BRITISH REGIONAL HEART STUDY

Q20 Physical examination protocol

1998-2000 rescreen.

British Regional Heart Study:
A follow-up study of cardiovascular risk factors and outcomes in older men

Funded: BHF
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11.0 Appendices

1.0 BACKGROUND

The British Regional Heart Study (BRHS) is a unique national prospective investigation into the fundamental causes of coronary heart disease (CHD), hypertension and stroke in men, including the reasons for the marked regional and social class variations in cardiovascular disease in Great Britain.

In 1978-80, 7735 men aged 40-59 were drawn at random from one general practice in each of 24 towns in England, Wales and Scotland (Appendix 3) and had a detailed assessment including a questionnaire on personal and family factors, an electrocardiogram, lung function tests and a blood sample for 24 biochemical and haematological measurements. Serum samples from men in 18 towns were deep frozen (-20°C) for later studies.

In 1998-2000, 20 years after baseline, a detailed remeasurement of the surviving men from the British Regional Heart Study cohort will be carried out. Remeasurement will be based on all 24 study towns. All survivors in those towns (N = 5800) will be invited to attend for remeasurement. Allowing a response rate of 80%, approximately 4500 men will be remeasured. The original General Practice or a Health Clinic in the town will be used as the survey base. Transport will be arranged for frail or disabled participants. Men who have migrated (i.e. moved) from the study towns will be invited for remeasurement, with a choice of attending either in the town where they were originally measured, or at the London Research Centre. Travel expenses will be paid

1.1 Who is invited to take part?

In each town all surviving men (approximately 250) who took part in the original BRHS in 1978-1980 and who are still alive have been invited to attend.

Participants who have migrated from the original town have been invited for remeasurement, either :

(a) returning to their original town
(b) going to another BRHS town which is closer and more convenient for them
(c) attend a London examination centre

Q20 invitation stationery – **(Appendix 1)**

1.2 Liaison with General Practices

The study will focus on the single General Practice in each town which was originally involved in the study and where participants were recruited from. Most study participants are still registered at these general practices.

By the time the Study Field Team visits a particular town, the town Practice will already have been visited by a member of the BRHS team and a meeting held with the Practice Staff to confirm the survey arrangements in the town. The survey will take place either within the Practice or (where this is not possible) in a local Health Clinic or other Health Authority premises.

1.3 Invitations to participants

The study participants have received a letter inviting them to take part in the study one month in advance. Where a participant is still registered with the original study Practice in the town,

the invitation letter is signed by the Practice partners. For participants who are no longer registered in the original study Practices, an invitation letter is organised by the BRHS manager and sent directly from the British Regional Heart Study directors.

The package received by the study participants will include:- **(Appendix 1)**

- the main invitation letter
- an appointment card (with tear-off reply slip)
- a questionnaire on Physical Activity and Diet (Yellow)
- an information sheet
- a reply paid envelope

The participants are asked to return:-

- the reply slip confirming, changing or declining their appointment
- the self administered Physical Activity and Diet questionnaire **(Appendix 2),** which aims to provide detailed information on the diet and physical activity patterns of the participants

In preparation for the survey visit they are asked:-

- to fast overnight or (in the case of appointments at or after 11.20) for about five and a half hours
- to wear clothing which is easily adjustable
- to bring reading glasses and their medications or a prescription list.

1.4 Framework of assessments being made

A team of three trained research nurses will comprise the field study team who will carry out all the physical measurements in the 24 BRHS study towns. In addition, at each examination centre there will be a receptionist, recruited locally, to meet and greet the participants and prepare the participants for the physical examination.

On arrival the participants will present themselves to the receptionist where each participant will:-

- be logged in and have documents prepared
- prepare for assessment (given a dressing gown etc)
- receive local anaesthetic cream (if required)
- receive the self-completed questionnaire and data sheet on a clipboard (all labelled)

The participants will proceed from the Receptionist to Workstation 1 and Workstation 2 in order, returning to the Receptionist before departing.

At Workstation 1, each Participant will:-

- have measurements of anthropometry
- blood pressure
- lung function/spirometry

Workstation 2, each Participant will:-

- be asked to provide information on medications
- have a resting electrocardiogram

- provide a blood sample
- be asked about consent for record tracing, result recording, blood storage

Workstation 3: The research nurse operating this station will have a participant contact free day and will prepare the blood aliquots and assist the receptionist as required

The participants will proceed from the Receptionist to Workstation 1 and Workstation 2 in order, returning to the Receptionist before departing.
The Research Nurses **will rotate** between the workstations daily.

Scheduled dates for the physical examination in each study town 1998/2000 (Appendix 3).
List of dates when the physical examination was carried out in each BRHS study town.

2.0 RECEPTIONIST PROCEDURES

2.1 Equipment needed for the Receptionist workstation

- Log Book
- The main 20 year follow-up survey questionnaire(Blue). **(Appendix 5)**
- Physical examination Data collection forms ('Data sheets') **(Appendix 4)**
  (blank forms will be at the back of the pack for unconfirmed appointment)
- Labels for questionnaire
- Appointment Schedule ***** = confirmed , **?** = Unconfirmed
- Removal list with name of current GP
- Travel Claim Cards for Removals to be reimbursed
- Repeat test request information in plastic folders
- Fasting instructions
- Clip boards x 6
- Pens, Stapler, Scissors, Ruler, Paper clips etc
- Emla cream, tegaderm, tourniquet, dish
- TNT receipts etc.
- Kettle, toaster, refreshments disposables etc
- Dressing gowns and hangers etc x 5
- 1 Folders for ECGs
- 1 Folder Data Sheets

Weekly Time sheets Approximately

- 8.45-12.45 Morning Session
- 1.45- 5.45 Afternoon Session
- 40 hours week due to early finish on Fridays

2.2 Receptionist Duties on screening site

Participant arrival – greet and ONLY THEN:

2.2.1. Participant Registration

- Enter participant's serial number, batch number, full name, and time of arrival in log book
- check the identity of each participant on arrival at the centre, including name, date of birth, address and G.P. - log any changes
- If the participant is a Removal (i.e. no longer in original study practice), check GP name & address on list supplied Tick if correct or amend
- log arrival time and any change of address/G.P.
- Prepare participant clipboard with:
  Data sheet and a labelled Main Questionnaire(blue)
  - If Participant is a Removal (i.e. no longer registered at original study practice) also attach a travel claim
- If a Participant does not attend - mark **DNA** (did not attend) on the appointment list in **red pen** with a line through his name

2.2.2 Preparation for assessment

Ask the Participant

- to remove clothes (above waist) and shoes and put on a dressing gown (and slippers if necessary)
- give him a carrier bag for his clothes (Recommend using bag as this prevents loss of belongings)
- to pass urine if needs to do so before being measured (we are not planning to test urine in the protocol)
- if they would like anaesthetic cream for the blood test
  - apply with dressing to the inside of the elbow or ask a research nurse for help.
  - if a vein can be easily identified in the left arm, Receptionist will apply local anaesthetic cream. If not, Research Nurse 3 will be called to advise.

- to complete the main 20 year follow-up survey questionnaire (Blue) while waiting for the Research nurse from Workstation 1
- The receptionist will direct the Participant to Research Nurse at Workstation 1

2.2.3 Participant documents

Each participant receives a clipboard with attached with documents which he takes with him to the different workstations:

- The physical examination data collection form(data sheet). DOB ticked.
- The main 20 year follow-up survey questionnaire (Blue) labeled with participant study number
- Prepare travel claim form for those living outside area (removals/migrants or special cases requested by participant)
- Information sheet for Repeat testing (1st week only, to be seen again in 2nd week)

2.2.4 Removals(migrants)

Participants who are no longer registered at the GP practice they wer**e** recruited from at baseline 1978-80)

Reimbursed for travel expenses

- We have offered to reimburse travel expenses if participant has moved to a new GP. Ask how much their travel has cost, put this on the travel claim form and attach to clipboard. The Research Nurse will give him the money at the end of the examination.
- On his return to reception the claim will be signed if he has accepted payment. Give the claim form a number and enter amount in petty cash book
- If participant has a receipt of travel expenses keep this on a green tag with the cards etc.

2.2.5 Repeat measurements (1st week only)

Repeat measurements. The importance of making replicate measurements in a section of the population to estimate the consequences of measurement imprecision is now well recognized. A random sample of 5% of all study participants will be invited for a complete repeat survey.

- At the end of their physical examination, if the participant has undergone **all** the assessments , he will be asked to volunteer to return for a repeat examination and blood test**.** If he agrees to return, then:
    - Give participant a new appointment and new serial and batch number from allocation at the end of the appointment listing, enter his name against this new number
    - Add fasting instructions to day slot allocation on card
    - Add the participant's details to the APPOINTMENT list, inform nurses at the end of the day
    - When this participant comes back for the repeat examination write his NEW SERIAL NUMBER / NEW BATCH NUMBER / OLD BATCH NUMBER and other details in RED ink.
- If No blood sample was obtained this participant should not be invited to return

2.2.6 Other information to be recorded in logbook

- If **no blood sample** was obtained this will be recorded on the front of the questionnaire by the nurse.
- Please put this in the logbook in **RED** ink e.g. No blood (Failed or Refused as appropriate)

2.2.7 End of Assessment: participant returns to Reception

The Receptionist will

- Take the clip board from the participant
- Ask the participant to get dressed
- Check that he has completed ALL PAGES in the main 20 year follow-up survey questionnaire (Blue). If not ask him to complete it before leaving.
- Ask if the questionnaire on Physical activity and Diet (Yellow) was returned via the post, issuing duplicate if necessary
- If participant willing to return for the repeat measurements- please provide appointment card see above
- Enter Log out time in logbook
- Give the Participant Tea / Coffee and Toast / Biscuits

2.2.8 Other Duties

- **Questionnaires -** file in serial number order
- **data sheets** - file in batch number order (3 digits)
- **ECG 's** folded in half and clipped together in attendance order
  with listings of ECG's on top the nurse will provide this for you

2.2.9 General

- Make tea & coffee for nurses and Participants
- Keep things organized
- Take delivery of Dry Ice on Mondays & Wednesdays. Keep receipts
- Give the TNT courier the blood samples, Research nurse 3 will the packed samples to you. Keep dockets

Purchases

- Buy Bread & Milk & Newspapers for busy days -
- Get money from Petty Cash - File receipts on a green tag and enter amount in the petty cash book

MEASUREMENT PROCEDURES/PROTOCOL

3.0 Workstation 1 PROCEDURES

Research Nurse for workstation 1 will be responsible for setting up and calibrating measurement equipment for this workstation. (q.v.)

3.1 CALIBRATION AND CHECKING OF INSTRUMENTS

The following calibration steps should be undertaken every morning:-

3.1.1 Stadiometer - Harpenden stadiometer

Please check recorded height of standard 1 metre ruler once instrument set up, and record result. (This ensures that recorder has not become displaced)

3.1.2 Scales - Soehnle digital electronic scales

The zero setting on the scales should be checked by pressing the reset button with the scales empty. This should be 00.0. The result should be recorded. If there is a problem:-

- check that correct adapter voltage (9.0 volts) is being used
- if persists, please discuss with base at earliest convenience

3.1.3 Spirometer/ Vitalograph

Instrument: Vitalograph Compact II instrument (Vitalograph Ltd, Buckingham, United Kingdom) , with calibration scyringe & printer paper, disposal mouthpieces, milton cleaning solution.

Please ensure that spirometer is turned on early and left to warm up before testing.

- Check paper supply.
- Enter 'set up' mode and go to 1 'accuracy + calibration'. when the machine invites you to blow air through the flowhead to equilibrate temperatures, please blow 3 litres through slowly, then 'continue'.
- Set ambient (room temperature) consulting the electronic thermometer.
- Pump 5 litres of air slowly (each litre must take more than 1 second) through the flowhead to calibrate and then 'exit'.
- Read in 5.00 as reference volume and enter.
- Update calibration if error is 1% or greater.
- 'Retest' by putting a further 5 litres of air through the flowhead. If error is 1% or greater update calibration again and retest one more time.
- If calibration will not settle, raise threshold for correction to 3%.
- When you have finished, move to main menu and to FVC test, and when the machine says 'perform blow', blow 1 litre through calibration syringe and record the result.

- **Recalibrate the Vitalograph as before for the afternoon Session.**

3.1.4 Dinamap blood pressure recorder

Instrument: Dinamap 1846 oscillometric blood pressure recorder & selection of BP cuffs, printer roll Insertion tape (CMS Ltd, London, United Kingdom).

- check paper supply in the printer
- put machine in auto mode and use set button to set interval between readings at one minute

The calibration procedure is as follows:-

- Set up the instrument with the adult cuff to be used in place. Insert the calibration kit on one of the cuff connector leads. Turn the instrument on with the SET button held in. The CYCLE display should show 88, and will continue to do so while the instrument is in calibration mode. The machine is now in calibration mode 1.
  Display read:- CYCLE 88
  MAP 0
  All others blank
- Inflate the cuff until the mercury column reads 200mm Hg (top of the mercury meniscus). RECORD the MAP result. Check that there are no leaks (pressure remains above 190mmHg for at least 10 seconds).
- Check MAP readings at 150, 100, 50, 0mmHg on the mercury column, RECORDING the result each time.
- If leaks in system:-
  - try replacing cuff with reserve
  - try replacing blue cable with spare

  If problem not solved discuss instrument servicing/replacement with Lead investigator Peter Whincup at earliest opportunity

3.2 MEASUREMENTS

Procedures with each participant will be as follows:-

- Research nurse will greet participant, checking identity on arrival and taking the clipboard with questionnaire and data sheet.
- participants should be asked to remove shoes and to remove any heavy or bulky item from pockets and place in a receptacle (bowl)

The measurements will be taken in order as follows:-

3.2.1 Height

Participants will be asked to stand on the stadiometer (on the feet placement card). The Research Nurse should check for the following points:-

- FEET: ankles should be together and resting on the bar at the back,
- ARMS: should be resting by sides, not behind or in front,
- HEAD: participant should be looking straight ahead (i.e. lower edge of orbit is in line with external auditory meatus [earhole])

Taking the measurement:

- The index fingers of both hands should then be placed below the mastoid process on

each side. During inspiration the increase in height should be maintained and during expiration gentle stretch should be applied. The measurement is recorded at the end of expiration. Care is needed to ensure that the participant does not stand on tiptoe.

- Record any problems which the participant has which may lead to underestimation of height in the `problem with height' box
- Reasons may include problem with **balance/standing OR problem with posture**

3.2.2 Weight

- Before standing on the scales ask participant for his estimate of his current weight, if known.
- Weight is recorded on the Soehnle digital electronic scales. Press button before asking participant to step on.
- Participant should stand reasonably straight if possible - leaning to one side (or forwards) can affect the weight recorded
- If the weight registered is between two 0.1 kg marks, take the lower one.

Then ask

- whether weight has changed in the past 3 years

If has changed, record

- whether change was intentional
- specific reasons for change
- what is the most that the participant has ever weighed?

3.2.3 Arm circumference (right arm)

- With the participant's right arm flexed to 90^{o}, identify the acromial process and the lower tip of the olecranon.
- Using the Holtain steel tape measure, identify the midpoint of the upper arm and mark with a felt tip pen.
- With the arm hanging loosely at the side the arm circumference should be measured at this point to the last completed millimetre.

3.2.4 Skinfold thicknesses (right side) using calipers

- Explain that you want to measure the thickness of the tissue behind the arm and shoulder.
- Measure the **triceps skinfold** at the midpoint of the upper arm as marked above. Measure the **subscapular skinfold** immediately below the tip of the scapula.
- In each case grasp the skinfold firmly (not too firmly!) and apply calipers immediately below fingers. Record reading as soon as caliper reading stabilizes.
- Record first measurements in each site and then repeat procedure.

3.2.5 Blood Pressure (right arm)

The participant should sit down at the measurement table and rest their right arm on the table. This will ensure that the participant is sitting with their upper arm at chest level.

- **Select the cuff size** in accordance with the measured arm circumference. Between 28.0 and 35.0cm use standard adult cuff. Less than 28.0 use small adult cuff, more than 35.0 use large adult cuff. The cuff should be placed around the upper arm with the bladder centre over the artery.

- **Check that the participant is familiar** with having his blood pressure taken (he should have had it done once!). Explain that:
    - you plan to take 4 measurements one minute apart, the first two sitting and the second two standing
    - the cuff will inflate and slowly deflate automatically
    - encourage the participant to keep the arm still and not to talk during measurement

- The Dinamap should have been set to take repeated measurements at one minute intervals. To take two measurements at one minute intervals, switch the AUTO/MANUAL switch to AUTO. The machine will immediately inflate the cuff and the first reading will begin.
- Once the cuff is deflating the participant should be warned that the machine will make a funny noise as it prints out the results.
- The second measurement will be made after a one minute interval on the Dinamap's automatic cycle. While waiting for the second measurement, the first result should be recorded and entries on
    - 'room temperature',
    - ethnic origin should be made, with a note on the
    - presence of alcohol and
    - presence of obvious dementia where appropriate.
- Once the second reading is complete, ask the participant to stand up, allowing the right arm to rest loosely by his side. Allow the instrument to continue with two additional measurements, and proceed with waist and hip measurements while these are being done.

3.2.6 Waist circumference

This should be measured with the participant standing with feet one foot apart on a marked template.

- The waist should be identified as the mid point between the iliac crest below and the lower edge of the ribs above, i.e. measured at the sides.
- Pass the tape around the waist (for large participants, ask them to help passing the tape around) and reinsert at front, positioning level at the waist.
- Ask participant to breathe out gently and record measurement at the end of expiration to the last completed millimetre.
- If you are not satisfied about the accuracy of your measurement, record a 1 in the appropriate coding box.
- Repeat the measurement

3.2.7 Hip circumference

This is measured by placing the tape measure around the hips at the point of maximum circumference.

- The tape should be horizontal and the gluteal muscles not contracted. Record to the last completed millimetre.
- If you are not satisfied about the accuracy of your measurement, record a 1 in the appropriate coding box.
- Repeat the measurement

3.2.8 Spirometry

- Preliminary explanation to participant. "This machine measures the size of the lungs. What I want you to do is to take a very big breath in and to blow out as hard and as long as you can, until your lungs are empty. Watch me."
- (Demonstration by nurse)
- Participant then practices: ensure that:-
    - full breath in
    - lips tightly around mouthpiece
    - long hard blow right to the end
- Before measurements made check about participant's use of inhaler and about the time of previous inhaler use.
- Before starting the test enter the participant's 6 digit serial number and press the 'enter' key in order to proceed.
- On the main menu press 'FVC test'. The machine will then say 'perform test', indicating that it is ready for the first blow.
- We want to record three definitive blows.
- Please ensure that you encourage the participant during the blow, particularly towards the end, by saying `blow...blow...blow' (or other agreed text).
- After each blow, press 'end test' to expedite results and then 'retest' to go on to the next test.
- The machine takes a short period to calculate results, after which FVC, FEVI and PEF figures will then be displayed on the screen. Once the results of each of the first two blows are displayed press 'retest' and the machine will display 'perform test' to indicate readiness for the next reading.
- Once the result of the third reading is recorded, check the `best test variation' which is recorded on the screen. If best test variation is more than 5% after 3 readings, please take an additional reading by pressing 'retest' again.
- If you are not satisfied that the participant has done an adequate blow on at least one of the readings, please enter 1 in the `problem' box.
- Once the 3 (4) readings are complete, press 'end test' to return to the main menu. Press 'print' and then 'selected' to print out the results. The printed output should be stapled onto the front of the data sheet in the space provided. Then press the 'new patient' category and agree to delete old patient's results. This will leave the machine waiting for the next participant's serial number to be entered in due course.

3.2.8.1 In the event of Vitalograph printer failure

Please record the number of readings and the best test variation directly from the screen before leaving the test screen. Then on main menu press option 5, display results and write down the other parameters on the data sheet.

3.2.9 Completion

- Participant should remain in dressing gown and proceed to Research Nurse 2
- Ensure that any possessions are restored or stored for collection later.
- Medicines/records should be taken through to Research Nurse 2.

3.2.10 At end of day

- General clearing-up
- Coding of questionnaires

4.0 Workstation 2 PROCEDURES

Research Nurse 2 will be responsible for setting up this station

4.1 Preparation

Research nurse on arrival to:

- Set up relevant equipment
- Prepare blood syringes and collection tubes for the morning and (if possible) afternoon session, following the appointment list for the day

4.2 Measurements

Research Nurse 2 will greet the participant, checking his identity on arrival and taking the data sheet and questionnaire.

4.2.1 Questionnaire (main 20 year follow-up survey questionnaire (Blue)) (Appendix 5)

- Ask the participant whether he had any problems filling in the questionnaire; check any specific items.
- Ask the participant to provide their medicines etc and to remove dressing gown and lie on the couch.
- Nurse will record medicine list on the questionnaire (question 18.0, 18.2) (**Appendix 5**) while the participant is undressing and will check the indications for medicines
- Final questions including time of last meal will also be recorded on the questionnaire (question 20.0) (**Appendix 5**) at this point.
- For the next stages the participant should lie as flat as is possible on the couch, though a pillow is quite acceptable.

4.2.2 Electrocardiogram

Instrument: ECG, Siemens Sicard 460- 12 lead ECG(crododile clips, electrodes, printer paper). ECG computer interpreted - University of Glasgow ECG core laboratory based at Glasgow Royal Infirmary. Analyzed and coded in accordance with Minnesota Coding definitions.

- Place ECG electrodes and record electrocardiogram (separate instructions will be required via PMacF).
- Remove the ECG electrodes and the local anaesthetic patch, to allow time for any local oedema to subside before blood taking.

Other measurements taken while participant is lying down on the couch.

4.2.3 Ankle oedema

- Check for presence of **ankle oedema** and for presence/absence of all four foot pulses.

4.2.4 Venous ulcers

- Check for presence of **venous ulcers** on the shin/above ankles

4.2.5 Bioelectrical impedance analysis (BIA)

Check whether participant has pacemaker

- If not, participant lies with legs, arms uncrossed, forearms pronated for **measurement of bioimpedance** as shown on instruction sheet (Bodystat).
- Nurse to **record coefficient(impedance value) only.**
- Bioelectrical impedance analysis (BIA) is a method for measuring body composition based on the rate at which an electrical **current** travels through the body measured in Ohms (Ω). Body fat (adipose tissue) causes greater resistance (impedance) than fat-free mass and slows the rate at which the current travels.
- Fat-free mass was determined by bioelectrical impedance analysis (BIA) using a Bodystat 500 (Bodystat Ltd, Douglas, uk) and the Deurenberg et al equation **https://link.springer.com/content/pdf/10.1007/s12603-013-0336-9.pdf**

4.2.6 Blood sampling

- The blood sample will be taken at the end of the examination, after the electrocardiogram is completed.
- The blood sample should be taken with the participant lying down.
- Check whether the participant has had previous problems with blood sampling.
- Alcohol swabs will be provided for skin cleaning - allow to dry after use.
- A tourniquet may be used throughout. Wear the rubber gloves provided for taking the sample. A 21 gauge butterfly needle (or Sarsted needle) should generally be used; a small supply of 23 gauge needles will also be supplied for exceptional use.
- If blood is not obtained at the first attempt, a single further attempt may be made in the opposite arm if the participant consents. No further attempt to obtain blood should be made.
- Each participant's blood collection tubes will be prepared in advance by the nurse in a polythene bag with an identification label on the front and individual tube labels throughout. Please check the label against the data sheet. The tube labels will have the participant's batch number and the full serial number for identification.

The first priority tubes are:-

green	citrate tube	code AE	9.0 ml
red	EDTA tube	code FJ	9.0 ml
yellow	fluoride tube	code K	2.7 ml
white	serum tube	code WN	9.0 ml

The second priority tubes are:

red	EDTA small	code T	2.7 ml
yellow	fluoride	code U	1.2 ml
white	serum tube	code OQ	9.0 ml

After venepuncture, raise participant's arm and encourage participant to press firmly on cotton wool pad to avoid bruising. Plasters are provided. Please check for elastoplast allergy - if present, use cotton wool and tape.

After venepuncture the tubes should be gently agitated and placed in a rack.
Please record:-

- the time of venepuncture
- the full success/partial success/failure of sampling
- if partial success, which of the `primary' collection tubes have no blood in them

- Samples can then be passed directly to Research Nurse 3 with the data sheet .

4.2.7 Written consent to follow-up study (Appendix 5)

- Nurse 2 should then take the participant through the consent procedure. (consent to follow-up participant's future health through his health records, passing on test result information to his G.P. and consent to storing his blood sample for future use). The consent form is contained in **question 21.0** of the ***20 year follow up questionnaire(blue questionnaire).*** **(Appendix 5).**

4.2.8 Participant expenses

- If the participant is a `migrant' (ie no longer registered at the original study General Practice), Nurse 2 should confirm travel expenses and reimburse as required.

4.2.9 Recruiting to the Repeatability/Variability Study

- If the participant is a non-migrant (i.e. registered at original study General Practice) seen during the first week of the study, she should ask whether he would be prepared to return for a further check during the second week of the study (measurements only, no questionnaire).

  Possible text
  "I wonder whether you would be willing to help us with our quality control procedures and return next week for a repeat measurement. This helps us to find out how much the measurements we are doing vary from day to day and helps us to assess how important the different factors are in relation to heart disease. Is there any possibility that you would be prepared to come back next week for a repeat check up (measurements and blood test, but no questionnaire)?"

  Nurse 2 will mark on her appointment list those participants invited for repeat and whether they agree. If the Participant agrees, Nurse 2 will give him a card to take to the receptionist. The receptionist will record the name of each participant in the log book and provide a new appointment card with fasting time recorded.

  A maximum of 12 participants per town should be recruited.

4.2.10 Research Nurse 2 tasks at the end of day

- Transmit ECGs to Glasgow

5.0 Workstation 3. Procedures

Equipment and consumables required:

BOC Dry Ice, Aliquot tubes & Caps, Electronic pipettes, manual pipettes, centrifuge, mini freezer, TNT courier labels, Camlab aliquot boxes, metaphosphoric acid.

Research Nurse 3 will be responsible for setting up this station. Research Nurse 3 will have sole responsibility for the organization and handling of blood samples. Gloves and aprons will be provided. Where necessary, she will assist the receptionist in placing local anaesthetic cream.

5.1 Prepare the blood aliquots

At the start of the day

Before the first samples come through there should be time:-

- to label sample tubes for the day
- to prime tube F with metaphosphoric acid. 1 ml of metaphosphoric acid is placed in the tube, using the accurate manual pipette. (The mixture is made up fresh each town, 10 grams of metaphosphoric acid to 100 ml of deionized water). By the end of the study this was made up weekly by the nurses.
- to collect dry ice for use during the day

5.2 Handling of blood samples for each Participant

5.2.1 Tubes T and U

- Tubes T and U will be set aside, as these are whole blood samples which do not require additional preparation. They should be stored in batch number order in a cool place (**not** frozen!) for TNT collection later in the day to be delivery overnight to Whittington Hospital.

5.2.2 Citrate tube (A-E), EDTA tube (F-J)

- Special priority should be given to the handling of the citrate tube (A-E), which is for coagulation factors. However, the handling of the EDTA tube (F-J) and the glucose tube (K) can take place alongside this one.

Centrifuging

After each pair of participants, the citrate tubes (A-E), the EDTA tube (F-J) and the glucose tube (K) should be spun at 3500 rpm for 5 minutes and then aliquotted. Ideally this will be completed within an hour of collection.

Aliquotting

Citrate tube A-E is aliquotted as follows:

Into 5 equal 1 ml aliquots A 1.0 ml, B 1.0 ml, C 1.0 ml, D 1.0 ml, E 1.0 ml [any extra to tube E] (use electronic pipette, discard pipette after use). Take particular care with this tube to ensure the buffy coat layer is untouched.

EDTA tube F-J is aliquotted as follows:-

Into F 0.5 ml using accurate manual pipette

Into G 0.5 ml, H 0.5 ml, I 2.0 ml, J 1.5 ml [any extra to tube J] (using electronic pipette, discard pipette after use)

Freezing
These aliquot tubes should be snap-frozen in dry ice once separated. Once snap frozen, they can then be placed in the separate boxes labelled A-J in the -20^{o}C freezers in batch number order.

Residues
The cell residues of the original citrate tube A-E and the EDTA tube F-J should be kept and placed in the freezer at convenience in bags of seven.

5.2.3 Glucose tube (K)

- The glucose tube K should be aliquotted using the hand bulb pipette into tube K (1.2 ml or so - tube slightly more than half full).
- They can then be transferred twice daily to the boxes labelled K in the -20^{o}C freezer in batch number order; no snap freezing is needed.
- The residue of the first tube K can be discarded.

5.2.4 Serum tubes W-N and O-Q

- As time permits, the serum tubes W-N and O-Q should be dealt with.
- They should be allowed a minimum of 30 minutes to settle before centrifuging.

Centrifuging
They should be centrifuged at 3500 rpm for 10 minutes. A small number of tubes may not separate well and require recentrifuging at 4000 rpm for a further 5-10 minutes.

Aliquotting
After centrifugation they should be sorted into pairs for each individual; each pair should be aliqotted in turn. The allocation of the two tubes should be sorted in order as follows; do W-N tube first, and aliquot as far as it will go. Serum from W-N and O-Q is interchangeable - can be used for top ups if needed. Both of these can be done with one electronic pipette.

- Tube W 1.5 ml, L 0.5 ml, M 0.5 ml, N 0.5 ml
- Tube O 1.0 ml, P 1.0ml, Q 1.0ml [any extra to tube Q].

Freezing
These aliquots should be transferred into the appropriate boxes L to O and placed in the -20 freezer and placed in batch number order; there is no need to snap freeze the samples.

Residues
The cell residues of the serum tubes L-N and O-Q can be discarded.

Blood aliquot plan is in **Appendix 6.**

5.3 Documentation of blood sampling

5.3.1 Paper printouts

For the tubes which will be most rapidly analysed, which are:-

- tube T (full blood count)- Whitington Hospital London N19- Couriered Daily
- tube U (carboxyHb) - Whitington Hospital London N19 - Couriered Daily
- tube W (biochemistry) - Chemical Pathology Royal Free Hospital NW3
- tube K (biochemistry) - Chemical Pathology Royal Free Hospital NW3

it will be necessary to mark on a paper printout which samples are not present. For tubes T and U this will need to be updated regularly and checked before samples collected at 2.00 p.m. The listing will need to be cut at the collection point and later afternoon samples will go with the following day's material.

A summary for each of tubes W and K will be needed for the whole town.
A summary of all other major tube categories will also be made.

5.3.2 Data sheet blood sample documentation

It is very important that we are aware of any tubes which have **not** been filled. These should be recorded on the data sheet as soon as possible. It will not be possible to make a final data sheet entry for each Participant until **all** that Participant's tubes are dealt with.

The default code will be `all tubes filled? yes = 1' No other entry will then be needed.

If all tubes filled? no = 2 we then need to mark the individual tubes which have **not** been filled. Tubes which have blood in, even if short, should be considered as filled for this purpose.

5.4 Problems - Insufficient sample.

Simply fill as many tubes as possible from what has been collected, in the usual order.

5.5 Problems - Blood into the wrong tubes.

The samples should be left in and the tubes relabelled in biro (not felt-tip) - (spare blank labels might be helpful).

5.6 At the end of the day

- Clean out centrifuges as needed
- Ensure that samples are all packed appropriately into their receiving boxes (tube A, tube B etc) and that no samples are still on dry ice.
- Set pipette buoys to charge
- Return the complete days set of completed datasheets to reception.

6.0 REPEAT MEASUREMENTS

A subset of non-migrant participants seen in the first week of the study will be asked to return for a repeat measurement during the second week of the study. The aim is to obtain remeasurements in about 5% of the total. This will involve 10 participants per town (suggest we attempt to recruit 12).

These participants will be recruited by RN2 from the outset. Need to be clear about who invited and who agreed. The Receptionist will book them an appointment at the end of the survey. For their second visit they will be provided with one of a pre-prepared list of supplementary serial numbers, which will be allocated by the Receptionist at the time of booking.

On the return visit, the Participant will skip the questionnaire, but will go through the remaining aspects of the measurement and blood taking procedure as before.

7.0 FEEDBACK OF RESULTS-

When the Participant consents, results will be fed back to the Participant's G.P. These will include :

- a verified copy of the ECG tracing
- Height, Weight
- Body mass index with cut offs as a comment,
  20 or less = underweight
  >20-25 = acceptable
  >25-30 = overweight
  >30 = obese

- BP : Systolic BP (mean of 2 readings, minus 8 mm)/ Diastolic BP (mean of 2 readings)

- **Blood results**
- Biochemistry include:
  Total, LDL, HDL cholesterol and triglycerides,
  Blood glucose urea k na creatinine urate
  tprotein alb bili alk phos ast = aspart transam alt = alanin transam
  ggt = gamma gt. (exclude mg ca corr ca po4)

- Haematology include: wbc, hb, platelets, rbc, hct, mcv, mch, mchc only

Abnormal values as defined by the laboratory will be indicated with a star next to the abnormal parameter. A copy of the ECG with report will be attached to this output.
A template of results sheet sent to participant's GP is in **Appendix 7.**

Protocol for reporting abnormal values requiring more urgent attention are summarized on the next section.

7.1 MARKEDLY ABNORMAL RESULTS

During study measurements- The only abnormalities which are likely to be identified during the study measurements are a high blood pressure reading or an abnormal electrocardiogram.

7.1.1 Action for high blood pressure readings

Comparability issue

Bear in mind that the Dinamap records systolic pressure about 8mm Hg higher than the mercury sphygmomanometer; diastolic readings are virtually identical. This is taken into account in the following recommendations, which refer to actual DINAMAP readings.

Diastolic pressure readings

Average 120mm Hg or more: severely raised
Average 100-119mm Hg: moderately raised

Systolic blood pressure readings

Average 210mm Hg or more: severely raised
Average 180-209mm Hg: moderately raised

Severely raised BP

If either systolic or diastolic pressure is **severely** raised, should tell the Participant:-
`Your blood pressure is **high** today. Has your blood pressure been high before, or have you received treatment for high blood pressure?'

(If no), `Blood pressure can vary from day to day, so that one high reading does not necessarily mean that you have high blood pressure.'

(All) `You would be well advised to arrange to see your doctor **within a week** to have a further check on your blood pressure. I will give you a card with a note of your blood readings today to give to your doctor' (Copy of card/ template for reporting abnormal results is in **Appendix 8**).

moderately raised BP

If either systolic or diastolic pressure is **moderately** raised, should tell the Participant:-

`Your blood pressure is **on the high side** today. Has your blood pressure been high before, or have you received treatment for high blood pressure?'

(If no), `Blood pressure can vary from day to day, so that one high reading does not necessarily mean that you have high blood pressure.'

(All) `You would be well advised to arrange to see your doctor **during the next two or three weeks** to have a further check on your blood pressure. I will give you a card with a note of your blood readings today to give to your doctor'

Direct notification of GP - to be discussed with participant (participant consent is required)

7.1.2 Abnormalities on Electrocardiograms

Always consider the state of the Participant first in interpretation. If the Participant is well and symptom-free, threshold for rapid action on the ECG will be higher.

If the ECG specifies `acute myocardial infarction' ask the Participant about recent chest pain, breathlessness or other symptoms of ill-health and about any previous history of heart trouble. Irrespective of answers to these questions, should refer Participant directly to G.P.

If the ECG specifies digoxin toxicity and the Participant is taking digoxin or any other cardiac glycoside, he should be referred to the G.P. directly.

7.1.3 Abnormalities on biochemical/haematological tests

Results which should be phoned through to the General Practitioner directly would include:-

- blood glucose above 15 mmol/L (provide urea and electrolytes also)
- blood urea above 20 mmol/L
- serum potassium below 2.5 mmol/L or above 6.0 mmol/L
- serum sodium below 120 mmol/L
- Haemoglobin below 8.0 g/dl; acute leukemia

8.0 PROTOCOL VIOLATIONS/DEPARTURES FROM PLAN

These will need to be dealt with as they arise. Details should be recorded in the study log book.

- If a member of staff is ill:-
  please phone base so that a replacement can be found as soon as possible and any other arrangements made

- Shortened Protocol
  if one nurse is out of action without replacement:-
  - Research Nurse 1 should omit skinfolds and do medication checks
  - Research Nurse 2 should do only the ECG, blood test and consents, sorting out bloods as time permits

9.0 ANSWERING QUESTIONS ABOUT THE STUDY

- What is the study for?
- What has the study shown so far?
- Will you want to see me again?
- Will these results be seen by my doctor?
- What happens if my tests are abnormal?

10. Ethical approval

Ethical approval for the physical examination was provided by both Local Research Ethics and London Multi-centre research ethics committee (MREC/02/2/91) – (**Appendix 9**)