TROPONIN I - CHECK-1

Quantitative determination of Troponin I in plasma or serum samples

Ref. 28071E (20 tests) / Ref. 28071E-10T (10 tests)

FOR EASY READER® AND EASY READER+® USE ONLY

I- INTENDED PURPOSE

The TROPONIN I -CHECK-1 is a rapid screening test for the detection of cardiac troponin I protein in whole blood, plasma or serum samples to be used as a tool by medical healthcare professionals assessing myocardial ischemia disorder such as acute myocardial infarction (AMI). The sole measurement of concentration or determination of troponin I is not sufficient to diagnose acute coronary syndrome or associated cardiac complications such as myocardial infarction.

II- 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, 8).

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 cTnl.

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-CHECK-1 is a rapid quantitative assay for the detection of cardiac Troponin I in plasma or serum 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 (T) and produces a pink-rose colour band.

In absence of troponin, there is no line in the reaction zone (T). The reaction mixture continues flowing through the absorbent device past the reactive zone (T) and control zone (C). Unbound conjugate binds to the reagents in the control zone (C), producing a pink-rose colour band, demonstrating that the reagents are functioning correctly.

III. TROPONIN I-CHECK-1 KIT COMPONENTS

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

1- TROPONIN I-CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes :	10	20
3- Instructions leaflet:	1	1

IV. STORAGE AND STABILITY

1- All TROPONIN I-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C).

2- Do not freeze the test kit.

3- TROPONIN I-CHECK-1 test is stable until the expiry date stated on the package label.

V. PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.

2- Read carefully the instructions before using this test.

3- 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.

4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

6- Avoid any contact between hands and eyes or nose during specimen collection and testing.

7- Do not use beyond the expiry date which appears on the package label.

8- Do not use a test from a damaged protective wrapper.

VI. SPECIMEN COLLECTION AND PREPARATION

1- TROPONIN I rapid test is performed on human serum or plasma.

2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).

3- If anticoagulant is needed, only citrate, EDTA or heparin should be used.

4- Each specimen should be treated as if potentially infectious.

5- 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.

6- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of diluting buffer (not provided but available upon request) before testing.

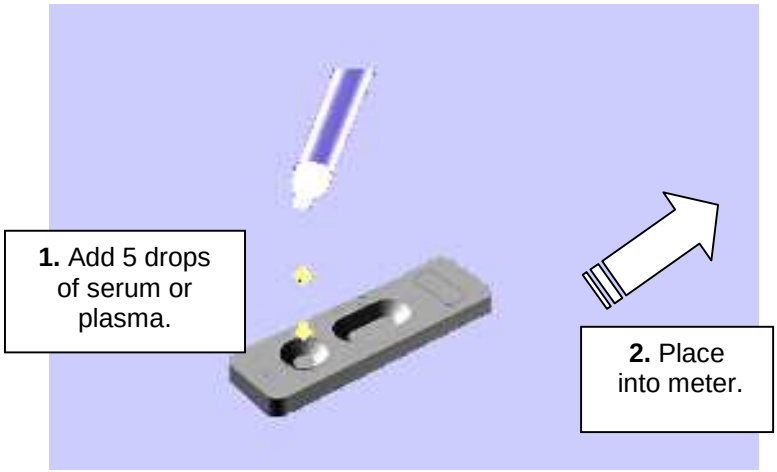
CE

VII. ASSAY PROCEDURE

<u>IMPORTANT</u>: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

<u>Follow the below instructions or refer to the picture n°1.</u>

1- Allow samples and TROPONIN I-CHECK-1 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 specimen and, by holding it vertically, dispense 5 drops (150 µL) of serum or plasma into sample well (▷).
5- Read the result **(in ng/mL**) after 20 minutes either using the immediate or countdown reading mode (see corresponding leaflet).
For general instructions describing how to use the VEDALAB's rapid test readers, refer to the corresponding leaflet.



Picture n°1

VIII. PERFORMANCES CHARACTERISTICS

a) Linearity

The linearity measuring range is 0-50 ng/mL.

b) Accuracy

1- <u>Standards</u>

A study has been performed on serum or plasma samples using five concentrations of DADE-BEHRING calibrators determined in duplicate on TROPONIN I-CHECK-1 rapid tests. The results show the good correlation of the values obtained with TROPONIN I-CHECK-1 on VEDALAB's reader.

Correlation figures : y = 1.159 x - 0.2435
r = 0.9992

2- <u>Human samples</u>

Using plasma samples, a field study has been performed on 67 patients with diagnosed AMI and 56 other patients with non AMI. The sensitivity and specificity of the TROPONIN I-CHECK-1 have been calculated to be respectively 94 % (CI 95% [87.7 – 98.6]*) and 91.1 % (IC 95% [79.4 – 97.4]*) when an AMI cut-off of 0.8 ng/mL was considered. For an AMI cut-off of 1 ng/mL, sensitivity and specificity have been found to be 91% (CI 95% [80.6 – 96.9]* for sensitivity and CI 95% [79.4 – 97.4]* for specificity).
*CI 95% : 95% confidence interval

c) Sensitivity

The detection limit is lower than 0.5 ng/mL. Any concentration around or higher than 0.8 ng/mL may suggest a possible AMI case (with a sensitivity of 94 % (CI 95% [87.7 – 98.6]*) (7) and further investigations should be made. Troponin I elevated levels are also observed in setting of systemic inflammatory response syndrome (SIRS) sepsis or septic shock (9).
*CI 95% : 95% confidence interval

d) Specificity

Negative sera assayed using the STRATUS-DADE analyser were found constantly negative using TROPONIN I-CHECK-1. No cross-reaction has been observed with the skeletal muscle Troponin I. A field evaluation study (7) has shown a specificity of 91.1 % (CI 95% [79.4 – 97.4]*) for a cut-off of 0.8 ng/mL.
*CI 95% : 95% confidence interval

e) Cross reactions

1- <u>Rheumatoid factor</u>

A negative serum sample spiked with 4 000 IU/mL of rheumatoid factor (RF) did not show any false positive result. Therefore there is no interference of RF in TROPONIN I-CHECK-1

2- <u>HAMA</u>

A negative serum sample spiked with human anti mouse antibodies (HAMA) of type 1 or type 2 did not show any false positive result. Therefore there is no interference of HAMA type 1 or type 2 in TROPONIN I-CHECK-1 at the tested concentration levels.

f) Interferences

1- Hemoglobin, bilirubin and triglycerides

Negative (0 ng/mL), weak positive (2ng/mL) and strong positive (20ng/mL) serum samples spiked wit hemoglobin (final concentration : 1.5 g/L), bilirubin (final concentration : 9mg/L) and triglycerides (final concentration : 2.5g/L) did not show any effect in samples status (positive or negative).

2- Anticoagulants

Plasma samples containing different anticoagulants were assayed indicating no matrix effect of citrate, heparin and EDTA.

g) Hook effect

No hook effect has been observed up to 5 µg/mLof human native troponin I.

h) Intra-assay reproducibility

Within run reproducibility was evaluated performing 25 replicates of three commercialy available references containing repectively 13.6; 30.1 and 70.7 ng/mL of troponin I determined with quantitative TROPONIN I-CHECK-1 test for VEDALAB's reader. The obtained CVs (coefficient of variation) were respectively equal to 8.9; 7.7 and 7.5%.

i) Inter-assay reproducibility

Between run reproducibility was evaluated performing 3 replicates of four serum samples containing 2, 5, 10 and 20 ng/mL of troponin I respectively measured using three different lots of TROPONIN I-CHECK-1 test and VEDALAB's reader.

The obtained CVs (coefficient of variation) were respectively equal to 14.8; 14, 10.8 and 12.3%.

IX. LIMITATIONS

1- As for any diagnostic procedure, the physician should confirm the data obtained using this test by other clinical methods.

2- Any Troponin I concentration close or higher than 0.8 ng/mL may suggest a possible AMI. 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- 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.

4- In case of delayed reading time, i.e. over 20-25 minutes, the test could also show sometimes positive results.

5- 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.

6- This format of test is to be only used with VEDALAB's rapid test readers.

7- If the reading time (20 minutes) is not strictly respected, wrong results will be obtained.

8- This format of test should not be used for visual reading.

9- As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/-25% should be considered for the final value and for the clinical significance of the result.

10- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

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.

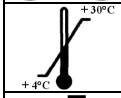
5- **Bodor G., Porter S., Ladenson J.** Human cardiac Troponin I measurement in suspected myocardial infarction with a double monoclonal antibody sandwich ELISA. Clin. Chem. 1990, 36 (6): 1103.

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

7- **Widijanti A., Fatonah S., Hernowati T.E.** Diagnostic value of quantitative rapid test cardiac troponin I in acute myocardial infraction patients. Department Clinical Pathologist Brawijaya University/Saiful Anwar Hospital in Malang Indonesia. 1-9. 2008.

8- **HAJAR,R.** Evolution of Myocardial Infarction and its Biomarkers : A Historical perspective. Heart Views. 17(4) : 167-172. 2016

9- **HUSSAIN, N**. Elevated cardiac Troponins in setting of Systemic Inflammatory Response Syndrome, Sepsis and septic shock. ISRN cardiology. Articles D723435. 2013

	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		

 Manufactured by VEDALAB - France

CHANGES DESCRIPTION

Changes type:

- N/A — Not Applicable (creation)
- Technical change — Addition, revision and/or removal of information related to the product.
- Administrative — Implementation of non-technical changes noticeable to the end-user.

Changes type	Change description
Technical change	- Chap VIII info c) sensitivity+ I) cross reaction (addition) + CI 95%+ e) cross reaction + f) interferences +g) hook effect + h) inter assay+ I) intra assay
Administrative	- Chap I (addition) - Chap X bibliographic references 8& 9 (addition)

Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.