Creatine Kinase Test Code: CK

Intended Use
For in vitro diagnostic use only

CARESIDE™ Creatine Kinase (CK) Cartridges are used with the CARESIDE Analyzer™ to quantitatively measure creatine kinase activity in anti-coagulated whole blood, plasma or serum specimens. The CARESIDE Creatine Kinase test aids in the diagnosis and treatment of patients with myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.

The CARESIDE Analyzer can use CK results together with CKMB results to calculate the %CKMB (CKMB/Total CK x 100).

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

Test Summary
Creatine kinase (CK), also known as creatine phosphokinase, is an enzyme consisting of two sub-units (termed B and M) that catalyzes the reversible phosphorylation of creatine by adenosine-triphosphate (ATP) to creatine phosphate (CrP) and adenosine-diphosphate (ADP). Total active CK is predominantly the summation of CK-BB, CK-MB and CK-MM isoenzymes. CK is distributed in various organs; the highest activities are found in skeletal muscle, heart, and brain. Considerably lower activities are present in the urinary bladder, stomach, ileum, colon, uterus, liver, erythrocytes, and kidney.

Measurement of total CK activity is important in the diagnosis of cardiac and skeletomuscular disorders, and is increased after muscle trauma, intramuscular injections, exercise, and in other conditions. The CK level is also increased after acute alcohol intoxication, surgery-induced muscle injury, drug overdoses and poisoning, trauma to muscle or brain, hypothermia, hyperthermia, Reye’s syndrome, infectious diseases, and hypothyroidism. Elevated CK activities have been described in all forms of muscular dystrophy as well as polymyositis, dermatomyositis, and other myopathies.

Test Explanation
Each CARESIDE Creatine Kinase cartridge consists of a CK-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 sample moves onto the spreading and substrate layer where it is spread evenly on the film before moving into the reaction layer where a reddish dye forms in the presence of CK.

As the cartridge spins, photodiodes measure film reflectance of light emitted from wavelength-specific light emitting diodes (LEDs) over a period of time. The instrument uses the time course of reflectance
measurements and the lot-specific standard curve to calculate CK activity.

**Test Principle**

The CARESIDE Creatine Kinase Test measures CK activity as the rate of conversion of creatine phosphate to creatine. As typically described, the measured reaction is the reverse direction of this reversible reaction. Some other tests measure CK activity in the forward direction as the rate of conversion of creatine to creatine phosphate. The two different methods yield clinically equivalent results when interpreted with respect to their own reference intervals although the results are different quantitatively.

The metered sample is distributed uniformly by the spreading and substrate layer. The sample moves through a reagent layer where N-acetylcysteine activates the CK. The ATP formed in the CK-catalyzed reaction of creatine phosphate and ADP then reacts with glucose in a hexokinase-catalyzed reaction. The resulting glucose-6-phosphate then leads to the formation of a diformazan dye in a coupled enzymatic reaction with NAD+. The rate of change of the color intensity of the resulting reddish dye, as measured by the amount of reflected light at 570 nanometers, directly relates to the CK activity in the sample.

**Test Reaction Sequence**

Creatine phosphate + ADP $\xrightarrow{\text{CK}}$ Creatine + ATP

ATP + Glucose $\xrightarrow{\text{Hexokinase}}$ ADP + G-6-P

G-6-P + NAD$^+$ $\xrightarrow{\text{G6PD}}$ 6-phosphogluconic acid + NADH + H$^+$

NTB + NADH $\xrightarrow{\text{Diaphorase}}$ Diformazan dye + NAD$^+$

**Test Element Architecture**

![Test Element Architecture Diagram]

**Test Cartridge Ingredients**

The active ingredients include creatine phosphate, ADP, glucose, hexokinase, glucose-6-phosphate dehydrogenase, NAD+, nitrotetrazolium blue, N-acetylcysteine, and diaphorase.

**Test Warnings and Precautions**

- For *in vitro* diagnostic use only
- Use the CK 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.
- Drugs can affect the measurement of CK activity.

**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 CK 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 preparations are required.

**Note:** When performing multiple tests to monitor an individual patient, always use the same specimen type.

**Whole blood**

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

Serum
Collect blood in the absence of anti-coagulant. Allow blood to fully clot at room temperature. Centrifuge the clotted blood and separate the serum promptly from the clot.

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

Specimen Handling and Preparation
All specimens may be stored at room temperature when tested within 4 hours of draw. If necessary, specimens may be refrigerated at 2 to 8°C\(^1\). If testing is delayed beyond 48 hours, serum or plasma specimens may be frozen at -20°C or colder for up to 1 month\(^1\). Avoid repeated freeze-thaw. Thoroughly mix any stored specimen before testing. Do not freeze whole blood.

Do not dilute specimens for routine testing. To obtain results for patients that have levels above the reportable range, dilute only serum or plasma samples. Do not dilute whole blood specimens. Use normal saline to perform a twofold dilution (1 part sample, 1 part diluent), and multiply the result by 2.

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 ±10\(\mu\)l 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 by wiping before 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 CK 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: U/L (CrP→Cr), 37°C, NAC
- Reportable Range: 20 to 1600 U/L

Expected Values (Reference Interval)
Distributions of CK activity from healthy subjects often demonstrate a positive skew\(^2\). Expected values and the upper limit of the reference interval are affected by population characteristics such as age, gender, race, lean body mass, and physical activity\(^1\).

The following is a total CK reference interval for serum from white adults\(^1\).

- Females: 26 to 140 U/L, 37°C
- Males: 38 to 174 U/L, 37°C

Consistent with the literature\(^1\), a reference interval with an increased upper reference limit (47 to 294 U/L, 37°C) was obtained using the CARESIDE Creatine Kinase test and non-parametric analysis of plasma specimens from a population of 41 ambulatory, healthy adults of mixed race in the United States.

It is recommended that each institution establish values for the population they serve.

Interpretation of Results\(^1\)
Elevated CK activities are sometimes used as a screen for myocardial infarction (MI). Elevated CK activities should be followed up by serial measurement of other more specific cardiac markers. The diagnosis of myocardial infarction should be based on clinical history, signs, symptoms, non-laboratory studies, and serial determination of cardiac markers.

When the CK and CK-MB values exceed their respective upper reference interval limits, the %CKMB should be evaluated. In myocardial infarction, the
%CKMB usually falls between 3% and 30%. Ratios of less than about 3% to 6% may indicate significant skeletal muscle damage (skeletal muscle contains a low percentage CK-MB). Ratios of greater than 30% may indicate the presence of CK-BB or macro CK which can manifest as false CK-MB elevations.

**Limitations**
Do not use hemolyzed specimens.

**Performance Characteristics**

**Detection Limit**
The CARESIDE CK demonstrated a detection limit of 20 U/L.

**Accuracy**
The CARESIDE CK concentration agrees with a coupled enzyme photometric method for creatine kinase using N-acetyl-L-cysteine reactivation.

![CK Method Comparison](image)

**Precision**
Human serum based controls (representing a low, middle and high CK 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>CARESIDE CK Precision</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Conc. (U/L)</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>Within-run SD (U/L)</td>
<td>2.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Within-run CV (%)</td>
<td>5.7</td>
<td>5.4</td>
</tr>
<tr>
<td>Total SD (U/L)</td>
<td>2.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Total CV (%)</td>
<td>6.0</td>
<td>7.9</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>Triglyceride</td>
<td>2000 mg/dL</td>
</tr>
</tbody>
</table>

**Bibliography**

**Technical Assistance**
If questions arise concerning 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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