Roche cobas®h 232 Troponin T METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing a cardiac Troponin T test using the Roche cobas h 232 analyser. The Roche cobas h 232 analyser can be used by healthcare professionals for measuring major cardiac blood markers. This document will be focusing on Troponin T, and further information on the other parameters can be found on their website.

2. HAZARDS

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal protective equipment should be worn when processing samples, performing maintenance and troubleshooting procedures.

3. CLINICAL SIGNIFICANCE

Acute Coronary Syndrome (ACS) is a term used to describe a group of conditions resulting from insufficient blood flow to the heart muscle.[1] These conditions range from atypical chest discomfort and non specific electrocardiographic changes to a large ST-segment elevation, myocardial infarction and cardiogenic shock.[2] Symptoms can include chest pain including tightness and heaviness in the chest, discomfort in the arms and upper body, shortness of breath and other constitutional symptoms including sweating, nausea and light-headedness.[1]

Diagnosis of ACS is based on a complete medical history, physical examination, electrocardiogram to evaluate the electrical activity of the heart and blood tests to evaluate the presence of biological markers resulting from cardiac cell injury.[1]

Troponin T & I are members of a group of cardiac regulatory proteins which function to regulate the calcium mediated interaction of muscle filaments actin and myosin resulting in contraction and relaxation of striated muscle.[4] Troponin T is almost exclusive to the myocardium, with small amounts expressed in skeletal muscle not detectable in current Troponin T assays.[4] Insufficient blood flow and oxygen supply to the heart muscle causes necrosis of the myocardium and subsequent release of Troponin T & I into the bloodstream.[5]

Troponin T in the bloodstream rises to detectable levels after 4-6 hours, peaks at 10-12 hours and can be detected for up to 14 days post infarction.[5,6] Troponin I is released from necrotic cardiac myocytes into the bloodstream within hours (~4-8 hours) after the onset of chest pain. The peak TnI concentration is generally reached in 12-48 hours.[7] Troponin I serum levels

can remain elevated for up to 4–7 days.[5]

The diagnostic utility of Troponin T & I to detect myocardial necrosis and to enable risk stratification in patients with ACS is well established.[5,8] Furthermore, the use of Troponin T as a prognostic indicator for recurrence of ischaemic events and death in ACS patients is increasing. [5,9]

Results from PoCT devices measuring Troponin T & I should always be used in conjunction with clinical presentation, history and other diagnostic information.

4.   TEST PRINCIPLE

The test strip contains two monoclonal antibodies specific to cardiac troponin T (cTnT) of which one is gold-labelled, the other biotinylated. The antibodies form a sandwich complex with the cTnT in the blood. Following removal of erythrocytes from the sample, plasma passes through the detection zone in which the gold-labelled cTnT sandwich complexes accumulate and the positive signal is displayed as a reddish line (the signal line). Excess gold-labelled antibodies accumulate along the control line, signalling that the test was valid. The intensity of the signal line increases in proportion to the troponin T concentration. The optical system of the cobas h 232 instrument detects the two lines and measures the intensity of the signal line. The integrated software converts the signal intensity to a quantitative result and shows it in the display.

4.1   Interference

No interference was observed **up to** the following concentrations for all analytes:

Bilirubin	20 mg/dL	Hemolysis (Hb)	200 mg/dL
Biotin	10 ng/mL	Lipaemia (triglycerides)	440 mmol/L
Rheumatoid factors	300 IU/mL		

- The assay is unaffected by haematocrit values between 30 – 50%
- In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration
- High concentrations of lipoic acid (e.g. in pharmaceuticals or as food additives) can lead to lower measurement values
- There is no high-dose hook effect at Troponin T concentrations < 200000 ng/L
- Very high concentrations of Troponin T may cause the control line to fail to appear and the instrument may display an error message.
- Patient samples containing heterophilic antibodies may react in immunoassays to give falsely elevated or decreased results
- Strong electromagnetic fields may interfere with the proper operation of the meter

Important! It is possible that other substances and/or factors not listed above may interfere with the test and cause false results.

4.2 Accuracy

C€ This product fulfills the requirements for Directive 98/79/EC on in vitro diagnostic medical devices.

A comparison of 3 lots of the Roche CARDIAC T Quantitative test with the Elecsys Troponin T test in a clinical patient population showed slopes between 0.80 and 1.20 in the majority of the method comparisons with a correlation coefficient of ≥ 0.9.

4.3 Precision

Repeatability was measured with 3 lots of the Roche CARDIAC T Quantitative tests and heparinised human blood. The majority of the variation coefficients were below 9 % over the entire measurement range. Intermediate precision was measured with the Roche CARDIAC Control Troponin T quality control in 5 different hospitals. The majority of the variation coefficients were below 11%.

5. INSTRUMENT

Product specifications
5.1 Operating Conditions and Technical Data

Temperature range	18° – 32°C
Relative humidity	10 - 85% (non-condensing)
Maximum altitude	4000m
Position	Place meter on a level, vibration-free surface while applying the sample until the necessary sample has been absorbed completely by the test strip
Measuring range	100 – 2,000 ng/L
Sample size	150 μL

Test time	12 minutes with 2 minutes for sample detection
Memory	500 test results with date, time and comments, 500 liquid control results, and 200 code chip records (100 test + 100 QC)
Barcode scanner	Yes
Interface	Infrared interface, LED/IRED Class 1 USB and Ethernet port; printer
Battery operation	Yes – handheld battery pack (rechargeable)
Mains connection	Yes - Input: 100-240 V (± 10%)/ 50-60Hz /400 mA, Output: 7.5 V DC / 1.7 A
Number of tests with fully charged battery	Approx. 10 tests
Safety class	Class III
Automatic power-off	Yes – Programmable 1 - 60 minutes
Dimensions	275 x 102 x 55 mm
Weight	650g incl. handheld battery pack and scanner

5.2   Storage and transport conditions

Temperature range Meter (In original container)	-25° to +70°C
Relative humidity	10 - 85% (non-condensing)

6.   SPECIMEN REQUIREMENTS

6.1   Sample Material
Venous whole blood stored in lithium or sodium heparin tubes without separating gel are acceptable. Blood collection tubes containing EDTA, citrate, sodium fluoride or other additives are not acceptable.
Samples are stable for 8 hours at room temperature. Do not refrigerate or freeze samples.

Venepuncture (see suitable anticoagulants above)
- Skin surface must be cleaned with an alcohol swab and dried well prior to collection to ensure there are no substances on the skin surface
- Ensure sample is properly mixed and at room temperature before testing
- Sample stability: 8 hours at room temperature. Do not refrigerate or freeze sample.

7. CARTRIDGES/REAGENTS

7.1 Storage and handling
- Test strips should be refrigerated at 2° - 8°C. DO NOT FREEZE.
- Test strips can be stored up to 1 week at room temperature at 15° - 25°C.
- Perform a test at temperatures between 18° – 32°C
- Use the test strips at 10 - 85% humidity. Do not store the test strips in high heat and moisture areas such as the bathroom or kitchen and keep away from direct sunlight.
- Test strips:
  - Can be used immediately after removal from the refrigerator
  - Must be used within 15 minutes once the pouch has been opened.
  - Must be discarded if they are past their use by date. Expired test strips can produce incorrect results.
  - Can be used until the printed use by date when they are stored and used correctly.

7.2 Storing information about test strips
Every pack of test strips includes a lot-specific code chip which provides information about the lot-specific properties of the test strip. On opening a new box of test strips, insert the code chip into the meter. If not inserted when starting a new lot, the instrument display prompts the user to insert the chip. To ensure that the code chip and test strip lot match, compare the lot number in the display with the number on the code chip.

8. CALIBRATION

The Roche CARDIAC T Quantitative test is calibrated against the Elecsys Troponin T hs test using serum. The instrument automatically reads in the lot-specific calibration data from the code chip; thus operator calibration is not necessary.

9. QUALITY CONTROL

Quality control material (perform as per your organisation's protocol)
Accurately testing known levels of Troponin T ensures that the system and your technique used in testing give accurate results on patient tests. The control solutions have defined (known) values. The results for these solutions must first fall within a certain acceptable range in order to allow valid patient testing.

A quality control test should be performed every time a new shipment of test strips are received, when a new lot number of test strips are used, if the clinical picture does not correlate with the patient test results, after major maintenance, and at a minimum of once a month.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparisons are encouraged.

The Roche Cobas h 232 uses the following methods for quality:
- Code chips
- IQC (Electronic QC)
- Control solutions

9.1 Coding the meter
- Use the new code chip that comes with every new box of test strips.
- Compare the code number on the chip with the corresponding code number on the box of test strips.
- Insert the code chip into the code chip slot, located at the top of the meter, until you feel it snap into place.
  NOTE: Do not force the code key into the meter; it only goes in one way.
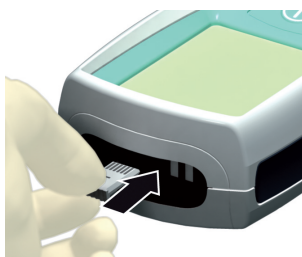
9.2 Electronic quality control (IQC)
The Roche CARDIAC IQC test serves as a performance check for the optical system of the cobas h 232 device. The IQC consists of two Troponin strips with already set positive results (one is a low positive and one is a high positive). The strips are reusable and test the internal mechanisms of the instrument to ensure the intensity of the positive line is read correctly. The IQC should be performed weekly, alternating between the two levels. Store the IQC strips unopened, at 2-30 °C up to the stated expiration date. After opening, store for up to 6 months.

- Bring the Roche CARDIAC IQC test strip to room temperature before starting the measurement
- From the main menu of the instrument select QC TEST
- When prompted remove one test strip from the container and closer the container immediately.
- Insert the IQC strip into the meter (when the instrument asks for a code chip, insert the code chip from the IQC box).
- The instrument will take approximately 20 seconds to perform the test and when completed the instrument will indicate if the test has passed or failed.
- Remove the test strip (low or high) from the device directly after the measurement is performed and place it quickly into its container to protect it from dust and moisture.

NOTE: Do not touch or wipe the signal line area of the test strip. Do not apply any sample material to the test strip. Do not expose the test strip to sunlight.

9.3   Running control solutions

The control solutions have two levels:
- Roche CARDIAC Control Troponin T quality control, level 1
- Roche CARDIAC Control Troponin T quality control, level 2
  - each with a lot-specific encoding chip

Store the controls at 2-8 °C and tightly capped when not in use.
The stability of the lyophilized control serum at 2-8 °C is up to the stated expiration date. Stability of the components in reconstituted control serum at 2-25 °C is 24 hours and at and below -20 °C is 12 weeks (can be frozen up to 5 times in the original vial). Frozen or refrigerated reconstituted control material must be brought to room temperature prior to use.

Preparing the control solution
- Carefully open a vial, avoiding the loss of lyophilized control serum
- Pipette in exactly 1.0 mL of distilled water.
- Carefully close the vial and dissolve the contents completely by occasional gentle swirling over 15 minutes.
  NOTE: Avoid the formation of foam

Inserting the test strip
- Turn the instrument on by pressing the On/Off button for longer than 5 seconds
- Wait for completion of the self-test
- Touch the QC Test button
- The test strip symbol prompts you to insert the test strip
- Remove the test strip from the foil package
  NOTE: Only remove the test strip from the foil package when you are ready to perform a test.
- Hold the test strip so that the application and test areas are facing up. Insert the test strip quickly into the test strip guide of the meter using a smooth, even motion. Slide the test strip in as far as it will go and a beep tone will indicate that the meter has detected the test strip.
  NOTE: If you are using a new test strip lot number and have not inserted the code chip yet, you will be prompted to do so now.
- If you are using new control material, remove the code chip (for the test strip), press "New" and insert the code chip that came with the control material
 **NOTE:** Alternatively, the QC Lot number is stored in the memory, and you can select the code for your current control material from the list.
- Select the QC level
- The thermometer symbol shows that the test strip is warming up, and the parameter and code chip number are also displayed on the screen.
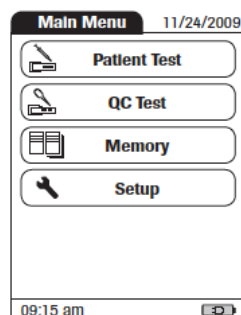
Applying the Control Solution
- When the warming up process is complete, a further beep tone sounds and a pipette icon appears on the screen
- Using a pipette or syringe apply exactly 150 µL (0.15ml) of control solution to the application area
  NOTE: You have 5 minutes to apply the entire sample to the application area. Do not use a sample that has air bubbles, and do not touch the pipette tip to the application zone.
- Touch the tick button to confirm that the sample has been applied. The meter will now have an hourglass symbol while it detects the sample.
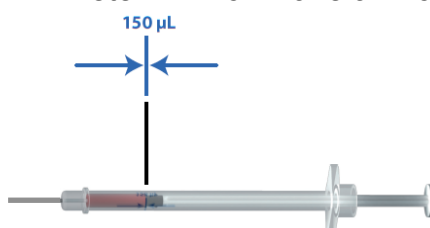
Results
- Once the sample has been detected, the actual measurement starts, and the countdown will begin.
  NOTE: Do not touch the test strip until the result is displayed on the screen
- The target value and range will be shown on the display along with "Pass" or "Fail" and automatically stored in the memory.

10. TEST PROCEDURE

Check the charge level on the screen. If the charge is low, connect the device to the power supply.

10.1 Code chip
The code chip provides the meter with important manufacturer-specific data that it needs to perform a Troponin T test. The code chip contains information about the test method, the lot number and the expiry date of the new test strips. The meter is ready to use once the code chip has been inserted. See

section 9.1 for details.

10.2   Performing the Test

Inserting the test strip

- Turn the instrument on by pressing the On/Off button for longer than 5 seconds.
- Wait for completion of the self-test.
- Touch the Patient Test button and enter the Patient ID using the touchscreen keypad or scan the patient barcode.
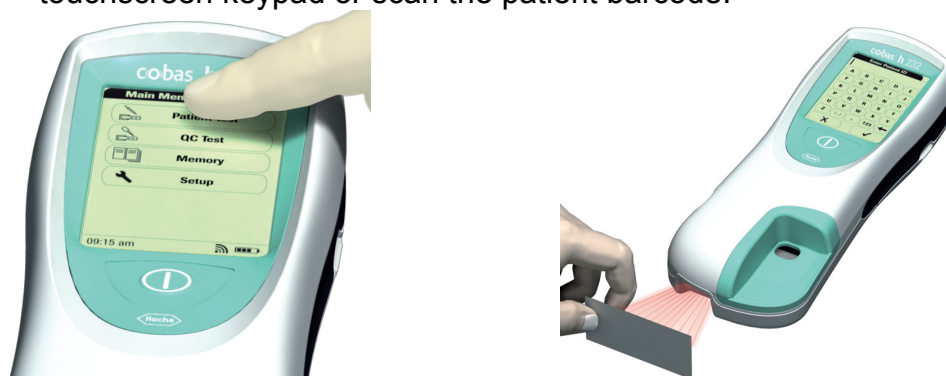
- The test strip symbol prompts you to insert the test strip. Remove the test strip from the foil package only when you are ready to perform a test.
- Hold the test strip so that the application and test areas are facing up. Insert the test strip quickly into the test strip guide of the meter using a smooth, even motion. Slide the test strip in as far as it will go and a beep tone will indicate that the meter has detected the test strip.
 **NOTE:** If you are using a new test strip lot number and have not inserted the code chip yet, you will be prompted to do so now.
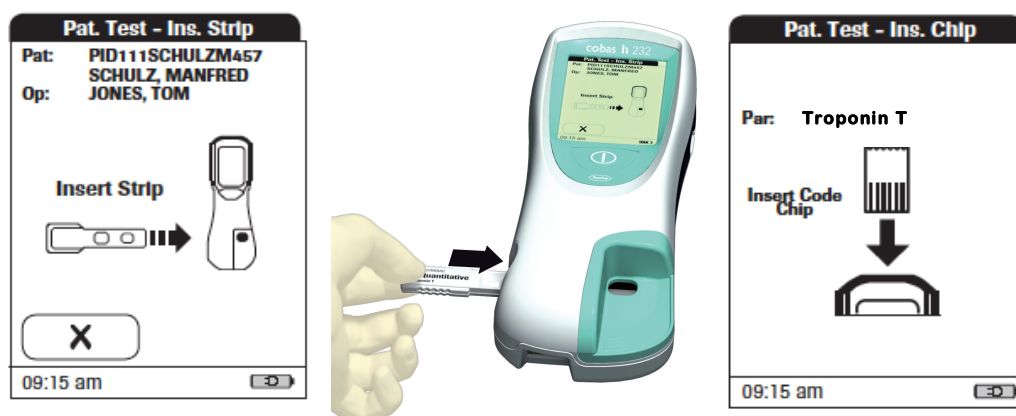

Applying the sample

- The thermometer symbol shows that the test strip is warming up, and the parameter and code chip number are also displayed on the screen.
- When the warming up process is complete, a further beep tone sounds and a pipette icon appears on the screen
- The meter is ready to perform the test and is waiting for the blood to be applied.

- Using a pipette or syringe apply exactly 150 µL (0.15ml) heparinised whole blood to the application area
  NOTE: You have 5 minutes to apply the entire blood sample to the application area. Do not use a sample that has air bubbles, and do not touch the pipette tip to the application zone.
- Touch the tick button to confirm that the sample has been applied. The meter will now have an hourglass symbol while it detects the sample.
  NOTE: Do not add more blood after the test has begun.
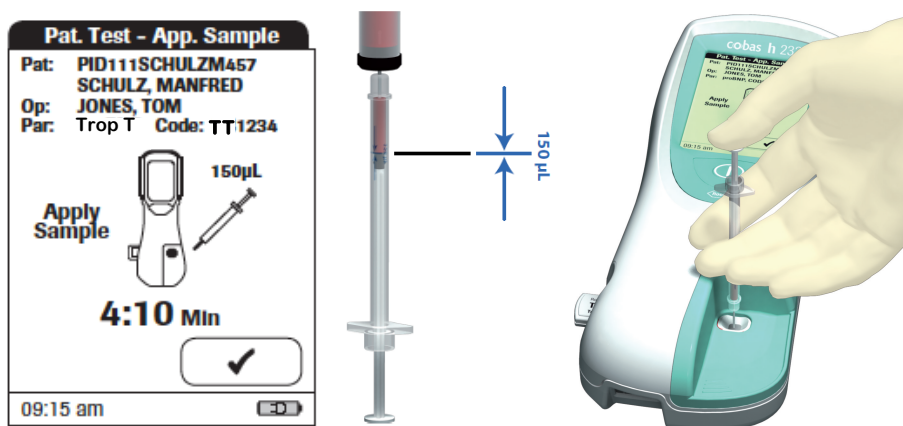
Results

- Once the sample has been detected, the actual measurement starts, and the countdown will begin.
  NOTE: Do not touch the test strip until the result is displayed on the screen
- The result will be shown on the display and automatically stored in the memory.
- Remove the test strip from the measurement chamber and turn off the meter by pressing the On/Off button for longer than 2 seconds.
  NOTE: A negative result should display 1 line and a positive result should display 2 lines in the reading window of the test strip. A visual check of the test strip following the test is recommended to double check the instrument as if too much blood is applied to the strip it can filter into the reading window affecting the results. The reading window should remain clear with only a tinge of pink. If too much blood is added it will turn red and the test should be repeated.
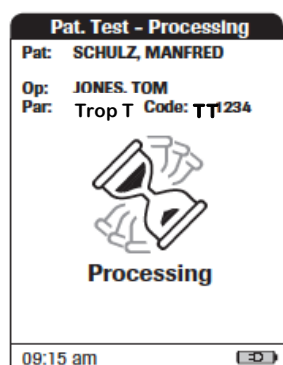
11. RESULTS

11.1 Expected values
The measuring range is 100 – 2000 ng/L.

11.2 Interpretation of results

Troponin T Concentration	Result Displayed	Comment
Below 50 ng/L	Trop T < 50 ng/L	Acute myocardial infarction not likely, but still possible; in context of clinical assessment repeat the test (e.g. after 3–6 h) to detect rising Troponin T levels.
Between 50 ng/L and 100 ng/L	Trop T 50 – 100 ng/L	Acute myocardial infarction possible, repeat the test to detect rising Troponin T levels in context of clinical assessment according to guidelines; search for differential diagnosis and other causes of Troponin T elevation.
Between 100 ng/L and 2000 ng/L	For example, Trop T 900 ng/L	Acute myocardial infarction likely; consider differential diagnosis for other causes of Troponin T elevation.
Above 2000 ng/L	Trop T > 2000 ng/L	Acute myocardial infarction very likely; consider differential diagnosis for other causes of Troponin T elevation.

11.3 Transferring Data to a printer or computer
- Using the infrared interface, you can send test results directly to a printer.
- To print the result, align the infrared sensors on both the instrument and printer and press the printer button.
  NOTE: The printer uses thermal paper and will fade over time. Results should be photocopied and stored in patient's notes.
- Using the data ports of the handheld base unit (docking station), you can upload stored test results to a PC/host system (e.g. cobas IT 1000 PoC data management system).
  NOTE: Enabling the connection to a computer disables the connection to a printer (and vice versa)

12. MAINTENANCE

- Turn off the meter before cleaning it. Unplug the power supply unit and remove the handheld battery pack.
- First remove any blood and other dirt using water or soapy water then disinfect the meter

- Use only the following items for cleaning: ordinary lint-free cotton buds, lint-free tissues
- Suitable cleaning agents include: ammonium chloride solution (2%), diluted bleach solution (1:10), mild soapy water, Dispatch ®, citric acid (2.5%), hydrogen peroxide (0.5%), sodium hypochlorite solution (0.6%), 70% isopropyl alcohol, CoaguWipe Bleach Towel (only used for cleaning the outside of the meter)

Cleaning the Sampling Area
- Remove the sample application cover by pulling it forward horizontally (in the direction of the arrow).
- In case of significant dirt or contamination, you can rinse the sample application cover (separately from the meter) under warm running water. Dry the sample application cover with a fresh tissue.
- Clean the outside of the meter with a lightly moistened tissue. Then dry the meter with a fresh tissue.

Cleaning Test Strip Guide
- Clean the easily accessible and visible pipetting field area of the test strip guide with a moistened cotton bud or tissue.
- Dry the test strip guide with a fresh tissue.
  NOTE: Do not insert any objects into the concealed areas of the measurement chamber as this might damage the optical components of the meter.
- Clean the membrane (small circle) in the visible area at the end of the test strip guide with a moistened cotton bud or tissue.
- Allow the inside of the test strip guide to dry for about 10 minutes.
- Re-attach the sample application cover to the housing and make sure that it snaps correctly into place.
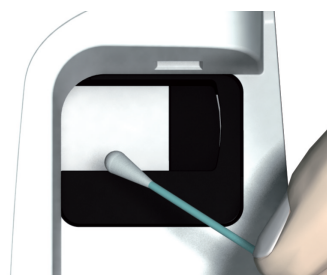
13. REFERENCES

This method has been adapted from the Roche cobas h 232 System Operator's Manual, test strips and control solution package inserts.

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