Atherosclerosis Risk in Communities Study Protocol

Manual 7

Blood Collection and Processing

Visit 3

Version 3.0

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FOREWORD

This manual, entitled Blood Collectiona and Processing, is one of a series of protocols and manuals of operation for the Atherosclerosis Risk in Communities (ARIC) Study. The complexity of the ARIC Study requires that a sizeable number of procedures be described, thus this rather extensive list of materials has been organized into the set of manuals listed below. Manual 1 provides the background, organization, and general objectives of the ARIC Study. Manuals 2 and 3 describe the operation of the Cohort and Surveillance Components of the study. Detailed Manuals of Operation for specific procedures, including those of reading centers and central laboratories, make up Manuals 4 through 11, 13 and 14. Manual 12 on Quality Assurance contains a general description of the study's approach to quality assurance as well as the details for quality assurance for the different study procedures.

ARIC Study Protocols and Manuals of Operation

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1	General Description and Study Management
2	Cohort Component Procedures
3	Surveillance Component Procedures
4	Pulmonary Function Assessment - (Retired)
5	Electrocardiography
6	Ultrasound Assessment a. Ultrasound Scanning b. Ultrasound B-mode Image Reading Protocol c. Distensibility Scanning Protocol - (Retired) d. Distensibility Reading Protocol - (Retired)
7	Blood Collection and Processing
8	Lipid and Lipoprotein Determinations
9	Hemostasis Determinations
10	Clinical Chemistry Determinations - (Retired)
11	Sitting Blood Pressure
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1. PURPOSE

The Atherosclerosis Risk in Communities (ARIC) study is a multidisciplinary study designed to measure risk factors for atherosclerosis and heart disease. It is a prospective study which sampled a large, randomly selected population and then will follow it for an extended period of time.

Nationally there are four field centers involved. Each of these field centers collects blood and processes it for shipment to three central laboratories. Each central laboratory performs specialized tests on the blood samples. The central laboratories include: a Central Lipid Laboratory at Baylor College of Medicine in Houston and a Central Hemostasis Laboratory at the University of Texas Medical School in Houston.

The Central Lipid Laboratory evaluates the lipid profiles of the participant including general tests for lipid content and glucose as well as other more specialized lipoprotein profiles. The Central Hemostasis Laboratory evaluates various blood coagulation factors including tests for platelet activation and natural inhibitors of blood clotting as well as more general tests of the hemostatic system. The hemostatic evaluation is made only on a selected subset of ARIC participants, though blood is collected and stored at the Hemostasis Laboratory for all ARIC participants. In addition to the central laboratories, each of the field centers has its own hematology laboratory which evaluates hematological parameters.

The foundation on which all of these tests is based is the blood samples that are collected and processed by the technicians at each of the field centers. Probably the most important step (and potentially the most variable) is the collection and field center processing of the blood samples. Laboratory tests can be repeated, but if the blood sample itself is not correctly drawn and processed, the laboratory results may be precise but may not be valid. In a study such as ARIC which may involve more than 40,000 samples over an extended period of at least 6 years, even a small amount of variability can have a statistically important effect. It is important that the study measure true differences between participants rather than (systematic) differences in blood drawing procedures. The ARIC Study depends on the field center technicians who perform the blood drawing and sample processing. It is important that these people be not only well trained and competent at drawing and processing the blood, but also willing to take pride and responsibility in their work.

2. PREPARATION

2.1 Participant Contact

Since the study depends on the voluntary return of participants over an extended period of time, every effort must be made to make the entire procedure as easy and painless as possible for the participants. The technicians must remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is for the technicians to be thoroughly knowledgeable about all aspects of the procedures. The ARIC study involves the collection at Visit 2 of approximately 53 ml of blood from each participant. A total of 8 tubes of blood of various sizes is collected. The smallest tubes contain less than a teaspoon (3.5 ml), while the largest tube contains slightly over 2 teaspoons (9.5 ml) of blood. Any participant who is concerned about the volume of blood should be reassured that the total amount of blood drawn is less than 2.5 ounces although it may look like more. The technician may also assure participants that they donate 9 times as much blood (450 ml) when they donate a pint of blood.

The technicians and the clerk should be properly attired in a white lab coat.

2.2 Staff Certification Requirements

Ideally the blood drawing and processing are performed by two certified ARIC technicians. The technicians complete a training course taught by certified laboratory staff. Each technician must complete the training and pass both written and practical exams before becoming ARIC certified. Recertification takes place annually and is authorized by the Hemostasis Laboratory.

2.3 Blood Collecting Trays and Tubes

Prior to venipuncture prepare two trays for each participant. One tray holds the Vacutainer tubes used in the blood collection. The other tray holds the various colored plastic tubes which contain the final whole blood, serum and plasma aliquots which are to be frozen and sent to the central laboratories for analysis. Both of these sets of tubes should be prelabeled with the appropriate code numbers for the participant. A list of equipment, suppliers, and vendors is provided in Appendix II.

2.3.1 Blood Collection Tray

First, the technicians organize and prepare the blood collection tray. The tray itself should be made of hard plastic which is unbreakable and can be easily cleaned. The tray has individual compartments which are filled with the following supplies as illustrated in Figure 1 Sample Tray.

- A test tube rack to hold the seven blood collection tubes which are drawn from each participant. One tube is kept in a separate rack on ice. These tubes are described in detail in the next section.
- Sterile, disposable 21 gauge butterfly needles.
- A plastic Vacutainer tube guide.

- Vacutainer Luer adapters.
- Sterile alcohol swabs.
- Gauze sponges.
- A tourniquet.
- Bandages ("Band Aids").
- An ice water bath filled with ice and water approximately 10 minutes before blood drawing.
- A stopwatch.

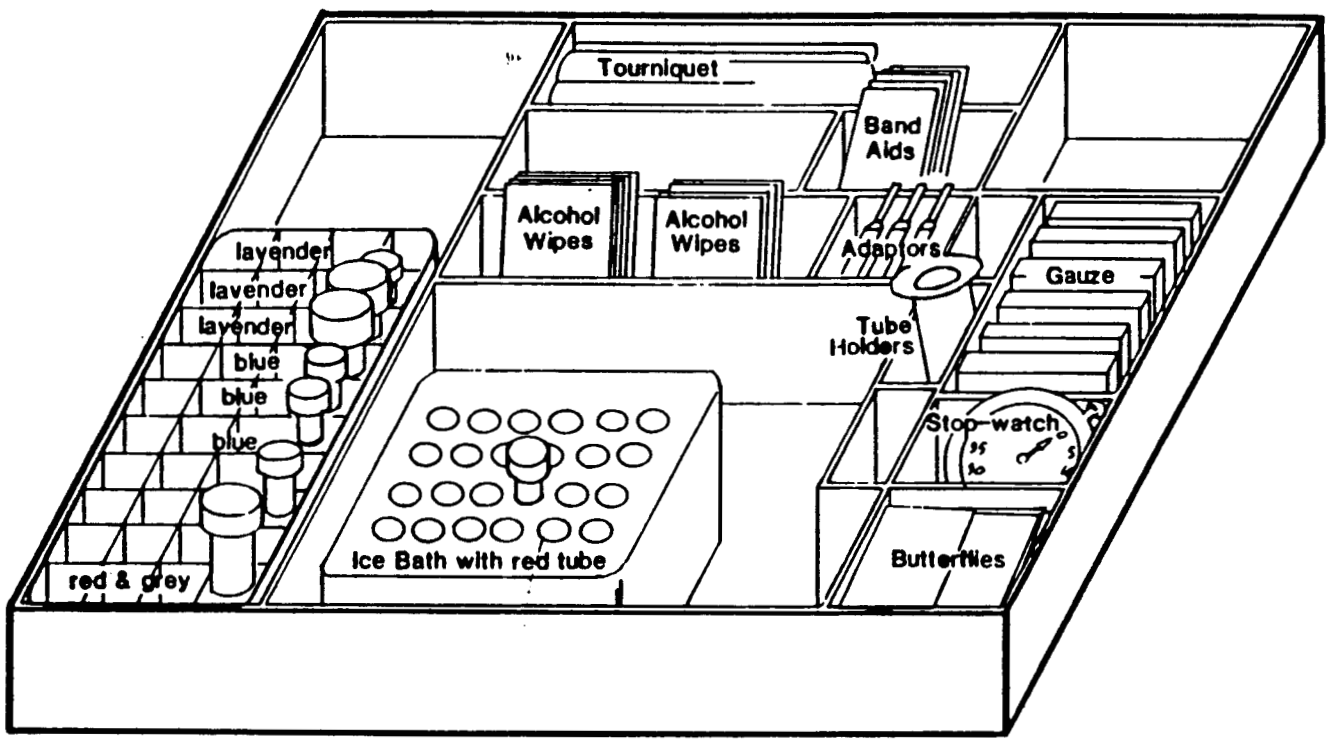
2.3.2 Blood Collection Tubes

About 53 ml of blood are drawn from each participant using eight Vacutainer tubes. Samples from these eight blood collection tubes are used in approximately 40 different biochemical and hematological assays. It is important that the technicians know more than just the arrangement of the blood collection tubes and the sequence of tube collection. They should also be familiar with the purpose of each tube, the type of anticoagulant in each tube and possible sources of error in the handling of each tube. These tubes are organized in the test rack in the following sequence.

Tube #1 is a 9.5 ml red and gray stoppered tube filled with 9.5 ml of blood. This tube does not contain anticoagulant. After drawing, the blood clots at room temperature for 30 minutes. After approximately 30 minutes, the tube is centrifuged and the serum is removed, frozen and stored for weekly shipment to the Central Hemostasis Laboratory in Houston.

Tube #2 is a 7 ml siliconized red stoppered tube injected with 0.7 ml of special anticoagulant by the field center technicians. The special anticoagulant mixture is provided by the Central Hemostasis Laboratory on a periodic basis. The mixture is kept at 4°C at all items. Tube #2 is filled with 6.3 ml of blood. The plasma from this tube is sent to the Central Hemostasis Laboratory for measurement of unstimulated levels of platelet and coagulation factors which may be present in the circulating blood. Since the plasma in this tube is used to measure unstimulated levels of these factors, it is extremely important that any potential in vitro activation be avoided. Less than flawless venipuncture can stimulate both the platelets and coagulation systems and even a small amount of activation can cause a large false positive elevation in these factors. For the same reasons, this tube is also chilled in an ice water bath for at least 10 minutes prior to sample collection, then immediately mixed after collection and returned to the ice bath. After centrifuging, the sample is filtered to remove any remaining platelets. Until the plasma is filtered, the remaining platelets can still cause a false elevation of some of the factors. The technicians should be aware of all of these potential pitfalls in the collection and processing of this tube.

Figure 1. Blood Sample Collection Tray



Tubes #3, #4 and #5 are 4.5 ml blue stoppered tubes contain 0.5 ml of 3.8% sodium citrate anticoagulant. Each tube is filled with 4.5 ml of blood. The plasma from these tubes is sent to the Central Hemostasis Laboratory for assay of some of the more generally measured coagulation factors and inhibitors. Since some of these factors are unstable, it is important that these tubes be kept cold in a refrigerated centrifuge until they are aliquotted into their respective tubes. Of all of the tubes which are centrifuged during Stage I, these tubes are aliquotted last. The rack containing these aliquots is transferred to the refrigerator immediately after plasma is aliquotted.

Tubes #6 and #7 are 10 ml lavender stoppered tubes containing the anticoagulant, EDTA. After each tube fills, invert it 8 times and place it in the ice water bath until the last tube is collected. Glucose determinations will be performed on the plasma from these tubes. Therefore, it is necessary to remove the plasma from the red cells in 30 minutes or less to reduce the possibility of falsely decreased levels of glucose. Tubes are then placed in the centrifuge for a 10 minute spin. The plasma from these tubes is sent to the Central Lipid Laboratory at Baylor College of Medicine in Houston.

Tube #8 is a 5.0 ml lavender stopped tube containing the anticoagulant EDTA. The tube remains at room temperature until the end of the draw. A hematology requisition is then completed, attached to the tube and the sample is stored in the refrigerator until being sent to the local hematology lab for each field center. This tube is used to assay for a standardized set of hematology tests.

2.4 Blood Collection Tubes: Labeling and Set-up

Eight tubes are drawn in the following sequence:

Tube #1:	9.5 ml red and gray stoppered tube
Tube #2:	7 ml siliconized red stoppered tube
Tube #3:	4.5 ml blue stoppered tube
Tube #4:	4.5 ml blue stoppered tube
Tube #5:	4.5 ml blue stoppered tube
Tube #6:	10 ml lavender stoppered tube
Tube #7:	10 ml lavender stoppered tube
Tube #8:	5.0 ml lavender stoppered tube

Strips of pre-numbered adhesive labels for each Vacutainer tube, each plastic microsample storage tube, the specimen bags and packing list are attached to the initial data collection forms. Apply labels to the blood collection tubes for each participant prior to blood collection. Write "6" on tube #6 so it won't be confused with tube #7. Arrange the set of tubes in a test tube rack. Check the identifying information on the form and label to make sure that the specimen belongs to the participant identified on the labels. The labeling of tubes for aliquots of specimens to be sent to the central laboratories can be done by a clerk working side by side with the venipuncture technician. The chance of mislabeling is minimized when only one person's specimens are handled at a time.

A number of ARIC participants are selected to donate duplicate samples for analysis. Duplicate samples are assigned their own ID number and shipped to the designated central laboratory one week later. This is described more completely in the Quality Control Section.

2.5 Sample Aliquot Tubes: Labeling and Set-up

The technician prepares a tray of the plastic freezer tubes which contains the final samples to be shipped to the central labs for each participant. Each type of sample tube or preparation has a corresponding color coded freezer tube. The technicians should be trained to organize the tray for the sample processing and aliquotting as follows:

2.5.1 Sample Tray

The tray itself should be a flexible sponge test tube rack which will fit tubes from 10-16 mm in diameter (see Figure 2). The tray has 5 rows and 10 columns. The columns are numbered 1-10 from left to right. The rows are lettered A-E from top to bottom.

2.5.2 Organization

The technicians need the following supplies for each sample tray:

6 - 1.5 ml yellow polypropylene microsample tubes
8 - white screw caps for 2 ml vials
9 - 1.5 ml blue polypropylene microsample tubes
20 - 2 ml white polypropylene screw top vials
10 - lavender screw caps for 2 ml vials
2 - brown screw caps for 2 ml vials
1 - Millipore Millex HA-4 filter
1 - 5 ml syringe
6 - plastic transfer pipettes

The colored plastic sample aliquot tubes are labeled with the participant ID number and arranged in the sample tray in the following order:

Col 1: white screw top vials in wells A-C
Col 2: white screw top vials in wells A-B
Col 3: yellow sample tubes in wells A-E.
Col 4: yellow sample tube in well A, a Millipore Filter in well C and the 5 ml syringe in well E.
Col 5: Blue sample tubes in wells A-C.
Col 6: Blue sample tubes in wells A-C.
Col 7: Blue sample tubes in wells A-C.
Col 8: 5 white screw top vials in wells A-E.
Col 9: 5 white screw top vials in wells A-E.
Col 10: 5 white screw top vials in wells A-E.

2.6 Preparation for Specimen Collection

Prepare for specimen collection in the following manner. Early morning, prior to drawing blood from the participants:

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.

2. Check that each Vacutainer tube is properly labeled with the appropriate participant number.

3. Check that the sample processing tray is properly equipped. Every item on the checklist must be ready and in its proper position.

4. Check that each sample aliquot tube is labeled with the appropriate participant identification number.

5. Perform quality control (Q.C.) check on centrifuge temperature (4°C ± 2°C).

6. Perform Q.C. check on refrigerator temperature (4°C ± 2°C).

7. Perform Q.C. check on freezer temperature (-70°C ± 10°C).

Approximately 10 minutes before scheduled participant arrival:

1. Fill ice bath 3/4 full with crushed ice.

2. Fill ice bath with cold water.

3. Place Tube #2 (7 ml red top) in ice bath to chill.

At participant arrival:

1. Check that the ID number on the tubes matches the participant ID.

2. Check that duplicate Quality Control tubes are prepared and labeled if needed.

2.7 Venipuncture Form

ID Number, contact year, and name should be entered on the Venipuncture form prior to the arrival of the participant.

3. VENIPUNCTURE

3.1 Precautions for Handling Blood Specimens and Guidelines for OSHA Bloodborne Pathogens Standards

NOTE: Please see Appendex XII (A-25) for specific guidelines and recommendations.

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 APPREHENSIVE 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.

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.

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

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

6. 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.

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.

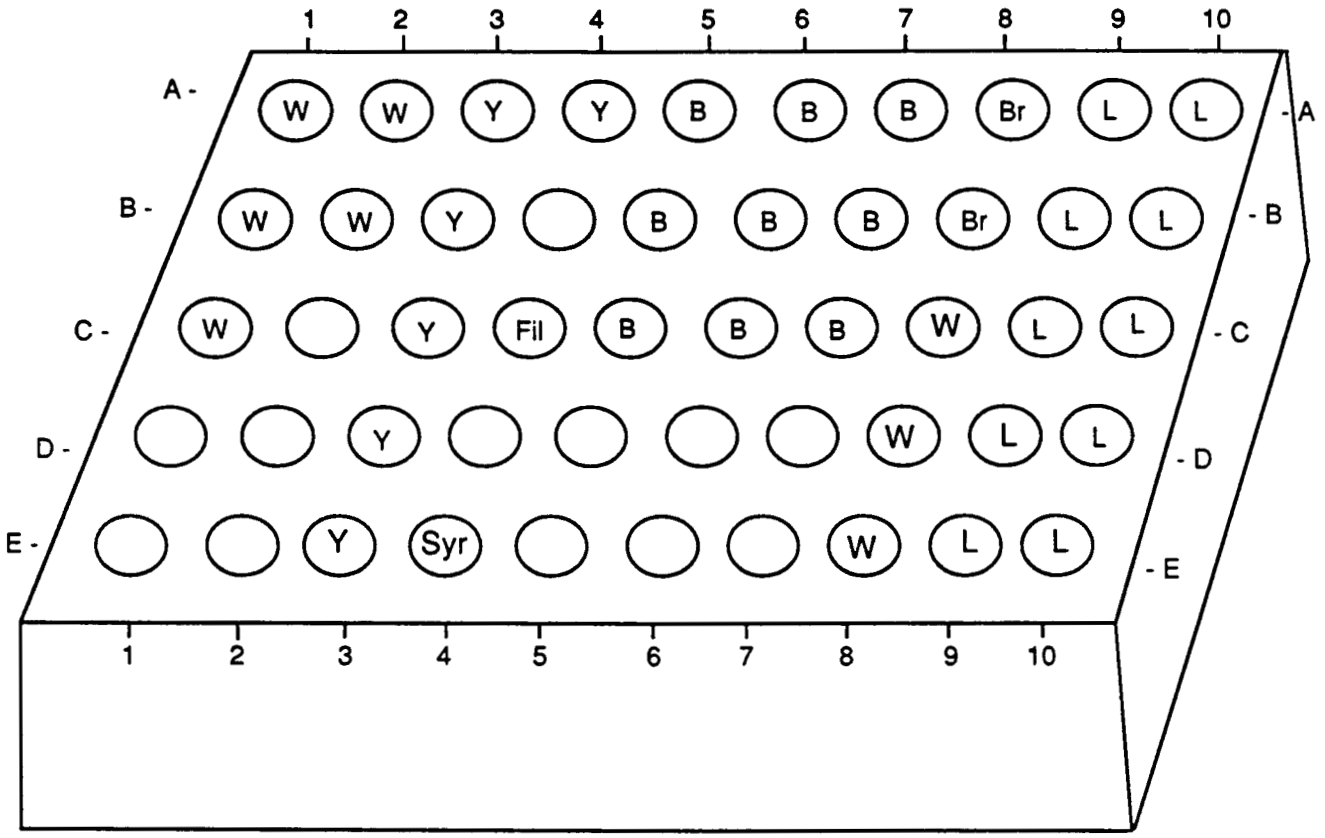
Draw Tube #6 (10 ml lavender top). Invert 8 times then place in ice bath.

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

Draw Tube #8 (5.0 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
B = Blue micro sample tube
L = White vial with lavendar screw cap
Br = White vial with brown screw cap
Syr = Syringe
Fil = Millipore Filter

4. BLOOD PROCESSING

Processing of the various blood samples is divided into 3 stages. Attention should be paid to the condition at which the sample tubes are kept prior to centrifuging and aliquotting. See Figures 3-5.

4.1. Stage One: Immediate Processing

At the conclusion of venipuncture, tube #1 and tube #8 are incubating at room temperature. Tubes #2, #3, #4, #5, #6, and #7 are in the ice water bath.

Remove tubes #2, #3, #4, #5, #6, and #7 from the ice bath and place them in the centrifuge cups. Balance the centrifuge, then, centrifuge tubes #2, #3, #4, #5, #6, and #7 at 3,000 x g for 10 minutes at 4C. Record on the Venipuncture Form the time at which these tubes began to spin.

Tube #1 remains incubating at room temperature. Store tube #8 in the refrigerator till shipment to the local field center hematology laboratory. Wait for centrifuge to come to a complete stop. Proceed to stage 2 processing.

4.2 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. Centrifuge revolutions per minute may vary from center to center depending on rotating radius of the centrifuge.

4.3 Stage Two:

Approximately 10 minutes after venipuncture.

4.3.1 Lavender Stoppered Tubes (Tubes #6 and #7)

1. Remove the sponge rack from the refrigerator. Remove lavender stoppered tubes (#6 and #7) from centrifuge. Allow red top (#2) and blue top tubes (#3, #4 and #5) to remain temporarily in the refrigerated centrifuge. Alternatively, remove all tubes from the centrifuge and place them in the ice bath.

2. Put tubes #6 and #7 in wells 8D and 8E of the sample preparation tray which contains sample tubes labeled with the corresponding participant number. Remove the stoppers.

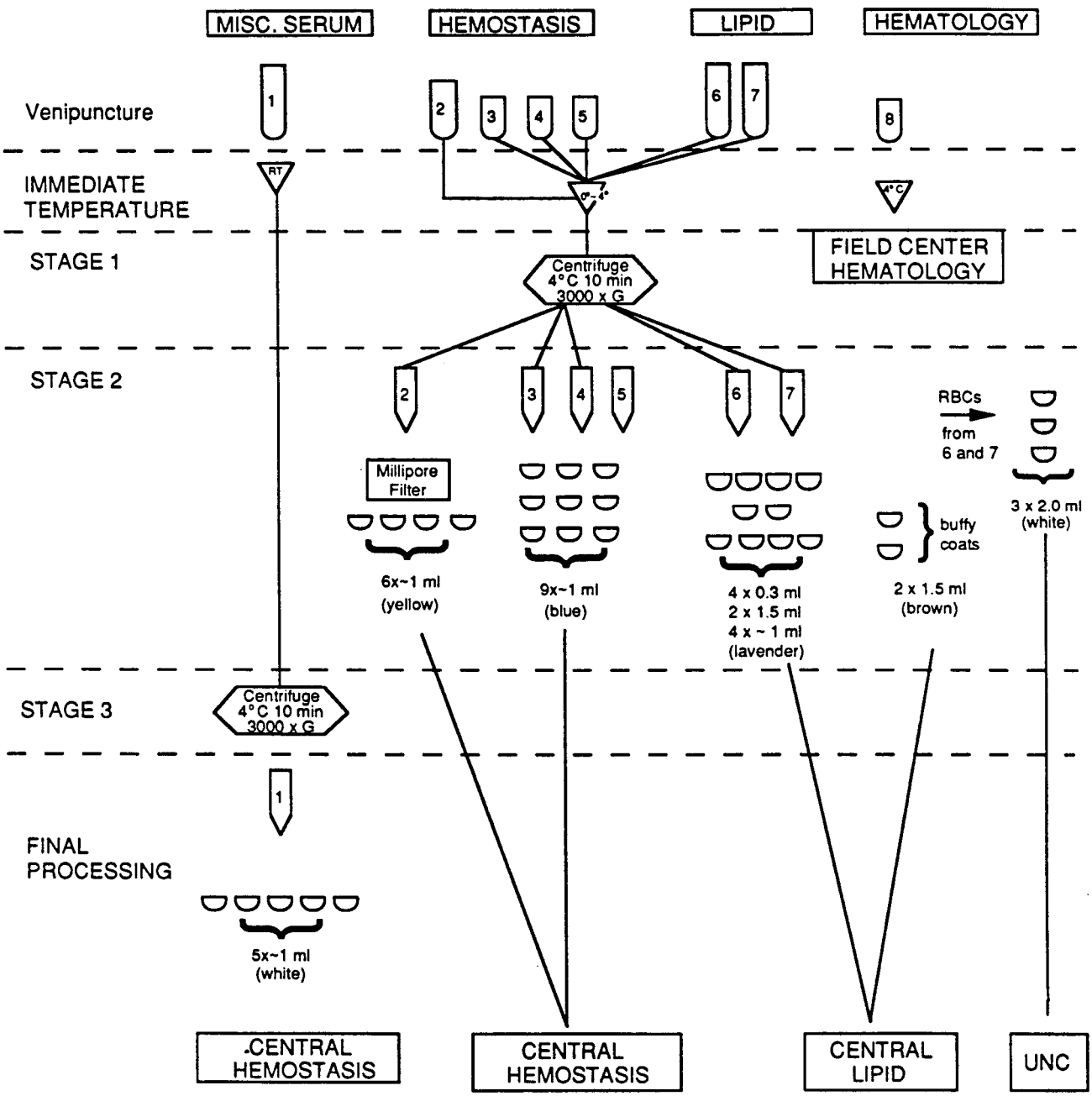
Figure 3.

SAMPLE PROCESSING FLOW SHEET

Venipuncture time 0:00 ▼ 0:00 - 0:15 ▼ 0:10 - 0:25 ▼ 0:30-0:45 ▼ 0:40-0:55 ▼ 0:45-0:60 ▼ <1:30 ▼

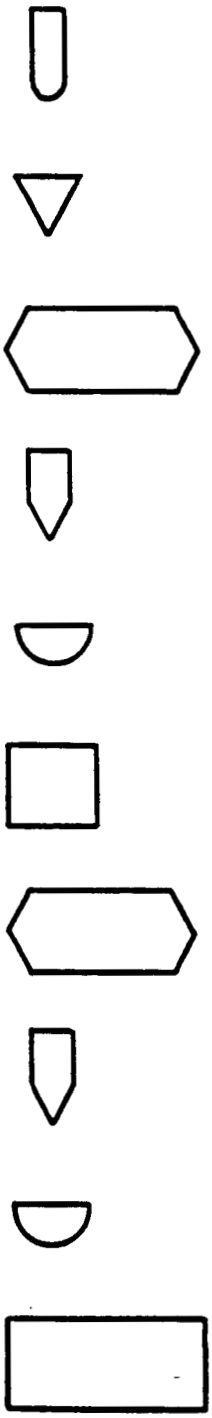
	Temperature after Venipuncture	Stage I	Stage II	Stage III	Draw Next Donors	Final Processing	Freeze	Packaging	Destination
Tube 1 Red and Gray 9.5 ml room temperature	Room temperature	Incubate at room temperature 30 minutes		Centrifuge 10 minutes at 4C 3000 xg		Aliquot serum into 5 white vials seal with white screw caps	5 white cap vials	5 white cap vials 1 red cap vial in 3" x 6" bag	Central Hemostasis
Tube 2 Red top 7 ml ice bath	Ice bath	Centrifuge 10 minutes at 4 C 3000 xg	Filter plasma into 6 yellow micro sample tubes	Refrigerate			6 yellow micro sample tubes	6 yellow tubes 9 blue tubes in 3" x 6" bag and 5 white capped vials in 2 3" x 6" bags	Central Hemostasis Laboratory
Tube 3, 4 & 5 Blue tops 4.5 ml room temperature	Ice bath	Centrifuge 10 minutes at 4 C 3000 xg	Aliquot plasma into 9 blue micro sample tubes	Refrigerate			9 Blue micro sample tubes		
Tube 6 & 7 Lavendar tops 10 ml room temperature	Ice bath		Aliquot plasma into 10 white vials seal with lavendar screw caps. Transfer buffy coats into 2 white vials seal with brown srew caps.	Refrigerate			10 lavender cap vials 2 brown cap vials 3 white cap vials	10 lavender cap vials 2 brown cap vials in 3" x 6" bag	Central Lipid Laboratory
Tube 8 Lavender top 3 ml room temperature	Room temperature	Refrigerate							Field Center Hematology Laboratory

Figure 4. Blood Sample Processing Sequence



Legend to Figure 4

Sample Processing Sequence



3. Using the plastic transfer pipet, and being careful not to disturb the red or white cell layers, remove the clear plasma supernatant. Inspect for hemolysis, then transfer plasma from one lavender top tube into the two sample tubes in wells 9E and 10E with approximately 1.5 ml of plasma. Using the same plastic pipet, transfer any remaining plasma from the lavender top tubes 6 and 7 equally into the four white screw top vials in wells 10A-10D. Using the automatic pipet, transfer 0.3 ml of plasma from the tube in well 10A to the tube in well 9A. Then using the same pipet tip, transfer 0.3 ml of plasma from the tube in well 10B to the tube in well 9B. Transfer 0.3 ml of plasma from the tube in 10C to the tube in 9C and transfer 0.3 ml plasma from the tube in 10D to the tube in 9D. To allow for expansion during freezing, do not fill any vials more than 3/4 full.

4. Fasten the lavender screw caps onto the white screw top vials in columns 9 and 10 and allow them to remain in the sponge rack.

5. Using a plastic transfer pipet, gently remove the white cell layer from each of the lavender blood collection tubes and transfer approximately 1.5 ml of cells from each tube into the white screw top vials in wells 8A and 8B.

6. Fasten the brown screw caps on the white screw top vials in wells 8A and 8B and allow them to remain in the sponge rack.

7. Use the plastic transfer pipet, that was used in transferring the buffy coat, to equally divide the remaining red blood cells into three 2 ml. white vials in wells 8C, 8D, and 8E. Fasten white screw tops onto the vials, and allow them to remain in the sponge rack. (See Appendix XI.)

8. Restopper the sample collection tubes 6 and 7 and discard them in a biohazard waste bag.

4.3.2 Red Stopper Tube (Tube #2)

1. Remove the red stoppered tube from the 4°C holding location. Place it in well 4B in front of the yellow sample aliquot tubes.

2. Pull out the plunger of the 5 ml syringe which is in well 4E.

3. Attach the male Luer connector of the syringe barrel to the female Luer connector of the Millipore HA-4 filter in well 4C.

4. Remove the stopper from the red top tube. Using a plastic transfer pipet, remove all of the plasma and transfer it to the syringe barrel which is attached to the Millipore filter. Be careful not to disturb the underlying buffy coat or red cells.

5. Position the lower part of the Millipore filter over the first yellow sample tube (well 3A).

6. Replace the plunger of the syringe. Filtered plasma will start to flow from the bottom.

7. Slowly depress the plunger of the syringe to continue filtering the plasma directly into the yellow sample aliquot tubes.

8. Divide the plasma evenly between all 6 yellow aliquot tubes.

9. Fasten the caps of the yellow aliquot tubes and allow them to remain in the sponge rack.

10. Restopper the red stopper blood collection tube and discard it into a biohazard waste bag.

4.3.3 Blue Stoppered Tubes (Tubes #3, #4 and #5)

1. Remove the 3 blue stoppered tubes from the refrigerated centrifuge. Place the tubes in wells 5E, 6E and 7E in front of the blue sample aliquot tubes. Remove the stoppers.

2. Using a plastic transfer pipet, transfer the plasma from one of the blue top tubes, in approximately equal aliquots, into each of the three blue sample aliquot tubes placed in the wells behind it. Repeat this process with plasma from the other blue top tubes.

3. Fasten the caps on the sample aliquot tubes and replace them in the sponge sample tray.

4. Replace the stoppers on the blue top blood collection tubes 3, 4, 5 and discard them in a biohazard bag. Place the entire sponge sample tray with all of the aliquot tubes into the 4°C refrigerator. Proceed to Stage 3 processing.

4.4 Stage Three

Stage three begins approximately 30 minutes after venipuncture.

As soon as possible after the 30 minutes timer goes off, (not longer than 45 minutes after blood collection), spin tube #1 at 3,000 x g for 10 minutes. Record time of beginning to spin on the Venipuncture form.

4.5 Final Processing

When the centrifuge has come to a complete stop, remove the sponge sample rack from the refrigerator.

4.5.1 Red and Gray Stoppered Tube (Tube #1)

1. Remove the red and gray top from the centrifuge and place it in well 1E of the sponge test tube rack in front of the three white sample tubes.

2. Remove the stopper from the tube. Use a plastic transfer pipet, aliquot the serum equally into the five white tubes in wells 1A-1C and wells 2A and 2B.

3. Fasten white screw caps on each of the vials in wells 1A-1C and wells 2A-2B.

4. Replace the stopper on the red and gray stoppered blood collection tube and discard it in a biohazard waste bag.

4.6 Freezing

When all of the blood collection tubes have been aliquotted into their respective microsample tubes and the microsample tubes have been replaced in the sponge rack, the entire rack is placed upright in the -70°C freezer for a minimum of 30 minutes. Samples must be placed into the freezer within 90 minutes from venipuncture time. Samples must be thoroughly frozen before packaging them for storage and shipping. Record the time that the samples are placed in the freezer on the Venipuncture form.

5. STORAGE AND SHIPPING

5.1. Packaging

Each participant's blood samples are packaged in freezer storage bags corresponding to the final destination of the tubes.

1. Label four 3" x 6" storage bags with the appropriate participant number.

2. Remove the sponge sample tray with the corresponding participant specimens from the -70°C freezer. Package quickly after this point to avoid thawing of the specimens.

5.1.1 Central Lipid Laboratory

Place the ten white screw top vials with lavender screw caps and the two white vials with brown screw caps into a prelabeled 3" x 6" storage bag. Again verify that tubes and bag are numbered correctly. Press the air out of the bag and seal. Place the bag in the Central Lipid Laboratory styrofoam box in the -70°C freezer and follow the storage directions described in Section 5.2.

5.1.2 Central Hemostasis Laboratory

Place the nine blue sample tubes and the six yellow sample tubes in one prelabeled 3"x 6" bag and the 5 white capped vials into a second prelabeled 3"x 6" storage bag. Press the air out of the bags and seal. Place the bag in the Central Hemostasis Laboratory styrofoam box in the -70°C freezer and follow the storage directions in section 5.2.

5.1.3 University of North Carolina

Place the three white screw top vials from the sponge (8C, 8D, 8E) into a prelabelled 3" x 6" storage bag. Verify that the tubes and bag are labelled correctly. Press the air out of the bag and seal. Place the bag in the University of North Carolina (UNC) styrofoam box in the -70°C freezer and follow the storage directions in Section 5.2.

5.2 Storage

Two boxes are placed in the -70°C freezer for temporary storage prior to their shipment to the three central laboratories. These boxes are labeled LIPID and HEMOSTASIS respectively.

The 3" x 6" bag containing the 10 lavender and 2 brown screw cap tubes is placed in the LIPID box.

The 3" x 6" bag containing the three white capped vials is placed in the UNC box.

The 2 3"x 6" bags containing the remaining eighteen tubes are placed in the HEMOSTASIS box.

All bags remain in their boxes until shipment to their respective labs.

5.3 Shipping

The samples remain in their styrofoam boxes at -70°C until they are shipped. All frozen sera collected and stored within the last work week are shipped to their respective clinical laboratories on Monday with the exception of Quality Control sera, as discussed in the Quality Control section, by overnight courier. Samples can be shipped on Tuesday if the field center is closed on Monday, but the contact person at each central laboratory must be notified that the shipment will arrive one day later than usual. There is no minimum shipping requirement; frozen samples are shipped weekly regardless of the number of specimens that have been frozen and stored within the last collection period.

5.3.1 Packaging Instructions

The bags of frozen serum samples for hemostasis and lipid labs, and the University of North Carolina, are packed and shipped in styrofoam boxes. See Figure 6. Packaging instructions are as follows:

1. Place a 3" layer (approximately 3 lbs.) of dry ice on the bottom of the styrofoam box.

2. Put half of the bags of sample tubes into a 1 gallon zip lock bag and seal. Place this bag in the styrofoam box on top of the dry ice.

3. Layer another 3 lbs. of dry ice on top of and around the sample bags.

4. Put the remaining sample bags into a second 1 gallon zip lock bag, seal, and place this bag on top of the dry ice.

5. Layer another 3 lbs. of dry ice on top of and around the sample bags.

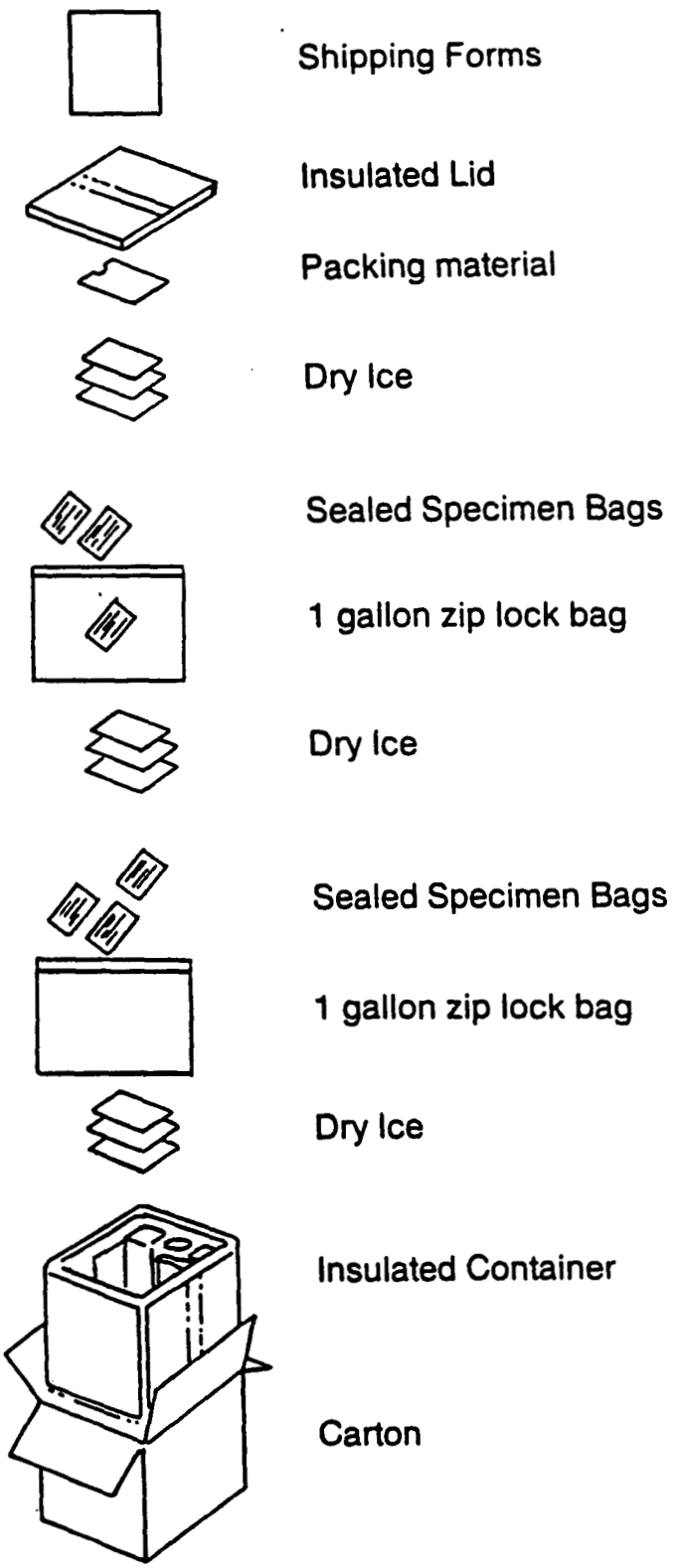
6. Place packing material on top of the dry ice to fill the box.

7. Place the paper shipping forms on top of the packing material. The shipping forms with instructions are shown in the Appendix.

8. Seal the box tightly with strapping tape.

9. Address the box and place it in a designated area for pickup.

Figure 5. Packing of Shipping Containers



5.3.2 Mailing Instructions

Shipping containers with frozen sera are sent to the respective central laboratories by overnight courier to ensure receipt within 24 hours and the empty styrofoam containers are returned to the field centers by UPS.

Field Centers in Hagerstown, Jackson, and Winston-Salem ship the specimens by Federal Express with a guaranteed delivery within 24 hours.

5.3.2.1 Hemostasis Laboratory

Shipping containers to the Central Hemostasis Laboratory are addressed as follows:

Central Hemostasis Laboratory
University of Texas Medical School
Room 7.035
6431 Fannin
Houston, Texas 77030
Telephone: (713) 792-5121

5.3.2.2 Central Lipid Laboratory

Shipping containers to the Central Lipid Laboratory are addressed as follows:

Central Lipid Laboratory
Atherosclerosis Clinical Laboratory
Methodist Hospital, Mail Station F701, Rm. F756
6565 Fannin
Houston, Texas 77030
Telephone: (713) 790-4351

5.3.2.3 University of North Carolina

Shipping containers to the University of North Carolina are addressed as follows:

Dr. Marilyn Vine
c/o Jenny Reid
McGavran-Greenberg Building, Room 2101C
School of Public Health, CB #7400
University of North Carolina
Chapel Hill, North Carolina 27599-7400
Telephone: (919) 966-7451

6. QUALITY CONTROL

6.1 Venipuncture and Equipment Records

Quality control procedures performed in ARIC central laboratories are addressed in Manuals 8-10. One component involves evaluation at the coordinating center of monthly mean values for each technician. This is informative because field centers select representative subsamples for examination each month. In the field centers there are two different aspects of quality control. One is the daily or monthly record of the performance of the equipment. This is most easily kept as a check sheet with the daily or monthly records, as described below. The other aspect of quality control is the record of the venipuncture which is part of each participant's records. See the Appendix III for a copy of this form and for instructions filling out the form. This record documents how long it takes to fill the standard 9.5 ml Vacutainer tube during the blood collection. It also shows the number of attempts it takes to get a good venipuncture and the code number of the technician who performs it. This record provides needed assurance that the blood was drawn in a standardized manner and that the equipment was functioning properly. Quality control is the best documentation that samples in each of the four field centers are being drawn and processed identically. Differences in the way the samples are collected or processed could potentially create a significant difference in assay results, which could make the laboratory results unusable. It is very important that the quality control records of the procedures and the equipment be properly maintained.

For the equipment, daily records should be kept on all refrigerators and freezers and the temperature of the refrigerated centrifuge must be recorded daily. See the Appendix V for a sample form. A sample Quality Control checklist is enclosed in this manual (see Appendix VI). The local blood processing certifier is to fill out this sheet monthly, certifying that daily checks have been performed properly and describing problems in this area. The certifier will also enter results of the monthly centrifuge check and equipment and supply check. The Monthly Quality Control checklists should be kept in a permanent file in the field centers.

6.2 Quality Control Duplicate Blood Samples

As part of the quality control program for laboratory determinations from blood samples (hemostasis, lipids and hematology), duplicate specimens are sent to the laboratories, with one half of each specimen pair sent under the participant's regular ARIC I.D., and the other half under a Quality Control Phantom Participant (Q.C.) I.D. The Q.C. I.D.s are not distinguishable from other ARIC I.D.s so that this forms a blinded external quality control program monitoring measurement variability.

To reduce the burden upon ARIC respondents, no one respondent is asked to contribute sufficient extra blood to make a complete set of duplicates for all laboratories. Instead, extra blood is drawn from several respondents and sent out under the same Q.C. I.D. For data analysis, results on each laboratory measurement are matched to the appropriate participant results.

All Q.C. samples are stored an extra week at the field centers and then sent to the central laboratories with a regular shipment.

The plan for processing blood samples from ARIC participants calls for processing blood tubes in cycles, with blood from one participant in each cycle.

To reduce the risk of confusing which Q.C. tube matches which real participant, Q.C. blood samples are drawn from only one member of each pair of participants whose blood is processed at the same time. Ideally each day is devoted to a fixed laboratory. For example, on Monday draw Tube 1 (miscellaneous serum); on Tuesday, draw Tube 2 (hemostasis); on Wednesday, Tubes 3,4 & 5 (hemostasis), on Thursday, draw tubes 6 & 7 (lipids and glucose); on Friday, draw any tubes that might have been missed earlier in the week due to holidays, no-shows, or other reasons.

6.2.1 Weekly Blood Q.C. Sample Checklist

The ARIC Field Center venipuncture technicians maintain a weekly checklist posted in their work area of the Q.C. samples to be drawn during the week. As each sample is drawn and processing completed, it is checked off. On Friday morning, this checklist is consulted to see if there were any additional samples needed to make up the complete set of Q.C. samples. An example of the checklist is given below.

Weekly Blood Q.C. Sample Checklist

Week:

Day	Tubes	Laboratory	Sample 1	Sample 2
Monday	1	Hemostasis (misc. serum)	_____	_____
Tuesday	2	Hemostasis	_____	_____
Wednesday	3,4,5	Hemostasis	_____	_____
Thursday	6,7	Lipids & glucose	_____	_____
Friday	Make-up any missed above			

6.2.2 Preparation for Drawing and Processing Q.C. Samples

Blood Drawing Tubes: Each morning the blood drawing technicians prepare extra blood collection tubes for the samples to be drawn that day. Each tube is labelled with one of the Q.C. I.D.s to be used that week. In addition, the technicians may wish to mark Q.C. tubes (other than Tube 8) "Q.C." in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. (Since Tube 8 goes to the Hematology Lab, it should not be marked as Q.C.). The Q.C. Tube #2 (7 ml red top) must be kept in the ice bath until use, as is the regular participant Tube #2. The other Q.C. tubes are set in the same rack used to hold the regular blood collection tubes, in a separate row from the other tubes.

Sample Aliquot Tubes: Each morning a separate foam block is prepared for each set of Q.C. blood tubes that the technician plans to draw that day. The foam block contains all the aliquot tubes needed to process the day's quality control samples. The tubes in each block are labelled in advance with one of the Q.C. I.D.s being used that week. Care must be taken during processing that the labels on the sample aliquot tubes match the label on the Q.C. blood collection tubes. Since only one Q.C. set is drawn in each blood collection cycle, only the foam block with I.D.s for that set is out in the work area at that time.

6.2.3 Drawing and Processing Q.C. Blood

Selecting Participants for Q.C. Blood Draw: Normally, the Q.C. samples are drawn from the first member of each group of participants whose blood is being processed simultaneously. Based upon the size of their veins, the difficulty of drawing the blood, and the apprehension a participant shows about the blood draw, the venipuncture technician may need to forego the drawing of the Q.C. tube from the first, and draw from the second member instead.

Order of Q.C. Tubes in Relation to Regular Blood Collection: The Q.C. Tube#2 follows immediately after the real Tube #2 in the draw. The other Q.C.tubes may be added at the end of the blood draw without harming the measurements. This procedure is followed to cause the least disruption of the collection of the regular blood samples. If the blood flow falls off at the end of the draw, so that it would be difficult to obtain the extra Q.C. tubes, a different participant is used to get this blood. A NEW NEEDLE STICK SHOULD NOT BE DONE JUST TO GET MORE BLOOD FOR Q.C. TOURNIQUET SHOULD NOT BE REAPPLIED AFTER INITIAL RELEASE.

Processing and Freezing Q.C. Blood: Q.C. blood samples are processed along with the regular blood samples. At certain points, the Q.C. blood samples must wait for processing until the regular blood samples have completed a particular step. For example, at Stage 2 of processing, Q.C. samples are not taken out of the refrigerated centrifuge until after the regular tubes #3, #4 and #5 have been aliquotted into sample vials and put in the refrigerator. After processing is completed for each Q.C. blood collection tube, the sample aliquot tubes are put into the -70°C for freezing (for a minimum of 30 minutes). After the samples are thoroughly frozen, they are put into a freezer storage bag and put into the freezer box corresponding to the destination of these tubes.

Since hemostasis tubes are collected from more than one participant, the samples from the first hemostasis tubes collected are still bagged as soon as they are frozen. A small holding box is set up in the freezer for bags with incomplete set of hemostasis samples. When a full set is completed, these bags are taken out of the holding box, zipped open and all the hemostasis tubes for a particular Q.C. I.D. rebagged together and put into the regular hemostasis freezer box.

Filling Out the Venipuncture Form for Q.C. Blood: Only one Venipuncture form is filled out for each set of blood samples for one Q.C. I.D. Information on the venipuncture attempts and filling time pertain to the participant contributing tube 2 and the rest of the form pertains to the participant contributing tubes 3, 4 and 5. Randomly add or subtract 5 minutes to the time when the blood draw began when filling out the Q.C. sample's venipuncture form and add 7 days to the date of blood drawing.

Logging the Match between Q.C. and Regular ARIC IDs and Reporting These to the Coordinating Center: The Q.C. Phantom Participant's folder is kept in the blood drawing area while the phantom I.D. is being used to draw Q.C. blood tubes. In the folder is the ARIC Quality Control Phantom Participant Form which is used to keep track of the match between Q.C. and regular ARIC "phantom" set. A sample copy is shown in Appendix VII. At the top of the log sheet is a space for the Q.C. Phantom Participant's I.D. As participants donate blood to make up a Q.C. set, labels with their I.D.s are added to the line corresponding to the tubes donated. This step must be done immediately after completing drawing blood for that participant, to minimize the chance of recording the wrong I.D. One such form is recorded for each Q.C. I.D. used. As soon as the full set of tubes is completed for each phantom participant (or at the end of the week, if any set is incomplete), the Q.C. phantom participants' folder with this form is given to the receptionist (or other person designated by the Study Coordinator). When all repeatability studies for the phantom are completed, the folder is processed like other participants' folders, except that the Q.C. phantom participant form is sent to the Coordinating Center and the field center keeps a photocopy of this form in the phantom's folder. Each regular participants's folder also holds an ARIC Participant Blood Q.C. Log Form to indicate whether any blood was drawn from that person for Q.C. I.D. to which they are matched. This sheet is filled out at the venipuncture station while the participant is there. A sample is shown in Appendix X.

7. TRAINING PROCEDURES

7.1 Technician Training and Evaluation

The technician must study ARIC Manual 7 and watch a few participant samples being processed. Then the technician may proceed to a mock drawing and mock processing of samples. Mock venipuncture is performed with the butterfly needle and Vacutainer system. A piece of latex tubing with a knot in one end leading to a glass of water is used as a target vein. Practice tubes are collected in the correct order, then placed at their proper positions and temperatures. The sample is processed from start to finish exactly as if real blood were being used. Each technician performs a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow the technician to become comfortable with the procedures before proceeding to live participants.

At this point the technicians are ready to practice on live volunteers. The technicians practice at least once with just one volunteer at a time and again process the blood entirely by themselves from start to finish. If the technicians do not feel comfortable, they can always go back and repeat the process with dummy tubes. If volunteers are available, it may be beneficial to repeat this several times. Any questions or problems that the technicians have must be solved before the technicians actually proceed to drawing the ARIC participants. Before the technicians draw blood from any ARIC participant, they must take and pass the practical and written tests included at the end of this manual. After passing the tests and depending on the written evaluation of their instructor, they may proceed either to drawing blood from the ARIC participants as part of a team, or to do more practice on live volunteers.

7.2 Clerk Training

The best way for the clerk or technicians to learn the forms and procedures is to go through each of them step by step. The clerk should carefully read and understand the Venipuncture Form. Examples of forms with correct and incorrect responses are compared and the clerk should recognize inappropriate responses. At this point, the clerk fills out a practice form making appropriate responses for himself, or for a fictional participant.

After becoming familiar with the participant information forms, the clerk learns to label the sample collection and freezing tubes. The trays for blood collection and for sample processing are set up by the field center technicians. Every tube in every tray must have a label attached with the appropriate participant code number. This number must be on all forms, results, tubes and shipping packages leaving the field center. The clerk needs to know the destination of each form, tube, and sample. A checklist is kept with the material from each participant, and the clerk needs to know what each item is on the list.

Before assisting in the blood collection, the procedure should be explained to the clerk. He or she should be familiar with the whole procedure even though he or she will not be actively participating in most of the process. The clerk is shown how to gently mix the tubes of blood and which are placed or replaced immediately in an ice bath. The clerk is also shown how to time the filling of tube #1 and where to record that on the participant's Venipuncture form. Once the first participants have been drawn, the clerk or technician makes sure that the blood drawing stations are set up for the next set of participants. A checklist is placed at each station to facilitate the preparation and completeness of each station.

Once comfortable with the mixing and placement of tubes, the pace required, and the preparation of the drawing stations, the clerk is ready to assist in the actual drawing procedure. If the field center does not have a clerk available to help during the blood collection and processing, the technicians must be able to perform the clerk's duties as well.

8. FIELD CENTER HEMATOLOGY SERVICES

8.1 Clinical Significance

Quantitation of the formed elements of the blood (erythrocytes -RBCs, leukocytes - WBCs, and platelets) is important in the ARIC study primarily so that the associations of the formed elements with atherosclerosis and its clinical manifestations can be studied. The association of elevated WBC count with the risk of cardiovascular disease requires confirmation. These determinations are also of value in recognizing asymptomatic disorders (e.g., anemia, leucocytosis, and thrombocytopenia) which may require the ARIC participant's referral to his usual source of care for further medical evaluation.

8.2 Principles of Quantitative Hematologic Determinations (1)

Procedure for counting circulating blood cells, whether manual or automated, all involve a sequence of (1) diluting the blood specimen, (2) aliquotting the diluted specimen into a measured volume, and (3) counting the cells in that volume.

All hospital based and independent laboratories now use automated instruments to count blood cells. These instruments work on either of two basic principles. In the first type of instrument employing electronic particle counting (e.g., the Coulter counter, Coulter Diagnostics, Hialeah, FL), blood cells pass through an aperture through which an electrical current is passed. The change in electrical resistance caused by the cell's passage is counted as a voltage pulse. Combinations of aperture size and threshold/window discrimination of voltage pulse height allow distinctions between erythrocytes, leukocytes, and platelets. The second type of instrument (e.g., Hemalog H-6000, Technicon Corporation, Tarrytown, NY) uses light-scattering from cells flowing through a counting chamber. Scattered light is detected by a photomultiplier tube, and cell number and size are evaluated as voltage pulses.

Automated hematology analyzers directly measure the cell counts for total RBCs, WBCs, and platelets. Total hemoglobin (Hb) is measured by the formation of hemoglobincyanide (HICN). The hematocrit (Hct) is calculated from the measurement of RBCs and either the calculated erythrocyte mean cell volume (Coulter Counter) or pattern of light-scattering (Hemalog H-6000). The hematocrit, as calculated by these automated analyzers, may differ from the hematocrit as determined directly by centrifugation ("Packed cell volume"). These differences are usually not significant in normal subjects with correct handling of samples.

Three red cell indices are calculated by automated hematology analyzers: (1) the mean cell volume (MCV), (2) the mean cell hemoglobin (MCH), and (3) the mean cell hemoglobin concentration (MCHC). The following formulas are used in these derivations:

$$(1) \quad \text{MCV (ul or fl)} = \frac{\text{Hct} \times 1000}{\text{RBCs (106/ul)}}$$

$$(2)\quad \text{MCH (pg)} = \frac{\text{Hb (g/L)}}{\text{RBC (106/ul)}}$$

$$(3)\quad \text{MCHC (g/dL)} = \frac{\text{Hb (g/Dl)}}{\text{Hct}}$$

fl = femtoliter
pg = picograms
g/Dl = grams per deciliter
ul = microliter
g/L = grams per liter

These indices are clinically useful in recognizing and classifying various types of anemias. If the primary erythrocytic measurements (Hct, RBC count and Hb) are normal, these indices will also be essentially normal.

8.3 General Operation of Field Center Hematology Studies

In contrast to the other types of laboratory determinations in the ARIC study which are performed at a central laboratory (e.g., coagulation, lipids), hematology procedures use specimens collected in EDTA which cannot be shipped to distant sites without jeopardizing sample stability and reducing reliability.

Each ARIC Field Center uses a local reference laboratory to perform the routine hematology procedures specified by the ARIC protocol. These laboratories are responsible for prompt specimen pickup, analysis, and result reporting. Although whole blood specimens collected in EDTA are stable for up to 24 hours at 4°C, it is desirable that ARIC specimens collected in the morning at the Field Center be analyzed that day by the reference laboratory. Specimens collected by the Field Centers in the afternoon are analyzed promptly after storage at 4°C. (EDTA is the only acceptable anticoagulant for samples to be analyzed for cell counts. [Heparin produces variable artifacts of cell size.] The professional staff at each Field Center periodically review the performance of the laboratory performing ARIC hematology studies, particularly in terms of the laboratory's quality control program for automated hematology.

8.4 Calibration and Interlaboratory Standardization

Each field center utilizes the services of one or more local hematology laboratories. Jackson uses the hematology laboratory at the University of Mississippi Medical Center seven days a week. The Jackson laboratory runs all ARIC hematology specimens on two instruments, the Coulter S + IV and the Technicon H-6000. Washington County uses the services of one hematology laboratory, the Hagerstown Medical Laboratory, to process samples during the week. The Minneapolis field center sends blood samples to one hematology laboratory which uses a Coulter S + IV. Forsyth County uses one hematology laboratory, Roche-Biomedical Laboratories, Inc., which uses a Technicon H-6000 for processing ARIC samples. A technical summary of type of instruments, calibration and quality control is provided in Table 1. Three of the four laboratories use the same type of hematology analyzer with similar calibration procedures (as shown in Table 1), thus reducing problems with interlaboratory standardization. Standardization of the processing of hematology specimens, however, remains problematic as illustrated (Savage; RA, 1985).

No stable reference materials are available for standardizing cell counts. The International Committee for Standardization in Hematology and the College of American Pathologists have both recognized that an automated hematology calibrator material that possesses the physical and chemical characteristics of fresh whole blood and is of sufficient stability to be analyzed by reference methods and distributed to hospital and outpatient laboratories of recalibration purposes is not now available nor is likely to appear because of the technical inadequacies of surrogates for white blood cells and platelets and to the compromises in matrix composition that are necessary in rendering the product stable for long-term analysis (Savage, RA, 1985).

Table 1. ARIC Field Center Hematology: Technical Summary

Field Center	Instrument	Calibration	Quality Control
Jackson, MS	Coulter S + IV	S-Cal	CAP Survey[1] Patient Samples
Washington County, MD	Coulter S + IV	S-Cal	CAP Survey Patient Samples
Minneapolis, MN	Coulter S + IV	S-Cal	CAP Survey
Forsyth County, NC	Technicon H-6000	Fisher Computrol	CAP Survey Medicare Survey Staff Samples

[1] CAP Survey - College of American Pathologists Survey

Laboratories currently use stabilized materials prepared by the manufacturers of automated hematology analyzers to calibrate their instruments. Thus, interlaboratory standardization in this area depends upon the widespread use of the calibrator.

8.5 Precision

Precision of cell counts within the laboratory relies upon (1) replicate determinations performed on the same specimen over a 24-hour period, (2) use for stabilized cell suspensions, (3) calculation of a "moving average" of all patient results, or (4) some combination of two or more of the preceding quality control methods (3). The precision (expressed as coefficient of variation (CV) of routine automated hematology assays and the minimum bias which can be detected by either replicate assays of fresh blood or serial assays of stabilized blood are summarized in Table 2 (modified from Bull, BS, 1982). Precision is better (and thus minimum detectable bias is less) for erythrocyte than for leucocyte or platelet counts.

Table 2. Precision of Routine Automated Hematology Assays

Routine Assay (CV)	Type of Whole Blood Control	Minimum Detectable Bias % Day	Week	Month
1. Cyanmethemoglobin (1%)	Fresh	2		
	Stabilized	2	2	2
2. RBC, by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
3. Hct or MCV by automated (<1%) counter	Fresh	2		
	Stabilized	2	4	7
4. WBC by automated (2%) counter	Fresh	6		
	Stabilized	6	8	8
5. Platelet count by (4%) automated counter	Fresh	12		
	Stabilized	12	15	15

Source: modification from Bull BS

Markedly abnormal hematology results are to be telephoned back to the Field Center, according to the criteria specified in ARIC Manual 2.

8.6 Accuracy of Automated Hematology Procedures

Interlaboratory comparability of these data can also be evaluated by mailed proficiency-testing samples in programs operated by external organizations. All the laboratories selected by the Field Centers participate in the hematology proficiency survey of the College of American Pathologists (CAP). Table 1 also shows the other quality control procedures used by each laboratory.

The major interlaboratory variable in automated hematology which these external proficiency programs have identified is that of the material used for calibration. As indicated previously, three of the four laboratories participating in the ARIC Field Center hematology studies use the same material.

8.7 Reporting of Results

The laboratories performing automated hematology for the ARIC Field Centers have the responsibility for reporting results formatted (either manually or electronically) for incorporation into the ARIC data base. Five data elements are included as hematology results in this data base:

1) Total hemoglobin (Hb)
2) Leucocyte (WBC) count
3) Platelet count
4) Hematocrit
5) Differential

8.8 References

1. Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: W.B. Saunders, 1985, pp. 586-601.

2. Savage RA. Calibration bias and imprecision for automated hematology analyzers. An evaluation of short-term bias resulting from calibration of an analyzer with S-Cal. AM J Clin Pathol 84:186-190, 1985.

3. Lappin TRJ, Farrington CL, Nelson MG, Merrett JD. Intralaboratory quality control of hematology. Comparison of two system. Am J Clin Pathol 72:426-431, 1979.

4. Bull BS. The use of patient values, calibrator and control materials in the routine laboratory. In Advances in Hematological Methods: The Blood Count. Van Assenfeldt OW, England JM, eds. Boca Raton: CRC Press, 1982, pp. 217-227.

Appendix I. Tests to be Performed

Central Hemostasis Laboratory (ARIC Manual Vol. 9) cases and controls

- Activated PTT
- Fibrinogen
- Factor VII
- Factor VIII C
- Von Willebrand Factor - Antigen
- Beta-thromboglobulin
- Platelet Factor 4
- Tissue Plasminogen Activator
- Protein C
- Antithrombin III
- Protein S (total & free)
- Plasminogen Activator Inhibitor
- D dimer
- Fibrinopeptide A

Central Lipid Laboratory (ARIC Manual Vol. 8)

- Total Cholesterol
- Total Triglycerides
- HDL Cholesterol
- LDL Cholesterol (calculated)
- Lipoprotein Lp (a)
- Glucose

Field Center Hematology Laboratory (ARIC Manual Vol. 7)

- Hematocrit
- White Blood Cell Count
- Platelet Count
- Hemoglobin
- Differential

Appendix II. Equipment and Supplies

Supplies provided by the central laboratories

1. Special anticoagulant mixture to be provided by Hemostasis Lab.
2. "Millipore" filters.

Supplies to be obtained by field centers

Supplier	Catalog No.	Description	Approx. No. per Week
Sarstedt	72.690.478	Yellow Microsample Tubes 500/pk	120
"	72.690.475	Blue Microsample Tubes 500/pk	270
"	65.716.008	Lavender Screw Caps 500/pk	300
"	65.716.009	Brown Screw Caps 500/pk	60
"	72.609	White screw top vials	540
"	65.716	White screw caps 500/pk	150
Fisher	11-676-21	Styrofoam boxes multipurpose biomailers (for mailing to Central Lipid & Central Chemistry Labs)	2
Fisher	03-530	Frozen Sample Shipper (Styrofoam boxes for mailing to Central Hemostasis Labs)	1

Miscellaneous Supplies

Abbott	4492	Butterfly Needles 40/box	45
S/P	B3035-12	Luer Adaptors BD #7226 100/pk	30
"	B3062-Swab	Alcohol Swabs 2,000/s	45
"	B-3063-5	Gauze Sponges 200/pk	90
"	B3062-191	Band Aids 100/pk	45
"	B3060-1	Tourniquets	
"	B3035-4	Vacutainer Tube Holders 10/pk	
"	PB214-12	Transfer Pipettes 500/pk	210
"	B1210-11	Freezer Bags 3" x 6" 250/pk	120
"	B1210-12	Freezer Bags 6" x 6" 250/pk	40
"		Freezer Bags 12" x 12"	
"	S9221-1	Sponge Tube Rack	
"	S9546-23G	Hypodermic Needles 23GA 100/pk	5
Unspecified	Vendor	Syringes 1 ml	1
Rainin	RT-200	Pipet tips 100-1000 l	90
		Dry Ice approximately 9 lbs. per shipping box	25 lbs.

Supplier	Catalog No.	Description	Approx. No. per Week
		Vacutainer Tubes 100/pk	
S/P	B2970-33	Serum Separator Red/Gray BD#6510	30
"	B2980-52	Red Stopper 7 ml BD#6431	30
"	B2994-94	Na Citrate, Blue D#6418	90
"	B2991-54	EDTA-Lavender 10 ml BD#6457	60
"	B2951-65/ B2911-51	EDTA-Lavender 5.0 ml BD# 367653 or 6452	30
Small Equipment Items			
Rainin	P-1000	Automatic Adjustable Pipet 100-1000	
S/P	C6548-3	Digital Stopwatch	
"	B29222-1	Blood Collection Trays	
"	T2050-1	Thermometers -20°C - +110°C	
"	B1796-Balance	Balance Harvard (Ohaus 1550SD)	
"	C6510-1	Timer - 3 channel digital	

Equipment purchased and maintained by field centers

1. Table-top refrigerated Centrifuge
2. Freezer (-70°C)
3. Refrigerator with crushed ice maker.

Appendix III. ARIC Venipuncture Form and QXQ Instructions

ARIC VENIPUNCTURE FORM

Atherosclerosis Risk in Communities

ID NUMBER: | | | | | | | | CONTACT YEAR: 0 7 FORM CODE: V E N VERSION: C 02/23/93

LAST NAME: | | | | | | | | | | | | INITIALS: | |

Public reporting burden for this collection of information is estimated to average 4 minutes, including time for reviewing instructions, gathering needed information and completing and reviewing the questionnaire. If you have comments regarding this burden, please send them to Attention: PRA Reports Clearance Officer, PHS, 721-B Hubert H. Humphrey Building, 200 Independence Avenue, SW, Washington, D.C. 20201, and to the Paperwork Reduction Project (0925-0281), Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, D.C. 20503.

INSTRUCTIONS: This form should be completed on paper during the participant's visit.

A. BLOOD DRAWING

1. Do you have any bleeding disorders? ..... Yes Y
   No N
   Don't Know D

   If Yes, specify in Item 13.

2. Date of blood drawing: ..... | | / | | / | |
   month day year

3.a. Time of blood drawing: ..... | | : | |
   h h m m

   b. AM or PM: ..... AM A
   PM P

4. Was all blood drawn before the snack? ..... Yes Y
   No N

   If No, specify non-fasting tubes on page 3.

5. Number of venipuncture attempts: ..... |

6. Filling time of Tube 1: ..... [ ][ ] seconds

7. Was the tourniquet reapplied? ..... Yes Y
No N

If Yes, specify on page 3.

8. Code number of phlebotomist: ..... [ ][ ][ ]

B. BLOOD PROCESSING

9.a. Time at which specimen Tubes 2-7 were spun: [ ][ ]:[ ][ ]
h h m m

b. AM or PM: ..... AM A
PM P

10.a. Time at which specimen Tube 1 was spun: [ ][ ]:[ ][ ]
h h m m

b. AM or PM: ..... AM A
PM P

11.a. Time at which specimens were placed in freezer: [ ][ ]:[ ][ ]
h h m m

b. AM or PM: ..... AM A
PM P

12. Code number of technician processing the blood: [ ][ ][ ]

13. Comments on blood drawing/processing: ..... Yes Y
No N

If Yes, Specify: _____

_____

14. Paper Incident Record (page 3) used? ..... Yes Y
No N

PLACE ARIC ID LABEL HERE.

VENIPUNCTURE INCIDENT RECORD

A. BLOOD DRAWING INCIDENTS: THIS LOG IS COMPLETED TO DOCUMENT PROBLEMS WITH THE VENIPUNCTURE. PLACE AN "X" IN BOXES CORRESPONDING TO THE TUBES IN WHICH BLOOD DRAWING PROBLEMS OCCURRED. IF A PROBLEM OTHER THAN THOSE LISTED OCCURRED, USE ITEM 6.

	Tubes							
	1	2	3	4	5	6	7	8
1. Sample not drawn								
2. Partial sample drawn								
3a. Tourniquet reapplied								
3b. Fist Clenching								
4. Needle movement								

5. Phlebotomist code: __ __ __

6. Other problems in blood drawing: _____

_____

B. BLOOD PROCESSING INCIDENTS: THIS LOG IS COMPLETED TO DOCUMENT PROBLEMS PROCESSING THE SPECIMENS. PLACE AN "X" IN BOXES CORRESPONDING TO THE TUBES IN WHICH PROCESSING PROBLEMS OCCURRED. IF A PROBLEM OTHER THAN THOSE LISTED OCCURRED, USE ITEM 13.

	Tubes							
	1	2	3	4	5	6	7	8
7. Broken tube								
8. Clotted								
9. Hemolyzed								
10. Lipemic								
11. Other Contamination								

12. Blood Processor Code: __ __ __

13. Other problems in blood processing: _____

_____

14. Date of procedures: __ __ / __ __ / __ __ .

ORIGINAL TO ARIC COORDINATING CENTER; COPIES TO CENTRAL LABS AND FIELD CENTER.

INSTRUCTIONS FOR VENIPUNCTURE FORM
VEN, VERSION C, 11/10/92
PREPARED 11/11/92

I. GENERAL INSTRUCTIONS

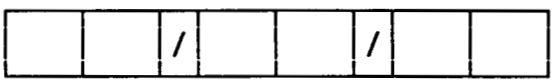
The Venipuncture Form should be completed during the participant's clinic visit to record the results of that procedure. Technicians performing venipuncture and blood processing must be certified and should have a working knowledge of the ARIC Blood Collection and Processing Manual of Operations. Technicians should also be familiar with and understand the document entitled "General Instructions for Completing Paper Forms" prior to completing this form. ID Number, Contact Year, and Name should be completed, as described in that document, prior to the arrival of the participant.

II. SPECIFIC INSTRUCTIONS

A. BLOOD DRAWING

1. If the participant has a bleeding disorder, consult with the field center physician, physician assistant or nurse practitioner before proceeding with the venipuncture. If the participant does not know whether he/she has a bleeding disorder, offer the explanation, "If you have a bleeding disorder you would have symptoms like excessive nose bleeds, or very easy bruising, or problems with bleeding after tooth extractions, or any type of surgery." If the participant is still unsure, consult with field center medical personnel before going on. Specify any bleeding disorders as briefly as possible in Item 13 of the Venipuncture Form.

2. Note the date of blood drawing on the form. Code in numbers using leading zeros where necessary to fill all fields. For example, May 3, 1993 would be entered as shown below:



If the participant is rescheduled for another day, the actual date when blood is drawn should be entered.

3. Note the time of venipuncture on the form. This is the time when the vein is punctured. Fill in the fields using leading zeroes where necessary and indicate AM or PM.

4. Check the participant's Itinerary Sheet, or ask the participant if he/she has had the clinic snack. If so, specify non-fasting tubes in Section A, question 6 of the Incident Record.

5. Include all venipuncture attempts by all phlebotomists. The same technician should not attempt a venipuncture more than twice.

6. Note the time required to fill tube 1. 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 donor's arm for the duration of the draw.

7. Do not reapply the tourniquet during tubes #2 - #5. Only reapply the tourniquet <u>after</u> tube #5, and only if this is necessary to spare the participant another stick. Specify which tubes correspond to the tourniquet reapplication in Section A of the Incident Record.

8. The phlebotomist who performed the blood drawing procedure must enter his/her code number in the fields provided. If more than one phlebotomist attempts to draw the blood, enter the code of the <u>first</u> phlebotomist.

B. BLOOD PROCESSING

9. Note the time at which the centrifuge containing these tubes began to spin. Fill in the fields using leading zeroes where necessary and indicate AM or PM.

10. Note the time at which the centrifuge containing this tube began to spin. Fill in the fields using leading zeroes where necessary and indicate AM or PM.

11. Note the time at which the samples were placed in the freezer. Fill in the fields using leading zeroes where necessary and indicate AM or PM.

12. Enter the code number of the technician who <u>began</u> processing the blood.

13. Include any clarifications or other information relevant to the assays being performed that are not included in the Incident Record, Fasting Tracking Form (FTR), Medication Survey Form (MSR), or the Health History Form (HHX). This information will be keyed into the Venipuncture DES record. Be as clear and concise as possible.

14. Answer "Y" if any problem occurred in either blood drawing or blood processing that necessitated use of the paper Incident Record attached to the venipuncture form. In such a case, attach the correct ARIC ID label on the original and make copies. Send original to the ARIC Coordinating Center and a copy to the pertinent central laboratory(ies). Place one copy in the participant's folder. Answer "N" if no such problems occurred. In this case, an Incident Record is unnecessary and therefore a copy need not be made.

Appendix IV. ARIC Shipping Forms

ARIC SHIPPING FORM

PART ONE (To be completed at Field Center)

TO: Name and address of Central Agency preprinted here.

FROM: Name and address of Field Center preprinted here.

SHIPMENT PACKED AND SEALED:

TIME: _ _ : _ _ AM PM DATE: _ _ / _ _ / _ _.

ARIC BATCH NUMBER: A R _ _ _ _ _ _

REPORTING PERIOD:

STARTING DATE: _ _ / _ _ / _ _

ENDING DATE:_ _ / _ _ / _ _

TOTAL NUMBER OF SPECIMENS ENCLOSED: _____

NUMBER OF CONTENTS PAGES ATTACHED: ____

COMMENTS CONCERNING SHIPMENT CONTENTS: _____

_____

INITIALS OF PERSON PACKING AND COMPLETING SHIPPING FORMS: _ _ _

****

PART TWO (To be completed at Central Agency)

SHIPMENT ARRIVED AT CENTRAL LABORATORY:

TIME: _ _ : _ _ AM PM DATE: _ _ / _ _ / _ _.

COMMENTS ON CONDITION OF SHIPMENT ON ARRIVAL: _____

_____

INITIALS OF PERSON UNPACKING SPECIMENS: _ _ _

ARIC SHIPPING FORM
CENTRAL HEMOSTASIS LABORATORY
UNIVERSITY OF TEXAS MEDICAL SCHOOL
64321 FANNIN
HOUSTON, TX 77030

SPECIMEN ID	VIAL COLOR	NUMBER OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
	BLUE			
	YELLOW			
	WHITE			
	BLUE			
	YELLOW			
	WHITE			
	BLUE			
	YELLOW			
	WHITE			
	BLUE			
	YELLOW			
	WHITE			
	BLUE			
	YELLOW			
	WHITE			
	BLUE			
	YELLOW			
	WHITE			

ARIC SHIPPING FORM
DR. WOLFGANG PATSCH
ATHEROSCLEROSIS CLINICAL LABORATORY
METHODIST HOSPITAL, MAIL STATION F701, ROOM F756
6565 FANNIN
HOUSTON, TX 77030

SPECIMEN ID	VIAL COLOR	NUMBER OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			
	LAVENDER			
	BROWN			

SHIPPING FORM INSTRUCTIONS

There are two types of shipping forms: (1) the face sheet and (2) the contents sheet(s). Both forms are computer generated and are included in every shipment. The FACE SHEET is a two part form (Part One on the top of the page; Part Two on the Bottom of the page). Part One (the top half) is completed by the field center.

The NAME AND ADDRESS of the SHIPPER (field center) and the RECIPIENT (central laboratory) are preprinted on each shipping form.

The date and time the SHIPMENT was PACKED AND SEALED is recorded.

The ARIC BATCH NUMBER is recorded. The batch number has eight (8) characters: ARabnnnn, where

fields 1 and 2 (AR):	The study code, A R
field 3 (a):	The code for the sending agency.
field 4 (b):	The code for the receiving agency.
fields 5 - 8 (nnnn):	The sequential shipping batch number, counting all batches shipped from "a" to "b" since the beginning of the project.

ARIC AGENCY CODES

Field Centers	F	Forsyth County, NC
	J	Jackson, MS
	M	Minneapolis, MN
	W	Washington County, MD
Central Agencies		
	D	ECG Computer Center - Halifax
	E	ECG Reading Center - Minneapolis
	H	Hemostasis Laboratory
	L	Lipid laboratory
	N	Program Office, NHLBI
	P	Pulmonary Function Reading Center
	U	Ultrasound Reading Center
	Z	Coordinating Center

The STARTING and ENDING DATE of the SHIPMENT PERIOD is recorded.

The TOTAL NUMBER OF SPECIMENS ENCLOSED in the shipping container is confirmed by the field center technician by both counting baggies and adding the numbers in the "specimen ID" column on the contents sheets.

The NUMBER OF CONTENTS PAGES ATTACHED is recorded. This varies depending on the number of samples in the shipment.

Remarks (peculiarities) about the shipment are written in COMMENTS CONCERNING SHIPMENT CONTENTS.

The INITIALS OF THE PERSON COMPLETING PART ONE OF THE SHIPMENT FORM are coded.

SHIPPING FORM INSTRUCTIONS (cont.)

Part Two of the SHIPPING FORM is completed by the receiver, in this case, the central laboratory.

The date and time the SHIPMENT ARRIVED at the Central Agency is recorded.

COMMENTS on the CONDITION of the SHIPMENT upon ARRIVAL are recorded, such as, shipment totally thawed.

The INITIALS OF THE PERSON COMPLETING PART TWO OF THE SHIPMENT form are coded.

The second shipping form is the CONTENTS SHEET. The contents sheet(s) is composed of an original and two copies. The original is sent to the central laboratory. The first copy is kept by the field center and the second copy is sent to the Coordinating Center.

Each central laboratory has its own specific contents sheet. More than one contents sheet may be used in each shipment, depending on the number of specimens enclosed. The number of pages attached and each page number are filled in at the top of the contents page, i.e., page 1 of 5. Except for the last two fields, this form is filled out at the field center as the specimens are collected and stored. In this case, the form must be checked against the specimens when packed for shipment.

The SPECIMEN ID number is entered in these spaces. This is most easily done by attaching one of the adhesive Participant ID number labels in the space provided. It is suggested that a second person check these IDs against the IDs on the vials to correct any errors.

The color of the appropriate vial(s) is recorded in COLOR VIAL. It is especially important when shipping the multiple vials to the Central Hemostasis and Central Lipid Laboratories.

The number of the same size and colored vial(s) with the same Participant ID number is recorded in NUMBER VIALS, e.g., there are 9 blue vials sent to the Hemostasis Laboratory.

COMMENTS on the quality of the specimens upon receipt are recorded at the central agency. These are optional, but should be Participant ID number specific, such as tube broken, thawed, etc.

The DATE and TIME of ARRIVAL of the specimens at the central agency are recorded to acknowledge receipt and for inventory purposes.

Appendix V. Checklist: Blood Drawing Station

CHECKLIST
BLOOD DRAWING STATION

Stopwatch

Alcohol Wipes

Gauze Squares

Vacutainer Holder

Butterfly Adaptor

Butterfly Needles

Tourniquet

Band Aids

Ice water bath

1 red and Gray Top 9.5 ml tube - labeled with donor ID

1 Red 7 ml tube with liquid anticoagulant - labeled in ice water bath

3 Blue 4.5 ml tubes - labeled

2 Lavender 10 ml tubes - labeled

1 Lavender 5.0 ml tube - labeled

Appendix VI. ARIC Daily Temperature Record

ARIC
DAILY TEMPERATURE RECORD

DATE M	D	Y	Freezer	Refrig	Room	Initials	DATE M	D	Y	Freezer	Refrig	Room	Initials

Appendix VII. ARIC Monthly Equipment Quality Control Checklist

CENTER _____ DATE _____

CERTIFIER _____ ID NUMBER _____

TECHNICIAN _____ ID NUMBER _____

SET UP

	(S)	(U)*	Comments
1. Daily QC records			
refrigerator temperature	___		_____
centrifuge temperature	___		_____
freezer temperature	___		_____
2. Equipment and Supplies			
refrigerated centrifuge	___		_____
refrigerator	___		_____
-70 C freezer	___		_____
stopwatch	___		_____
timer	___		_____
ice bath	___		_____
butterfly needles with adapter	___		_____
syringe	___		_____
Millipore filter	___		_____
Vacutainer hub	___		_____
tourniquet	___		_____
Vacutainer tubes	___		_____
other	___		_____

* (S) = Satisfactory / (U) = Unsatisfactory

Appendix VIII. ARIC Venipuncture and Processing Procedures Certification Checklist

CENTER _____ DATE _____

CERTIFIER _____ ID NUMBER _____

TECHNICIAN _____ ID NUMBER _____

VENIPUNCTURE	(S)	(U)*	Comments
1. Labels checked	___		_____
2. Participant prepared and procedure explained.	___		_____
3. Venipuncture Form filled.	___		_____
4. Tourniquet application and release	___		_____
5. Venipuncture technique	___		_____
6. Tube collection sequence	___		_____
7. Inversion technique	___		_____
8. Tube incubation location	___		_____
9. Stasis obtained	___		_____
10. Needle disposal	___		_____
PROCESSING			
1. Knowledge of centrifuge operation	___		_____
2. Aliquotting supply set-up	___		_____
3. Stage I tube spin	___		_____
4. Stage II aliquotting	___		_____
5. Stage III tube spin	___		_____
6. Vials sealed	___		_____
7. Final processing stage	___		_____
8. V-Form completed	___		_____
9. Freezer organization	___		_____
10. Time constraints	___		_____
11. Disposal of contaminated supplies	___		_____
PACKAGING AND SHIPPING			
1. Specimens bagged	___		_____
2. Adequate dry ice used in shipping	___		_____
3. Shipping paperwork	___		_____

* (S) = Satisfactory / (U) = Unsatisfactory

Appendix IX. Sample Exams for Certification

PRACTICAL EXAM FOR ARIC BLOOD DRAWING TECHNICIAN

1. Place the following 8 blood collection tubes in the correct set-up order and location for the venipuncture: 1-9.5 ml red and gray top; 1-7 ml red top with anticoagulant; 3-4.5 ml blue tops; 2-10 ml lavender tops and 1-5.0 ml lavender top.

2. Specify which tube(s) go into the an ice bath after collection. How long before collection and how long after collection should the tubes remain on ice?

3. Remove the appropriate tubes from the tray, balance them and place them in the centrifuge. How long should they spin? At what speed?

4. Set up a sponge tray with the appropriate number, color an order of each color microsample tube, Millipore filter and 5 ml syringe.

5. Place the collection tubes in front of their respective colored sample tubes. Describe what further processing is required of each collection tube before it is aliquotted into its respective sample tube.

6. Divide the colored sample tubes and place them in bags according to their final destination.

7. Describe the quality control for each piece of equipment.

SAMPLE
WRITTEN EXAM

1. Which tube(s) contains a special mixture of enzyme inhibitors and antiplatelet anticoagulants?

   a) The 9.5 ml red and gray top
   b) The 7 ml red top
   c) The 10 ml lavender
   d) The 4.5 ml blue tops

2. The serum in the white capped vials are sent to which is sent to which laboratory?

   a) Chemistry
   b) Lipid
   c) Hemostasis
   d) None

3. The contents of which tube(s) are the most sensitive to differences in venipuncture?

   a) The 7 ml red top
   b) The 5.0 ml lavender
   c) The 4.5 ml blue tops
   d) The 10 ml lavender tops

4. Which tube(s) needs to be filtered to remove remaining platelets from plasma?

   a) The 5.0 ml lavender top
   b) The 4.5 ml top
   c) The 7 ml red top
   d) None of the above

5. Which tube is drawn last?

   a) A 5.0 ml lavender top
   b) A 4.5 ml blue top
   c) A 7 ml red and yellow top
   d) An 9.5 ml red and gray top

6. Which tube(s) contain unstable factors that must be kept cold while being processed?

   a) The 5.0 ml lavender top
   b) The 4.5 ml blue top
   c) The 10 ml lavender
   d) The 9.5 red and gray top

7. What type of study(ies) will the 10 ml lavender top tubes be used for?

   a) Chemistry
   b) Lipid
   c) Coagulation
   d) Hemoglobin A1C

8. What lab does the serum from the 9.5 ml red and gray top tube go to?

   a) Clinical Chemistry laboratory
   b) Central Lipid laboratory
   c) Central Hemostasis laboratory
   d) Field Center Hematology laboratory

9. When is the tourniquet removed?

   a) after tube #1 fills
   b) after tube #2 fills
   c) after all tubes fill
   d) it does not matter

10. Which tube (s) are the buffy coat taken from?

   a) 7 ml red top
   b) 4.5 ml blue top
   c) 9.5 ml red/grey top
   d) 10 ml lavender top

True or False

11. The factors being analyzed from the 4.5 ml blue tops tube are stable for up to 2 hours at 37°C?

12. The 7 ml red top tube has a special anticoagulant to prevent enzymatic breakdown of lipids?

13. The Central Lipid laboratory samples are less sensitive to venipuncture technique than the Hemostasis Laboratories.

Appendix X. ARIC Quality Control Phantom Participant and Non-Participant ID Form

ARIC QUALITY CONTROL PHANTOM PARTICIPANT AND NON-PARTICIPANT ID FORM

Form Code: PNPA Record Type: 028

Note: This form should be sent to the Coordinating Center: (1) the same week it is filled out for a non-participant or monitoring ID, or (2) within two weeks of the first entry for a QC phantom.

Phantom Participant
ID Number _____ Contact Year: _____

This ID is for (circle one): P A QC Phantom Participant
M An ID used for Monitoring Visit
N An ID used for a Non-Participant

Date ID Assigned: ____ /____ /____ ID of Person Assigning ID: _____

Venipuncture Phantom QC Log

Tube	Matching Participant ID	Date Drawn (Mo/ Day/ Yr)	Technician ID
1	_____	____/____/____	_____
2	_____	____/____/____	_____
3, 4 & 5	_____	____/____/____	_____
6 & 7	_____	____/____/____	_____
8	_____	____/____/____	_____

Anthropometry Phantom QC Log

Procedure	Matching Real Participant ID	Date of Meas. (Mo/Day/Yr)	Technician ID
Measurement Group D (triceps skinfold, subscapular skinfold, wrist breadth)	_____	____/____/____	_____
Measurement Group C (waist girth, hip girth, unadjusted sitting height, stool height)	_____	____/____/____	_____

Appendix XI. Collection and Storage of Red Blood Cells

ARIC - VISIT 2
COLLECTION AND STORAGE OF RED BLOOD CELLS

On September 5, 1990 the ARIC Steering Committee approved the collection of red blood cells (RBC) at the four ARIC field centers and their long-term storage at the Department of Epidemiology at the University of North Carolina (UNC). Dr. Marilyn F. Vine is responsible for the storage and inventory of RBC samples at UNC.

RBCs are collected from regular ARIC participants and those participating in the Postprandial and Lipoprotein and Atherosclerosis (PPL) Study. The collection of RBCs does not result in any changes to the blood drawing procedures at the ARIC field centers. Additional procedures, however, are required during the second and final stages of sample processing (refer to ARIC Manual 7). As the introduction of the PPL Study into ARIC-Visit 2 has resulted in two sets of sample processing directions, the following instructions for processing RBCs are divided into two sections: (1) regular ARIC participants and (2) ARIC-PPL study participants.

1. FIELD CENTER PROCEDURES FOR REGULAR ARIC PARTICIPANTS

a. Drawing the Sample

RBCs are obtained from the lipid tubes. Draw the lipid tubes, numbers 6 and 7, according to the protocol in Section 3.4 in Manual 7.

b. Processing and Storing the Sample

When preparing the sponge, label three additional 2 ml white vials with the participant's ARIC ID label. Place these tubes in wells 8C, 8D, and 8E. During stage 2, after transferring the buffy coat to the two white vials with the brown screw caps (steps 1-7 in Section 4.3.1 of Manual 7), use a plastic transfer pipet to equally divide the remaining red blood cells into three 2 ml white vials (in wells 8C, 8D, and 8E). Fasten white screw tops onto the vials and continue with the directions for freezing the other samples at -70 degrees centigrade. Plastic storage bags for RBC samples are labelled by hand "RBCs for UNC" and with the participant's ARIC ID label. Once frozen, each participant's three vials of RBCs are stored in their own separate plastic bag and kept frozen until shipment to UNC once a month.

c. Shipping of Samples to the University of North Carolina

The bags of frozen RBCs for UNC are packed and shipped in the same styrofoam boxes which have served as storage containers in the freezers. Packing instructions are identical for RBCs from regular ARIC and ARIC-PPL participants and follow the same instructions as those for the other ARIC frozen specimens.

1. Place a 2" layer (approximately 2 lbs.) of dry ice on the bottom of the styrofoam box.

2. Put half of the bags of sample tubes into a 1 gallon zip lock bag and seal. Place this bag in the styrofoam box on top of the dry ice.

3. Layer another 2 lbs. of dry ice on top of and around the sample bags.

4. Put the remaining sample bags into a second 1 gallon zip lock bag, seal, and place this bag on top of the dry ice.

5. Layer another 2 lbs of dry ice on top of and around the sample bags.

6. Place packing material on top of the dry ice to fill the box.

7. Place the paper shipping forms in a plastic bag and lay it on top of the packing material.

8. Seal the box tightly with strapping tape.

9. Address the box and place it in the designated area for pick up.

RBCs are shipped to UNC once a month on a staggered schedule. The shipping schedule is listed below in Table 1.

Table 1. Shipping Schedule for Frozen Red Blood Cells to UNC

Field Center	Shipping Schedule
Forsyth County, NC	1st Monday of each month
Jackson, MS	2nd Monday of each month
Minneapolis, MN	3rd Monday of each month
Washington County, MD	4th Monday of each month

Shipping containers are sent by overnight courier to arrive within 24 hours to UNC at the below address. The empty styrofoam containers are returned periodically to their respective field centers, via UPS.

Dr. Marilyn F. Vine
c/o Jinny Reid
McGavran-Greenberg Building, Room 2101C
School of Public Health, CB# 7400
University of North Carolina
Chapel Hill, NC 27599-7400

Telephone: 919-966-7451

2. FIELD CENTER PROCEDURES FOR ARIC-PPL PARTICIPANTS

The procedures for drawing and processing RBCs are included in the PPL protocol and are outlined here for convenience.

a. Drawing the Sample

RBCs are obtained from the PPL-lipid tubes. Draw the three lipid tubes; RBCs are obtained from the first two tubes (numbers P1 and P2), according to the PPL protocol in Section 5.5.

b. Processing and Storing the Sample

When preparing the sponge prior to venipuncture, place three white screw top vials (labelled with PPL participant IDs) in wells 9A, 9B and 9C. During stage 2, after transferring the buffy coat to the two white vials with the brown screw caps (Section 5.5.1 in the PPL Protocol), use a sterile disposable plastic transfer pipet to equally divide the remaining red blood cells into three 2 ml white vials in wells 9A, 9B, and 9C. Fasten white screw tops onto the vials and continue with the directions for freezing the other samples at -70 degrees centigrade. Plastic storage bags for RBC samples are labelled by hand "RBCs for UNC" and with the participant's PPL ID label. Once frozen, each participant's three vials of RBCs are stored in their own separate plastic bag and kept frozen until shipment to UNC once a month.

c. Shipping of Samples to the University of North Carolina

Follow the procedures above in Section 1.c for shipping RBCs from ARIC participants. RBCs from ARIC-PPL participants are stored in their own small plastic bags and should be transferred to one (or more if necessary) large zip lock plastic bag labelled "RBCs for PPL participants" for shipping.

POSTPRANDIAL LIPOPROTEINS AND ATHEROSCLEROSIS (PPL) STUDY
ARIC CENTRAL LIPID LABORATORY
Methodist Hospital, Mail Station F701, Room F756
6565 Fannin
Houston, TX 77030

PAIR MEMBER	SPECIMEN ID LABEL	# ORANGE TOP TUBES 0 hr	3.5 hr	8 hr	# RED TOP TUBE @ 8 hr	DATE DRAWN	PPL INCIDENT FORM	UNPACKING TIME	CONDITION UPON ARRIVAL
___	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)					__/__/__	(circle) Y N	__:__ A P	

POSTPRANDIAL LIPOPROTEINS AND ATHEROSCLEROSIS (PPL) STUDY

ARIC CENTRAL HEMOSTASIS LABORATORY
University of Texas Medical School
6431 Fannin
Houston, TX 77030

PAIR MEMBER	SPECIMEN ID LABEL	# BLUE VIALS AT 3.5 HOUR	DATE DRAWN	PPL INCIDENT FORM	UNPACKING TIME	CONDITION UPON ARRIVAL
___	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
___	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
	(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	

ARIC - RED BLOOD CELL ANCILLARY STUDIES
DR. MARILYN VINE, C/O JINNY REID
McGAVRAN-GREENBERG BUILDING, ROOM 2101C
SCHOOL OF PUBLIC HEALTH, CB# 7400
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, NC 27599-7400
919-966-7451

SPECIMEN ID LABEL	#WHITE VIALS AT FASTING	DATE DRAWN	ARIC INCIDENT FORM	UNPACKING TIME	CONDITION UPON ARRIVAL
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	
(Place ID label here)		__/__/__	(circle) Y N	__:__ A P	

Appendix XII. Guidelines for OSHA Bloodborne Pathogens Standards

GUIDELINES FOR OSHA BLOODBORNE PATHOGENS STANDARDS

There shall be a written exposure control plan. The plan should be designed to eliminate or minimize employee exposure. The effectiveness of this plan shall be based on the adoption of Universal Precautions as a form of infection control. The plan shall include, but not be limited to identification in writing of tasks, procedures, and personnel classification where occupational exposures may occur. Occupational exposure is defined as "Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with infectious material that may result from the performance of an employee's duties." The plan shall contain procedures to accurately report exposure incidents. Circumstances of exposure shall be documented. The plan shall contain procedures for evaluating the circumstances of exposure. The goal is to identify and correct the situations the lead to the exposure incident. Employees' exposure determinations in terms of job classification and tasks performed shall be evaluated. The goal is to determine what procedures involve occupational risk in order to ascertain what measures can be taken to eliminate or reduce that risk. The plan shall set forth a time table for implementing other measures that will be used to reduce risk (engineering and work practice control, vaccinations, training and education). The exposure control plan shall be accessible to all employees.

There shall be engineering and work practice control plans.

A. Engineering Controls:

Engineering controls act on the source of the hazard by the installation of physical devices such as sharps containers and protective shields.

Sharps containers: Needles will not be capped or bent. Contaminated needles will be deposited in a sharps container. The container shall be leak-proof on the sides and bottom, puncture resistant, closable, and color coded red-red/orange or should display the universal biohazard sign. The container for contaminated sharps shall not be reused. Sharps containers must be easily accessible to personnel and located as close as possible to the area of use. They must be maintained in an upright position and not allowed to overfill.

Handwashing Facilities: The employer shall provide handwashing facilities readily accessible to employees.

Personal Protective Equipment (PPEs): PPEs shall be provided to the employee at no cost. Lab coats/gowns are considered PPEs if they are used to prevent an employees' uniform or street clothing from becoming contaminated with infectious materials. We recommend gloving for all venipuncture and blood processing ("double gloving" is not necessary). Gloves and disposable gowns should be changed upon contamination. Non-disposable lab coats/gowns shall be changed upon contamination. Other PPEs (e.g. goggles, face masks, face shields) shall be worn when facial contamination is reasonably anticipated (splashing, spraying, creation of droplets). PPEs shall be removed before leaving the work area and shall be replaced or repaired as necessary.

Work practice controls reduce the likelihood of exposure by altering the method in which a task is performed (mouth pipetting, recapping of needles).

All procedures involving blood shall be performed in such a manner as to eliminate splashing, spraying or creation of droplets. There shall be no eating, drinking, or application of cosmetics where infectious materials are stored or handled, or where procedures involving infectious material are performed. Food and drink shall not be stored in areas where infectious materials are present. There shall be no pipetting by mouth. Contaminated sharps or needles shall not be recapped or bent. All storage places for infectious materials (refrigerators, freezers, centrifuges) shall be clearly labeled with the universal red/red-orange biohazard sign. Any infectious materials shipped out of the facility shall be clearly labeled with the biohazard sign.

Housekeeping: There shall be a written schedule for cleaning. The plan should identify what surfaces will be cleaned, and identify an appropriate disinfectant to be used. Work areas will be cleaned after the completion of procedures, upon contamination, and at the end of the work shift.

Training Program: <u>There shall be a formal training program.</u> The training program shall be offered during work hours and provided at no cost to the employee. The training program must be offered upon assignment and annually. Additional training shall be provided when modifications of procedures or tasks are such that new exposures are created. The program shall include, but not be limited to, discussion of bloodborne pathogens and their transmission and epidemiology, an explanation of the exposure control plan, an explanation of the methods used to identify tasks and other procedures that may lead to exposure, and explanation of methods used to prevent exposure, including PPEs, engineering controls, and work practice controls. There shall be information on the Hepatitis B vaccine, to include discussions on the efficacy, safety, administration, and benefits of the vaccine. There shall be explanation of the procedures to follow in the case of an exposure incident. There shall be information concerning post-exposure and follow-up evaluations. There shall be ample opportunity for questions and answers. The trainer must be knowledgeable with the subject matter contained in the program.

<u>Hepatitis B Vaccination:</u>

Hepatitis B Vaccination shall be made available to all employees who have occupational exposure, at no cost to the employee. It shall be made available to the employee after training and within 10 days of the initial assignment. The employer shall not make participation in a screening program a prerequisite for receiving the vaccination. The employee will be considered exempt if they have previously received the complete vaccination series, antibody testing has revealed that the employee is immune, or if the vaccine is contraindicated for medical reasons. The employee can decline the vaccination. If the employee initially declines but reconsiders at a later date, the vaccine shall be made available to that employee. If the employee declines a statement shall be signed. The formal statement is in appendix A of the regulations. If a booster is deemed necessary in the future, the booster shall be made available.

Post-exposure Evaluation and Follow-up:

Upon the report of an exposure incident, the employer shall make available to the employee a confidential medical evaluation and follow-up that shall include, but not be limited to:

The evaluation and follow-up shall be provided by a Healthcare Professional. Documentation of the route of the exposure and the circumstances under which the exposure occurred. Identification of the source individual unless prohibited by law. The source individuals blood shall be tested for HBV and HIV as soon as feasible and upon consent. If consent is not legally required, the source individuals shall be tested and the results documented. Results shall be made available to the exposed employee and the employee shall be informed of the applicable laws and regulations concerning disclosure. Upon consent the exposed employee's blood shall be collected and tested for HIV and HBV. If the employee does not give consent for HIV testing, the sample shall be held for 90 days. If during that time the exposed employee gives consent the testing shall be completed as soon as feasible. Post-exposure prophylaxis shall be offered when medically indicated, as recommended by the US Public Health Service.

Recordkeeping:

Medical Records: Medical records for employees with occupation exposure must be kept for the duration of employment plus 30 years. The records shall be kept confidential. The medical record shall be made available to the exposed employee. The medical record shall not be disclosed to any person in or outside of the work place without the employee's express written consent or as required by law. The medical records are not available to the employer.

Training Records: Training records shall include the training dates, a summary of the training program, names and job titles of all persons attending the program, and the name, job title, and qualifications of the individual conducting the program. The records shall be kept for the duration of employment plus 3 years.