CARDIAC TROPONIN I (cTnI) RAPID TEST CASSETTE
Troponin I immunochromatographic rapid test in whole blood, serum or plasma samples

I. PRINCIPLE

Troponin I (TnI) is one of the thin filament-associated regulatory proteins of muscle (1). It is encoded by three different genes that are differentially expressed by the various muscle tissues, resulting in slow – and fast skeletal and cardiac TnI isoforms (2). The unique amino acid sequence of cTnI makes it an ideal candidate for the laboratory detection of acute myocardial infarction (AMI) and has facilitated the development of monoclonal antibodies that do not cross-react with skeletal muscle troponins (3). Published studies from various groups have demonstrated the utility of cTnI measurement for detection of AMI (3, 4, 6, 7). CK-MB and cTnI both elevated beyond normal reference limits within 4-6 hours after infarction. Typical reference limits were, as reported by Bodor *et al* (3), 6.7 ng/mL for CK-MB and 3.1 ng/mL for cTnI. Likewise, each report sites similar time frames for the peak values of CK-MB and cTnI : CK-MB peaked in 13-15 hours, cTnI in 11-15 hours. Typical ranges were 39 –185 ng/mL for CK-MB and 18.5 –188 ng/mL for cTnI (5). However, CK-MB level returns to normal after 36-48 hours, while levels of cTnI remains elevated for up to 6-10 days. The level of cTnI is very low in normal healthy people, and not detected in patients with skeletal muscle injury. Therefore, cTnI is a specific marker for diagnosis of AMI.

TROPONIN I - (cTnI) is a rapid qualitative assay for the detection of cardiac troponin I in serum, plasma or whole blood samples. The method employs a unique combination of monoclonal dye conjugate and polyclonal solid phase antibodies to identify troponin in the test samples with a high degree of sensitivity. As the test sample flows through the absorbent device, the antibody-dye conjugate binds to the troponin forming an antibody-antigen complex. This complex binds to the anti troponin antibody in the reaction zone and produces a pink-rose colour band when troponin concentration is higher than 1ng/mL. If the concentration of troponin is lower than 1ng/mL, there is no line in the reaction zone. The reaction mixture continues flowing through the absorbent device past the reactive zone and control zone. Unbound conjugate binds to the reagents in the control zone, producing a pink-rose colour band, demonstrating that the reagents are functioning correctly.

II. TROPONIN I - (cTnI) KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- TROPONIN I - (cTnI) test units	10	20
2- Disposable plastic pipettes	10	20
3- Diluent in dropper bottle	3 mL	5 mL
4- Instructions leaflet	1	1

5- Positive control (optional): a freeze-dried preparation is optionally available, as a positive control (1 x 0.25 mL). It should be reconstituted with 0.25 mL of distilled water and produces an assay result equivalent to that produced by positive specimens (i.e. pink color) and should be kept at +2°C to +8°C after reconstitution.

III. STORAGE AND STABILITY

1- All TROPONIN I - (cTnI) kit components should be stored at room temperature (+4°C to +30°C).
2- Do not freeze the test kit.
3- TROPONIN I - (cTnI) is stable until the expiry date stated on the package label.

IV. PRECAUTIONS

1- For *in vitro* diagnostic use and professional use only.
2- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1 % solution of Sodium hypochlorite for one hour before disposal.
3- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
4- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
5- Avoid any contact between hands and eyes or nose during specimens collection and testing.
6- Do not use beyond the expiry date which appears on the package label.
7- When the test is performed with whole blood samples, only fresh samples should be used (< 4 hours).
8- Do not use a test from a damaged protective wrapper.

V. SPECIMEN COLLECTION AND PREPARATION

Serum, plasma (citrate, EDTA or heparin) or whole blood.
1- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid heamolysis).
Each specimen should be treated as if potentially infectious.
2- Whole blood samples should be tested immediately (< 4 hours).
3- If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.

CE

VI. ASSAY PROCEDURE

a) Samples

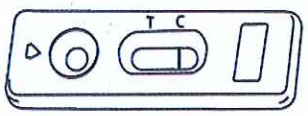
1- Allow samples and TROPONIN I - (cTnI) test devices to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- Label device with the patient's name or control number.
4- Fill the serum dropper with specimens (serum, plasma or whole blood) and by holding it vertically, dispense one drop (25µL) of serum or plasma into sample well (▷). If whole blood is used, dispense 2 drops (50 µL) into sample well (▷) **and wait for the whole blood sample to be completely absorbed before adding diluent.**
5- Add exactly 4 full drops of diluent (150 µL) into the sample well (▷).
6- Read the results between 15 and 20 minutes after the sample dispensing.

b) Positive control (optional)

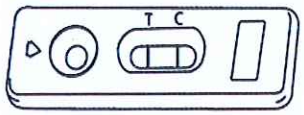
1- Remove the test device from the pouch .
2- After reconstitution, add 25 µL of the positive control into the sample well of the reaction device.
3- Using the dropper bottle, add exactly 4 full drops of diluent in the sample well.
4- Read the results of the test after 15 to 20 minutes.

VII. READING TEST RESULTS

Negative
One coloured band appears in the control zone (C).



Positive
Two clearly distinguishable bands appear indicating a positive result.



Inconclusive: If no band at all appears the test is inconclusive. In this case, it is recommended that the test be repeated immediately or a fresh specimen obtained and tested within 4 hours.

VIII. PERFORMANCES CHARACTERISTICS

A) Sensitivity
Negative human sera (pre-assayed using the STRATUS-DADE analyser) were spiked with cTnI either complexed (I.T.C.) or purified at different concentrations.
The results show that TROPONIN I - (cTnI) is able to specifically detect the cTnI at a minimum concentration of 1 ng/mL.
The protein is equally recognized either in troponin complexed form (T.I.C. or I.C.; T.I.C.: ternary complex cTnT, cTnI, TnC; I.C.: binary complex cTnI, TnC) or in purified form (7).

B) Specificity
Negative sera assayed using the STRATUS-DADE analyser were found constantly negative using TROPONIN I - (cTnI).
No cross-reaction has been observed with the skeletal muscle Troponin I.

C) Interferences
No interference was observed with the following substances:

- Bilirubin (10 mg/dL)
- Hemoglobin (250 mg/dL)
- Triglyceride (1 000 mg/dL)

No interference was observed on negative serum containing CRP concentrations up to 96 mg/mL and RF concentrations up to 3,072 IU/mL.
Whole blood and plasma samples with different anticoagulants were assayed indicating no matrix effect for citrate, EDTA and heparin.

D) Hook effect
No hook effect has been observed up to 5 µg/mL both for complexed and purified form of Troponin I.

E) Precision
1- Intra-assay
Within run precision was determined by using 5 replicates of blood or serum samples, either positive or negative for Troponin I. The negative and positive values were correctly identified 100% of time.

2- Inter-assay
Between run precision was determined by using the same Troponin I specimens in three different lots of reaction devices. Again, the negative and positive values were identified 100% of time.

IX. LIMITATIONS

1- As for any diagnostic procedure, the physician should confirm the data obtained using this test by other clinical methods.
2- TROPONIN I - (cTnI) is designed to yield a positive result for cTnI concentrations at 1.0 ng/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, 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.
3- TROPONIN I - (cTnI) is only providing qualitative results.
4- **Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples.**
5- In case of high RF (rheumatoid factor) or CRP (C-reactive protein) concentrations (high levels indicate acute infections), the test could exceptionally show a positive result.
6- In case of delayed reading time, i.e. over 20-25 minutes, the test could also show sometimes positive results.
7- The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However, high level of HAMA could give falsely positive results.

X- BIBLIOGRAPHY

1- **Perry S.V.** The regulation of contractile activity in muscle. Biochem Soc Trans 1979; 7; 593-617.

2- **Bucher E.A., Maisonpierre P.C., Konieczny S.F., Emerson C.P. Jr.** Expression of the Troponin complex genes : transcriptional coactivation during myoblast differentiation and independent control in heart and skeletal muscles. Mol Cell Biol 1988;8 : 4134-42.

3- **Bodor G.S., Porter S., Landt Y., Ladenson J.H.** Development of monoclonal antibodies for an assay of cardiac Troponin-I and preliminary results in suspected cases of myocardial infarction. Clin Chem 1992; 38: 2203-14.

4- **Cummins B., Auckland M.L., Cummins P.** Cardiac-specific Troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J 1987; 113 : 1333-44.

5- **Bodor G., Porter S., Ladenson J.** Human cardiac Troponin I measurement in suspected myocardial infarction with a double monoclonal antibody sandwich ELISA. Clin. Chem. 1990, 36 (6) : 1103.

6- **Apple F.S., Wu A.** Myocardial infarction redefined : role of cardiac troponin testing. Clinical Chemistry. Vol. 47 no.3. 2001.

7- **Bates K.J., Hall E.M. et al.** Circulating Immunoreactive Cardiac Troponin Forms Determined by Gel Filtration Chromatography after Acute Myocardial Infarction. Clinical Chemistry. Vol. 56 no.6. 952-958.2010.

Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by	2	Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged		Manufacturer		Consult Instructions for Use

Hangzhou AllTest Biotech Co., Ltd.
#550, Yinhai Street
Hangzhou Economic & Technological Development Area
Hangzhou - 310018, P. R. China
www.alltests.com.cn

CE

EC REP
MedNet GmbH
Borkstrasse 10
48163 Muenster
Germany