METHOD FOR THE MEASUREMENT OF BEDSIDE TROPONIN T (cobas h232)

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BEDSIDE TROPONIN T

1. PURPOSE AND SCOPE.

This method is to be used for the determination of Troponin T in human blood.

2. HAZARDS.

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Gloves should be worn when processing samples.

Quality Control
As human source material, should be treated as potentially infectious. (Although having been tested for HBsAg and HIV, this does not guarantee that all infective units have been detected!)

3. CLINICAL.

Cardiac Troponin T is a regulatory contractile protein that is only released following damage to myocardial cells and is not raised in patients with skeletal muscle damage/disease. Cardiac Troponin T is released only in situations where myocardial cell damage takes place such as Acute Coronary Syndromes (ACS) and the release into the blood takes place within 3-6 hours of the myocardial damage occurring. Cardiac Troponin T has a diagnostic window that extends from 3 hours to 14 days. Situations other than ACS where cardiac Troponin T may be raised include myocarditis, cardiac failure, pulmonary embolism, chronic renal failure, septic shock and dermatomyositis (reflecting myocyte damage)

4. PRINCIPLE.

Test Strips
The test contains two monoclonal antibodies specific for cardiac Troponin T: one gold-labelled, the other biotinylated. The antibodies form a sandwich complex with any cardiac Troponin T that is present in the blood sample. Erythrocytes are removed from the sample and the plasma passes through the detection zone, in which the Troponin T sandwich complexes accumulate along a line of streptavidin, appearing as a red signal line. Excess gold-labelled antibodies gather along the control line, signalling visually that the test was valid. The signal line increases in intensity in proportion to the cardiac Troponin T concentration.

The optical system of the cobas h232 instrument recognises the two lines and measures the intensity of the signal line. Integrated system software converts the intensity reading to a quantitative result, which is then displayed in the window.

cobas h232
The cobas h232 instrument detects test strips signal lines by means of a CCD photosensor with imaging lens. The signal line increases in intensity in proportion to the concentration of Troponin T. Integrated system software converts the signal intensity to a quantitative result, which is then displayed in the window. The coding chip contained in every test strip pack and a bar code on the underside of the test strip include all test and lot-specific information for automated quantitative evaluation with the instrument.

5. INSTRUMENT.

cobas h232 (Roche Diagnostics). The cobas h232 instrument uses a rechargeable battery pack which is supplied with the instrument. As the instrument has no direct power supply, it should be regularly charged in the downloader/charger to ensure that the instrument is sufficiently charged for testing.

6. REFERENCE SOURCE OF METHOD

Adapted from the method described in the Roche cobas h232 operator’s manual (November, 2006).
7. **CONSUMABLE STORAGE**

Troponin T test strips should be refrigerated at all times when not in use (2-8°C). Liquid quality control material should be stored in the freezer if it is not able to be tested immediately. Lyophilised quality control material should be refrigerated until use.

8. **SAMPLE COLLECTION AND STORAGE.**

Blood is collected in a **Lithium Heparin tube with no gel.** Heparin is the only anticoagulant allowed. Samples are stable for 8 hours at room temperature. **DO NOT REFRIGERATE OR FREEZE SAMPLES.**

9. **STANDARDS.**

Each lot of Cardiac T Quantitative test strips is calibrated against Elecsys Troponin T from Roche Diagnostics (laboratory based method). The coding chip programs the instrument with calibration data unique to that lot of strips, so calibration by the user is not required. Coding chip for each lot number must be read by the cobas h232 instrument before sticks can be used (instrument will prompt user to insert coding chip if this is not done).

10. **QUALITY CONTROLS.**

Cardiac control for Troponin T consists of a lyophilised sample based on human sera containing slightly elevated Troponin T concentrations. Quality control should be run monthly. To run a quality control sample you select Patient Test, but scan the QC barcode for the patient ID. The rest of the procedure is identical to that of a patient test. Results are to be recorded in the Troponin folder and faxed to iCCnet CHSA.

**Preparation of control material:**

Open the bottle of control very carefully, avoiding loss of any lyophilised material. Carefully add exactly 0.5 ml of distilled water. Close the bottle carefully and allow the contents to dissolve for 5-10 minutes by occasionally gently swirling the contents whilst avoiding foam formation.

Each bottle of Troponin control solution should be used once. The expected result for the Troponin internal quality control is indicated by iCCnet CHSA scientific staff. If the result falls outside the expected result, repeat the test. If the result is outside the expected result again, contact iCCnet CHSA technical support on (08) 8378 2208.

11. **IQC.**

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. The results are to be recorded in the Troponin folder.

**Method:**

From the main menu of the instrument select **QC TEST** and when prompted insert one of the IQC strips (if the instrument asks for a code chip, insert code chip from the IQC box). The instrument will take approximately 1 minute to perform the test and when completed the instrument will indicate if the test has passed or failed. If a fail message is displayed, repeat test. If the test fails again contact iCCnet CHSA technical support on (08) 8378 2208.
12. **TEST PROCEDURE (patient)**

1. Turn the instrument on by pressing the On/Off button for longer than 5 seconds.

2. Wait for completion of the self test. *The display reads:*

3. Touch the **Patient Test** button and enter the Patient ID. *The display will now read:*

4. The above test strip symbol prompts you to insert the test strip.

5. 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 (as indicated below). Slide the test strip in as far as it will go, a beep tone indicates that the meter has detected the test strip.

   *note: Exposure to external influence (e.g. humidity) may deteriorate the test strips. Only remove test strips from their foil packaging when you are ready to perform the test.*

6. 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.

7. The thermometer symbol shows that the test strip is warming up. *The display reads:*

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**TESTING PROCEDURE**

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PCT_CT.FM2 v2
8. When the warming up process is complete, a further beep tone indicates you can now apply the sample. The display reads:

![Image of sample application]

9. Using a pipette or syringe apply exactly 150 µl (0.15ml) heparinised whole blood as shown below:

![Image of blood sample application]

10. Touch the /checkbld button to confirm that the sample has been applied. The meter will now have an hourglass symbol while it processes the sample. Once the sample has been detected, the actual measurement begins, which will take 12 minutes. If the instrument detects that the sample is positive for Troponin T it will indicate this on the display as shown below:

![Image of positive result]

11. The Troponin T result will be shown on the display and automatically stored in the memory. To print the result, align the infrared sensors on both the instrument and printer and press .

12. 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 (≥ 100ng/L) 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 too much blood added to the strip 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.
### RESULTS.

Results obtained by the cobas h232 are displayed in table below.

<table>
<thead>
<tr>
<th>Troponin T Concentration</th>
<th>Result Format</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 50 ng/L</td>
<td>Trop T &lt;50 ng/L</td>
<td>Negative for Troponin T. ACS protocol should be followed closely to rule out acute coronary syndrome.</td>
</tr>
<tr>
<td>Between 50 ng/L and 100 ng/l</td>
<td>Trop T 50-100 ng/L</td>
<td>This is an intermediate result and the cardiac Troponin T concentration may continue to change. Patient should be monitored closely and RTropT test repeated as per the ACS Protocol.</td>
</tr>
<tr>
<td>Between 100 ng/L and 2000 ng/l</td>
<td>e.g. &quot;Trop T 270 ng/L&quot;</td>
<td>This is a positive result for Troponin T indicating cardiac muscle damage.</td>
</tr>
<tr>
<td>Above 2000 ng/L</td>
<td>Trop T &gt; 2000 ng/L</td>
<td>This is a strong positive result for Troponin T indicating massive cardiac muscle damage.</td>
</tr>
</tbody>
</table>

### MAINTENANCE.

Monthly or as per needed. Wipe any blood off instrument using an appropriate alcohol or detergent wipe. Remove sample application cover to wipe blood off this.

NOTE: Do not use alcohol wipe on display screen.

### REFERENCE RANGE.

Reference Limits: < 100 ng/L.

### ASSOCIATED HAZARDS.

SUSPECTED CONTAMINATION WITH ANY OF THE FOLLOWING SHOULD BE REPORTED TO YOUR SECTION SAFETY OFFICER OR SUPERVISOR.

1. **Processed human serum ie QC’s:**
   As human source material, should be treated as potentially infectious (although having been tested for HBsAg and HIV, this does not guarantee that all infective units have been detected!).

2. **Patient samples:**
   As human source material, should be treated as potentially infectious.