Aspartate aminotransferase (AST/GOT)-Liquizyme (1 + 1)
E.C.2.6.1.1.

**Intended Use**

Spectrum Diagnostics Liquizyme AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum on both automated and manual systems.

**Background**

The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues such as myocardial infarction, viral hepatitis and muscular dystrophy. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the sera of patients with coronary and hepatobiliary diseases.

**Method**

Kinetic method according to the International Federation of Clinical Chemistry (IFCC) (3).

**Assay Principle**

The series of the reaction involved in the assay system is as follows:

1. The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.

   \[ \text{L-Aspartate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{2-Oxoglutarate} \]

2. Oxaloacetate in presence of NADH and malate dehydrogenase (MDH), is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD.

   \[ \text{Oxaloacetate} + \text{MDH} + \text{NADH} \rightarrow \text{Malate} + \text{NAD}^+ \]

3. Addition of lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

   \[ \text{Sample Pyruvate} + \text{LDH} \rightarrow \text{L-Lactate} + \text{NAD}^+ \]

**Reagents**

**Reagent 1 (R1 Buffer/Enzymes)**

- Tris buffer (pH 7.7) 80 mmol/L
- L-Aspartate 450 mmol/L
- MDH $\geq$ 900 U/L
- LDH $\geq$ 2000 U/L
- Sodium Hydroxide 300 mmol/L
- Sodium Azide 8 mmol/L

**Irritant (Xi):** R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S337/39: Wear suitable gloves and eye/face protection.

**Reagent 2 (R2 Coenzyme)**

- NADH $\geq$ 0.06 mmol/L
- 2-Oxoglutarate 4 mmol/L
- Sodium Azide 8 mmol/L

For further information, refer to the Aspartate aminotransferase reagent material safety data sheet.

**Precautions and Warnings**

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Reagent Preparation, Storage and Stability**

All reagents are stable until expiration date stated on label when stored refrigerated at 2–8°C. Working solution can be prepared by adding equal volumes from R1 and R2; Stability: 2 days at 2 – 8°C.

**Deterioration**

Do not use liquizyme AST reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm.

**Specimen Collection and Preservation**

Use nonheparinized serum. Heparin and EDTA are the only acceptable anticoagulants. The biological half-life of AST in serum is 17 hours.

**Stability:** 1 day at 15 – 25°C; 7 days at 4 – 8°C; 12 weeks at -20°C.

**System Parameters**

- Wavelength: 340 nm (334 – 365 nm)
- Optical path: 1 cm
- Assay type: Kinetic
- Direction: decrease
- Sample: Reagent Ratio e.g.: Reagent volume 1 : 10
- Sample volume: 1 ml
- Equilibration time: 30 seconds.
- Read time: 1 to 3 minutes
- Zero adjustment: Against air
- Reagent Blank Limits: Low 1.00 AU
- High 2.5 AU
- Sensitivity: 5 U/L
- Linearity: 400 U/L

**Procedure**

**Pipette in a test tube:**

| Working solution | 1.0 (or 0.5 ml R1 + 0.5 ml R2)  |
| Specimen | 100 μl |
| Mix, read initial absorbance after 30 seconds, and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (μA/min).

**Calculation**

To calculate the AST/GOT activity use the following formula:

\[ U/L = \frac{1780 \times \Delta A 334}{nm/\min} \]

\[ U/L = \frac{1746 \times \Delta A 340}{nm/\min} \]

\[ U/L = \frac{3235 \times \Delta A 365}{nm/\min} \]
Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>32</td>
<td>135</td>
</tr>
<tr>
<td>SD</td>
<td>1.2</td>
<td>1.27</td>
</tr>
<tr>
<td>CV%</td>
<td>4.1</td>
<td>0.99</td>
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</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>34</td>
<td>136</td>
</tr>
<tr>
<td>SD</td>
<td>1.6</td>
<td>1.43</td>
</tr>
<tr>
<td>CV%</td>
<td>4.5</td>
<td>1.16</td>
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</table>

Methods Comparison
A comparison between Spectrum Diagnostics AST (1+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.98 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity
The reaction is linear up to AST concentration of 400 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interfering Substances

<table>
<thead>
<tr>
<th>Serum, plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
</tr>
<tr>
<td>Erythrocyte contamination elevate results, since AST activities in erythrocytes are 15 times higher than those in normal sera.</td>
</tr>
<tr>
<td>Icterus</td>
</tr>
<tr>
<td>No significant interference.</td>
</tr>
<tr>
<td>Lipemia</td>
</tr>
<tr>
<td>Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.</td>
</tr>
<tr>
<td>Anticoagulants</td>
</tr>
<tr>
<td>Citrate and fluoride inhibit the enzyme activity.</td>
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</tbody>
</table>

Drugs
Calcium dobesilate and doxycycline HCL cause artificially low AST values at the tested drug level.

Expected values

<table>
<thead>
<tr>
<th></th>
<th>37 °C</th>
<th>30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>up to 31 U/L</td>
<td>up to 21 U/L</td>
</tr>
<tr>
<td>Males</td>
<td>up to 37 U/L</td>
<td>up to 25 U/L</td>
</tr>
<tr>
<td></td>
<td>(up to 0.52 µKat/L)</td>
<td>(up to 0.35 µKat/L)</td>
</tr>
<tr>
<td></td>
<td>(up to 0.62 µKat/L)</td>
<td>(up to 0.42 µKat/L)</td>
</tr>
</tbody>
</table>

Temperature conversion factor is 1.37 (25→37 °C) and 2.04 (25→30 °C).

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range
5 – 400 U/L.

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

SS6: dispose of this material and its container at hazardous or special waste collection point.

SS6: avoid release in environment. Refer to special instructions/safety data sheets.

References

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tbody>
<tr>
<td>259 001</td>
<td>2 x 25 ml</td>
</tr>
<tr>
<td>259 002</td>
<td>4 x 25 ml</td>
</tr>
<tr>
<td>259 003</td>
<td>2 x 100 ml</td>
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</tbody>
</table>

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EC REP

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