Roche cobas® h 232 D-Dimer METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing a D-Dimer using the Roche cobas h 232 analyser. The Roche cobas h 232 analyser can be used by healthcare professional for measuring major cardiac blood markers. This document will be focusing on D-Dimer, and further information on the other parameters can be found on the company's website.

2. HAZARDS

Patient Samples

All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Protective Personal equipment should be worn when processing samples, quality control testing and maintenance procedures.

3. CLINICAL SIGNIFICANCE

Venous thromboembolism is a term used for both deep venous thrombosis (DVT), which involves clot formation in the deep veins of the body and a pulmonary embolism (PE), where the clot can break off and travel to the lungs.[1] PEs originate from proximal DVT of the leg (popliteal, femoral, or iliac veins), with upper extremity DVT less common but also may lead to PE, especially in the presence of a venous catheter. [2]

Classic symptoms of DVT include swelling, pain, and discoloration in the affected extremity. Physical examination may reveal the palpable cord of a thrombosed vein, unilateral oedema, warmth, and superficial venous dilation. However, these symptoms are of low predictive value and can occur in other conditions such as musculoskeletal injury, cellulitis, and venous insufficiency.

Diagnosis of DVT is based on the Wells clinical prediction rule, which provides a reliable estimate of the pretest probability of DVT; Doppler ultrasonography; helical computed tomography (CT); contrast venography; Impedance plethysmography and D-dimer tests.[1,2] Furthermore, the diagnosis of PE is based on clinical examination and a determination of risk factors; pulmonary angiography; ventilation-perfusion scanning; helical CT scanning; and D-Dimer Tests. [1]

D-Dimer is a degradation product of crosslinked fibrin.[3] The D-Dimer concentration is a measure of fibrinolytic activity of plasmin in the bloodstream.[4] D-Dimer concentrations are raised in the setting of acute deep vein thrombosis (increased blood clotting and fibrinolytic activity); however, an elevated D-Dimer concentration is insufficient to establish the diagnosis of PE because such values are both nonspecific and commonly present in hospitalised patients.[2,3] Despite this low specificity, the sensitivity and negative

predictive value of the sensitive d -dimer assay in patients with suspected VTE is extremely high, where a low d-dimer concentration (<0.5 ug/ml) can be useful in ruling out VTE in low risk patients. [3,4]

Various PoCT devices measure D-Dimer and results should always be used in conjunction with clinical presentation, history and other diagnostic information.

4. TEST PRINCIPLE

The test strip contains two monoclonal antibodies against fibrin degradation products, which contain the D-Dimer molecule. One of the antibodies is gold-labelled and the other biotinylated. The antibodies form a sandwich complex with the D-Dimer in the lithium or sodium heparinised blood. Following removal of erythrocytes from the sample, plasma passes through the detection zone in which the gold-labelled D-Dimer 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 D-Dimer 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	342 µmol/L	Hemolysis (Hb)	200 mg/dL
Biotin	30 ng/mL	Lipaemia (triglycerides)	5.31 mmol/L
Rheumatoid factors	79 IU/mL	Haematocrit	26 – 56%

- High concentrations of lipoic acid (e.g. in pharmaceuticals or as food additives) can lead to lower measurement values
- Very high d-dimer concentrations (approx. > 5 0 µg/mL) can lead to lowered values (hook effect). However, this effect does not result in false normal values. Alternatively, the control line may fail to appear and the instrument may display an error message. In this case, determination must be carried out using another method.
- High concentrations of d-fragments as may appear under thrombolytic therapy can lead to lower measurement values.
- Patient samples containing heterophilic antibodies(definition required) which could react in immunoassays to give falsely elevated or decreased results

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

$C\epsilon$ This product fulfills the requirements for Directive 98/79/EC on in vitro

diagnostic medical devices.

A comparison using 3 different lots of the Roche CARDIAC D-Dimer test with the Tina-quant D-Dimer test in a clinial patient population showed slopes between 0.94 and 1.03 in the majority of the method comparisons. The majority of the correlations in these method comparisons were ≥ 0.93.

4.3   Precision
Within-series imprecision was measured with 3 lots of Roche CARDIAC D-Dimer tests and heparinised human blood. The majority of the variation coefficients were below 11 %. Day-to-day imprecision was measured with the Roche CARDIAC Control D-Dimer quality control in 5 different hospitals. The majority of the variation coefficients were below 10 % (level 1) and below 17 % (level 2).

5.   INSTRUMENT

Product specifications
5.1   Operating Conditions and Technical Data

Temperature range	$18^{o} - 32^{o}C$
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	0.1 – 4 µg/mL
Sample size	150 µL
Test time	8 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 recommended. Capillary blood, serum or plasma is not allowed.

Venepuncture (see suitable anticoagulants above)
- **Skin surface must be cleaned with an alcohol swab and dried well prior to collection to ensure there is no substances are 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^oC
- 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.
  - Are discarded if they are past the 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 lot of test strip vials includes a code strip. Each code strip belongs to a single lot number and provides important information about the lot-specific properties of the test strip. On opening a new box of test strips, insert the new code strip into the meter. Ensure the code stored matches the code of the test strips in use.

8.   CALIBRATION

Each test strip lot of the Roche CARDIAC D-Dimer test is calibrated against the Tina-quant D-Dimer test from Roche Diagnostics. The instrument automatically reads in the lot-specific calibration data from the code chip, eliminating the need for calibration by the user. Calibration has been performed so that the results obtained are comparable to those obtained using the Tina-quant D-Dimer reference method with citrate plasma as the sample material.

9.   QUALITY CONTROL

Quality control material (perform as per your organisation protocol)

Accurately testing known levels of D-Dimer 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 and External Quality Assurance Program is not available, monthly lab comparison is encouraged.

9.1 Laboratory Comparison

If your policy states you must perform laboratory comparison then perform a venepuncture sample for the laboratory and run sample on the Roche Cobas h232. Collection of both samples should occur at the same time. Record and compare the results ensuring they are in acceptable range for your organization

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

9.2 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.
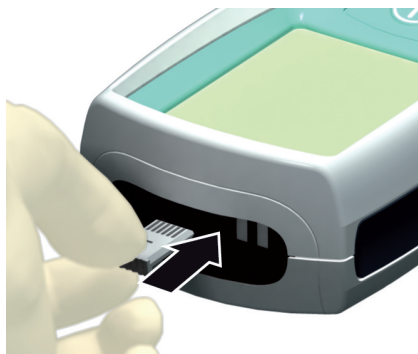
9.3 Electronic quality control (IQC)

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 or after opening, store 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 close the container immediately.
*   Insert the IQC strip into the meter (when the instrument asks for a code chip, insert 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 the 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.

9.4   Running control solutions

The control solutions has two level:

- Roche CARDIAC Control D-Dimer quality control, level 1
- Roche CARDIAC Control D-Dimer quality control, level 2
each with a lot-specific encoding chip

Store the control at 2-8 °C and tightly capped when not in use.
The stability of the lyophilized control serum is 2-8 °C up to the stated expiration date. Stability of components in reconstituted control serum at 2-25 °C is 24 hours and at and below -20 °C: 6 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 within 15 minutes.
NOTE: Avoid the formation of foam

Inserting test strip

- Turn the instrument on by pressing the On/Off button for longer than 5 seconds. Self test will be performed
- 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 indicates 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 a 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 is also displayed on the screen.
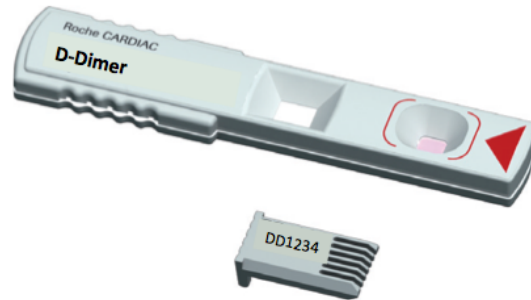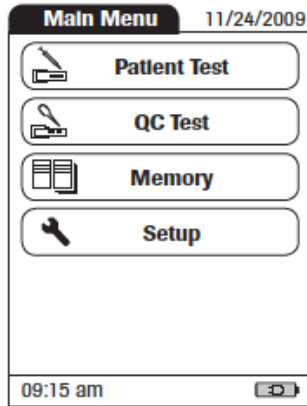
Applying the Control Solution

- When the warming up process is complete, a further beep tone and 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.
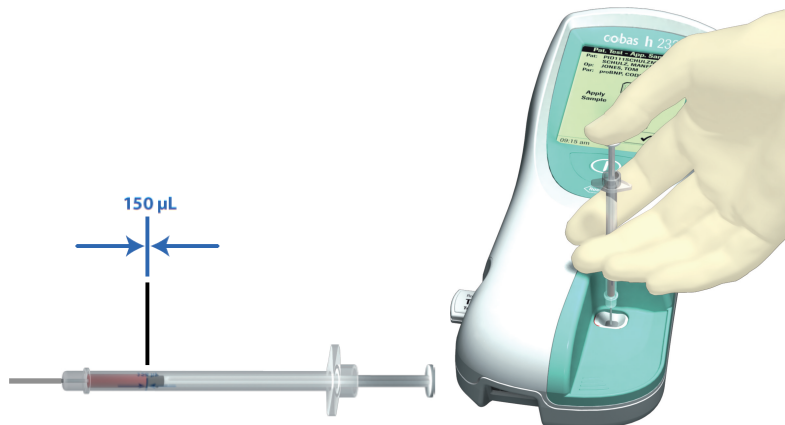
Results

- Once the sample has been detected, the actual measurement begins, 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 D-Dimer 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. Self test will be performed
- Touch the Patient Test button and enter the Patient ID using the touchscreen keypad or scan the patient barcode.
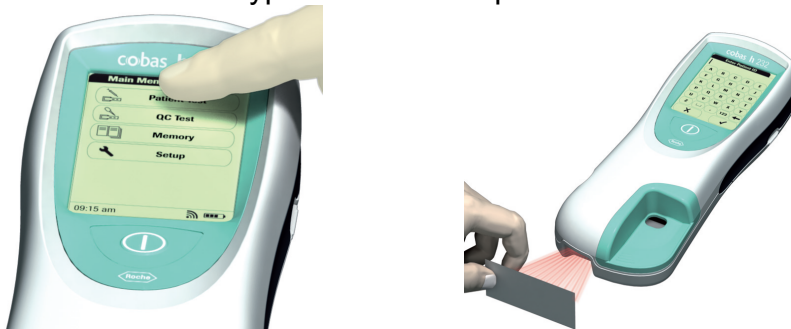
- The test strip symbol prompts you to insert the test strip. Remove the test strip from the foil package only when you are ready to perform a test.
- Hold the test strip so that the application and test areas are facing up. Insert the test strip quickly into the test strip guide of the meter using a 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 is also displayed on the screen.
- When the warming up process is complete, a further beep tone and 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.
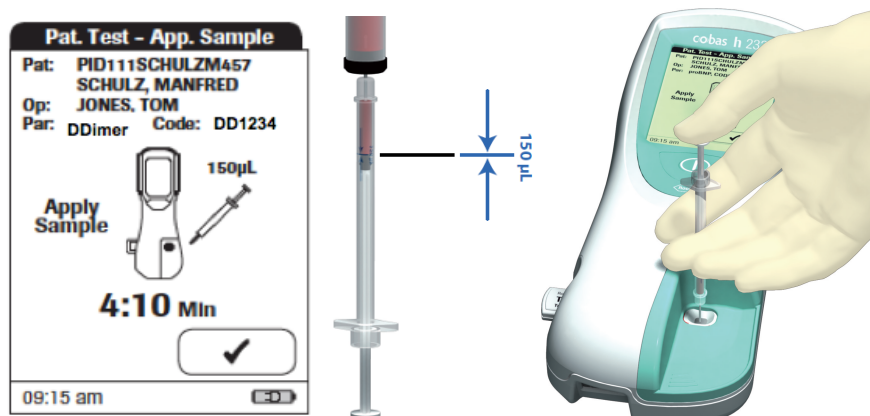
Results

- Once the sample has been detected, the actual measurement begins, 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 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.
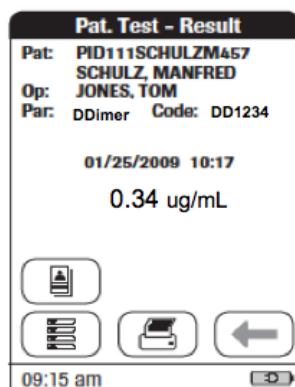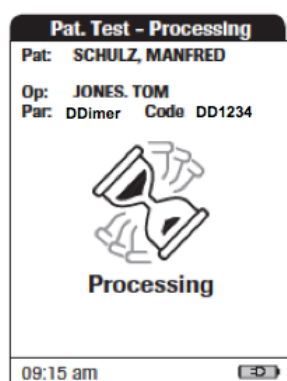
11. RESULTS

11.1 Expected values
The measuring range is 0.1- 4 µg/mL. Values above or below this range will are displayed as >4µg/mL or <0.1 µg/ml, respectively.

11.2 Interpretation of results

D-Dimer Concentration	Remarks
<0.5 ug/ml	Pulmonary embolism/ DVT unlikely. If clinical suspicion remains, follows standard investigation procedures.
>0.5 ug/ml	Pulmonary embolism/ DVT cannot be ruled out. Follow standard investigation procedures for the investigation of pulmonary embolism/ DVT.

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 (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.
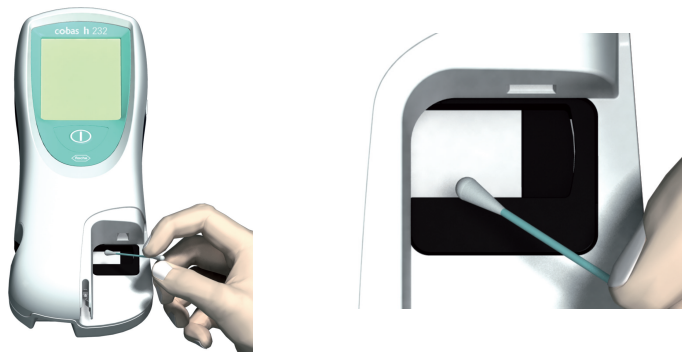
13.   REFERENCES

This method has been adapted from the Roche cobas h232 System Operator's Manual, test strips and control solution package inserts.

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2. Fancher, TL, White RH & Kravitz, R 2004, 'Combined use of rapid D-dimer testing and estimation of clinical probability in the diagnosis of deep vein thrombosis: systematic review', BMJ, pp. 329 – 821.
3. Harrison, A 2005, 'Evaluation and management of the acutely dyspneic patient: the role of biomarkers", American Journal of Emergency Medicine, vol. 23, pp. 371–378.
4. Roche Diagnostics, 2007 ,'Roche CARDIAC D-Dimer', Cobas h232 Test strip package insert, Mannheim, Germany.