procedural errors. Use Assayed Control Serum Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R93083)

PROCEDURAL LIMITATIONS
The assay is linear from 0.42 to 800 mg/dL when a sample to reagent ratio is 1 to 44. Samples with glucose concentrations higher than 800 mg/dL should be diluted with distilled or deionized water or 0.9% NaCl and assayed again. Correct the results for the dilution factor.

EXPECTED VALUES
The conventional fasting reference range for glucose is 74 – 106 mg/dL.6
It is recommended that each laboratory establish its own reference range.

PERFORMANCE CHARACTERISTICS7
ACCURACY
The following table lists the data obtained in a comparison of the Cliniqa Glucose UV reagent (y) with a similar glucose hexokinase reagent (x) run on an automated analyzer. Values ranged from 9 to 718 mg/dL.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>131</th>
<th>151</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>y-intercept</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>

PRECISION
Studies were performed on an automated analyzer according to the guidelines of NCCLS Publication EPS-T.5

<table>
<thead>
<tr>
<th>Level 1</th>
<th></th>
<th>Level 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dL)</td>
<td>87</td>
<td>303</td>
<td></td>
</tr>
<tr>
<td>CV% within-run</td>
<td>1.6</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>CV% total</td>
<td>2.3</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

SENSITIVITY
The sensitivity of this assay, defined by the change in absorbance per cm pathlength per mg/dL glucose in the sample, is 5 mA/cm per mg/dL. Analytical sensitivity is 0.42 mg/dL for a standard automated application.

REFERENCES
7. Data on file at Cliniqa Inc.

Sample Suggested Application Procedure for Automated Analyzers
It is recommended that each laboratory verify assay performance, including potential for sample carryover. Suggested and Validated Application Procedures are available for specific Automated Analyzers. Please contact Technical Service for additional information.

Refer to the Instrument Manufacturer’s Manual regarding the following:

- Installation procedures and requirements
- Principles of Operation
- Performance characteristics and specifications
- Operation instructions
- Calibration procedures including materials/or equipment to be used
- Operational precautions, limitations and hazards
- Service and maintenance

ASSAY PROCEDURE
An example of standard automated application:

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>340 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>3.0 µL (+64 µL H2O)</td>
</tr>
<tr>
<td>R-1 Volume</td>
<td>100 µL</td>
</tr>
<tr>
<td>R-2 Volume</td>
<td>32 µL (+3 µL H2O)</td>
</tr>
</tbody>
</table>

37°C, 0.5 minutes (read blank) 37°C, read at 5.0 minutes

Enter the respective concentrations of the calibrators

For in vitro diagnostic use

See package insert for proper use

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RE-ORDER INFORMATION
Glucose UV Liquid Reagent

Catalog No.
R84682

Made in the USA