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AMICHECK-TROP I™ WB

Rapid test for Troponin I -WB
DEVICE

INTENDED USE
AMICHECK TROP I-WB™ is a rapid, two-site sandwich immunoassay for the detection of human cardiac Troponin I (cTnI) levels in human whole blood, serum and plasma.

SUMMARY
Discovered by Ebashi, Troponins are regulatory proteins in cardiac muscle that modulate the interaction between actin and myosin, during the calcium-mediated contraction of cardiac muscle. Three distinct tissue specific isoforms of Troponin I have been identified, two in skeletal muscle and one in cardiac muscle. The cardiac isoform of Troponin I (cTnI) has an additional sequence of 31 amino acids at the N terminal end that accounts for cardiac specificity, with a molecular weight of 22.5 kDa. This absolute specificity of Troponin I for cardiac tissue makes it an ideal biomarker for myocardial injury.
Clinical study results have demonstrated that elevated levels of cardiac Troponin I (cTnI) are detectable in blood stream within 4 to 6 hours after the onset of chest pain, reach peak concentration in approximately 12 hours and remain elevated for 3-10 days following acute myocardial infarction. Thus cardiac Troponin I (cTnI) meets all the criterion laid down by National Academy of Clinical Biochemistry (NACB) for an ideal cardiac biomarker in early identification and risk stratification of patients with chest pain suggestive of ischaemia and identification of patients that present after infarction.

PRINCIPLE
AMICHECK TROP I-WB™ utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad contains two components-Agglutinating sera for cTnI conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. As the test sample flows through the membrane assembly of the device, the highly specific Agglutinating sera for cTnI - colloidal gold conjugate complexes with cTnI in the sample and travels on the membrane due to capillary action along with rabbit globulin-colloidal gold conjugate. This sample moves further on the membrane to the test region (T) where it is immobilized by another specific Agglutinating sera for cTnI coated on the membrane leading to the formation of a pink-purple band. A detectable coloured band is formed if cTnI level is equal to or greater than 0.3 ng/ml. The absence of this coloured band in the test region indicates cTnI concentration is less than 0.3 ng/ml.
The unreacted conjugate along with unbound complex if any, move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated on the membrane at the control region (C) forming a pink-purple coloured band. The control band formation is based on the 'Rabbit /Agglutinating sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band acts as a procedural control and serves to validate test results.

REAGENTS AND MATERIALS SUPPLIED
AMICHECK TROP I-WB™ kit contains:

A. Individual pouches, each containing -
   1. DEVICE : Membrane assembly pre-dispensed with Agglutinating sera for cTnI-colloidal gold conjugate, rabbit globulin- colloidal gold conjugate, Agglutinating sera for cTnI and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. Desiccant pouch.

B. PIPETTE : Disposable Plastic Sample Applicator.
C. BUF : Sample running buffer in a dropper bottle.
D. Package Insert.

REF	506010010	506010003
Σ	10T	3T

STORAGE AND STABILITY
The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE. After first opening of the sample running buffer bottle it can be stored between 4°C to 30°C for the remaining duration of its shelf life.

NOTES
1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use beyond expiry date.

4. Do not intermix the reagents from different lots.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
7. Do not re-use the test device.
8. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

1. **AMICHECK TROP I-WB**™ uses human whole blood, serum and plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. **For using whole blood as a sample.** EDTA or Heparin or oxalate can be used as a suitable anticoagulant.
4. Test specimen should be used immediately and should not be frozen. Do not use haemolysed, clotted or contaminated specimens as lipaemic and turbid specimen may lead to erroneous results.

IMPORTANCE OF SEQUENTIAL TESTING

Immediately after a cardiac event, the damaged myocardial cells start releasing cardiac Troponin I (cTnI) in circulation and their level rises in a time specific manner. Since patients present at varying times for testing following the onset of chest pain in a cardiac event, it is necessary to perform sequential testing for optimal diagnostic accuracy.

A protocol for measuring cardiac Troponin I (cTnI) levels requires testing at admission or 3 hours after onset of chest pain and at 6 and 9 hours. Modification may be necessary depending upon specific clinical situation. Hence sequential testing of cardiac Troponin I (cTnI), together with ECG results and patient history and symptoms are necessary for differential diagnosis between acute myocardial infarction and unstable angina pectoris.

The positive and negative likelihood ratios correspond to the clinical concepts of ruling in and ruling out disease. Thus, a higher positive likelihood ratio means that a test result is better for ruling in disease when positive, and a lower negative likelihood ratio means that a test result is better for ruling out disease when negative. Examination of likelihood ratios reveals that levels of cardiac Troponin I (cTnI) are very useful at ruling out AMI when the value is negative at 10 or more hours from the onset of chest pain. However, a negative test value early in the course of episode of chest pain does very little to reduce the likelihood of AMI. A positive cardiac Troponin I (cTnI) value after 6 or more hours after the onset of chest pain appears to be very useful at ruling in AMI. Thus a negative cardiac Troponin I (cTnI) level identifies patient at low risk for adverse cardiac events.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the **AMICHECK TROP I-WB**™ kit components to room temperature before testing.
2. Open the foil pouch by tearing along the "notch".
3. Retrieve the device and desiccant pouch. Check the colour of the desiccant. It should be blue, if it has turned colourless or pink, discard the device and use another device.
4. ***Once opened, the device must be used immediately.***
5. Tighten the cap of the sample running buffer bottle provided with the kit in clockwise direction to pierce the buffer bottle nozzle.
6. Label the device with specimen identity.
7. Place the testing device on a flat horizontal surface.
8. Holding the sample applicator (provided in the kit) vertically, carefully dispense **two drops** of specimen into the sample port 'A'.
9. Add **four drops** of sample running buffer in buffer port 'B'.
10. At the end of **20 minutes,** read results as follows:

	**Negative Result** Presence of a pink-purple coloured band at Control region (C) indicates absence of cTnI.
	**Positive Result** Presence of pink-purple coloured band at the Test region (T) in addition to control band (C) indicates presence of cTnI.
 	**Invalid Result** The test is invalid if no pink-purple coloured band is visible at twenty minutes. The test should also be considered invalid if only test band appears with absence of control band. Verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

AMICHECK TROP I-WB™ detects cardiac Troponin I at a concentration of ≥ 0.3 ng/ml.

Internal Evaluation

The performance of **AMICHECK TROP I-WB**™ was evaluated in comparison with a commercially available rapid test using a panel of 240nos. Troponin I negative specimens (serum, plasma and whole blood) collected from healthy individuals (free from any symptoms of cardiac arrest) and Troponin I positive control obtained from **Fapon International Limited (CTNI Recombinant Antigen, cat # GRNCTNIN101)** diluted to obtain concentrations of ≥ 0.3ng/ml Troponin I. The results of the evaluation were as follows:

SPECIMEN DATA	TOTAL	AMICHECK TROP I-WB™	Commercially Available Rapid Test
Troponin I Negative specimens	**240**	**240**	**240**
No. of serum samples	80	80	80
No. of plasma samples	80	80	80
No. of whole blood samples	80	80	80
Troponin I Positive Control			
1ng/ml to 10ng/ml	90	90	90
<1ng/ml	30	30	30
0.3ng/ml	30	30	30

Based on this evaluation :
Sensitivity of **AMICHECK TROP I-WB**™ - 100%
Specificity of **AMICHECK TROP I-WB**™ - 100%

External Evaluation

In an independent study conducted performance of **AMICHECK TROP I-WB**™ was evaluated using a panel of 120 samples in comparison with a commercially available rapid test. The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	AMICHECK TROP I-WB™	Commercial Rapid Test
No. of specimen tested	120	120	120
No. of Positive specimens	12	12	12
No. of Negative specimens	108	108	108

Based on this evaluation :
Sensitivity of **AMICHECK TROP I-WB**™ - 100%
Specificity of **AMICHECK TROP I-WB**™ - 100%

LIMITATIONS OF THE TEST

1. Sequential testing of cTnI is important for diagnosing patients presenting with an evolving AMI. Diagnosis should not be made based on a single test result.
2. Samples with normal CK-MB levels and positive **AMICHECK TROP I-WB**™ result may occur in a patient with unstable angina pectoris and probably reflects a micro infarct not detected by CK-MB test .
3. Unstable angina pectoris and Non ST segment elevation myocardial infarction(NSTEMI) are closely related cardiac manifestations. Samples with normal CK-MB level and non-diagnostic ECG change, but positive test results indicates a subset of high-risk acute coronary syndrome patients and are classified under NSTEMI.
4. All serum cardiac enzyme markers may be positive with rhabdomyolysis, however cTnI is only slightly elevated despite significant elevations in both CK and CK-MB test.
5. cTnI levels may rarely rise in skeletal muscle disorders and renal failure.
6. cTnI levels may rise in other cardiac conditions causing myocardial damage namely myocarditis, cardiac contusion, recent cardiac surgery or catheterization.
7. cTnI is present only in cardiac tissue; cTnI levels in blood stream are extremely low in normal healthy individuals.
8. cTnI levels are elevated upto 8 days, hence re infarction may not be detected.
9. Interference due to heterophile antibodies, rheumatoid factors and other nonanalyte substances in test sample, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **AMICHECK TROP I-WB**™ uses sufficient amount of blocking agents to inhibit majority of these interference, nevertheless, some samples with high titers may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogeneous artifact and lead to appropriate investigation.
10. Presence of a band at the test region even if low in intensity or formation is a positive result .

11. The deliberate slow reaction kinetics of **AMICHECK TROP I-WB**™ is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.
12. 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 laboratory findings have been evaluated.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Do not reuse
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	BUF — Sample Running Buffer
Use by	REF — Catalogue Number	DEVICE — Device	Do not use if package is damaged
Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	PIPETTE — Disposable Plastic Sample Applicator	
EC REP — Authorised Representative in the European Community	Xn NaN, R22, S23-46-61 — Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.		

Manufactured by:

Zephyr Biomedicals

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EC REP

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