Reagent kit for the quantitative determination of glucose concentration in serum and liquor. Enzymatic colorimetric method (GOD/POD/PAP).

Determination of glucose concentration is important in the diagnosis and treatment of disorders of carbohydrate metabolism. Values higher or lower than the reference are of diagnostic significance. The levels are increased in diabetes mellitus, hyperthyroidism and in the hyperactivity of the pituitary gland. Decreased levels are observed in cases of overproduction of insulin by the pancreas, with tumors of the pancreas, as well as with hypofunction of the organs involved in glucose synthesis and carbohydrate metabolism.

Principle
Glucose oxidase (GOD) converts the sample glucose into gluconate. The Hydrogen peroxide (H₂O₂) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable using Trinder indicator reaction at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample.

Glucose+O₂ $\rightarrow$ GOD $\rightarrow$ Gluconic acid+H₂O₂

$2\text{H}_2\text{O}_2+\text{Phenol}+4\text{Aminoantipyrine}$ $\rightarrow$ POD $\rightarrow$ Red quinone+$4\text{H}_2\text{O}$

Reference values
Serum: 3.89-5.84 mmol/l (70-105 mg/dl)
Cerebrospinal fluid: 2.78-3.89 mmol/l (50-70 mg/dl)

It is recommended that each laboratory should assign its own normal range.

Reagents
1. Reagent (R1)
   - Phosphatase buffer, pH 7.40 100 mmol/l
   - Phenol 10 mmol/l
   - 4-Aminoantipyrine 0.3 mmol/l
   - Glucose oxidase 10000 U/l
   - Peroxidase 700 U/l

2. Glucose standard

Precautions
Discard cloudy reagent. Avoid contamination by using clean laboratory material (pipettes, plastic vials for analyzers, ...). The reagent contains sodium azide (0.1 %). To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of unlabelled reagent.

Sample
Serum free of haemolysis.
Cerebrospinal fluid.

PROCEDURE

Preparation and stability of working reagent
The reagent is ready for use. If the absorbance of working reagent is higher than 0.1 at 492 nm the reagent cannot be used.

Assay conditions
- Wavelength: 505 (492-520) nm
- Temperature: 37 °C
- Cuvette: 1 cm light path
- Method: endpoint (increasing)
- Read against: reagent blank

Mix and measure the absorbance (A) after a five-minute incubation.

<table>
<thead>
<tr>
<th>Pipette into cuvette</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>10µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>10µl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix and measure the absorbance (A) after a five-minute incubation.

Calibration

1. Distilled water
2. Glucose standard Cat. No.: 50411 or Roche C.F.A.S. (Calibrator for automated system)

Random Calibration Serum Level I or Random Calibration Serum Level II

Calibration frequency
Two-point calibration is recommended:
- after reagent lot change;
- as required following quality control procedures.

Calculation

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}
\]

A = Absorbance
C = Concentration

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA
The following data were obtained using the Olympus 400 analyzer (37°C).

Linearity
The test is linear up to 40 mmol/l (720 mg/dl) glucose concentration.

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.019 mmol/l (0,34mg/dl) Glucose concentration at 492 nm.

Precision

<table>
<thead>
<tr>
<th>Reproducibility</th>
<th>Sample</th>
<th>Average concentration (mmol/l)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample I</td>
<td>5.54</td>
<td>0.08</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>sample II</td>
<td>13.9</td>
<td>0.16</td>
<td>1.17</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Repeatability</th>
<th>Sample</th>
<th>Average concentration (mmol/l)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample I</td>
<td>4.5</td>
<td>0.04</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>sample II</td>
<td>15.4</td>
<td>0.12</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

Correlation
Comparative studies were done to compare our reagent with another commercial Glucose PAP reagent. The results from these studies are detailed below.

Correlation coefficient:
\[ r = 0.9999 \]
Linear regression:
\[ y = (0.980x+0.099) \times 10^3 \]

Specificity
Bilirubin 855 µmol/l (50 mg/dl), lipid 1000 mg/dl and ascorbic acid 0.14 mmol/l (25 mg/dl) don’t interfere with the assay up to the given levels.

Note
With this assay the determination of glucose concentration in urine is not acceptable, because ascorbic acid influences the measurement. The reference method of glucose determination is the hexokinase and the glucose-6-phosphate-dehydrogenase (HK/G-6-PDH) UV test (It is also suitable for the determination of glucose concentration in urine). Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels: