

ATHEROSCLEROSIS RISK IN COMMUNITIES STUDY PROTOCOL

MANUAL 7

BLOOD COLLECTION AND PROCESSING

VISIT 4

Version 4.0

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**For copies, please contact:
ARIC Coordinating Center
Department of Biostatistics
CB #8030, Suite 203, NationsBank Plaza
The University of North Carolina
Chapel Hill, NC 27514-4145**

FOREWORD

This manual, entitled Blood Collection 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 and 13 through 15. Manual 12 on Quality Assurance contains a general description of the study's approach to quality assurance as well as the details for quality control for the different study procedures.

ARIC STUDY PROTOCOLS AND MANUALS OF OPERATION

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3	Cohort and Community Surveillance
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5	Electrocardiography
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1.0 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 ARIC field centers: Forsyth County, NC; Jackson, MS, Washington Co., MD; selected suburbs of Minneapolis, collected from all study participants at the fourth cohort examination (Visit 4). Specimens are processed at the field centers for shipment to, analysis, and long-term storage at four central laboratories: the ARIC Hemostasis Laboratory at the University of Texas Medical School in Houston, TX (blood and urine samples); the ARIC Lipid Laboratory at Baylor College of Medicine in Houston, TX (blood samples only), the Minneapolis ARIC Field Center in Golden Valley, MN (urine samples only); and the University of North Carolina (UNC) School of Dentistry in Chapel Hill, NC.

Blood samples are stored and evaluated at the central lipid and hemostasis laboratories in Houston, TX and the UNC School of Dentistry in Chapel Hill, NC. 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. Serum is collected for the Dental study for assays of the immune response to oral infections, and circulating markers of inflammatory response.

One freshly voided urine specimen is collected from each participant at the field center and separated into three samples. Two samples, one for the determination of creatinine and one for the determination of albumin, are stored at the Minneapolis ARIC field center. One urine sample is stored at the ARIC Hemostasis Laboratory for the measurement of hemostatic metabolites.

The procedures for the collection, processing and shipment of blood samples and urine samples are described in separate sections within this manual of operations.

The foundation on which all of these tests is based is the blood and urine 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. For example, 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 60,000 samples over an extended period of 12 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.

2.0 PREPARATION OF THE BLOOD SAMPLES

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 4 of approximately 60 ml of blood from each participant. A total of 7 tubes of blood of various sizes are routinely collected on the majority of ARIC participants. Exceptions include participants who are ineligible for the oral glucose tolerance test (OGTT) who do not have the 2 hour post-load glucose tube drawn, participants from field centers which do not have a local laboratory provide a hematology panel, and participants who have one extra tube drawn for quality control analyses. The smallest tubes contain less than a teaspoon (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 laboratory assistant should be properly attired in a clean lab coat.

2.2 Staff Certification Requirements

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 pre-labeled with the appropriate code numbers for the participant. A list of equipment, suppliers, and vendors is provided in Appendix I. A checklist of supplies for the blood drawing workstation 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. These tubes are described in detail in the next section.
- Sterile, disposable 21 gauge butterfly needles
- A plastic Vacutainer holder
- Vacutainer Luer adapters
- Sterile alcohol swabs
- Gauze sponges
- A tourniquet
- Bandages ("Band Aids") and paper tape
- An ice water bath filled with ice and water approximately 10 minutes before blood drawing

2.3.2 Blood Collection Tubes

About 60 ml of blood are drawn from each participant using seven Vacutainer tubes. The tubes for the minimum ARIC study are identified as tubes 1-5, with tube 5 labeled with a barcoded dental label. Tube 6 is an optional hematology and tube 7 is for the oral glucose tolerance test. [Samples from these 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 4.5 ml blue stoppered tube containing 0.5 ml of 3.8% sodium citrate anticoagulant. This tube is filled with 4.5 ml of blood and inverted eight times. The plasma from this tube 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 this tube be kept cold in a refrigerated centrifuge or placed in an icewater bath until it is aliquotted into their respective tubes. Of all of the tubes which are centrifuged during Stage I, this tube is aliquotted last. The rack containing these aliquots is transferred to the refrigerator immediately after plasma is aliquotted.

Tube #3 and #4 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 and buffy coats are sent to the Central Lipid Laboratory at Baylor College of Medicine in Houston.

Tube #5 is a 9.5 ml red and gray stoppered tube. 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 for 10 minutes and the serum is removed, elicited into 4 red screw cap microfuge tubes, frozen at -70° to -80° C, and stored for bi-weekly shipment to the Dental Research Center at the University of North Carolina.

Tube #6 is an optional 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 for a standardized set of hematology tests.

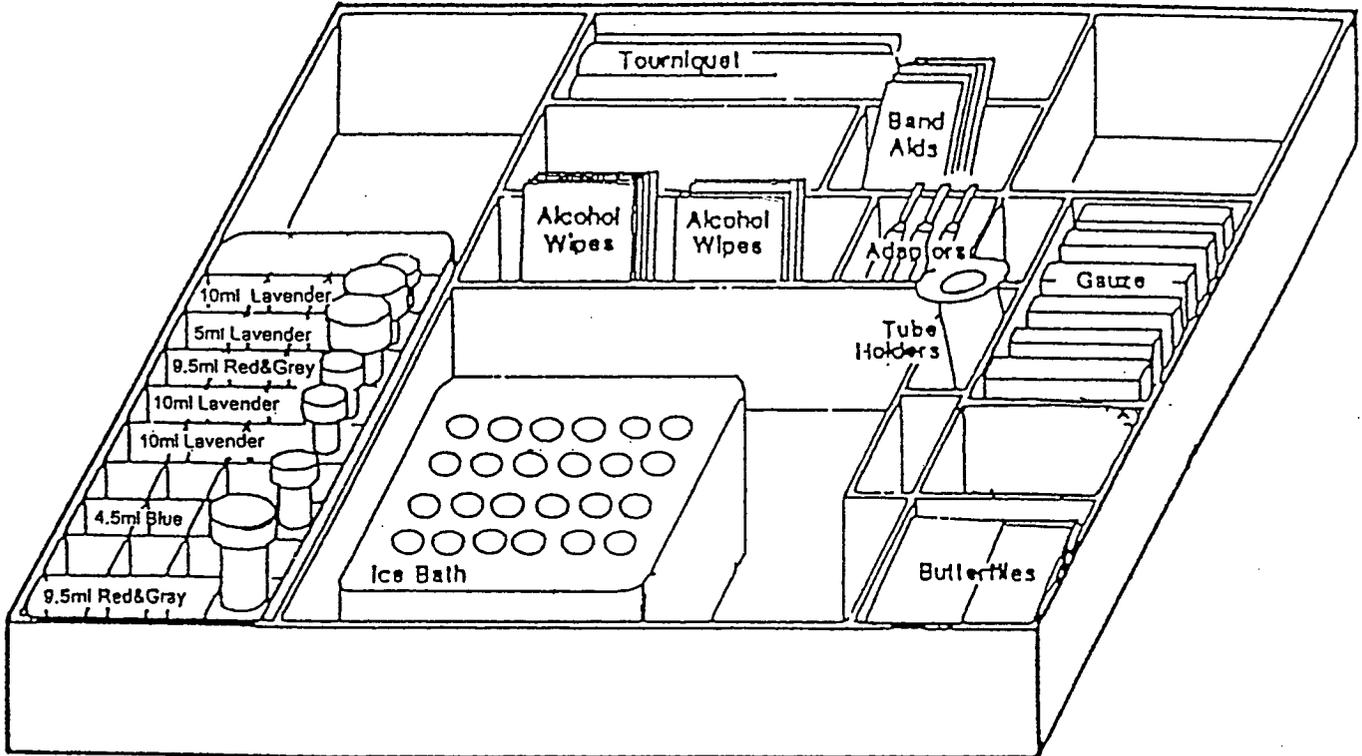
Tube #7 is 10 ml lavender stoppered tube for OGTT containing the anticoagulant, EDTA. It is drawn 2 hours (\pm 10 minutes) after the participant began to drink the glucola. After the tube fills, invert it 8 times and place it in the ice water bath. Record the time at which the tube was drawn on the Oral Glucose Tolerance Administration Form (Appendix XIV). A glucose determination will be performed on the plasma from this tube. 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. The tube is then placed in the centrifuge for a 10 minute spin. The plasma from this tube is sent to the Central Lipid Laboratory at Baylor College of Medicine in Houston.

2.4 Blood Collection Tubes: Labeling and Set-up

Seven (or six, since tube #6 is optional) tubes are drawn in the following sequence:

Tube #1:	9.5 ml red and gray stoppered tube
Tube #2:	4.5 ml blue stoppered tube
Tube #3:	10 ml lavender stoppered tube
Tube #4:	10 ml lavender stoppered tube
Tube #5:	9.5 ml red, gray stoppered tube - Dental Study
Tube #6:	5 ml lavender stoppered tube - optional hematology
Tube #7:	10 ml lavender stoppered tube - 2 hour OGTT sample

Figure 1. Blood Sample Collection Tray



2.4.1 Blood Samples for the Lipid and Hemostasis Laboratories

For blood samples that are sent to the ARIC Lipid and Hemostasis laboratories, strips of pre-numbered adhesive ARIC participant ID 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 24 hours prior to blood collection. Write "3" on tube #3 so it won't be confused with tube #4. 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.

2.4.2 Blood Samples for the ARIC Dental Study

For blood samples to be sent to the UNC School of Dentistry, strips of adhesive ARIC Dental Study participant ID labels are distributed to each field center from the ARIC Dental Study. These labels are pre-printed with a readable numeric participant ID and its bar code equivalent. The ARIC Dental Study IDs include an expanded ARIC participant ID which begins and ends with an additional sequence of characters. The code begins with "AR", followed by the code letter for each site (F for Forsyth, J for Jackson, M for Minnesota, and W for Washington County). The regular 6 digit ARIC participant ID follows the initial three letters. Labels for serum samples end with a 3 digit code: "321", "322", "323" or "324". The "3" in the third position from the end indicates a serum sample. The "21", "22", "23" and "24" uniquely numbers the serum sample aliquot for each participant. For example, the ARIC Dental Study ID of "ARF123456321" has the "AR" for ARIC, the "F" for Forsyth County, the 6 digit ARIC participant ID (123456), and the three digit suffix which distinguishes one serum aliquot from another. Additional, pre-printed ARIC Dental Study labels with a "000" suffix are also included in the strips of labels sent to each field center. Except as described below in the section on Replacement Labels, labels ending in "000" are only used to identify paper forms.

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.

2.4.3 Replacement Labels for the ARIC Dental Study

When a bar-coded participant ID label for an ARIC Dental Study storage tube is missing or has been accidentally destroyed, replacement bar codes can be re-printed and shipped to the field centers on an "as-needed" basis. Labels can be printed at the UNC School of Dentistry as late as 5:00pm EST the afternoon preceding a participant's scheduled ARIC exam, for delivery to the field center by priority Federal Express the following morning. Frances Smith at the University of North Carolina School of Dentistry serves as the contact person for ordering replacement labels (919-962-0233).

When a shortage of Dental Study labels with the terminal digits which uniquely identify a participant's four aliquots ("321", "322", "323" and "324") is not

recognized with sufficient time for replacement labels to be shipped to a field center, the participant's bar-coded labels with the terminal "000" digits may be substituted and applied to the storage vial. (The "000" labels are normally reserved for paperwork or temporary identification of a sample.) These "000" replacement labels are corrected by hand with a black Sharpie marker to indicate the appropriate suffix ("321", "322", "323" or "324"), and a line drawn across the bar code with a black Sharpie marker to indicate to the receiving laboratory at the UNC School of Dentistry that the labels have been substituted. Only a black Sharpie permanent marker can be used when marking tubes or labels.

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:

- 5 - 1.5 ml yellow polypropylene microsample tubes
- 5 - white screw caps for 2 ml vials
- 3 - 1.5 ml blue polypropylene microsample tubes
- 21 - 2 ml white polypropylene screw top vials
- 10 - lavender screw caps for 2 ml vials
- 4 - red screw caps for 2 ml vials
- 2 - brown screw caps for 2 ml vials
- 6 - plastic transfer pipettes

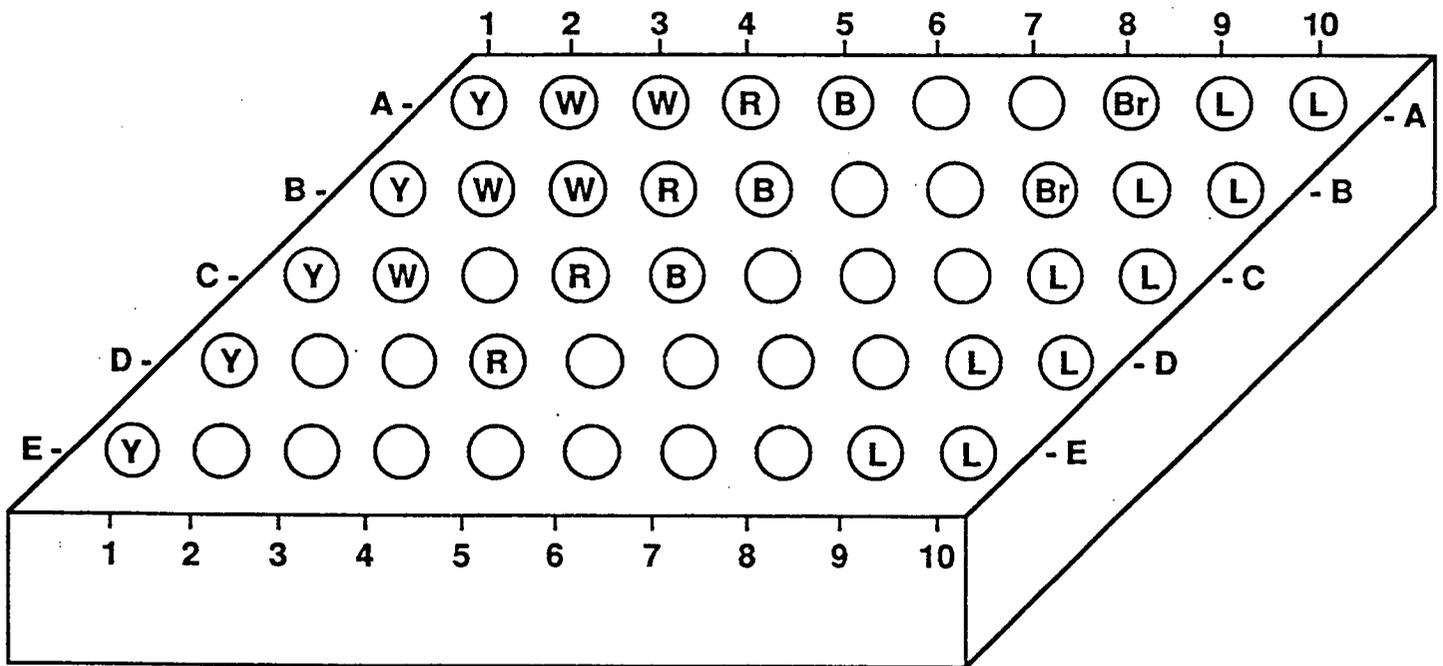
The plastic sample aliquot tubes are labeled with the study-specific participant ID number. Note that the 2.0 ml tubes for the ARIC dental study samples are labelled with the bar-coded IDs. All other tubes have the standard ARIC participant ID labels. Blood for the dental study assays is collected on all ARIC participants, even if they do not have the dental examination.

The labelled aliquot tubes are arranged in the sample tray, and matched up with the attached diagram (Figure 2) in the following order:

- Col 1: 5 yellow micro sample tubes in wells A-E
- Col 2: 3 white vials with white screw caps in wells A-C
- Col 3: 2 white vials with white screw caps in wells A-B
- Col 4: 4 white vials with red screw caps in wells A-D.
- Col 5: 3 blue micro sample tubes in wells A-C.

- Col 8: 2 white vials with brown screw cap in wells A-B.*
Col 9: 5 white vials with lavender screw caps in wells A-E.
Col 10: 5 white vials with lavender screw caps in wells A-E.

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 lavender screw cap**
- Br = White vial with brown screw cap**
- R = White vial with red screw cap**

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 ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$).
6. Perform Q.C. check on refrigerator temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$).
7. Perform Q.C. check on freezer temperature ($-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$).

Approximately 10 minutes before scheduled participant arrival:

1. Fill ice bath 3/4 full with crushed ice.
2. Fill ice bath with cold water.

At participant arrival:

1. Check that the ID number on the tubes matches the participant ID on the Laboratory Form (Appendix III).
2. Check that duplicate Quality Control tubes are prepared and labeled if needed.

2.7 Laboratory Form

ID Number, contact year, and name should be entered on the Laboratory form (Appendix III) prior to the arrival of the participant.

3.0 VENIPUNCTURE

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

NOTE: Please see Appendix XIII (A-35) 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 OSHA approved cleaning solution 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 containers 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 (with their consent) and that they will be contacted if clinically important tests are abnormal.

Continue to fill in The ARIC Laboratory Form (see Appendix III). Always verify participant, by verbally asking: "What is your full name, please?". 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 Laboratory 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. Since HRVs are performed prior to venipuncture in Visit 4, the participant should be in the sitting position 5 minutes prior to venipuncture to the extent that it is feasible.

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 12 (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. 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 tube 2. A tourniquet may be reapplied during tubes 3, 4, 5 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 Laboratory form and Shipping form.
6. 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.
7. 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.
8. 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 #2, restick the participant beginning with tube 1. Discard all tubes from the previous attempt. If blood flow stops after tube #2, 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.

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. Remove tourniquet.

Draw Tube #2 (4.5 ml blue top). Gently invert 8 times then immediately place in ice bath.

Draw Tube #3 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #4 (10 ml lavender top). Invert 8 times then place in ice bath.

Draw Tube #5 (9.5 ml red and gray top). Invert 8 times then place at room temperature and start 30 minute timer.

Draw Tube #6 (optional)(5 ml lavender top). Invert 8 times then place at room temperature.

Draw tube #7 (after 2 hours have elapsed) (OGTT)(10 ml lavender top). Invert 8 times then place in ice bath.

Finish venipuncture.

4.0 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, tubes #1, 5 and tube #6 are incubating at room temperature. Tubes #2, #3, #4 are in the ice water bath.

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

Tubes #1 and 5 remains incubating at room temperature. Store tube #6 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 #3 and #4)

1. Remove the sponge rack from the refrigerator. Remove lavender stoppered tubes (#3 and #4) from centrifuge. Allow blue top (#2) to remain temporarily in the refrigerated centrifuge. Alternatively, remove all tubes from the centrifuge and place them in the ice bath.
2. Put tubes #3 and #4 in wells 8D and 8E of the sample preparation tray which contains sample tubes labeled with the corresponding participant number. Remove the stoppers.
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 approximately 1.5 ml of plasma from one lavender top tube into the two sample tubes in wells 9E and 10E. Using the same plastic pipet, transfer any remaining plasma from the lavender top tubes 3 and 4 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.

GLUCOLA

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 2:00

	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 x g		Aliquot serum into 5 white vials seal with white screw caps	5 white cap vials	5 white cap vials in 3" x 6" bag	Central Hemostasis
Tube 2 Blue tops 4.5 ml room temperature	Ice bath	Centrifuge 10 minutes at 4 C 3000 x g	Aliquot plasma into 3 blue micro sample tubes	Refrigerate			3 Blue micro sample tubes	3 blue tubes in 3" X 6" bag	Central Hemostasis Laboratory
Tube 3 & 4 Lavendar tops 10 ml room temperature	Ice bath	Centrifuge 10 minutes at 4 C 3000 x g	Aliquot plasma into 10 white vials seal with lavendar screw caps. Transfer buffy coats into 2 white vials; seal with brown screw caps.	Refrigerate			10 lavender cap vials 2 brown cap vials	10 lavender cap vials 2 brown cap vials in 3" x 6" bag	Central Lipid Laboratory
Tube 5 Red and gray 9.5ml room temperature	Room temperature	Incubate at room temperature 30 minutes		Centrifuge 10 minutes at 4C 3000 x g		Aliquot serum into 4 white vials with red screw caps	4 red cap vials	4 red cap vials into box	UNC Dental Laboratory
Tube 6 Lavender top 5 ml	Room temperature	Refrigerate	OPTIONAL COLLECTION AT FIELD CENTERS						Field Center Hematology Lab
Tube 7 2 hr OGTT Lavendar top 10 ml	Ice Bath	Centrifuge 10 minutes at 4 C 3000 x g	Aliquot plasma into 5 yellow micro sample tubes				5 yellow micro sample tubes	5 yellow micro sample tubes in 3" x 6" bag into 6x6 bag with lipids	Central Lipid Laboratory

Figure 3. Sample Processing Flow Sheet

Figure 4 : Blood Sample Processing Sequence

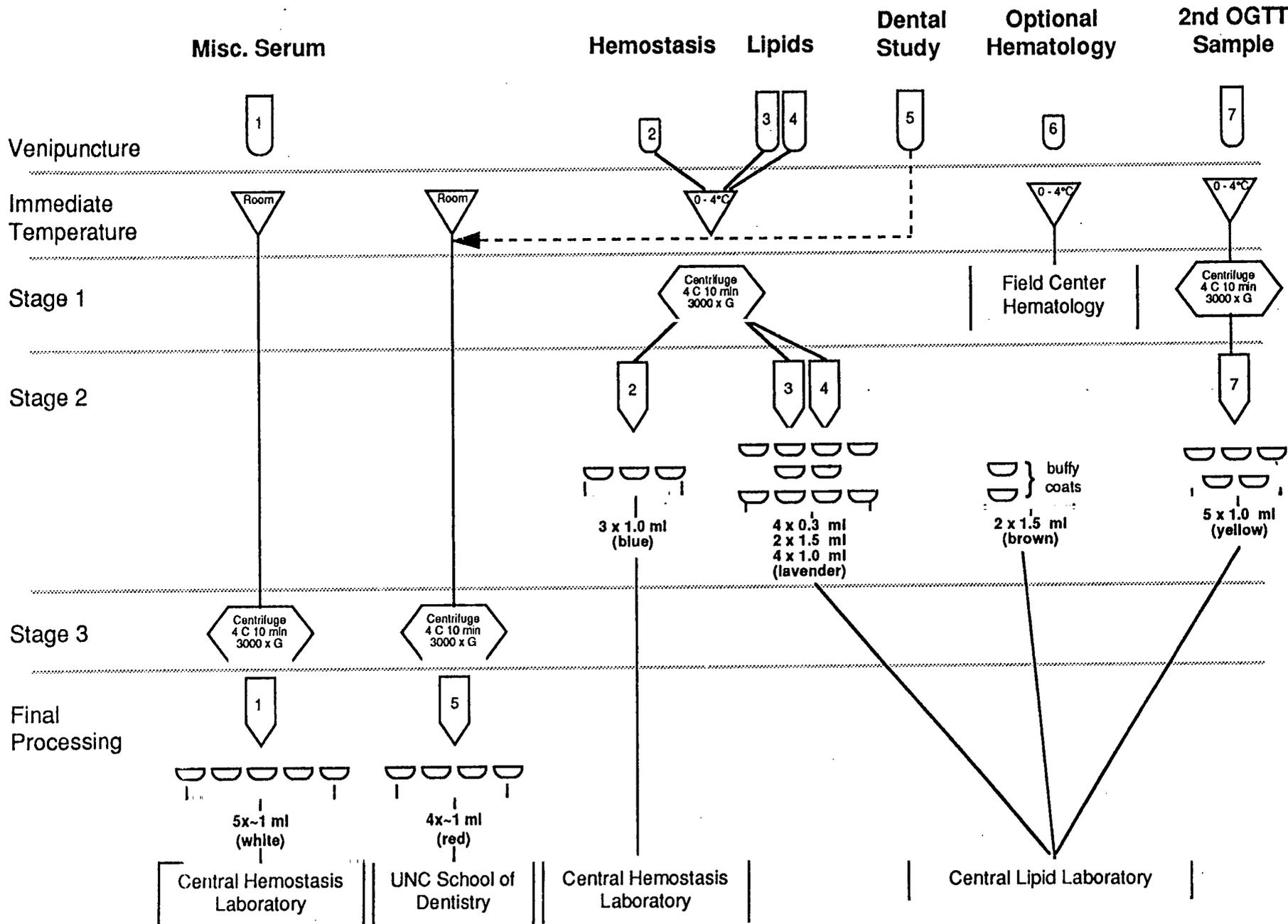
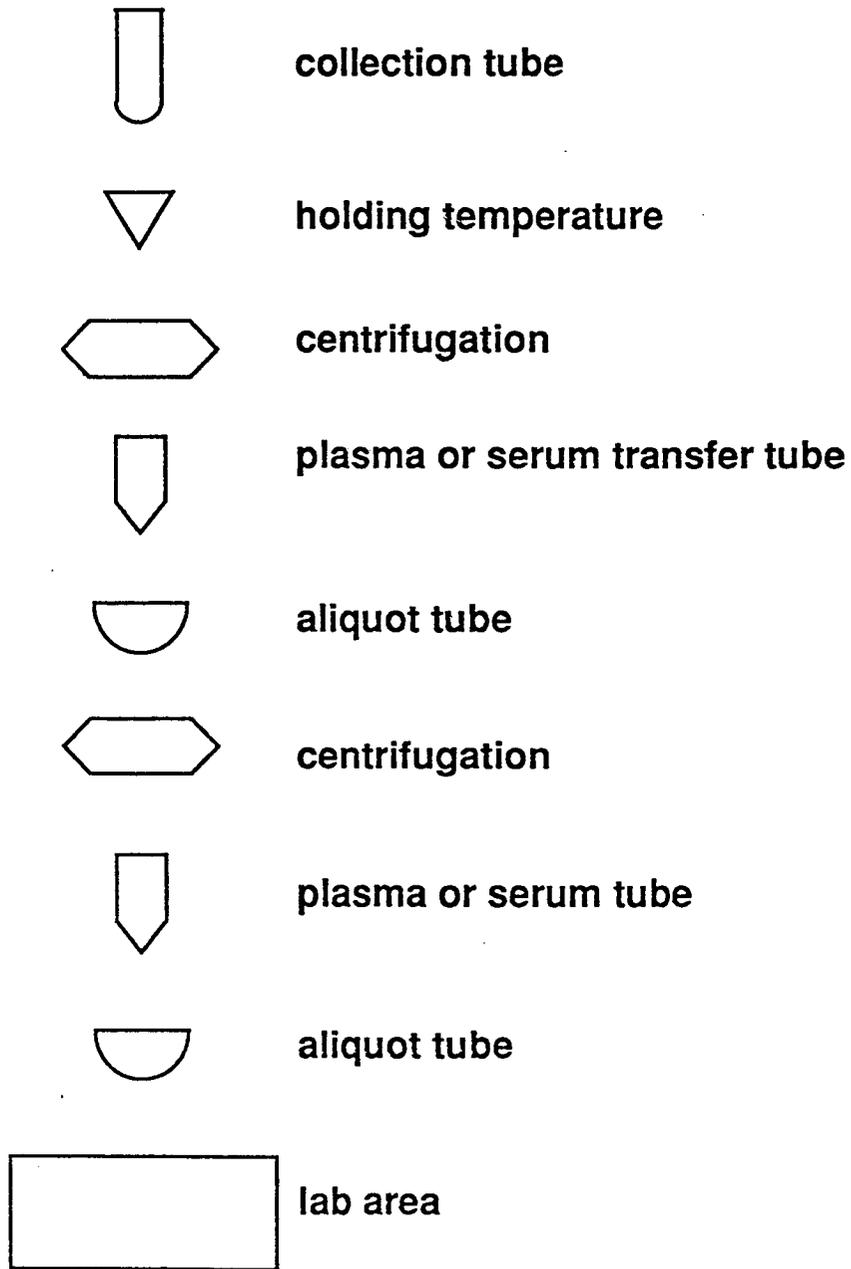


Figure 4. Blood Sample Processing Sequence

Legend to Figure 4

Sample Processing Sequence



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.

Restopper the sample collection tubes 3 and 4 and discard them in a biohazard waste container.

4.3.2 Blue Stoppered Tube (Tube #2)

1. Remove the blue stoppered tube from the refrigerated centrifuge. Place the tube in well 5E in front of the blue sample aliquot tubes. Remove the stoppers.
2. Using a plastic transfer pipet, transfer *all but approximately 1 ml* of the plasma from the blue top tube, in approximately equal aliquots, into each of the three blue sample aliquot tubes placed in the wells behind it.
3. Fasten the caps on the sample aliquot tubes and replace them in the sponge sample tray.
4. Replace the stopper on the blue top blood collection tube 3 and discard it in a biohazard container. 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 tubes #1 and 5 at 3,000 x g for 10 minutes. Record time of beginning to spin Tube #1 on the Laboratory 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 Tubes (Tubes #1 and 5)

1. Remove the 1st red and gray top tube from the centrifuge and place it in well 2E of the sponge test tube rack in front of the three white sample tubes, and *then tube 5 and place it in well 4E.*
2. Remove the stopper from the tube. Use a plastic transfer pipette, aliquot the serum equally into the five white tubes in wells 2A-2C and wells 3A and 3B.

3. Fasten white screw caps on each of the vials in wells 2A-2C and wells 3A-3B.
4. Replace the stopper on the red and gray stoppered blood collection tube and discard it in a biohazard waste container.
5. Remove stopper from tube #5 and using plastic transfer pipette aliquot serum into the four white tubes in wells 4A-4D.
6. Fasten red screw caps on each of the vials.
7. Replace the stopper of #5 and discard it in biohazard container.

4.6 OGTT Lavender Stoppered Tube (tube #7)

1. Remove the sponge rack from the freezer. Remove tube #7 (lavender stoppered tube) from centrifuge.
2. Put tube #7 in well 2E of the (OGTT)sample preparation tray. Remove the stopper.
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 equally into each of the yellow microsample tubes in wells 1A-1E.
4. Fasten the caps on the sample aliquot tubes and replace them in the sponge.
5. Replace the stopper on the lavender top tube #6 and discard into biohazard container.
6. Replace the sponge in -70° freezer.

4.7 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 Laboratory form.

5.0 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 three 3x 6 storage bags and one 6x6 storage bag with the appropriate participant number. (This should be done the day before blood collection.)
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 10 white vials with lavender screw caps and the 2 white vials with brown screw caps into a 3x6 bag and 5 yellow *micro sample tubes* into another pre-labeled 3x6 storage bag. Again verify that tubes and bag are numbered correctly. Press the air out of the bag and seal. Place both bags into a 6x6 storage bag. 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 three blue sample tubes and the 5 white capped vials into a third pre-labeled 3x6 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

When all of the blood collection tubes have been aliquotted into their respective microsample tubes and the appropriate colored caps attached, the entire rack is placed upright into the -70°C freezer. After a minimum of 30 minutes, frozen samples are packaged for storage and shipping. Place the four red screw top tubes from the sponge (4A - 4D) into a 2" X 5" fiberboard box with a 10 X 10 grid, labeled with starting date for that box and collection center name. Assign left/right and top/bottom sides of the box for orientation purposes. Begin filling the box in the upper left corner of the box, continuing with successive patients from left to right. Upon completely filling the box, there should be 25 participants per box. Storage and shipping forms should be immediately completed to match orientation of patient samples in boxes.

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 6x6 bag containing the 10 lavender and 2 brown screw cap tubes and 5 yellow micro sample tubes are placed in the LIPID box.

The 3x6 bag containing the remaining eight 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. For Lipid and Hemostasis Laboratory samples, there are 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.

Shipping containers with frozen sera are sent to UNC by Federal Express Priority Overnight to ensure receipt within 24 hours. Empty styrofoam containers are returned to the field centers by UPS. Ship frozen sera in the fiberboard boxes used for storage. Arrange with the Dental Team for combined shipping of all specimens for dental analysis in the same shipping container. All samples should be contained in white fiberboard boxes, labeled "GCF", "Plaque", or "Serum" to designate contents. There is no minimum shipping requirement. Ship all samples accumulated since the previous shipment, shipping on the first and third Mondays of each month. Samples can be shipped on Tuesday if the field center is closed on Monday, but the receiving laboratory at UNC must be notified of the change in shipping.

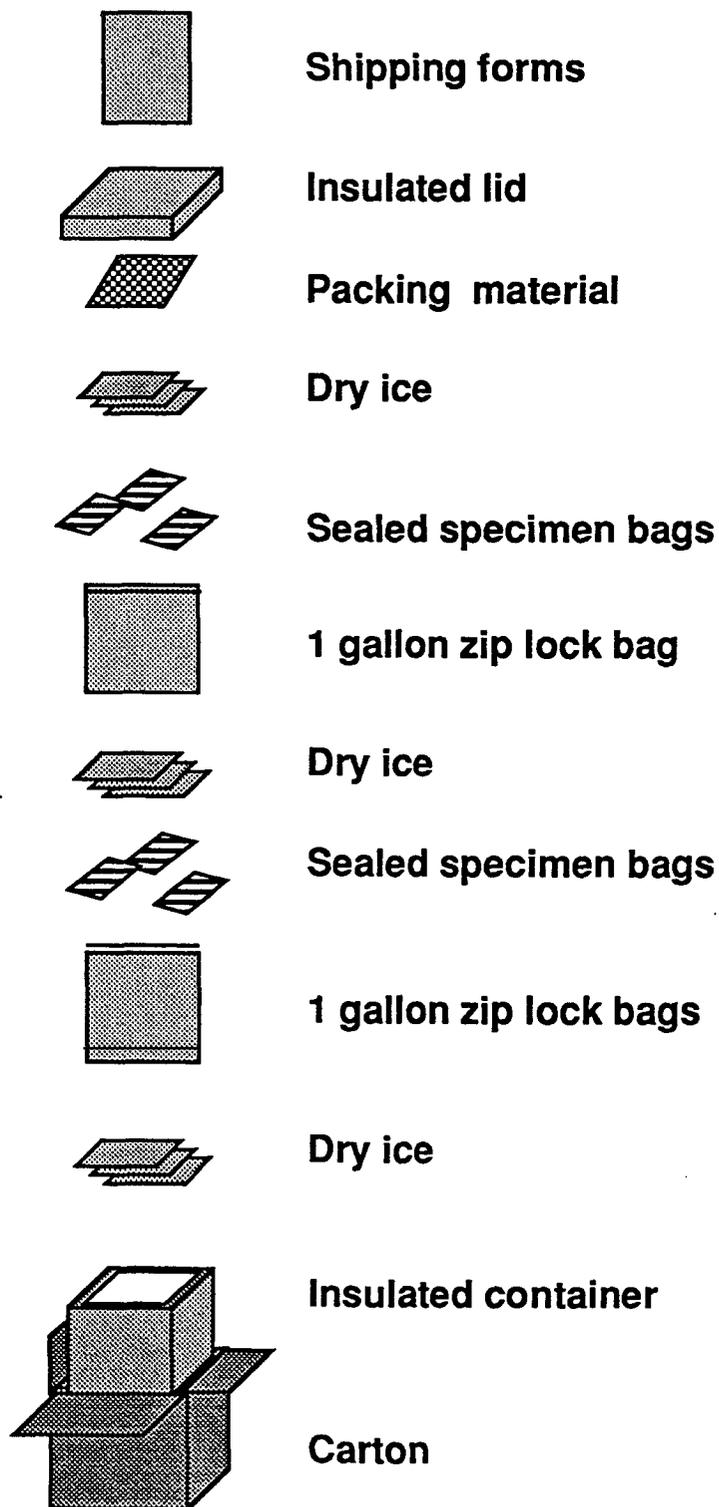
5.3.1 Packaging Instructions for Lipid and Hemostasis Laboratory Samples

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 5. 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 insulated lid. The shipping forms with instructions are shown in Appendix IV.
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 to Lipid and Hemostasis Laboratories



5.3.2 Packaging Instructions for ARIC Dental Study Samples

1. Place a 3" layer of dry ice (approximately 3 lbs.) on the bottom of the styrofoam box.
2. Place the white fiberboard boxes containing specimens on the layer of dry ice.
3. If boxes are stacked greater than 5" deep (more than 2 boxes deep), place additional dry ice between the layers of boxes.
4. Top with an additional 3" of dry ice.
5. Place the paper shipping forms (Appendix IV) and dental exam forms (GCF and Plaque acquisition form) on top of the dry ice.
6. Seal the box tightly with strapping tape.
7. Alternately, paperwork may be placed on top of the styrofoam cover if an outer shell cardboard box is used.
8. Attach Federal Express forms to the outside of the package, specifying:
 - Section 4 - Priority Overnight
 - Section 5 - Other packaging
 - Section 6 - Does NOT contain dangerous goods, DO check the dry ice box and fill in number of packages X weight of dry ice (i.e. 1 X 7 kg).
9. Shipping containers to the University of North Carolina Dental Research Center are addressed as follows:

Francis Smith
 UNC School of Dentistry
 226 Dental Research
 Chapel Hill, NC 27599-7455
 Telephone: (919) 962-0633

5.3.3 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.

5.3.3.1 Hemostasis Laboratory

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

Nena Aleksic
Division of Hematology
ARIC Central Hemostasis Laboratory
 University of Texas Medical School

Room 5.434
6431 Fannin
Houston, Texas 77030
Telephone: (713) 500-6779

5.3.3.2 Central Lipid Laboratory

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

Louis Smith, MD
ARIC Central Lipid Laboratory
Atherosclerosis Clinical Laboratory
MS F701, Room F756
6565 Fannin
Houston, Texas 77030
Telephone: (713) 790-4351

5.3.3.3 University of North Carolina

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

Frances W. Smith
Dental Research Center
CB # 7455, 226 DRC
University of North Carolina
Chapel Hill, North Carolina 27599-7400
Telephone: (919) 966-7451

6.0 URINE PROCESSING

Three urine samples are prepared from the urine specimen, one for the measurement of hemostatic metabolites, one for the determination of creatinine and one for the determination of albumin. Because the addition of acid/alkaline substances (used to adjust the pH of the urine) disrupts the measurement of creatinine, the sample for the measurement of creatinine **MUST** be elicited first. After the aliquots for creatinine and albumin have been completed, 40 ml of the remaining sample are transferred into the centrifuge tube to be shipped to the Hemostasis Laboratory (regardless of whether the pH has been adjusted).

Labelled urine samples should be placed in the designated specimen refrigerator for storage prior to processing as soon as possible after the specimen has been voided. This can be done either by the participant or an ARIC staff member, as determined by local option. However, procedures need to be set up at each field center to verify that urine samples are not inadvertently left out at room temperature.

Urine samples need to be processed and frozen as soon as possible, and within a maximum of 12 hours of collection. In the interim, urine samples need to be refrigerated, and may remain at room temperature for a maximum of 4 hours. Optimally, they should be refrigerated as soon as possible after the specimen has been voided and left there until processed. Urine samples that have remained at room temperature for more than 4 hours, or are not processed and placed in the freezer within 12 hours of collection are thrown out. In that case, Item 17 on the Laboratory Form (Urine sample collected?) is coded NO, an explanatory notelog is completed for that question, and the skip pattern instructions on the form are followed.

6.1 Labeling of Aliquoting Vials

The technician prepares the work area by laying out a plastic transfer pipette, the two 3.5 ml aliquotting vials, and the 50 ml centrifuge tube. An ID label is affixed to each specimen vial. The cryovial with the yellow cap insert is used for the CREATININE specimen and the blue cap tube for the ALBUMIN. The orange top centrifuge tube is used for the specimen shipped to the Hemostasis Laboratory. ID labels are placed horizontally on the aliquotting vials (as is the standard for ARIC).

6.1.1 Sample Preparation of Creatinine

Pipette 3 ml of the collected urine into the 3.5 ml cryovial with the YELLOW cap insert for the CREATININE vial.

6.1.2 Sample Preparation of Albumin

Label a plastic graduate cylinder with one of the participant's ID labels. Transfer 100 ml of the collected urine into the graduated cylinder. Measure the urine pH with pH paper. If the value is 7.0, aliquot 3 ml of urine into the ALBUMIN vial (BLUE cap insert), cap and close it.

If the urine is acidic (pH below 7.0), wearing gloves and protective eyewear or working under the lab top shield, add 3 to 4 drops of sodium hydroxide (3N) to the 100 ml of urine in the plastic graduated cylinder, and mix with a stirring rod. Alternatively, parafilm may be used to cover cylinder and invert after adding acid. Measure the pH again. If the value is 7.0, aliquot the sample. If the urine is alkaline (pH above 7.0), wearing gloves and protective eyewear, add 3 drops of hydrochloric acid (3N) into the plastic graduated cylinder, and mix with the stirring rod. Check the pH again. Continue this process by adding one drop at a time, stirring after each addition, until the proper pH is reached. When the proper pH is reached (7.0), aliquot 3 ml of urine into the albumin vial with the BLUE top. (See Appendix XVI for reagent preparation).

6.1.3 Sample Preparation of Hemostatic Metabolites

Transfer 40 ml of urine from the graduated cylinder into the 50 ml, orange-cap centrifuge tube.

Complete the LABORATORY (LABB) Form (Appendix III), noting the adequacy of the volume and whether the creatinine, albumin and hemostatic metabolite samples were successfully processed. Dispose of the remaining urine. Rinse the plastic graduated cylinders with Liqui-Nox, followed by a rinse with distilled water. Freeze all samples immediately, in the upright position.

6.1.4 Procedures for Small Urine Samples

If the urine sample is inadequate to process the three samples, check to see if a second sample was provided. If there is a second sample and it (in and of itself) is adequate for processing, use the second sample (record the time voided on the Laboratory form based on that sample) and discard the first sample. If neither are adequate, combine the specimens, and transcribe the latest voiding time on the Laboratory form. If there appears to be inadequate urine for the creatinine and albumin specimens, split the sample into these two vials, and indicate on the laboratory form that no urine is to be shipped to the Hemostasis Laboratory because of insufficient volume. If the volume of urine available in the graduated cylinder after completing the creatinine and albumin specimens is greater than 5 ml, transfer all the remaining urine into the centrifuge tube (up to 40 ml), for shipment to the Hemostasis Laboratory.

For total urine samples less than 100 ml, there is concern about the dilutional effect of any substances added for pH adjustment. Therefore, the sodium hydroxide or hydrochloric acid should be added one drop at a time to bring the sample to the proper pH.

6.1.5 Procedures for Collection of Urine Samples Contaminated with Blood

Although urine samples contaminated with blood will affect the measurement of albumin and make pH titration more difficult, these specimens should not be thrown out. All urine samples collected from ARIC participants that have adequate volume of processing are kept, including those that are (appear to be) contaminated. Documentation of the contamination, however, is useful. Use the note log associated with the first question for urine collection (Item 17) on the Laboratory (LABB) form. When the urine sample is collected (Item

17=YES), but the urine sample is, or appears to be, contaminated, enter "sample contaminated with blood" in the note log for Item 17.

6.2 Storage Instructions for Urine Samples

6.2.1 Storage Instructions for Creatinine and Albumin Samples

1. Freeze all urine sample aliquots immediately at -70° C in the upright position.
2. Order storage and shipping boxes supplied by Baxter Diagnostics (Figure 3.5 and Appendix 3.2.a), which have spaces for 81 vials. Number each box with an initial letter for your field center (F, J, M, W). Use consecutive numbers from the range assigned to the field centers: (Forsyth County, 100-199; Jackson, 200-299; Minneapolis, 300-399; Washington County, 400-499). This letter and number are written in big letters on all sides of the box.
3. Within a storage/shipping box, pack the samples in order of the date drawn, putting a single participant's two specimens (CREAT and ALB) side-by-side in a row. Participant One's CREAT sample is placed in position 1 and the ALB sample in position 2. The CREAT and ALB vials of Participant Two are placed in positions 3 and 4, respectively. Position 81 is always empty.
4. Record the Box and Position numbers on the participant's LABORATORY (LABB) Form (Appendix III).
5. Record the IDs in each grid of the box (Figure 6) on a Box Log Form (see Appendix VI).

6.2.2 Storage Instructions for Hemostatic Metabolite Samples

1. All 40 ml sample aliquots are frozen in the upright position at -70° C for a minimum of $1\frac{1}{2}$ hours to allow for thorough freezing.
2. One week's worth of specimens are stored frozen at the field center.

6.3 Shipping Instructions

Creatinine and albumin are shipped to the ARIC Minneapolis field center when there are four full boxes filled (Figure 6).

Minneapolis ARIC Field Center
Suite 204 Bassett Creek Medical Ctr
5851 Duluth Street
Golden Valley, MN 55422-3956

Contact Person: Carolyn Campbell
(612) 627-4253

Hemostasis metabolite samples are shipped weekly to the ARIC Hemostasis Lab.

University of Texas
Medical School at Houston
6431 Fannin, MSB 5.434
Houston, TX 77030

Contact Person: Nena Aleksic
(713) 500-6779

Packaging the Creatinine and Albumin Urine Sample Storage Box

The packaging and shipping of frozen samples is the responsibility of each field center.

1. Samples must be frozen for at least 24 hours before they are packaged to be shipped.
2. The respective Shipping Sheet (Appendix IV) is enclosed with each shipment.
3. Samples are shipped by air courier so that they arrive at the laboratory by 10:30am the following morning. At least 10 pounds of dry ice is necessary to keep samples frozen for up to 48 hours; 15-20 pounds is recommended. Containers are returned as soon as possible.
4. Because creatinine and albumin samples are shipped to the Minneapolis field center on a non-standardized schedule, the field center technician notifies the Minneapolis field center contact person to expect a shipment either the day of shipping or the morning on which the shipment should arrive. If the shipment is not received by noon, the contact person notifies the field center immediately. Field center staff initiate a trace on the missing samples in order to have them located before thawing occurs.

Because the hemostatic metabolite samples are shipped weekly to the Hemostasis Laboratory on a predetermined schedule, the contact person at the hemostasis Laboratory notifies the field center if a shipment is not received by noon on the expected day. Modification in shipping schedules are made between the Hemostasis Laboratory and the field centers when shipping does not occur on the expected schedule, e.g., the shipment or receipt of specimens date is a holiday.

5. Each shipment is labelled "CONTENTS TO REMAIN FROZEN/DRY ICE LABEL".
6. The creatinine and albumin vials are shipped in the storage boxes (see Figure 6) in which they have already been stored at the field center.
7. The boxes with frozen samples for creatinine and albumin are placed upright in an insulated shipping container and surrounded with dry ice pellets. (It is not necessary to place the boxes in ziplock plastic bags before packing in dry ice.)
8. The tubes for the Hemostasis Laboratory are placed in two ziplock plastic bags or one 9x12 Bitran bag.

Figure 6. Grid for Urine Sample Storage Box

1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63
64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	

6.4 Training

Training in the collection, processing, storage and shipping of urine specimens for laboratory technicians is provided centrally, or locally for new staff by a certified laboratory technician at each field center.

6.5 Certification

The lead technician is certified at central training by the central trainer; other technicians are certified either at central training or by the lead technician at the field center. Recertification is done bi-annually (January and July) by observation.

6.6 Quality Control

In addition to annual recertification authorized by the Hemostasis Laboratory, protocol adherence in the performance of each procedure is reviewed at least biannually by the lead technician and annually by Coordinating Center field center monitors. Deviation from protocol and possible remedial actions are discussed with study coordinators and staff at that time. Major deviations are brought to the attention of the Cohort Operations Committee.

6.7 Quality Control Duplicate Urine Samples

As part of the quality control program for laboratory determinations from urine samples (albumin/creatinine and hemostatic metabolites), duplicate specimens are sent to the respective laboratories (ARIC Minneapolis Field Center and the ARIC Hemostasis Laboratory), with one half of each specimen pair sent under the participant's regular ARIC ID, and the other half under a Quality Control (QC) Participant ID. The QC IDs are not distinguishable from the other ARIC IDs so that this forms a blinded, external quality control program for monitoring measurement variability.

On Tuesday of each week, two 3.5 ml phantom QC urine samples are collected, processed and stored for shipment to the ARIC Minneapolis Field Center for albumin/creatinine quality control and one 40 ml urine sample is collected, processed and stored for shipment to the ARIC Hemostasis Laboratory for hemostatic metabolite quality control. See Appendix VIII for the Weekly Urine QC Sample Checklist.

In order for the QC urine samples to be tracked by the Coordinating Center, Section D of the Laboratory Form should be completed.

7.0 QUALITY CONTROL

7.1 Venipuncture and Equipment Records

Quality control procedures performed in ARIC central Hemostasis and Lipid laboratories are addressed in Manuals 8-9. 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. 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 Appendix IX for a sample form. In addition, the actual speed of the centrifuge needs to be checked and recorded monthly with a tachometer. A sample Quality Control checklist is enclosed in this manual (see Appendix X). 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.

7.2 Quality Control Duplicate Blood Samples

As part of the quality control program for laboratory determinations from blood samples (hemostasis, lipids), 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 (See Appendix XV).

To reduce the burden upon ARIC participants, no one person is asked to contribute sufficient extra blood to make a complete set of duplicates for all laboratories. Instead, extra blood is drawn from several participants 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 (lipid), on Thursday, draw tube 7 (OGTT); on Friday, and draw any tubes that might have been missed earlier in the week due to holidays, no-shows, or other reasons.

7.2.1 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:

<u>Day</u>	<u>Tubes</u>	<u>Laboratory</u>	<u>Sample 1</u>	<u>Sample 2</u>
Monday	1	Hemostasis (misc. serum.)	_____	_____
Tuesday	2	Hemostasis	_____	_____
Wednesday	3,4	Lipid	_____	_____
Thursday	7	Lipid (OGTT)	_____	_____
Friday	Make-up any missed above			

7.2.2 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 labeled with one of the Q.C. I.D.s to be used that week, in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. The 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 labeled 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.

7.2.3 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. 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 tube #2 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.

Filling Out the Laboratory Form for Q.C. Blood:
No filling out of a Laboratory form is necessary.

8.0 TRAINING PROCEDURES

8.1 Technician Training and Evaluation

Technicians/phlebotomists must study ARIC Manual 7 and watch a few participant samples being processed. Then they 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. Phlebotomists perform a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow trainees to become comfortable with the procedures before proceeding to live participants.

At this point the technicians/phlebotomists are ready to practice on live volunteers. They practice at least once with just one volunteer at a time and again process the blood entirely by themselves from start to finish. Trainees who do not feel comfortable 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 trainees have must be solved before proceeding to drawing ARIC participants. Before trainees draw blood from any ARIC participant, they must take and pass the practical and written tests (Appendix XII) 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.

8.2 Laboratory Assistant Training

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

After becoming familiar with the participant information forms, the Laboratory assistant 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 Laboratory assistant needs to know the destination of each form, tube, and sample. A checklist (Appendix II) is kept with the material from each participant, and the Laboratory assistant needs to know what each item is on the list.

Before assisting in the blood collection, the procedure should be explained to the laboratory assistant. 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 person is shown how to gently mix the tubes of blood and which are placed or replaced immediately in an ice bath. Once the first participants have been drawn, the laboratory assistant 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 laboratory assistant is ready to assist in the actual drawing procedure.

9.0 FIELD CENTER HEMATOLOGY SERVICES

9.1 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. At Visit 4, hematology studies are done at local option.

9.2 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 that chooses to have hematology studies done 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, and provide the Coordinating Center with documentation of their local hematology laboratory QC programs.

9.3 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. Three data elements are included as hematology results, which are reported to participants, along with laboratory specific reference ranges to support alert and referral procedures at the individual field centers.

- 1) Total hemoglobin (Hb)
- 2) Leukocyte (WBC) count
- 3) Hematocrit

APPENDIX I. EQUIPMENT AND SUPPLIES

Supplies to be obtained by field centers

<u>Supplier</u>	<u>Catalog No.</u>	<u>Description</u>	<u>Approx. No. per Week</u>
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)	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"	
"	S92221-1	Sponge Tube Rack	
Rainin	RT-200	Pipet tips 100-1000 ul	90
		Dry Ice approximately 10 lbs. per shipping box	25 lbs.
Fisher	11-678-24A	Fiberboard boxes (dental) 12/pk	
	11678-24C	Box divider (dental) 12/pk for 100 vial box	

<u>Supplier</u>	<u>Catalog</u>	<u>No. Description</u>	<u>Approx. No. per Week</u>
		<u>Vacutainer Tubes 100/pk</u>	
S/P	B2970-33	Serum Separator Red/Gray BD#6510	60
"	B2994-94	Na Citrate, Blue D#6418	30
"	B2991-54	EDTA-Lavender 10 ml BD#6457	90
"	B2951-65/	EDTA-Lavender 5.0 ml	30

Small Equipment Items

Rainin	P-1000	Automatic Adjustable Pipet 100-1000
	PT-200	Pipette tips 1000ul
"	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 and crushed ice maker

SUPPLY LIST FOR URINE COMPONENT

CATALOG
NUMBERDESCRIPTION

CURTIN MATHESON SCIENTIFIC, INC.

057-12 55 ML TRANSFER PIPETS (graduated to .25 ml) (PK/500)
 366-38 550ML CLEAR POLYPROPYLENE CENTRIFUGE TUBE, PLUG SEAL (SCREW)
 CAP (Corning)
 024-990 10 OZ POLYSTYRENE, NONSTERILE SPECIMEN CONTAINER (CS/500)
 024-991 POLYSTYRENE LIDS (for specimen containers) (CS/500)

FISHER SCIENTIFIC

14-850-10G pH PAPER (pH RANGE 3.0-7.5) (CS/10)
 08-570-21D 100 ML GRADUATED CYLINDER (CS/8)
 12-565-162N 3.6 internally threaded vials 400/pk 4 pks/cs
 12-565-246 yellow cryo cap inserts 4 pks/cs
 12-565-243 blue cryo cap inserts 4 pks/cs

BAXTER DIAGNOSTICS, INC.

R3890-7 FIBERBOARD BOX AND COVER (3" X 5½" X 5½")
 R3894-7 BOX DIVIDER (81 vial size)

URINE SUPPLIES

VENDORS

Baxter Diagnostics, Inc.
1210 Waukegan Road
MP82
McGaw Park, IL 60085

Phone: (800) 234-5227

Curtin Matheson Scientific, Inc.
2 North Point Drive
Suite 300
Houston, TX 77060

Phone: (800) 650-0650 (industrial)

Curtin Matheson Scientific, Inc.
955 Cobb Place Blvd.
Kennesaw, GZ 30144-6802

Phone: (800) 241-7670 (biomedical)
FAX: (404) 590-9014

Fisher Scientific
711 Forbes Avenue
Pittsburgh, PA 15219-4785

Phone: (800) 766-7000
FAX: (800) 926-1166

Sarstedt, Inc.
P.O. Box 468
Newton, NC 28658-0468

Phone: (800) 257-5101
FAX: (704) 564-4003

GENERAL SUPPLIES

General Supplies

Droppers
Disposable gloves
Glass stirring rods
Liqui-Nox
Distilled Water
Parafilm

Shipping Supplies

Dry ice
Biohazard labels
Insulated shipping containers

APPENDIX II. CHECKLIST FOR BLOOD DRAWING WORKSTATION

Sterile, disposable 21 gauge butterfly needles

Plastic Vacutainer holder

Vacutainer Luer adapters

Sterile alcohol swabs

Gauze sponges

A tourniquet

Bandages ("band aids")

An ice bath filled with ice and water approximately 10 minutes before blood drawing

- 2 Red and gray top 9.5 ml tubes; one labeled with ARIC participant ID; one labeled with Dental Study barcode participant ID
- 1 Blue 4.5 ml tube, labeled with ARIC participant ID
- 3 Lavender 10.0 ml tubes, labeled with ARIC participant ID (3rd lavender tube, only if 2 hour post glucose load OGTT is to be drawn)
- 1 Lavender 5.0 ml tube, labeled with ARIC participant ID for optional Hematology

5. Was fasting blood drawn before the glucola/snack? Yes Y
 No N

6. Number of venipuncture attempts:

7. Was the tourniquet reapplied? Yes Y
 No N

8. Phlebotomist ID:

C. BLOOD PROCESSING

9.a. Time at which specimen tubes 2-4 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 specimen tubes 1-4 were placed in freezer: :
 h h : m m

b. AM or PM: AM A
 PM P

12.a. Time at which specimen Tube 6 was spun: :
 h h : m m

b. AM or PM AM A
 PM P

13.a. Time at which specimen Tube 6 was placed in the freezer? :
 h h : m m

b. AM or PM AM A
 PM P

14. Technician ID for fasting samples:
15. Code number of technician processing
post-glucose load samples:
16. Comments on blood drawing/processing: Yes Y
No N

If Yes, Specify: _____

D. URINE SAMPLE

17. Urine sample collected? Yes Y
 No N

18. Date of urine sample: / /
m m / d d / y y

19.a. Time of urine sample: :
h h : m m

b. AM or PM AM A
 PM P

20. Volume adequate for processing? Yes Y
 No N

21. Creatinine/Albumin RECORD box number.

- 22.a. Creatinine vial processed? Yes Y
 No N

b. Creatinine POSITION number.

- 23.a. Albumin vial processed? Yes Y
 No N

b. Albumin POSITION number.

24. Hemostasis vial processed? Yes Y
 No N

25. Technician ID for urine samples.

(LABB screen 1 of 4)

A. MEDICAL HISTORY

1. Has a doctor ever said you had any of the following?

a~ Kidney stones?

Yes (Y), No (N) or Unknown (U)

b~ Any other kidney disease, apart from a temporary infection?

Yes (Y), No (N)* or Unknown (U)*

c~ Have you ever had a kidney transplant or been treated with dialysis for more than 6 months?

Yes (Y) or No (N)

B. FASTING BLOOD DRAWING

2~ Do you have any bleeding disorders?

Yes (Y), No (N) or Unknown (U)

3~ Date of blood drawing:
mm/dd/yy

4.a~ Time of fasting blood drawing:
hh:mm

b~ AM (A) or PM (P)

(LABB screen 2 of 4)

5~ Was fasting blood drawn before the glucola/snack?

Yes (Y) or No (N)

6~ Number of venipuncture attempts:

7~ Was the tourniquet reapplied?

Yes (Y) or No (N)

8~ Phlebotomist ID:

C. BLOOD PROCESSING

9.a~ Time at which specimen tubes 2-4 were spun:
hh:mm

b~ AM (A) or PM (P)

10.a~ Time at which specimen Tube 1 was spun:
hh:mm

b~ AM (A) or PM (P)

(LABB screen 3 of 4)

11.a~ Time at which specimen tubes
1-4 were placed in the
freezer? _____ █
hh:mm

b~ AM (A) or PM (P) _ █

12.a~ Time at which specimen Tube 6
was spun: _____ █
hh:mm

b~ AM (A) or (PM) _ █

13.a~ Time at which specimen Tube 6
was placed in the
freezer? _____ █
hh:mm

b~ AM (A) or PM (P) _ █

14~ Technician ID for fasting
samples: _____ █

15~ Code number of technician
processing post-glucose
load samples: _____ █

16~ Comments on blood
drawing/processing: _ █

Yes (Y) or No (N)
[IF YES, RECORD ON NOTE LOG]

D. URINE SAMPLE

17~ Urine sample collected? _ █

Yes (Y) or No (N)*

(LABB screen 4 of 4)

18~ Date of urine sample: _____ █
mm/dd/yy

19.a~ Time of urine sample: _____ █
hh:mm

b~ AM (A) or PM (P) _ █

20~ Volume adequate for
processing? _ █

Yes (Y) or No (N)*

21~ Creatinine/Albumin RECORD box
number: _____ █

22.a~ Creatinine vial processed? _ █

Yes (Y) or No (N)*

b~ Creatinine POSITION number: _____ █

23.a~ Albumin vial processed? _ █

Yes (Y) or No (N)*

b~ Albumin POSITION number: _____ █

24~ Hemostasis vial processed? _ █

Yes (Y) or No (N)

25~ Technician ID for
urine samples: _____ █

INSTRUCTIONS FOR LABORATORY FORM
LAB, VERSION B, 03/26/96
PREPARED 03/26/96

I. GENERAL INSTRUCTIONS

The LABORATORY Form is completed during the participant's clinic visit to record information on the collection and processing of blood and urine samples. Technicians performing venipuncture and processing blood and urine samples must be certified and should have a working knowledge of the relevant Manuals 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. MEDICAL HISTORY

- 1.a The set of questions of kidney disease are repeated from the AFU Medical History form to update information that was collected previously. A positive response requires a physician diagnosis. The time frame is anytime prior to this interview. Read the question. It should not be necessary to define kidney stones. Continue with item 1.b.
- 1.b Examples of other kidney (renal) diseases are kidney failure, diabetic kidney disease. If NO or UNKNOWN, go to Item 2. If YES, continue with Item 1.c.
- 1.c Read question and record response.

B. FASTING BLOOD DRAWING

2. 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 16.
3. 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:

0	5	/	0	3	/	9	3
month			day			year	

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

4. 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.
5. Check the participant's Itinerary Sheet, or ask the participant if he/she has had the glucola or the clinic snack.
6. Include all venipuncture attempts by all phlebotomists. The same technician should not attempt a venipuncture more than twice.
7. Do not reapply the tourniquet during tubes #2 - #4. Only reapply the tourniquet after tube #4, and only if this is necessary to spare the participant another stick. Specify if a tourniquet reapplication occurred in Item 16.
8. The phlebotomist who performed the fasting blood drawing procedure enters his/her code number in the fields provided. If more than one phlebotomist attempts to draw the blood, enter the code of the first phlebotomist.

C. BLOOD PROCESSING

9. Note the time at which the centrifuge containing tubes 2-4 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 tube #1 began to spin. Fill in the fields using leading zeroes where necessary and indicate AM or PM.
11. Note the time at which samples from tubes 1-4 were placed in the freezer. Fill in the fields using leading zeroes where necessary and indicate AM or PM.
12. Note the time at which the centrifuge containing Tube 6 began to spin. Fill in the fields using leading zeroes where necessary and indicate AM or PM.
13. Note the time at which the sample from Tube 6 was placed in the freezer. Fill in the fields using leading zeroes where necessary and indicate AM or PM.
14. Enter the code number of the technician who began processing the fasting blood samples (tubes 1-4).
15. Enter the code number of the technician who processed the 2 hour post-glucose load sample (tube 6).

16. Include any clarifications or other information relevant to the assays being performed that are not included in the 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.

D. URINE SAMPLE

17. Indicate whether a urine sample was collected. If NO, go to Item 25 and enter your technician ID. If YES, continue:
18. Enter the date on which the urine sample was collected using the standard date format.
19. Transcribe from the participant ID or TIME label on the urine sample container time (in hours and minutes) at which the urine sample was voided. Fill in the fields using leading zeroes where necessary and indicate AM or PM. If the participant voided twice, transcribe the latest time.
20. If urine sample is small, split between the creatinine and albumin vials. If sample is too small to process, select NO and go to Item 25.
21. Enter the RECORD (storage and shipping) BOX Number for the creatinine and albumin samples.
22. If the creatinine sample cannot be processed, select NO (Item 22:a) and go to Item 23.a. If creatinine is processed, record YES (Item 22.a) and the POSITION number of the creatinine aliquot vial in the storage and shipping box (Item 22.b).
23. If the albumin sample cannot be processed, select NO (Item 23.a) and go to Item 24. If albumin is processed, record YES (Item 23.a) and the POSITION number of the albumin aliquot vial in the storage and shipping box (Item 23.b).
24. If the urine sample for the Hemostasis Laboratory cannot be processed, select NO. If this urine sample is processed, select YES. Continue with Item 25.
25. Enter the code number of the technician who processed the urine samples.

APPENDIX IV

A - 15

ARIC VISIT 4 SHIPPING FORM
 CENTRAL HEMOSTASIS LABORATORY
 UNIVERSITY OF TEXAS MEDICAL SCHOOL
 6431 FANNIN
 HOUSTON, TX 77030

BATCH: AR ____ H ____

DATE: _____

PAGE: __ OF __

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

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Use one or more of the following codes to indicate problems and incidents for the pertinent ID and specimen:

BLOOD DRAWING

ND - sample not drawn FC - fist clenching
 PD - partial sample drawn NM - needle movement
 TR - tourniquet reapplied

BLOOD PROCESSING

BT - broken tube LP - lipemic
 CL - clotted OC - other contamination
 HM - hemolyzed

ARIC SHIPPING FORM

BATCH: AR ____ H. ____

PAGE: ____ OF ____

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

VERSION 4.0 4/96

Use one or more of the following codes to indicate problems and incidents for the pertinent ID and specimen:

BLOOD DRAWING

ND - sample not drawn FC - fist clenching
 PD - partial sample drawn NM - needle movement
 TR - tourniquet reapplied

BLOOD PROCESSING

BT - broken tube LP - lipemic
 CL - clotted OC - other contamination
 HM - hemolyzed

ARIC VISIT 4 SHIPPING FORM
 DR. LOUIS SMITH
 ATHROSCLEROSIS CLINICAL LABORATORY
 METHODIST HOSPITAL, MAIL STATION F701, ROOM F756
 6565 FANNIN
 HOUSTON, TX 77030

BATCH: AR ___ L _____

DATE: _____

PAGE: ___ OF ___

LAB USE ONLY	SPECIMEN ID	VIAL COLOR	NUMBER OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			

VERSION 4.0 4/96

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BLOOD PROCESSING

BT - broken tube LP - lipemic
 CL - clotted OC - other contamination
 HM - hemolyzed

ARIC SHIPPING FORM

LAB USE ONLY	SPECIMEN ID	VIAL COLOR	NUMBER OF VIALS	FIELD CENTER COMMENTS	CONDITION ON ARRIVAL
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			
		LAVENDER			
		BROWN			
		YELLOW			

VERSION 4.0 4/96

Use one or more of the following codes to indicate problems and incidents for the pertinent ID and specimen:

BLOOD DRAWING

- ND - sample not drawn
- PD - partial sample drawn
- TR - tourniquet reapplied
- FC - fist clenching
- NM - needle movement

BLOOD PROCESSING

- BT - broken tube
- CL - clotted
- HM - hemolyzed
- LP - lipemic
- OC - other contamination

**Storage and Shipping Form
for Dental Serum**

Date Shipped _____

Position	Date Stored	Participant ID	Position	Date Stored	Participant ID	Comments
1 - 4			53 - 56			
5 - 8			57 - 60			
9 - 12			61 - 64			
13 - 16			65 - 68			
17 - 20			69 - 72			
21 - 24			73 - 76			
25 - 28			77 - 80			
29 - 32			81 - 84			
33 - 36			85 - 88			
37 - 40			89 - 92			
41 - 44			93 - 96			
45 - 48			97 - 100			
49 - 52						

ARIC SHIPPING (FACE) FORMS

APPENDIX V

ARIC Shipping Form

Part I (to be completed at field center)

A - 20

TO: Dr. Louis Smith
Atherosclerosis Clinical Lab
Methodist Hospital
Mail Station F701, Room F756
6565 Fannin Street
Houston TX 77030

FROM: (YOUR ADDRESS HERE)

Shipment packed and sealed:

Time: ___ : ___ (a.m./p.m.)

Date: ___ / ___ / ___

ARIC Batch Number: A R ___ L ___

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 II (to be completed at Central Agency)

Shipment Arrived at Central Laboratory:

Time: ___ : ___ (a.m./p.m.) Date: ___ / ___ / ___

Comments on condition of shipment on arrival: _____

Initials of person unpacking specimens: _____

ARIC SHIPPING (FACE) FORM

A - 21

Part I (to be completed at field center)

TO: Central Hemostasis Lab
6431 Fannin Street
Houston TX 77030

FROM:
(YOUR ADDRESS HERE)

Shipment packed and sealed:

Time: ___ : ___ (a.m./p.m.) Date: ___ / ___ / ___

ARIC batch number A R ___ H ___ _ _ _ _

Reporting period:

Starting date: ___ / ___ / ___ Ending date: ___ / ___ / ___

Total number specimens enclosed: _____

Number of contents pages attached: _____

Other remarks concerning shipment contents: _____

Initials of person packing and completing shipping forms: _____

.....

Part II (to be completed at Central Agency)

Shipment arrived at laboratory:

Time: ___ : ___ (a.m./p.m.) Date: ___ / ___ / ___

Comments on condition of total shipment on arrival: _____

Initials of person unpacking specimens: _____

TO: Dental Research Center
School of Dentistry, Campus Box 7455
226 DRC
UNC
Chapel Hill NC 22599-7455

FROM: Forsyth County ARIC
2060 Beach Street
Winston-Salem NC 27103

Reporting Period:

Starting Date: _____ - _____ - _____

Ending Date: _____ - _____ - _____

TOTAL number specimens enclosed: _____

Other remarks concerning shipment contents: _____

Initials: _____

~~~~~  
.....

Arrived at Laboratory: Time: \_\_\_\_ : \_\_\_\_ a.m. / p.m. Date: \_\_\_\_\_  
Comments on condition of total shipment on arrival : \_\_\_\_\_

Initials: \_\_\_\_\_

# ARIC

A - 23

## URINE SHIPPING SHEET

To: Nena Aleksic, ARIC Central Hemostasis Laboratory

From: (circle one): F J M W

### A. Field Center Package date and time

1.a. Date:

|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
|   |   | / |   |   | / |   |   |
| M | M | / | D | D | / | Y | Y |

b. Time:

|   |   |   |   |   |
|---|---|---|---|---|
|   |   | : |   |   |
| H | H | : | M | M |

2. Number of bags enclosed: (attach all logs)

|  |  |
|--|--|
|  |  |
|--|--|

bags

3. Remarks:

---

---

---

4. Field Center Technician ID:

|  |  |  |
|--|--|--|
|  |  |  |
|--|--|--|

### B. Arrival at lab

S.a. Date:

|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
|   |   | / |   |   | / |   |   |
| M | M | / | D | D | / | Y | Y |

b. Time:

|   |   |   |   |   |
|---|---|---|---|---|
|   |   | : |   |   |
| H | H | : | M | M |

6. Remarks:

---

---

---

7. Laboratory Technician Initials:

|  |  |  |
|--|--|--|
|  |  |  |
|--|--|--|

LAB: TO CONFIRM ARRIVAL, FAX THIS FORM BACK TO FIELD CENTER

# ARIC

URINE SHIPPING SHEET

To: Carolyne Campbell, Minneapolis ARIC Field Center

From: (circle one): F J M W

A. Field Center Package date and time

1.a. Date:

|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
|   |   | / |   |   | / |   |   |
| M | M |   | D | D |   | Y | Y |

b. Time:

|   |   |   |   |   |
|---|---|---|---|---|
|   |   | : |   |   |
| H | H |   | M | M |

2. Number of boxes enclosed: (attach all box logs)

|  |  |
|--|--|
|  |  |
|--|--|

boxes

3. Remarks: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

4. Field Center Technician ID:

|  |  |  |
|--|--|--|
|  |  |  |
|--|--|--|

B. Arrival at lab

5.a. Date:

|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
|   |   | / |   |   | / |   |   |
| M | M |   | D | D |   | Y | Y |

b. Time:

|   |   |   |   |   |
|---|---|---|---|---|
|   |   | : |   |   |
| H | H |   | M | M |

6. Remarks: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

7. Laboratory Technician Initials:

|  |  |  |
|--|--|--|
|  |  |  |
|--|--|--|

LAB: TO CONFIRM ARRIVAL, FAX THIS FORM BACK TO FIELD CENTER

## APPENDIX VI

**Creatinine and Albumin:  
Box Logging Form, Laboratory Form, and Shipping Sheet**

For each box of stored creatinine and albumin samples, record on the Box Logging Form the location of the two specimens for each ID. Write the box number at the top. Write or insert an ID label for each participant whose sample is stored. Put these in a notebook until specimens are shipped.

Complete and key the LABORATORY Form for each participant.

When shipping, complete the Shipping Sheet as follows:

1. Enter the field center, date and time shipment was packed and sealed at the field center.
2. Enter the number of boxes in this shipment and their numbers.
3. Add any remarks and tech ID.

Send with the shipment a photocopy of the Shipping sheet with one copy of each Box Logging Form corresponding to this sheet. The laboratory will FAX back the Shipping Sheet as a confirmation.

**Hemostasis Sample: Logging Form, and Shipping Sheet**

When shipping, complete the Shipping Sheet as follows:

1. Enter the field center, date and time shipment was packed and sealed at the field center.
2. Enter the number of boxes in this shipment and their numbers.
3. Add any remarks and tech ID.

Send with a shipment a photocopy of the Shipping Sheet with one copy of each Box Logging Form corresponding to this sheet.

APPENDIX VI

A - 26

# ARIC

## URINE BOX and POSITION LOGGING FORM

BOX NUMBER: \_\_\_\_\_

| POSITION | ID | POSITION | ID | POSITION | ID    |
|----------|----|----------|----|----------|-------|
| 01-02    |    | 29-30    |    | 57-58    |       |
| 03-04    |    | 31-32    |    | 59-60    |       |
| 05-06    |    | 33-34    |    | 61-62    |       |
| 07-08    |    | 35-36    |    | 63-64    |       |
| 09-10    |    | 37-38    |    | 65-66    |       |
| 11-12    |    | 39-40    |    | 67-68    |       |
| 13-14    |    | 41-42    |    | 69-70    |       |
| 15-16    |    | 43-44    |    | 71-72    |       |
| 17-18    |    | 45-46    |    | 73-74    |       |
| 19-20    |    | 47-48    |    | 75-76    |       |
| 21-22    |    | 49-50    |    | 77-78    |       |
| 23-24    |    | 51-52    |    | 79-80    |       |
| 25-26    |    | 53-54    |    | 81       | EMPTY |
| 27-28    |    | 55-56    |    |          |       |



### WEEKLY URINE QC SAMPLE CHECKLIST

Week: \_\_\_\_\_

| DAY | URINE SAMPLES   | LAB         | DATE        | TECH ID | PHANTOM LABEL | PARTICIPANT ID |
|-----|-----------------|-------------|-------------|---------|---------------|----------------|
| TU  | 2 tubes, 3.5 ml | Minneapolis | ___/___/___ | -----   |               |                |
|     | 1 tube, 40 ml   | Hemostasis  | ___/___     | -----   |               |                |

Week: \_\_\_\_\_

| DAY | URINE SAMPLES   | LAB         | DATE        | TECH ID | PHANTOM LABEL | PARTICIPANT ID |
|-----|-----------------|-------------|-------------|---------|---------------|----------------|
| TU  | 2 tubes, 3.5 ml | Minneapolis | ___/___/___ | -----   |               |                |
|     | 1 tube, 40 ml   | Hemostasis  | ___/___     | -----   |               |                |

Week: \_\_\_\_\_

| DAY | URINE SAMPLES   | LAB         | DATE        | TECH ID | PHANTOM LABEL | PARTICIPANT ID |
|-----|-----------------|-------------|-------------|---------|---------------|----------------|
| TU  | 2 tubes, 3.5 ml | Minneapolis | ___/___/___ | -----   |               |                |
|     | 1 tube, 40 ml   | Hemostasis  | ___/___     | -----   |               |                |

Week: \_\_\_\_\_

| DAY | URINE SAMPLES   | LAB         | DATE        | TECH ID | PHANTOM LABEL | PARTICIPANT ID |
|-----|-----------------|-------------|-------------|---------|---------------|----------------|
| TU  | 2 tubes, 3.5 ml | Minneapolis | ___/___/___ | -----   |               |                |
|     | 1 tube, 40 ml   | Hemostasis  | ___/___     | -----   |               |                |

APPENDIX VIII  
WEEKLY URINE QC SAMPLE CHECKLIST



**APPENDIX X**  
**ARIC Monthly Equipment Quality Control Checklist**

|                  |                 |
|------------------|-----------------|
| CENTER _____     |                 |
| DATE _____       |                 |
| CERTIFIER _____  | ID NUMBER _____ |
| TECHNICIAN _____ | ID NUMBER _____ |

(S)atisfactory/(U)nsatisfactory                      Comments

SET UP

- |                                |       |       |
|--------------------------------|-------|-------|
| 1. Daily QC records            |       |       |
| refrigerator temperature       | _____ | _____ |
| centrifuge temperature         | _____ | _____ |
| freezer temperature            | _____ | _____ |
| 2. Monthly QC records          |       |       |
| centrifuge tachometer check    | _____ | _____ |
| 3. Equipment and Supplies      |       |       |
| refrigerated centrifuge        | _____ | _____ |
| refrigerator                   | _____ | _____ |
| -70 C freezer                  | _____ | _____ |
| timer                          | _____ | _____ |
| ice bath                       | _____ | _____ |
| butterfly needles with adapter | _____ | _____ |
| syringe                        | _____ | _____ |
| Vacutainer hub                 | _____ | _____ |
| tourniquet                     | _____ | _____ |
| Vacutainer tubes               | _____ | _____ |
| other                          | _____ | _____ |

**APPENDIX XI  
ARIC VENIPUNCTURE AND PROCESSING PROCEDURED  
CERTIFICATION CHECKLIST**

**VENIPUNCTURE**

- |     |                                               |       |       |
|-----|-----------------------------------------------|-------|-------|
| 1.  | Labels checked                                | _____ | _____ |
| 2.  | Participant prepared and procedure explained. | _____ | _____ |
| 3.  | LAB 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.  | Lab 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                | _____ | _____ |

**Appendix XII**  
**Sample Exams for Certification**

**PRACTICAL EXAM FOR ARIC BLOOD DRAWING TECHNICIAN**

1. Place the following 7 blood collection tubes in the correct set-up order and location for the venipuncture: 2-9.5 ml red and gray top; 1-4.5 ml blue top; 3-10 ml lavender tops and 1-5.0 ml lavender top.
2. Specify which tube(s) go into the 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 and order of each color microsample tube.
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 of 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
  - 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?
  - b) The 5.0 ml lavender
  - c) The 4.5 ml blue tops
  - d) The 10 ml lavender tops
  
4. Which tube is drawn last?
  - a) A 5.0 ml lavender top
  - b) A 4.5 ml blue top
  - d) An 9.5 ml red and gray top
  
5. 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
  
6. What type of study(ies) will the 10 ml lavender top tubes be used for?
  - a) Chemistry
  - b) Lipid
  - c) Coagulation
  - d) Hemoglobin A1C
  
7. What label 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
  
8. 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

A - 34

9. Which tube(s) are the buffy coat taken from?
- b) 4.5 ml blue top
  - c) 9.5 ml red/grey top
  - d) 10 ml lavender top

TRUE OR FALSE

10. The factors being analyzed from the 4.5 ml blue tops tube are stable for up to 2 hours at 37°C.
11. The Central Lipid laboratory samples are less sensitive to venipuncture technique than the Hemostasis Laboratories.

APPENDIX XIII  
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 that lead to the exposure incident. Employees' exposure determinations in terms of job classification and tasks involve occupational risk in order to ascertain what measure 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/gown are considered PPEs if they are used to prevent an employee's 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. 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 the procedures, upon contamination, and at the end of the work shift.

**Training Program:** There shall be a formal training program. 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, and explanation of the 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 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.

#### Hepatitis B Vaccination:

Hepatitis B Vaccination shall be made available to all employees who have occupational exposure, at not 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 the 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 expressed 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.





(GTSB screen 1 of 3)

1~ [REFER TO PIN SHEET; DO NOT READ TO PARTICIPANT]  
 Was participant treated for diabetes in Visit 3? \_

Yes (Y)\* or NO (N)

[IF "YES", EXCLUDE AND SKIP TO EXCLUSION STATEMENT]

2~ Do you regularly take medication to control diabetes (high blood sugar)? \_

Yes (Y)\* or No (N)

[IF "YES", EXCLUDE AND SKIP TO EXCLUSION STATEMENT.]

3~ [REFER TO FASTING FORM; DO NOT READ TO PARTICIPANT]  
 Has participant fasted at least 10 hours? \_

Yes (Y) or NO (N)\*

[IF "NO", EXCLUDE AND SKIP TO EXCLUSION STATEMENT.]

4~ Have you had surgery to remove part of your stomach or small intestine? \_

Yes (Y)\*, No (N) or Unknown (U)

[IF "YES", EXCLUDE AND SKIP TO EXCLUSION STATEMENT]

(GTSB screen 2 of 3)

5~ Are you on kidney dialysis? \_

Yes (Y)\* or No (N)

[IF "YES", EXCLUDE AND SKIP TO EXCLUSION STATEMENT]

\*\*\*\*\*

OZ~  EXCLUSION STATEMENT  
 Because you (SELECT THE RELEVANT STATEMENT BELOW)

- are taking medication to control diabetes,
- have not been able to fast for 10 hours,
- have had part of your stomach removed,
- are on kidney dialysis,

it may not be useful or safe for you to participate in this portion of the study.

[GO TO ITEM 7]

\*\*\*\*\*

(GTSB screen 3 of 3)

6~ Are you willing to participate in  
the glucose tolerance test? \_ █

Yes (Y) or No (N)

7~ Date of data collection: \_\_\_\_\_ █  
mm/dd/yy

8~ Method of data collection: \_ █

Computer (C) or Paper (P)

9~ Code number of person completing  
this form: \_\_\_\_\_ █

# ARIC

Atherosclerosis Risk in Communities

## APPENDIX XV QUALITY CONTROL PHANTOM PARTICIPANT AND NON-PARTICIPANT ID FORM

Form Code: PNPDP (12/21/95)

Note: This form should be collected on paper and entered into the Data Entry System (DES) within two weeks of the first assignment for a QC phantom. The paper form should be stored in the Field Center.

Phantom Participant

ID Number: \_\_\_\_\_ Contact Year: 10

1. 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

2. Date ID assigned: \_\_\_/\_\_\_/\_\_\_  
mm dd yy

3. Code number of person assigning phantom ID: \_\_\_\_\_

| <u>Procedure</u>         | <u>Matching ARIC Participant ID</u><br>a. | <u>Date Drawn (Mo/Day/Yr)</u><br>b. | <u>Technician ID</u><br>c. |
|--------------------------|-------------------------------------------|-------------------------------------|----------------------------|
| <u>Venipuncture</u>      |                                           |                                     |                            |
| 4. Tube 1                | _____                                     | ___/___/___                         | _____                      |
| 5. Tube 2                | _____                                     | ___/___/___                         | _____                      |
| 6. Tubes 3 & 4           | _____                                     | ___/___/___                         | _____                      |
| 7. Tube 6                | _____                                     | ___/___/___                         | _____                      |
| 8. <u>Anthropometry</u>  | _____                                     | ___/___/___                         | _____                      |
| 9. <u>Urine Specimen</u> | _____                                     | ___/___/___                         | _____                      |

(PNPD screen 1 of 1)

1~ This ID is for:       
 QC Phantom Participant (P), Monitoring Visit ID (M), or Non-Participant ID (N)

2~ Date ID assigned:       
 mm/dd/yy

3~ Code number of person assigning phantom ID:     

| Procedure         | Matching ARIC<br>Participant ID | Date Drawn<br>(mm/dd/yy) | Technician<br>ID |
|-------------------|---------------------------------|--------------------------|------------------|
| 4. Tube 1         | a~ _____                        | b~ _____                 | c~ _____         |
| 5. Tube 2         | a~ _____                        | b~ _____                 | c~ _____         |
| 6. Tubes 3&4      | a~ _____                        | b~ _____                 | c~ _____         |
| 7. Tube 6         | a~ _____                        | b~ _____                 | c~ _____         |
| 8. Anthropometry  | a~ _____                        | b~ _____                 | c~ _____         |
| 9. Urine Specimen | a~ _____                        | b~ _____                 | c~ _____         |

ARIC PARTICIPANT Q.C. LOG FORM

Participant I.D. (use adhesive label) \_\_\_\_\_

Was blood drawn from this participant for Q.C.?    Y    N

If so, which Tube(s) was drawn for Q.C.?

Tube 1 \_\_\_\_\_    Tube 2 \_\_\_\_\_    Tubes 3,4 \_\_\_\_\_    Tube 7 \_\_\_\_\_

I.D. of Q.C. phantom participant matching this participant:

\_\_\_\_\_

WEEK \_\_\_\_\_

| <u>DAY</u> | <u>TUBES</u> | <u>LABORATORY</u> |       |
|------------|--------------|-------------------|-------|
| MONDAY     | 1            | HEMOSTASIS        | _____ |
| TUESDAY    | 2            | HEMOSTASIS        | _____ |
| WEDNESDAY  | 3,4          | LIPID             | _____ |
| THURSDAY   | 7            | LIPID             | _____ |

**APPENDIX XVI  
REAGENT PREPARATION FOR URINE COMPONENT**

3N HCL

3N HCL should be available for purchase; no additional preparation should be necessary.

3N Sodium Hydroxide (NaOH) from 5 N NaOH

In a 100 ml volumetric flask, add 60 ml of 5N NaOH to approximately 30 ml of distilled water. Cover, mix, and fill to the line with distilled water. Remix. The solution is stable for one year at room temperature in a plastic bottle.

\*\*\* NOTE \*\*\*

REAGENT PREPARATION CAN BE ARRANGED THROUGH A LOCAL MEDICAL OR RESEARCH LABORATORY, OR A COMMERCIAL LABORATORY WITH GOOD QUALITY CONTROL STANDARDS, OTHERWISE, IF MIXED IN THE ARIC FIELD CENTER LABORATORY.

REAGENT PREPARATION MUST BE DONE IN A FUME HOOD WEARING APPROPRIATE EYE AND SKIN PROTECTION.

LABEL ALL REAGENTS APPROPRIATELY WITH NAME AND CONCENTRATION OF SOLUTION, DATE OF PREPARATION, EXPIRATION DATE, AND NAME OF PERSON PREPARING THE SOLUTION.

HCL AND NaOH ARE CORROSIVE AGENTS AND MUST BE LABELED AS SUCH!!!