3. **VENIPUNCTURE**

3.1 **Precautions for Handling Blood Specimens**

Handle all specimens as potentially infectious for laboratory workers. Transmissions of the infectious agents associated with hepatitis and the acquired immunodeficiency syndrome (AIDS) via "needlestick" skin punctures have been documented.

Where feasible, wear disposable plastic gloves when collecting and processing specimens. Alternatively, wash hands thoroughly with disinfectant soap prior to leaving the work area. Cover skin cuts or abrasions.

If the phlebotomist accidentally sustains a contaminated needle stick, clean the wound thoroughly with soap and water and notify the ARIC physician. Store needles in a locked cabinet when the clinic is closed.

Use 0.1% sodium hypochlorite (household bleach) to clean up any spills of blood, plasma, or serum. Use this solution to clean up all laboratory work surfaces at the completion of work activities.

Dispose of all needles and tubing in puncture-resistant containers for safe disposal.

Do not perform any pipetting by mouth; especially of any blood, serum, or plasma.

Avoid formation of potentially infectious aerosols by careful pipetting and centrifugation.

Place all used Vacutainer tubes and blood products in biohazard bags for disposal.

3.2 **Phlebotomy Room**

The blood drawing takes place in an isolated room or participants are separated by room dividers. The room is equipped with all of the necessary blood drawing supplies. A separate counter or work table is equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator and freezer should be nearby.

3.3 **Participant Preparation**

Informed consent must be obtained by the receptionist (see ARIC Manual 2) before drawing blood. This procedure is followed to ensure that the participants understand the purpose of blood drawing and the possible complications of venipuncture. A standard informed consent has been prepared for this study.
With regard to laboratory procedures, the consent statement informs study participants that although there may be some minor discomfort, their blood (2.5 ounces) will be drawn by trained technicians. The consent statement also states that a copy of the test results is sent to their physicians and that they will be contacted if clinically important tests are abnormal, if so desired by the participant.

Continue to fill in The ARIC Venipuncture Form. (see appendix III). The subject is asked whether he/she has a bleeding disorder before the blood is drawn. If such a disorder is present, ask the subject whether he/she has had blood drawn previously and if so, whether he/she had any problems with excessive bleeding or bruising at the venipuncture site. If the participant has a history of venipuncture problems, the participant's blood should be drawn only if approved by a physician. If blood is to be drawn, fill in date and time on the Venipuncture form and whether participant has had the clinic snack.

Standardize blood drawing to the sitting position. It is difficult to standardize the length of time that a person is in the sitting position prior to venipuncture, but to the extent that it is feasible, this should be attempted.

Perform venipuncture with a 21 gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small thin walled needle which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for participants will be the contact with the technicians who draw the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure, e.g., "I am going to be drawing about 2.5 ounces of blood. This blood will be used in tests for lipids and cholesterol and blood clotting factors. We hope to be able to use the results of these tests to predict who might have a greater risk of heart attacks."

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPEHENSIVE ABOUT HAVING BLOOD DRAWN. Do not under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood.

3.4 Venipuncture

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). Use a tourniquet to increase venous filling. This makes the veins more prominent and easier to enter. PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer
than two (2) minutes. To do so may result in hemoconcentration or a variation in blood test values. Instructions for the reapplication of a tourniquet are given on page 16 (Item 6) and page 17 (Item 5). If a tourniquet must be applied for preliminary vein selection, it should be released and reapplied after a wait of two minutes. If the patient has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

1. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

2. Tuck the end of the tourniquet under the last round.

3. If a velcro tourniquet is used, adhere the ends to each other.

Identify vein: Palpate and trace the path of veins several times with the index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, and roll easily. If superficial veins are not readily apparent, have the participant close his fist. Lowering the extremity over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.

Cleanse the venipuncture site.

1. Remove alcohol prep from its sterile package.

2. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.

3. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the patient when the venipuncture is performed.

4. If venipuncture becomes difficult, the vein may need to be touched again with your hand. If this happens, the site is cleansed again with alcohol.

Assemble the butterfly-Vacutainer set.

1. Attach the Luer adaptor to the Vacutainer holder.

2. Attach the Luer end of the butterfly needle set to the Luer adaptor.

3. Place the #1 red and gray stoppered tube in the Vacutainer holder being careful not to break the vacuum.

Perform venipuncture.

1. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches (2.5 or 5.0 cm) below the venipuncture site.

2. With the needle bevel upward, enter the vein in a smooth continuous motion.
3. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.

4. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

5. Start a timer to measure the flow rate of blood into the first blood collection tube. This time is entered on the Venipuncture form. If the flow rate in the tube is so slow that blood does not fill the first collection tube within 36 seconds, stop the blood collection and repeat on the other arm. If blood is flowing freely, the butterfly needle may be taped to the participant's arm for the duration of the draw.

6. Remove the tourniquet after tube 1 fills. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. During the procedure, do not allow the contents of the tube to contact the stopper. Do not reapply tourniquet for tubes 2-5. A tourniquet may be reapplied during tubes 6-8 to spare the participant a restick, but the tourniquet must not be on for more than 2 minutes. When the tourniquet is reapplied, this is noted on the Venipuncture form and the Incident Log must be filled out.

7. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.

8. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the Vacutainer and attach another without removing needle from vein.

9. When the blood flow ceases, remove the tube from the holder. The shutoff valve recovers the point, stopping blood flow until the next tube is inserted (if necessary).

If a blood sample is not forthcoming, the following manipulations may be helpful.

1. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
4. The same technician should not attempt a venipuncture more than twice. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box. Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

5. If blood flow stops before collecting tube 5, restick the participant beginning with tube 1. Discard all tubes from the previous attempt. If blood flow stops after tube 5, restick the participant, but collect only the unfilled tubes from the previous attempt. A tourniquet may be applied in this case but should be released if possible as soon as blood flows into the first EDTA tube. As always, the tourniquet must never be on for longer than two minutes.

Bandaging the arm.
1. Under normal conditions:
   a. Slip the gauze pad down over the site, continuing mild pressure.
   b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

2. If the participant continues to bleed:
   a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
   b. Wrap a gauze bandage tightly around the arm over the pad.
   c. Tell the participant to leave the bandage on for at least 15 minutes.

PRECAUTIONS - WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAWING.
1. Have the person remain in the chair, if necessary have him/her sit with head between knees.
2. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds.
3. Provide the person with a basin if he/she feels nauseous.
4. Have the person stay seated until the color returns and he/she feels better.
5. Place a cold wet cloth on the back of the person's neck.
6. If the person faints, use smelling salt to revive.
7. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member, who will advise you on further action.

3.5 Blood Mixing During Venipuncture

To invert tubes, hold the tube horizontal to the floor. Slowly tip the stopper end down while watching the air bubble rise to the butt. (1st inversion) When the bubble reaches the butt, the tube should be at approximately a 22 degree angle to the floor with the center of the tube at the fulcrum. Now, lower the butt end while watching the bubble float to the stopper. Again, the tube should be at a 22 degree angle to the floor with the center of the tube at the fulcrum. (2nd inversion) Lower the stopper end again when the bubble reaches the stopper. This is the third inversion. Invert each tube eight times. Eight inversions should take 6-13 seconds.

Draw tube #1 (9.5 ml red and gray top). Gently invert 8 times. Place the tube in a rack at room temperature. Note and record the amount of time it takes for the tube to fill with blood. If this is greater than 36 seconds, the blood flow is not adequate and the venipuncture must be repeated. Remove tourniquet.

Draw Tube #2 (7 ml red top). Gently invert 8 times then immediately replace in ice bath.

Draw Tube #3 (4.5 ml blue top). Invert 8 times then place in ice bath.

Draw Tube #4 (4.5 ml blue top). Invert 8 times then place in ice bath.

Draw Tube #5 (4.5 ml blue top). Invert 8 times then place in ice bath.

Draw Tube #6 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #7 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #8 (3.5 ml lavender top). Invert 8 times then replace in rack at room temperature.

Finish venipuncture.
Figure 2. Blood Sample Storage Tray

W = White vial with white screw cap
Y = Yellow micro sample tube
R = White vial with red screw cap
B = Blue micro sample tube
L = White vial with lavendar screw cap
Br = White vial with brown screw cap
Syr = Syringe
Fil = Millipore Filter