STUDY OF OSTEOPOROTIC FRACTURES (V4)

Protocol for BRI Serum Specimens

1. Introduction

The purpose of serum samples is to allow us to test for markers of bone loss in women who lose bone most rapidly. Some markers for bone resorption and bone formation are found in the serum while other markers of bone loss are being developed. We will preserve the samples for several years until the best potential markers have been developed. Then we will use the sample to look for differences in the levels of these markers between those SOF participants who fracture and those who do not.

We will draw enough blood to get one 4 mL tube of serum from each participant at the same time as the blood is drawn for the calcium 45 test.

2. Equipment

Gloves, disposable, non-sterile
4 ml cryotubes for storage and shipping.
Horizontal centrifuge
10 ml red-top tubes, non-coated (BD #6441), 2-3 per subject
Vacutainer set-ups; 20 or 21 g needles
Wooden applicator sticks
Color cap inserts (white) for the 4 mL cryotubes
Non-self defrosting freezer, -20 C, dry ice
Plastic disposable transfer pipettes (with built in bulbs)
Cryotube storage boxes (100 cell); Scientific Products #R3890-7;
100 cell cryotube inserts; Scientific Products #R3890-8
Insulated shipping boxes for sending cryotubes to BRI; Scientific Products #M1066-12

3. Procedures

A. Venipuncture

PUT ON GLOVES (not mandatory if easier to receive blood w/o) – clinic decision – own risk

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) Draw blood using the vacutainer system (2 10mL tubes for Creighton and 2 10 mL tubes for BRI). For detailed instructions, use those supplied with the vacutainer tubes. A syringe may be used for participants with veins that are too small or fragile for the vacutainer system.

B. BLOOD PROCESSING - RED TOP TUBES

PUT ON GLOVES

a) Allow the filled red-top tubes to stand at room temperature for 20 - 40 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 pipet (the clot may sometimes stick to plastic). Use a new pipet for each subject. Transfer the serum to prelabeled (see e. below) cryotube with white stopper insert. Fill the cryotube up to the line that is already marked on the tube (this yields 3.6 to 4 mL). Do not go above this line because expansion space is needed when the serum freezes. If there is not enough serum to fill the tube, fill it as much as possible.

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 cryotube 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) Insert the white insert into the serum cryotube cap. 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 -20 C freezer.

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

4. Temporary Storage and Shipping

a) After samples have been frozen by placing cryotubes upright on a -20 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 sent to BRI. (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".) Orient the box so that the oval holes along the bottom of the box are facing toward you on one side and away from you on the other side. In a clearly visible spot in the upper right cornet 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 -20C in the storage box until 100 cryotubes have been filled and frozen. This should take about four weeks.

e) When the box is ready for shipping, record on the grid form the dates over which the samples were collected, your clinic, the date the box was shipped to BRI and number of tubes being shipped.

f) Send one copy of the form to BRI with the box and keep one copy yourself.

g) When the box arrives at BRI, it will be assigned a unique identifier and placed into storage. A copy of the grid with the identifier will be sent to the coordinating center.

h) A box of samples should be shipped in an insulated shipping box on dry ice by a 24 hour air carrier (such as Fed Ex). To ensure that the samples can be received at BRI the next day, ship Monday - Wednesday only. The insulated shipping box and method can be chosen by each center. However, the shipping box must provide insulation and have inner dimensions sufficiently large to handle 1 storage box (o.d. 5.25 x 5.25.x 4.75 inches) and 5-10 lbs of chipped dry ice to keep the samples cold even if shipment is interrupted for a day or two. (If in doubt, err on the side of too much dry ice.)

i) Serum should be shipped to:

Don Dover
Biomedical Research Institute
12264 Wilkins Ave
Rockville, Maryland 20852

5. Summary of Important Rules

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

b) The tourniquet must be released before blood is drawn.

c) 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.

d) The red-top vacutainer tubes must be kept at room temperature for at least 20 miuntes but no longer than 40 minutes before centrifugation.

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