PROTOCOL FOR BRI BLOTTERS

1. Introduction

This protocol should be used for all participants who have NOT previously had DNA specimens collected.

We will draw enough blood to get 0.5 ml from each participant.

2. Equipment:

MINNEAPOLIS, PITTSBURGH, PORTLAND

- Gloves, disposable, non-sterile
- 6 ml yellow top vacutainer tubes (Fisher #02-684-29), containing acid citrate dextrose (ACD)-1 per participant
- 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 cc syringes-one per participant
- Plastic disposable transfer pipets (with built-in bulbs)
- Filter paper: Whatman BFC180 3 by 3 inch bloodstain cards, 1 per participant
- Drierite: Indicator Mesh #8
- ZipLoc[™] plastic bags (food storage size)
- Airtight plastic container for storage of ZipLoc[™] bags
- Whatman BFC180 bloodstain card: Can be ordered from June Fitz (Fitzco, Inc.) at 1-800-367-8760, ext. 101

BALTIMORE

- Gloves, disposable, non-sterile
- Unistik 2 disposable fingerstick Lancet device (Fisher #22-032913), 1 per participant
- Microtainer capillary blood collector with EDTA and Microgard closure (Fisher #02-669-33), 1 per participant
- Alcohol prep pad
- Bandage
- Filter paper: Whatman BFC180 3 by 3 inch bloodstain cards, 1 per participant
- Drierite: 5lbs Indicator Mesh #8
- ZipLoc[™] plastic bags (food storage size)
- Airtight plastic container for storage of ZipLoc[™] bags
- Whatman BFC180 bloodstain card: Can be ordered from June Fitz (Fitzco, Inc.) at 1-800-367-8760, ext. 101

3. Procedures:

A. Venipuncture (Minneapolis, Portland, Pittsburgh)

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 yellow-top 6 ml, Fisher #02-684-29). 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 yellow-top tubes a few times to allow the anticoagulant to mix with the blood.
- **g**) Blood in the yellow-top tubes will not clot, but may settle. This is not a problem.

Fingerstick (Baltimore)

PUT ON GLOVES.

- a) Use alcohol pad to wipe participant's middle finger.
- b) Before drawing the blood, a preprinted label showing the participant's ID code should be placed on each capillary blood collector. It is essential to check the ID code on each collector 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) Using the fingerstick Lancet device (Fisher #22-032913) to collect blood sample. Put the device firmly against their fingertip. Push in the yellow cap to set, remoe the cap and then sample. For detailed instructions, use those supplied with the fingerstick device.
- d) Hang hand on the side, gently milk finger but avoid excessive squeezing. Use the FloTop collector on the top of the capillary blood collector (Fisher #02-669-33) to let the blood flow into the tube smoothly. The collector can hold 0.25 to 0.5 cc blood. Fill the tube as much as possible.
- e) When the collection is done, use alcohol pad to wipe participant's middle finger. Apply bandage.

B. Processing the Blotters (ALL CLINICS)

PUT ON GLOVES.

- a) Label the corner of a 3 in. by 3 in. piece of filter paper with the subject's ID and name code, using a #2 pencil.
- b) Draw about ½ cc whole blood from a yellow-top tube with a plastic pipette or pour blood from the capillary tube (Baltimore). 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 tsp 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 inside 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 entire batch of specimens will be shipped to BRI once all Visit 8 participants have been completed. Include a list of all ID's with the specimens.
- i) Send a copy of the list of ID's to Lily Lui at the Coordinating Center.
- **j**) Minneapolis, Pittsburgh and Portland-discard the remaining blood in the yellow-top tube or process for your own purpose.