Aldolase

Kinetic determination of aldolase in serum and plasma

<table>
<thead>
<tr>
<th>REAGENT 1: (lyophilized) 5 x 20 mL</th>
<th>REAGENT 2: (lyophilized) 2 x 1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>REAGENT 3: 1 x 0.5 mL</td>
<td></td>
</tr>
</tbody>
</table>

REAGENT 3 contains Ammonium Sulphate

R36/37/38: IRRITATING to eyes, respiratory system and skin
S26: in case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36: wear suitable protective clothing

PRECAUTIONS IN USE

- In addition to the possible risk indications regarding the reactive components, reagents may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 88/379/EEC directives and following modifications and amendments about classification, labelling and packaging of dangerous preparations (reagents). However, it is recommended to handle reagents carefully, to avoid ingestion and contact with eyes, skin and mucous membranes and to use laboratory reagents according to good laboratory practice.

SUMMARY

Serum aldolase determinations have been of greatest clinical interest in primary diseases of skeletal muscle. High increases of enzyme may be noted in case of Duchenne-type muscular dystrophy. Lesser degrees of enzyme elevation are encountered in dermatomyositis and polymyositis.

PRINCIPLE

Aldolase converts fructose-1,6-diphosphate (F-1,6-DP) to glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DAP). The addition of triosephosphate isomerase (TPI), glycerol-phosphate dehydrogenase (GDH) and NADH converts the dihydroxyacetone phosphate to glycerol-1-phosphate. The rate of the aldolase reaction is measured by the decrease in absorbance at 340 nm as a consequence of the conversion of NADH to NAD\(^+\).

REAGENTS

Reagents, stored at 2-8 °C in unopened vials, are stable up to expiry date indicated on the package.

Components of the kit and initial concentration of reactive components (see "Preparation of reagent solutions"):

- **REAGENT 1**: lyophilized
  - collidine buffer 51 mmol/L, pH 7.4, mono-iodoacetate 0.27 mmol/L, F-1.6-DP 2.7 mmol/L, sodium azide < 0.1%
- **REAGENT 2**: lyophilized
  - NADH 12 mmol/L
- **REAGENT 3**: ready to use
  - GDH ≥ 80 U/mL, TPI ≥ 1.0 kU/mL, LDH ≥ 150 U/mL, ammonium sulphate ≥ 40% (w/v)

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

NOTES AND LIMITATIONS

1) In case of A1 absorbance < 0.95 dilute the sample 1 + 1 with normal saline and re-assay. Multiply the result by 2 (see "ANALYTICAL PROCEDURE").
2) CONVERSION FACTOR:
   - Aldolase activity at 25 °C (U/L) = [ aldolase activity at 37 °C (U/L) ] x 0.41
   - Aldolase activity at 37 °C (U/L) = [ aldolase activity at 25 °C (U/L) ] x 2.44

Preparation of reagent solutions

**Solution R1**: reconstitute the contents of each vial of REAGENT 1 with 20 mL of distilled water; let stand for about 10 minutes and gently mix. Stability: 15 days at 2-8 °C, if contamination avoided and vial recapped immediately after use.

**Solution R2**: reconstitute the contents of each vial of REAGENT 2 with 1 mL of distilled water; let stand for about 10 minutes and gently mix. Stability: 30 days at 2-8 °C, if contamination avoided and vial recapped immediately after use.

**REAGENT 3**: ready to use. Reagent in unopened vial is stable up to expiry date indicated on the package. Stability: 120 days at 2-8 °C after opening, if contamination avoided and vial recapped immediately after use.

QUALITY CONTROL

The use of control materials at different levels of analyte is recommended to verify test accuracy.

17003 p.1/2
SAMPLE
Serum, plasma (heparin, EDTA). Avoid hemolysed samples. Collect samples in accordance with the NCCLS procedure reported in bibliography (1). Stability of the sample: 15 days at 2-8 °C.

Instrumentation and materials required but not provided
• Usual laboratory equipment
• Filters photometer or spectrophotometer
• Normal saline (NaCl 9 g/L)

ANALYTICAL PROCEDURE
Wavelength: 340 (334-365) nm
Pathlength: 1 cm
Temperature: 37 °C
Sample/Solution R1/Solution R2/REAGENT 3: 1/12.5/0.25/0.05
Reaction: fixed-time (decrease)

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

<table>
<thead>
<tr>
<th>Put into cuvette:</th>
<th>Sample blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>0.2 mL</td>
<td>0.2 mL</td>
</tr>
<tr>
<td>Solution R1</td>
<td>-</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Normal saline</td>
<td>2.5 mL</td>
<td>-</td>
</tr>
<tr>
<td>Solution R2</td>
<td>-</td>
<td>0.05 mL</td>
</tr>
<tr>
<td>REAGENT 3</td>
<td>-</td>
<td>0.01 mL</td>
</tr>
</tbody>
</table>

Mix carefully and incubate for 5 minutes at working temperature. Read the absorbance A1 against Sample Blank. Incubate at 37 °C for exactly 20 minutes after the first reading and read the absorbance A2 against Sample Blank.

If A1 < 0.95 dilute the sample 1 + 1 with normal saline and re-assay. Multiply the result by 2

CALCULATION
(A1 - A2) x F = aldolase activity in U/L
F = 54.8 at 340 nm   F = 101.5 at 365 nm   F = 55.8 at 334 nm

This F factor is valid only for the above-mentioned working conditions (wavelength, sample volume, final reaction volume and pathlength).

REFERENCE VALUES
Serum/Plasma:
≤ 7.6 U/L (37 °C)
≤ 3.1 U/L (25 °C)

It is recommended that each laboratory establish its own expected range.

PERFORMANCES (determined on automatic analyzer)
Measuring range: 1.0 - 30.0 U/L. Samples with concentration higher than 30.0 U/L must be diluted 1:10 with normal saline and result multiplied by 10.
Intra-Assay Precision: it was determined on 20 replicates of each control (tested 3 levels - L1/L2/L3). Results were as follows: L1: average 6.81 U/L, SD 0.10, CV% 1.40 / L2: average 7.87 U/L, SD 0.20, CV% 2.59 / L3: average 18.39 U/L, SD 0.17, CV% 0.91.
Sensitivity: 1.0 U/L. Sensitivity was calculated on 20 replicates of normal saline and reported as the "mean zero value + 3 SD".
Accuracy: this test (y) was compared with a commercially available method (x). Results were as follows:
SERUM: N = 57, r = 0.98214, y = 1.0536 x -0.43104

WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY