

**Study Protocol for the Singapore Consortium for  
Cohort Studies (SCCS)  
(DRAFT)  
Updated September 6<sup>th</sup> 2007**

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### List of Partner Organizations:

Singapore Tissue Network (STN)  
National Healthcare Group Polyclinics (NHGP)  
National University Hospital (NUH)  
Alexandra Hospital (AH)  
Tan Tock Seng Hospital (TTSH)  
National Research Laboratory (NRL)  
Singapore Eye Research Institute (SERI)

## PROTOCOL SUMMARY

### 1.1 Introduction

Many diseases develop due to a variety of complex interplay between our innate susceptibility (genes) and lifestyle exposures (environment). In Singapore, there is a unique opportunity, not only to study the effect of genes and environment on the risk of developing diseases, but to do so in three different ethnic backgrounds so as to take into account ethnic differences in genes or environment that may come into play with the development of diseases.

Singapore is a multiethnic population with three main ethnic groups, Chinese (76%), Malays (14%), and Indians (9%)<sup>1</sup>. Singapore is unique in that it consists of three ethnic groups that have relatively same exposure levels. As such, Singapore has developed a biomedical sciences (BMS) initiative to exploit its unique position as a country that can represent more than 60% of Asia's peoples. Singapore currently hosts six of the top ten pharmaceutical conglomerates, key industry players and a growing base of medical technology companies within Tuas Biomedical Park, a 183-hectare BMS-dedicated site slated for expansion to double its current capacity due to increasing industry demand. Singapore also features a dedicated Research & Development complex, the Biopolis, which is home to five biomedical public research institutions and laboratories from the Agency of Science Technology and Research (A\*STAR). The Biopolis offers a "plug and play" infrastructure for pharmaceutical and biotechnology companies to share scientific facilities and services, facilitating cross-disciplinary research and public-private collaborations for the advancement of the field and enhancement of business.

Singapore, therefore, has much to offer by the development of a cohort study to study the interaction between genes and environment in disease development among Chinese, Malays, and Indians. Information obtained from the study could be applicable to India, China and much of South East Asia. The study of Type 2 diabetes mellitus (T2DM) is of particular importance in Singapore. Previous studies conducted in Singapore suggest that Asian Indians are three times more likely to develop T2DM than Chinese and Malays at a given BMI<sup>2,3</sup>. When Asian Indians do develop T2DM, the risk of developing ischemic heart disease (IHD) is also higher than in Chinese and Malays. Striking ethnic differences in the incidence of ischaemic heart disease is also seen in the Singapore population<sup>2</sup>. Singapore, with its ethnically diverse population is therefore poised to conduct a cohort study with T2DM as well as cardiovascular disease as the focus of the study.

T2DM, a multi-factorial disease, is a major public health problem that has reached epidemic proportions with nearly 200 million patients worldwide<sup>4</sup>. In Singapore, the prevalence of diabetes, consistently reported in the past three National Health Surveys<sup>5,6</sup> as being in excess of 8%, making Singapore one of the five countries with the highest percentage of diabetics worldwide. Thus far, studies focusing on genetics or environment alone have not been able to explain much of the variance of T2DM manifested in the different ethnic groups. Gene-environment interactions (GEI), with a particular focus on the dietary patterns associated with the different ethnic groups would be an ideal method

of identifying the complex multifactorial pathogenesis of T2DM, which may, in turn, accelerate the development of new clinical therapies through the identification of novel molecular targets.

Rapid socio-economic progress has resulted in Singapore having a high rate of IHD mortality that rivals that in the United States<sup>4</sup>. However, this has not affected the three ethnic groups equally<sup>2,5,7-9</sup>. Indians have a three-fold increase in risk of IHD<sup>2,5,7-9</sup> as compared to Chinese, while Malays exhibit an intermediate risk. However, the differences in serum lipid profiles for all three ethnic groups do not explain the increase in risk. Malays have been found to have the highest levels of LDL cholesterol<sup>10</sup> with Chinese having the lowest levels of LDL cholesterol. On the other hand, Indians have the lowest levels of HDL cholesterol<sup>5,10,11</sup>, while Chinese have the highest levels of HDL cholesterol. Thus far, obesity (particularly central obesity), insulin resistance, diabetes mellitus and low HDL cholesterol are the risk factors that have been found to be significantly different between the ethnic groups. However, even combined, they do not explain the drastic increase in IHD risk for Indians. Ascertaining the presence of gene environment interactions between the three different ethnic groups could help identify the contributions of genetic and lifestyle factors to the pathogenesis of IHD.

## **1.2 Background on studies**

The Singapore Consortium of Cohort Studies (SCCS) is a research initiative funded by the Biomedical Research Council (BMRC). This large prospective study aims to investigate the genetic and lifestyle factors affecting the risk of developing various chronic diseases such as diabetes, cancer, and heart disease. These diseases are of particular importance in Singapore as they have the highest mortality/morbidity rates in the general population<sup>12</sup>. The involvement of a large proportion of the Singaporean population in the cohort studies will ensure that the prolonged follow-up of these subjects which will be used to derive genetic and environmental risk factors will accurately reflect the average risks of the general population. Moreover, the involvement of members of the three main ethnic groups (with an over sampling of ethnic minorities) will ensure that ethnicity-specific risk factors can be identified.

While the study of the factors associated with IHD remains the primary focus of the SCCS, there remain a number of secondary aims that this study will address. The reliable collection of exposure information for a large number of people will facilitate the study of numerous diseases such as breast and colorectal cancers, as well as cerebrovascular diseases. Cancers and cerebrovascular diseases combined account for more than one third of deaths in Singapore in 2006<sup>12</sup>. With sufficient follow-up time, nested case-control studies with breast cancer, colorectal cancer, and cerebrovascular diseases as outcomes will be well powered to detect any gene-environment interactions that may affect the pathogenesis of these diseases. The SCCS will also be a rich source of readily available controls for case-control studies conducted with other disease focuses in mind.

The SCCS is composed of two major arms, a diabetic cohort (DC), and a multi-ethnic cohort (MEC). The DC will build on the existing NUS Diabetes Cohort Study of 3900 diabetic patients who were previously recruited. Additionally, diabetic patients will continue to be recruited at local polyclinics. The MEC is a cohort of normal people aged 21 to 75 who will be followed up in 3-5 year intervals for the development of a wide range of diseases. The MEC builds on two existing cohort studies, the Singapore Prospective Study Program (SP2), and the Singapore Cardiovascular Cohort Study 2 (SCCS2). At the end of July 2007, we have a total of 5214 subjects in the MEC and 4003 patients in the DC.

### 1.2.1 Objectives summarized

- To study gene-environment interactions in the causation of major chronic illnesses like IHD, DM and cancers, among the three major ethnic groups.
- The study of gene-treatment interactions in the pathogenesis of diabetic complications.
- To provide a large variety of healthy controls who can be matched with regards to age or ethnicity for current ongoing case-control studies.

### 1.2.1 Background on the NUS Diabetic Cohort (NDC)

The NDC was designed to study the genetic and environmental risk factors of diabetic nephropathy. T2DM subjects, regardless of race or co-existing medical conditions (hypertension, hyperlipidemia, pre-existing diabetic complications) were included. Subjects using traditional medicine concomitantly were also included in this study. Subjects with mental illness, as well as those with clinically obvious non-diabetic kidney disease (such as polycystic kidney disease) were excluded. Subjects with type 1 diabetes or diabetes mellitus resulting from endocrinopathies were also excluded.

Subjects were recruited from polyclinics, Alexandra Hospital, Tan Tock Seng Hospital, and National University Hospital. Apart from questionnaire data, detailed clinical, biochemical, and treatment information was obtained from the case notes of each patient.

### 1.2.2 Background on the Singapore Prospective Study Program (SP2)

The SP2 is a cohort study that is designed to examine the pathogenesis of cardiovascular and metabolic diseases (hypertension, dyslipidemia, obesity, and diabetes mellitus) in Singapore). The primary objective of the SP2 involved examining the differential role of genetic variants and environmental factors in altering lipid and lipoprotein concentrations in the three different ethnic groups. The secondary objective of the SP2 was the recalibration of the Framingham Coronary Heart Disease predictive function for use in an Asian population.

The SP2 recalled the subjects studied in the 1998 National Health Survey<sup>6</sup> to collect additional information on clinical, biochemical, dietary and genetic parameters. 4018 patients were recruited and are currently being followed up.

### 1.2.3 Background on the Singapore Cardiovascular Cohort Study 2 (SCCS2)

The SCCS2 is a cohort study designed to identify environmental risk factors and genetic factors in cardiovascular and metabolic diseases, determine the optimal definitions of obesity and glucose intolerance for the identification of persons at risk for T2DM of cardiovascular diseases (CVD) in Asian subjects, and to assess the public health impact of these definitions by determining the prevalence and risk associated with metabolic syndrome (MS) in Singapore. MS was taken to represent a constellation of metabolic abnormalities including obesity, hypertension, and dyslipidemia.

The SCCS2 recalled subjects from the 1992 National Health Survey<sup>5</sup>, the Singapore Thyroid Heart Study<sup>7</sup> (1982-1985), and the National University of Singapore Heart Study<sup>11</sup> (1993-1995) to collect additional information on clinical, biochemical, dietary and genetic parameters. 4,426 patients were recruited and followed up for a mean of 9.6 years.

Results from the SCCS2 suggest that Asian Indians are more susceptible to T2DM than Chinese and Malays at a given<sup>2,3</sup>. When Asian Indians do develop T2DM, the risk of developing ischemic heart disease (IHD) is also higher than in Chinese and Malays. Therefore the SCCS2 recommends that prevention of T2DM in the Asian Indians is important to check the rising rates of IHD in Asia. In other research<sup>13</sup>, the SCCS2 has showed that using a definition of MS which does not include obesity as a requirement fails to identify a fairly large proportion of individuals who would otherwise be classified as having MS.

### **1.3 Gene environment interactions**

Recent developments in high throughput technologies in genomics analyses at a relatively low-cost have made it feasible and possible for population-based genetic research to be conducted. It has presented the opportunity for biomedical researchers to address questions on the complex contributions of genes, the environment, and gene-gene and gene-environment interactions to health. The studies of these interactions at a population level should lead to better prevention, diagnosis, and treatment of complex diseases. Studies conducted to date have found it genetic or environmental effects alone cannot explain most of the disease patterns observed.

Diabetes mellitus, ischemic heart disease and most cancers are multi-factorial diseases involving complex gene-environment interactions. An ideal methodology for identifying factors related to disease is a prospective cohort study which allows for the measurement of environmental exposures and collection of biological markers before disease symptoms even appear. This reduces bias and subjects in the study are not limited to survivors.

Preliminary studies involving gene-diet interactions have had promising results. The Framingham offspring study found a significant gene-nutrient interaction<sup>14</sup>. It showed that the -514C/T polymorphism and measures of HDL-C metabolism depended on the amount and type of fat consumed. Specifically, that the T allele of the polymorphism was only associated with higher HDL-C and size in subjects consuming less than 30% of their energy from fat.

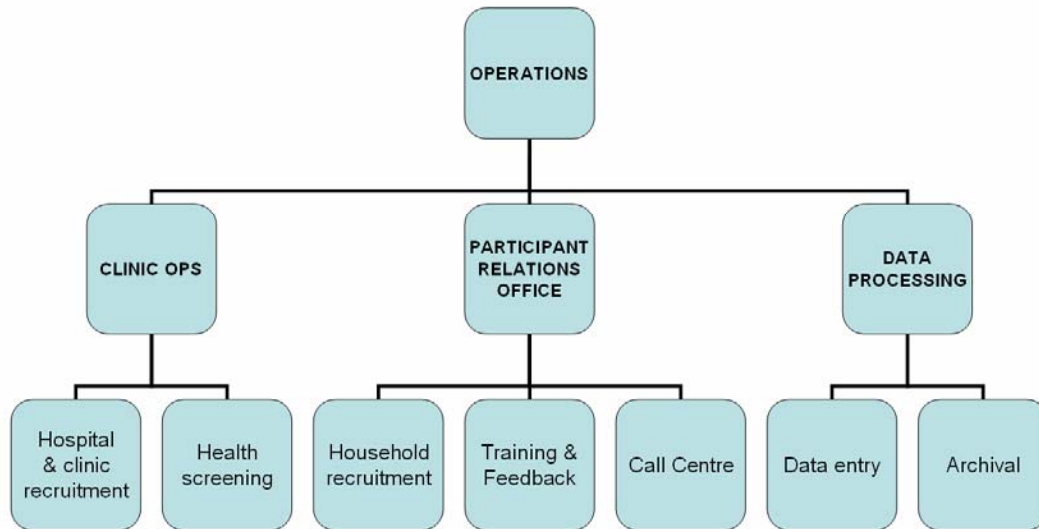
Consistent with the findings from the Framingham study, researchers in Singapore have found that the TT genotype was associated with a more atherogenic lipoprotein phenotype with increasing dietary fat intake<sup>15</sup>. Unlike the Framingham study, in which the main lipid parameter that was affected was the serum HDL cholesterol concentration, the Singaporean study showed that the lipid parameter that was affected was the serum triglyceride concentration. The gene-diet interaction with serum HDL concentrations that was observed in the Framingham study was only statistically significant in the Asian Indian population in Singapore.

## Study Operations

### 1.5.1 General Organization of the study

The SCCS is a major project of the Center for Molecular Epidemiology, part of the Yong Loo Lin School of Medicine at the National University of Singapore. Currently, 68 staff members are working on various aspects of the SCCS. The organizational structure of the team is summarized in the figure below.

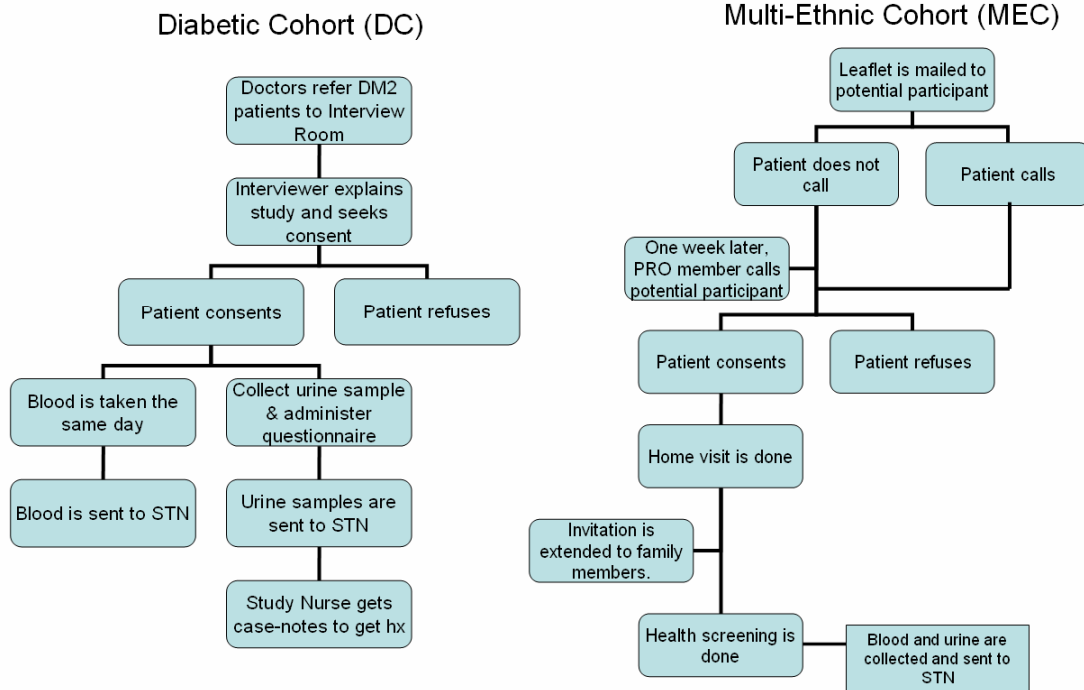
## The SCCS team



### 1.5.2. Recruitment of DC

For the DC, during their regular visits, diabetic patients presenting at Ang Mo Kio and Choa Chu Kang Polyclinics, as well as Alexandra Hospital, will be identified by their attending physicians and referred to a SCCS research nurse who will then inform them of the study. With consent, the trained staff will interview the participant and ask for consent to extract further information from the patient's case notes. Polyclinic staff will assist with biospecimen collection. Recruitment flow of the DC is summarized in the figure below.

# Recruitment flowchart for MEC and DC



For the MEC, subjects who were part of the National Survey for Neurological Diseases<sup>16</sup> (n=15,906), were mailed an invitation to participate in the SCCS. The recruitment procedure is detailed above. During the home visit, a detailed family history was taken at the time of the home visit. Subsequently all participants of the home visit (the primary respondent, as well as family members recruited) were invited to attend a health screening free of charge. Consent was again taken for the collection of biospecimens during the 1 to 2 hour long health screening. Subjects were given a summary sheet of their BMI and blood pressure results, as well as a copy of their consent form to take home at the end of their visit.

### 1.5.3 Information and Biospecimens collected

Detailed questionnaires detailing the subject’s lifestyle which is relevant to their diabetic and kidney conditions (such as smoking history) and family history of diabetes is being captured. Informed consent is obtained for the questionnaire, for further case notes abstraction from their medical records, linkage to national disease registries, and the use of their blood and other biological samples for the purpose of future research.

Fasting venous blood was collected in both the DC and MEC subjects (see table below for breakdown of use. Subjects with high or abnormal levels of blood or urine results

were called and alerted to their test results. If necessary (fasting glucose 6.1-6.9mmol), additional appointments were scheduled for oral glucose tolerance tests (OGTTs) to confirm glucose tolerance.

<b>Diabetic Cohort</b>			<b>Multi-Ethnic Cohort</b>	
Use	Vol. (mls)	<b>Tube</b>	Vol. (mls)	Use
<u>Blood</u>		<b>EDTA</b>		
DNA, RBC, Plasma,	10		10	DNA, RBC, Plasma,
Whole blood		<b>Plain tube no. 1</b>	10	Whole blood
Serum storage	5	<b>CPT</b>	8	Serum storage
		<b>Plain tube no. 2</b>	5	Lymphocytes
		<b>Fluoride Oxalate</b>	2	Lipid panel,
				creatinine
<b>Total</b>	<b>15</b>		<b>35</b>	Fasting glucose
<u>Urine</u>		<b>Falcon tube</b>		
Urine storage,	25		25	Urine storage,
dipstick analysis				dipstick analysis

Other measures captured during the health screening include retinal photographs, anthropologic measurements (weight, height, and waist circumference), Blood pressure (sitting arm, prone arms and ankles), and foot sensation measurements.

#### 1.5.4 Transport and storage of biospecimens

Samples are maintained on cold gel packs at 4°C immediately after extraction. Vans pick up and transport the biospecimens at 2-3 hour intervals after extraction. The tubes are packed in foam boxes lined with foil and maintained at 4°C with cold gel packs. Samples are all processed at the Singapore Tissue Network (STN). The maximum time between extraction and storage is 4 hours. On arrival at STN, whole blood, red blood cells, DNA and plasma will be extracted from blood in EDTA tube. Extracted DNA, whole blood, plasma and Red blood cells will be stored in -80°C freezer at STN. Serum is isolated from the plain tube and stored in the -80°C freezer at STN. If CPT tubes are used, lymphocytes are isolated, and 1ml of lymphocytes are transferred to 2 cryovials which are then stored in liquid nitrogen. Urine is aliquoted and stored at the -80°C freezer at STN.

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Standardization of measurements

**The health screening for the SCCS will be conducted in the Singapore Eye Research Institute, level 5 of the Singapore National Eye Centre.**

Health screening will be conducted from 0800-1100. The system will need to handle up to 20 subjects per day.

**The health screening for the SCCS will be conducted in four sections.**

- A Registration and Baseline Measurement
- B Measurement of ankle brachial index, vibration sense and fine touch.
- C Retinal photography
- D Blood sample screening and Blood Glucose measurement.

## A Registration and Baseline Measurement (Rm 1)

**Requisite:** Data Collection Form (blank)  
Barcode system – system include barcode reader, printer and notebook PC (provided by SCCS project management)  
Writing material- pen, pencil, writing pad etc  
Plastic folder (coloured- to differentiate from SiMES study subjects)  
Water resistant sticky labels.  
SCCS Health Examination checklist Forms  
Non- elastic measuring tape X 5 pcs

### Action

- 1 Receive and greet the subject.
- 2 Enter your assigned investigator code on the appropriate column.
- 3 Verify the subject's identity by his NRIC  
  
Briefly describe and explain the sequence of events and what will take place to the subject.
- 4 **Take consent** for blood taking, pupil dilation, use of NRIC number, and also for the storage of remaining blood for future studies. Place the consent for in the plastic folder that the patient will carry with them for the remainder of the health screening
- 5 Scan the subject's NRIC with the barcode reader. The information will be displayed on the notebook PC. If not, enter the subject's particulars manually.
- 6 Activate the printer to print a specified amount of sticky labels with subject ID and initials
- 7 Paste a printed label on a blank SCCS data collection form and place the form in a coloured plastic folder. The plastic folder should contain:
  - a. The subject's health examination checklist
  - b. An adequate amount of sticky label with the subject's ID printed on it.
- 8 Take measurements for the following:
  1. Height
  2. Weight
  3. Hip measurement
  4. Waist measurement
- 9 The **height measurement** has to be done with a wall-mounted scale reading device. The subject has to remove his/ her shoes and stand with heels together (feet 45 degrees to each other), knees straight, and heels buttocks and shoulder blades against the wall.

Arms and shoulders should be relaxed and hanging loosely by the sides of the body. Position subject's head with the "Frankfurt" plane, (the Frankfurt plane is the line between the external auditory meatus and the orbital bone below the eye.) being horizontal. Lower and press the headpiece firmly on the crown of the head. Record to the nearest decimal point. In cm

- 10 **Weight measurement:** The subject has to remove any objects such as keys and coins, pager or mobile phone, etc. for accurate weight measurement. Place the subject's item into the plastic container provided. The plastic container should be in plain sight of the subject. Record weight in kg to one decimal place
- 11 **Hip measurement-** point of measurement is set at the trochanter of the femur. Record the hip circumference in cm to 1 decimal point
- 12 **Waist measurement-** point of measurement is set as mid point between the last rib and the iliac crest. The waist is measured with the subject holding his/her breath in light expiration. Record the waist circumference in cm to 1 decimal point
- 13 **Blood pressure measurement** - Palpate the radial pulse by placing the 2<sup>nd</sup> and 3<sup>rd</sup> fingers over the subject's radial artery. When checking the pulse, the investigator's thumb should not be used. Establish peak inflation level by attaching the cuff to standard manometer and slowly inflate the cuff while palpating the radial artery until the pulse can no longer be felt. Inflate an additional 20mmHg. Place the bell of the stethoscope on brachial artery; slowly deflate cuff (approximately 2mm/ second while listening. Continue listening at the brachial artery until the cuff is completely deflated. Record 1<sup>st</sup> and 5<sup>th</sup> stages of Korotkoff sounds (onset and disappearance)
- 14 Record to the nearest 2mm Hg. Do not round the BP reading to the nearest 10mmHg.
- 16 Alert the supervisor if subject's blood pressure exceeds 180 mmHg SBP or 100 mmHg DBP.
- 15 Upon completion of the baseline measurement, the subject will be handed the coloured plastic folder containing, the health examination form, and sticky labels. The subject will then be directed to the next station where the eye examination is conducted.

## **B Measurement of ankle brachial index, vibration sense and fine touch (Rm 2)**

<b>Requisite</b>	Blood pressure monitoring device for arm and ankle Biothesiometer testing device Writing material Non sterile examination gloves Alcohol laced cleaning material
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### **Action**

- 1 Ensure that the monitoring device is in a working condition.
- 2 Greet the subject and guide the subject to the to lie prone on the examination bed.  
Ensure that the subject is comfortable
- 3 Explain what is about to take place. Retrieve the plastic folder from the subject. Enter your assigned investigator code into the designated column.
- 4 Request the subject to roll up his/ her right sleeve to expose the forearm. Shoes and socks should be removed at the same time. Roll up the subjects trousers to expose the ankles.
- 5 Attach the blood pressure cuff to the right arm. If the right arm cannot be used to measure blood pressure, then the cuff should be attached to the left.
- 6 Measure systolic blood pressure in the arm using the doppler probe
- 7 Place a cuff around the right ankle and record the systolic blood pressure using the doppler probe on the posterior tibial artery. If you are unable to detect the posterior tibial pulse with the doppler probe, then use the dorsalis pedis pulse
- 8 Repeat the systolic blood pressure measurement for the left ankle.
- 9 Measure the neuroethisiometer readings at the apex of the 1<sup>st</sup> toe and also medial malleoli of both ankles
- 10 Document the monofilament sensation at the 5 points of the foot as described. Record the number (out of 5) of points that the subject is able to feel for each foot.
- 11 Repeat the systolic blood pressure measurements in the right arm, right ankle and left ankle.
- 12 Record the findings on the health examination form.
- 13 Hand the form in the plastic folder back to the subject and direct the subject to the next station.

## **C Retinal photography (Rm 3)**

### **Action**

- 1 Greet the subject and guide the subject to the couch. Ensure that the subject is comfortable
- 2 Check pupils for the presence of glaucoma.
- 3 If subject has high blood pressure or glaucoma, do not administer dilating eye drops. Else, warn the subjects that the drops may sting, then proceed to administer 2-3 drops per pupil.
- 4 After 10-15 minutes, direct the subject to the seat and proceed with the retinal photography
- 5 Files should be saved using a file name that incorporates the SP2 study ID which will be found of the sticky labels that a subject is carrying.
- 6 Hand the form in the plastic folder back to the subject and direct the subject to the next station.

## **D Blood sample screening and Blood Glucose measurement (Rm 4)**

<b>Requisite</b>	Plain tubes prepared with streptokinase added EDTA tubes (10ml type) Plain tubes (with gel) Flouride Oxylate tubes EDTA tube (3ml) CPT tube (10 ml) Syringes (10 and 5 cc types) Hypodermic needles (21 and 23 FG) Vacutainer Blood collection set. Alcohol swabs Plaster strips Ice Box Examination gloves
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### **Action**

- 1 Ensure that all the necessary items are ready before receiving the subject.
- 2 Receive and greet the subject. Guide the subject to the chair. Retrieve the plastic folder from the subject
- 3 Ask the subject if he/ she is on an anti- clotting therapy, for e.g. warfarin. If so, special care needs to be taken to ensure prolonged pressure on the injection site to prevent bleeding
- 4 Gather the necessary blood tubes and label them with sticky labels containing the subject's ID. Place the excess remainder back in the plastic folder. Place a sticky label onto the Collective List of subject that will be needed to document all the samples taken for the day. The same form will be used for handing and taking over from the NRL staff.
- 5 Check to see if the patient has given consent for blood taking and for storage of blood specimens. If no consent has been given, then no blood should be taken.
- 6 Explain to the subject what is about to take place.
- 7 Give fair warning before taking the blood sample.
- 8 Ensure that the workstation is always kept clean and free from blood spill.
- 9 Proceed with the procedure to extract blood sample from the subject.
- 10 Apply some pressure on the entry point to stop excessive bleeding.
- 11 Apply a plaster strip onto the wound and inform the subject that he may remove the

plaster strip after 30 minutes. Do not allow him to leave the station if the puncture wound is still bleeding.

- 12 Provide the subject with some light refreshment.
- 13 The subject is then directed back to Rm1 where he is given a copy of his blood pressure measurements and BMI. Subject is notified if his BMI places him in an “at risk” category
- 14 Staff check to ensure all data has been recorded before the subject leaves for home.