
PROTOCOL FOR COLLECTING WHOLE BLOOD AND BLOTTERS

I. WHOLE BLOOD (for DNA collection)

1. Introduction

This protocol is to be used for **all** participants who have given consent for their blood to be used for DNA analysis at the Visit 8 clinic visit.

The purpose of whole blood samples is to allow us to extract the DNA from the white blood cells for genetic assays.

We will draw **10 ml** of blood if possible.

2. Equipment:

A. For whole blood specimens:

- Gloves, disposable, non-sterile
- Non-self-defrosting freezers, -20°C and -70°C
- dry ice
- 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)
- 10 ml EDTA tubes (lavender-top), 1 per subject (Fisher # 02-683-84)
- 10 ml syringes-one per participant
- 5 ml cryotubes (for specimen storage) – 2 per participant (Fisher # 09-761-74)
- Plastic disposable transfer pipets (with built-in bulbs)
- Cryotube storage boxes (81 cells) (Fisher # 11-678-24B); 81-cell cryotube inserts (Fisher # 13-989-218)
- Sterile normal saline (a liter should last a long time; keep refrigerated)

B. For blood blotters:

- Filter paper: Whatman BFC180 3×3 inch bloodstain cards, 1 per subject
- Drierite: Indicator Mesh #8
- ZipLoc™ plastic bags (food storage size)
- Airtight plastic container for storage of ZipLoc™ bags

3. Procedures:

A. Venipuncture

PUT ON GLOVES

- a) The participant must have been seated for 10 minutes before venipuncture. This standardizes the degree of orthostatic hemoconcentration.
- b) 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.
- c) 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.
- d) Remove tourniquet.
- e) Draw blood using the vacutainer system (one 10 ml lavender-top tube). 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.
- f) Gently turn the lavender-top tubes a few times to allow the anticoagulant to mix with the blood. Place the tube in crushed ice if it is not going to be processed immediately..
- g) 1/2 ml of whole blood will be used for the blood blotter. The remaining whole blood will be separated by pouring whole blood into two 5 ml cryotube(s).
- h) 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.

B. Solutions for Some Common Problems

- If unable to obtain a full 10 ml of blood for the lavender-top tubes, obtain as much as possible and process what you have.

C. Temporary Cryotube Storage

- 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.
- Use the cryotube storage box grid for recording the position of cryotubes, by ID number, within the storage boxes. (This is a back-up identification system in case the ID numbers on the tube are obliterated after prolonged storage at -70°C.) 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.
- 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.
- Store samples at -20°C in the storage box until 81 cryotubes have been filled and frozen. When the storage box is full, move the box to a -70°C freezer for long term storage.

II. WHOLE BLOOD ON BLOTTERS FOR PCR ANALYSIS

1. Procedures:

- a) Label the corner of a 3 in. by 3 in. piece of filter paper with the subjects ID and namecode, using a #2 pencil.
- b) Draw about ½ ml of whole blood from the lavender-top tube with a plastic pipette. If blood has settled, mix thoroughly before pipetting.
- c) Decant the ½ cc of whole blood onto the filter paper. Hold up a corner of the filter paper while decanting so that the blood doesn't soak through to the paper towel. The Whatman bloodstain cards will have four circles intended for use as a reference. However, the blood can simply be dropped into the center of the card, and need not be applied within the circles.
- d) Place the whole blood/filter paper on a paper towel and allow to air dry.
- e) When the blood is completely dry, place the numbered filter paper in a ZipLoc™ plastic bag. The plastic bag should contain 2 tspn Drierite. Store about 50 pieces of filter paper in each plastic bag, clipped (plastic clip) together in ID order. Record the ID numbers contained in each ZipLoc™ bag on a piece of paper taped to the insides of the bag and visible from without.

- f) Store the plastic bags in the airtight plastic container along with 1 Tbsp Drierite for each bag. Store the plastic container in the refrigerator.
- g) Monitor the Drierite in the baggies and the plastic container each week. If the Drierite changes from blue to pink-red in color, replace it with a fresh sample. The Drierite may be recycled by warming it in an oven until it turns blue again.
- h) The specimens will be stored locally at -70°C .
- i) Send a spreadsheet including all ID's to Lily Lui at the Coordinating Center at the end of Visit 8.