**Intended Use**

*For in vitro diagnostic use only*

CARESIDE™ Alkaline Phosphatase (ALP) cartridges are used with the CARESIDE Analyzer™ to quantitatively measure the enzyme activity of alkaline phosphatase in anticoagulated whole blood, plasma or serum specimens. The CARESIDE Alkaline Phosphatase test aids in the diagnosis and treatment of patients with diseases of the liver, pancreas, bone, parathyroid, and intestines.

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

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**Test Summary**

Alkaline phosphatase consists of a group of at least five isoenzymes that catalyze the hydrolysis of phosphate mono-esters optimally at an alkaline pH. These isoenzymes are found in varying proportions in different tissues. ALP isoenzymes are found in the kidney, small intestine, osteoblasts, placenta, and liver. Significant elevations of ALP concentrations in the blood result from hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Modest ALP elevation occurs in pregnancy, congestive heart failure, ulcerative colitis, intra-abdominal infections, Hodgkin’s disease and other malignancies. Certain drugs can cause elevation in ALP activity in the blood *in vivo*.

**Test Explanation**

Each CARESIDE Alkaline Phosphatase cartridge consists of an alkaline phosphatase-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. In the spreading and substrate layer the sample distributes evenly on the film and the enzymatic conversion of the substrate into a yellow dye occurs in the presence of alkaline phosphatase.

As the cartridge spins, photodiodes measure film reflectance of light emitted by wavelength-specific light-emitting diodes (LEDs) over a fixed time period. The instrument uses the reflectance measurements and the lot-specific standard curve to calculate alkaline phosphatase activity.
Test Principle
The metered sample is distributed uniformly by the spreading and substrate layer. Under alkaline conditions, ALP catalyzes the conversion of p-nitrophenyl phosphate to p-nitrophenol. p-Nitrophenol diffuses through the detection layer and into the underlying buffer where it converts non-enzymatically to p-nitrophenoxide which has an intense yellow color. The rate of change of color intensity of the yellow dye, as measured by the amount of reflected light at 425 nanometers, directly relates to the alkaline phosphatase activity in the sample.

Test Reaction Sequence
p-Nitrophenyl Phosphate

\[ \text{ALP} \downarrow \]

p-Nitrophenol + Phosphoric Acid

Test Element Architecture

Test Cartridge Ingredients
The active ingredient is p-nitrophenyl phosphate.

Test Warnings and Precautions
- For *in vitro* diagnostic use only
- Use the alkaline phosphatase 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 the film; 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™ System.

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

Collect blood specimens by standard venipuncture technique.

Patient Preparation
No special preparations are required. Do not use grossly hemolyzed or icteric specimens.

Whole blood
Collect whole blood in blood collection tubes containing sodium heparin. Do not use EDTA, sodium fluoride, citric acid, oxalic acid, or monoiidoacetic acid. Mix anticoagulant according to manufacturer’s instructions allowing sufficient time for anticoagulation to occur.

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

Specimen Handling and Preparation
All specimen types may be stored at room temperature when tested within 4 hours of draw. If
necessary, specimens may be refrigerated at 2 to 8°C. If testing is delayed beyond 48 hours, serum or plasma specimens may be frozen at -20°C or colder for at least one month. Thoroughly mix any stored specimen before testing. Do not freeze whole blood.

Do not dilute samples for routine testing. To obtain results for patients that have results above the reportable range, dilute only serum or plasma samples. Do not dilute whole blood. Use normal saline to perform a five-fold dilution (1 part sample, 4 parts diluent), and multiply the result by 5.

**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 on 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 before proceeding.
3. Close the cartridge lid firmly until it clicks indicating 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 alkaline phosphatase controls. Contact CARESIDE Technical Service for a list of commercial control products compatible with the CARESIDE Analyzer. 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.

- **Reporting Units (37°C):** U/L
- **Reportable Range:** 30 to 2500 U/L

**Expected Values (Reference Interval)**
The following reference interval was obtained by non-parametric analysis of plasma specimens from a population of 61 healthy adults in the United States using the CARESIDE Alkaline Phosphatase test:

**Central 95% interval:** 40 - 140 U/L

The observed reference interval is similar to a published consensus reference interval for adult serum of 40 to 150 U/L at 37°C.

Expected values may vary depending upon the population of subjects and the test method. It is recommended that each institution establish values for the population they serve using the CARESIDE Alkaline Phosphatase.

**Limitations**
1. Do not use hemolyzed specimens or samples because ALP contamination from red cells will occur causing falsely elevated results.
2. Specimens from dialysis patients may show falsely low results.
3. 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 Alkaline Phosphatase cartridge has a lower detection limit of 30 U/L.
Alkaline Phosphatase (ALP) CARESIDE Analyzer™ System

Accuracy
The CARESIDE ALP agrees with a liquid phase kinetic photometric method for alkaline phosphatase using p-nitrophenylphosphate as substrate.

ALP Method Comparison

Precision
Human serum based controls (representing a low, middle and high alkaline phosphatase 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.

CARESIDE Alkaline Phosphatase Precision

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean Conc. (U/L)</th>
<th>Within-run SD (U/L)</th>
<th>Within-run CV (%)</th>
<th>Total SD (U/L)</th>
<th>Total CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>47.4</td>
<td>3.08</td>
<td>6.5</td>
<td>4.65</td>
<td>9.8</td>
</tr>
<tr>
<td>Level 2</td>
<td>208</td>
<td>16.4</td>
<td>7.9</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Level 3</td>
<td>380</td>
<td>36.6</td>
<td>9.6</td>
<td>37.4</td>
<td>9.8</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>Hemoglobin</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1500 mg/dL</td>
</tr>
</tbody>
</table>

Bibliography

Technical Assistance
If questions arise concerning the CARESIDE or the CARESIDE Analyzer or 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:

Telephone: (888) 698-CARE (2273)
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07-Jun-2000