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General Testing Comments

Vascular Studies will be performed at the BHF Glasgow Cardiovascular Research Centre.

The order in which tests should be taken is as follows.

1. Pre-test preparation and baseline measurements.
2. Pulse wave analysis
3. Pulse wave velocity
4. Carotid intima media thickness
5. Brachial flow mediated dilatation
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AI</td>
<td>Augmentation index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>cIMT</td>
<td>Carotid intima-media thickness.</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
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<tr>
<td>PWA</td>
<td>Pulse wave analysis</td>
</tr>
<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
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</table>
Pre-test preparation and baseline measurements.

Scope
Pre-test questionnaire to identify factors that may influence the performance of the test or the test results. Recording of basic pre-test parameters including heart rate, blood pressure, height and weight.

Duration
Approximately 20min

Health and Safety
Small risk of discomfort or bruising from venepuncture.

Equipment and Supplies
Vascular CRF
Weighing scales
Measuring stand
Urine sample collection pot
Needle, vacutainer, alcohol swab, cotton bud, small plaster.
Bottles for blood samples.
Bed for patient positioning.

Procedure
1. Complete pre-test questions using vascular CRF
   a. This will allow the patient to rest and acclimatise to the room.
2. Record the blood pressure.
3. Record height in metres.
   a. Patient should remove shoes.
4. Record weight
   a. Patient should remove shoes and heavy outer clothing.
5. Obtain blood sample if required.
6. Obtain urine sample if required (this can be done at a time convenient to the patient).

Blood Samples

<table>
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<tr>
<th>Sample</th>
<th>Measuring</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mL serum</td>
<td>Routine biochemistry</td>
</tr>
<tr>
<td>2.5mL whole blood EDTA</td>
<td>RNA extraction</td>
</tr>
<tr>
<td>2.5mL whole blood EDTA</td>
<td>Markers of oxidative stress</td>
</tr>
<tr>
<td>5mL whole blood</td>
<td>DNA extraction</td>
</tr>
<tr>
<td>2.5mL whole blood EDTA</td>
<td>Routine haematology</td>
</tr>
</tbody>
</table>

Urine Sample
Midstream urine (20mL) collected and stored.
Dipstick urine performed.
Pulse Wave Analysis

Scope and application
This standard operating procedure describes the procedure for the operation of the SphygmoCor Pulse wave analysis system. This non-invasive test is used to phenotype the peripheral vascular system and calculates cardiovascular risk.

Summary of method
A micromanometer-tipped probe (Sphygmocor, AtCor Medical Pty Ltd, Australia) is placed on the surface of the skin overlying the radial artery. For accurate readings, this is applied with a light pressure so that the transmural forces within the vessel are perpendicular to the arterial surface. The peripheral radial pulse wave is continuously recorded and the mean of 10 pulse waves was analysed. Integral Sphygmocor software uses a validated transfer function and radial artery waveform to calculate the aortic pulse waveform (Chen, Circulation 1997). The augmentation pressure index, a measure of systemic arterial stiffness, is calculated by the difference between the second and first systolic peaks (P2-P1), and expressed as a percentage of the pulse pressure.

Equipment and Supplies
Bed for appropriate positioning.
Blood pressure monitor.
SphygmoCor system
Tonometer (SPT-301B, AtCOR/Millar)
Laptop
External USB/CD/harddrive.
Serial cable.

Duration of test
Approximately 15min
Rest: 10min
Lying BP measurement: 1 min
Test: 4min

Health and Safety
A non-invasive and safe procedure.

Quality Control
Equipment is maintained by the BHF Glasgow Cardiovascular Research Centre.
Procedure

1. **PATIENT POSITIONING**
   - Lie the patient flat in bed
   - Remove watches, bracelets or tight fitting tops.
   - Position the wrist at the same level of the heart.
   - Allow the patient to rest in this position for 10min.
   - Explain the procedure to the subject.

2. **SETTING UP THE SOFTWARE**
   - Switch on the main SphygmoCor unit (switch is at the back).
   - Turn on laptop and select CV RESEARCH
   - Right click on SCOR symbol
     - Run as
     - Select “following user”
     - User: Admin
     - Password: BHFGCRC2005
   - Message from windows may appear
     - Select yes if on windows XP
     - Select no if on windows 7
   - The system opens up in the default “DATA” database.
   - In the PATIENT SCREEN, select DATABASE MANAGER from the SYSTEM MENU.
   - Highlight the database you want to select and press the SELECT button.
   - Press OK and the newly selected database is open for use.

3. **NEW PATIENT**
   - If the subject is already in the database, click on their details.
   - Click CREATE NEW in patient tool bar
   - Enter patient details in the PATIENT EDIT panel
     - Initials
     - Study number
     - Date of birth and sex
     - Add notes e.g. “can only do PWA on right wrist” “radical mastectomy on left”
     - Press UPDATE.
   - Select STUDY
     - Ensure on PWA
   - Go to the STUDY DEFINITION AREA.
     - Select the artery by clicking RADIAL check box.
   - Measure a lying blood pressure.
   - Enter patient parameters
     - Blood pressure (Mandatory)
     - Height and weight (optional)
     - Add additional notes e.g. study visit 1/2/3.
   - Insert operator initials.
4. **RECORDING A STUDY**

- Press **CAPTURE DETAILS** (top right of study screen)
- Record data
  - Place probe over the radial artery on the non-dominant arm.
  - Wrist should be slightly dorsiflexed, hold tonometer over strongest pulse.
  - Find a good trace. This will display in the **SIGNAL DETAIL** area.
  - Record at least a full screen of similar pulse waves (within the **SIGNAL FOR PROCESSING AREA**) – at least 12 seconds.
- Press the space bar to stop recording or click **OK** button.

5. **EXAMINE THE REPORT AND ASSESS QUALITY**

- The **REPORT SCREEN** will open.
- Ensure the patient data is correct. Use the **MODIFY** tool-bar button to modify parameters.
- Examine quality on the basis of the following...
- What is the heart rate variability and is the patient in sinus rhythm?
  - If yes – continue with analysis
  - If no – cannot continue with analysis
- Does the pulse wave look like a normal pulse wave?
  - If yes – continue
  - If no – try again to identify the pulse wave.
- Are all **QUALITY CONTROL INDICES** displayed in **GREEN** or white?

<table>
<thead>
<tr>
<th>Quality Control requirements</th>
<th>Reason it might be poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pulse height ≥ 80</td>
<td>Signal too weak</td>
</tr>
<tr>
<td>Pulse height variation 5%</td>
<td>Variable amounts of pressure have been applied by the operator during the study.</td>
</tr>
<tr>
<td>Diastolic variation 5%</td>
<td>Tremor from patient or the operator</td>
</tr>
<tr>
<td>Shape deviation 2</td>
<td></td>
</tr>
<tr>
<td>Operator index &gt;80%</td>
<td>Overall combination of above.</td>
</tr>
</tbody>
</table>

- If yes: meets quality control
- If no, displayed in **RED**: need to repeat testing

- Is the **OPERATOR INDEX** more than >80%?
  - If yes: meets quality control
  - If no: need to repeat testing.

- Inspect the shape of the peripheral waveform.
  - It will change according to age of patient and CV risk factors.
  - Initial upstroke should be sharp, then rise to an initial peak and a second shoulder which is the peak of the reflected wave. The end of ejection is a point of closure of the aortic valve and is shown by the notch marked incisura.
  - Waveforms that do not have these features are more dome shaped and reflect incomplete positioning of the tonometer.
- If the report does not meet quality control settings you should perform the study again by returning to the **STUDY SCREEN** and pressing **CAPTURE DATA**.
- If you are unable to record a study that meets quality control guidelines, record the best possible and record reason in study notes.
- Record a **second study** as above.
6. **DOCUMENTATION**

- Recalculate using **REPORT** button if wrong BP or parameters.
- Record the **BP** and **time** of study in the CRF.
- Record the **AI**, **aortic pressure**, and **Alx@75** in the CRF.
- Repeat analysis if concerns about quality.

7. **SAVING DATA**

- Go to **REPORT SCREEN**
- **EXPORT** button
- Export to As Text to import data into a spreadsheet program
  - Use the select button to choose the folder you wish to export to.
  - Press export.
  - Press the Close button.
- Export As Graphic to save the report as a JPG graphic file.
- To Export all measurements for a patient access the Patient Screen and click the Export Option.
Pulse Wave Velocity

Scope and application
This standard operating procedure describes the procedure for the operation of the SphygmoCor Pulse wave analysis system. This non-invasive test is used to phenotype the peripheral vascular system and calculates cardiovascular risk. Carotid-femoral pulse wave velocity is the gold-standard for measurement of arterial stiffness, and has predictive value in epidemiological studies (Laurent, 2006).

Summary of method
Three ECG stickers are placed on the chest. The pressure probe is placed sequentially over the right common carotid artery, and then the right femoral artery to identify the pulse wave. The systolic upstroke, or foot, of the pressure wave was identified by an integral algorithm within the Sphygmocor software. Wave transit time was calculated by the system software, using the R wave as a reference frame. Surface distance between the two recording sites is determined with a measuring tape. A single measure of pulse wave velocity (m/s) is generated.

Equipment and Supplies
Bed for appropriate positioning.
Blood pressure monitor
SphygmoCor monitor.
Tonometer (SPT-301B, AtCOR/Millar)
Laptop
External USB/CD/harddrive.
3 ECG leads
3 ECG stickers.
Tape measure.

Duration of test
Approximately 10min.

Health and Safety
Safe procedure
Non-invasive
Patient may feel uncomfortable during identification of femoral artery in groin.

Quality Control
Equipment is maintained by the BHF Glasgow Cardiovascular Research Centre.
Procedure

1. PATIENT POSITIONING

- Perform in the same position as PWA.
- Loosen clothing around femoral artery to allow identification of pulse wave. Subject may need to remove trousers. Ensure patient comfort and dignity.
- Place ECG stickers on subject’s chest or arms
  - RA = WHITE
  - LA = BLACK
  - LL = RED
- Attach the ECG leads.
- Use one of these positions (Figure 1):

2. MEASUREMENTS

- Identify the sternal notch (A on Figure 2)
- Identify the carotid pulsation on left (B on figure 2)
- Measure from the carotid to sternal notch (usually around 90-100mm)
- Record in mm in CRF
- Measure from sternal notch to umbilicus (C in Figure 2) and record in mm.
- Measure from the umbilicus to the femoral pulsation (D in Figure 2) and record in mm.
- Add the latter two measurements together.
- Figure 2:

```
Proximal measurement = A to B
Distal measurement = (A to C) + (C to D)
```
3. SETTING UP THE SOFTWARE

- You should already be in the correct database and patient.
- If not see SOP for PWA for SETTING UP SOFTWARE and NEW PATIENT.

4. NEW PWV

- Select PWV whilst in PATIENT SCREEN
- Enter measurements.
  - Proximal: Carotid to sternal notch
  - Distal: Sternal notch to femoral
- Insert operator initials.
- Select the appropriate site for Site A – Femoral.
- Select the appropriate site for Site B – Carotid.
- Enter the blood pressure.
- Ensure that the capture time in seconds is set at 10 for both sites A and B.
- Ensure the PWV algorithm is set for Intersecting tangents.
- Enter medication, notes or anthropometric measurements if required.

5. RECORspacing A STUDY

- Press CAPTURE Detailees (Site A – femoral)
  - Ensure the ECG is being recorded.
  - Identify the femoral
    - Midway between the anterior superior iliac spine and the front of the pubic bone, with the thigh flexed at the hip joint.
  - Place probe over the femoral artery.
  - Find a good trace (does not need to be perfect) and a constant pressure waveform.
  - Record at least a full screen of similar pulse waves (minimum 12 seconds).
  - Press the space bar to stop recording.
- A message will appear
  - “Data successfully captured at site A. Do you wish to proceed to site B?”
  - Click ok.
- Site B
  - Ensure the ECG is being recorded.
  - Place probe over the carotid.
  - The patient should be lying with their head tilted slightly back and to the side.
  - Find a good trace (does not need to be perfect) and a constant pressure waveform.
  - Record at least a full screen of similar pulse waves (minimum 12 seconds)
  - Press the space bar to stop recording
- A report will then be generated.
6. DATA QUALITY

- Are the numbers for carotid and femoral pulse wave in green?
  - If yes: meets quality control
  - If no: need to repeat testing if possible.
- Is the standard deviation less than 10% of the mean PWV?
  - If yes: meets quality control
  - If no: need to repeat testing.
- Pulse wave velocity measurements should be rejected if a red warning message such as “inconclusive” appears.

7. REPEAT

- Repeat steps 5 and 6 to record a second study.

8. DOCUMENTATION

- Repeat analysis if concerns about quality (but this is a robust reading).
- Complete CRF including the PWV MEAN and STANDARD DEVIATION

9. SAVING DATA

- Go to REPORT SCREEN
- EXPORT button
- Export to As Text to import data into a spreadsheet program
  - Use the select button to choose the folder you wish to export to.
  - Press export.
  - Press the Close button.
- Export As Graphic to save the report as a JPG graphic file.
- To Export all measurements for a patient access the Patient Screen and click the Export Option.
Carotid Intima Media Thickness

Scope and application
This standard operating procedure describes the correct procedure for the recording of carotid intima-media thickness (CIMT).

Summary of method
B-mode ultrasonography of the right carotid artery is performed with the patient supine and their head extended and positioned slightly to the left. On a longitudinal image of the carotid, the far wall of the common carotid is visualised and an image saved in reference to the R wave of the ECG. Analysis is performed off-line.

Equipment and Supplies
Ultrasound machine
Linear array vascular probe
Ultrasound jelly
Couch
CD for scan recording.
Meijer Arc and wedge pillow

Duration of test
Approximately 25mins.
Preparation time:2min
Scanning time: 15-20min
Form completion:2min

Health and Safety
This is a safe procedure. Some patients may feel some discomfort at the neck but the procedure can be stopped immediately. Abnormal carotid arteries may be identified on occasion requiring further follow up.

Quality Control
Equipment is maintained by the BHF Glasgow Cardiovascular Research Centre.

Procedure
1. PATIENT POSITIONING
   - Lie the patient flat in bed.
   - Remove necklaces and expose neck area.
   - Apply ECG stickers to right arm, left arm and lower abdomen on left.
   - Position head to left, resting on wedge pillow or towel if preferred, ensure comfortable.

2. SETTING UP THE SOFTWARE
   - Switch on the Acuson Sequoia by pushing the button below monitor on the left.
   - To enter subject identifiers press BEGIN/END button in top left corner of control panel.
   - Enter subject initials in “Patient Name” field.
• Press TAB or ENTER to move to next field.
• Enter STUDY NUMBER in “Patient ID” field.
• Scroll down “Study Type” enter as “CAROTID”.
• Scroll down “Exam Preset” enter as “CAROTID”.
• Enter date of birth and gender.
• Enter sonographer ID (initials).
• Press “BEGIN IMAGING” on soft key on base of monitor.
• Adjust DEPTH to 40mm (larger necks may require greater depth – if so, note the depth used on CRF).
• Adjust frequency to 8.00MHz.

Make sure patient in correct position, reclined comfortably with head turned to left. The common carotid artery (CCA), carotid bulb (BULB) and internal carotid artery (ICA) will be scanned for this study on both the right and left sides using an “ear-to-ear” approach.

3. Identify the distal RIGHT COMMON CAROTID ARTERY

• Label image: press CLEAR TEXT button and press soft touch monitor button on bottom left of screen to choose the “Text 2” option which shows text to the right of the picture.
• Move cursor over the text area with the track ball and type “R_CCA”.
• Apply gel to probe and approach neck on “ear-to-ear” plane.
• Identify the distal CCA, just proximal to the dilatation, in RES4 (i.e. block 4x4cm).
• Press RES (Regional expansion selection) button.
• RES 2x2cm box appears.
• Move box to area of interest with track ball.
• Press RES button again to select this box.
• To store an image, once real time image satisfactory press GAIN/FREEZE/RUN (ideally after 5-10secs of holding a good image position).
• Rotate GAIN/FREEZE/RUN wheel to find optimal still image between QRS complexes (on “R” wave).
• Press CALIPERS ON and use track ball to move calliper to desired position.
• On CCA image, place caliper mark at start of bulb ~1cm below it. Click LEFT mouse button to mark it.
• To store image press IMAGE STORE.
• Press the CINE button.
• The image should play over in a loop. If it happens to be frozen, press GAIN/FREEZE/RUN to unfreeze.
• When the white line marker appears at left side of screen, or alternatively when the thick grey marker appears at the right side of the screen, press “CLIP STORE” to save the heartbeat clip.
• Once finished storing press CINE again.
• If screen happens to be frozen press GAIN/FREEZE/RUN to unfreeze.
• Press RES again to leave RES mode.
4. **REPEAT ABOVE RCCA PROTOCOL (no.3) for:**

Right carotid bulb (labelled R_BULB) and Right internal carotid artery (labelled R_ICA)

5. **RIGHT INTERNAL CAROTID Doppler and Pulse Wave**

- Identify the right internal carotid region in RES4 (arterial wall distal to the flow divider).
- Press **D COLOUR** for Doppler.
- Press **PW** button.
- Move cursor to position Doppler gate in entrance of ICA.
- Use trackball to adjust position to optimise trace.
- Press **ANGLE** knob and adjust angle to be parallel to blood flow.
- Use **GAIN** button (top left of board) to increase amplitude of trace if required.
- Press **GAIN/FREEZE/RUN** to freeze image once thick grey line has moved to right end of screen.
- Press **CALIPERS ON**.
- Use trackball to position caliper on peak of Doppler trace.
- Double check that angle is set correctly and position cursor over highest peak.
- Press **IMAGE STORE** to store the image.
- **If velocity is >1.25m/s inform the study doctor as further routine investigation may be required.**
- To exit press **GAIN/FREEZE/RUN** to unfreeze, then **PW** followed by **D COLOUR**.
- To review images before exiting press **REVIEW**.
- To end examinations press **BEGIN/END** and press **START NEW PATIENT** soft key.
6. REPEAT STEPS 3, 4, 5, on LEFT side using “ear-to-ear” approach for LEFT COMMON CAROTID ARTERY.

7. SAVING THE STUDY
   - Press STUDY UTILITY.
   - Ok to close present study – Say Yes
   - Identify study recorded.
   - Save to disc.
Brachial Flow Mediated Dilatation

Scope and application
This standard operating procedure describes the correct method for performing brachial flow mediated dilation, for assessment of endothelial function.

Summary of method
A supine patient lies with their arm outstretched. A blood pressure cuff is placed on the forearm. The brachial artery is identified in the longitudinal plane above the antecubital fossa and fixed in position with a stereotactic frame. A baseline image is obtained for 3min, and flow velocity is recorded. The cuff is occluded for 5min. At the end of this 5 min period, the cuff is deflated.

Equipment and Supplies
Ultrasound machine
Linear array probe
Blood pressure cuff
Two towels
Snake arm (stereotactic frame)
Table
Bed
Ultrasound jelly
ECG stickers and leads.
Timer

Duration of test
Approximately 25min (15min set up and baseline recording, 5min occlusion, 5min post-occlusion).

Health and Safety
May be associated with some arm discomfort at the site of cuff inflation. The arm will feel numb and tingling. On cuff deflation arm can feel tingly and hot. However this is a safe and well tolerated procedure

Quality Control
Equipment is maintained by the BHF Glasgow Cardiovascular Research Centre.
Procedure

1. PATIENT POSITIONING
   a. Lie the patient flat in bed and rest for 10min.
   b. Remove watches, bracelets or tight fitting tops.
   c. Apply ECG stickers to right arm, left arm and lower abdomen on left.
   d. Position the right arm out to the side at 90° across two towels on the examining table. Ensure the position is comfortable and that the upper arm is resting on the examining table.
   e. Place a blood pressure cuff on the forearm. Do not inflate.
   f. Ensure the fine tuners on the snake arm are set at 15mm and the contrast buttons are in the middle.

Figure 1: Cuff and probe position (adapted from Corretti et al, J Am Coll Cardiol. 2002;39(2):257-265)

7. SETTING UP THE SOFTWARE
   - Press BEGIN/END to bring up the PATIENT DEMOGRAPHIC PAGE.
   - Fill in the required details
     - Initials, ID, Add visit number into Comments.
   - Go to first drop down box and use the cursor and selectors (mouse ball and buttons on either side).
   - Select CV ARTERY and then on the second drop down box and select BRACHIAL.
   - Press BEGIN IMAGE.
   - The image screen will appear
   - Ensure frequency set to 8Mhz
   - Increase EDGE to +3.
   - Set DEPTH to 30mm (this can be changed if required for optimum image)
   - Apply ECG leads and check the trace. Use GAIN (on left hand side) to make this larger or smaller.
8. FINDING THE BRACHIAL ARTERY
   a. Use the linear vascular probe.
   b. Apply ultrasound jelly to the upper arm.
   c. Press D COLOR and make the box bigger with the mouse ball and cursor.
   d. Detect the brachial artery in longitudinal axis 2-15cm above antecubital fossa.
   e. Move position arrow up to the vessel lumen using POSITION.
   f. Once the image is okay, secure the snake arm.
   g. Fine tune to focus the image.
   h. Change the contrast to darken the lumen and brighten the walls.

Figure 2: Brachial artery image (adapted from Corretti et al, J Am Coll Cardiol. 2002;39(2):257-265) and D COLOR button.

9. OBTAINING A BASELINE IMAGE
   - Press CLEAR TEXT and type BASELINE on to the screen. Press CLEAR TEXT again.
   - Press PULSE WAVE (PW) and then move the green cursor to the middle of the artery so the green lines are parallel with the vessel walls.
   - Press the ANGLE button and then turn it so the green lines are parallel with the vessel walls.
   - Press the GATE button until the two lines meet in the middle.
   - Record the angle in the CRF (should be between 70-90°)
   - Press SCALE button to make sure the pulse wave velocity peaks are visible but not taking up too much of the screen.
   - Once the pulse wave peaks have gone all the way to the end of the screen, press IMAGE STORE.
   - Then press the BASELINE button to increase the baseline as this will allow the pulse wave enlargement after the cuff has been deflated.
   - Then come out of pulse wave by pressing PULSE WAVE (PW) button again.
   - Make sure image is still okay and remove D COLOR.
   - Then press TRIGGER (TRIG) and then CLIP STORE in that order.
   - Record for 3 minutes and then press CLIP STORE and TRIGGER in that order to stop recording.
10. CUFF INFLATION AND POST-CUFF IMAGE.
   - Press CLEAR TEXT again and move the cursor over to where you wrote BASELINE and change it to POST CUFF.
   - Put to D-COLOR back on. Check the image and optimise with fine tuning if required.
   - Inflate the cuff for 5 minutes. Warn the patient and give them an idea of time left during the 5min.
   - Before the cuff is deflated go into PULSE WAVE (PW) screen and move the green cursor back into the centre of the artery.
   - Deflate the cuff and wait for a full screen of pulse waves and press IMAGE STORE.
   - Come out of PW, remove D COLOR, press TRIGGER and then CLIP STORE.
     - You have 20-30 seconds to do this bit so you can miss out on pulse wave measurement if the image has moved too much. Recording vessel diameter is the most important.
   - Optimise the image now if required.
   - Record for 5min.
   - Press CLIP STORE and TRIGGER in that order to stop recording.
   - Remove the cuff from the patient.

11. SAVING THE STUDY
   - Press STUDY UTILITY.
   - Ok to close present study – Say Yes
   - Identify study recorded.
   - Save to disc.
   - Return the fine adjustors to 15mm.
References


SphygmoCor System Operators Manual

SphygmCor PWA/PWV Software Guide