

## **CHAPTER 10 VASCULAR FUNCTION STUDIES: DETAILED PROTOCOLS FOR PERFORMING THE STUDIES**

### **10.1 Equipment and Supplies**

The following equipment and supplies are needed for the Vascular Function studies:

1. Examination table or bed with adjustable height
2. Sonographer chair with adjustable height
3. Ultrasound system approved by HFM Study Vascular Function Core (Boston University)
4. SunTech 247 Blood Pressure Device for Automated and Manual Measurement (see Chapter 10, Appendix 10.1 for users manual)
5. Custom Hokanson 3.25" x 22" BP cuff with quick release sphygmomanometer
6. Back-up CDs or DVDs
7. EKG leads
8. Nitroglycerin 0.4mg SL
9. Tape Measurer
10. Medical Tape
11. Towels
12. SphygmoCor Device
13. Hokanson EC6 Plethysograph Device with NIVP3 software, rapid cuff inflator, SC10D arm cuff, upper limb strain gauge set and contoured foam blocks

### **10.2 Scheduling Studies and Subject Preparation**

#### **10.2.1 Timing of Studies:**

The Vascular Function Studies must be performed before the fistula creation surgery in order for a subject to continue to participate in the study. The studies should be performed as close as possible to the date of surgery and preferably no more than 45 days before surgery. It is not necessary for the 3 types of Vascular Function Studies to be performed on the same day; however, to reduce the burden to the subject it is recommended that they be performed at one session if possible.

#### **10.2.2 Subject Preparation:**

Subjects will be given the following instructions in preparation for the studies:

1. Do not eat or drink other than water for at least 6 hours before the studies.
2. Do not smoke for at least 6 hours before the studies.
3. Do not exercise after midnight prior to the studies.
4. Participants will continue their usual medications, except that those taking insulin will be instructed to take one half their usual dose of insulin because they will be fasting prior to vascular testing

Women of child-bearing potential must have a negative pregnancy test within 48 hours of the studies in order to undergo the NMD component of FMD/NMD. If pregnant, nitroglycerin will not be administered and the NMD component of the testing will not be performed.

### **10.3 Subject Arrival and Setup**

#### **10.3.1 Order of Studies**

If all studies are being performed in the same session the order of the measurements and studies should be:

1. Blood pressure and heart rate per minute
2. Venous Occlusion Plethysmography
3. Arterial PWV
4. Brachial artery FMD/NMD.

The FMD/NMD studies should be the last studies performed for a subject on a given day.

#### **10.3.2 Subject Arrival, Setup, and Blood Pressure / Heart rate per minute**

1. Confirm that informed consent form has been obtained.
2. Confirm that the subject has followed instructions about fasting, smoking, and exercise. Subjects should be instructed not to eat or drink anything besides water. However, the sites should go ahead and do the vascular testing, even if the participant has not complied with the instructions. The time and date of last food, liquids, smoking or exercise will be recorded on Form 210.

*Note that we prefer subjects to be fasting at least 6 hours before the exam, however, if a subject has eaten or drank anything but water >2 hours from the start time of the study you may proceed. Do not proceed if subject has eaten or drank anything but water <2 hours from the start time of the study. If the subject cannot tolerate fasting throughout the entire exam and needs to eat, study should be aborted for that day and all tests should be rescheduled.*

*Note that if a patient has exercised or smoked after midnight, proceed with vascular testing and make a note on Form 210.*

3. The subject will lie supine on the ultrasound exam table/bed for 10 minutes. During this period, ECG leads are placed.
4. The vascular testing (plethysmography, PWV, and FMD) will be performed on the surgical arm. Every effort should be made to measure blood pressure and collect blood from the non-surgical arm. If blood pressure measurement or blood collection must be performed on the same arm (**e.g. subject has only one arm, history of mastectomy, functioning arteriovenous fistula or graft in one arm, etc.**), **at least 30 minutes should be allowed to elapse between measurement of blood pressure and/or blood collection and the ultrasound study.**

***Note that it is always preferable to perform vascular tests on the surgical arm. However, if there is a patent or failing fistula in the surgical arm, please use the non-surgical arm.***

***If there are any immediate questions regarding this issue, please call Mai at 267-566-3142.***

5. Initiate completion of **Form 210** (Getting Ready for Vascular Function Studies).
6. After 10 minutes of rest, measure blood pressure and pulse with the SunTech 247 blood pressure device using the non-ultrasound (non-surgical) arm. Repeat blood pressure and heart rate per minute three times, waiting at least one minute between readings. Record measurements on **Form 210** (Getting Ready for Vascular Function Studies).

#### **10.4. Venous Occlusion Plethysmography**

##### **10.4.1 Venous Occlusion Plethysmography – Patient Setup**

1. Position the subject supine, with the **surgical** arm on the same side as the plethysmograph.
2. Measure the circumference of the subject's wrist in cm, positioning the measuring tape on top of the ulnar styloid process. Record measurement on **Form 211** (Venous Occlusion Plethysmography).
3. Measure the subject's elbow circumference, with the arm straight, position the measuring tape on top of the medial epicondyle. Record measurement **on Form 211**.
4. Measure the distance of the subject's forearm, between the medial epicondyle and ulnar styloid process. Record measurement **on Form 211**.
5. Measure the distance from the base of the patient's palm to the tip of their middle finger. Record measurement on **Form 211**.
6. Find the widest portion of the patient's forearm and mark the spot with a marker. Using the mark you just made, measure the circumference of the subject's forearm and record measurement on **Form 211**. Subtract 4 cm from the circumference of the subject's forearm and use the strain gauge that correlates to that number. For example, if a subject's forearm circumference is 22 cm, use the 18 cm strain gauge. Record the strain gauge used for the subject **on Form 211**.

***Note that the forearm strain gauge set contains 8 gauges from 16 to 30 cm in 2 cm increments. It is always better to use the next smallest strain gauge down if the exact size you need is not available. For example, if the patient's forearm measures 23 cm use the 18 cm strain gauge, as there is no 19 cm gauge available.***

7. Place the SC10D rapid inflator arm cuff around the subject's upper arm and connect to cuff inflator.
8. Position the subject's wrist on the contoured foam block and prop up the upper arm with two of the foam wedges. Make sure that the forearm is positioned above the level of the heart and hanging freely between the contoured foam and foam

- wedges. It is important keep the forearm clear from any obstructions (i.e.: blankets, sheets) as they will disturb the strain gauge readings and skew the data.
9. Following the marks you made on the patient's forearm for the strain gauge measurement, place the strain gauge on the patient's forearm. You may use a piece of medical tape to hold down the black head of the gauge, but never tape over the mercury portion. Make sure that after you attach the strain gauge, the mercury filled portions are not touching each other or the cable that connects the gauge to the EC6.
  10. Inform the subject that he/she should remain absolutely still during the entire procedure.

#### 10.4.2 Venous Occlusion Plethysmography: EC6 Program Set Up

1. Open the NIVP3 software on your computer
2. Enter the Subject ID into the dialog box and press enter, reconfirm information and press enter. Enter X, X into the portion where a name is required.
3. A patient information screen will open up, please select gender and enter date of birth. Then in the studies menu click on **Lower Venous Tests**
4. Once you have clicked the Lower Venous button, please click on **MVO** and this will put the number 1 to the right hand side of MVO. Now click on **Begin Tests** button
5. "**Begin Tests**" button opens a screen that allows you to edit the test protocol and begin taking readings
  - a. Click on **Edit MVO Protocol**
  - b. Under **Protocol Name/Description** specify SPG and choose SPG under **Instrument Mode**
  - c. Make sure **VO, MOV2, and VO/(T1/2)** are checked
  - d. Next you will set the parameters for the MVO readings
    - Set the first **Baseline** reading to 10 (the amount of time, in seconds, that the software runs to record baseline), set **Inflate** to 180 (the amount of time the cuff will stay inflated) and **Deflate** to 5 (the amount of time recorded during deflation).
    - Modify all five stages to reflect the same times for **Baseline, Inflate** and **Deflate**
    - Click **Close** to exit the screen, then click either **Left or Right** to denote the arm are you are testing
    - Under the **Left or Right** box there is a drop down menu, re-select **SPG** from this menu in order for the software to recognize the changes you to the MVO Protocol.

#### 10.4.3 Venous Occlusion Plethysmography: Obtaining Measurements

1. After editing is complete (see 10.4.2), continue in the Begin Test screen, and click on **Take Reading**
  - a. The program will ask you to zero the instrument

- To zero the instrument flip the **Balance** switch down on the EC6 machine
- Click **OK** after balancing device
- b. The test screen will open after hitting **OK** - you now have the opportunity to make sure all the settings on the EC6 are correct.
  - Make sure the EC6 is set to **Venous**
  - The range is subjective and is normally set on 1% or .5%. It is suggested to begin on 1%. Position the waveform on the screen with the position knob on the EC6 so that it is towards the bottom 10% of the waveform screen.

**Note that if the range is set to 1% on the EC6 it must also be set to 1% on the test screen. If not, simply click Range and select correct value.**

- Calibrate to 1% by flipping the calibrating switch to **1%** on the EC6 and then balance the device again.

**Note that by balancing the device, the waveform tracing may move up, remember to reset it down to the bottom 10% of the screen before beginning the test.**

- c. Remind the patient to keep absolutely still
  1. Set the automatic cuff inflator dial to 20mmHg
  2. Begin taking readings by pressing the **spacebar**
  3. You will see a timer in the bottom right hand corner. This timer will count down every stage of the test. Upon pressing the spacebar, the 10 second baseline counter will begin. When the counter reaches 0 seconds, inflate the cuff.
    - a. Once you inflate the cuff you will notice the waveform tracing begin to increase, this will continue until the forearm volume has stabilized for the given venous pressure. When stabilization is reached the waveform will plateau.
    - b. When the counter reaches 3 minutes, cuff will automatically deflate
      - The timer will automatically switch to the deflation timer or 5 seconds and stop tracing after the 5 seconds have recorded.
      - Review the waveform acquisition. The left hand cursor should be at baseline right before the cuff inflated and the right hand cursor should be at the cuff deflation point. If the cursors are out of place you can manually move them to the correct position. The other line connected by two points is the measurement of the outflow 2 second after cuff inflation.
    - c. Press the **space bar** to continue, it will bring you back to the original screen. Record on **Form 211** the values listed under the columns labeled **CAP% AND MVO**
    - d. After recording the value immediately press **Take Reading** to move on to the next “stage” of the test
      - You must wait until the counter reaches 1 minute 50 seconds to begin the next reading, while waiting set the automatic cuff inflator to 30mmHg.
    - e. Once the counter reads 1 minute 50 seconds, hit the spacebar to begin baseline acquisition for 10 seconds.

- f. When the counter reaches 0 seconds inflate the cuff.
- g. When the counter reaches 3 minutes deflate the cuff and after the 5 second deflation period press the spacebar to move on.
- h. Record on **Form 211** the values listed under the columns labeled **CAP% AND MVO** and repeat the inflation and deflation process with 40mmHg, 50mmHg and 60mmHg

*Note that the subject's hand may tingle when the cuff is inflated to the higher pressures; please assure the patient that this is normal and safe.*

*Note that the subject's readings may go beyond the waveform box at the higher venous pressures. If you notice that the waveform tracing nears the top of the screen at a previous mmHg, please set the range to the next highest level for the next test. For example, if the subject's waveform tracing nears the top of the waveform box at 50mmHg, set the range to 2% before inflating to 60mmHg. If you change the range to 2%, remember to also set the EC6 to 2% and to recalibrate and rebalance the EC6 as well.*

*Note - if a patient has a negative MVO value, try the following to correct the problem: Readjust the cuff on the patient's arm ensuring it is snug against the patient's skin. Place a small folded hand towel underneath the foam block that supports the upper arm to ensure that the forearm is above the level of the patient's heart.*

4. Please note – if for any reason you need to repeat a test for a given venous pressure, simply cancel the data acquisition by selecting cancel from the patient tab at the top left of the screen.
5. After completing plethysmography, disconnect subject from Hokanson EC6 device and proceed to SphygmoCor.

## **10.5 Arterial Pulse Wave Velocity**

### **10.5.1 SphygmoCor Set up**

1. Open SphygmoCor program and click **Create New** patient button and enter subject ID and demographics, then click **Update** to accept changes.
2. Attach three EKG leads to the subject, one on each forearm and one under the patient's right clavicle.
3. Click **PWV** from the left side menu and then click the **Study** tab from the top menu.
4. **You will first collect the Carotid-Radial Data.**

### **10.5.2 Measuring Carotid-Radial PWV**

1. After clicking the **Study** tab the study screen will appear. Enter the average of the three blood pressure values recorded on **Form 210** into the appropriate fields. Select **radial** for Site A and **carotid** for Site B.
2. Using the tape measurer, measure the distance between the subject's radial pulse (site A) on their surgical arm and suprasternal notch (remember to have the

patient position their arm straight out to their side). **Enter this value into the corresponding distances section on the SphygmoCor screen, remembering to multiply the number by 10 to convert it to mm.**

3. Measure the distance between the subject's carotid pulse (site B) and suprasternal notch, record the value on the distances section on the SphygmoCor screen.

**Note that all measurements should be on the same side as the surgical arm unless the subject has a functioning hemodialysis AV access in that arm**

4. After entering the subject's distances and blood pressure, enter the study start time on **Form 212** and click **Capture Data** to proceed.
5. Locate and monitor the radial pulse, keeping your hand still and applying consistent pressure. Continue to monitor the radial pulse until you attain 10 waves that are similar in amplitude and have well defined feet with clean and sharp upstrokes. Also, make sure that the signal strength is  $\geq$  or  $=$  to 300. Press the **spacebar or OK** to capture data.
6. Repeat procedure with the carotid pulse. (Note – recall during training that the most important portion of the waveform for the carotid data is a well defined foot).
7. After capturing the carotid data, check to see that the standard deviation of the Pulse Wave Velocity is less than or equal to 10% (located in the lower right-hand corner of the screen).
  - a. If the standard deviation is greater than 10%, you must repeat the entire procedure.
  - b. If the standard deviation is less than or equal to 10% proceed to check your "Operator Index":
    - Create a PWA by selecting **Create PWA** button on the PWV Report screen. It will ask you if you want to create a PWA using site A, choose **Yes, Ok** then **No** when it asks if you would like to create a PWA using the data from site B.
    - Click **Patient** button, then select **PWA** from the side.
    - Click **Report** button, check to see that the "Operator Index", located under "Quality Control", is greater than 75 and that all the numbers located by the "Operator Index" are green.
      - If the "Operator Index" is less than 75, you must repeat the PWA.
      - If the "Operator Index" is greater than or equal to 75, you may proceed to capture the carotid-femoral measurements.

## 8. You will next measure Carotid-Femoral Data

### 10.5.3 Measuring Carotid-Femoral PWV

1. Re-select the patient and select **PWV** from the drop down menu click on the **Study** button, enter the last blood pressure values recorded and select. Select femoral for Site A and carotid for Site B.

2. Using a measuring tape, measure the distance between the subject's femoral pulse and suprasternal notch. *Enter this value into the corresponding distances section on the SphygmoCor screen, remembering to multiply the number by 10 to convert it to mm.*
3. After entering the subject's distances and blood pressure, click *Capture Data* to proceed.
4. Locate and monitor the femoral pulse, keeping your hand still and applying consistent pressure. Continue to monitor the femoral pulse until you attain 10 waves that are similar in amplitude with well defined feet. Press the spacebar or *OK* to capture data.
5. Repeat procedure with the carotid pulse.
6. After capturing the carotid data, check to see that the standard deviation of the Pulse Wave Velocity is less than or equal to 10% (located in the lower right-hand corner of the screen).
  - a. If the standard deviation is greater than 10%, you must repeat the entire procedure.
7. After completing the test complete the remainder of **Form 212** and return the completed form to the personnel responsible for keying it into the DCC database. For additional instructions on how to fill out Form 212, please see the figures below:

### Pulse Wave Velocity (PWV) Report



Figure 1. The letters correspond to variables on HFM Study Form #212, question 10.

The figure pictured above illustrates where to find 10. a. “Carotid-Radial Pulse Wave Velocity CR-PWV (m/sec)” or 10. b. “Carotid-Femoral Pulse Wave Velocity CF-PWV (m/sec)”, depending on which PWV report you are viewing. Thus, if you have just completed the Carotid-Radial measurements, and are looking at the report, the PWV value circled in the picture above will be the Carotid-Radial PWV. **The pulse wave velocity value only corresponds to the reading you have just completed.** The figure also illustrates where to find 10. k. “Standard Deviation Carotid-Radial (%)” or 10. l. “Standard Deviation Carotid-Femoral (%)”. Just like the PWV value, the standard deviation % only corresponds to the reading you have just completed.

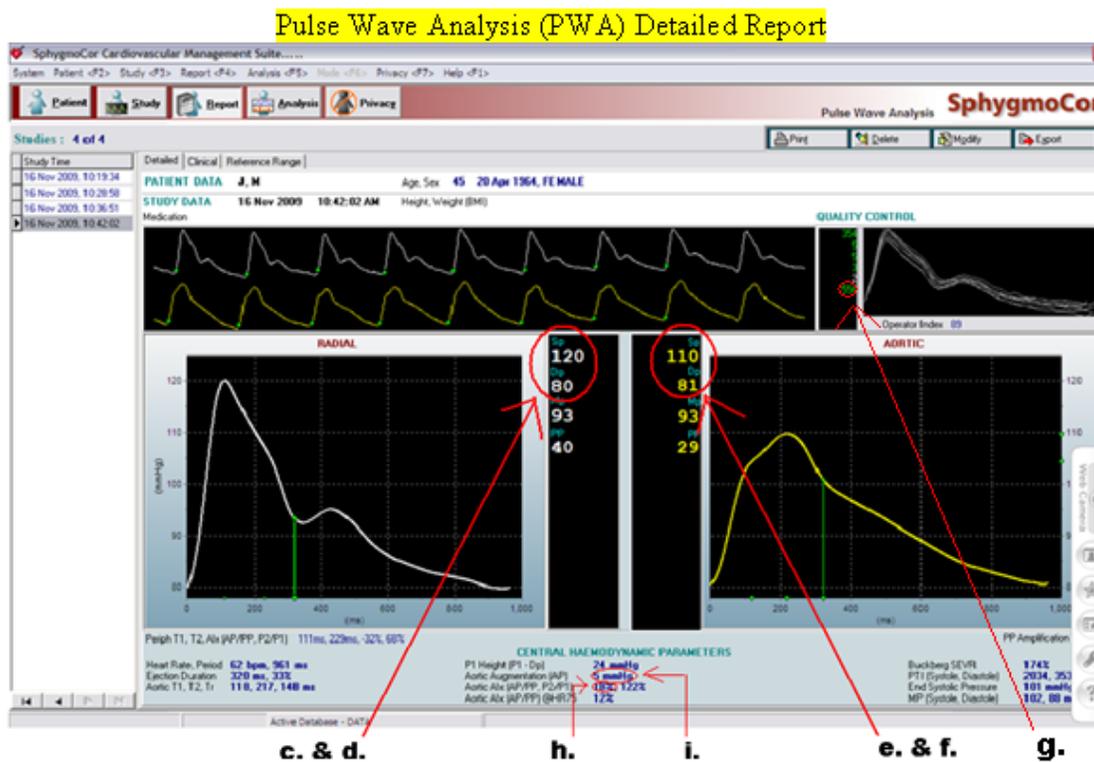


Figure 2. The letters correspond to variables on HFM Study Form #212, question 10. The figure pictured above illustrates where to find 10. c. “Radial Systolic BP P\_SP (mmHg)” and 10. d. “Radial Diastolic BP P\_DP (mmHg)”. Please note the radial pressures are the same as the blood pressures you enter into the system. The figure also illustrates where to find 10. e. “Central Systolic BP C\_SP (mmHg)”, 10. f. “Central Diastolic BP C\_DP (mmHg)”, 10. h. “Augmentation Index AIx (%)” and 10. i.

“Augmented Pressure AG (mmHg)” and 10. g. “dP/dT P\_Max\_DPDT (mmHg/sec).

### Clinical Report

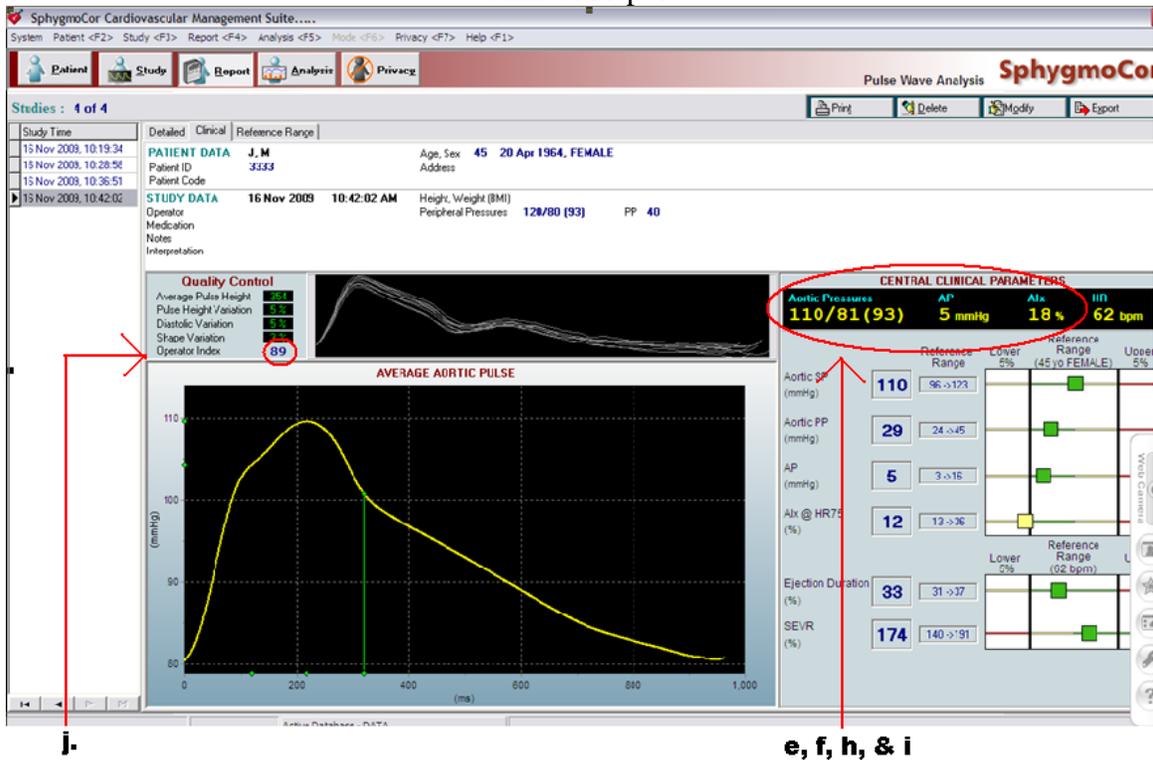


Figure 3. The letters correspond to variables on HFM Study Form #212, question 10. The figure pictured above illustrates where to find 10. j. “Operator Index”. Please note that in order to get an operator index, you must create a PWA after finishing your Carotid-Radial PWV measurements. To do this, click on the **Create PWA** tab on the PWV report, it will ask you if you want to create a PWA using the first site, click **Yes, OK** then **No** when it asks to create a PWA using site B. The figure above also shows that 10. e, f, h and i can also be found on this page, as well.

## **10.6 Brachial Artery Flow-Mediated Dilation (FMD) and Nitroglycerin-Mediated Dilation (NMD)**

### **10.6.1 Subject Set-Up and Baseline 2D and Doppler Acquisition**

1. Attach the custom narrow gauge Hokanson cuff to the upper extremity of the subject's surgical arm and place a rolled towel beneath the patient's wrist slightly elevating the subject's wrist allows for adequate venous return.
2. Connect ECG leads from the ultrasound machine to the subject.
3. Insert DVD depending on your mode of back-up into the ultrasound machine.
4. Select Brachial Artery presets (The Core laboratory will work with site personnel to establish suitable presets for the ultrasound system).
5. Enter subject's study ID number into ultrasound system  
*Note that the subject's name, medical record number, or date of birth should not be entered into the ultrasound system.*
6. Confirm that date and time are correct on the screen.
7. Confirm that a clear ECG tracing is visible at bottom of screen.
8. Confirm that the calibration scale is clearly visible on one side of image.
9. Find brachial artery, confirm that arterial flow is present.
10. If necessary, reposition arm and hand position to obtain optimal 2D image.
11. **Image quality is the highest priority and the sonographer should take sufficient time to obtain the best possible image. The features of an optimal image include:**
  - a. The artery lies horizontally on the screen
  - b. The artery is vertically and horizontally centered on the screen
  - c. The visualized segment is straight
  - d. A clear media-adventia hypoechoic line (M-line) is well visualized on both near and far walls over at least 1 cm in length.
  - e. The lumen is sonolucent without shadowing in the center of the lumen
  - f. An identifiable landmark is seen.
12. After obtaining optimal image switch into PW and set up Doppler. Set the gate to the width of the arterial lumen and position it so that the center of the sample volume is in the center of the brachial artery lumen and the angle is parallel to arterial flow. After setting up the Doppler switch out PW and return to 2D mode.
13. Please set your ultrasound's clip store or capture presets to capture the appropriate amount of time for each section of the ultrasound.
14. Acquire 15 seconds of baseline 2D images; be sure to hold the view still during this process.
15. Switch into PW and acquire 10 cardiac cycles. Remain in PW mode and proceed to cuff inflation.

### 10.6.2 Cuff Inflation

1. The sonographer tells the subject: “I am now going to inflate the cuff on your arm. It will feel very tight and you may notice that your hand and arm are numb. Please remain absolutely still.
2. Inflate the cuff to 200 mmHg or 50 mmHg above the systolic blood pressure (whichever is higher). As soon as the artery is occluded begin 5 minute timer. Keep ultrasound system in PW mode throughout the duration of the occlusion.
3. The transducer position should be held steady throughout the 5-minute inflation period using soft-tissue landmarks. The artery may collapse and become invisible.
4. Four minutes after cuff inflation the sonographer tells the subject: “You have one minute more to go. Please remain absolutely still.
5. Ten seconds before cuff release tell the subject: “I am going to release the cuff; you will feel a warm flush in your arm. Please remain absolutely still.

### 10.6.3 Deflation Scan

1. Exactly five minutes after cuff inflation, release the cuff.
2. Immediately after cuff deflation, the sonographer should obtain 10 beats of PW Doppler signal to capture the peak hyperemic flow signal.
3. 15 seconds after cuff release, switch to 2D mode, even if good Doppler images were not obtained
4. Center the arterial image in the center of the screen and use the previously identified landmark to align the previously scanned portion of the artery in the middle of the screen. Hold the vessel in the center of the screen for **1 minute 15 seconds**.

*Note that the 2-D sequence is the most crucial, and every effort should be made to hold the image motionless during this entire period. If there is difficulty obtaining the Doppler signal, the attempt should be abandoned after 15 seconds, allowing the sonographer to capture the much more important 2-D image.*

### 10.6.4 Immediate Check of Data Quality

1. Before proceeding to the Nitroglycerin portion of the study, the sonographer will review the tape and address the following issues:
  - a. Ensure that the study was recorded.
  - b. Ensure that the image quality is good as described above. Clear intima at the near and far walls and a lumen free of shadowing are particularly important.
  - c. Ensure that a common portion of the artery was visualized at baseline and after cuff deflation.
  - d. Ensure that there are 10 beats of Doppler recorded at baseline and after cuff release.
  - e. Ensure that hyperemia has occurred.

*If any component is missing or inadequate, the patient should be asked to wait for at least two hours and the cuff occlusion and deflation scan should be repeated. This repeat study should be completed and verified as adequate before nitroglycerin is administered.*

### 10.6.5 Nitroglycerin Component

1. Omit the nitroglycerin component if:

If any of the baseline Systolic blood pressures are less than 100 mmHg

**OR**

The subject has a history of nitrate intolerance or migraine headaches

**OR**

The subject has used sildenafil (Viagra), vardenafil (Levitra) or tadalafil (Cialis) in the past 7 days.

1. If nitroglycerin is going to be administered, allow the subject to rest for 10 minutes.
2. Enter new heading into the ultrasound system to denote the nitroglycerin portion of the scan.
3. Obtain pre-nitroglycerin images (15 seconds) of the same segment of brachial artery used for flow-mediated dilation, using the soft tissue landmarks.
4. Warn the subject: "I am going to give you a nitroglycerin tablet under your tongue. Hold it in your mouth and allow it to melt. You will feel it burning, and then you will feel a generalized flushed feeling throughout your body. You may experience pounding in your head or a headache. If you do, it will pass in a few minutes.
5. Administer sublingual nitroglycerin 0.4 mg.
6. Allow the nitroglycerin to dissolve for 2:45, then after confirming with the patient that the nitroglycerin has dissolved, begin capturing 2D images of the brachial artery for one minute.

*Note that a physician should be readily available to assist should any complications due to Nitroglycerin administration occur, however, it is not required for the physician to be present when administering the drug. However, you should follow your lab's and hospital's guidelines for administering medication.*

*Note that the NTG portion is extremely important to the study and every effort should be made to collect this data as long as the patient qualifies for the medication.*

### 10.6.6 Study Wrap-Up

1. Measure post nitroglycerin blood pressure. If systolic pressure is less than 100 mm Hg, keep the subject supine until the blood pressure is greater than 100 mmHg.
2. Have the subject sit on the edge of the examination table/bed until any dizziness passes, and then assist him/her to a standing position and be sure that he/she can ambulate without problems.
3. Complete **Form 213** and include a copy of the form with the images sent to the **Vascular Function Central Reading Facility**.
4. Save study to DVD/CD in the DICOM format, copy it and send it by overnight courier to the Core Laboratory at Boston University for analysis (record date sent to BU on **Form 213**. Please keep the copy of the disc at your lab and back it up on a hard drive. Note – for the first five patients please send the ultrasound studies via overnight delivery as soon as you perform them, please review

Chapter 11.1 for instructions on shipping data. After that you may wait until the CD or DVD is full to send the disc to be read.

***Note that a patient must complete a minimum of two of the three vascular tests to continue in the study.***