Risk Factors for Hip and Colles Fracture Study

Clinical Center Laboratory Procedures

Index

A. General Objectives

1. Obtain at least 15 ml of serum from each participant using four 10 ml vacutainer tubes.
2. Distribute this serum into three labeled Nunc cryotubes.
3. Freeze serum at -20°C until one storage box is filled (81 cryotubes), i.e. about 1 week.
4. Ship one storage box of frozen serum on dry ice in insulated shipping containers to:

Dr. James Leef
Director
Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852
Tel: (301) 881-3300

B. Procedures. N.B. Precaution: There should be no chalk boards in the collection, processing or storage areas. Chalk dust is calcium carbonate. It will contaminate blood with calcium and falsely elevate serum calcium concentrations.

1. Blood Collection

a. Obtain blood between 8:00 a.m. and 2:00 p.m. and record the time.

b. Before the participant is scheduled for the clinic visit, inform her of the need for restricted food intake. The goal is to avoid obtaining a lipemic serum sample. To do this, it is important to prohibit the intake of any foodstuff containing fat. Thus, breakfast or lunch should be limited to foods such as:

(1) Coffee or tea with sugar and non-fat milk. Regular milk or low-fat (1% and 2%) milk are not permitted.
(2) Toast or bread without butter or margarine. Sweet rolls or rolls containing fat (i.e., croissants) are not permitted.
(3) Breakfast cereals (i.e., corn flakes, bran, etc.) with non-fat milk only.
(4) Any fruit or tomato juice.
(5) Salads without dressing.

c. Venipuncture:

(1) The participant must have been seated for 10 minutes before venipuncture. This standardizes the degree of orthostatic hemoconcentration.

(2) Before drawing the blood, a label showing the participant's ID code should be placed on each vacutainer tube. The pre-printed labels for vacutainer tubes will be provided by the Coordinating Center. 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.

(3) 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.

(4) Draw blood using the vacutainer system (Becton-Dickinson #6441, red-top, non-coated, 10 ml, 16x100 mm). For detailed instructions, use those supplied with the vacutainer tubes. After the needle is securely placed in the vein, remove the tourniquet or deflate the blood pressure cuff. Fill the four 10 ml red-top tubes as completely as possible with blood. A syringe may be used for participants with veins that are too small or fragile for the vacutainer system.

(5) If a full red top vacutainer of blood is not obtained, collect a full replacement tube immediately.

2. Blood Processing.

a. Allow the filled red-top tubes to stand at room temperature for at least 60 minutes but for no more than 120 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 10 ml tubes of blood for centrifugation. Use a horizontal centrifuge; angle heads are not satisfactory.

c. Centrifuge the blood for 10 minutes at <u>room temperature</u> 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 a 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 (borosilicate glass pasteur). Use a new pipet for each vacutainer tube. Transfer the serum to prelabeled (see e. below) cryotubes. Fill each cryotube up to the line that is already marked on the tube (this yields 4.5 to 5.0 ml). Do not go above the line because expansion space is needed when the serum freezes. Fill each cryotube in turn; if there is not enough serum to fill the third tube, fill it as much as possible. Conversely, if there is more serum available than the three cryotubes can accomodate, save the extra serum at -70°C for the cardiology study per Dr. Kuller's protocol (Pittsburgh PI).

e. Each cryotube should be individually labeled and filled with serum. Use a pen with permanent ink. Dr. Leef has recommended that "Sharpies" be used to print the ID codes. 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. The chance of error is increased by the latter procedure.

f. If a serum sample 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. When a specimen is judged to be badly hemolyzed, another set of four vacutainer tubes should be obtained. This may necessitate rescheduling a blood collection if the participant has already left the clinic. However, for safety's sake, the other three vacutainer tubes from the first sample should be processed and stored at -20°C until a new collection of four vacutainer tubes can be obtained.

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 -20°C freezer.

3. Temporary Serum Storage and Shipment

a. After serum has 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. Use the grids on the last pages of this protocol to keep a record of which ID codes were included in which boxes.

b. Store serum at -20°C in the storage box until 81 cryotubes have been filled and frozen. This should take about a week.

c. A box of serum should then be shipped in an insulated shipping box on dry ice by a 24 hour-air carrier (such as Federal Express). To ensure that the samples can be received at BRI the next day, ship Monday - Thursday only. The 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.)

d. Serum should be shipped to: Dr. James Leef, Director
Biomedical Research Institute
12111 Parklawn Drive
Rockville, Maryland 20852

C. SUMMARY OF IMPORTANT RULES

1. The participant must be seated for 10 minutes prior to venipuncture.

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

3. The tourniquet must be released before blood is drawn.

4. 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 serum.

5. The vacutainer tubes must be kept at room temperature for at least 60 minutes but no longer than 120 minutes before centrifugation.

6. Each cryotube is filled to the line with serum. Hemolized serum is to be discarded and after another sample obtained.

7. Serum is frozen by placing cryotubes upright on a -20°C freezer shelf.

REASONS FOR FAILURE TO OBTAIN SUFFICIENT SERUM

1. Original vacutainers not properly filled.

2. Insufficient time allowed for adequate clot formation. Sample not at room temperature.

3. Failure to free clot from tube sides before centrifugation.

4. Failure of centrifuge to develop required relative centrifugal force.

SUMMARY OF SUPPLIES

1. Blood drawing supplies

   a. Blood pressure cuff; any reasonably accurate device is recommended for use as a tourniquet.

   b. Tourniquets; Banquet Adult Tourniquet, Scientific or equivalent, approximately 4 required per center.

   c. Vacutainer supplies

      (1) Vacutainer tubes; Becton Dickinson # 6441, non-coated 10 ml red top, 16 x 100 mm; these tubes are carried by most major distributors, e.g. American Scientific Products (B2982-54), VWR Scientific (BD6441), Fisher Scientific (02-683-60), Curtin-Matheson (393-357).

      (2) Disposable needles; 20 or 21 gauge, 1-1/2 inch

      (3) Vacutainer holders, approximately 10 required per center

2. Serum processing and storage

a. Centrifuge with horizontal head capable of developing an RCF of 1000xg.

b. Disposable transfer pipets (borosilicate pasteur), 5 3/4", approximately 5000 required per center

c. Rubber bulbs for use with disposable transfer pipets.

d. Applicator sticks, wooden

e. Freezer, -20°C (non-self defrosting)

3. Shipping supplies

a. Insulated boxes; a suggested shipping box has inner dimensions of 8" x 6" x 12" and is manufactured by Polyfoam Packers Corporation (Wheeling, IL [312] 398-0110 or [800] 323-7442) Catalogue #324, $83.35 for one case of 12. It is also carried by Fisher Scientific. BRI is willing to recycle the shipping boxes. It is suggested that each center have a stock of 12. This number may change depending on the shipping boxes' durability under repeated 3rd or 4th class mailing conditions.

b. Dry ice; 5 to 10 lbs per shipment, chipped.

c. Overnight carrier; the carrier you choose will depend on local contracts and prices. The carrier must be able to accomodate dry ice packages and guarantee delivery within 24 hours.

4. TO BE PURCHASED BY COORDINATING CENTER

a. Nunc cryotubes; 5 ml, approximately 7200 needed per center, i.e., 24 cases of 300. These cryotubes must all be from the same lot in order to control for any trace calcium contamination. A sufficient number from one lot have been set aside for all centers.

b. Nunc storage boxes; 5 x 5 x 3 inches (the tops fit loosely or 'telescope' to accomodate the longer cryotubes). Nunc inserts; 81 spaces (9 x 9). Boxes and inserts sold as a set, approximately 90 sets needed per center.

September 29, 1986

SOF Clinical Centers Laboratory Procedures

Addenda

1. Blood Collection

Before blood drawing, each participant should be asked what she ate for breakfast (and lunch for P.M. participants). If the dietary instructions were not followed, reschedule the participant for a blood draw at a later date, and urge her to follow the dietary instructions at that time. However, if the participant does not want to or cannot reschedule, delay the blood draw until just before lunch (or until later in the afternoon for PM participants i.e. 2-2:30 P.M.). Keep a list of the ID numbers of participants whose blood was drawn even though they did not follow the dietary instructions. At the first data system update in October or November 1986 we will add a data field for this serum variable.

In special instances, it's all right to use a blood pressure cuff or tourniquet during the blood draw to maintain blood flow for participants who otherwise would not provide a full complement of full red-top tubes.

2. Blood processing

For participants whose centrifuged blood does not yield three cryotubes of serum, it is possible to recentrifuge the blood to obtain additional serum. Detailed procedures for recentrifuging will be provided by Sarah Sanchez of UCSF. If you have any questions about this, please call Sarah at 415-750-2089.

3. Temporary storage and shipping

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 back-up identification system in case the ID numbers on the tube are obliterated after prolonged storage at -180 degrees.)

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

As the filled tubes are placed into the slots formed by the inserts, write the ID number which is on the tube into the corresponding box on the paper grid.

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

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

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.

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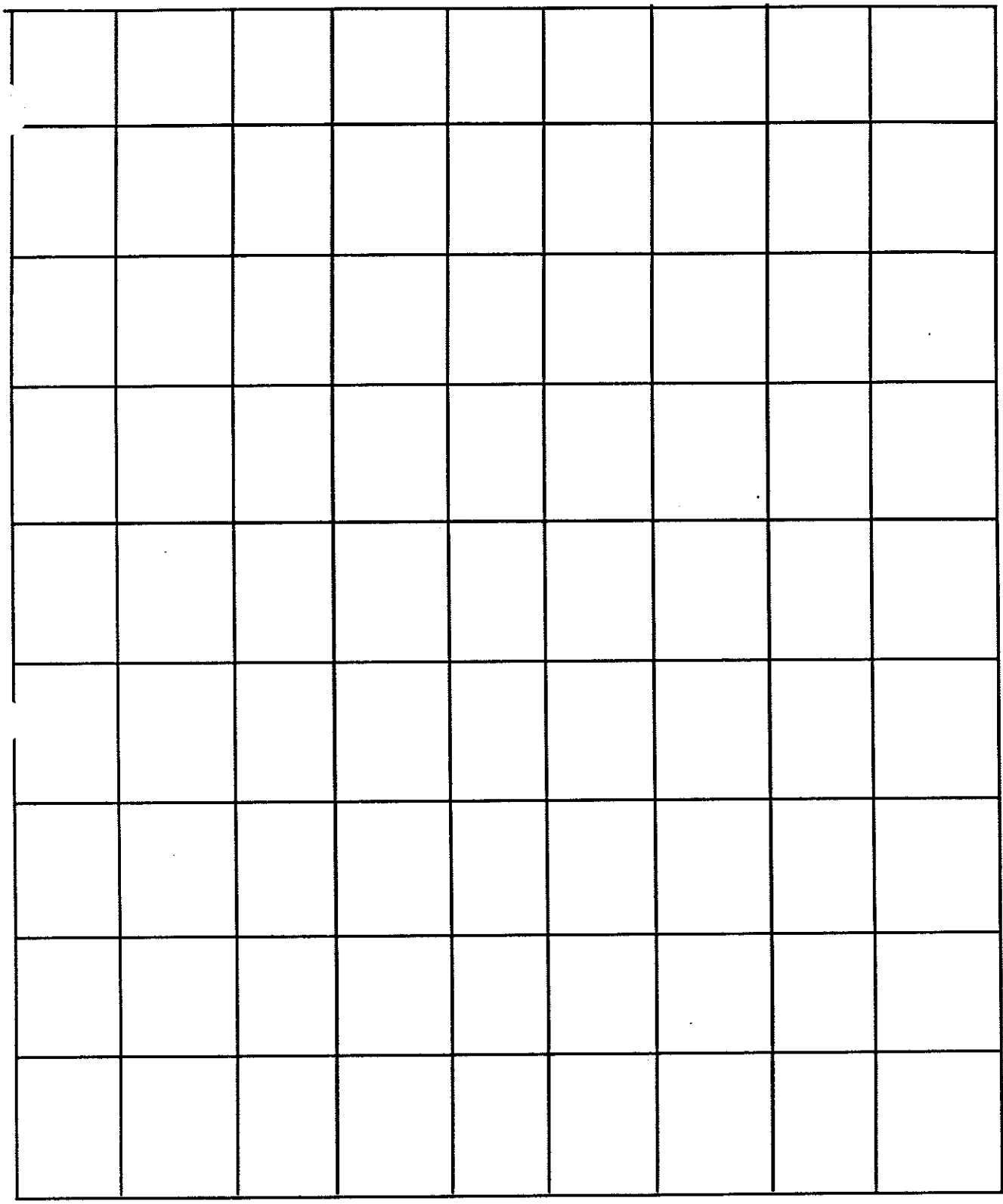

SOF 9/29/86

Box ID Number_____

Collection dates _____ to _____ Clinic _____

Date sent to BRI _____ # of tubes _____