SERUM SPECIMENS

1. Introduction

Serum samples may be collected from participants attending clinic visit and can be used to test for biomarkers, hormones or other metabolites that may be related to aging, physical and cognitive function and sleep disturbances in elderly women. Whole blood will also be collected and be used to conduct genetic studies. We may preserve the samples for several years until the best potential markers have been developed. We will draw enough blood for eight 0.5-ml cryovials of serum from each participant and two 4.5-ml cryovial of whole blood.

2. Equipment

- 4.5 ml cryovial #12-565-161 N, 1 per subject
- 1 ml cryovial for storage. (Fisher #12-565-169N), 8 per subject
- Gloves, disposable, non-sterile
- Horizontal centrifuge
- 10 ml red-top tubes, non-coated (Fisher #02-683-60), 2 per subject
- 5 ml EDTA Purple top tubes (Fisher #02-685-2C), 2 per subject
- Vacutainer set-ups; 20 or 21 g needles:
  - Needle holder (Fisher #02-665-110)
  - Needle (20g- Fisher #02-665-31, 21g- Fisher #02-665-21)
  - Butterfly needle (21g) #02-655-42
- Wooden applicator sticks (Fisher #01-340)
- Non-self defrosting freezer, -70 C
- Plastic disposable transfer pipettes (with built in bulbs) (Fisher #13-711-5A)
- Cryotube 2” storage boxes (100 cell); Fisher #11-678-24A and 3” storage boxes # 11-678-24B
- 100 cell cryotube inserts; Fisher #11-678-24C

3. Procedures

A. Venipuncture

PUT ON GLOVES

a) Before drawing the blood, a preprinted label showing the participant's ID code should be placed on each vacutainer tube. It is essential to then check the ID code on each tube to ensure that the specimen being collected belongs to the participant. This can best be done by holding the tube next to the ID number on the participant's chart and calling out the number. Then ask the participant to say her name aloud and verify it against the name on the chart.

b) Draw blood from an antecubital vein whenever possible. Use a tourniquet to produce venous distention so that a needle can be inserted. A blood pressure cuff inflated midway between systolic and diastolic blood pressure is most effective and is highly recommended. Do not leave the tourniquet in place for more than 2

minutes. This avoids excessive hemoconcentration. If the 2 minute interval is exceeded, abandon the arm temporarily and attempt to obtain the specimen from the other arm.

c) Remove tourniquet after 2 mins.

d**)** Draw blood using the vacutainer system—(2 10ml red top and 2 5ml purple top).

e) A syringe may also be used in the event of fragile veins.

B. BLOOD PROCESSING - RED TOP TUBES

PUT ON GLOVES

a) Allow the filled red-top tubes to stand at room temperature for 60-90 minutes. This procedure is necessary to allow an adequate clot to form.

b) After clot formation and before centrifugation, remove the red-top stoppers and gently free the clot from the sides of the tube with a clean plain wooden applicator stick. Replace the stoppers. Balance the tubes of blood for centrifugation. Use a horizontal centrifuge; angle heads are not satisfactory.

c) Centrifuge the blood for 10 minutes at room temperature at a setting known to yield a relative centrifugal force (RCF) of at least 1000 **x** g at the bottom of the tubes. The table below gives those combinations of centrifuge speed in revolutions per minute (rpm) and rotating radius (r) that will yield an RCF value of 1000 **x** g. RPM should be read from a tachometer or rev counter when the centrifuge is normally loaded. Radius (r) is measured in centimeters from the center of the rotor shaft to the bottom of the vacutainer tube when the tube is in a horizontal position.

r (cm)	12	14	16	18	20	22.5	26
rpm	2800	2600	2400	2250	2100	2000	1900

Do not use a brake to slow down the centrifuge.

d) Remove serum from the clot by aspiration with a clean transfer pipette (the clot may sometimes stick to plastic). Use a new pipette for each subject. Transfer the serum into 8 separate pre-labeled (see e. below) cryotubes. Fill the cryotubes up to approximately 0.5 ml per tube.

e) Each cryotube should be individually labeled with the ppt ID and filled with serum. Use a pen with permanent ink. "Sharpies" work best. Keep the labeled cryotubes away from solvents such as alcohol or acetone as these will erase the ID code. Before transferring the serum, the vacutainer tube and cryotubes should be held side by side and the numbers read aloud to check that the ID code

numbers match. Do not set up production lines of labeled empty cryotubes. This increases the chance of error.

f) If the serum is reddish in color, determine if it is hemolyzed or simply contaminated with red blood cells. One can tell the difference by recentrifuging the vacutainer tube. This will pellet any contaminating red cells and the serum will clear. If the sample is hemolyzed the red color will remain in the serum. If the patient is still in the clinic, another red-top tube should be obtained. Otherwise, the hemolyzed sample should be processed.

g) Some caution should be used in capping the cryotubes. Screw the caps on firmly to secure them tightly against the rubber gasket, but do not apply an extreme amount of pressure. To promote rapid freezing, place the cryotubes upright in a footless metal rack that is in contact with a shelf in a -70 C freezer.

h) Blood processing should be completed and tubes placed in cold storage within two hours of collection.

C. BLOOD PROCESSING - PURPLE TOP TUBES (one to freeze and store and one for CBC's)

a) draw (2) 5 ml purple top tubes and invert several times.

b) Pipet (1) 5 ml purple top into 1 4.5ml cryovial and freeze it at -70.

c) Process other 5ml purple top for the CBC's. Your local lab will tell you if they want 2 slides and the tube or just the tube. Label and send to local lab for CBC and DIFF.

4. Site Storage

a) After samples have been frozen by placing cryotubes upright on a -70 C shelf (overnight), place cryotubes in ID numerical order into a storage box using the inserts.

b) Use the cryotube storage box grid for recording the position of cryotubes, by ID number, within the shipping boxes. (This is a backup identification system in case the ID numbers on the tube are obliterated after prolonged storage at -180 degrees). As the filled tubes are placed into the slots formed by inserts, write the ID number which is on the tube into the corresponding box on the paper grid.

c) Since the box does not have a definite up or down, right or left, you will have to mark the upper right corner of the cardboard box and the insert. (The paper grid is already marked "upper right" and "upper left".) In a clearly visible spot in

the upper right corner of the box and the insert (to the right and away from you), punch a hole in the cardboard with a single hole paper punch.

d) Store samples at -70C in the storage box.

5. Summary of Important Rules

a) The tourniquet must not be in place for more than 2 minutes.

b) Vacutainer tubes and cryotubes must be pre-labeled with the participant's ID code number. Vacutainer tube ID numbers must be checked with the participant's chart immediately before venipuncture. Cryotube ID numbers must be checked with each respective vacutainer tube before transferring the samples.

c) The red-top vacutainer tubes must be kept at room temperature for at least 60 minutes but no longer than 90 minutes before centrifugation.

d) Blood processing should be completed and serum stored in the freezer within 2 hours of collection.

6. Alert Values: CBC Results

There are two levels of alert values for the CBC measurement: immediate referrals and alerts. CBC results requiring immediate referral are provided below; abnormal CBC results which do not fall within the values designated for immediate referrals will be considered alerts, as determined locally by site investigators. The protocols for immediate referrals and alerts will be determined locally, at each clinical site.

Immediate referrals are potential emergencies which may require immediate notification of the participant and (if so requested by the participant) her primary physician or other available health care provider. These are findings made upon reviewing the CBC results from the lab, following the clinic or home visit. Depending on the specific protocol for your site, the site staff may contact the SOF study investigator for a clinical diagnostic assessment, determine whether immediate referral is indicated or may contact the participant directly with a referral to her physician or to an emergency room. With participant's consent (obtained verbally at the time of the immediate referral), the study physician would contact the participant's referring physician directly.

<u>CBC results requiring immediate referral:</u>

- Hematocrit < 30% or >60%
- Total White Blood Count > 20,000 x10 ^{6}/L (or >20 x 10 ^{9}/L)
- Absolute Neutrophil Count <1,000 x10 ^{6}/L (or <1 x 10 ^{9}/L)
- Platelet Count < 50,000 or > 1,000,000 x10 ^{6}/L (or <50 or >1000 x 10 ^{9}/L)

Alerts are related to abnormal CBC results that do not fall within the values designated for immediate referrals which may require medical attention but

generally not on an emergency basis. In most cases, notification of the participant should be sent by mail within 10 days. However, certain alerts may require more immediate attention at the discretion (and responsibility) of the study physician.