**Intended Use**

Enzymatic UV test (hexokinase method) for the quantitative determination of glucose in human serum, plasma, urine, haemolysate and cerebrospinal fluid on Beckman Coulter analysers. For *in vitro* diagnostic use only.

Glucose reagent OSR6521 for use on the AU2700 and AU5400 systems only.

**Summary**

In the fasting state, blood sugar levels are regulated by the liver, which ensures that levels are maintained within precise limits. The rapid and precise manner in which fasting blood sugar levels are regulated is in marked contrast to the rapid increase in blood sugar, which occurs during ingestion of carbohydrates. A fall in blood glucose to a critical level (approximately 2.5 mM) leads to dysfunction of the central nervous system. This manifests as hypoglycaemia, and is characterised by muscle weakness, lack of coordination and mental confusion. Further decrease in blood glucose levels leads to hypoglycaemic coma. Blood glucose concentrations show intra-individual fluctuations, which are dependent on muscular activity and the time interval since food intake. These fluctuations are increased further where there is dysregulation, such as occurs in a number of pathological conditions in which blood glucose may be elevated (hyperglycaemia) or depressed (hypoglycaemia). Hyperglycaemia most commonly occurs as a result of a deficiency in either the amount or efficiency of insulin, a condition known as diabetes mellitus. This disease is characterised by the elevation of blood glucose to such an extent that the renal threshold is exceeded and sugar appears in the urine (glycosuria). Blood glucose measurement is used as a screening test for diabetes mellitus, where there is suspected hyperglycaemia, monitoring of therapy in diabetes mellitus, evaluation of carbohydrate metabolism, for example in gestational diabetes acute hepatitis, acute pancreatitis and Addison’s disease. Hypoglycaemia is associated with a range of pathological conditions including neonatal respiratory distress syndrome, toxoaemia of pregnancy, congenital enzyme defects, Reye’s syndrome, alcohol ingestion, hepatic dysfunction, insulin-producing pancreatic tumours (insulinomas), insulin antibodies, nonpancreatic neoplasms, sepsicaemia and chronic renal failure. CSF glucose may be low or undetectable in patients with acute bacterial, cryptococcal, tubular or carcinomatous meningitis, or in cerebral abscess, probably due to consumption of glucose by leucocytes or other rapidly metabolising cells. In meningitis or encephalitis due to viral infections, it is usually normal.

**Test Principle**

Glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidises glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD$^+$ to NADH. The increase in absorbance at 340nm is proportional to the glucose concentration in the sample.

**Reaction principle**

\[
\begin{align*}
\text{Glucose} + \text{ATP} & \xrightarrow{\text{HK, Mg}^{2+}} \text{Glucose-6-phosphate} + \text{ADP} \\
\text{Glucose-6-Phosphate} + \text{NAD}^+ & \xrightarrow{\text{G6P-DH}} \text{Gluconate-6-P} + \text{NADH} + \text{H}^+
\end{align*}
\]

**Contents, Reagent Composition in the Test**

Final concentration of reactive ingredients:

- PIPES buffer (pH 7.6) 24.0 mmol/L
- ATP $\geq$ 2.0 mmol/L
- NAD$^+$ $\geq$ 1.32 mmol/L
- Mg$^{2+}$ 2.37 mmol/L
- Hexokinase $\geq$ 0.59 kU/L
- G6P-DH $\geq$ 1.58 kU/L
- Preservative

**Precautions and Warnings**

Exercise the normal precautions required for handling all laboratory reagents.

To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Dispose of all waste material in accordance with local guidelines.

**Reagent Preparation**

The reagents are ready for use and can be placed directly on board the instrument.

**Storage and Stability**

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 30 days.

**Specimen**

Serum, EDTA or heparinised plasma. To minimise loss of glucose through glycolysis serum should be removed from red cells as soon as possible. Specimens that cannot be rapidly separated should be collected into tubes containing fluoride, moniodoacetate or mannose. Glucose in stabilised haemolysate and plasma is stable for up to 7 days when stored at 2…8°C and 2 days when stored at 15…25°C. Icteric and strongly lipemic samples should be avoided.

Urine: Fresh, random collections are recommended for urine specimens. Stable in urine for 2 hours when stored at 2…25°C. Analyse as soon as possible.

Cerebrospinal fluid: Process immediately to avoid falsely low results.
Test Procedure
Refer to the appropriate User Guide and Setting Sheet for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

Calibration
Serum/plasma/haemolysate/CSF: Use System Calibrator Cat. No. 66300.
Urine: Use Urine Calibrator Cat. No. ODC0025.
The glucose values of both calibrators are traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 965.
Recalibrate the assay every 30 days, or when the following occur:
Changes in reagent lot or significant shift in control values;
Major preventative maintenance was performed on the analyser or a critical part was replaced.

Quality Control
Serum/plasma/haemolysate: controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.
Urine: Biorad Liquichek Urine Chemistry Controls Cat. No. 397 and 398 or other control materials with values determined by this Beckman Coulter system may be used.
CSF: Control materials with values determined by this Beckman Coulter system may be used.
Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed.
The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.
If any trends or sudden shifts in values are detected, review all operating parameters.
Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

Calculation
The Beckman Coulter analysers automatically compute the glucose concentration of each sample.

Reference Intervals

Serum/Plasma (fasting) Adults 4.1 – 5.9 mmol/L (74 – 106 mg/dL)
Children 3.3 – 5.6 mmol/L (60 – 100 mg/dL)
Haemolysate Adults 3.3 – 5.5 mmol/L (60 – 100 mg/dL)
Urine 0.1 – 0.8 mmol/L (1 – 15 mg/dL)
CSF Adult 2.2 – 3.9 mmol/L (40 – 70 mg/dL)

The generally accepted cut-off levels for the diagnosis of diabetes are:

(a) random plasma glucose of ≥ 11.1 mmol/L
(b) fasting plasma glucose (FPG) ≥ 7.0 mmol/L or
(c) 2-h postload glucose ≥ 11.1 mmol/L during an oral glucose tolerance test (OGTT).
If any one of these criteria is met, results must be confirmed by repeat testing on a subsequent day, unless there is unequivocal hyperglycaemia with acute metabolic decompensation.
Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Specific performance characteristics
Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

Linearity
The test is linear within a concentration range of 0.6 – 45.0 mmol/L (10 – 800 mg/dL) for serum, plasma, haemolysate and CSF. The test is linear within a concentration range of 0 – 45 mmol/L (1 – 800 mg/dL) for urine.

Precision
The following data was obtained on an AU600 using 3 serum pools analysed over 10 days.

<table>
<thead>
<tr>
<th>n = 60</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean mmol/L</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>3.27</td>
<td>0.02</td>
<td>0.70</td>
</tr>
<tr>
<td>6.27</td>
<td>0.03</td>
<td>0.54</td>
</tr>
<tr>
<td>16.36</td>
<td>0.08</td>
<td>0.51</td>
</tr>
</tbody>
</table>

The following data was obtained on an AU640 using 3 urine pools analysed over 20 days.

<table>
<thead>
<tr>
<th>n = 80</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean mmol/L</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>0.46</td>
<td>0.01</td>
<td>1.39</td>
</tr>
<tr>
<td>11.40</td>
<td>0.09</td>
<td>0.82</td>
</tr>
<tr>
<td>42.45</td>
<td>0.13</td>
<td>0.31</td>
</tr>
</tbody>
</table>

The following data was obtained on an AU2700 using 3 haemolysate analysed over 20 days.

<table>
<thead>
<tr>
<th>n = 80</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean mmol/L</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>2.25</td>
<td>0.05</td>
<td>2.30</td>
</tr>
<tr>
<td>5.94</td>
<td>0.09</td>
<td>1.54</td>
</tr>
<tr>
<td>18.6</td>
<td>0.12</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Sensitivity
The lowest detectable level, using serum settings, on an AU600 analyser was estimated at 0.04 mmol/L.
The lowest detectable level, using urine settings, on an AU2700 analyser was estimated at 0.04 mmol/L.
The lowest detectable level represents the lowest measurable level of glucose that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.
Method Comparison
Patient serum samples were used to compare this Glucose assay OSR6121 on the AU600 against another commercially available glucose assay. Results of linear regression analysis were as follows:

\[ y = 1.037x - 0.081 \quad r = 0.998 \quad n = 117 \quad \text{Sample range} = 0.3 - 43.3 \text{ mmol/L} \]

Patient urine samples were used to compare this Glucose assay OSR6121 on the AU2700 against another commercially available glucose assay. Results of linear regression analysis were as follows:

\[ y = 1.001x - 0.008 \quad r = 1.000 \quad n = 120 \quad \text{Sample range} = 0.06 - 26.23 \text{ mmol/L} \]

Patient CSF samples were used to compare this Glucose assay OSR6121 on the AU600 against another commercially available glucose assay. Results of linear regression analysis were as follows:

\[ y = 0.97x - 0.02 \quad r = 0.991 \quad n = 101 \quad \text{Sample range} = 1.8 - 7.7 \text{ mmol/L} \]

Interfering Substances
Results of serum studies conducted to evaluate the susceptibility of the method to interference were as follows:
- Ascorbate: Interference less than 3% up to 20 mg/dL ascorbate
- Icterus: Interference less than 10% up to 40 mg/dL or 684 µmol/L bilirubin
- Haemolysis: Interference less than 3% up to 5 g/L haemoglobin
- Lipemia: Interference less than 10% up to 700 mg/dL Intralipid®

Results of urine studies conducted to evaluate the susceptibility of the method to interference were as follows:
- Ascorbate: Interference less than 3% up to 50 mg/dL ascorbate
- Icterus: Interference less than 3% up to 40 mg/dL or 684 µmol/L bilirubin

Results of haemolysate studies conducted to evaluate the susceptibility of the method to interference were as follows:
- Haemolysis: Interference less than 20% up to 150 g/L haemoglobin
- Icterus: Interference less than 10% up to 16 mg/dL or 273.6 µmol/L bilirubin
- Lipemia: Interference less than 10% up to 700 mg/dL Intralipid®

In very rare cases gammopathy, especially monoclonal IgM (Waldenström’s macroglobulinemia), may cause unreliable results.
Refer to Young10 for further information on interfering substances.

Setting Sheet Footnotes
# User defined  ¤  Analyser default value
† System Calibrator Cat. No.: 66300/ Urine Calibrator Cat. No.: ODC0025
* Values set for working in SI units (mmol/L). To work in mg/dL multiply by 18.
‡ Pretreat all samples: 20 µL sample and 1000 µL haemolysing reagent.

BIBLIOGRAPHY