# **RESPONSE** BIOMEDICAL

## INSTRUCTIONS FOR USE

## C1101-1.3

# **RAMP®** Troponin I

### WARNING!

For in vitro diagnotic use only Failure to follow RAMP® test procedures may result in invalid and/or erroneous results. Read the entire Instructions For Use prior to performing test.

## INTENDED USE

The RAMP® Troponin I Assay is a quantitative immunochromatographic test indicated for use as an in vitro diagnostic product used to measure cardiac troponin I levels in EDTA whole blood. Measurement of cardiac troponin I aids in the rapid diagnosis of acute myocardial infarction (AMI). The RAMP® Troponin I Assay is intended to be used only to prioritize patient management for those suspected of AMI.

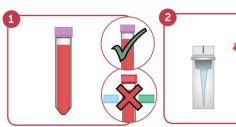
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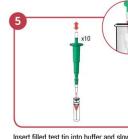
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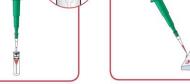
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## **Running a test**



Collect EDTA whole blood sample for testing. Prepare instrument to run test.



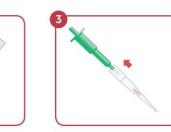


Insert filled test tip into buffer and slowly depress plunger 10 times to fully mix.

Transfer 75 µL of mixed sample into test cartridge well.

Place buffer vial upright on level surface

and remove cap.



to the transfer device.

read result.

Open foil pouch and firmly attach test tip Depress plunger and insert test tip into EDTA whole blood sample. Gently release plunger to draw blood into test tip.



### Discard all used components.

Immediately insert cartridge into RAMP® instrument port. When test is finished,

#### SUMMARY AND EXPLANATION

Troponin is the contractile regulatory protein of striated muscles. This protein complex is comprised of three distinct polypeptides that are involved in calcium regulation: troponin C, I and T. Troponin I (Tnl) is the subunit that inhibits actomyosin ATPase activity [1,2]. The cardiac isoform of TnI is not expressed in any type of skeletal muscle and is tissue- specific for the myocardium, making it an excellent biochemical marker for detection of myocardial injury [2-5]. Studies of patients with AMI have demonstrated early release of TnI into the blood stream after the onset of chest pain, reaching peak concentrations at 14 to 36 hours. The levels remain elevated for 3 to 7 days after infarction [5,6]. Measurement of TnI levels therefore provides a sensitive and specific determination of myocardial injury over a wide time window

Elevated levels of cardiac-specific troponins convey prognostic information beyond that supplied by the patient's clinical signs and symptoms, the electrocardiogram (ECG) at presentation, and the pre-discharge exercise test [7]. Antman, et al. reported that patients with elevated levels of TnI had a statistically significant increase in mortality (p<0.001) when compared to patients without TnI elevations [8]. Further, the study provided data supporting a quantitative relationship between the TnI level and the risk of death in acute coronary syndrome (ACS) patients (p<0.001). Additional work has demonstrated increases in other non-fatal cardiac events such as non-fatal myocardial infarction, congestive heart failure, and urgent revascularization with increasing levels of TnI [9-11].

The ability of TnI to be measured at the low-end of the concentration range allows therapeutic intervention to be considered at any elevation above the normal range. Patients that present with no ST-elevation on their ECG but who have even a slight elevation in TnI or TnT may receive a greater treatment benefit from certain drugs such as GP IIb/IIIa inhibitors or low molecular weight heparins [12-14].

Other conditions such as blunt trauma or myocarditis that is not secondary to ischemic coronary artery disease can also lead to myocardial injury and result in increased TnI blood levels. These clinical factors should be considered when interpreting test results, and the TnI levels should be used in conjunction with clinical signs and symptoms and ECG changes [7].

#### TEST PRINCIPLE

The RAMP® Troponin I test is a quantitative immunochromatographic test for the determination of TnI in EDTA whole blood. The EDTA whole blood is mixed with buffer and antibody-coated, labeled particles, and applied into the sample well of the test cartridge. The red blood cells are retained in the sample pad, and the separated plasma migrates along the strip. Fluorescent-dyed particles coated with anti-TnI antibodies bind to TnI, if present in the sample. As the sample migrates along the strip. Thi-bound particles are captured at the detection zone, and excess fluorescent-dyed particles are captured at the control zone.

The RAMP® instrument then measures the amount of fluorescence emitted by the complexes bound at the detection zone and at the control zone. Using a ratio between the two fluorescence values, a quantitative reading is calculated. For further information on the use of the instrument, refer to the RAMP® Operator's Manual.

#### REAGENTS

- The RAMP® test kit contains all the reagents necessary for the quantification of Troponin I in EDTA whole blood.
- The sample buffer contains phosphate buffer, animal protein, surfactant, and ProClin® 300 / ProClin<sup>®</sup> 950 as preservatives.

#### WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use, For US customers, the RAMP® Troponin I test must be operated in a laboratory setting when used with the RAMP® 200.
- For use by gualified personnel per local, state, or Federal regulations or accrediting agency requirements
- Read the entire instructions for use (IFU) prior to use. Directions should be read and followed carefully, or invalid or erroneous results may occur.
- Do not interchange or mix components of different RAMP® tests, RAMP® lots or components from other manufacturers
  - Do not use the kit or any kit component beyond the stated expiry date
- Do not use any visibly damaged components
- Do not insert a cartridge on which blood or any other fluid is spilled into the instrument. .
- Disposal of all waste materials should be in accordance with local guidelines.
- . Exercise standard precautions required for handling all laboratory reagents and patient samples
- The device contains material of animal origin and should be handled as a potential biohazard
- The sample buffer provided contains ProClin®, a potential skin sensitizer. Avoid spilling or splashing reagents containing ProClin® on skin or clothing. In case of contact, thoroughly flush with water.

#### STORAGE AND STABILITY

Store at 2 to 8°C (35 to 46°F). Do not freeze.

#### Stability

Unopened at 2 to 8°C (35 to 46°F)	Up to the stated expiration date
When stored at 15 to 25°C (59 to 77°F)	14 days

#### SAMPLE COLLECTION & PREPARATION

- Use ONLY EDTA Whole Blood (Plastic K2EDTA tubes are recommended). Other sample types and anticoagulants have not been evaluated
- Avoid blood samples that show gross hemolysis as these may interfere with the test and cause erroneous results. If this occurs, another blood sample should be obtained and tested
- Testing must be completed within 2 hours of phlebotomy. However, if this is not possible, the EDTA whole blood can be stored for up to 2 days at 2 to 8°C. If stored, allow blood samples to equilibrate to 18 to 25°C for at least 15 minutes prior to use.

#### MATERIALS PROVIDED

- 25 pouches, each containing 1 RAMP® test cartridge and 1 test tip
- 25 RAMP<sup>®</sup> buffer vials
- 1 transfer device for 75 ul
- 1 lot card
- 1 instructions for use (IEU)

#### MATERIALS REQUIRED (BUT NOT PROVIDED)

- REF: C1100 RAMP® Reader instrument: or
- REF: C2100 RAMP<sup>®</sup> 200 instrument control module, and ٠ REF: C3100 RAMP® 200 instrument test module
- ٠ REF: C2003 RAMP® Cardiac Controls (optional)
- Optional accessories such as RAMP<sup>®</sup> printer and/or barcode scanner
- Specimen collection tubes: EDTA (Venous Whole Blood)
- Use only the listed RAMP® instruments with this test.

#### LOT CARD CALIBRATION

Each RAMP® test kit includes a lot card that is individually packaged in an anti-static pouch. The lot card provides information specific to the kit test cartridge lot, including lot number. expiration date, and standard curve information. For further details on loading lot-specific information, see the RAMP® instrument Operator's Manual. No additional calibration beyond insertion of the lot card is necessary. This operation is required only once per test kit lot.

For each new lot, remove the lot card from its pouch and insert it into the lot card slot on the instrument. Once the lot card has been uploaded, return to its pouch and do not discard. Avoid touching the contacts at the end of the lot card.

#### PROCEDURE

Prior to sample preparation allow all components to come to room temperature for at least 15 minutes

- Keep the test cartridge and test tip in the sealed foil pouch until ready for use. Once opened, test cartridges and test tips must be used or discarded within 60 minutes.
- The test cartridge, test tip, and buffer vial should be discarded after a single-use. Do not ٠ reuse
- Prepare RAMP<sup>®</sup> instrument for test cartridge, Refer to the RAMP<sup>®</sup> Operator's Manual 1. for detailed instructions on Starting a Test.
- Ensure that the EDTA whole blood sample is well mixed by gentle inversion.
- 3 Uncap the buffer vial and place upright on a clean, dry level surface, or in a holder.
- 4. Open a test pouch and remove the test cartridge and tip. Place the test cartridge on a clean, level surface. Firmly attach the test tip to the supplied transfer device.
- 5. Before inserting the test tip into the sample, fully depress the transfer device plunger. Insert tip into sample and fully release plunger. The test tip should fill with 75 µL of 6
- blood
- 7. Immediately transfer the filled test tip into the buffer vial close to, but not touching, the hottom
- 8. Mix sample slowly by fully pressing and releasing the plunger 10 times; while keeping the tip submerged in the buffer for optimal mixing and to minimize air bubbles.
- Once mixing is complete, draw 75 μL of sample into the test tip by releasing the plunger one final time and immediately dispense liquid into the sample well of the test cartridge. Small droplets may remain in the tip: this is expected.
- 10. Immediately insert the test cartridge fully into the instrument and press until firm resistance is felt
- 11. The instrument will draw the cartridge in and test development will begin.
- 12. The instrument will analyze the cartridge and report the result in approximately 19 minutes
- 13. Record the result, if required. For additional information on printing and/or uploading results, please refer to the Operator's Manual.

 $(\epsilon)$ IVD

Remove the used test cartridge and discard all used test components according to local 14. biohazard procedures. DO NOT reuse

For additional information on the general operation and troubleshooting of the instrument, please refer to the RAMP® Operator's Manual.

#### QUALITY CONTROL

Refer to the RAMP® Operator's Manual for full details on guality control operation and troubleshooting.

#### SYSTEM QUALITY CONTROL

The RAMP® instrument has error checking and self-diagnostic functions (Internal Quality Control (IQC)) that assure system integrity. These include algorithms and measurements used to confirm acceptable operator technique, sample handling, and test performance. Frequency of IQC may be programmed at desired intervals. Valid results are displayed only after all performance requirements have been met.

#### PROCEDURAL CONTROLS

- Each RAMP® test has built-in controls. Test cartridges have a control zone that is scanned as part of the test protocol to ensure proper sample flow
- Control limits for each lot of test cartridges are established during the manufacturing process and are incorporated in the test-specific lot parameters. If a control result does not meet specifications, the sample result is not reported and a message is displayed.

#### LIQUID QUALITY CONTROL (LQC)

- It is recommended that quality control materials be run with the RAMP® test in conformance with Federal, state and local requirements for quality control testing.
- While the running of commercial control materials are recommended, it is not a requirement to use, or assure, performance of the RAMP® test unless specified by local regulations or institutional requirements.
- To run a LQC sample, follow the instructions under the "Procedure" section in this IFU. Treat the control as a whole blood sample

#### TEST RUN MESSAGES

When the RAMP® instrument is unable to continue a specific task it will emit an audio alarm and display a message. Refer to the RAMP® Operator's Manual 'Troubleshooting Guide' section for a full description of all messages. If repeated tests give unexpected results, contact Response Biomedical Technical Support for assistance

#### LIMITATIONS

- For diagnostic purposes, the patient's medical history, clinical examination and other findings should always be assessed in conjunction with the RAMP® test results. A test result that is inconsistent with the clinical signs and symptoms should be interpreted with caution; the results of the RAMP® TnI test are not to be used to classify the extent of myocardial necrosis.
- Factors such as technical or procedural errors or the presence of substances in blood specimens other than those that have been evaluated (see Interference section of this IFU), may interfere with the RAMP® test and cause erroneous results.
- As with any immunoassay, patient specimens may contain heterophilic antibodies that may result in either falsely elevated or depressed results. Presence of these antibodies may be due to elevated levels of rheumatoid factor, treatment with mouse monoclonal antibodies for diagnostic or therapeutic purposes, or other undetermined factors. The RAMP® test has been formulated to reduce the effects of heterophilic antibodies, but complete elimination of heterophilic interference from all samples cannot be guaranteed
- Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner (U.S. only).

#### TEST CUT-OFF AND EXPECTED VALUES

One hundred and eighty (180) normal individuals were enrolled in the Expected Values clinical trial. The 99% reference range of results was < 0.10 ng/mL for the RAMP® Troponin I test.

Each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, determine its own reference ranges. The RAMP® Troponin I test is intended to be used only to prioritize patient management for those suspected of AMI

#### PERFORMANCE CHARACTERISTICS

#### MEASUREMENT RANGE

0.10 to 32 ng/mL.

Tnl values below the 20% functional sensitivity should be reported as less than < 0.10 ng/mL instead of the numerical value. TnI levels in excess of 32 ng/mL are reported as greater than > 32 ng/mL.

#### HOOK EFFECT

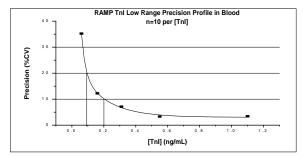
No high dose hook effect was observed for the RAMP® Troponin I test up to the highest level tested (500 ng/mL Tnl).

#### DETECTION LIMIT

The lower limit of detection (LLD) is defined as the analyte concentration corresponding to the mean (n=20) plus 2 standard deviations of the zero. The LLD of the RAMP® Troponin L test is 0.03 ng/mL TnI, the lowest TnI level that can be distinguished from zero.

Another characteristic of an analytical measurement is the functional sensitivity, which is defined as the TnI level at which the test method displays a particular percent coefficient of variation (%CV). Estimates of the 20% and 10% functional sensitivities for the RAMP® Troponin I test were determined from whole blood estimates. The 20% and 10% functional sensitivities are 0.10 ng/mL and 0.21 ng/mL Tnl, respectively. Tnl values below the 20% functional sensitivity should be reported as less than (<) 0.10 ng/mL, instead of the numerical value.

#### Tnl Low Range Precision Profile in blood, n=10 per [Tnl]

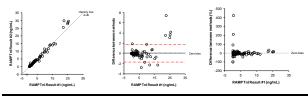


#### PRECISION

The within-run and total precision of the RAMP® Troponin I test were determined using the NCCLS EP-5 protocol by one operator assaying duplicates of control materials and human plasma pools twice each day over 10 days. The mean, standard deviation and % CV were calculated for each reported concentration of TnI.

	Tnl Standards					
	Mean Concentration [ng/mL]					
	5.01	1.05	0.70	0.40	0.29	0.22
Within-run [%]	8.3%	8.7%	6.5%	5.3%	9.3%	7.2%
Total [%]	8.3%	10.0%	8.4%	7.4%	10.0%	11.4%

#### **Clinical Site Evaluations of Analytical Performance:** Standard Error of the Estimate between Runs



n	128	
bias	0.084	
95 % CI	-0.104 to 0.271	
95 % limits of agreement		95 % CI
lower	-2.014	-2.331 to -1.697
upper	2.181	1.864 to 2.498

One hundred and eighty-four (184) subjects were enrolled in the precision study. Of these, 55 were normal individuals (28 males and 27 females) and 129 were patients suspected of AMI based on the individual hospital criteria (76 males and 53 females). The samples were selected from those obtained during the method comparison clinical trial. The samples were stored refrigerated for up to one day between analyses. The data were reviewed and one outlier was removed

Correlation (linear regression) for replicate Result 2 vs Result 1 for RAMP® Troponin I is presented below. The standard error of the estimate is Sy.x = 0.94.

Population	n	Sy.x	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Combined	183	0.79	1.086	-0.153	0.989
Suspect AMI	128	0.94	1.093	-0.246	0.988

#### LINFARITY

Th antigen concentrations of 0.86, 1.72, 3.44, 6.88, 13.75, and 27.50 ng/mL were prepared in normal donor EDTA blood. The linearity and percent recovery were determined by assaying

five replicates of each concentration and baseline. The mean, standard deviation and %CV of replicates were calculated for each concentration. Linear regression analysis of actual Th concentration versus expected TnI concentration resulted with an R = 0.997 and a slope of 1.019 with an offset of 0.279. The recovery of spiked TnI antigen at the five concentrations ranged from 95 to 115% with an average of 105%.

#### INTERFERENCE

Potentially interfering substances were evaluated by spiking different concentrations of potential interferents into normal donor EDTA whole blood with Tol added. Different blood samples were used for each potential interferent. Interference was evaluated by calculating the TnI concentration of potential interferent-spiked blood, expressed as a percentage of the Thi concentration of the un-spiked (no potential interferent) blood sample. No evidence of cross-reactivity or interference was observed for hemoglobin, triglyceride, bilirubin, cholesterol, or heparin at levels of up to 1500 mg/dL, 3000 mg/dL, 80 mg/dL, 500 mg/dL, and 66 IU/mL, respectively. No trend was observed in the TnI predictions as the concentration of potential interferent was increased.

#### ANALYTICAL SPECIFICITY

Potentially cross-reactive substances were evaluated by spiking different concentrations of each potential cross-reactant into normal donor EDTA whole blood. Skeletal Troponin I, Cardiac Troponin T and Cardiac Troponin C all tested up to 1000 ng/mL appear to have no cross-reactivity with the RAMP® Troponin I test. Human anti-mouse antibodies (HAMA), human anti-goat antibodies (HAGA), human anti-rabbit antibodies (HARA) and Rheumatoid Factor (RhF) appear to have minimal cross-reactivity with the RAMP® Troponin I test.

### CLINICAL EVALUATIONS

#### METHOD COMPARISON

365 subjects were enrolled in the method comparison clinical trial. Of these subjects, 180 were normal individuals (84 males and 96 females) and 185 were suspected of acute myocardial infarct (AMI) based on the individual hospital criteria (115 males and 70 females). EDTA and heparin whole blood samples were obtained for each of these subjects. All normal subjects were consented. Waste samples were used for the subjects suspected of AMI. An aliquot of the EDTA whole blood sample was taken for the RAMP® Troponin I test and heparinized plasma was prepared for the Dade Behring Dimension Cardiac Troponin-I Flex Assay. To accommodate the differing reportable ranges of the RAMP® Troponin I and the Dimension Cardiac Troponin-I Flex Assay, the data was winsorized, and then examined for outliers. One outlier was removed from the suspect AMI population. The correlation data is presented in the table below

Population	n	Sy.x	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Combined	364	0.94	0.456	0.011	0.988
Suspect AMI	184	1.33	0.456	0.025	0.986

#### **CLINICAL SENSITIVITY & SPECIFICITY**

The sensitivity, specificity, and percent agreement of all samples were calculated comparing a clinical cutoff of 0.3 ng/mL TnI for the RAMP® Troponin I test to the published clinical cutoff of 0.6 ng/mL TnI presented in the Dade Dimension package insert. The RAMP® Troponin I test demonstrates good sensitivity, specificity, and percent agreement when compared with this reference method. The data is presented in the table below.

	n	[%]	s.e.ª	95% Cl <sup>b</sup>	
Sensitivity	136	94.85	1.90	91.14	98.57
Specificity	229	98.25	0.87	96.56	99.95
PV <sup>c</sup> +	133	96.99	1.48	94.09	99.90
PV -	232	96.98	1.12	94.78	99.18
Concordance	365	96.99	0.90	95.23	98.74

a) s.e = Standard error b) CI = Confidence interval c) PV = Predictive value

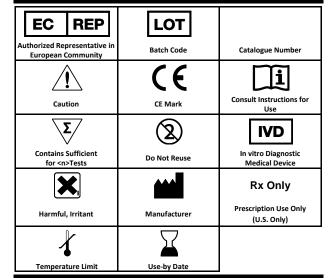
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#### GLOSSARY OF SYMBOLS



#### **PRODUCT SUPPORT / ASSISTANCE**

If you have any questions regarding the use of this product please contact Response Biomedical Corp. Technical Support:

- Within US or Canada (+1.866.525.7267)
- Outside US or Canada (+1.604.219.6119)
- By email at techsupport@responsebio.com

#### MANUFACTURER

## Sector Response Biomedical





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