Aspartate Aminotransferase
Test Code: AST

Intended Use
For in vitro diagnostic use only

CARESIDE™ Aspartate Aminotransferase (AST) cartridges are used with the CARESIDE Analyzer™ to quantitatively measure aspartate aminotransferase activity in anti-coagulated whole blood, plasma or serum specimens. The CARESIDE AST test aids in the diagnosis and treatment of various heart and liver diseases. Aspartate aminotransferase is also referred to as aspartate transaminase.

The CARESIDE Analyzer can use AST results together with alanine aminotransferase (ALT) results to calculate the ratio of ALT to AST.

CARESIDE AST, a single use cartridge, aids in specimen separation and delivers a measured volume of sample to a dry film to initiate the measurement of AST activity. The film cartridge (patent pending) contains all reagents necessary to measure AST activity.

Test Summary
Aspartate aminotransferase (AST), formerly known as serum glutamate oxaloacetate transaminase (GOT or SGOT), is an enzyme involved in the metabolism of amino acids. The principal sources of AST appearing in blood are heart, liver, skeletal muscle, kidney, and erythrocytes. AST measurement is important in myocardial infarction, hepatic parenchymal disease, and muscle disease.

Serum AST levels are elevated in viral hepatitis, toxic hepatitis, metastatic hepatic carcinoma, cirrhosis, and in infectious or other inflammatory conditions affecting the liver. In hepatocellular disease other than viral hepatitis, the ratio of ALT to AST is usually less than 1. This ratio, called the DeRitis ratio, is often reversed in viral hepatitis. Elevated AST activity appears after myocardial infarction: AST levels increase in 6 to 8 hours after chest pain onset, peak after 18 to 24 hours, and fall to within the reference interval for healthy individuals by day five. In muscular dystrophy and dermatomyositis, AST levels increase up to eight-fold. AST values ≥ 300 U/L may be due to acute hepatocellular injury, viral hepatitis, or toxic hepatitis. Other causes of increased AST such as alcoholic hepatitis, myocardial infarction, and muscular dystrophy are associated with values lower than 300 U/L.

Test Explanation
Each CARESIDE AST cartridge consists of an aspartate aminotransferase-specific multi-layer reagent film mounted in a plastic base with a hinged lid. The user introduces the patient sample into the cartridge Sample Well, closes the lid and inserts the cartridge into the CARESIDE Analyzer.

Once loaded, the CARESIDE Analyzer scans the cartridge barcode and spins the cartridge to move the sample from the Sample Well into the cartridge channels and chambers. While spinning, the cartridge and the contained specimen are warmed to 37°C. As the cartridge continues to spin, the blood cells (if present) are separated from the plasma/serum and the cells accumulate in the separation well. Any excess sample flows into an overflow well, leaving the required volume in the metering passage.

The sample in the metering passage is automatically dispensed onto the multi-layer reagent film. The spreading and substrate layer distributes the sample evenly on the film, and the sample moves through a
reagent layer and into a reaction layer where a green dye forms in the presence of AST.

As the cartridge spins, photodiodes measure reflectance of light emitted by wavelength-specific light-emitting diodes (LEDs) at fixed times. The instrument uses the reflectance measurements and the lot-specific standard curve to calculate AST activity.

**Test Principle**

The metered sample is distributed uniformly by the spreading layer. The AST catalyzes the reaction of L-aspartate and $\alpha$-ketoglutaric acid without supplementation with the cofactor pyridoxal 5-phosphate (P-5'-P) to form oxaloacetic acid and L-glutamic acid. The oxaloacetic acid is converted to pyruvic acid and CO$_2$ by oxaloacetate decarboxylase (OAC) in the reagent layer. Pyruvate oxidase (PO) in the reaction layer then catalyzes the reaction of pyruvic acid with oxygen and phosphoric acid to form hydrogen peroxide, carbon dioxide, and acetyl phosphate. Finally, the hydrogen peroxide oxidizes a diarylimidazole leuco dye in a peroxidase (POD) catalyzed reaction to form a green dye and water. The color intensity, as measured by the amount of light reflected at 615 nanometers, directly relates to the AST activity in the sample.

**Test Reaction Sequence**

\[
\text{L-asparaginate} + \alpha \text{-ketoglutaric acid} \xrightarrow{\text{AST}} \text{Oxaloacetic acid} + \text{L-glutamic acid}
\]
\[
\text{Oxaloacetic acid} \xrightarrow{\text{OAC}} \text{Pyruvic acid} + \text{CO}_2
\]
\[
\text{Pyruvic acid} + \text{O}_2 + \text{phosphoric acid} \xrightarrow{\text{PO}} \text{H}_2\text{O}_2 + \text{CO}_2 + \text{acetyl phosphate}
\]
\[
\text{Diarylimidazole leuco dye} + \text{H}_2\text{O}_2 \xrightarrow{\text{POD}} \text{Green dye} + \text{H}_2\text{O}
\]

**Test Cartridge Ingredients**

The active ingredients are L-aspartate, $\alpha$-ketoglutaric acid, potassium phosphate, oxaloacetate decarboxylase, pyruvate oxidase, diarylimidazole leuco dye, and horseradish peroxidase.

**Test Warnings and Precautions**

- For *in vitro* diagnostic use only.
- Use the AST cartridge within 15 minutes of opening.
- Handle all patient samples, QC materials of human origin, and dosed test cartridges using universal precautions.
- Protect reagent film from contamination or excessive light. Do not touch; touching the film can cause optical errors.
- Do not reuse cartridges.
- Specimens should be free from precipitates.

**Test Cartridge Storage & Preparation**

Cartridges are stable until the date printed on the cartridge label when stored at 2 to 8°C (36 to 46°F). Do not use if the package has been opened or damaged, or if the package seal is not intact. Unused cartridges may be returned to refrigerated storage if they are unopened.

**Test Calibration**

No user calibration is required. Each cartridge lot is factory calibrated and the calibration information is read automatically by the CARESIDE Analyzer.

**Specimen Collection**

The CARESIDE AST Cartridge is intended for use with appropriately collected whole blood, plasma or serum, and requires 90 ±10µL sample per test cartridge.

**Patient Preparation**

No special patient preparation is necessary.

**Whole blood**

Collect whole blood in blood collection tubes containing heparin. Do not use EDTA, sodium fluoride, citric acid, oxalic acid, or monothioglycolic acid. Mix anticoagulant according to manufacturer’s instructions allowing sufficient time for anticoagulation to occur. Promptly use or process the whole blood.
Serum
Collect whole blood in the absence of anticoagulant. Allow blood to fully clot at room temperature. Centrifuge the clotted blood and separate the serum promptly from the clot.

Plasma
Collect whole blood using anticoagulant as described under Whole Blood, above. Promptly centrifuge the blood and separate the plasma from the cells.

Specimen Handling and Preparation
If analysis is not performed within 4 hours, refrigerate specimens at 2 to 8°C. Serum AST is stable for 3 days at 2 to 8 °C. Serum or plasma specimens should be frozen if stored longer than 3 days. Thoroughly mix stored specimens before testing. Do not freeze whole blood.

Do not dilute specimens for routine testing. To obtain results for patients that have AST levels above the reportable range, dilute only serum or plasma specimens. Do not dilute whole blood specimens. Use normal saline to perform initial dilutions (1 part sample, 4 parts diluent), and multiply the result by 5. Where necessary, use saline to further dilute the diluted sample (1 part diluted sample, 4 parts diluent), and multiply the result by 25.

Test Procedures
1. Select the test to be performed on the CARESIDE Analyzer.
2. To dose the cartridge, lift the test cartridge lid by lifting up of the side clips. To the Sample Well apply 90 ±10μl of sample or fill the well internally. An exact volume is not required, since the test cartridge will meter the correct volume. Do not allow the sample to spill over the well. Any sample outside of the well must be completely removed proceeding.
3. Close the cartridge lid firmly until it clicks indicating complete closure.
4. Load the dosed test cartridge into the CARESIDE Analyzer and press “Confirm” (see the CARESIDE Analyzer Operator’s Manual for more information).

Quality Control
Each CARESIDE test cartridge barcode contains all required calibration information. The CARESIDE Analyzer scans the barcode, continuously performs quality control self-checks during operation, and alerts and prevents the user from proceeding if an unacceptable condition exists.

At a frequency determined appropriate by the user’s institution, evaluate instrument performance using two levels of AST controls. Contact CARESIDE Technical Service for a list of commercial control products compatible with the CARESIDE Analyzer System. Each institution should establish quality control ranges using the CARESIDE Analyzer and monitor test performance using a Levey-Jennings chart (See CARESIDE Analyzer Operator’s Manual).

Results
Results require no user calculation and appear on the CARESIDE Analyzer screen, as they become available.

<table>
<thead>
<tr>
<th>Reporting Units (37°C)</th>
<th>U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reportable Range</td>
<td>10 to 1000 U/L</td>
</tr>
</tbody>
</table>

Expected Values (Reference Interval)
The following reference interval was obtained by non-parametric analysis of plasma specimens from a population of 37 healthy adults in the United States using the CARESIDE AST test:

| Reference Interval | Less than 32 U/L |

This interval is similar to a published reference interval (10 to 40 U/L) for serum specimens from healthy adults performed under similar conditions. Expected values may vary depending upon the population of subjects and the testing method. It is recommended that each institution establish values for the population it serves.

Limitations
1. Do not use hemolyzed specimens. AST released from hemolyzed red cells causes falsely elevated results.
2. A result outside the reportable range or any result not consistent with the clinical picture should be repeated to confirm.
Performance Characteristics

Detection Limit
The CARESIDE AST cartridge demonstrated a lower detection limit of 10 U/L.

Accuracy
The CARESIDE AST activity agrees with an enzymatic photometric method for aspartate aminotransferase using pyridoxal phosphate activation.

![AST Method Comparison](image)

Precision
Human serum based controls (representing a low, middle and high AST activity) were tested in duplicate in at least 20 runs over a period of at least 3 days. Results are shown in the following table.

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Conc. (U/L)</td>
<td>58.3</td>
<td>134</td>
<td>283</td>
</tr>
<tr>
<td>Within-run SD (U/L)</td>
<td>3.0</td>
<td>9.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Within-run CV (%)</td>
<td>5.1</td>
<td>6.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Total SD (U/L)</td>
<td>2.7</td>
<td>9.1</td>
<td>19.3</td>
</tr>
<tr>
<td>Total CV (%)</td>
<td>4.7</td>
<td>6.8</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Actual precision may vary from site-to-site and from results indicated in this test insert. Each facility should verify that on-site product precision is appropriate for the site.

Interference
No significant interference was observed up to the levels indicated for the following substances:

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>3000 mg/dL</td>
</tr>
</tbody>
</table>

Bibliography

Technical Assistance
If questions arise concerning the CARESIDE Analyzer and test cartridges, please contact CARESIDE Technical Service.

Telephone: (888) 698-CARE (2273)  
(310) 338-6767
Fax: (310) 338-6789

Ordering Information
For product ordering or general information, please contact CARESIDE Customer Service.

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