

Health-Chem Diagnostics, LLC

3341 S.W. 15th Street – Pompano Beach, FL 33069 - USA – Phone: (954) 979-3845 – Fax: (954) 979-7997
Website: www.healthchemdiagnostics.com

ONE STEP CARDIAC AMI COMBO TEST

*Immunoassay for the Qualitative Determination of
Troponin I and Troponin I/T/C Complex, CKMB and Myoglobin*

INTENDED USE

The **One Step Troponin I & Troponin I/T/C Complex, CKMB and Myoglobin Test** is a panel of rapid immunoassay for the qualitative determination of Tni, CKMB and Myoglobin.

SUMMARY AND BIOLOGICAL PRINCIPLE OF THE ASSAY

Cardiac Troponin I (cTni), CKMB and Myoglobin are three specific protein markers found in cardiac muscles. These proteins and their complexes are released into blood circulation soon after the onset of acute myocardial infarction (AMI) and can be detected in as early as 6 hours and for up to 15 days. Troponin I has a molecular weight of 22.5 kilodaltons. Together with Troponin T (TnT) and Troponin C (TnC), Tni forms a troponin complex in the heart. Tni is found in skeletal muscles (sTni) as well, but it differs in its amino acid composition from cardiac Tni so that these two Tni can be distinguished immunologically. Not only is Tni a specific protein marker for AMI, its elevated level also varies from 6 to 50 ng/ml for 60-80 hours after AMI.

The CKMB is an isoform of the enzyme Creatine Kinase with MW85,745. When heart cells are damaged, it is released into the blood rapidly. The CKMB level of normal serum is less than 5 ng/ml; peak levels of CKMB can be 21 ng/ml or higher.

Myoglobin is a low molecular weight cytoplasm protein. When muscle cells are damaged, it is released into the blood more rapidly than any other myocardial markers; this makes it an important marker for the early diagnosis of AMI. The myoglobin level in normal serum ranges from 30 to 90

ng/ml. Elevated levels could be detected approximately 1 hour after the onset of AMI at 200 ng/ml or higher. A peak as high as 900 ng/ml can be reached within 4-8 hours after the onset of AMI, but that level usually returns to normal within 12 hours.

The **One Step Troponin I & Troponin I/T/C Complex, CKMB and Myoglobin Test** is an easy and fast method that does not require instrumentation such as ECG. The AMI test system uses unique antibodies; one pair selectively identifies free cTni, and one pair selectively identifies cTni-T-C complex with a high degree of sensitivity. The CKMB test system has one pair of antibodies to identify CKMB; there is also one pair of antibodies to identify Myoglobin in the Myo test system.

Principle of the Procedure

The **One Step Troponin I & Troponin I/T/C Complex, CKMB and Myoglobin Test** uses a sandwich immunoassay system and the innumochromatographic detection assay, to be performed in one assay. If cTni/cTni-T-C or CKMB or Myoglobin is present in the test sample in concentrations above the detection level, a labeled specific monoclonal antibody-dye complex forms. This complex is then captured by another specific monoclonal antibody immobilized in the Test Zone (T) of the membrane, producing a visible pink-rose color band on the membrane. The color intensity will depend on the concentration of the antigen in the sample. However, a color band will always appear at the control zone (C).

STORAGE AND STABILITY

1. Store as packaged in the sealed pouch at 2-30°C.

2. The test device must remain in the sealed pouch until time of use.
3. DO NOT FREEZE.
4. Do not use beyond the expiration date.

PRECAUTIONS

1. For professional *In Vitro* Diagnostic use only.
2. The test device should remain in the sealed pouch until time of use. Do not use after the expiration date.
3. All serum or plasma specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
4. The test device should be discarded in a proper biohazard container after testing.
5. Avoid cross-contamination of serum samples by using a new specimen pipette for each sample.

INSTRUCTIONS: SPECIMEN COLLECTION

1. Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods.

Specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C.

2. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

MATERIALS PROVIDED

1. Test cards individually foil pouched with a desiccant
2. Plastic dropper
3. Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

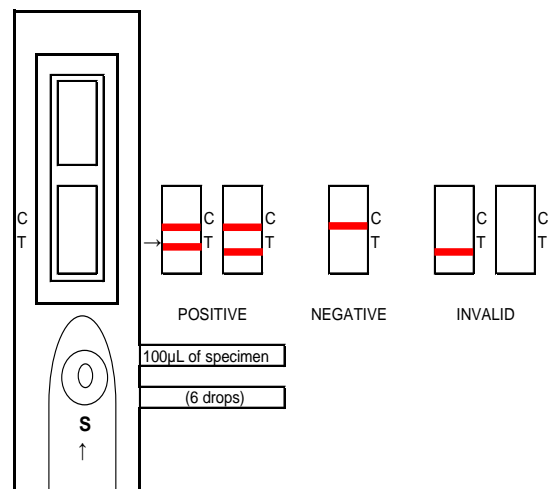
1. Timer/clock
2. Pipette
3. Controls

ASSAY PROCEDURE

1. Read package insert carefully before testing. Allow the test devices, whole blood, serum or plasma to

equilibrate to room temperature (15-30°C) prior to testing. Do not open pouches until ready to perform the assay.

2. Remove the test device from the foil pouch and use it as soon as possible.
3. Place the test device on a clean and level surface. Hold the dropper provided vertically and transfer **6 drops** of specimen to the specimen well (S) in the test device.
4. As soon as the sample reaches the view window, start timing.



5. Read results: Positive results will show up in less than 10 minutes. Negative results need to be read after 15 minutes.

Results must be discarded after 15 minutes due to possible false positives.

INTERPRETATION OF RESULTS

There are 3 test lines (T) and 1 control line ©, as shown below:

C = Control; Myo = Myoglobin;
CKMB = Creatine Kinase MB; Tni = Troponin I

1. **Positive:**

Two colored lines should be observed in the viewing window. The line in the test region (T) is the probe line; the line in the control region (C) is the control line, which is used to validate the test results. The color intensity of the test line may be weaker or stronger than that of the control line.

2. **Negative:**

The control line appears in the test window, but the test line is not visible.

3. **Invalid:**

No line appears in the control region. Under no circumstances should a positive sample be identified until the control line forms in the viewing area. If the control line does not form, the test is invalid and the assay should be repeated.

LIMITATIONS OF PROCEDURE

1. The test is for *in vitro* diagnostic use only.
2. Although the test is very accurate in detecting an elevation of analytes, a low incidence of false positive results can occur, especially in lysis samples.
3. A re-test may be needed after 6-8 hours if only one weak line appeared.
4. Troponin I & Troponin I/T/C Complex, CKMB and Myoglobin Test only provides qualitative result. A quantitative method must be used to determine the cTni concentration.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

The **One-Step Troponin I Test** designed to yield a positive result for cTni concentrations at 0.5ng/ml or greater. The time required for blood cTni level to reach the upper limit of normal has been found to be 4-6 hours after the onset of symptoms. CTni level reaches the maximum concentration after 12-24 hours of the onset, and then remains elevated for 6-10 days in some cases. Therefore, a negative result within the first hours of the onset of symptoms does not rule out AMI with certainty. If suspected, repeat the test at appropriate intervals.

PERFORMANCE CHARACTERISTICS

Sensitivity & Precision

The sensitivities of the One-Step cTni test are as follows:

- Tni: 1 ng/ml
- CKMB: 5 ng/ml
- Myoglobin: 70 ng/ml

The precision of the One-Step cTni test was determined using replicate assays of samples from three different patient pools, with kits from three different production lots. Each specimen sample was run through ten parallel assays. The data demonstrated 100% precision for the duplicates of each sample and 100% precision using the test kits from different lots.

Accuracy

Cut off	Special
0.5ng/ml	99.9%
0.2ng/ml	97%
0.1ng/ml	93%

Interference testing

The following substances were added to the negative control and 0.5ng/ml Troponin I spiked serum samples. No interference was found with any of the substances at the following concentrations;

Substance	Concentration
Bilirubin	10 mg/ml
Cholesterol	800 mg/ml
Hemoglobin	250 mg/ml
Triglyceride	250 mg/ml
sTnI	1000ng/ml
cTnT	1000ng/ml
cTnC	1000ng/ml
sTnI	1000ng/ml

Manufactured in the USA by:
HEALTH-CHEM DIAGNOSTICS LLC,
US FDA & ISO Certified Facilities
3341 SW 15th STREET, POMPANO BEACH, FL - USA
www.healthchemdiagnostics.com
Certified ISO CMDCAS 13485:2003



FM77504 - Quality Award
FDA Registration No.: 1048532