Creatine Kinase MB
Test Code: CKMB

Intended Use
For in vitro diagnostic use only

CARESIDE™ Creatine Kinase MB (CKMB) cartridges are used with the CARESIDE Analyzer™ to quantitatively measure creatine kinase-MB activity in whole blood, plasma or serum specimens. The CARESIDE CKMB test aids in the diagnosis and treatment of patients with myocardial infarction, myocarditis, Duchenne-type muscular dystrophy, polymyositis, rhabdomyolysis, and other myocardial and myopathic disorders.

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

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

Test Summary
Creatine kinase MB is an isoenzyme of creatine kinase (CK), also known as creatine phosphokinase. CK consists of two subunits (termed B and M) that catalyzes the reversible phosphorylation of creatine (Cr) by adenosine-triphosphate (ATP) to form creatine phosphate (CrP) and adenosine-diphosphate (ADP). Most active CK occurs as CK-BB, CK-MB and CK-MM isoenzymes. CK isoenzymes are found in several tissues. CK-BB is the predominant isoenzyme found in the brain, prostate, gut, lung, bladder, uterus, placenta and thyroid. Both CK-MB and CK-MM are found in the heart muscle, while CK-MM is the predominant form found in skeletal muscle.

The measurement of total CK and its various isomers is important in the diagnosis of several diseases, especially myocardial infarction. Other cardiac conditions such as angina pectoris, cardiogenic shock, electrical countershock, tachycardia, myocarditis and congestive heart failure have been reported as having a low occurrence of elevated total CK and CK-MB. Cardiac trauma resulting from heart surgery will cause an elevation in total CK and CK-MB so as to mask elevations subsequent to intraoperative myocardial infarction.

In myocardial infarction, the first 4 to 6 hours are characterized by a rise in the total blood CK activity, reaching a peak value between 18 and 30 hours and returning to normal by the third day. This initial rise is followed by a rise in the CK-MB fraction. After about 6 hours, CK-MB levels typically begin to rise and peak after about 18 hours, reaching levels of 10 to 25 times the upper reference limit. With successful reperfusion, CK-MB typically peaks about 12 hours earlier and peak activities are two to four times greater than in non-reperfused patients. It should be noted that CK-MB breaks down faster than CK-MM. Therefore, CK-MB activity returns to normal levels 48 to 72 hours post-infarction. As an indicator and measure of suspected myocardial damage, determination of CK-MB is commonly performed upon admission and every 6 to 12 hours thereafter.

Test Explanation
Each CARESIDE CKMB cartridge consists of a CK-MB 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 layer the sample distributes evenly on the film
before it moves into a reaction layer where a reddish dye
forms in the presence of CK-MB activity.

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

**Test Principle**
The CARESIDE CKMB test measures CK-MB activity
using the immunoinhibition method. In the spreading
and substrate layer, the CK-M activity, but not the CK-B
activity, is inhibited by the antibody to the CK-M subunit.
N-acetylcysteine (NAC) activates the CK-B, whose
activity is then measured. CK-B activity is quantitatively
related to the original CK-MB activity.

The CK-B catalyzed reaction of creatine phosphate and
ADP yields creatine and ATP. The ATP then reacts with
endogenous glucose in a hexokinase (HK) catalyzed
reaction. The resulting glucose-6-phosphate (G-6-P) 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 specimen
CK-MB activity.

**Test Reaction Sequence**

\[
\begin{align*}
\text{CK-MM, CK-MB} & \quad \rightarrow \quad \text{anti-CK-M antibody} \\
\text{Creatine phosphate} + \text{ADP} & \quad \rightarrow \quad \text{CK-B NK, Mg}^{2+} \\
\text{ATP} + \text{Glucose} & \quad \rightarrow \quad \text{Hexokinase} \\
\text{G-6-P} + \text{NAD}^+ & \quad \rightarrow \quad \text{G6PDH} \\
\text{NTB} + \text{NADH} & \quad \rightarrow \quad \text{Diaphorase} \\
\end{align*}
\]

**Test Cartridge Ingredients**
The active ingredients include anti-human CK-M goat
polyclonal antibody, creatine phosphate, ADP, glucose,
hexokinase, glucose-6-phosphate dehydrogenase, NAD$^+$,
nitrotetrazolium blue, and diaphorase.

**Test Warnings and Precautions**
- For in vitro diagnostic use only
- Use the CARESIDE CKMB cartridge within 2
  minutes of opening. Discard any cartridge that
  has been opened for more than 2 minutes prior to
  insertion into the CARESIDE Analyzer. Minimize
  the exposure of the cartridge to light.
- Handle all patient samples, QC materials of human
  origin, and dosed test cartridges using universal
  precautions$^2$.
- Protect reagent film from contamination. Do not
  touch the film; touching the film can cause optical
  errors.
- Do not reuse cartridges.
- Specimens should be free from precipitates.
- Drugs can affect the measurement of CK activity$^3$.
- Use the same specimen type for serial specimen
  analysis.

**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 routine 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 CKMB cartridge is intended for use with
appropriately collected whole blood, plasma or serum,
and requires 90 ±10 µL sample per test cartridge.
Collect blood using standard venipuncture techniques
collected$^4$.

**Patient Preparation**
No special preparations are required.

**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’s 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 and samples 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 72 hours, serum or plasma samples may be frozen at -20°C or colder for up to 1 month. Avoid repeated freeze-thaw. 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 levels above the reportable range, dilute only serum or plasma samples. Do not dilute whole blood. Use normal saline to perform a two-fold 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 µl 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 specimen to spill over the well. Any specimen outside of the well must be completely removed 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-MB 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, 37 °C
Reportable Range: 5 to 300 U/L

Expected Values (Reference Interval)
The following reference interval was obtained by non-parametric analysis of plasma specimens from a population of 48 ambulatory, healthy adults in the United States of mixed gender and race using the CARESIDE CKMB test.

Reference interval: less than 17 U/L

The observed reference interval is similar to published serum reference intervals for other commercial methods. Expected values may vary depending upon the population of subjects. It is recommended that each institution establish values for the population they serve.

Interpretation of Results
Elevated CK-MB activities are sometimes used as an indicator of myocardial infarction (MI). Single determinations of CK-MB are of limited value because CK-MB elevations above the upper reference limit may persist for only a short time period. The diagnosis of myocardial infarction should be based on clinical history, signs, symptoms, non-laboratory studies, and serial determination of CK-MB as well as other laboratory tests.

When the CK value exceeds the upper reference limit, the %CKMB should also be evaluated. In myocardial infarction, the %CKMB usually falls between 3% and 30%. %CKMB of less than 6% may indicate significant skeletal muscle damage (skeletal muscle contains a low percentage CK-MB). %CKMB of greater than 30% may indicate other conditions such as polymyositis or the presence of CK-BB or “macro-CK” (CK complexes or oligomeric mitochondrial CK respectively) which can manifest as false CK-MB elevations. Additional cardiac marker tests are sometimes used to confirm CK-MB results when CK-MB is used to screen for myocardial infarction or when indicated to follow-up %CK-MB results > 30%.
Limitations

1. Specimens with glucose concentrations of less than 31 mg/dL should not be used because the measurement system relies upon endogenous glucose.
2. Do not use hemolyzed or lipemic specimens.
3. Specimens in which CK-MB constitutes 30% or more of the total CK activity should be re-tested using electrophoresis.
4. CARESIDE CKMB cartridges will measure CK-BB giving a falsely elevated result in those patients whose specimens contain CK-BB. These patients may be identified by their characteristic %CK-MB of > 30%.
5. CARESIDE CKMB cartridges will measure type 1 or type 2 “macro-CK” and can result in false positive results based upon CK cutoffs. “Macro-CK” occurs in less than about 3% of hospitalized patients, and can generally be recognized by a lack of the characteristic rise and fall in CK-MB values in serial specimens despite displaying elevated %CKMB.
6. Specimens with levels of ascorbic acid above 5 mg/dL may decrease CKMB results.
7. A result not consistent with the clinical picture should be repeated to confirm.

Performance Characteristics

Detection Limit
The CARESIDE CKMB cartridge demonstrated a detection limit of 5 U/L.

Accuracy
The CARESIDE CKMB agrees with an immunoinhibition and N-acetylcysteine activation method for CKMB.

<table>
<thead>
<tr>
<th>Intercept (U/L)</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
<th>Range of values (U/L)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.84</td>
<td>0.99</td>
<td>1.00</td>
<td>7 to 300</td>
<td>36</td>
</tr>
</tbody>
</table>

Precision

Human serum based controls (representing a low, middle and high CKMB 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>Interferent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>5 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 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)
(310) 338-6767
Fax: (310) 338-6789

CARESIDE, Inc.
6100 Bristol Parkway
Culver City, CA 90230
PI-CKMB-B
07-Jun-2000