RESPONSE BIOMEDICAL

INSTRUCTIONS FOR USE

C1103-1.5

RAMP® Myoglobin

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® Myoglobin Assay is a quantitative immunochromatographic test indicated for use as an *in vitro* diagnostic product used to measure myoglobin levels in EDTA whole blood. Measurement of myoglobin aids in the rapid diagnosis of acute myocardial infarction.

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IVD CE

24-HOUR TECHNICAL SUPPORT

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RESPONSE CORPORATE OFFICE

Tel: 1-888-591-5577 (toll free) Tel: 1-604-456-6010 (int'l)

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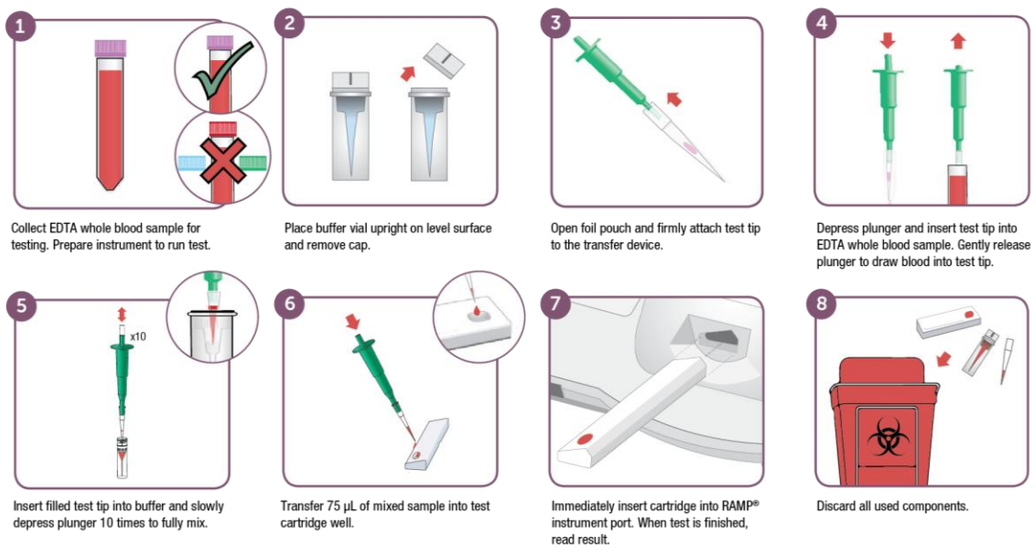
Running a test



SUMMARY AND EXPLANATION

Myoglobin is an intracellular heme protein, which is present in all types of muscle cells, including the heart. It has a molecular weight of approximately 17.8 kDa. Following myocardial injury or cellular necrosis, myoglobin is quickly released into the bloodstream.

Although elevated levels of myoglobin may be detected as early as 1 hour after an Acute Myocardial Infarction (AMI), myoglobin is most beneficial as a cardiac marker when measured 2–12 hours following an AMI. Myoglobin levels peak at 6 to 9 hours post infarction and return to normal within 24 to 36 hours [1,2]. Sequential negative myoglobin test results are often used to rule out AMI. A doubling of the myoglobin level within 1 to 2 hours provides strong evidence for AMI, even when the results are still in the normal range [3].

Myoglobin levels may be elevated as a result of muscle damage from trauma, surgery, exercise, and ischemia. Elevations may also be seen with degenerative diseases of the muscle. In the absence of conditions known to cause muscle damage, and in light of the patients' medical history, an elevated myoglobin level could indicate myocardial injury.

TEST PRINCIPLE

The RAMP® Myoglobin test is a quantitative immunochromatographic test for the determination of Myoglobin 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-myoglobin antibodies bind to myoglobin, if present in the sample. As the sample migrates along the strip, myoglobin-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 Myoglobin in EDTA whole blood.
- The sample buffer contains phosphate buffer, animal protein, surfactant, and ProClin® 300 / ProClin® 950 as preservatives.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use in a point-of-care or emergency testing or in a rapid response/stat laboratory setting. For US customers, the RAMP® Myoglobin test must be operated in a laboratory setting when used with the RAMP® 200.
- For use by qualified 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 K_2EDTA 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® buffer vials
- 1 transfer device for 75 µL
- 1 lot card
- 1 instructions for use (IFU)

MATERIALS REQUIRED (BUT NOT PROVIDED)

- REF: C1100 RAMP® Reader instrument; or
- REF: C2100 RAMP® 200 instrument control module, and
  REF: C3100 RAMP® 200 instrument test module
- REF: C2003/C5003 RAMP® Cardiac Controls (optional)
- Optional accessories such as RAMP® 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 reuse.

1. Prepare RAMP® instrument for test cartridge. Refer to the RAMP® Operator's Manual for detailed instructions on Starting a Test.
2. 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.
6. Insert tip into sample and fully release plunger. The test tip should fill with 75 µL of blood.
7. Immediately transfer the filled test tip into the buffer vial close to, but not touching, the bottom.
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.
9. 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 10 minutes.
13. Record the result, if required. For additional information on printing and/or uploading results, please refer to the Operator's Manual.
14. Remove the used test cartridge and discard all used test components according to local 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 quality 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

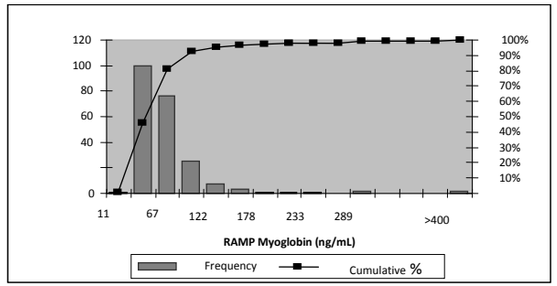
When the RAMP® instrument is unable to continue a specific task it will emit an audio alarm and display a message. Refer to the RAMP® Operator's Manual 'Troubleshooting Guide' section for a full description of all messages. If repeated tests give unexpected results, contact Response Biomedical Technical 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.
- 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.
- Myoglobin is not a cardiac-specific protein, and therefore myoglobin levels may be affected by other conditions that produce muscle damage.
- 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

Whole blood samples from healthy individuals divided equally across gender were tested. The lower (LLN) and upper (ULN) limits for normal range were defined as the 5th and 95th percentile values, respectively. The distribution of the results obtained is represented in the histogram below:



	n	LLN [ng/mL]	ULN [ng/mL]
Total	196	19.15	99.3

Each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, determine its own reference ranges.

PERFORMANCE CHARACTERISTICS

MEASUREMENT RANGE

2.4 to 400 ng/mL

Myoglobin levels in excess of 400 ng/mL are reported as greater than > 400 ng/mL; values less than 2.4 ng/mL should be reported as < 2.4 ng/mL.

HOOK EFFECT

No high dose hook effect was observed for the RAMP® Myoglobin test up to the highest level tested (8000 ng/mL myoglobin).

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 is 2.4 ng/mL myoglobin.

PRECISION

The within-run, between-run and total precision of the RAMP® Myoglobin test were determined by testing duplicates of each standard twice each day over 10 days. The mean, standard deviation and % CV were calculated for each reported concentration of myoglobin.

	Myoglobin Standards Mean Concentration [ng/mL]		
	50	100	200
Within-run [%]	13.0	6.7	6.5
Between-run [%]	13.8	9.7	9.1
Total [%]	14.3	10.4	10.6

DUPLICATE PRECISION COMPARISON AT CLINICAL SITES

Subjects enrolled in the precision study were a subset of the subjects enrolled to the method comparison study. 184 total subjects were enrolled and five (5) subjects were excluded due to a low signal result, where a repeat sample was not available. Of the 179 remaining subjects, 77 were normal individuals (40 males and 37 females) and 102 were suspected of AMI based on the individual hospital criteria (63 males and 39 females). Two data points were excluded following an outlier analysis. Data correlation for the RAMP® Myoglobin test replicate result 2 vs. result 1 is shown:

Population	n	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Suspect AMI	102	0.9750	1.9026	0.986
Normal	77	0.9013	3.7143	0.967
Total	179	0.9702	0.9349	0.968

LINEARITY

Discrete myoglobin antigen concentrations of 10, 50, 75, 100, 150, 200, and 350 ng/mL were prepared in a serum matrix. Linearity was determined by testing five replicates of each standard. The mean, standard deviation and %CV of replicates were calculated for each concentration. Linear regression analysis of actual myoglobin concentration versus expected myoglobin concentration results in an R = 0.998 and a slope of 1.07 with an offset of 1.143.

INTERFERENCE

Potentially interfering substances were evaluated by spiking different concentrations of interferents in normal donor EDTA whole blood containing 100 ng/mL of myoglobin. Different blood samples were used for each interferent. Interference was evaluated by calculating the myoglobin concentration of interferent-spiked blood, expressed as a percentage of the myoglobin concentration of the un-spiked (no interferent) blood sample. Hemoglobin, triglycerides, bilirubin, cholesterol, and coumidin at levels representing high physiological concentrations were tested for possible interference. No interference was observed when tested at the concentrations up to, and including those shown in the following table:

Compound	Concentration
Hemoglobin	2000 mg/dL
Triglyceride	3000 mg/dL
Bilirubin	60 mg/dL
Cholesterol	2000 mg/dL
Coumidin	200 µg/mL

ANALYTICAL SPECIFICITY

Samples containing rheumatoid factor at levels greater than 1300 Rf IU/mL may interfere with the test and cause erroneous results. If this occurs, another specimen should be obtained and tested by an alternate method.

CLINICAL EVALUATIONS

METHOD COMPARISON

Four hundred twenty-nine (429) subjects were enrolled in the method comparison clinical trial. Fourteen (14) subjects were excluded due to a low signal result, where a repeat sample was not available. Of the 415 remaining subjects, 196 were normal individuals (92 males and 104 females) and 219 were suspected of AMI based on the individual hospital criteria (131 males and 88 females). All normal subjects were consented. An EDTA whole blood sample was obtained for each of these subjects. Waste sample was used for the subjects suspected of AMI. An aliquot of the EDTA whole blood sample was taken for the RAMP® test and EDTA plasma was prepared for the Beckman ACCESS Myoglobin assay. The samples were stored refrigerated for up to five days between analyses. Data was winsorized to adjust for differing reportable ranges and 3 data points were excluded following an outlier analysis. Data correlation for the RAMP® Myoglobin test versus the Beckman ACCESS Myoglobin assay is presented:

Population	n	Slope	Intercept [ng/mL]	Correlation Coefficient [r]
Suspect AMI	219	1.0059	29.576	0.928
Normal	196	1.3831	15.609	0.889
Total	415	1.0309	25.905	0.932

CLINICAL SENSITIVITY & SPECIFICITY

Not evaluated.

REFERENCES

[1]. Vaananen HK, Syrjala H, Rahkila P, *et al.* Serum carbonic anhydrase III and myoglobin concentrations in acute myocardial infarction. *Clin Chem* 1990 Apr; 36(4): 635-8

[2]. Gibler WB, Gibler CD, Weinshenker E *et al*. Myoglobin as an early indicator of acute myocardial infarction. *Ann Emerg Med* 1987; 16(8): 851-6

[3]. Tucker JF, Collins RA, Anderson AJ, *et al*. Value of Serial Myoglobin Levels in the Early Diagnosis of Patients Admitted for Acute Myocardial Infarction. *Ann Emerg Med* 1994; 24(4): 704-8

GLOSSARY OF SYMBOLS

EC REP Authorized Representative in European Community	LOT Batch Code	Catalogue Number
Caution	CE Mark	Consult Instructions for Use
Contains Sufficient for <n>Tests	Do Not Reuse	IVD In vitro Diagnostic Medical Device
Harmful, Irritant	Manufacturer	Rx Only Prescription Use Only (U.S. Only)
Temperature Limit	Use-by Date	

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

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