

# Liquid Glucose (Hexokinase) Reagent Set

#### Intended Use

For the in vitro quantitative measurement of glucose in serum.

## **Test Summary**

The measurement of glucose concentrations in biological fluids has been well documented. Glucose testing can be diagnostically significant in diabetes, hypoglycemia, and various adrenal and pituitary disorders.

Enzymatic methods for the measurement of glucose were first described by Keilin and Hartree.<sup>(1)</sup> The U.S. Food and Drug Administration has proposed as the reference method for glucose a totally enzymatic procedure using hexokinase and glucose-6-phosphate dehydrogenase.<sup>(2)</sup> Passey, et.al.<sup>(3)</sup> have critically reviewed ten glucose methods and have used the hexokinase procedure as the reference method.

## Principle

Glucose + ATP 
$$\longrightarrow$$
 G<sub>6</sub>P + ADP  
Mg<sup>2+</sup>  
G<sub>6</sub>PDH

G<sub>6</sub>P + NAD<sup>+</sup> ----- ≻ 6-Phosphogluconate + NADH + H<sup>+</sup>

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) producing 6-phosphogluconate and NADH.

The formation of NADH causes increase in absorbance at 340 nm which is directly proportional the concentration of glucose in the sample.

## Reagents

Glucose Reagent: A buffered solution containing 2 mmol/L nicotinamide adenine dinucleotide, 4 mmol/L adenosine triphosphate, 2 mmol/L magnesium, > 2000 U/L hexokinase (yeast), > 4000 U/L glucose-6-phosphate dehydrogenase (microbial), stabilizers, and preservatives.

## Warnings and Precautions for Use

S24/25: Avoid contact with skin and eyes. See Material Safety Data Sheet for additional information.

## **Reagent Preparation, Storage and Stability**

Reagents are ready for use.

Supplied reagent is stable at 2-8°C until expiry date. Stability claims are based on real time studies

## **Reagent Deterioration**

The reagent solution should be clear. Turbidity would indicate deterioration.

## Disposal

Reagents must be disposed of in accordance with all Federal, State, and local regulations.

## **Specimen Collection and Storage**

- 1. Fresh, clear, unhemolyzed serum. Serum should be separated from cells as soon as possible to minimize glucose decomposition by glycolysis.
- In properly handled samples, glucose concentrations are stable for up to 3 days at 4°C.<sup>(4)</sup>

## Analytical Specificity (CLSI EP7)<sup>(5)</sup>

Cross contamination studies have not been performed on automated instruments. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Interferences from icterus, lipemia, and hemolysis were evaluated for this method on a Roche/Hitachi 704 $^{\otimes}$  analyzer.

| Concentration of Analyte |            | Substance  | Concentration of interferent where |   |  |
|--------------------------|------------|------------|------------------------------------|---|--|
| Conventional<br>Units    | SI Units   | Tested     | interference is insignificant      |   |  |
| 96 mg/dL                 | 5.3 mmol/L | Hemoglobin | 1000 mg/dL                         | 155 µmol/L  |  |
| 99 mg/dL                 | 5.5 mmol/L | Bilirubin  | 20 mg/dL                           | 342 µmol/L  |  |
| 91 mg/dL                 | 5.0 mmol/L | Intralipid | 100 mg/dL                          | 300 mg/dL<br>(3.4 mmol/L)<br>Simulated<br>triglycerides |  |

When assaying turbid or lipemic samples, it is recommended that a serum blank correction be performed. The blank can be prepared using 25  $\mu$ L of sample and 2.5mL of deionized water. The absorbance of this solution is determined at 340 nm and subtracted from the absorbance of that sample with reagent.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S.  $^{(6)}$ 

The information presented above is based on results from the manufacturer's studies and is current at the date of publication.

## **Materials Provided**

Glucose (Hexokinase) Reagent.

## Materials Required but not Provided

- 1. Mindray BS-200 Analyzer
- 2. BS-200 Operation manual
- 3. Chemistry Calibrator, catalog number C7506-50
- 4. Chemistry Control, catalog number C7592-100

## **Test Conditions**

For the data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:100 and a wavelength reading of 340 nm.

## Mindray BS-200 Test Parameters

| Test:         | GLU      | R1:                              | 300          |  |  |
|---------------|----------|----------------------------------|--------------|--|--|
| No.:          | 018      | R2:                              | 0            |  |  |
| Full Name:    | Glucose  | Sample Volume:                   | 3            |  |  |
| Standard No.: |          | R1 Blank:                        |              |  |  |
| Reac.Type:    | Endpoint | Mixed Rgt. Blank:                |              |  |  |
| Pri. Wave:    | 340nm    | Linearity Range:                 | 0 - 600      |  |  |
| Sec. Wave:    | 405nm    | Linearity Limit:                 |              |  |  |
| Direction:    | Increase | Substrate Limit:                 |              |  |  |
| Reac. Time:   | 0 / 12   | Factor:<br>Compensate: Slope 1.0 | Intercept: 0 |  |  |
| Incuba.Time:  |          | Prozone check                    |              |  |  |
| Unit:         | mg/dl    | q1: q2: q3: q4:                  |              |  |  |
| Precision:    | Integer  | PC: Abs:                         |              |  |  |

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## **Calibration Parameters**

| Rule: Two-p  | oint linear | Calibrator 1: | Deionized Water |  |
|--|-------------|---------------|-----------------|--|
| Sensitivity:   |             | Calibrator 2: | Chem Cal        |  |
| Replicates:  | 2           | Calibrator 3: |                 |  |
| Rule: Two-p<br>Sensitivity:<br>Replicates:<br>Interval (day):<br>Difference Limit: |             | Calibrator 4: |                 |  |
| Difference Limit:  |             | Calibrator 5: |                 |  |
| SD:  |             | Calibrator 6: |                 |  |
| Blank Response:  |             |               |                 |  |
| Error Limit:   |             |               |                 |  |
| Correlation Coefficie  | nt: 0       |               |                 |  |

#### Limitations

A sample with a glucose concentration exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

## Calibration

Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used.

## **Quality Control**

A normal and abnormal concentration control should be analyzed as required in accordance with local, state and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

## Calculations

The analyzer automatically calculates the glucose concentration of each sample.

## **Reference Intervals**<sup>(4)</sup>

70-105 mg/dL (3.9-5.8 mmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

## **Performance Characteristics**

Data presented was collected on a Roche/Hitachi $^{\textcircled{\text{B}}}$  704 analyzer unless otherwise stated.

#### RESULTS

Glucose concentration is reported as mg/dL (mmol/L).

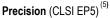
#### Reportable Range (CLSI EP6) (5)

The linearity of the procedure described is 600 mg/dL (33.3 mmol/L). The lower limit of detection of the procedure described is 0.6 mg/dL (0.03 mmol/L). This data results in a reportable range of 0.6 to 600 mg/dL (0.03 to 33.3 mmol/L).

## Accuracy (CLSI EP9)<sup>(5)</sup>

The performance of this method (y) was compared with the performance of asimilar glucose method (x) on a Hitachi<sup>®</sup> 704. Fifty patient serum samples ranging from 38 to 295 mg/dL (2.1 to 16.4 mmol/L) were tested and gave a correlation coefficient of 0.9992. Linear regression analysis gave the following equation:

This method = 0.9849 (reference method) + 2.3 mg/dL (0.13 mmol/L).

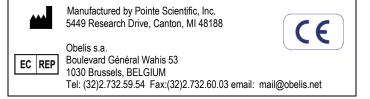


Data was collected on two concentrations of a control sera using a single lot of reagent in forty runs conducted over twenty days.

| Concentration |        | Total SD |        | Total | Within Run SD |        | Within     |
|---------------|--------|----------|--------|-------|---------------|--------|------------|
| mg/dL         | mmol/L | mg/dL    | mmol/L | CV%   | mg/dL         | mmol/L | run<br>CV% |
| 89            | 4.9    | 1.1      | 0.06   | 1.3   | 0.4           | 0.02   | 0.4        |
| 257           | 14.3   | 3.1      | 0.17   | 1.2   | 1.1           | 0.06   | 0.4        |

## References

- 1. Keilin, D. Hartree, E.F., Biochem. J. 42, 250 (1948).
- United States Department of Health, Administration. In Vitro Diagnostic Products for Human Use, Proposed Establishment of Glucose, Fed. Regist. 39, No. 126, 24136-24147 (1974).
- Passey, R.B., Gillum, R.L., Fuller, J.B., Urry, F.M., Giles, M.L., Evaluation and Comparison of Ten Glucose Methods and the Reference Method Recommended inthe Proposed Product Class Standard (1974), Clin. Chem. 23, 131-139 (1977).
- Burtis, C.A., Ashwood, E.R., Editors, *Tietz Textbook of Clinical Chemistry, Second Edition*, W.B. Saunders Company, Philadelphia, PA (1994).
- CLSI Method Evaluation Protocols, Clinical and Laboratory Standards Institute, Wayne, PA.
- Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington (1990).





Temperature limitation

LOT Lot and batch code

REF Catalog number

CE mark

Manufacturer

IVD In vitro diagnostic medical device

**EC REP** Authorized representative in the European Community

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