**GLUCOSE (Oxidase) REAGENT SET**

For the COLORIMETRIC determination of glucose in serum and plasma

**SUMMARY AND EXPLANATION**

Enzymatic methods, for the determination of glucose were first described by Kelin and Hartree in 1948. The use of the combination of glucose oxidase and peroxidase in the reagent, for glucose determination was introduced by Keston and modified, among others by Teller and Trinder. The disease most commonly associated with the above normal level of blood glucose Diabetes mellitus (Hyperglycemia). Other conditions which result in elevated blood glucose levels are: Disorders of the thyroid or Pituitary gland, Cushing disease, Traumatic injuries and mental stress. Low blood glucose levels (Hypoglycemia) may indicate hyperinsulin which can result from a pancreatic lesion or from an insulin overdose. Chronic depression of blood glucose levels can be caused by hormonal deficiencies, involving the Adrenal, Pituitary or Thyroid glands. The present enzymatic method is based on a modification of the method of Trinder.

**PRINCIPLE**

D-Glucose in the sample is oxidized by the enzyme glucose oxidase (GOD), to gluconic acid and hydrogen peroxide in the presence of peroxidase (POD) reacts with p-Hydroxybenzene sulfonate (p-HBS) and 4-aminoantipyrine (4-AAP) to form a quinoneminine complex.

Glucose + H2O + O2 GOD → Gluconic + H2O Acid

H2O2 + 4-AAP + p-HBS POD → Quinoneminine + H2O Dye

The intensity of the color formed is proportional to the glucose concentration sample.

**REAGENT**

1. Glucose Enzyme Reagent: contain, after reconstitution, Glucose Oxidase 15 U/mL, Peroxidase 2 U/mL; 4-Aminoantipyrine 0.4 mmol/L; p-Hydroxybenzene sulfonate 10 mmol/L; Buffers and stabilizers

2. Glucose Standard: 100 g/dL. Contains Glucose in Benzoic Acid Solution

**REAGENT PREPARATION:**

Reconstitute the reagent by adding the amount of distilled water indicated on the vial. Swirl gently to dissolve the content.

**PRECAUTION:**

For In Vitro Diagnostic Use. Do not pipette by mouth. Reagent might be harmful if swallowed. The enzyme reagent should not be used if there is indication that moisture has entered the vial. If upon reconstitution the enzyme reagent shows a strong pink color, the reagent may have become contaminated and should not be used.

**STORAGE AND STABILITY**

Protect from light. Store at 2°-8°C. All reagent are stable until the date appearing on the label. N.B. Reconstituted reagent is stable for at last 60 days when stored at 2°-8°C.

**SPECIMEN COLLECTION AND PREPARATION**

Serum or Plasma EDTA, Citrate, Heparin or Oxalate separated from the red cells within 20-30 minutes is suitable. Longer contact with red cells can result in a reduction of glucose levels due to glycolysis. At room temperature, glucose in whole blood samples decreases due to glycolysis at the rate of about 5% per hour. Fluoride (10 mg/dL of blood) may be used as an anticoagulant agent.

Glucose in serum or plasma is stable for 8 hours at room temperature or 24 hours when refrigerated. Samples may be frozen for up to two weeks.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Chemistry Analyzer or spectrophotometer with appropriate well, suitable for measurement at 490-520 nm.
2. Constant heat source at 37°C.
3. Timing Device.
4. Accurate dispensing device for measuring reagent, sample, standard and control such as different calibrated pipettes.
5. Distilled water for reagent reconstitution.

**PROCEDURE (AUTOMATED)**

Refer to specific instrument application for instruction.

**PROCEDURE (MANUAL)**

<table>
<thead>
<tr>
<th>Tube</th>
<th>Blank</th>
<th>Standard</th>
<th>Control</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (mL)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Distilled Water (mL)</td>
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<tr>
<td>Standard (mL)</td>
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<td>0.01</td>
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<td>---</td>
</tr>
<tr>
<td>Control (mL)</td>
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<td>---</td>
<td>0.01</td>
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<tr>
<td>Unknown (mL)</td>
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<td>---</td>
<td>---</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mix all tube. and incubate at 37°C for 10 minutes

Zero the instrument, set at 500 nm with the reagent blank. Read absorbance of the Standard, Control and unknown(s).

**NOTE:**

1. For instruments requiring a volume greater than 1.0 mL for accurate reading, use 3 mL of reagent and 0.020 mL of sample. Perform the test as directed above.
2. For direct read-out instruments set value of standard concentration and read the sample concentrations directly.

**STABILITY OF FINAL REACTION**

Final color is stable for 30 minutes at room temperature.

**Calculations**

The following equation is used to determine unknown concentran:

\[ \text{Unknown Abs} = \frac{x}{\text{Standard Abs}} \times \text{Glucose mg/dL} \]

**Example**

<table>
<thead>
<tr>
<th>Unknown Abs</th>
<th>Standard Abs</th>
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<tbody>
<tr>
<td>0.285</td>
<td>0.285 X 100 = 79 mg/dL</td>
<td>0.360</td>
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</tbody>
</table>

**EXPECTED VALUES**

Adult fasting levels: 70-110 mg/dL

It is recommended that each laboratory establish its own normal range values.

**QUALITY CONTROL**

The regular use of control serum is recommended as part of a quality control program.

**LIMITATION OF THE METHOD**

The method is linear to at least 500 mg/dL. Glucose Samples containing glucose levels above 500 mg/dL should be diluted with distilled water and reassayed. Multiply the result by the dilution factor.

Highly icteric or lipemic samples may require a blank correction, using the same volume of sample with isotonic saline in the place of the reagent. Szaze et. al. examine the effects of 43 drugs on glucose assay by the Trinder reaction and determined that none of them had a noticeable effect on the reaction, specifically when the levels of drug were obtained from in vivo specimens. Young et. al. publish a comprehensive list of the effects of drugs on clinical laboratory tests, including glucose assays.

**PERFORMANCE CHARACTERISTICS**

Linear regression analysis of 50 serum samples with glucose levels ranging from 61-393 mg/dL was performed comparing the present method to an enzymatic colorimetric glucose method commercially available with the following results.

\[ y \text{ (ours) } = 0.986 x + 2.6 \]

Within-run precision was found to be ±1.7% for a mean value of 168 mg/dL and as determined by analyzing by duplicate determinations 30 sera with glucose levels between 45 and 380 mg/dL.

**REFERENCES**


**WARRANTY**

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**Protocol Parameters for**

<table>
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<th>ATAC 6000</th>
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<tr>
<td>Filter 2</td>
<td>630</td>
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</table>
FLOWCELL TEMP.: 37 : 37
MAXIMUM NORM.: 110 : 200
MINIMUM NORM.: 70 : 120
UNIT: mg/dl : mg/dl
STANDARD: 98* : 165*
FACTOR: 1 : 0
NO. OF WASHES: 2.. : 2..
NO of Bead Washes: -- : 0
KIN. READ TIME: 0 : 0
GRAPH PRINTOUT: N : N
TEST LIMIT: 500 : 500
MAX. ABS. DELTA: 0 : 0

Note: See package insert for reagent preparation, storage and stability, and performance claims. Check the Calibrator insert for proper standard value.

Protocol Parameters for AKUSTAT 1020/1021

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<tr>
<td>High Value:</td>
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</table>

* Check Package Insert for proper standard value.
** As determined by particular laboratory.

Check with us for Protocol Parameters for other instruments.