Manual 7
Biospecimen Collection and Processing
Visit 11

Version 1.1
August 22, 2024

Biospecimen Collection and Processing
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0. MANUAL REVISIONS

Version Number	Date	Author	Section(s)	Description of Update
0.1	11/10/2023		All	Initial Draft for Review for V11 Biospecimen.
0.2	1/2/2024		All	Updated draft for review for V11 biospecimen.
0.3	1/16/2024		All	Add information to collect C4R aliquot at V11.
1.0	3/6/2024	Valint, Lavallee	5.1	Add information about shipping C4R aliquot at V11.
	6/14/2024	Azara	3.6	Add information about acceptability of partial draws

1. PURPOSE

1.1 Biospecimen Collection and Processing

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 that has randomly sampled a selected population and follows participants for an extended period.

Blood and urine specimens donated by the study participants at each of the four ARIC field centers are processed at the field centers for shipment to, analysis by, and long-term storage at three central laboratories: the ARIC DNA Laboratory at the University of Texas Medical School in Houston, TX; the ARIC Atherosclerosis Laboratory at Baylor College of Medicine in Houston, TX; and the ARIC Clinical Chemistry Laboratory at the University of Minnesota in Minneapolis, MN.

The Atherosclerosis Clinical Laboratory (ACRL) performs assays related to lipid metabolism (lipid profile: total cholesterol, triglyceride, direct hdl-c, calculated non-hdl-c, and calculated ldl-c), and inflammation (hs-C-Reactive Protein). The Clinical Chemistry Laboratory performs assays related to renal function, glycation and general chemistry (serum creatinine and albumin; urine albumin and creatinine; cystatin c; hemoglobin A1C; hemoglobin and platelets; magnesium; potassium). A complete list of the tests performed and their expected values is located in Appendix 1.

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

Laboratory tests are performed on specimen samples that are collected and processed by the technicians at each of the four ARIC field centers. Probably the most important step in this process (and potentially the most difficult to standardize) is the collection and field center processing of the blood samples. Laboratory tests can be repeated, but if the blood sample itself is not correctly drawn, labeled, and processed, the laboratory results may not be accurate even if the laboratory assays are precise. For the study to succeed, it is important that variation in measurement values reflect true differences between the study participants rather than differences in blood drawing or processing procedures. Thus, it is important that all field center technicians are well-trained, certified, and fully compliant with the protocol for drawing and processing the specimens in the field. Field center technicians should also be willing to take pride and responsibility in their work.

2. PREPARATION

2.1 Participant Contact

Since participation in this study is voluntary, every effort must be made to make the entire procedure as easy and painless as possible for participants. 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 collects five tubes of blood which is approximately 53 – 63 mL of blood from each participant (up to 90 mL is allowed per IRB protocol). The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 4 tablespoons, although it may look like more to them. The technician may also assure participants that they donate almost 10 times as much blood (450 mL) when they donate a pint of blood.

2.2 Staff Certification Requirements

Blood drawing and processing are performed by a certified ARIC technician(s) at each field center. 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 may be required annually or after taking a significant break from any area of biospecimen collection and is authorized by the supervisory panel.

Once the primary staff are trained and certified in all areas of biospecimen collection, processing and shipping, alternate staff may be trained and certified by the trainer(s) in individual components of the biospecimen collection work scope. Partially trained personnel are restricted to work only in the specific area for which they have been certified. Monthly performances in the specifically trained areas are required for training maintenance.

Partial or component training areas are grouped into three areas listed below. Note that all collection area trained technicians must be either certified phlebotomists, medical technologists, medical assistants, nurses, or other qualified personnel.

1. Collection:
    a. Blood drawing
    b. Tube mixing
    c. Types of tubes and sample types
    d. Biospecimen and Phantom Form
    e. QC tube(s) collection and documentation
    f. Internal lab record log
    g. Proper handling of each sample/tube type (ice or room temperature)
    h. Centrifugation
    i. Urine collection

j. Safety (blood borne hazards, needle disposal, etc.)

2. Processing Includes:

    a. Labeling for both QC and regular participant blood draws,
    b. How to fill out all related forms.
    c. Types of tubes and sample types
    d. Centrifugation
    e. Separation of plasma from EDTA tubes
    f. Separation of serum from clot
    g. Pooling of specimens per sample type
    h. Aliquoting urine and blood specimens
        1. Add BHT to designated EDTA aliquots
        2. Cap aliquots with appropriate cap color
    i. Safety

3. Shipping Includes:

    a. Sorting aliquots per intended destination (ACRL or UMN)
    b. Bagging blood and urine aliquots
    c. Double bagging where necessary
    d. Local and FedEx guidelines and regulations
    e. Biweekly shipment packaging
    f. FedEx notifications
    g. Addresses to ship to
    h. Documents to include in the biomailer

Partial certification requires a written examination and practical application observation by the certifying personnel for each specific area of training.

2.3 Blood Collection Trays and Tubes

One day prior to a scheduled participant visit, the technician prepares two trays: one to hold the blood collection tubes, another to hold the plastic vials which will hold the whole blood, serum, plasma, and urine aliquots until they are frozen and ultimately transferred to the Atherosclerosis Laboratory (ACRL), the University of Minnesota Laboratory (UMN) and the University of Texas Genetics Laboratory for analysis. Label these sets of tubes with the appropriate ID numbers for the participant. A list of equipment, suppliers, and vendors is provided in Appendix 4.

2.3.1 Blood Collection Tray

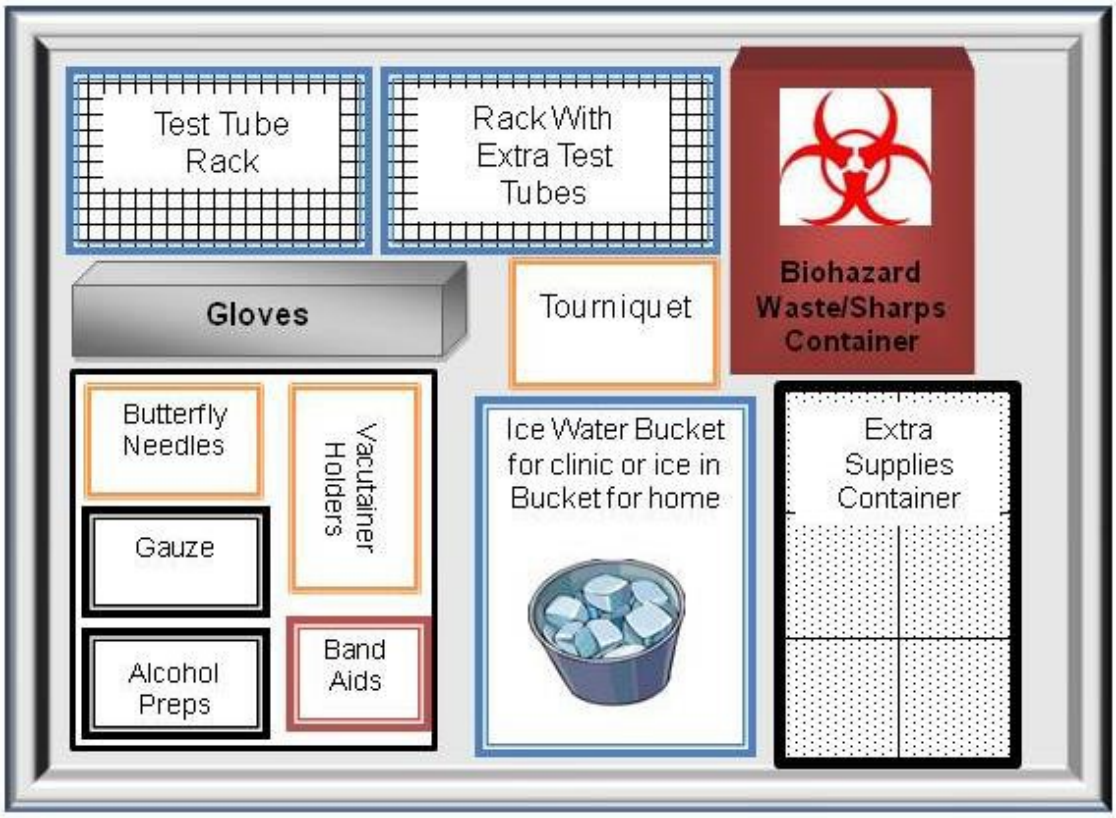
First, the technician organizes and prepares the blood collection tray. The blood collection tray is made of hard unbreakable plastic or other suitable material that can be

easily cleaned. The tray has individual compartments that are filled with the following supplies:

- test tube rack that holds at least 10 blood collection tubes (described in the next section)
- sterile, disposable 21 and 23 gauge butterfly needles with 12” tubing
- plastic vacutainer holders with Luer adapters
- sterile alcohol swabs
- gauze sponges
- tourniquet
- bandages ("Band Aids") or Coban

Ice packs and wash cloths should be readily available in the blood collection area for participants who become faint during the blood collection.

Figure 1. Clinic and Home Visit Blood Collection Supply Tray



2.3.2 Blood Collection Tubes

Technicians must be familiar with the arrangement of blood collection tubes, the order in which the tubes are to be filled, the type of anticoagulant in each tube, and the possible sources of error in handling each tube. These tubes are organized in the test tube rack in the following sequence:

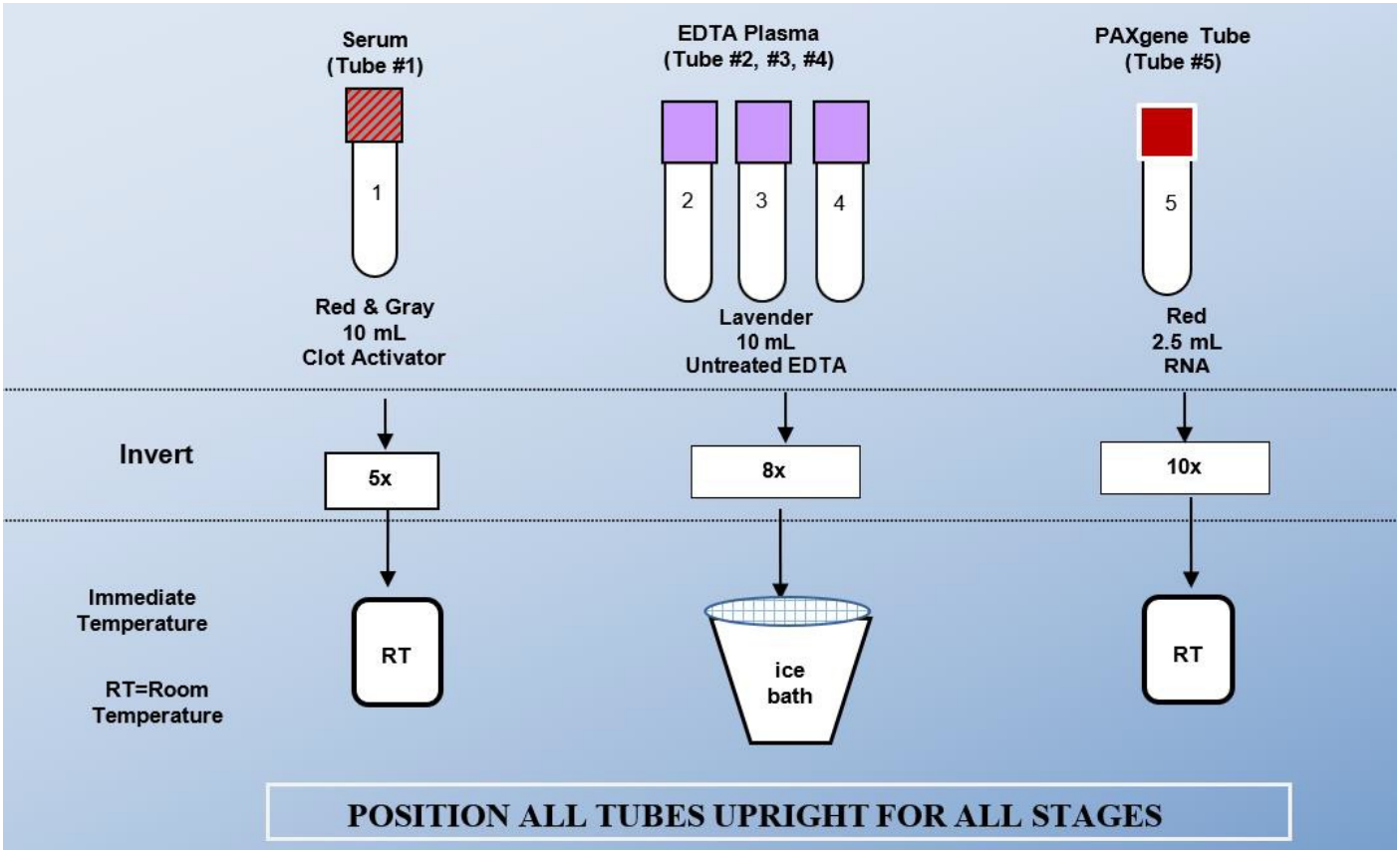
Tube #1 is a 10 mL red/gray stoppered tube. Although this tube does not contain anticoagulant, it does have a clot activator and therefore requires mixing following collection. The serum from this tube will be used for testing creatinine and other tests measured in the UMN Chemistry Laboratory.

Tubes #2, #3, and #4 are 10 mL lavender-stoppered tubes containing K_2 EDTA anticoagulant. The plasma from these tubes is used for several analytical tests including lipids, HS CRP and other tests measured in the ACRL. The white cells or buffy coats, taken from tubes 2 and 3, will be used to isolate DNA in the UT-Genetics Laboratory. Whole blood (0.5 mL) is taken from tube # 2 and tube #3 for hemoglobin A1c testing in the UMN Laboratory.

Tube #5 is a 2.5 mL red-stoppered PAXgene tube containing anticoagulant and lymphocyte stabilizers. (The PAXgene tube is the size of a 10 mL collection tube, but because of the liquid stabilizers, only 2.5 mL of blood is collected.) These tubes must be filled completely to standardize the blood to liquid anticoagulant ratio. Partially filled tubes will result in erroneous test results. RNA will be isolated from the lymphocytes and used for gene expression studies. Because there is a large volume of liquid in this tube, be sure to hold the tube below the participant's arm during collection. (There is a risk, although extremely small, that the liquid in the tube could flow into the participant's vein if the tube is not held below the arm during collection.)

A note on supply availability: During ARIC Visit 9 and 10, supply shortages impacted Field Center ability to obtain 10mL SST serum tubes and 10 mL EDTA tubes. 8.5mL SST serum tubes and 8.5 mL EDTA tubes were substituted if 10mL equivalents were not available. At Visit 11, supply shortages of these materials are no longer anticipated and 10mL tubes should be used for all specimen collection.

Figure 2. Order of Draw



Order of Draw Figure Key	
RT	Room Temperature (18-25°C)
Tube #1	SST, Serum (Red/ Grey Top)
Tubes #2, #3, #4	EDTA Plasma (Lavender Top) #2, #3=0.5 mL whole blood taken for A1c; #2, #3, #4 = buffy coats taken
Tube #5	PAXgene Specialty (Red Top)
TOTAL DRAW= 52.5 mL [MAX DRAW VOL.= 90 mL]	

2.3.3 Blood Collection Tubes: Labeling and Set-Up

Blood collection tubes can be set up in advance of the participant visit.

1. Apply barcoded ARIC ID “paper” labels to tubes 1-5.
2. Apply a barcoded ARIC ID “cryo” label to the PAXgene tube since this will be placed in the freezer. Place the labels on the tubes vertically, with the bar-code oriented from the bottom of the tube to the top of the tube (see Figure 3. Example of a properly positioned ARIC Aliquot Label.). Handle only one participant's specimens at a time so the chance of mislabeling is minimized.
3. Apply the barcoded ARIC ID “cryo” label to the 2.0 mL vials. Note that C4R label replaces the SR1 label for the serum aliquots. Keep the SR1 label on the participant’s sheet, unless it is needed. If there is only one serum aliquot from a participant, then that one aliquot will be labeled with a SR1 label.
4. Apply a barcode ARIC ID “paper” label to each page of the Biospecimen Collection Forms (BIO).
5. Arrange the blood collection tubes in the test tube rack in the same order in which they are to be collected. The five tubes are collected in the following order:

Tube #1: 10 mL red/grey stoppered tube (Serum)
Tube #2: 10 mL lavender stoppered tube (EDTA)
Tube #3: 10 mL lavender stoppered tube (EDTA)
Tube #4: 10 mL lavender stoppered tube (EDTA)
Tube #5: 2.5 mL red stoppered PAXgene tube

Figure 3. Example of a properly positioned ARIC Aliquot Label.

Approximately 30% of ARIC participants will be asked to donate one additional tube of blood for quality control purposes. The duplicate sample will be assigned a different ID number, called a Phantom ID, and shipped to the Central Laboratories. This quality control procedure is described more completely in Chapter 6.

2.3.4 Sample Aliquot Trays

The technician prepares a tray of the plastic freezer 2.0 mL cryovials, which will contain the aliquots to be shipped to the laboratories. Each type of serum/plasma/urine cryovial has a corresponding color-coded screw cap that fits onto it. The technicians are trained to organize the tray for the sample processing and aliquoting as follows:

The tray itself should be a flexible sponge rack or hard plastic aliquot rack, which will fit tubes from 10mm in diameter. The tray has 5 rows and up to 10 columns. The columns are numbered 1-10 from left to right. The rows are lettered A-E from top to bottom.

Cleaning instruction for Aliquot Trays

NOTE: Wear face shield, gloves, and coat or disposable gown for this procedure.

1. Perform this procedure daily or sooner if there is noticeable contamination.
2. Make a solution of 10% bleach by adding 1 part of household bleach to 9 parts of tap water in a bucket. Make this fresh each day.
3. Submerge the racks in the bleach solution.
4. Rinse under running tap water.
5. Air dry overnight.

Day one preparations include sample and aliquot trays set-up for each participant.

2.3.5 Organization

The technicians need the following supplies for each sample tray. Supplies are organized in the order of centrifugation and processing.

12 – 2.0 mL polypropylene cryovials (lavender top)
4 – 2.0 mL polypropylene cryovials (green top)
3 – 2.0 mL polypropylene cryovials (brown top)
8 – 2.0 mL polypropylene cryovials (red top)
2 – 2.0 mL polypropylene cryovials (black top)
6 – 2.0 mL polypropylene cryovials (yellow top)

During visits 9 and 10, due to COVID-19, some tops were difficult to obtain. At Visit 11, supply issues have resolved and sites should make effort to use supplies as indicated. In the rare case that supply is not available, a violet top may be substituted for the lavender top, a clear top may be substituted for the brown top, and a blue top may be substituted for the black top. If any of the above substitutions are made, staff must also comment on the biospecimen shipping and receiving form before shipment.

2.3.6 Labeling

Vertically label the plastic sample aliquot tubes with the barcoded ARIC ID “cryo” labels and arrange in the sample aliquot trays in the following order (see Figure 3 Aliquot Tray 1 Layout and Figure 4 Aliquot Tray 2 Layout):

Tray 1 (for stages 1-3 processing):

Col 1: 2.0 mL cryovials Green Caps (rows A-B); **Black** Cap (row C-D)
Col 2: 2.0 mL cryovials Green Caps (rows A-B); Brown Cap (row C-E)
Col 3: 2.0 mL cryovials Lavender Caps (rows A-E)
Col 4: 2.0 mL cryovials Lavender Caps (rows A-E)
Col 5: 2.0 mL cryovials Lavender Caps (rows A-B);
Col 6: 2.0 mL cryovials Empty (rows A-E)
Col 7: 2.0 mL cryovials Red caps (rows A-E)*
Col 8: 2.0 mL cryovials Red caps (rows A-C)
Col 9: Empty (rows A-E)
Col 10: Empty (rows A-E)

Tray 2 (for stage 4 processing):

Col 1: 2.0 mL cryovials Yellow caps (rows A-E)
Col 2: 2.0 mL cryovial Yellow cap (rows A)

*Note that 1 of the 8 serum aliquots will be labeled using a C4R label provided on the scheduled participant label sheet. The C4R labeled serum aliquot will be for the UMN lab. The C4R cryo label will be used in place of the SR1 cryo label, if there is more than 1 serum aliquot for that participant.

In case the participant only has one serum aliquot, then the **C4R label will not be used.** Instead, if the participant only has one serum aliquot, then label the aliquot with a SR1.

Figure 4. Aliquot tray 1 layout for stage 1-3 processing.

col / row	1	2	3	4	5	6	7	8	9	10
A	0.5 mL BHT treated plasma, Tubes #2,#3,#4	0.5 mL BHT treated plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma Tubes #2,#3,#4	*Empty*	0.5 mL serum, Tube #1	0.5 mL serum, Tube #1	*Empty*	*Empty*
B	0.5 mL BHT treated plasma, Tubes #2,#3,#4	0.5 mL BHT treated plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma Tubes #2,#3,#4	*Empty*	0.5 mL serum, Tube #1	0.5 mL serum, Tube #1	*Empty*	*Empty*
C	Whole Blood (before spin) Tube #2	Buffy Coat, Tube #2	0.5 mL EDTA plasma, Tubes #2,#3,#4	0.5 mL EDTA plasma, Tubes #2,#3,#4	*Empty*	*Empty*	0.5 mL serum, Tube #1	0.5 mL serum, Tube #1	*Empty*	*Empty*
D	Whole Blood (before spin) Tube #3	Buffy Coat, Tube #3	0.5 mL EDTA plasma Tubes #2,#3,#4	0.5 mL EDTA plasma Tubes #2,#3,#4	*Empty*	*Empty*	0.5 mL serum, Tube #1	*Empty*	*Empty*	*Empty*
E	*Empty*	Buffy Coat, Tube #4	0.5 mL EDTA plasma Tubes #2,#3,#4	0.5 mL EDTA plasma Tubes #2,#3,#4	*Empty*	*Empty*	0.5 mL serum, Tube #1	*Empty*	*Empty*	*Empty*

Figure 5. Aliquot tray 2 layout for stage 4 processing.

Col Row	1	2	3	4	5	6	7	8	9	10
A	1.5 mL Urine no pH adj.	1.5 mL Urine no pH adj	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	1.5 mL Urine no pH adj	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	1.5 mL Urine no pH adj	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
D	1.5 mL Urine no pH adj	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	1.5 mL Urine no pH adj	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

2.3.7 Preparation for Specimen Collection

In the 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 correct ARIC barcode ID label.
3. Check that the sample aliquot trays are properly equipped. Every item on the checklist must be ready and in its proper position.
4. Check that each aliquot storage container is labeled with the correct ARIC barcode ID label.
5. Perform and record quality control (QC) check on centrifuge temperature (4°C ± 2°C).
6. Perform and record QC check on refrigerator temperature (4°C ± 2°C).
7. Perform and record QC check on freezer temperature (-80°C± 5°C)
8. Perform and record QC check on room temperature. 20°C ±5°C

Approximately 10 minutes before scheduled participant arrival (clinic):

1. Fill ice bath ¾ full with crushed ice (clinic visit), or place sponge/rack in ice bucket and fill with crushed ice (home visit).
2. (Clinic visit) Place cold water into ice bath.

2.3.8 At Participant Arrival

1. Confirm the match between the participant name and the ARIC ID number on the blood collection tubes, urine specimen, aliquot vials and the Biospecimen Collection Form.
2. Check that duplicate Quality Control tubes are prepared and labeled (affix only the QC Phantom label, *do not* place the donor participant's label on the tube or the form), if needed.

3.6 Biospecimen Collection form

At the completion of specimen collection and processing, complete the Biospecimen Collection Form. If there are any deviations from the routine collection or processing protocol, record them on the Biospecimen Collection Form. This form is entered on paper first and then entered into the Carolina Data Acquisition and Report Tool (CDART) using the participant ID (CDART is the Coordinating Center's web-based Data Management System). A copy is sent to the ACRL Laboratory and the University of Minnesota (UMN) Laboratory with the biweekly sample shipment. File and maintain the original paper form until close out of study.

3. VENIPUNCTURE PROCEDURE

3.1 Precautions for Handling Blood Specimens

Handle all specimens as potentially infectious. The two primary blood borne diseases are hepatitis B and the acquired immune deficiency syndrome (AIDS). It has been demonstrated that the viruses which cause these conditions can be transmitted following contact of a tainted blood sample through "broken skin" or intact mucous membrane (mouth, eyes, or nose) or as a result of an inadvertent needle stick. Examples of "broken skin" include open cuts, nicks and abrasions, dermatitis, and acne.

The Occupational Safety and Health Administration (OSHA) rules mandate that technicians always wear disposable protective gloves when collecting and processing specimens. When performing a venipuncture, the protective gloves worn by the phlebotomist must be intact (e.g., a fingertip cannot be torn off the glove in order to locate a venipuncture site). If the phlebotomist accidentally sustains a contaminated needle stick, clean the wound thoroughly with disinfectant soap and water, notify a supervisor, and consult a physician.

Never take lab coats worn during the collection and processing of samples outside of the laboratory area except for laundering. Before leaving the laboratory, the technician will remove the lab coat and disposable gloves and wash hands with a disinfectant soap. Waterless anti-bacterial hand wash should be carried to the home and long-term care facilities (Belt Clip Purell Mini Pump for personal use, cat # ML1258, vendor Market Lab, or similar).

Use OSHA-approved cleaning solution to clean up any spills of blood, plasma, or serum. Use this solution to clean all laboratory work surfaces at the completion of work activities. 10% bleach can be freshly made and used. For non-clinic visits a fresh mix bleach system (cat. # ML0109, vendor Market Lab) can be used.

Figure 6. Example of fresh bleach mixture system.

OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers. Do not attempt to bend, break, or recap any needle before discarding it. Discard the butterfly set following each specimen collection. Do not perform any pipetting by mouth; especially of any blood, serum, plasma or urine.

Avoid formation of potentially infectious aerosols when removing the rubber stoppers from vacutainer tubes. In addition to wearing protective gloves, hold a piece of gauze over the stopper while slowly removing it from the tube. Creation of aerosols can also be diminished by careful pipetting and centrifugation techniques. Further steps to minimize infection risk while processing samples are described in the OSHA regulations stated in the Federal Register of December 6, 1991 (Vol. 56, No. 235, page 64177). Wear a mask in combination with an eye protection device, such as goggles or glasses with solid side shields or a chin-length face shield when working with potentially infectious materials that have the potential for splashing, spraying, or spattering. An alternative to these devices would be a desk-mounted clear plastic shield, which would offer similar protection from possible infectious splashes or sprays.

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

3.2 Phlebotomy Room

Clinic Visit

The blood drawing takes place in an isolated room or in a room with dividers. The room is equipped with all necessary blood drawing supplies. A separate work area is equipped with all supplies that are used in the blood processing. The centrifuge, refrigerator, and freezer should be nearby.

Home Visit

Assess the patient's home environment upon arrival for a suitable location to draw the participant's blood (i.e. a table). Ask permission to use this area. Place a liner on the table for protection and proceed.

It is ideal to use an arm wedge to facilitate the blood draw (see picture below).

Figure 7. Arm wedge for blood draw.

Long-term Care Facility

If the patient is bed ridden with the permission of the facility management and if it is not harmful to the patient, position the bed to a sitting position by raising the back of the bed and proceed.

If the patient is able to sit in a chair, ask permission to complete the draw from a chair using a suitable means as a table and proceed.

3.3 Participant Preparation

Informed consent must be obtained before drawing any blood, to ensure that the participants understand the purpose and possible complications of the venipuncture procedure. A standard informed consent has been prepared for this study. The consent statement informs study participants that although there may be some minor discomfort, their blood (about 4 Tablespoons) will be drawn by trained technicians.

Complete the Biospecimen Collection Form with the participant. 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 the site PI or use logical judgement. If blood is to be drawn, fill in date and time on the Biospecimen Collection Form.

The participant should be seated during the blood draw. It is difficult to standardize the length of time that a person is in the sitting position prior to venipuncture, but to the extent possible, attempt to have the participant sit for a minimum of 5 minutes.

Perform venipuncture with a 21-gauge butterfly needle and 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. A 23-gauge needle may be used if the participant has small veins or if drawing from a hand vein. The butterfly has a small thin-walled needle that 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. Give the participant 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 ounces of blood (or about 4 tablespoons). This blood will be used in tests for lipids (or fats), cholesterol, and kidney function. We hope to be able to use the results of these tests to predict who might have a greater risk of heart disease."

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 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. If the participant is very anxious, he/she may lie down during the blood collection. A reclining individual will undergo an extra vascular water shift, resulting in a dilutional effect on lipid values. If this option is taken, note it in the Venipuncture/Processing Incident section of the Biospecimen Form. Having a second

technician in the room to distract the participant with interesting conversation is often a helpful technique.

3.4 Venipuncture

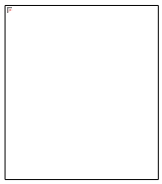
Have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). Use a tourniquet to increase venous filling. This makes the veins more prominent and easier to enter. The preferred arm to draw from is the left arm. Use the right arm only if blood collection is not possible from the left arm. This does not mean you must stick the left arm. Only do so if an adequate vein is apparent.

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 minutes. Doing so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection, and it remains on the arm for longer than two minutes, it should be released and reapplied after a wait of one minute. Instruct the participant that he/she should not clench their fist prior to the venipuncture. Doing so could cause fluctuations in the results in several of the analytes being measured. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

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

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

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



Note that it is acceptable to reapply the tourniquet if the blood flow slows. One method is, when first applying the tourniquet, place it in the "rabbit" (release ends upward) position. After releasing the knot, drape the tourniquet ends (criss-cross) on the arm allowing it to hang loosely rather than removing it. This may facilitate re-application timing. Multiple re-applications may be necessary

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, lowering the extremity over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.

Assemble the butterfly-vacutainer set.

1. Attach the Luer adapter to the vacutainer holder if not already attached.

2. Attach the Luer end of the butterfly needle set to the Luer adapter if not already pre-assembled.

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 a gloved finger. If this happens, cleanse the gloved finger with alcohol first.

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. Once blood appears in the butterfly tubing, place tube #1 (10 mL red/gray top) into the vacutainer holder. 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.

4. 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. DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.

5. Remove the tourniquet after tube #1 fills. Once the draw has started, do not change the position of a tube until it is withdrawn from the needle. The tourniquet may be reapplied if blood flow is slow without it. When the tourniquet is reapplied, note this on the Biospecimen Collection Form and fill out the Incident Log.

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 tube and attach another without removing needle from vein.

8. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the point, stopping blood flow until the next tube is inserted (if necessary). Gently invert tubes which require mixing: #1 – five times; tubes #2, #3, #4 – eight times and tube #5 – ten times. Immediately following removal of the tubes from the adapter place tubes #1 and #5 at room temperature and tubes #2, #3, and #4 into the ice water bath or refrigerator (4-8˚C).

9. When collecting tube #5, hold the PAXgene tube vertically, below the donor's arm. Allow at least 10 seconds for the blood draw to take place. The blood will slow from a stream to a drip. Ensure that the blood has stopped flowing before removing the tube from the holder. It may be helpful to count blood drops after the stream has slowed which will ensure the minimum amount of time has been achieved. Gently invert 10 times and transfer to -80°C freezer

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

- 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.

- 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. The same technician should not attempt a venipuncture more than twice (once in each arm). If a third attempt is necessary, a different phlebotomist should attempt the venipuncture following the same guidelines. <u>If a third attempt is unsuccessful the study participant is asked whether s/he would like to have staff make another attempt to obtain blood for the tests to be done by the study.</u> <u>If affirmative, at the conclusion of phlebotomy the participant is asked to sign a brief form stating that additional venipuncture was authorized (to be filed with the participant's informed consent). Note that this is a site specific form.</u>

- 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.

- To remove the needle, <u>lightly</u> 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. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent bruising.

- If the blood flow stops before all tubes are filled, repeat the venipuncture on the participant beginning with the first unfilled tube. Tubes #2-#5 should be completely filled in order to perform the analyses. However, if you are unable to fill the tubes completely, partially filled tubes #1-#4 will be accepted. Document any partial fills on Question 10 the BIO form. As always, the tourniquet should never be on for longer than two minutes. (See section 3.6 for handling incomplete or "short" draws).

Bandaging the arm.

1. Under normal conditions:

    a. Keep the gauze pad over the site, continuing mild pressure.
    b. Apply an adhesive bandage over the venipuncture site after making sure that blood flow has stopped.
    c. Tell the participant to leave the bandage on for at least 15 minutes.

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 an adhesive bandage tightly around the arm over the pad.
    c. Tell the participant to leave the bandage on for at least 15 minutes.

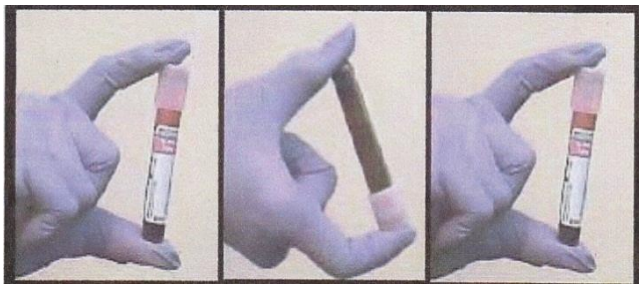
PRECAUTIONS - WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAW:

1. Have the person remain in the chair and if the chair can be reclined tilt the chair back.
2. Provide the person with a basin if he/she feels nauseous.
3. Have the person stay seated until the color returns and he/she feels better.
4. Have someone stay with the person to prevent them from falling and injuring themselves if they should faint.
5. Place a cold wet cloth on the back of the person's neck or on their forehead.
6. Once the episode has passed, some fruit juice may be given to the participant to counteract any possible hypoglycemia due to their fast.
7. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.5 Blood Tube Mixing and Storage During Venipuncture

All tubes must be mixed. Tubes #2 - #4 need to be mixed with the anticoagulant to prevent clotting. Even Tube #1, which does not contain an anticoagulant, does have a clot activator that needs to be mixed with the blood. Begin by holding the tube horizontal to the floor. Gently tip the stopper end down while watching the air bubble rise to the butt. Now, lower the butt end slightly while watching the bubble float to the stopper (1st complete inversion). Invert Tube #1 five times, Tubes #2-#4 eight times, and Tube #5 ten times. Each inversion should take about 1 second.

Figure 8. Tube mixing technique.



Tube #1: 10 mL red and gray-stoppered tube containing a clot activator.

Gently invert 5 times immediately after collection. Allow the blood to clot at room temperature for 30 minutes after collection. Then centrifuge, remove the serum, freeze and store at -80ºC for biweekly shipment to the Atherosclerosis and UMN Laboratories. Note that vial that is usually labeled "SR1" will be labeled using the provided "C4R" cryolabel, unless there is only 1 x serum aliquot. In case there is only 1 x serum aliquot, then the SR1 label will be used.

Tube #2: 10 mL lavender-stoppered tube contains EDTA anticoagulant.

Invert gently eight times, place in ice water bath until centrifugation. This tube must be re-mixed immediately prior to making the whole blood aliquot; mix by inverting gently **20** times. Remove 0.5 mL of whole blood from tube #2 and place in cryovial tube with black cap. Re-stopper the tube after taking out the aliquot of whole blood. This tube will be centrifuged with tubes #3 and #4. The plasma from tube #2 is used for lipid determination and other studies. The white blood cells from this tube will be used to isolate DNA.

Tube #3: 10 mL lavender-stoppered tube contains EDTA anticoagulant.

Invert gently eight times, place in ice water bath until centrifugation. This tube must be re-mixed immediately prior to making the whole blood aliquot; mix by inverting gently **20** times. Remove 0.5 mL of whole blood from tube #2 and place in cryovial tube with black cap. The plasma from tube #3 is used for lipid determination and other studies. The white blood cells from this tube will be used to isolate DNA. Therefore, do not discard the cells from this tube.

Tube #4: 10 mL lavender-stoppered tube contains EDTA anticoagulant.

Invert gently eight times immediately after collection. Place the tube in an ice water bath until centrifugation. The white blood cells from this tube will be used to isolate DNA. Therefore, do not discard the cells from this tube.

Tube #5: 2.5 mL red-stoppered PAXgene (PAX) tube for RNA preservation.
Invert gently 10 times immediately after collection. Put it in a wire rack in an upright position in the -80°C freezer. DO NOT use a Styrofoam rack or holder to freeze since it could cause the PAXgene tube to crack.

3.6 Partial Biospecimen Collection Procedures for Clinic and Home Visits[AL1]

A partial biospecimen collection for the ARIC Visit 11 Study is defined as a collection set consisting of less than the desired five tubes, (1) SST Serum, (3) EDTA Plasma, and (1) PAXgene and 10 mL of urine. This rule applies to both the clinic and home collections.

Incomplete or partial collections are acceptable or unacceptable under the following conditions.

a) Blood

- If no blood is collected, then send urine specimens anyway as a partial collection set and document on the BIO Form[AL2].
- If only the first tube is collected after the allowed number of attempts (3) by two individuals (an additional attempt is allowed if consent is obtained), then document on the BIO Form, process, and ship.
- If the partial collection includes at least (1) SST serum tube and (1-3) EDTA plasma tubes, then document on the BIO Form, process, and ship.
- If you are unable to fill the tubes completely, partially filled tubes #1-#4 will be accepted. Document any partial fills on Question 10 of the BIO form.

b) Urine:

- If no urine is collected, send the blood specimens anyway as a partial collection set and document on the BIO Form.
- If < 5.0 mL is collected, discard and do not send any urine for this participant.
- If < 9.0 mL is collected dispense aliquots of 1.0 mL rather than 1.5 mL. Try to fill all 6 aliquots, but if not possible, fill at least 3 aliquots. Document on the BIO Form.

3.7 Transfer of Specimens Collected at Home or Long-term Care Facility:

Once the samples are collected at the home visit, the room temperature samples will be transported in a secured cooler and the other samples will be transported in the secured cooler containing the ice to prevent accidental spillage. All needles will be disposed of in a small Sharps container.

3.8 Processing of Specimens Collected at Home or Long-term Care Facility:

All samples should be returned to the field center within an 8-hour time frame. Because the collection is made outside the clinic in either the participant's home or a long-term care facility the 90-minute processing window cannot be met. The maximum time of 8 hours was determined to be the most acceptable time for most tests to be performed. If this timeline will drastically affect the test results, that particular test will not be done on the non-clinic patient. Item 0d on the Biospecimen Form indicates whether the specimens were collected from a clinic or non-clinic participant.

All sample types can be centrifuged together since the serum will have surpassed the amount of time needed for clotting. Other than centrifugation, the processing should follow the same order and steps as with the clinic patients.

4. BLOOD AND URINE PROCESSING

4.1 Stage One: Immediate Processing

4.1.1 Specimens collected in-clinic:

After completion of venipuncture:

1. Tube #1 remains at room temperature for 30-45 minutes to allow the blood to clot (blood at 4°C clots extremely slowly). Set a timer for 30 minutes as a reminder to centrifuge these tubes.

2. Remove tube #2 from the ice water bath (or bucket) and invert *gently* **20** times, immediately prior to removing 0.5 mL whole blood into a cryovial vial located in the Aliquot Tray 1, column 5, row C. Re-cap tube #2 and replace it in the ice water bath. Repeat for tube #3, removing the tube from the ice water bath, gently inverting 20 times, then removing 0.5 mL whole blood into a cryovial vial located in the Aliquot Tray 1, column 5, row D. Recap tube #3 and replace it in the ice water bath. Place a black screw cap on each whole blood aliquot and store in the refrigerator. These aliquots of whole blood are for Hemoglobin A1c, hemoglobin and platelets and will be shipped to UMN for measurement.

3. As soon as possible after step 2, and within 15 minutes of collection, invert Tubes #2, #3, and #4, (2x) and place tubes in the centrifuge buckets in a balanced manner (see description of balancing the centrifuge in 4.1.1 “Operating the Centrifuge”). Spin these tubes at 3,000 x *g* for 10 minutes at 4° C. Record on the Biospecimen Collection form the time at which these tubes began to spin.

4. Tube #5 is placed in a wire rack in an upright position in the -80° C freezer until packaging for shipping. DO NOT place in a Styrofoam rack or holder to freeze.

4.1.2 Specimens collected in Home or Long Term Care Facility:

After completion of venipuncture:

1. Place tube #1 upright in a holder and into a transport cooler without ice (maintaining the tube at “room temperature” until processing). Since the tube will sit longer than 45 minutes, timing before centrifuging is not necessary.

2. Place tube #2, #3, and #4 upright in a holder in a transport cooler with ice. Upon arrival at the clinic, remove tube #2 and #3 from the transport cooler and invert *gently* **20** times, immediately prior to removing two 0.5 mL whole blood into a cryovial vial located in the Aliquot Tray 1, column 1, row C and D, one aliquot from each tube. Place a black screw cap on each aliquot and store in the refrigerator. These aliquots of whole blood are for Hemoglobin A1c, hemoglobin and platelets and will be shipped to UMN for measurement. Re-cap tube #2 and #3 and store in the refrigerator.

3. As soon as possible after step 2, invert Tubes #2, #3, and #4, (2x) and place tubes in the centrifuge buckets in a balanced manner (see description of balancing the centrifuge in 4.1.1 “Operating the Centrifuge”). Spin these tubes at 3,000 x *g* for 10 minutes at 4° C. Record on the Biospecimen Collection form the time at which these tubes began to spin.

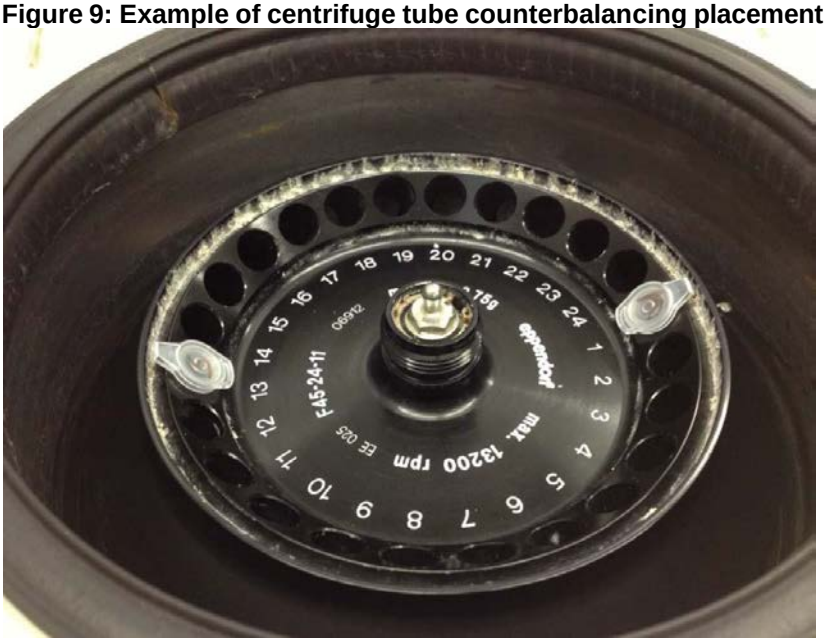
4. Tube #5 is placed in a wire rack in a transport cooler with ice. Upon arrival, place the wire rack holding the sample in an upright position in the -80° C freezer until packaging for shipping. DO NOT place in a Styrofoam rack or holder to freeze.

4.1.3 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. In order to achieve a 3000 x *g* centrifugal force (rcf) within the centrifuge, the corresponding revolutions per minute (RPM) may vary from centrifuge to centrifuge depending on radius of the centrifuge's rotor. Consult the centrifuge's operating manual for the appropriate RPM for each centrifuge. If the field center's centrifuge is not capable of creating a 3000 rcf, increase the centrifugation time until the rcf-minutes total 30,000. If, for example, the maximum force is 2000 rcf, then increase the time from 10 to 15 minutes. To balance the centrifuge, place tubes of the same size and with equal volume of blood as determined visually in opposite positions in the bucket adaptors. For tubes of blood that do not have another tube of equivalent blood volume, use a “balance tube” of

the same size containing an equivalent volume of water. Ensure that the tubes are directly across from one another before operating the centrifuge. Wait for centrifuge to come to a complete stop before opening the lid. Proceed to stage 2 processing.

Figure 9: Example of centrifuge tube counterbalancing placement



4.2 Stage Two: Processing of Plasma

Stage two begins approximately 15 minutes after venipuncture. Eye/face protection, gloves and lab coat must be used for all blood processing. All other rules regarding the safe blood specimen handling must also be observed.

Remove the aliquot trays from the refrigerator and place in an ice water bath on the bench behind the collection tube racks until completion of the collection tubes processing. Keeping the aliquot trays cold during collection tube processing is very important. In the case that the processing facility does not have access to suitable ice, the rack may be placed in a cold-water bath that has been stored in the refrigerator overnight.

When removing the plasma after centrifugation do not disturb the white blood cells layer, also called the buffy coat, which forms a thin layer between the upper plasma layer and the lower layer of packed red blood cells. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube using the initial processing conditions. Indicate on Item 15 and 16 of the Biospecimen Collection form that the tube was re-centrifuged.

Aspiration of the lipid layer that may float to the surface after centrifugation could also adversely affect the test results. Thus, it is critical that only the clear plasma or serum between the buffy coat and the upper lipid layer be aspirated when preparing these sample aliquots. If lipids floated to the top of the plasma, indicate on Item 15 and 16 of the Biospecimen Collection form “lipids present on top of plasma/serum were not pipetted”.

1. Remove tubes #2, #3, and #4 from the centrifuge and place them in a wire rack in front of the sample aliquot tray. Remove the stoppers.

2. Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear plasma supernatant from tubes # 2 - #4 and pipet it into a 50 mL graduated centrifuge tube

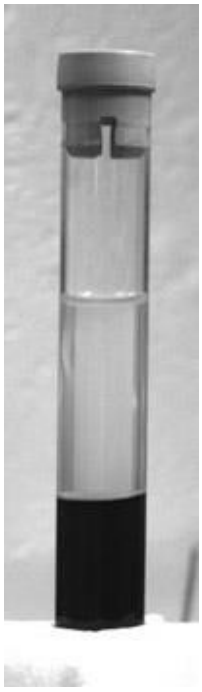
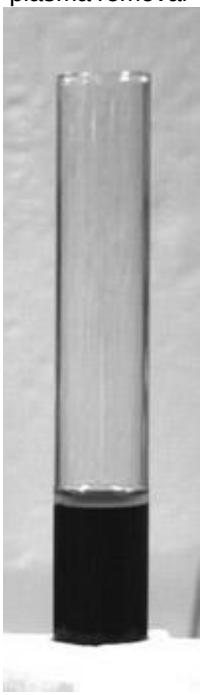
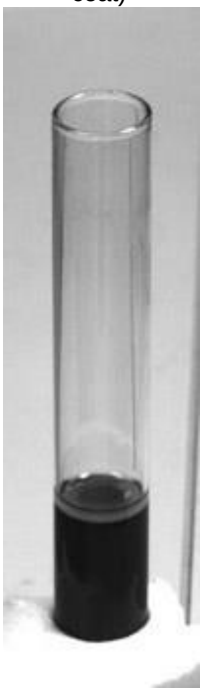
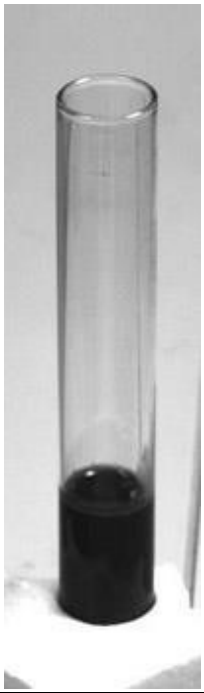
3. Using the same plastic transfer pipette, slowly aspirate the remaining plasma (minimum amount), buffy coat layer, and some of the red cells from tube #2. (Do not let the buffy coat aspirate into the bulb of the disposable pipette.) Transfer this to the 2.0 mL cryovial in position 2C. Repeat these steps for tube #3 and place the buffy coat into the 2.0 mL cryovial in position 2D. Repeat these steps for tube #4 and place the buffy coat into the 2.0 mL cryovial in position 2E. Fasten the brown screw caps onto these cryovials.

4. Re-stopper tubes #2 - #4 and discard in biohazard waste container.

5. Using a 1.0 mL pipettor (set to 0.5 mL), pipet from the 50 mL graduated centrifuge tube containing the plasma from tubes #2-4 and aspirate/dispense 0.5 mL of plasma into the cryovials in positions (refer to Figure 3: Aliquot Tray 1 Layout) column 1 rows A-B; column 2 rows A-B; column 3 rows A-E; column 4 rows A-E; column 5 rows A-B. You may need to change tips if bubbles occur. **Only for field centers that are participating in local storage:** Place the cap on the 50 mL graduated centrifuge tube to later distribute the remaining plasma equally into the appropriate number of 2.0 mL cryovials for local storage.

6. Add 10 µL of BHT solution using a 20 µL pipette set to 10uL to each of the four aliquots in column 1 rows A-B and column 2 rows A-B (change tips between each addition). With each addition, mix with the tip 3x (aspirate/dispense motions). Fasten the green and lavender screw caps onto the cryovials in columns 1-6 according to tray layout.

Figure 10. Appearance of blood samples during recovery of WBC's.

Whole blood in the collection tube	Blood after centrifugation	WBCs and RBCs after plasma removal	Top view of the WBCs (buffy coat)	Top view of sample after WBC removal
				

4.3 Stage Three: Processing of Serum

Stage three begins approximately 30 minutes after venipuncture for clinic participants. For samples collected in home or long term care facility, the serum samples may be centrifuged together with the plasma samples since all samples have sat longer than 45 minutes and the serum samples have clotted. If the plasma sample is centrifuged with the serum samples, then stage three processing can start at step two below immediately following the completion of stage two processing.

1. As close to 30 minutes after venipuncture as possible, and no longer than 45 minutes after venipuncture, spin the red stoppered tube #1 at 3,000 x *g* for 10 minutes at room temperature. Record the time when centrifugation begins on the Biospecimen Collection form. (**Stage 2 or Stage 4 processing can be done while these tubes are centrifuging**.)

2. Once the centrifuge comes to a complete stop, remove the tube and place it in a rack in front of the sample aliquot tray 1. Remove the stopper.

3. Set the 1.0 mL pipettor to 0.5 mL and pipette serum from Tube #1 into 2.0mL cryovials (see figure 3 aliquot tray 1 layout diagram) in column 7 rows A-E and column 8 rows A-C. Place the red screw caps on these vials.

4. Re-stopper tube #1 and discard it in a biohazard waste container.

Immediately following stage 4 urine processing, place the aliquot trays in the -80° C freezer. The aliquots should freeze in an upright position so that the material does not freeze in the cap. Record the time these aliquots are placed in the freezer on the Biospecimen Collection form.

4.4 Urine Collection and Processing

4.4.1 Urine Collection

A urine sample is collected from each participant (preferably) at the beginning of the clinical exam. After participants complete the reception work station activities, they are informed about the urine collection. The urine specimen is collected whenever the participant needs to void. If the participant has not voided by the time of the exit interview, the participant is asked to void at that time.

A copy of urine collection instructions should be given to the patient during the interview period to facilitate collection when the patient is ready to collect the sample. Read through the instructions with the patient making sure that he/she understands how to collect the specimen. Note that the use of a urine collection hat can make urine collection easier for some female participants.

HOW TO COLLECT YOUR URINE SAMPLE

Female Cleansing Instructions

- Wash hands thoroughly with soap and water.
- Unscrew the cap from the labeled specimen cup.

1. Stand in a squatting position over the toilet. Separate the folds of skin around the urinary opening.

2. Cleanse the area around the opening with the first towelette provided.

3. Repeat using a second clean towelette.

4. Urinate the first portion of urine in the toilet.

5. As you continue to urinate, bring the collection cup into the midstream to collect the urine sample.

6. Do not touch the inside or lip of the cup.

7. Urinate any excess urine into the toilet.

8. Replace the cap on the Urine Collection Cup.

9. Return the sample to the healthcare worker.

HOW TO COLLECT YOUR URINE SAMPLE

Male Cleansing Instructions

- Wash hands thoroughly with soap and water.
- Unscrew the cap from the labeled specimen cup.

1. Cleanse the end of the penis with the first towelette beginning at the urethral opening and working away from it (the foreskin of an uncircumcised male must be retracted).

2. Repeat using a second clean towelette.

3. Urinate the first portion of urine in the toilet.

4. As you continue to urinate, bring the collection cup into the midstream to collect the urine sample.

5. Do not touch the inside or lip of the cup.

6. Urinate any excess urine into the toilet.

7. Replace the cap onto the Urine Collection Cup.

8. Return the sample to the healthcare worker

A specimen cup (labeled with the participant's ID), cup lid, 2 towelettes, and a TIME VOIDED label are provided by the staff member working with the participant at that time. The participant is instructed to:

1. void in the cup, filling it halfway if possible, and place the lid securely on top of the container,
2. record the time of voiding on the label, and
3. bring the specimen cup back to the staff member, OR
4. place the sample container in a refrigerator designated for urine samples, and report to a staff member that the specimen has been collected, depending on locally approved OSHA regulations.

Bathrooms are equipped with a wall clock and pencils for participants to use in recording the time of voiding on the label. The staff member verifies the participant has written the "time voided" on the label and assesses the adequacy of the sample for processing. At least 10 mL of urine is recommended for processing, 15 mL is optimal for a complete processing set. If the first voided sample is insufficient (<8mL), the participant is requested to void again in a clean container prior to leaving the field center. A note is made on the participant's itinerary sheet that a second sample is needed. A note can also be made on the participant's first sample that a second sample is needed. The optimal time for the collection of the second specimen is after the snack. The instructions for providing the urine sample are repeated to the participant at that time.

Prior to processing, the laboratory staff records whether a urine sample was obtained and transcribes the collection time of the urine void onto each participant's Biospecimen Collection form.

Labeled urine samples should be placed in the designated specimen refrigerator for storage prior to processing and as soon as possible after the specimen has been voided. This can be done either by the participant or a 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 may be left at room temperature for a maximum of 4 hours.

Refrigerated urine samples need to be processed and frozen as soon as possible, and within 12 hours of collection. A comment is placed in Item 11 of the Biospecimen Collection form if a urine "sample has remained at room temperature for more than 4 hours", or "is not processed and placed in the freezer within 12 hours of collection".

Non-clinic samples should be placed in the cooler with ice until they reach the clinic for processing.

4.4.2 Stage Four: Urine Processing

The technician prepares the work area by laying out a pipettor and bringing forward the aliquot tray in the ice-water bath. Keeping the aliquot tray as cold as possible is very important since bacteria grows quickly in urine samples. A barcoded ARICID label was affixed to each specimen cryovials during day one preparation. ID labels are placed vertically on the cryovials, as on the blood vials. Urine processing is the same for both clinic and non-clinic specimens.

Eye protection, gloves and lab coat must be used for all urine processing. All other rules regarding the safe blood specimen handling must be observed when processing urines.

1. Mix the urine container by inverting three times.

2. Record the date and time of collection, the time of processing, and the processing technician's code on the Biospecimen Collection form A 1 and 2, B 3 and 4

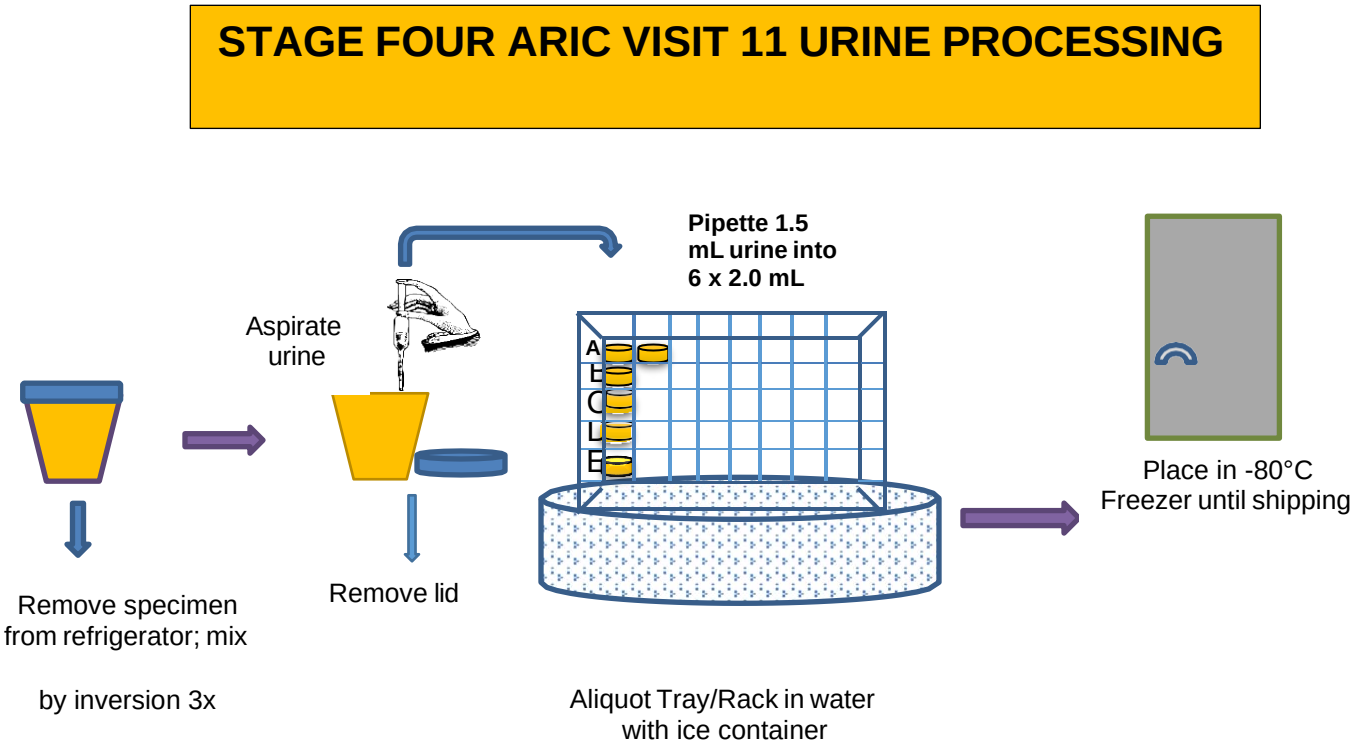
3. Using a 1.0 mL pipettor set to 1.0 mL, aspirate and dispense into six pre-labeled cryovials in the aliquot tray 2 positions column 1 row A-E and column 2 row A.

4. Set the 1.0 mL pipette to 0.5 mL and repeat dispensing into the six pre-labeled cryovials in the aliquot tray 2 positions column 1 row A-E and column 2 row A. Place the yellow screw caps on the urine vials.

5. Immediately after processing, transfer the aliquot trays with the blood and urine aliquots to the -80° C freezer.

6. Once the specimens are safely stored in the freezer, the urine remaining in the collection container may be discarded. The urine can be poured down a sink with copious amounts of water, or it can be flushed down a toilet. The empty collection container is discarded in accordance with local biosafety guidelines.

Figure 11. Stage four ARIC Visit 11 urine processing

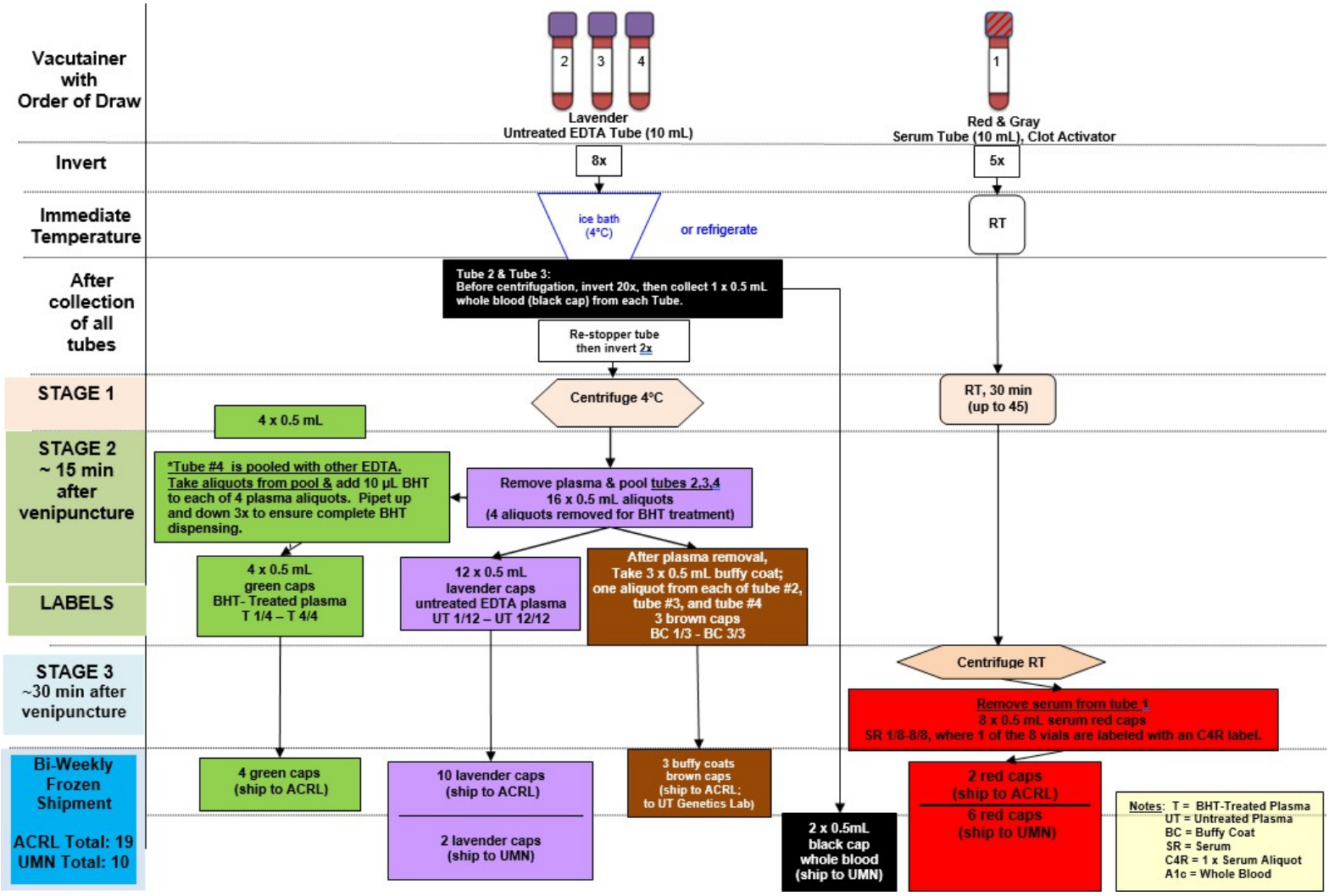


If the volume of urine sample is inadequate to process at least three of the six sample aliquots, check to see if a second sample was provided. If there is a second sample and it (in and of itself) is adequate for processing, use the second sample (record the time voided on the Biospecimen Collection form based on that sample) and discard the first sample. If neither is adequate, combine the specimens, and transcribe the latest voiding time on the Biospecimen Collection form.

4.5 Overview of Specimen Processing

A summary overview of the protocol steps for the collection and processing of urine and blood specimens is presented in Figure 5a and Figure 5b. (Stage 4 Urine Processing and Stage 3 Blood Specimen Processing, flow diagrams). The order of blood processing after tube collection begins with tube 5.

Figure 12. Blood specimen processing flow diagram



4.6 Freezing

When all of the blood and urine specimens have been aliquoted into their respective cryovials and the cryovials have been replaced in the wire test tube rack, the entire rack is placed upright in the -80° C freezer until packaging. Samples must be placed into the freezer within 90 minutes from venipuncture time. PAXgene tubes should be placed upright in the wire test tube racks to allow for homogenous freezing. Samples must be thoroughly frozen before packaging them for storage and shipping. Record the time that the aliquots are placed in the freezer on the Biospecimen Collection Form. Package the samples for each participant once frozen. This includes tube #5.

4.7 Labeling Aliquots

There are a total of 35 aliquots designated for ARIC at visit 11. Each aliquot should be labeled with a pre-printed ARIC aliquot label during processing. Orient each label such that the left of the label is aligned with the cap and the right of the label is aligned with the end. An example label is shown below:

If labels are damaged or otherwise unavailable, create a temporary label with subject ID, type of aliquot, volume, and visit information displayed until a replacement can be created. Hold all samples for a participant at the field center until all aliquots are properly labeled. Do not ship any improperly labeled aliquots until the labels can be corrected. Contact ARIChelp for guidance on receiving new labels.

Pre-printed ARIC label sheets also contain an extra label for an additional C4R serum aliquot.

5. STORAGE AND SHIPPING (FOR FROZEN SPECIMENS)

5.1 Packaging Frozen Specimens

Remove the sample aliquot tray from the -80° C freezer. Package quickly after this point to avoid thawing of the specimens. Each participant's serum, plasma, whole blood, urine samples, and PAXgene tubes are packaged in freezer storage bags by subject ID and the lab they are being shipped to. Label one (1) 4" x 6" primary bag per ID with the participant ID and "ACRL" Label one (1) 4" x 6" primary bag per ID with the participant ID and "UMN". Label as many large secondary shipping bags as needed with "ACRL". Label as many large secondary shipping bags as needed with "UMN." Each secondary bag will hold about 10 primary bags. ARIC Generation 2 samples may be shipped in the same shipping box as ARIC samples following the packaging instructions in 5.1.1. All ARIC Generation 2 samples, including PAXgene tubes, should be stored in secondary shipping bags, separately from ARIC samples, and labeled with "GEN2" on the outside. This will assist the lab staff in differentiating between ARIC samples and ARIC Generation 2 samples.

5.1.1 Packaging Frozen Specimens

1. For each participant place (2) red cap cryovials, (0.5 mL), (4) green cap cryovials (0.5 mL), a total of 10 (0.5 mL) lavender cap cryovials, (2) yellow cap cryovials (1.5 mL), (3) brown cap cryovials and (1) PAXgene tube wrapped in bubble wrap to cushion in primary bag #1 for ACRL. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
2. For each participant place (6) red cap cryovials (1 of the 6 vials should be labeled with the C4R Serum label and placed in a separate biohazard bag labeled C4R within the primary bag #1), (2) lavender cap cryovials (0.5mL), (2) black cap cryovial (0.5 mL), (4) yellow caps cryovials (1.5 mL) in primary bag #1 for UMN. Verify that the bags are labeled correctly and the C4R sample is with the other ARIC samples for that participant. Add an absorbent pad, press the air out of the bag and seal.
4. Place the primary bag(s) #1 for ACRL into a large secondary shipping bag(s) for ACRL. Place primary bag(s) #1 for UMN into a large secondary shipping bag(s) for UMN. Press the air out of the bags and seal.
5. Place the large shipping bags in the -80°C freezer until they are ready for shipping.

Complete the shipping log with appropriate information for these samples.

5.1.2 Packaging and Mailing Instructions for Biweekly Shipment of Specimens

The bag(s) of frozen sera, plasma, whole blood, urine samples, and PAXgene tubes are packed and shipped in Styrofoam boxes. Packaging instructions (See Figure 6) are as follows:

1. Place a layer of dry ice on the bottom of the Styrofoam box.

2. Put one-half of the large bags of cryovials into the Styrofoam box on top of the dry ice.

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

4. Put the remaining sample bags into the Styrofoam box on top of the dry ice.

5. Layer more dry ice on top of and around the sample bags. The amount of dry ice in the shipping should be sufficient to maintain the temperature for at least 48 hours.

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

7. Insert the paper shipping forms and any unused aliquot labels into a 12" x 12" bag and tape to the top of the biomailer lid. For the shipment to the ACRL laboratory also include a copy of the BIO form. For any partial collection where there are fewer than the expected number of aliquots being shipped, also include any unused cryo labels for that participant with the shipping form.

8. Secure the flaps of the outer shipping box with tape (criss-cross, making a plus sign, DO NOT completely seal with tape). Affix “Biological Substance, Category B” label adjacent to “UN 3373” label and a completely filled out FedEx dry ice label to outside of box.

9. Affix the FedEx airbill to the outside of the box. Record the site address and telephone number in section 1. (Do NOT use the billable stamp on dry ice shipments.) Contact FedEx (1-800-GO-FEDEX) for pickup.

10. If necessary, more than one box may have to be shipped biweekly

5.1.3 Shipping

The samples remain at -80° C until they are shipped. All frozen plasma, serum, whole blood, urine, and PAXgene tubes and phantom aliquots collected and stored within the last two work weeks are shipped to the Laboratories on Tuesdays following the schedule in Appendix 5. Samples may be shipped on Wednesday if the Field Center is closed on

Monday, but the contact person at the Laboratories must be notified that the shipment will arrive one day later than usual. There is no minimum shipping requirement; frozen samples are shipped biweekly regardless of the number of specimens that have been frozen and stored within the last collection period. Weigh all packages before shipping, if possible. It is important to record an accurate weight on the FedEx airbill. Do not over-estimate the package weight.

Whenever packages are shipped to the Laboratories, send an e-mail message containing the tracking number and date of shipment to Ron Hoogeveen (ronh@bcm.edu) and mailto:Liza Azara (azara@bcm.edu), Jaime Lavallee (fale0014@umn.edu) and Malini Udtha (HGC_Lab@uth.tmc.edu). Any questions about shipments to the UMN lab should be directed to Matt Price (price425@umn.edu)

All shipping containers are sent to the Laboratories by "Priority Overnight" shipping NOT "First Overnight" since First Overnight is very expensive, to ensure receipt within 24 hours. The empty Styrofoam containers are recycled by returning them to the Field Centers via ground transportation.

Shipping containers to the Laboratories are addressed as follows:

ARIC Central Laboratory

Care of: Liza Azara / Ron Hoogeveen
Atherosclerosis Clinical Research Laboratory (ACRL)
Baylor College of Medicine
One Baylor Plaza
Anderson Building, Room 515B
Mail Station: 285
Houston, Texas 77030
Telephone (713) 798-3407
Fax: (713) 798-7400
azara@bcm.edu / ronh@bcm.edu

ARIC Chemistry Laboratory

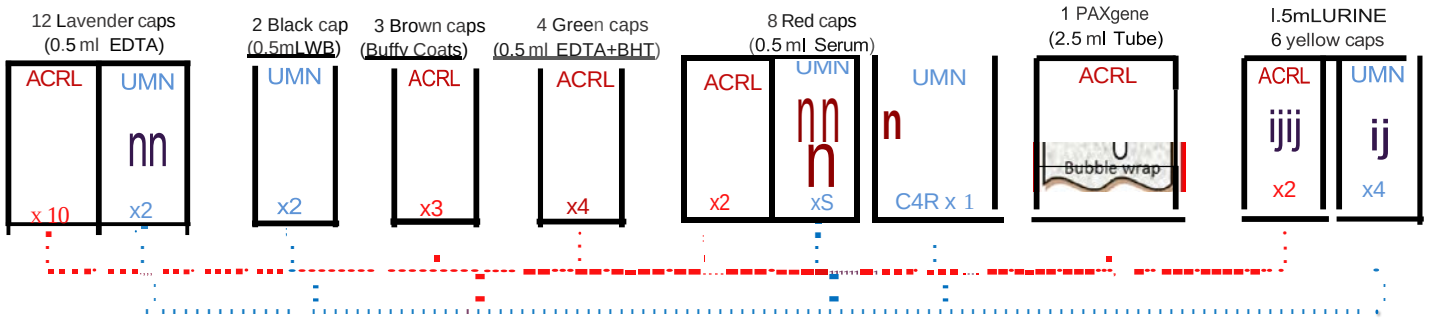
Jaime Lavallee/ARIC V11
University of MN/ARDL
1200 Washington Ave S Ste 175
Minneapolis, MN 55415
Telephone: (612) 625-5040
Fax: (612) 625-4142
fale0014@umn.edu

Be sure to use the appropriate ARIC visit in your address to ensure samples are processed correctly upon arrival at the laboratory.

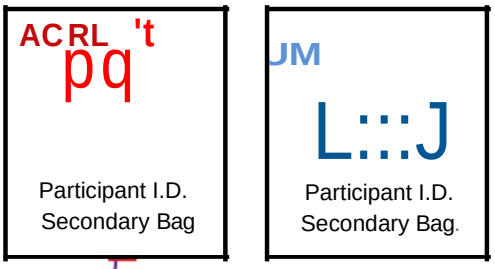
Figure 13. Shipping diagram.

Step 1. Bagging (Per Participant) — ON DRY ICE

a. Sort samples into respective primary bags (ACRL & UMN) all sample types for EACH participant



b. Place primary sample bags into the ACRL and MN secondary storage bags respectively; add absorbent pads.



2 Individual Biomailers

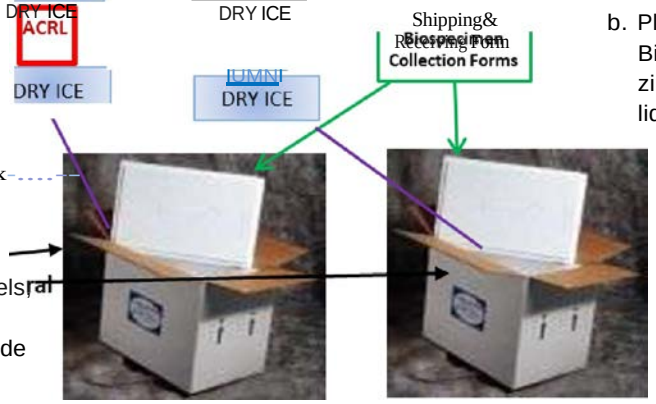
c. Place ACRL samples into a biomailer& ship to ACRL

d. Place UMN samples into a biomailer& ship to UMN

Step 2. Shipping

a. Add layer of dry ice to bottom of biomailers, followed by specimen bags, then another **layer** of dry ice on top. (use sufficient amt. of dry ice to sustain, esp. during hot weather months.

Suggested amount of 30-40 lbs for lg. box mailings.)



b. Place Biospecimen Collection Forms and the Bi-Weekly Shipping & Receiving Form in a zip lock, then tape to top of ACRL biomailer lid & UMN biomailer lid.

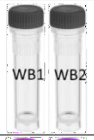
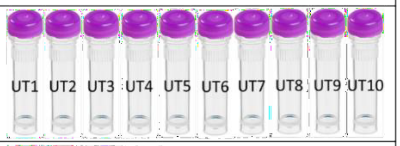
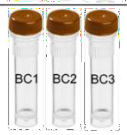
c. Seal boxes with tape, affix dry ice labels, biological substance labels, contents description labels, and completed Fede Express air bills

d. Have Fed-Ex pick up for Priority Overnight delivery by 10:30 a.m.

Figure 14. Example packaging for Visit 11 UMN aliquots.

The bag shown on the bottom should contain the participant's C4R serum aliquot. Note the bag is labeled "C4R" with sharpie. The bag shown on the top should contain the participant's other five serum aliquots for the UMN lab. Both bags should be labeled with a UMN ARIC participant label. Once the bags have been filled, the bag on the bottom should be placed inside the bag on the top.

Figure 15. Visit 11 Aliquot Yield

ARIC EXAM 11						Vial Amount / Aliquots for:	
Tube	**Sample Type**	**Vial Cap Color**	**Vial Amount**	**Aliquots Label Used**	**Volume per Vial**	**UMN Chemistry Lab (ARDL)**	**BAYLOR Lipid Lab (ACRL)**
Tube 2, 3, 4 Full Draw (Before Centrifugation)	Whole Blood Aliquot	Black	2	WB1 – WB2	0.5 mL		
Pooled Tube 2, 3, 4 Full Draw	Untreated EDTA Plasma	Lavender	12	UT1 – UT12	0.5 mL		
Pooled Tube 2, 3, 4 Full Draw	Treated EDTA Plasma	Green	4	T1 – T4	0.5 mL		
Tube 2, 3, 4 Full Draw (After Supernatant Removed)	Buffy Coat	Brown	3	BC1 – BC3	Varies		
Tube 1 Full Draw	Serum	Red	8	C4R*; SR2-SR8	0.5 mL		
Urine Collection Cup Full Draw	Urine	Yellow	8	UR1 – UR6	1.5 mL		
Tube 5 Full Draw	PAXgene RNA (Whole Blood)	Red	1	PAX	2.5 mL		

6. QUALITY CONTROL

The first step in quality assurance for blood drawing is the training and certification process. Other steps include maintaining logs of equipment checks, observation of technicians (by other technicians and by CC staff on monitoring visits) as they go through the sequence of steps in blood drawing and processing; review of the condition of samples received at central laboratories for problems in shipment; and periodic analysis of the study data for signs of problems in drawing or processing, such as hemolysis or delays in completing processing.

In addition to the Biospecimen Quality Control procedures discussed in this chapter, ARIC Manual 12 details additional study wide Quality Control and Assurance measures.

6.1 Personnel Training and Certification

To be certified, technicians complete Webex/ Zoom training taught by certified laboratory staff which includes biospecimen (blood, urine) collection, processing, packaging and shipping as well as quality control measures such as phantom specimen collection. Each technician must complete the training and pass both written and practical exams before becoming certified for the ARIC study. Certification requirements for personnel are:

- Collection, processing, and shipping specimens for 3 volunteers under the supervision of the certified lead technician at the field center, and
- Completion and submission to the CC of the written exam.

Only certified phlebotomists should be hired. ARIC training is specific to the collection and processing procedures of the study.

6.1.1 Training Procedures

Technicians will be trained in the phlebotomy procedure by their respective institutions. Many institutions now require their phlebotomists to have national certification. The study does not provide phlebotomy training.

A check list of the venipuncture and processing procedures that ARIC technicians must know and be prepared to demonstrate is listed in the “Checklist for Observation of Biospecimen Collection and Processing”. The technicians must study the ARIC Biospecimen Collection and Processing Manual and pre-training slides providing basic knowledge prior to actual training session.

During training, the technician will be walked through various procedures before participating in a practicum for drawing and processing samples. During the practicum, the technician will perform venipuncture and collect practice tubes in the correct order and place them in their proper positions. Technicians will process samples from beginning to end, providing them with hands-on experience and allowing them to become comfortable with the procedures, before they are certified to collect ARIC samples.

Any questions or problems must be addressed before the technicians proceed to collect biospecimen samples from ARIC participants. Before technicians draw blood from any ARIC participant, they must take and pass the practical and written exams provided on the ARIC website. After passing the exam and dependent on the written evaluation of their instructor, they may either proceed to drawing blood from the ARIC participants as part of a team or continue to practice on volunteers.

ARIC visit 9/10 slides will be updated and used for training/retraining all V11 lab personnel and used to supplement the lab manual. Certified personnel will perform the training/retraining locally, incorporating revisions and updates. Some personnel may be retained from visit 9/10 to complete visit 11, however they must retake and pass the practical and written exams before recertification. The manual for biospecimen collection and processing along with the slides and webinar replaces a centralized "training site" for all four centers in one setting.

6.2 Venipuncture and equipment records

There are two different aspects of venipuncture and equipment quality control. One is the daily or monthly record of the performance of the refrigeration equipment, and centrifuge. Daily and monthly measurements (e.g., temperatures) are recorded on a log, as described below. The other aspect of quality control is documentation of problems with blood collection and processing which is part of each participant's record. (See Biospecimen Collection Form, Items 10, 11, 15, and 16.)

- all or some blood samples not drawn
- tourniquet reapplied
- fist clenching
- needle movement
- participant reclining
- broken tubes
- clotted tubes
- hemolyzed serum or plasma
- lipemic serum or plasma
- other processing problems

This record provides documentation that blood was drawn in a standardized manner and that the equipment was functioning properly. This quality control documentation is the best evidence 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 seriously compromise the laboratory test data. It is very important that the quality control records of the procedures and the equipment be properly maintained.

Daily, log the temperatures of the laboratory, all refrigerators, freezers, and refrigerated centrifuges, In addition, check and record the actual speed of the centrifuge annually with a tachometer. (This is usually performed by a biomedical engineer.) These checks are

summarized onto the Summary of Observation and Equipment Checklist (see the QC appendix of Manual 2) quarterly and sent to the CC.

6.3 Monitoring by the Central Laboratories

The laboratories review the drawing and processing time, as recorded on the Biospecimen Collection Form. If there are extreme values that raise questions about the validity of laboratory results, the field center is alerted to the problem. If a value is considered suspicious or an “outlier”, the Biospecimen Collection Form is reviewed for any collection/processing discrepancies, and if there are concerns related to the collection/processing time the field center is notified by the lab. Monitoring is described in more detail in the laboratory manual.

6.4 Quality Control Duplicate Blood Samples

As part of the overall quality control program for laboratory determinations from blood and urine samples, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under the participant's regular ARIC participant ID number, and the other half under a Quality Control Phantom Participant (QC) ID number. Do not send QC phantom form to laboratories.

To reduce the burden on participants, only one extra tube of blood is drawn from select participants and processed under a Phantom ID. For data analysis, results of each laboratory measurement are matched to the appropriate participant results at the Coordinating Center from the QC Phantom ID Form that is completed by Field Center technicians.

The QC blood samples are collected in sequential order (cycling back to Tube #1 after QC Tube #5 has been collected). Each Field Center will collect QC samples from approximately 5% of each specimen. The Snapshot Report in CDART as well as the participant’s BIO form will show whether a participant has been selected for lab QC.

6.4.1 Blood and Urine QC Sample Checklist

The venipuncture technicians maintain a Phantom Tracking sheet (found on the ARIC website) in their work area of the QC samples to be drawn. As each sample is drawn and processing completed, it is marked as such.

6.4.2 Preparation for Drawing and Processing QC Samples

Blood Drawing Tubes: Each morning (or the afternoon before) the blood drawing technician(s) prepares the extra blood collection tube(s) any QC sample(s) to be drawn that day. Each tube is labeled with the QC ID number to be used for that participant. In addition, the technicians may wish to mark QC blood drawing tubes "QC" in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. **However, since the laboratories are blinded to the samples that are meant for QC, duplicate tube #5 should not be marked differently from the regular participant sample.** QC tube #5 is sent as is to the ACRL

to be delivered to the UT Laboratory. All QC tubes are set in the same rack used to hold the regular blood collection tubes in a separate row from the other tubes.

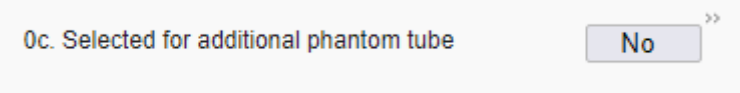
Sample Aliquot Tubes: Each morning (or the afternoon before) separate sample aliquot trays are prepared for any QC blood vials that the technician will process that day. The tray contains all the aliquot vials needed to process the quality control sample. The tubes in each block are labeled in advance with the QC ID number. Care must be taken during processing that the labels on the sample aliquot tubes match the label on the QC blood collection tubes.

For the duplicate urine sample, six extra cryovials for the urine QC duplicates are set out and labeled with the urine QC ID number.

6.4.3 Collecting and Processing QC Blood and Urine

Selecting Participants for QC Blood Draw: Technicians should attempt to collect a phantom (QC) sample from every participant selected for lab QC as indicated in the "General Appointment Information" section of the "Participant Snapshot Report" OR on item 0c of the BIO form (shown below). In some cases, even when the participant is selected for a QC sample, the size of the participant's veins, difficulty drawing the other specimens, or apprehension surrounding blood draw makes drawing the QC tube prohibitive. If the technician determines that a participant cannot give a QC tube, that tube is instead collected from the next participant selected for additional phantom tube. No participant should give more than one QC tube.

Figure 16. BIO form indicator of participant QC selection



Order of QC Tubes in Relation to Regular Blood Collection: Draw the QC tubes after the other tubes have been collected. This procedure is followed to cause the least disruption of the collection of the regular blood samples. If the blood flow slows at the end of the draw such that it would be difficult to obtain the extra QC tube, do not attempt to obtain a QC sample from that participant. Instead, this QC sample should be collected from the next participant selected to give a QC sample. DO NOT PERFORM A NEW NEEDLE STICK JUST TO GET MORE BLOOD FOR A QC SPECIMEN. DO NOT REAPPLY THE TOURNIQUET AFTER INITIAL RELEASE.

Processing and Freezing QC Blood: Process the QC blood samples along with the regular blood samples. After processing is completed for each QC blood collection tube, the sample aliquot tubes are put into the -80° C freezer along with QC tube #5. QC tube #5 is stored identically to the regular PAXgene tubes, however the label will be identified with a phantom number. Other tubes will have aliquots taken and then discarded. After the samples are thoroughly frozen, they are put into a freezer storage bag and put into the freezer box.

The six urine QC samples are placed into the freezer at the same time as their matched participant pair.

Logging the Match between QC and Regular ARIC ID's and Reporting these to the Coordinating Center: The ARIC Phantom Tracking Sheet and PHT forms are used to keep track of the match between the QC and regular ARIC specimens. As participants donate blood to make up a QC set, labels with their participant ID numbers and their Phantom numbers are added to the Phantom Tracking Sheet corresponding to the tubes donated. This step must be done immediately after completion of drawing blood for that participant, to minimize the chance of recording the wrong ID number. Information regarding the Phantom Collection is transferred to the Coordinating Center by keying the Phantom form (PHT) into the data management system. Do not send a hardcopy of the Phantom form (PHT) or the Phantom Tracking Sheet to the ACRL or UMN Laboratories because it will unblind the masked QC analysis of the samples. Do not place a phantom label on the hardcopy of the Biospecimen (BIO) form for any participant selected for QC. File and maintain the original paper PHT form until study closure checks are made.

Shipping QC Samples: QC Samples should be shipped following the instructions in Chapter 5. All QC samples collected under a single phantom ID should be bagged together and separately from any regular blood draw samples. This is done to maintain confidentiality of the phantom ID numbers. To maintain blinding on the shipping log, enter the true date of specimen collection, but use “08:00AM” as the time of collection for all QC samples, regardless of the time of day when the sample was drawn.

6.5 Laboratory Biospecimen Processing

6.5.1 Procedures for Laboratory Analyte Determinations

Blood samples are collected and processed at the field centers for shipment to a laboratory for analysis of several analytical tests. In the present section, the emphasis is on quality assurance in the central laboratories, beginning with the receipt of samples. These matters receive careful and detailed discussion in the laboratory manual, which covers procedures for: receiving samples and storing them at a proper temperature until analysis; schedules of equipment maintenance; storage and handling of reagents, calibration standards, and quality control materials; internal and external quality control programs; and transcription and reporting of measurement results. This section discusses reporting on the effectiveness of laboratory quality assurance procedures and utilization for quality control of (1) analyses of study data and (2) blind replicate samples from participants sent to the laboratory.

6.5.2 Receiving Samples at Laboratory

At the laboratories, a record in the local database is created using the participant ID number for each specimen when it arrives. It is important in handling ARIC frozen blood samples to avoid any unnecessary exposure to room temperature. Clear procedures for

unpacking specimens upon arrival are set out in the laboratory protocols to minimize such exposure. While awaiting analysis, specimens are to be kept in storage at -80^{0}C. The laboratory has provisions for (1) prompt detection of power failure or of failure of a freezer to maintain the proper temperature, including both local alarms and alarm signals to a pager monitored by laboratory personnel if a problem develops after hours; (2) back-up freezers in the event of a freezer failure and backup power supplies in the event of power failure; (3) plans for the use of dry ice to maintain the sample temperature until any problems with the freezer can be repaired.

6.5.3 Maintenance Procedures at the Laboratories

Maintenance procedures for laboratory equipment are fully specified in the laboratory protocols or in manufacturers' manuals referenced in the protocols. Technicians are fully instructed in these procedures.

A regular schedule is set up for routine maintenance procedures, with logbooks kept on their performance. The laboratory supervisors review these logs on a regular basis to verify that proper maintenance procedures are being carried out according to the schedule set and that any special maintenance procedures needed are carried out.

The laboratory protocol fully specifies the reagents used, the sources from which they are procured, and the procedures used to prepare and store reagents to guarantee the stability of the reagent and the accuracy of the assay. The laboratory protocol also fully specifies the sources of calibration standards and quality control materials, the procedures used to prepare and store calibration standards and quality control materials, to guarantee the stability of the material and the accuracy of the assay. To maintain the comparability of measurements using new and old reagents, calibration standards and controls, an overlap period is carried out, during which concentration values for the new reagent, calibrator or control are determined and compared to the material that which is being replaced.

6.5.4 Internal Quality Control

The laboratories maintain an internal quality control program involving the analysis of multiple samples from quality control pools in each analysis run in which ARIC study samples are analyzed. Results for these samples are used to decide whether the measurement process is in control and whether the results on the study samples will be accepted or whether the measurements should be repeated after taking corrective action.

Internal quality control procedures monitor analytical performance of the test relative to medical goals and alert analysts to unsatisfactory analytical performance. Quality control statistics are used to make judgments about the quality of analytical results, whether system correction is necessary, whether patient data should be accepted or rejected, and for estimating performance parameters which can be compared to analytical and medical goals. Testing is monitored by two control samples analyzed per run for each batch of samples. A permanent standard deviation (SD) and coefficient of variation (CV) is

determined by analyzing the material on 50 separate days. The mean for new lots of material is established by analyzing the material on 15-20 separate days. The SD and CV from the data collected over 15-20 days is used to monitor the permanently established SD. Quality control results are plotted on Levy-Jennings plots and acceptability (i.e. in statistical control) is determined using three Westgard rules (1-2s, 1-3s, and 2-2s). Documentation is made on the control charts when there is a change in reagent lot numbers, any action is taken due to unacceptable control results, and when other pertinent information is observed.

6.5.5 External Quality Control

For many of the assays performed in the ARIC study, the laboratories participate in various standardization or certification programs run by outside agencies, such as the College of American Pathologists or the CDC Lipid Standardization Program. The laboratories should continue to maintain acceptable results in these programs and promptly provide the CC with copies of any reports on their performance generated by these programs. Should any of the results achieved in these programs appear problematic, they are reviewed by the CC and the Quality Control Committee or Laboratory Committee together with other quality control information on the assay in question to determine what action is appropriate.

6.5.6 C4R Processing and Shipment

The sample processing protocol for the ARIC V11 will have a serum aliquot "C4R" label. The ARIC lab committee has approved 1 of the 8 serum aliquots obtained from Tube #1 to be labeled with a C4R label. The appropriate C4R labels will be provided on the label sheet dedicated for each ARIC participant. Each field center will label one of the eight serum aliquots (0.5mL) of the participants set with the provided C4R label found on the label sheet. Note that C4R label replaces the SR1 label for the serum aliquots. If there is only one serum aliquot from a participant, then that one aliquot will be labeled with a SR1 label. See Figure 15 below.

The C4R serum aliquots are destined for the chemistry lab at UMN and should be placed in a separate bag inside the biweekly biomailers (for easy identification).

Figure 17. Serum Aliquot with a C4R label

ARIC EXAM 11		Serum Aliquots for:	
SERUM	Aliquot Labels Used	UMN Chemistry Lab (ARDL)	BAYLOR Lipid Lab (ACRL)
Tube 1 Full Draw	8 Aliquots C4R*; SR2-SR8		

Note that C4R label replaces the SR1 label for the serum aliquots. Keep the SR1 label on the participant's label sheet. If there is only one serum aliquot from a participant, then that one aliquot will be labeled with a* **SR1 *label.*

7. LABORATORY DATA TRANSFER

The Atherosclerosis Laboratory and the University of Minnesota Laboratory have the responsibility for reporting results to the Coordinating Center. All test results are transmitted to the Coordinating Center in .csv file format. This transmission occurs biweekly. A selected group of these tests is reported to the field centers to be distributed to the participants. In addition to this group of tests, any test result exceeding its ARIC-defined "alert range" is also included in the report. This data transfer is achieved through file transfer protocol (FTP) or use of a Coordinating Center upload facility that is accessed through the web-based Data Management System, CDART (the Carolina Data Acquisition and Reporting Tool). (See Appendix 1 for complete analyte details for ARIC Visit 11)

If a lab discovers errors in any previously transferred ARIC data files, the following steps are taken to resolve the issue:

- The lab will notify the CC within 3 days of identifying a problem. This will help the CC to prevent incorrect results from being sent to ARIC participants when applicable.
- The lab will provide an explanation of values that are changing (including analytes affected) and identify ARIC participants (or batch) involved.
- The CC will identify whether results already sent to participants are impacted. If results are majorly impacted, the CC will facilitate a discussion with ARIC PIs about how to handle the updated records.
- Once the corrected file is approved for transfer, the CC will provide instructions for transferring the corrected file.
- After the data is updated, the CC will run a comparison to verify that the expected changes were the only values changed and that the spread of values is consistent with unchanged data.

8. REPORTING RESULTS

The Laboratories have the responsibility for reporting results to the Coordinating Center within one month of sample receipt. In order to see if the Coordinating Center has received and processed the lab results for a participant, the field center can run the “Results Status Report” using CDART, the ARIC data management system. This report displays the receipt dates of information from the Central Laboratories and reading centers. Tests reported to the participants will be available to the field centers via a report in CDART called the “Summary of Results Report.” Any tests included in this report whose results exceed their alert range will be flagged appropriately. Reference ranges can be found in Appendix 2.

9. LOCAL FIELD CENTER ALIQUOTS

Field centers will not store local aliquots for ARIC Visit 11. Visit 11 labels including local aliquots may have been printed due to timing of this decision. Do not create local aliquots.

10. APPENDICES

Appendix 1. Laboratory tests ARIC Visit 11

Sample Type - Visit 11 Test	Suitable for home visit?	Lab	Fasting	Volume of specimen needed	Machine (Method) –	**Report to Participant	**Participant Alert
Whole Blood - HbA1c	Yes	UMN	No	0.5 mL	Tosoh G8 (HPLC)	**YES**	No
Whole Blood - Hemoglobin	Yes	UMN	No		Sysmex XS-1000i	**YES**	No
Urine – Albumin	Yes	UMN	No	1.5 mL	Roche Cobas 8000	No	No
Urine - Creatinine	Yes	UMN	No		Roche Cobas 8000	No	No
***Urine – calculated ACR**	-	-	-	N/A	Calculated value	**YES**	**YES**
****Serum - Glucose**	Yes	UMN	No	0.5 mL	Roche Cobas 8000	No	No
Serum - Creatinine	Yes	UMN	No		Roche Cobas 8000	**YES**	**YES**
Serum - Albumin	Yes	UMN	No		Roche Cobas 8000	**YES**	No
Serum - Cystatin C	Yes	UMN	No		Roche Cobas 8000	No	No
Serum - Potassium	Yes	UMN	No		Roche Cobas 8000	**YES**	**YES**
Serum - Magnesium	Yes	UMN	No		Roche Cobas 8000	**YES**	No
Serum - Fructosamine (Roche)	Yes	UMN	No		Roche Cobas 8000	No	No
Serum - Glycated Albumin (Asahi)	Yes	UMN	No		Roche Cobas 8000	No	No
Serum - 1,5-anhydroglucitol (GlycoMark)	Yes	UMN	No		Roche Cobas 8000	No	No
****eGFR**	Yes	UMN	-	N/A	Calculated value	**YES**	**YES**
Plasma - hs-CRP	Yes	ACRL	No	0.5 mL	Beckman Coulter AU480	No	No
Plasma - lipids TC	Yes	ACRL	No		Beckman Coulter AU480	**YES**	No
Plasma - lipids HDL-C	Yes	ACRL	**YES**		Beckman Coulter AU480	**YES**	No
Plasma - lipids TG	Yes	ACRL	**YES**		Beckman Coulter AU480	No	**YES**
***Plasma – lipids calculated LDL-C**	Yes	ACRL	-	N/A	Calculated value	No	No
***Plasma – lipids calculated non-HDL-C**	Yes	ACRL	-	N/A	Calculated value	**YES**	No

*ACR – albumin-to-creatinine ratio is calculated. LDL-cholesterol is calculated from total cholesterol, HDL-cholesterol and triglycerides. Non-HDL-cholesterol is calculated from total cholesterol and HDL-cholesterol. ACR, HDL- cholesterol, and non-HDL-cholesterol will be reported to the participants.

** The column for “Report to Participant” indicates test results that will be included in a final summary report to each participant. The column “Participant Alert” indicates alerts that should be immediately communicated with a participant depending on the test result value.
*** Since the participant is not giving a fasting sample, the glucose value is not reportable. HbA1c is reported to participants instead.
**** eGFR - estimated glomerular filtration rate is a calculated value. ARIC estimates kidney function, called estimated glomerular filtration rate (eGFR), following national guidelines. In 2022 there was a recommendation to switch to a slightly updated equation so ARIC reporting at Visit 10 follows this recommendation. The change in equation usually results in slightly lower estimates among African-American participants and slightly higher estimates among other participants for the same serum creatinine, age and sex.

Appendix 2. Laboratory Test Reference Ranges

Description	Unit	Reference Ranges
HbA1c	%	Normal: <5.7
		Pre- diabetes: 5.7 – 6.4
		Diabetes: >6.4
Hemoglobin	g/dL	Female: 11.7 – 15.7
		Male: 13.3 – 17.7
Urine Albumin	mg/L	<20
Urine Creatinine	mg/dL	Males: 40 – 278
		Females: 29 – 226
Urine - Calculated ACR	mm/g Cr	<30
Serum - Creatinine	mg/dL	Females: 0.4 – 1.1
		Males: 0.5 – 1.2
Serum - Albumin	g/dL	3.5 – 5.2
Serum - Cystatin C	mg/L	0.51 – 1.05
Serum - Potassium	mmol/L	3.5 – 5.1
Serum - Magnesium	mg/dL	1.6 – 2.6
High Sensitivity C-Reactive Protein	mg/L	<2.5
Total cholesterol	mg/dL	<200 desirable
		200-239 borderline
		≥240 high
High Density Lipoprotein-Cholesterol	mg/dL	Males: >55 favorable
		Females: >65 favorable
Total Triglycerides	mg/dL	<150
		150-199 borderline high
Calculated Low Density Lipoprotein-Cholesterol	mg/dL	<100 optimal
		100-129 near optimal
Calculated non-High Density Lipoprotein-Cholesterol	mg/dL	<130 desirable
		139-159 borderline high

Appendix 3. Summary of Aliquots Created

Number of aliquots for each specimen type sent to each lab for participant and QC sets.

ACRL		UMN		Aliquot Type	Color	Tube # (Participant Set)	Tube # (QC Set[1])
Complete Participant Set	QC Set[1]	Complete Participant Set	QC Set[1]				
2	2	6	6	Serum	Red	1	1
4	3	--	--	EDTA + BHT Plasma	Green	Pooled 2,3,4	4
10	6	2	2	EDTA Plasma	Lavender	Pooled 2,3,4	2,3[2] (not pooled, 4 aliquots from each tube)
2	2	4	4	Urine	Yellow	n/a	n/a
3	1	--	--	Buffy Coat	Brown	2,3,4	3
--	--	2	1	Whole Blood	Black	2, 3	2

The PAXgene tube is not processed or aliquoted per protocol. There is one PAXgene tube collected in a complete participant set and one PAXgene tube collected as part of a QC set. Both the participant and QC tubes are immediately frozen and then sent to the ACRL with the remaining participant set.

1 This table refers to a "QC set" for easy direct comparison between the number of aliquots per type obtained from a regular participant draw and from the QC draw. However, each blood specimen tube in the "QC set" is collected from a different participant and assigned a different phantom ID. Only the urine sample and tube 1 will have the same phantom ID for the QC set because these are collected from the same participant.

2 In the regular participant set, tubes 2, 3, and 4 are pooled to obtain the proper number of aliquots for the EDTA + BHT plasma and EDTA plasma type aliquots. Since tubes come from different participants in the "QC set," tubes 2,3, and 4 will not be pooled. 4 aliquots will be created from each of tubes 2 and 3 and 3 aliquots will be created from tube 4.

Appendix 4. Equipment and Supplies

Description	Vendor	Vendor Specific?	Cat#	Unit	Notes
Shipping					
Biomailers					ACRL will ship to the Field Centers
Pax tube mailers/wrap					ACRL will provide instructions for procurement.
Primary size zip lock bags	Local				Primary containment for cryovials.
Secondary zip lock bags	Local				Secondary containment for cryovials.
Dry ice	Local				Order enough in case delivery of samples is delayed!
IATA approved Dry Ice shipping labels	VWR		10029-350	pk 500	If not provided by shipping company. FedEx will supply upon request
IATA approved UN3373/ Category B labels	VWR		10029-360	pk 500	If not provided by shipping company. FedEx will supply upon request
Absorbent sheeting					Something absorbent is required in case tubes thaw and leak during shipping. It is up to the individual site to choose absorbent material.
Phlebotomy & Safety Supplies					
21 gauge Safety-lok bld collection set w holder	VWR	***	BD 89005-534	cs/200	This size works well for most participants. Can order parts separately
23 gauge Safety-lok bld collection set w holder	VWR	***	BD 89005-532	cs/200	This size is better for fragile veins especially in the hand. Parts can be ordered separately.
Gauze	VWR or local		82004-740	cs/5000	2"x2" is a recommended size.
Tourniquets	VWR or local		89511-830	pk/100	No Latex please!
Bandaids	Local				caution: may tear fragile skin
Coban wrap	VWR or local		10024-084	36 rolls/cs	We like coban for fragile skin.
Alcohol pads	VWR or local		15648-916	bx/200	
10 mL red/gray serum SST/clot activator vacutainer tube	VWR	X	BD 367985	pkg 100	
10 mL lavender EDTA vac. tube	VWR	X	BD366643	pkg 100	
2.5 mL red PAXgene vac tube	VWR	X	77776-026	case 100	
Lab Coats	Local				disposable or otherwise
****Full Face Shields	Fisher or local		19-181-600A	25/bx	A different protective covering for your eyes and mouth may also be acceptable. See your institution's requirements. This is good laboratory practice due to COVID-19.

Description	Vendor	Vendor Specific?	Cat#	Unit	Notes
****Integrated Mask & Eyeshield	Fisher		18-999-4821	100/cs	Highly recommended but if unavailable consult your institution for similar product.
****Safety Splash Goggle	Fisher		19-065-423	10/cs	May be used with a face shield and mask
gloves (small)	Local				Pick your favorite brand, no latex.
gloves (medium)	Local				Pick your favorite brand, no latex.
gloves (large)	Local				Pick your favorite brand, no latex.
gloves (x-large)	Local				Pick your favorite brand, no latex.
Biohazard disposal bags	Local				
Sharps Container	Local				
Spill Kit	Local				
Hand Sanitizer	Local				Use most protective 90% alcohol. 70% and greater are next acceptable.
Surface Guard Liners (home collections)	Market Lab		ML2660-BL	500/pk	Something similar is also acceptable. Used for home draws.
*Phlebotomy Wedge Anti-Microbial Coating	Market Lab		ML9623	each	Optional
Processing Supplies					
**Racks for aliquotting	Thomas Scientific		1159V62	Pk/5	Useful to hold cryovials for aliquots. Comes in different colors, this # is clear
**Racks to hold tubes	Local or Thomas Scientific		9259L60	each	See cat. For different sizes to hold Vacutainer Tubes. This size will hold your 10 mL tubes & the 2.5 mL. Order only if you need.
Set-up trays	Local or Thomas Scientific		0209W55	each	Whatever works for your site
Water bath trays	Local or Thomas Scientific		1198Z44	12/CS	This is an example. You may use a pan from your local stores.
Timers (2 or more)	Local				Suggested:1 for each participant whose samples are processed at the same time.
10% bleach or other disinfectant	local				Make 10% bleach up daily! It degrades quickly.
urine cups	local or VWR		89508-712	CS/100	Up to site preference. Does not need to be sterile.
Castile soap towelettes	local or VWR		15648-924	pkg/100	Used for clean catch urine collection.
**Screw-cap vials (2.0 mL)	Fisher	X	02-681-343	500/bag	Used for blood and urine aliquots. Alternative: Thomas Scientific Cat. #1149J78
**Screw caps -lavender	Fisher	X	02-681-366	500/bag	Used for EDTA aliquots, tubes 2,3,4. Alternative: Thomas Scientific Cat. #1149Y23

Description	Vendor	Vendor Specific?	Cat#	Unit	Notes
					Due to COVID-19 supply issues violet caps may be substituted for lavender as long as the substitution is indicated on the shipping form.
**Screw caps -red	Fisher	X	02-681-361	500/bag	For SST aliquots, tubes 1. Alternative: Thomas Scientific Cat. #1149Y22
**Screw caps -green	Fisher	X	02-681-363	500/bag	Used for BHT-treated EDTA aliquots. Alternative: Thomas Scientific Cat. #1149Y20
**Screw caps -yellow	Fisher	X	02-681-360	500/bag	Used for urine aliquots. Alternative: Thomas Scientific Cat. #1149Y24
**Screw caps -brown	Sarstedt	X	65.716.009	1000/bag	Used for buffy coats, tubes 2 and 3. Due to COVID-19 supply issues clear caps may be substituted for brown as long as the substitution is indicated on the shipping form.
**Screw caps -black	Sarstedt	X	65.716.007	1000/bag	Used for A1c aliquot of whole blood, tube 2. Due to COVID-19 supply issues blue caps may be substituted for black as long as the substitution is indicated on the shipping form.
**200 µL pipette tips-racks	Local or Vendor of Choice				Choose whatever works for your pipettor. Filtered tips are acceptable.
**200 µL pipette tips-refills	Local or Vendor of Choice				Choose whatever works for your pipettor. Filtered tips are acceptable.
**1000 µL pipette tips	Local or Vendor of Choice				Choose whatever works for your pipettor. Filtered tips are acceptable.
**BioPipettor (100 µL)	Local or Vendor of Choice				Choose the size that will work best for you. May have from previous visits, re-calibrate if using an existing pipettor.
**or: BioPipettor (200 µL)	Local or Vendor of Choice				Choose the size that will work best for you. May have from previous visits, re-calibrate if using an existing pipettor.
**BioPipettor (1000 µL)	Local or Vendor of Choice				May also use a repeat pipettor of your choice. May have from previous visits, re-calibrate if using an existing pipettor.
disposable transfer pipets 5 mL plastic	VWR or local		414004-001	500/box	Used to take off buffy coats

Description	Vendor	Vendor Specific?	Cat#	Unit	Notes
**50 mL conical tubes in rack, sterile	local or Thomas Scientific		20A00R753	500/CS	To pool plasma from tubes 2, 3, 4 prior to aliquoting. Doesn't have to be sterile.
Bench paper with plastic backing--20" x300'	Local or VWR		51138-500	2 rolls/CS	Great in case of spills; cut to size.

X—It is recommended to order this specific item made by this manufacturer. However, due to restrictions related to the COVID-19 pandemic, a similar item made by a different manufacturer is acceptable.
* Optional
**Each center will need to obtain a quote from their vendor of choice.
***Please note that you may be able to obtain many of these items from different vendors at better pricing or from your institutional medical supplies center. This list is a guide of the supplies necessary for biospecimen collection, processing, and shipping. Other items may be added as needed. The Centers are responsible for all supplies except Biomailers, which will be provided by Baylor (ACRL). ****These items are either/or choices in the event one or the other is not available by vendor or institution. Phenix is now under the name “Thomas Scientific.”

Equipment purchased and maintained by Field Centers:
Table-top centrifuge with swinging buckets, refrigerated, and capable of producing 3,000 x *g*
Freezer capable of maintaining -80˚ C with at least 5 cu ft storage
Refrigerator 4˚ C

Appendix 5. Specimen Shipping Schedules for Visit 11

ARIC Visit 11 Shipping Dates for all Field Centers (2024-2025)				
MONTH-YEAR	**1ST SHIPMENT**	**2ND SHIPMENT**	**3rd SHIPMENT***	**COMMENTS**
March 2024	Tuesday, March 19	--	--	
April 2024	Tuesday, April 2,	Tuesday, April 16,	Tuesday, April 30	
May 2024	Tuesday, May 14	Tuesday, May 28	--	
June 2024	Tuesday, June 11	Tuesday, June 25	--	
July 2024	Tuesday, July 9	Tuesday, July 23	--	
August 2024	Tuesday, August 6	Tuesday, August 20	--	
September 2024	Tuesday, September 3	Tuesday, September 17	--	
October 2024	Tuesday, October 1	Tuesday, October 15	Tuesday, October 29	
November 2024	Tuesday, November 12	--	--	Due to the Thanksgiving holiday, the next shipment date is 3 weeks away from the November shipment date.
December 2024	Tuesday, December 3	Tuesday, December 17	--	
January 2025	Tuesday, January 7	Wednesday, January 22	--	The second shipment date is shifted to January 22nd to account for the MLK holiday.
February 2025	Tuesday, February 4	Tuesday, February 18	--	
March 2025	Tuesday, March 4	Tuesday, March 18	--	
April 2025	Tuesday, April 1	Tuesday, April 15	Tuesday, April 29	
May 2025	Tuesday, May 13	Wednesday, May 28	--	The second shipment date is shifted to May 28th to account for the Memorial Day holiday.
June 2025	Tuesday, June 10	Tuesday, June 24	--	
July 2025	Tuesday, July 8	Tuesday, July 22	--	Tuesday, July 22 is the last planned shipment day for Visit 11.