**Intended Use**

System reagent for the quantitative determination of α-Amylase in human serum and urine on Beckman Coulter AU analyzers.

**Summary**

α-Amylase (EC 3.2.1.1) is found primarily in the pancreas and salivary glands. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis.¹

Amylase activity in serum tends to increase rapidly after an attack of pancreatitis and may be demonstrated as early as six to eight hours after its onset. Levels stay elevated for one to three days and then return rapidly to normal, reflecting the efficient renal clearance of the enzyme. Decreased amylase levels have been found in abscesses of the liver, acute hepatocellular damage, cirrhosis, cancer of the liver and bile duct and cholecystitis.¹

Amylase is a relatively small protein and is therefore filtered readily into the urine. The enzyme can be found in increased concentrations in the urine for longer periods of time than in the serum. An amylase content determined on a 2-hour urine collection is an excellent test for detecting pancreatitis.

**Methodology**

This Amylase procedure utilizes 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG₃) as substrate.² This substrate reacts directly with α-Amylase and does not require the presence of ancillary enzymes. The release of 2-chloro-4-nitrophenol (CNP) from the substrate and the resulting absorbance increase per minute is directly related to the α-Amylase activity in the sample. The resulting increase in absorbance can be measured spectrophotometrically at 410/480nm.

\[
\text{CNPG}_3 \xrightarrow{\alpha-\text{Amylase}} \text{CNP} + \text{CNPG}_2 + \text{Glucose} + \text{Maltotriose}
\]

**System Information**

For AU400/400e/480, AU600/640/640e/680 and AU2700/5400 Beckman Coulter Analyzers.

**Reagents**

Final concentration of reactive ingredients:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES (pH 6.05)</td>
<td>36.1 mmol/L</td>
</tr>
<tr>
<td>Calcium Acetate</td>
<td>3.60 mmol/L</td>
</tr>
<tr>
<td>NaCl</td>
<td>37.2 mmol/L</td>
</tr>
<tr>
<td>Potassium Thiocyanate</td>
<td>253 mmol/L</td>
</tr>
<tr>
<td>CNPG₃</td>
<td>1.63 mmol/L</td>
</tr>
</tbody>
</table>

Also contains preservatives.

**Precautions**

1. For in vitro diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

**Preparation of Reagents**

The α-Amylase reagent is ready for use. No preparation is required.

**Storage and Stability**

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 8°C.
2. The opened reagent is stable for 30 days when stored in the refrigerated compartment of the analyzer.

**Indications of Deterioration**

Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

**Specimen Collection and Preparation**

Serum or heparinized plasma free from hemolysis is the recommended specimen. Separate from blood cells as soon as possible.

Non-acidified urines with random or timed collections are valid specimens.

**Sample Storage and Stability**

Amylase is stable in serum for one week when stored at 15 - 25°C and for up to one month when stored at 2 - 8°C.³

In urine, an acid pH may make the enzyme less stable; therefore, pH should be adjusted to approximately 7.0 before storage.

**Interfering Substances**

1. Do not pipette the reagent or sample by mouth to avoid salivary amylase contamination. Laboratory studies have shown that chelating agents such as the anticoagulants citrate and EDTA will interfere with this method.⁴
2. Results of studies³ show that the following substances interfere with this amylase procedure.
Amylase

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 20 mg/dL Bilirubin
Hemolysis: No significant interference up to 250 mg/dL Hemolysate
Lipemia: No significant interference up to 1000 mg/dL Intralipid

* Intralipid, manufactured by KabiVitrum Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User’s Guide appropriate to the analyzer.

Materials Provided

α-Amylase Reagent

Stability of Final Reaction Mixture

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration

Calibration of this amylase procedure is based upon the theoretical extinction coefficient for CNPG₃, which has a molar absorptivity of 11,320 at 410/480 nm.

Quality Control

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure. Appropriate qualified urine controls should be established and utilized during urine analysis.

Results

Automatically printed out for each sample in U/L at 37°C.

Dynamic Range

The Amylase procedure is linear from 10 to 2000 U/L for serum determinations and to 1500 U/L for urine determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Expected Values

Serum: 29 - 103 U/L
Urine: 5 - 27 U/hour

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Amylase Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision

Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision for serum samples is less than 5% CV and total precision is less than 10% CV. Assays of control sera and pooled urine were carried out and data reduced following CLSI guidelines above.

<table>
<thead>
<tr>
<th>Serum</th>
<th>N = 100</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, U/L</td>
<td>SD</td>
<td>CV%</td>
<td>SD</td>
</tr>
<tr>
<td>102.7</td>
<td>0.8</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>412</td>
<td>3.1</td>
<td>0.8</td>
<td>7.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine</th>
<th>N = 100</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, U/L</td>
<td>SD</td>
<td>CV%</td>
<td>SD</td>
</tr>
<tr>
<td>29.9</td>
<td>0.5</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>136.1</td>
<td>1.2</td>
<td>0.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Method Comparison

Patient samples were used to compare this Amylase Reagent. The table below demonstrates representative performance on the AU analyzers.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>X Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU640</td>
<td>AU600</td>
</tr>
<tr>
<td>Slope</td>
<td>0.976</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.5</td>
</tr>
<tr>
<td>Correlation Coeff. (r)</td>
<td>1.000</td>
</tr>
<tr>
<td>No. of Samples (n)</td>
<td>180</td>
</tr>
<tr>
<td>Range (U/L)</td>
<td>11-1646</td>
</tr>
</tbody>
</table>
Amylase

Urine

Urine samples were used to compare this Amylase Reagent. The table below demonstrates representative performance on the AU analyzers.

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coeff. (r)</th>
<th>No. of Samples (n)</th>
<th>Range (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU640</td>
<td>0.978</td>
<td>+1.9</td>
<td>1.000</td>
<td>195</td>
<td>11-1312</td>
</tr>
<tr>
<td>AU600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity

Typical change in absorbance per minute for 1 U/L of amylase is 0.11 mAbsorbance.

References

7. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
9. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA