Trial Procedures Manual

Effect of A Reduction in GFR after Nephrectomy on Arterial Stiffness (EARNEST)

REC Number: 13/EE/0015

Protocol Version: v1.0 – 01 Nov 12

Chief Investigator:	Dr Ian B. Wilkinson Clinical Pharmacology Unit, Box 110 Addenbrooke's Hospital Hills Road Cambridge CB2 0QQ
Telephone:	01223 336898
Trial Sponsor:	Cambridge University Hospitals NHS Foundation Trust & the University of Cambridge
Co-ordinator:	katan.patel@addenbrookes.nhs.uk Tel: 01223 349 243
SAE Reporting:	
Email	cctu@addenbrookes.nhs.uk
FAX	01223 256623 (Fax)
Medical Monitor:	Professor John Cockcroft Department of Cardiology, Wales Heart Research Institute, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN United Kingdom
Telephone:	029 2074 3489
Email:	cockcroftJR@cardiff.ac.uk

Contents

Trial Procedures Manual guideline

This trial procedures manual is a supplement to the EARNEST protocol. It is not a replacement for the protocol but provides detailed instructions on how to carry out key aspects of the study. Please read it carefully in conjunction with the protocol and the regulatory guidelines that govern Good Clinical Practice.

Trial Procedures

Donor patients will be recruited from all patients undergoing donor nephrectomy at local sites. Control patients will be recruited from clinics, advertisements, outside institutions and any local volunteer databases.

Following identification by the local PI and clinical care team of potentially eligible patients, all patients will be given a minimum of 24 hours to read the Patient Information Sheet and decide whether they wish to participate in the study.

A signed, written informed consent form must be obtained from the patient prior to any study-specific procedures or assessments. Refer to Figure 1 for an overview of the initial procedure flowchart which documents the patient recruitment and registration process. For timing schedule of all assessments please refer to the Time and Events Table.

An overview of the study assessments is presented in Figure 2. The manual provides a detailed description of procedures for EARNEST study assessments and laboratory procedures. However, there may be instances where local practises and guidelines should be followed.

Figure 1. Initial procedure flowchart

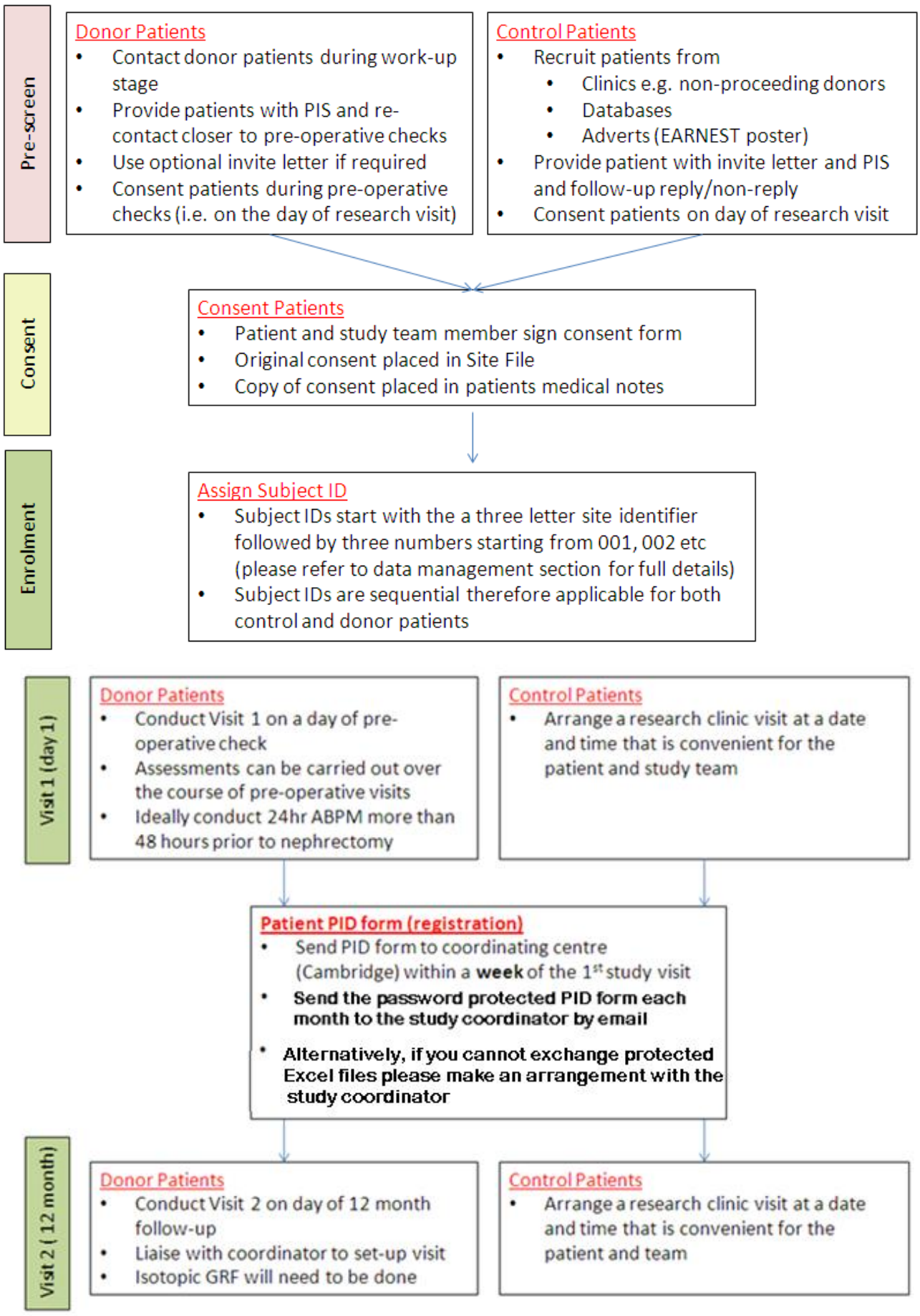
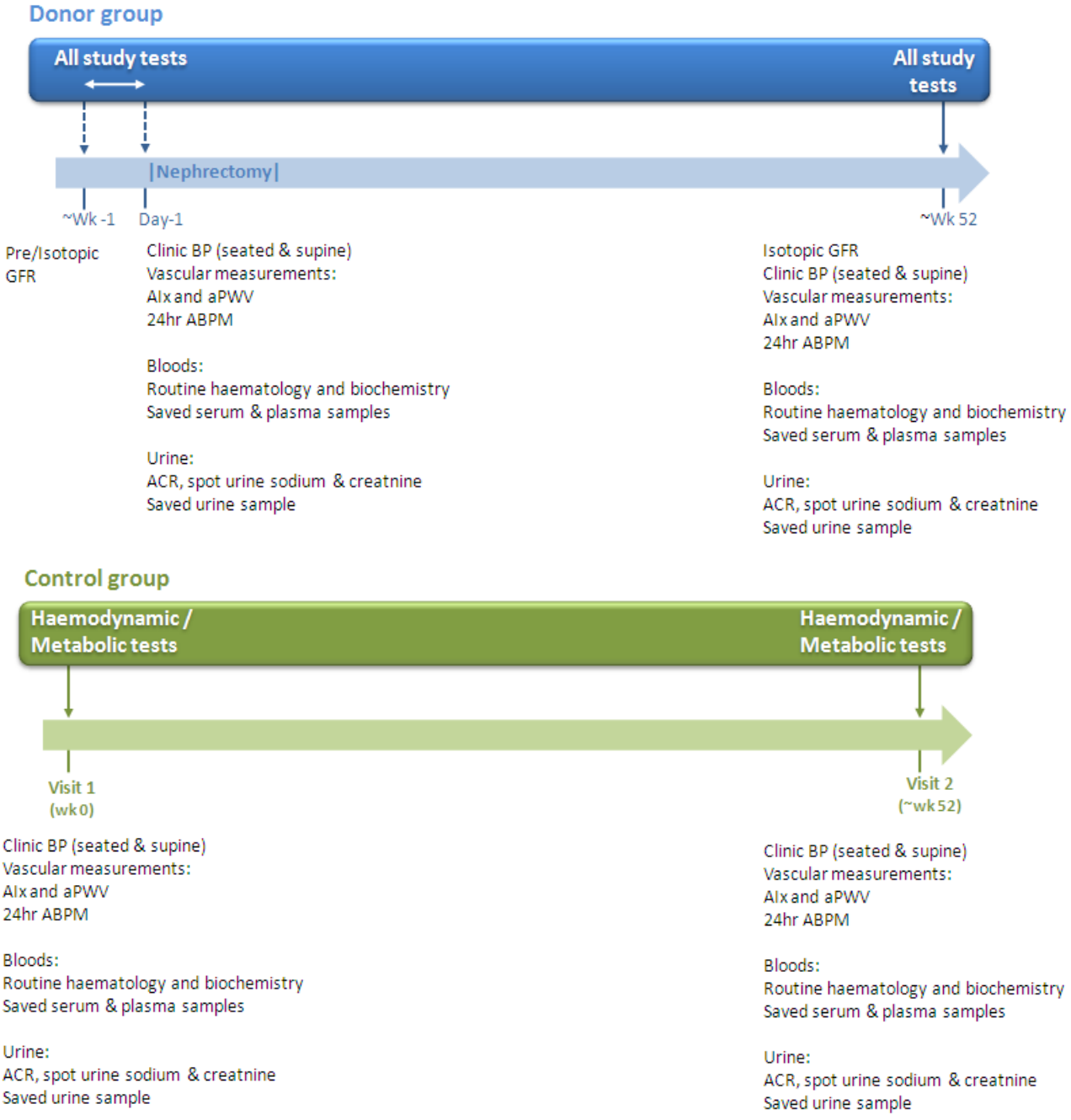


Figure 2. Study schematic



Patient Recruitment

A sufficient number of kidney donor and control patients will be enrolled across the participating sites, so that 800 participants with data suitable for the primary statistical analysis (approximately 400 per cohort) complete the trial.

Donor patients

Donor patients can be contacted during the work-up stage and provided with an invitation letter (if required) and Patient Information Sheet (PIS). Patients should be followed-up before their pre-operative visits and if interested advised about EARNEST consent and study assessments being conducted during these visits.

Control patients

Control patients may be recruited through clinics, databases and adverts. Patients should be given an invitation letter and a PIS. The study teams should follow-up patients and arrange the first research visit at a time that is convenient for both the patient and study team.

Informed Consent

The investigator or designee will obtain informed, written consent from each patient before participation and performance of any protocol specific procedures. A contact number for the study team will be provided on the PIS to enable participants to ask any questions they may have. The voluntary nature of participation and the ability to withdraw an individual's consent at any time will be emphasized.

The informed consent form used for this trial and any change made during the course of this trial, must be prospectively approved by the REC and local R&D be undertaken by the lead coordinating site in Cambridge. Patients who do not fully understand the information provided will not be enrolled on to the study.

The Informed Consent 'script' should contain (at minimum) the following points:

- Background/Purpose
- Why the patient has been invited
- Brief overview of the study – multi-site, approx 800 patients, Sponsor
- Brief descriptions of the procedures
- Benefits and disadvantages
- What happens to the results
- What if there is a problem – contact details/out of hours
- GP to be informed
- Female patients; clarify pregnancy status
- State the patient can withdraw at any time for any reason which they do not need to give
- Any questions the patient may have
- Carefully read the consent form and initial in each box
- Travel expenses, how and when this will be paid

The study doctor/delegated member of the study team will then go through the inclusion/exclusion criteria form.

Patients will be eligible for inclusion into the study only if all of the following criteria are met:

Inclusion criteria

Donor Group

- A donor group will be recruited from all patients undergoing donor nephrectomy.

Control groups

- A contemporaneous control group will be recruited from clinics, advertisements within and outside the institutions and from any local volunteer databases.

Exclusion criteria

Donor and Control Group

- These will be the current nationally set exclusion criteria for donors (same criteria for control patients) and include age and Glomerular filtration rate (GFR) cut-off (Table 1), diabetes mellitus, any history of cardiovascular or pulmonary disease (that would contraindicate kidney donation), evidence of hypertensive end-organ damage, known LV dysfunction (including ejection fraction < 40%) and atrial fibrillation.

Table 1. Acceptable GFR/ eGFR by age

Age (years)	Acceptable GFR/ eGFR at time of study visit 1 (mL/min/1.73m^2)
Up to 46	80
50	77
60	68
70	59
80	50

Donor group

- Pregnancy at one-year follow-up visit

Data management

Patients can be contacted beforehand by giving them the patient invitation letter (separate letter for donor and control patients are provided). It may be helpful to keep a record of the patients that are contacted in the EARNEST subject invitation log (Optional - figure 3).

Figure 3. EARNEST subject invitation log

Cambridge Clinical Trials Unit Box 111 FRM001/EARNEST

Subject Invitation Log Page ___ of ___

Study Title:	EARNEST		
Investigator:		Site name/no.:	
R&D Number:	A092761	REC no.:	13/EE/0015

	Initials	Date of Birth	Date Invitation sent	Date of Response	Response	Mode of response	Future clinic visit scheduled?	Reason for non acceptance or non participation
1					☐ Interested ☐ Not Interested	☐ Postal Mail ☐ Phone ☐ In Person ☐ Other _____	☐ Yes ☐ No If yes, date _____	

Once the patient has signed the Informed Consent form, they can begin their research assessments. The subject details will need to be included on the mandatory screening log (Figure 4). If the patient signs consent but withdraws from the study at any time then this will be documented in the appropriate columns in the screening log.

Figure 4. Example of the patient screening log

Subject Screening Log FRM002/EARNEST

Page ___ of ___

Study Title:	EARNEST		
Investigator:		Site name/no.:	
R&D Number:	A092761	REC no.:	13/EE/0015

	Initials	Date of Birth	Date of Consent	Subject ID no.	Donor or Control?	Donor changed to Control?*	For patient *discontinued* during trial*:	
							Date of discontinuation	Reason:
1						☐ Yes ☐ No		

Patient Identifiable Information form (PID)

The EARNEST study coordinator will send each study site a password encrypted Excel sheet for the site to complete. The form must be completed for each patient that is consented so that the coordination can register the site for UKCRN accruals. This form will also be used to register patients for long-term follow-up.

Alternatively, if password encrypted files cannot be exchanged then please arrange a method (e.g. post or fax) to get this information with the study coordinator.

CRFs (working documents)

Patient study assessment data will be entered onto the CRFs (working documents) provided. There are 2 sets of CRFs, one for the baseline visit (V1) and one for the 12 month follow-up visit (V2). On the first page full details of the participant and site need to be entered and whether they are a control or donor patient (Figure 5).

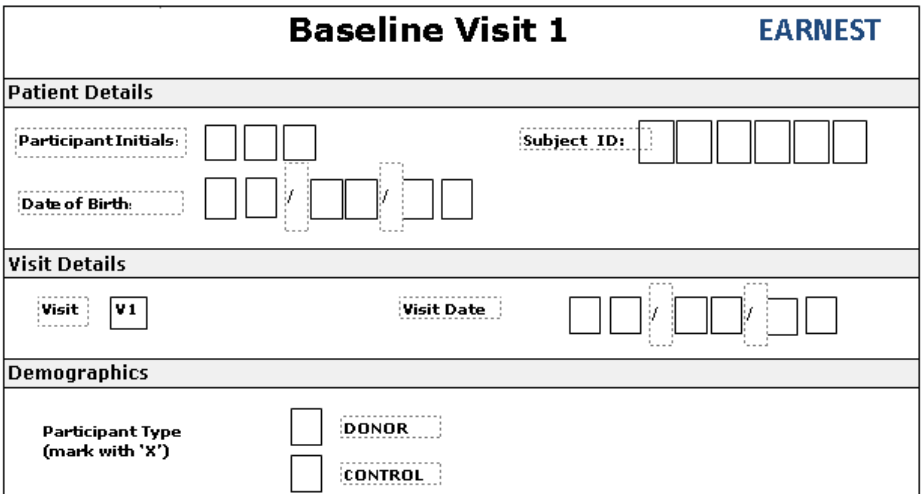
Subject IDs

The Subject ID is the patient trial number, specific for that patient throughout the trial. This ID will be used for all the subsequent documentation. **Subject ID numbers** for any site will start with the site identifier (see below) e.g. **CAM** followed by a three digit sequential numbering system **001, 002, 003** etc. The subject IDs e.g. **CAM001, CAM002** are applicable to both donors and controls.

1) Addenbrooke's, Cambridge – **CAM**
2) Queen Elizabeth Medical Centre, Birmingham – **BAM**
3) St Georges Hospital, London – **GST**
4) Western Infirmary, Glasgow – **GLA**
5) University Hospitals Coventry and Warwick – **COV**
6) Bristol NHS trust – **BRI**

If your site is not in the list above then please contact the coordinating centre

Figure 5. Screenshot of part of the first page of the CRF



For the following assessments please fill in the CRF and attach printouts directly to the working document (for that visit):

1) **24hr ABPM summary printout**

EARNEST database

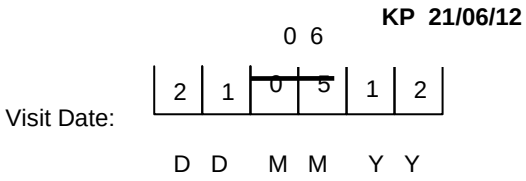
When the EARNEST database is completed, sites will be notified and data should be entered from the CRF into the database as soon as possible.

Data from other studies

Data collected from previous related studies (KARMA and CRIB-DONOR) will be entered onto the EARNEST database.

General instructions for completing CRF (working documents)

- Working documents must only be completed by the CI/PI and study team members delegated the task.
- Please complete the working documents using black ink, writing with consistency, completeness, logic and legibility.
- Corrections should be crossed-through once, initialled, dated and the appropriate correction made.



- The working documents must be completed and signed at the bottom of the form by the designee or Investigator promptly.
- Dates are to be entered in a dd/mm/yy format unless specified otherwise
- If a result is **missing**, enter in the results column '**ND**', i.e. not done.
- If the value is unknown then use '**NK**' for not known.
- Fill in boxes ☐ with a tick **√ or 'X'**.
- Where printouts of source documents are available they should be attached to the working document for that visit.

Study measurements

The time and events table below provides an overview of the study assessments that need to be completed for this study.

Table 2. The Time and Events table

DONOR GROUP	Visit 1 (Baseline)	Visit 2 (Research Clinic Visit)
Approximate visit schedule	**Wk -6/Day -1**	**Wk 52**
Informed Consent	X	
Demographics and history	X	X^
Isotopic GFR	X*	X
Haemodynamic tests Office BP 24h ABPM **Arterial Stiffness:** aPWV, AIx	 X X X	 X X X
Blood sampling - FBC, U&E, LFT, Bone profile, PTH, TFT, uric acid (urate), Mg, CRP	X	X
Blood storage – serum and plasma	X	X
Urine sampling – ACR, Urine Na and creat, β-HCG**	X	X
Urine storage - biomarkers	X	X

^ Medical history since V1

* This may be done earlier

**Applicable to women of child-bearing potential – if required

CONTROL GROUP	Visit 1	Visit 2
Approximate visit schedule	**Wk 1**	**Wk 52**
Informed Consent	X	
Demographics and history	X	X^
Haemodynamic tests Office BP 24h ABPM **Arterial Stiffness:** aPWV, AIx	 X X X	 X X X
Blood sampling - FBC, U&E, LFT, Bone profile, PTH, TFT, uric acid (urate), Mg, CRP	X	X
Blood storage – serum and plasma biomarkers	X	X
Urine sampling – ACR, Urine Na and Creat, β-HCG*	X	X
Urine storage – urine biomarkers	X	X

^ Medical history since V1

* Applicable to women of child-bearing potential

Detailed guidance for individual assessments are provided below.

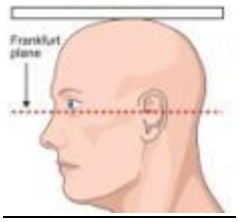
Anthropometry Measurements – Height and weight

Height and weight measurements will be carried out for both groups of patients. The following equipment should be used:

- Weighing Scales
- Wall height chart/stadiometer

Height

- The subject is asked to remove their shoes.
- Subject stands against height wall chart/ stadiometer, with their feet shoulder width apart.
- Subject's heels, bottom, shoulder blades and head should be in contact with the wall chart/stadiometer. If applicable, ask subject to take their hair out of their pony tail to facilitate this.
- Ask subject to look straight forward so that the Frankfurt plane (auriculo-orbital plane) is horizontal.



- Stadiometer is held level against top of subject's head. Height is recorded in centimetres.

Weight

- The subject is asked to remove shoes and socks and any heavy items of clothing/ objects inside of clothing.
- Scales are 'zeroed'. Subject stands on scales and weight is recorded in kilos.

Office Blood Pressure and heart rate

The first measurement will be made following at least **5 minutes of seated or supine rest**. Two further readings should be taken with at least one minute between readings. All measurements should be made using the same arm and cuff size. Blood pressure is measured in millimetres of mercury (mmHg) and is always quoted as the SBP over the DBP, for example 120/80mmHg.

Equipment

- A BHS-validated oscillometric sphygmomanometer blood pressure monitor will be used, as this has been validated according to BHS guidelines.
- Cuffs used should be of a size appropriate to the patient. If arm circumference exceeds 33cm, a large cuff should be used.

The device should be used in accordance with the manufacturers' instructions and the blood pressure reading should be recorded as soon as it has been taken.

Preparation

- Use a regularly calibrated device
- Rest the patient in the measurement position for a minimum of five minutes before measuring blood pressure.
- Ask patient to remove tight fitting clothing
- The observer should outline the procedure briefly, in particular, s/he should mention that some discomfort may be felt when the cuff is inflated, and that the measurement will be made several times.
- The cuff is to be applied to the non-dominant arm of the patient
- Support patients arm at heart level with his or her hand relaxed – arm to be supported (e.g. on a table) and the cuff to be placed in the middle of the upper arm and the bottom edge of the cuff being 2-3cms above the ante-cubital fossa. The cuff should fit firmly, and be well secured.
- Use correct sized cuff. For smaller adult arm (circumference up to 33cm) use a cuff 12cm x 23cm. For most adults (circumference up to 42cm) use cuff 13cm x 35cm.
- Encircle the rubber bladder around the least three quarters of the arm circumference
- Three readings should be taken. At least one minute should elapse between readings.

Seated

Ask the participant to rest in a seated position for a minimum of 5 minutes before measuring blood pressure. Two further readings should be taken with at least one minute between readings.

Supine

Ask the participant to rest in a supine position for a minimum of 5 minutes before measuring blood pressure. Two further readings should be taken with at least one minute between readings.

Recording the Data

- The blood pressure reading should be recorded as soon as it has been taken.
- The arm in which the pressure is being recorded, the position of the patient, and the size of cuff used should also be noted.
- **The average of the last two readings will be used as the recorded blood pressure (refer to CRF).**

Trouble-shooting

The following are possible reasons behind errors in blood pressure readings:

1. Defective equipment (e.g. leaky tubing or valve)
2. Too rapid deflation
3. Using wrong cuff size
4. Cuff not at same level as heart
5. Poor technique
6. Digit preference, rounding up to a 5 or 10 mmHg
7. Observer bias – such as expecting a young subject's blood pressure to be normal.

Arterial Haemodynamics

Measuring Blood pressure and Arterial Stiffness

The aim of this assessment is to use the SphygmoCor system to non-invasively record central blood pressure and arterial stiffness.

Equipment list

- SphygmoCor System (including tonometer, ECG leads and connecting cables) with computer/laptop
- ECG stickers (with 'studs')
- **Callipers** are preferable but a tape measure may be used if you are unable to source – **the same measurement device should be used at both visits**
- Semi-automated oscillometric blood pressure monitor with medium and large cuff
- Bed or couch and chair for operator (one of which should be height adjustable)

Associated Documents

- SphygmoCor® Operator's Manual (AtCor Medical Ltd)

Procedure

PREPARATION

- Patient should be supine
- Connect the SphygmoCor module to the computer/laptop using the serial/USB connector
- Connect power supply to SphygmoCor system and check power is 'ON'
- Connect tonometer to SphygmoCor (if not already connected)
- Connect the ECG cable to the SphygmoCor system (if not already connected)
- Double click on 'SphygmoCor' icon on the desktop
- Select the database associated with the EARNEST study by clicking on 'System', scroll down to 'Database Manager', select the required database, click OK.
- In the Study screen, select 'new patient'

- **How to create the EARNEST database on the SphygmoCor:**
- 1. Turn on the SphygmoCor and computer.
- 2. Double click on the SphygmoCor icon on the desktop.
- 3. A box appears on the screen asking about 'optimising the database' – click on NO.
- 4. Click on the 'System' button (top left of the screen), a drop down menu will appear.
- 5. Double click on 'Database Manager'

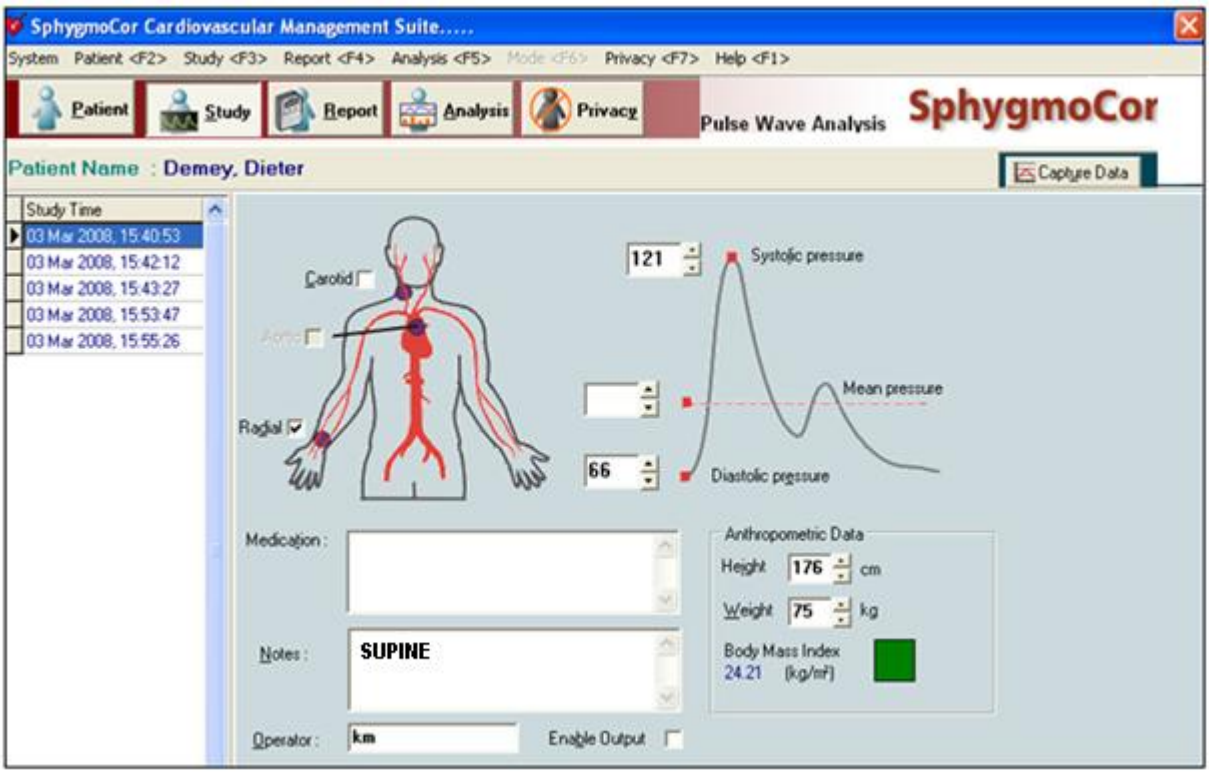
- 6. A box appears; enter EARNEST into both 'Name' and 'Description' fields.
- 7. Click on 'Create new button'.
- Your EARNEST database has now been created.

Enter participant's details into the database and confirm if details are correct, Select 'PWA' from the menu. Click on 'study' to go to the study screen.

RECORDING DATA

- Enter the averaged systolic and diastolic supine pressures into the relevant fields in the study screen (see Figure 6)
- Ensure that 'Radial' is checked and enter 'Supine' into the Notes field (Figure 6). NB it is not necessary to enter the patient's height and weight.
- Click on 'Capture Data' to initiate a recording

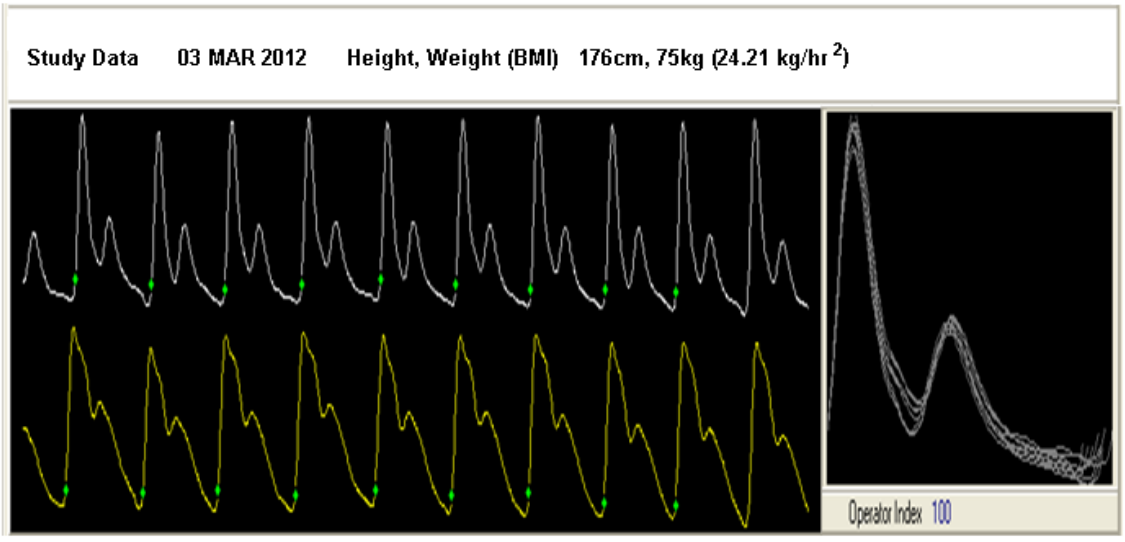
Figure 6. Screenshot of the SphygmoCor data system



- In the **same** arm in which blood pressure was measured, palpate the radial artery and locate the strongest pulse of the artery using your index finger
- The best results are obtained if the wrist is bent outward in the 'dorsiflex' position (whilst supporting the wrist). This pushes the artery towards the surface of the skin thus making access easier.

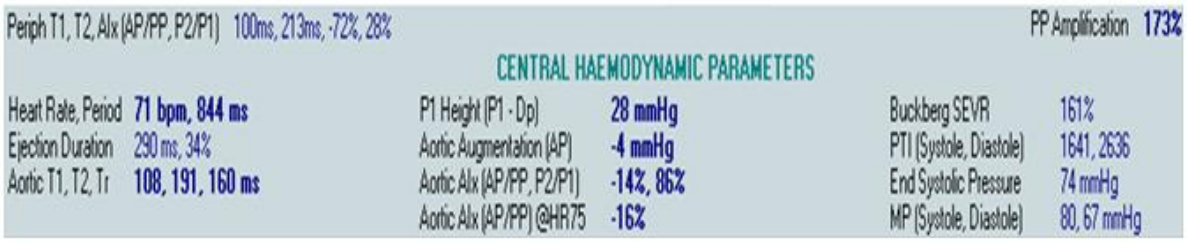
- Using the tonometer, obtain a steady waveform from the artery. Remember to press gently and steadily so that the waveform is displayed completely within the screen. If the trace disappears from the screen, the operator is pressing too hard or not hard enough.
- Adjust the tonometer slightly backwards and forwards across the artery to ensure the strongest, smoothest signal. As you move away from the centre of the artery the waveform becomes smaller and noisier.
- Once you have obtained a good waveform, you will notice that it is consistent, large and moving across the screen in a steady vertical position
- Allow for 10-15 seconds of steady and good quality waveforms
- Press the SPACE bar on the laptop/computer to acquire the data
- The study report screen will then appear automatically
- Check the quality control indices and the operator index (Figure 7). The first three indices should all appear in green and the operator index should ideally be >80.

Figure 7. Example of results output



- Take a second reading by pressing 'F3' to return to the study screen and 'Enter' to initiate a second data capture. Again, check the quality control indices and operator index. Also check the Aortic AIx values on the duplicate readings (Figure 8).

Figure 8. Example of results output



- If the quality is poor or if Aortic AIx differs by >4 percentage points, take a third measurement, delete the poor quality reading and keep the two closest readings.

To record on paper CRFs (see page 22):

- Central systolic and diastolic blood pressure (sphygmocor; two readings and the average)
- Mean arterial Pressure (MAP; sphygmocor; two readings and the average)
- Aortic AIx (sphygmocor; two readings and the average)

NB: Three (3) brachial BP readings are required (enter average of last two) and two (2) high quality sphygmocor readings (central BP, MAP and AIx) are required.

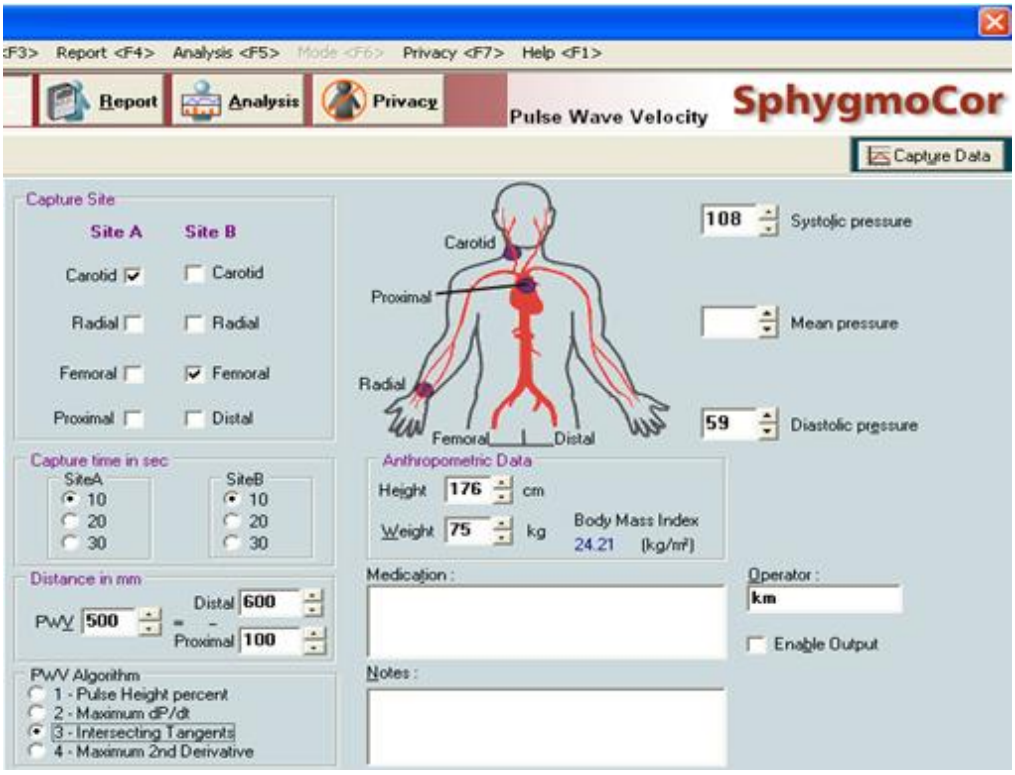
PWV readings

- In the meantime:
- In SphygmoCor, go to the 'Patient' screen, select 'PWV' and then go to the 'Study' screen (i.e. it is not possible to change the mode from 'PWA' to 'PWV' without returning to the 'Patient' screen)
- Attach ECG electrodes to the participant
- Palpate the carotid and femoral arteries and mark the skin overlying the strongest part of the pulse
- Measure and record the PWV distances with a measure tape or calliper:-

1. Distal: Suprasternal Notch (the notch at the top of the sternum) to the distal (femoral artery) site.
2. Proximal: Distance from the suprasternal notch to the proximal (carotid artery) site.

- Enter these values into the fields labelled 'Distances in mm' in the sphygmocor Study screen (Figure 9). Also, ensure that the carotid and femoral boxes are checked under 'Site A' and 'Site B' (Site A refers to whichever site is measured first so if you start at the carotid artery, then Site A should = carotid and site B=femoral). Finally ensure that under 'PWV algorithm', option 3 (Intersecting Tangents) is checked.

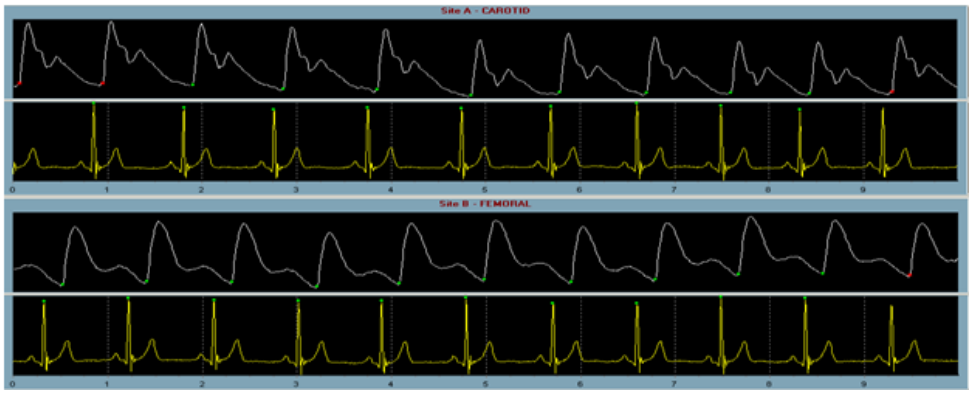
Figure 9. Screenshot of data entry page



- Enter the averaged supine systolic and diastolic pressures into the relevant fields in the study screen (Figure 9)
- Click on 'Capture Data' to initiate a recording.
- Ensure that the ECG trace is clear and not distorted by artefact. Also ensure that there is a clear R wave (necessary for gating of waveforms)
- Place the probe over the first marked site (e.g. the carotid artery, if this is listed as 'Site A') and make small adjustments to obtain a smooth waveform, as outlined in the steps above
- Allow for 10-15 seconds of steady and good quality waveforms and then press the spacebar. A window will appear stating that Site A was captured successfully and asking if you want to proceed with data capture at Site B. Click 'Yes'.
- Place the probe over the second marked site (e.g. the femoral artery, if this is listed as 'Site B') and make small adjustments to obtain a smooth waveform. Again, allow for 10-15 seconds steady and good quality waveforms and then press the spacebar. The study report screen will then appear.

- Visually check the quality of the waveforms and take note of the PWV value and SD (Figure 10).
- Take a second reading by pressing 'F3' to return to the study screen and 'Enter' to initiate a second data capture. Again, check the quality of the waveforms and take note of the PWV value and SD. If the PWV differs by >0.5m/s, or the SD by >1.0m/s, take another reading. Ideally, delete poor quality readings and keep the closest two (if within 0.5m/s) or if readings are of good quality, yet different values, keep 3-4 readings and average them.

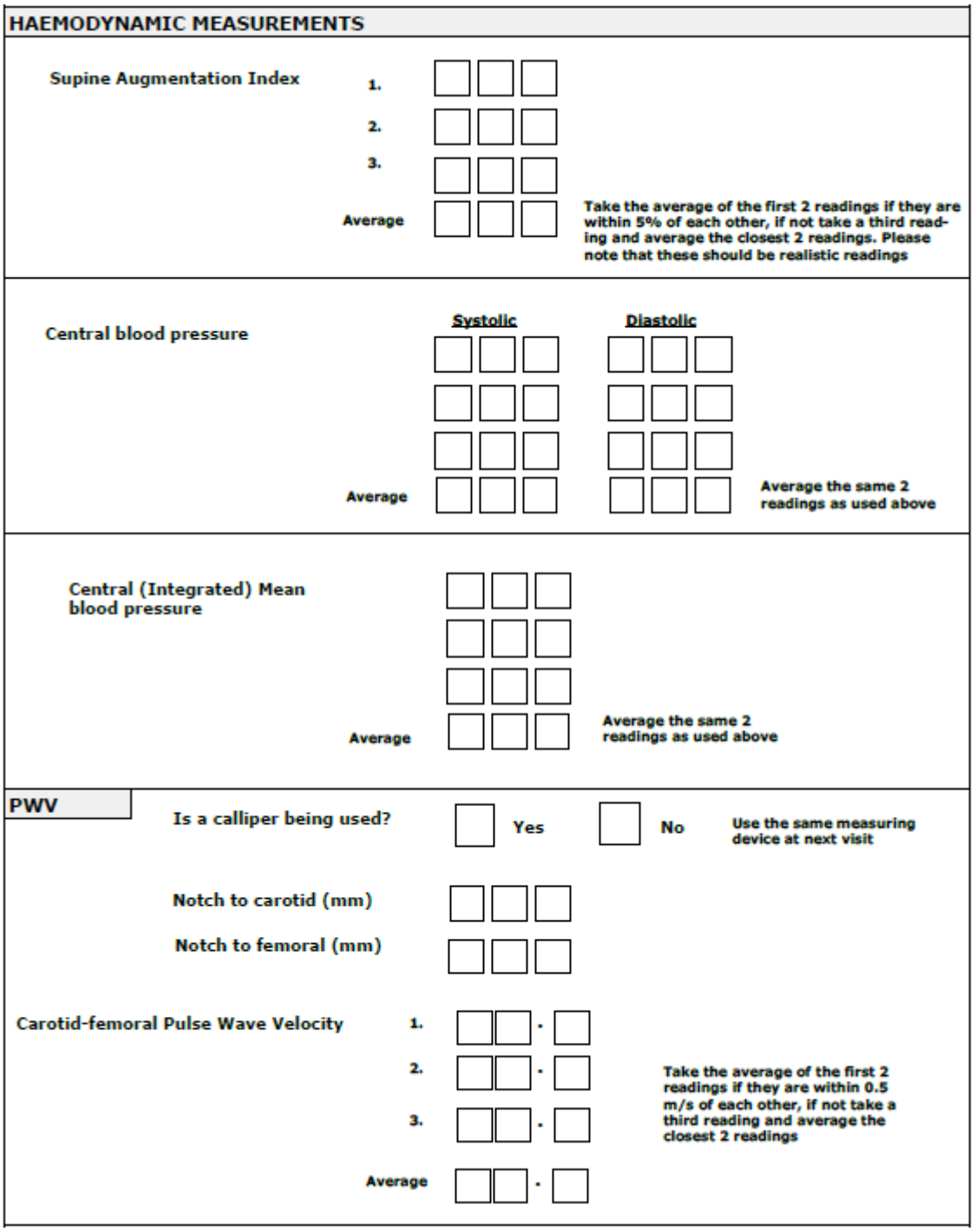
Figure 10. Example of waveform output readings



To record on source working documents refer to Figure 12 and below:

- Path Lengths in mm: Proximal (Notch-Carotid); Distal (Notch-Femoral)
- PWV (SphygmoCor; two readings and the average)

Figure 11. Screenshot of data entry page for haemodynamic measurements

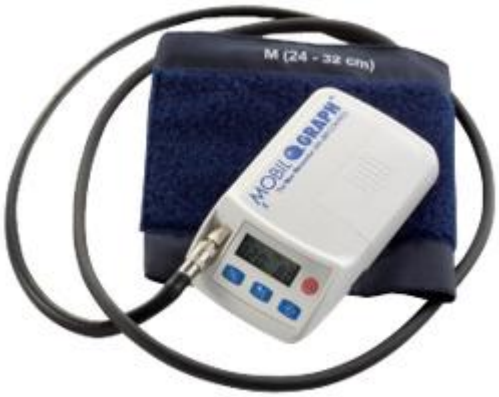


24 hr ABPM

Recording of 24 hour brachial and central blood pressure, cardiac output and other indices derived from pulse wave analysis (PWA).

Equipment list

- Mobilograph machine
- Blood pressure cuff
- Charged batteries



Method

Preparing the Monitor for Use

- Open HMS server program for Mobilograph on computer in nurses' office (equipped with blue tooth transmitter/receiver).
- Click on 'create new patient' icon. Enter subject ID, name and date of birth. Click on 'save' icon.
- Click anywhere in the 'patient data box', enter age, sex, height and weight.
- Insert newly charged batteries into Mobilograph.
- Turn machine on by pressing 'red button'. Once the display shows the time, press down the left hand button continuously whilst pressing the middle button (the display should flash 'bt'), then press the right hand button ('bt' is shown on the display). Blue tooth is now activated.
- A new window appears on the computer, select 'prepare device'.
- Click on the icon to 'set clock of device' to synchronise clock to computer time.
- Check that 'protocol 11' is displayed on computer. (**Program** to record measurements every 30 minutes between 08.00am and 22.00pm and every 60 minutes between 22.00pm and 08.00am). Timing of measurements can be amended as per study protocol.
- Click on icon 'send patient id'.
- Click 'close'.
- Exit software program.
- Attach correct size blood pressure cuff to patient. Connect cuff to monitor.
- After the patient has been seated for 5 minutes press the left hand button on the Mobilograph to activate the monitor.

- Inform the patient that prior to the cuff inflating between 08.00am and 22.00pm, the monitor will bleep. Advise the patient to remain still during inflation. (Between 22.00pm and 08.00am, the cuff will inflate as programmed but will not bleep).
- Advise the patient to contact the unit if they experience any problems with the monitor.
- After 24 hours of monitoring, advise the patient to press the 'red button' to turn monitor off, alternatively – advise the patient to carefully remove the batteries to turn off the monitor.

Downloading the Data

- Open HMS server program for Mobilograph and select patient from list. Turn Mobilograph monitor on and activate blue tooth as above.
- A new window appears on the computer, select 'Read-out of values'.
- Click on 'OK' for the next 4 boxes that appear on computer, unless you want to change any of the data.
- To print a copy of the results, click on the 'blood pressure' tab then click on date of measurement then click on the 'print' icon and **save as PDF**.
- **Fill in CRF and attach printout summary**

Recharging the Batteries

- Remove rechargeable batteries from the monitor and place in the battery recharger provided with the equipment.
- Leave batteries in charger until the red light turns green. Batteries are now fully recharged.

Returning monitors

- At both visits people should be encouraged to return ABPMs at hospital visits or in person but if not possible they should be supplied with a prepaid padded addressed envelope.

Measurement of isotopic GFR (kidney donors only)

Pre-donation

Isotopic GFR is likely to have been organized as part of the clinical pathway of living kidney donation prior to enrollment into EARNEST. As part of informed consent it should be explained to donor participants that this information will be used. The clinical information should be obtained and recorded in the paper CRFs.

1-year follow-up

This needs to be organized by the trial physician at the local radiology department, ideally to coincide with the one-year follow-up visit to minimize participant inconvenience.

All women of child-bearing age should have a pregnancy test prior to undergoing the scan unless they decline to.

The participants should receive an information sheet from the radiology department regarding their scan prior to their hospital visit. Please remind them to eat a light meat-free breakfast.

Recording

The following information should be recorded.

Baseline:

Absolute iGFR

Normalised iGFR for body-surface area

Percentage of kidney function on left and right side

Follow-up

Absolute iGFR

Normalised iGFR for body-surface area

Laboratory guidelines

Laboratory tests should be performed at Visit 1 (baseline) and Visit 2 (12 month follow-up) during the study as indicated in the Time and Events Table (Table 2).

Biochemistry laboratory tests

1) **Blood** will be drawn into the appropriate tubes (as dictated by local laboratory guidelines) for analysis of the following parameters:

- Full blood count
- Liver function tests
- Urea & electrolytes
- Bone profile
- PTH (Parathyroid Hormone)
- TFT (Thyroid function tests)
- Urate
- Mg
- CRP

Samples for these analyses should be taken into the appropriate tubes as designated by the local analysis laboratory. Samples should then be labelled and delivered to the laboratory following the appropriate local procedures.

The blood tubes for the various tests require collection in the correct 'order of draw' otherwise the cross contamination can produce incorrect results. Please follow local guidelines.

Note: for eGFR ensure that the patient has had a meat free diet on the day of test

2) **Urine** sample will be collected for ACR and spot urine sodium and creatinine and sent to biochemistry (labelling analysis guidelines as for bloods above)

Samples for storage

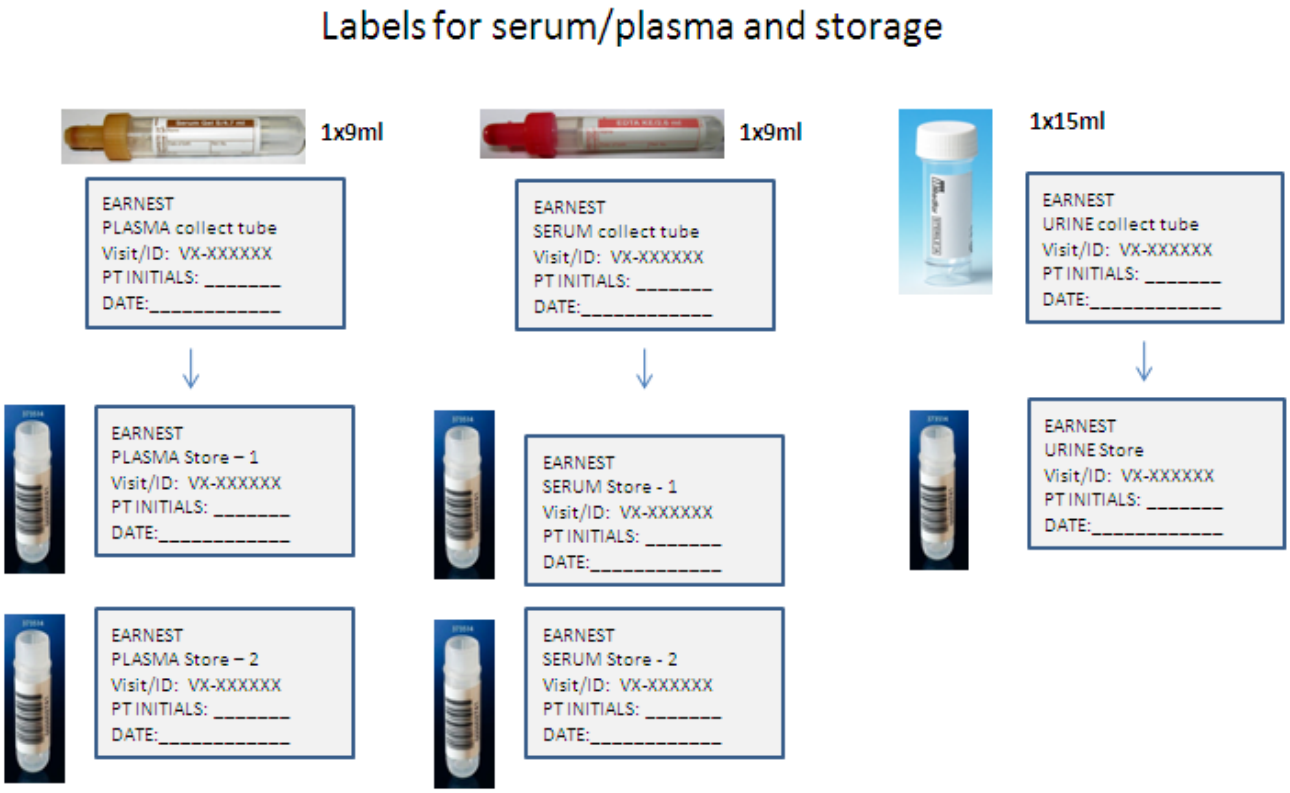
In addition to the biochemistry samples outlined above, additional **blood (plasma and serum) and urine** will be collected:

1) 1 x EDTA tube – collect ~9 mL
2) 1 x Serum tube – collect ~9mL
3) 1 x Universal – collect minimum of 5mL

Blood tubes and universal tubes are provided by the participating site and labels will be provided by the coordinating centre as below.

Blood tube labels for storage samples

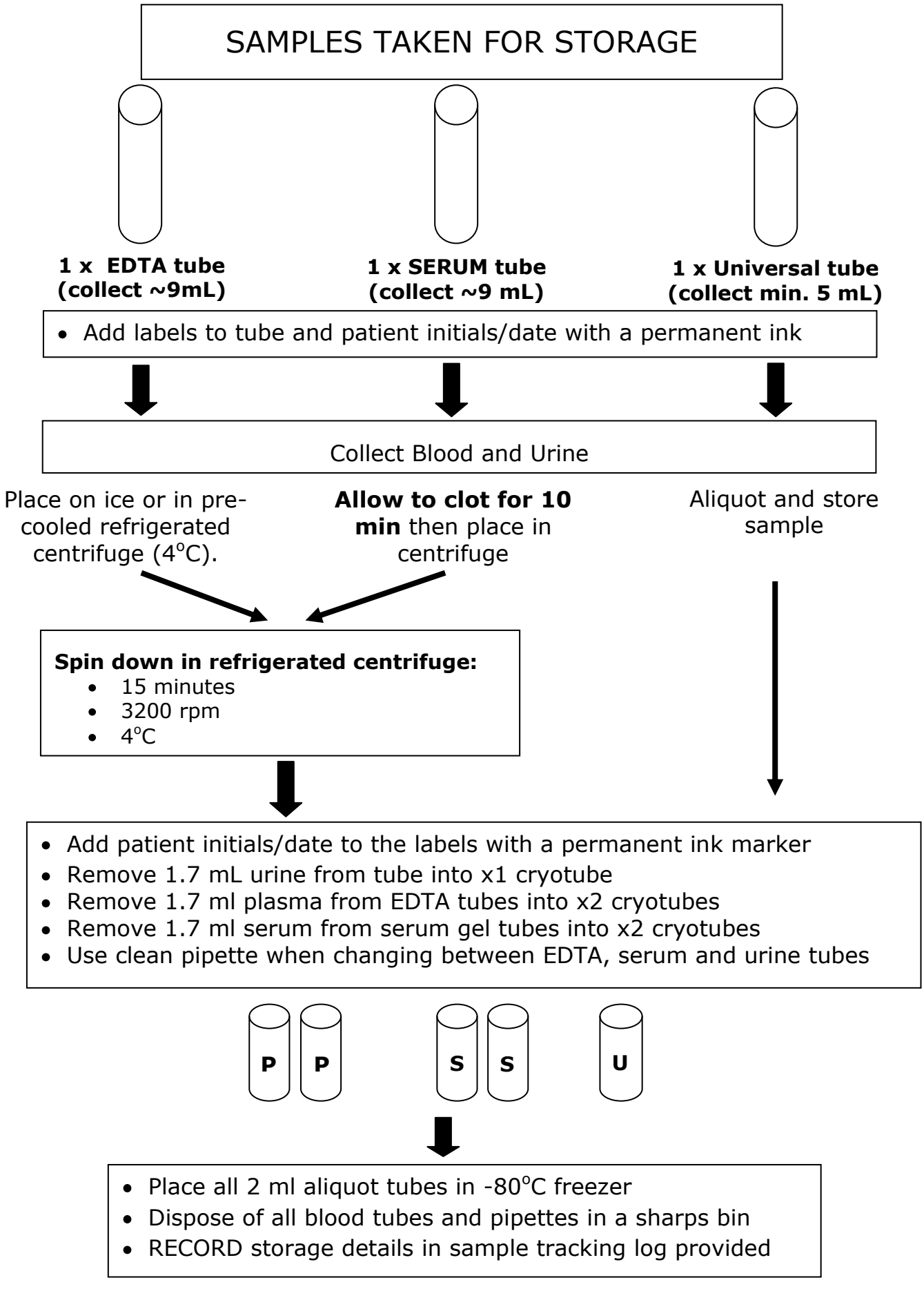
The following labels will be provided by the coordinating centre for blood/universal collection tubes and cryo vials (serum/plasma/urine storage only).



Each site will be provided with labels for 50 patients in the first instance. Labels will need to be filled in with the patient initials and date. A spare set of labels will be provided with the Visit/ID and site left blank. These can be used to replace labels where an error has occurred.

Sample processing for storage samples

Please see below on how to process and store samples:



Sample tracking form

Use the sample tracking forms provided as part of the ISF to record storage and shipping details. Use 1 form for each visit.

Sample Storage Log FRM003/EARNEST

Trial Name: **EARNEST** VISIT^ 1 ☐ 2 ☐

Subject ID No	Sample ID	Sample type	Collection Date+	Collection Time (24hr)	Time Processed (24hr)	Time Stored (24hr)	Sample Storage Temp (°C)	Location	Staff Initials
	URINE	Urine		:	:	:			
	PLASMA-1	Plasma		:	:	:			
	PLASMA-2	Plasma		:	:	:			
	SERUM-1	Plasma		:	:	:			
	SERUM-2	Serum		:	:	:			

^Tick or 'X' applicable visit
+Collection date: If not done then enter 'ND' and reason

SAMPLE SHIPPING			
Date of Shipment	Courier	Shipping destination and reference	Staff Initials

Site guidelines

Standardisation – Staff and Equipment

Delegated study staff will be appropriately trained to undertake each assessment and will be provided with this investigator's manual prior to the start of the study. Study staff should undergo training at each site to ensure standardization and precision. Equipment will be calibrated at baseline and throughout the study at specific intervals.

Safety Reporting

Only SAE's relating to study procedures will be reported – **please inform the CI initially** if you suspect an SAE. SAE forms are located in the ISF. The study team at the participating sites should report the SAE's to the Sponsor using either one of the following methods:

i) E-mail address – cctu@addenbrookes.nhs.uk
ii) Fax - 01223 256623

Please follow the reporting timelines below:

Action	Timeline
Reporting SAE to Sponsor by sending it to CCTU	24 hours of Investigator awareness
Returning query responses to CCTU	As advised in query, depending on the nature of query and urgency

Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal results that are found in any clinical/study assessment will be reviewed by the local team and fed back to the participants and GP where the abnormality is deemed clinically relevant. Any further investigation or treatment required will be provided by the usual NHS clinical route.

GCP training

All study staff must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this study. This training should be updated every 2 years or in accordance with your Trust's policy.