DRAFT Manual 7
Biospecimen Collection and Processing
Visit 6

Version 1.2

Biospecimen Collection and Processing
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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 which sampled a large, randomly selected population and then will follow it for an extended period of time.

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, 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 Laboratory performs assays related to lipid metabolism (lipid profile), glucose and insulin, inflammation (hs-CRP) cardiac performance (NT-proBNP, hs troponin T, hs TnI, Galectin 3). The Clinical Chemistry Laboratory performs assays related to renal function and oxidative stress (creatinine, urine albumin and urine creatinine,), glucose, hemoglobin A1C, and a set of analytes in a subset). Glucose and insulin will not be measured on samples collected at the homes or long-term care facilities due to the potential delays between collection and processing. 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, fully compliant with the protocol for drawing and processing the specimens in the field, and also 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 eight tubes of blood which is approximately 73-83 mL of blood from each participant (up to 90 mL is allowed). The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 5 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 takes place annually and is authorized by the supervisory personnel.

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.

1. Collection: (must be a certified phlebotomist, medical technologist, medical assistant, nurse, or other qualified personnel) includes the following training.
    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. How each sample/tube type are handled (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. Removal of whole blood aliquot for HbA1c
e. Aliquoting
    - Color of caps from which tubes
    - Urine aliquotting
f. Safety

3. Shipping Includes:

    a. Sorting aliquots per intended destination (ACRL or UMN)
    b. Bagging blood and urine aliquots
    c. Double bagging
    d. Local and FedEx guidelines and regulations
    e. Weekly shipments packaging
    f. FedEx notifications
    g. Addresses to ship to
    h. What 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 3.

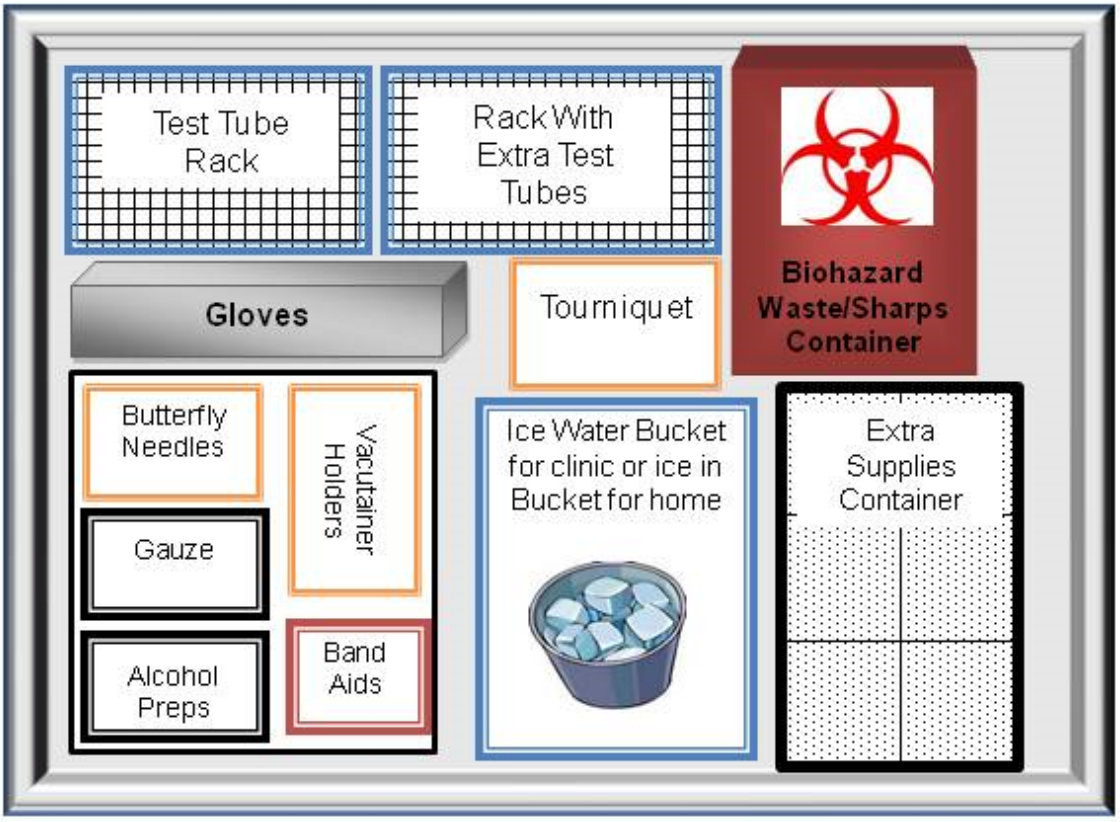
2.3.1 Blood Collection Tray

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, or 23 gauge butterfly needles with 12” tubing
- plastic vacutainer holders with Luer adapters
- sterile alcohol swabs
- gauze sponges
- tourniquet
- bandages ("Band Aids")

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: Drawing of Clinic and Home Visit Blood Collection Tray Filled With List Above



2.3.2 Blood Collection Tubes

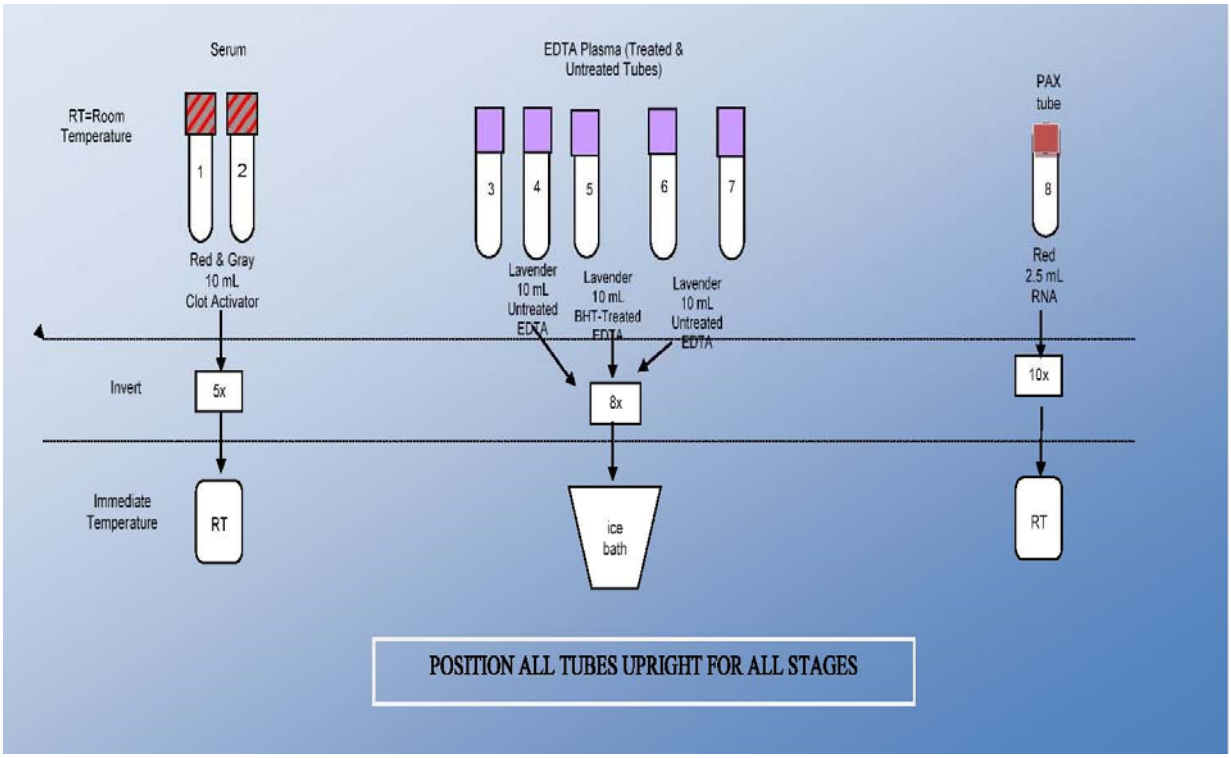
Technicians must be familiar with: the arrangement of blood collection tubes, the order in which the tubes are to be filled, the type of anticoagulant in each tube, and the possible sources of error in handling each tube. These tubes are organized in the test tube rack in the following sequence:

Tubes #1 and #2 are 10 mL red/gray stoppered tubes. Although these tubes do not contain anticoagulant, they do have a clot activator and therefore require mixing following collection. The serum from these tubes will be used for testing creatinine and other tests measured in the UMN Chemistry Laboratory.

Tubes #3, #4, #5, #6 and #7 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 will be used to isolate DNA in the UT-Genetics Laboratory. Whole blood (0.5 mL) is taken from tube # 3 for hemoglobin A1c testing in the UMN Laboratory.

Tube #8 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 in order 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.)

Figure 2 Order of Draw



RT= Room Temperature (18-25°C)

Tubes 1-2: SST, Serum (Red/Gray Top)
Tubes 3-4 EDTA Plasma (Lavender Top) * #3=0.5 mL taken for A1c
Tube 5: EDTA Plasma (Lavender Top) BHT treated
Tube 6-7: EDTA Plasma (Lavender Top)
Tube 8: PAXgene (Red Top)

TOTAL DRAW= 72.5 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 1-7. Apply a barcoded ARIC ID "cyro" label to the paxgene tube since this but 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. Handle only one participant's specimens at a time so the chance of mislabeling is minimized.
2. Apply a barcode ARIC ID "paper" label to each page of the Biospecimen Collection Forms (BIO).

3. Arrange the blood collection tubes in the test tube rack in the same order in which they are to be collected. The eight tubes are collected in the following order:

Tube #1:	10 mL red/grey stoppered tube (Serum)
Tube #2:	10 mL red/grey stoppered tube (Serum)
Tube #3:	10 mL lavender stoppered tube (EDTA)
Tube #4:	10 mL lavender stoppered tube (EDTA)
Tube #5:	10 mL lavender stoppered tube (BHT-treated EDTA)
Tube #6:	10 mL lavender stoppered tube (EDTA)
Tube #7:	10 mL lavender stoppered tube (EDTA)
Tube #8:	2.5 mL red stoppered Paxgene tube

Note: BHT is a preservative and prevents the oxidation of lipids

A number of ARIC participants will be asked to donate one to two additional tube(s) 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 below, in Sections 6.2 – 6.4.

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 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 safety glasses and gloves 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 a sample and aliquot tray 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.

19 – 2.0 mL polypropylene cryovials (lavender top)
3 – 2.0 mL polypropylene cryovials (green top)
2 – 2.0 mL polypropylene cryovials (brown top)
6 – 2.0 mL polypropylene cryovials (red top)
1 – 2.0 mL polypropylene cryovials (black top)
6 – 2.0 mL polypropylene cryovials (yellow top)

2.3.6 Labeling

Vertically label the plastic sample aliquot tubes with the barcoded ARIC ID "cryo" labels and arrange in the sample aliquot tray in the following order (see Figure 1. Aliquot Tray Layout):

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

Figure 3. Aliquot Tray Layout

Aliquot Tray Layout **(Stages 1 - 4 Processing)**

Col Row	1	2	3	4	5	6	7	8	9	10
A	1.5 mL plasma, Tube #5 BHT	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	1.5 mL EDTA plasma Tubes #3,4,6,7	1.5 mL EDTA plasma Tubes #3,4,6,7	EMPTY	1.0 mL serum Tubes #1,2	EMPTY	1.5 mL Urine
B	1.5 mL plasma, Tube #5 BHT	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	1.5 mL EDTA plasma Tubes #3,4,6,7	1.5 mL EDTA plasma Tubes #3,4,6,7	EMPTY	1.0 mL serum Tubes #1,2	EMPTY	1.5 mL Urine
C	1.5 mL plasma, Tube #5 BHT	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	EMPTY	1.5 mL EDTA plasma Tubes #3,4,6,7	1.5 mL EDTA plasma Tubes #3,4,6,7	EMPTY	1.0 mL serum Tubes #1,2	EMPTY	1.5 mL Urine
D	EMPTY	0.5 mL plasma, Tubes #3,4,6,7	0.5 mL plasma, Tubes #3,4,6,7	EMPTY	1.5 mL EDTA plasma Tubes #3,4,6,7	EMPTY	EMPTY	1.0 mL serum Tubes #1,2	EMPTY	1.5 mL Urine
E	Buffy Coat, Tube # 3	Buffy Coat, Tube #4	0.5 mL plasma, Tubes #3,4,6,7	HbA1c Tube 3 (before spin)	1.5 mL EDTA plasma Tubes #3,4,6,7	EMPTY	1.0 mL serum Tubes #1,2	1.0 mL serum Tubes #1,2	1.5 mL Urine	1.5 mL Urine

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.

Approximately 10 minutes before scheduled participant arrival (clinic):

1. Fill ice bath 3/4 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. (see Appendix 4).
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.

2.4 Biospecimen Collection form

At the completion of specimen collection and processing, complete the Biospecimen Collection Form (**Appendix 4**). If there are any deviations from the routine collection or processing protocol, record them on the Biospecimen Collection Form (**Appendix 4**). This form is entered on paper first and then entered into the DMS using the participant ID. A copy is sent to the ACRL Laboratory and the University of Minnesota (MN) Laboratory with the bi-weekly 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 of 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).

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.

Illustration: Picture of Fresh Bleach Mix 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 of the necessary blood drawing supplies. A separate work area is equipped with all of the 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).

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, ask permission to 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 two ounces) will be drawn by trained technicians.

Complete the Biospecimen Collection Form with the participant (**Appendix 4**). 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. 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 5 tablespoons). This blood will be used in tests for lipids (or fats), cholesterol, and blood clotting factors. We hope to be able to use the results of these tests to predict who might have a greater risk of heart 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 nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. 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 (**Appendix 4**). 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 two minutes. 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.

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 and #2) five times and (tube# 3 through tube #7) eight times and tube #8, ten times. Immediately following removal of the tubes from the adapter place them at room temperature except for tubes #3, #4, #5, #6, and #7 which are placed into the ice water bath.

9. When collecting tube #8, 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 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.

- 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, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box. 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 of the tubes are filled, repeat the venipuncture on the participant beginning with the first unfilled tube. Tubes #3 - #7 must be completely filled in order to perform the analyses. 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. Slip the gauze pad down over the site, continuing mild pressure.

   b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

2. If the participant continues to bleed:

   a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.

   b. Wrap a gauze bandage tightly around the arm over the pad.

   c. Tell the participant to leave the bandage on for at least 15 minutes.

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

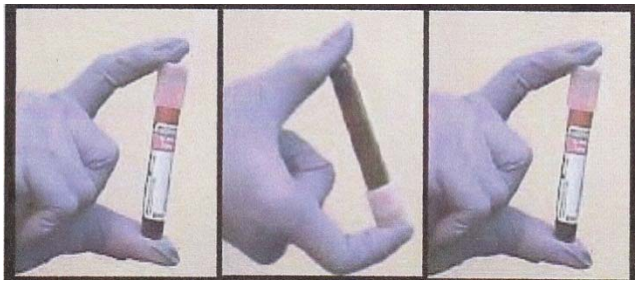
4. Have someone stay with the person to prevent them from falling and injuring themselves if they should faint.

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 in order 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 with the anticoagulant to prevent clotting. Even tubes #1 and #2 that do not contain an anticoagulant, 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 (1st inversion). Now, lower the butt end slightly while watching the bubble float to the stopper (2nd inversion). Lower the stopper end again when the bubble reaches the stopper. This is the third inversion. Invert tubes 1 and 2 five times, tubes 3-7 eight times, and tube 8, ten times. Eight inversions should take 6 to 8 seconds.



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 bi-weekly shipment to the Atherosclerosis and UMN Laboratories.

Tube #2: 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 bi-weekly shipment to the Atherosclerosis and UMN Laboratories.

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

Invert gently eight times, place in ice water bath until centrifugation. Remove 0.5 mL of whole blood from tube #3 and place in cryovial tube with black lid. 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, place in ice water bath until centrifugation. The plasma from tube #4 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 #5: 10 mL lavender-stoppered tube contains EDTA anticoagulant plus BHT is added later.

Invert gently 8 times immediately after collection. Place the tube in an ice water bath until centrifugation. BHT will be added at the aliquoting step.

Tube #6: 10 mL lavender-stoppered tube contains EDTA anticoagulant.

Invert gently 8 times immediately after collection. Place the tube in an ice water bath until centrifugation.

Tube #7: 10 mL lavender-stoppered tube contains EDTA anticoagulant.

Invert gently 8 times immediately after collection. Place the tube in an ice water bath until centrifugation.

Tube #8: 2.5 mL red-stoppered Paxgene (PAX) tube for RNA preservation.
Invert gently 10 times immediately after collection. Put it in a rack in the -80 freezer

3.6 Partial Biospecimen Collection Procedures for Clinic and Home Visits

A partial biospecimen collection for the ARIC Visit 6 Study is defined as a collection set consisting of less than the desired eight tubes, (2) SST Serum, (5) 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 only the first tube is collected after the allowed number of attempts (4) by two individuals, discard and do not send any specimens for this participant.
- If the partial collection includes at least (1) SST serum tube and (1-2) EDTA plasma tubes process and ship documenting on 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 (< 8.0 mL) discard and do not send any urine for this participant
- If 8 mL is collected dispense aliquots of 1.0 mL rather than 1.5 mL using the same number (6) of 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.

All samples should be returned to the field center within an 8-hour time frame. Processing should continue the same as a clinic blood draw.

4. BLOOD AND URINE PROCESSING FOR CLINIC AND HOME VISITS

4.1 Stage One: Immediate Processing

After completion of venipuncture:

1. Tubes #1 and #2 remain 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 #3 from the ice water bath (or bucket) and invert gently 8 times, remove 0.5 mL whole blood into a cryovial vial located in the Aliquot Tray, column 4 row E. Place a black screw cap on and store in the refrigerator. This aliquot of whole blood is for Hemoglobin A1c and will be shipped to MN for measurement. Re-cap tube #3 and replace it in the ice water bath.

3. As soon as possible, or within 15 minutes of collection, invert tubes, #3, #4, #5, #6, and #7 2x and place tubes in the centrifuge buckets in a balanced manner (see description of balancing the centrifuge in 4.2 “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 #8 is placed in an upright position in the -80° C freezer until packaging for shipping.

4.1.1 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. Wait for centrifuge to come to a complete stop before opening the lid. Proceed to stage 2 processing.

4.2 Stage Two: Processing of Plasma

Stage two begins approximately 15 minutes after venipuncture. Eye 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 ice water bath on the bench behind the collection tube racks until completion of the collection tubes processing.

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 Item15 and 16 of the Biospecimen Collection form (Appendix 4) 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 (Appendix 4) “lipids present on top of plasma/serum were not pipetted”.

1. Remove tubes #3, #4, #5, #6, and #7 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 # 3 - #7and pipet it into a 50mL graduated centrifuge tube

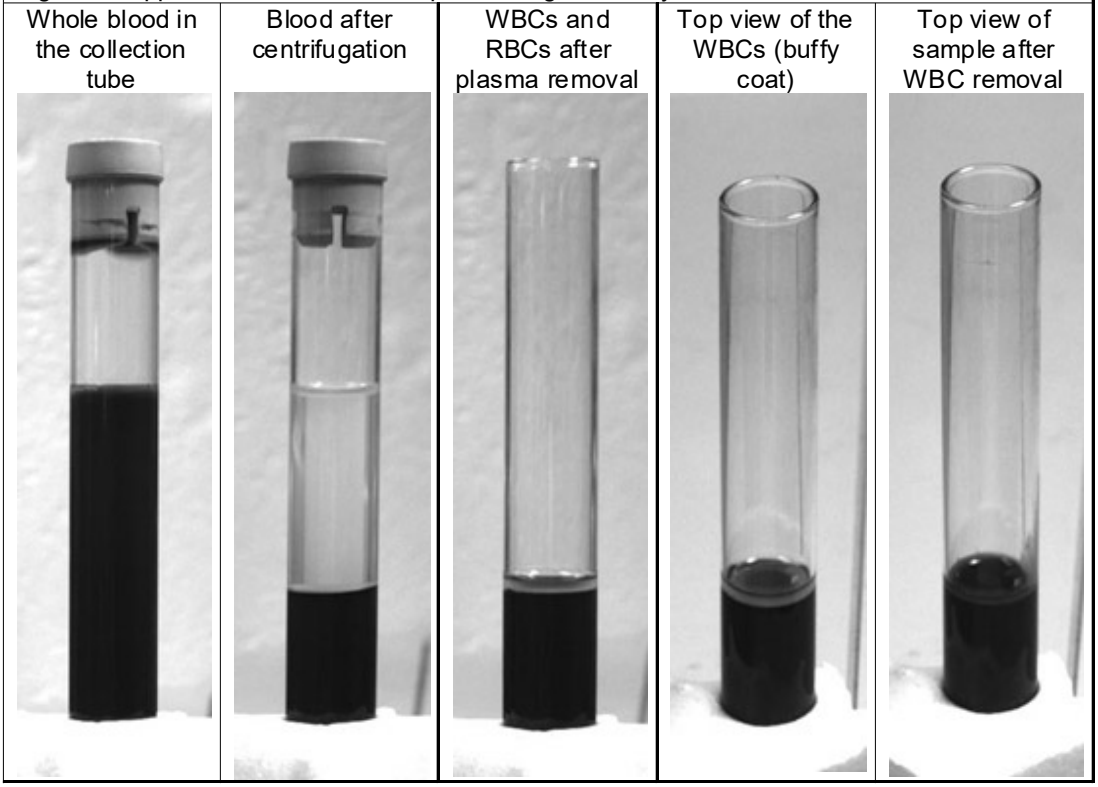
3. Using the same plastic transfer pipette, slowly aspirate the remaining plasma (minimum amount), buffy coat layer, and some of the red cells from tube #3. (Do not let the buffy coat aspirate into the bulb of the disposable pipette.) Transfer this to the 2.0 mL cryovial in position 1E. Repeat these 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 #3 - #7 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 #3-7 aspirate Dispense 0.5 mL of plasma into the cryovials in positions (refer to figure 2: Aliquot Tray Layout) column1 rows A-C; column 2 rows A-D; column 3 rows A-E; column 4 rows A-B; column 5 rows A-E and column 6 rows A-C. You may need to change tips if bubbles occur. Set the 1.0 mL pipet to 1.0 mL and dispense 1.0 mL to cryovials in column 1rows A-C; column 5 rows A-E and column 6 rows A-C. 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 30 µL of BHT solution using a 100 µL pipette set to 30uL to each of the three aliquots in column 1 rows A-C (change tips between each addition). With each addition, mix with the tip 3x (aspirate/dispense motions). Fasten the lavender and green screw caps onto the cryovials in columns 1-6 according to tray layout.
7. Using a plastic transfer pipette, remove the buffy coat (up to 0.5 mL) from tube #3 and transfer into (1) 2.0 mL cryovial in column 1 row E and repeat steps from tube #4 transferring into (1) 2.0 mL cryovial in column 2 row E. Fasten the brown screw caps onto these vials.
8. Re-stopper tubes #3, #4, #5, #6 and #7 and discard them into a biohazard waste bag.

Figure 1. Appearance of Blood Samples during Recovery of WBCs



4.3 Stage Three: Processing of Serum

Stage three begins approximately 30 minutes after venipuncture.

1. As close to 30 minutes after venipuncture as possible, and no longer than 45 minutes after venipuncture, spin the red stoppered tubes #1 and #2 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. When the centrifuge has come to a complete stop, remove tubes and place them in a wire rack in front of the sample aliquot tray 1. Remove the stoppers.

3. Using a plastic transfer pipet, withdraw serum from tubes #1 and #2. Place serum into the 15 mL graduated centrifuge tube. Set the 1.0 mL pipetor to 1.0 mL and pipette serum from 15mL graduated centrifuge tube into 2.0mL cryovials (see figure 2 aliquot tray layout diagram) in column 7 row E and column 8 rows A-E. Place the red screw caps on these vials.

4. Re-stopper tubes #1 and #2 and discard them 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.

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 half way 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 9 mL of urine is required for processing, 15 mL is optimal for a complete processing set. If insufficient, 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 by the staff person who observes the placement of the participant's urine specimen in the refrigerator. 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 (Appendix 4, Items 1 and 2).

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 Item11 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".

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 water bath. 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.

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

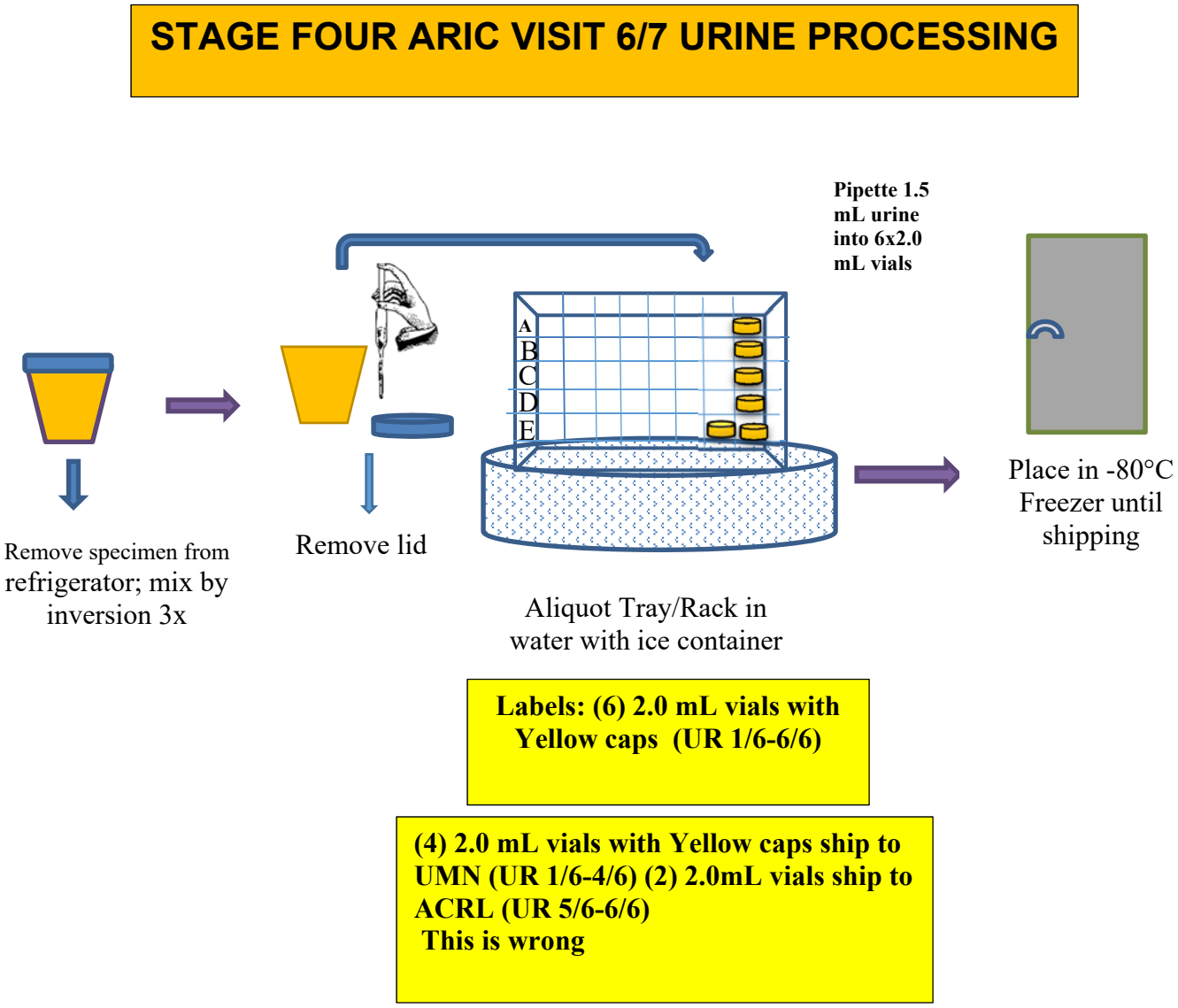
3. Using a 1.0 mL pipetter set to 1.0 mL, aspirate and dispense into six pre-labeled cryovials in the aliquot tray positions column 9 row E and column 10 rows A-E.

4. Set the 1.0 mL pipette to 0.5 mL and repeat dispensing into the six pre-labeled cryovials in the aliquot tray positions column 9 row E and column 10 rows A-E. 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 4. Stage Four ARIC Visit 6 Urine Processing

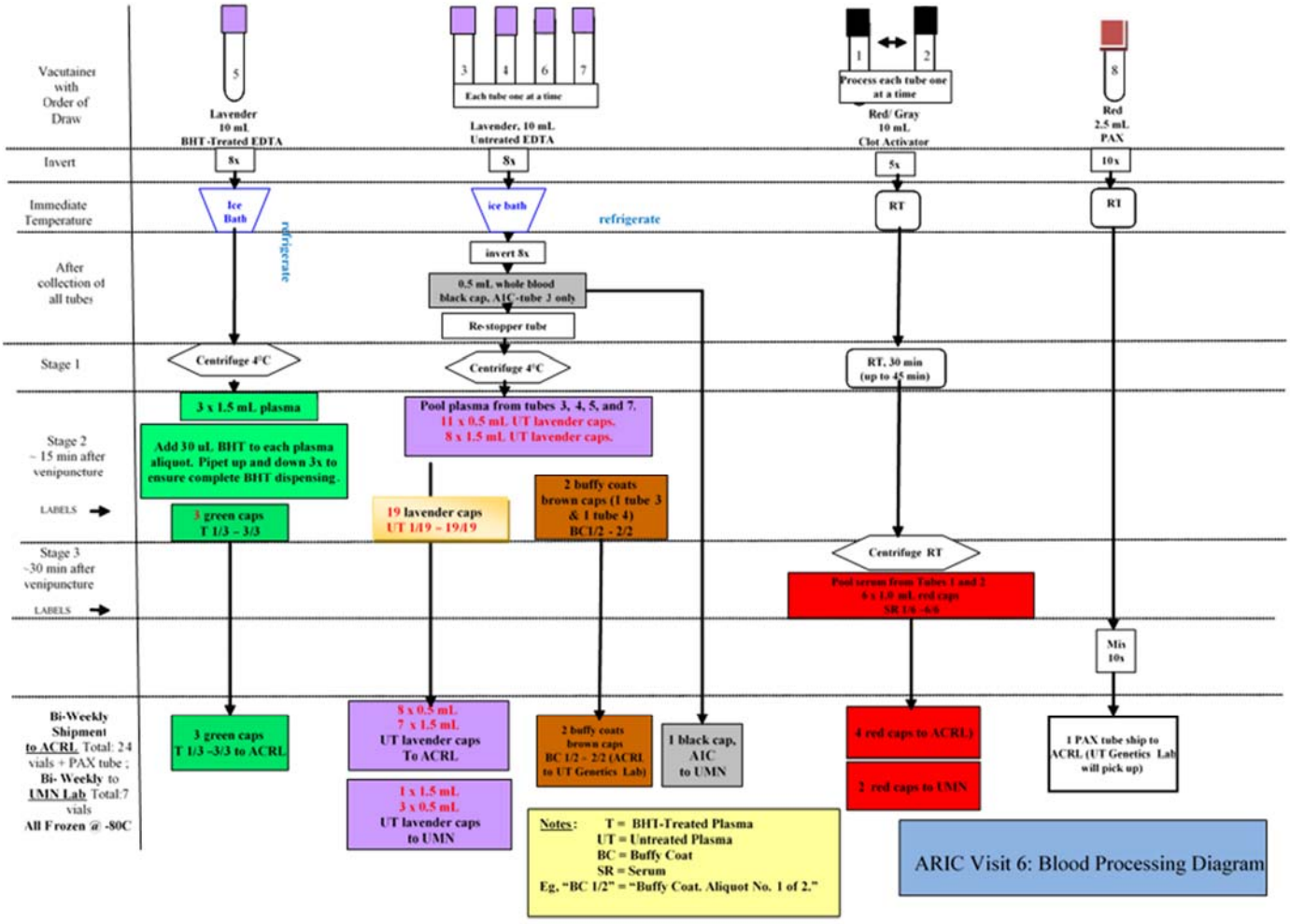


If the volume of urine sample is inadequate to process at least three of the six sample aliquots, check to see if a second sample was provided. If there is a second sample and it (in and of itself) is adequate for processing, use the second sample (record the time voided on the Biospecimen Collection 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 Collection

A summary overview of the protocol steps for the collection and processing of blood and urine specimens is presented in Figure 5. (Specimen Processing Flow Diagram). The order of blood processing after tube collection begins with tube 5.

Figure 5. Specimen Processing Flow Diagram



4.6 Freezing

When all of the blood and urine specimens have been aliquotted into their respective cryovials and the cryovials have been replaced in the aliquot tray/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. 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 #8.

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 according to their specimen type and the lab they are being shipped to. Label six (6) 4" x 6" primary bags with the participant ID and "ACRL" Label three (3) 4" x 6" primary bags with the participant ID and "UMN". Label one (1) large shipping bag with the participant ID and "ACRL". Label one (1) large shipping bag with the Participant ID and "UMN"

5.1.1 Packaging Frozen Specimens

1. Place (4) red cap cryovials in primary bag #1 for ARCL. Place (2) red cap cryovials in primary bag #1 for UMN. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
2. Place (3) green cap cryovials into primary bag #2 for ARCL. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
3. Place a total of 15 (8 x 0.5ml & 7 x 1.5mL) lavender cap cryovials into primary bag #3 for ACRL. Place a total of 4 (3 x 0.5mL and 1 x 1.5mL) lavender cap cryovials in primary bag #2 for UMN. Also place (1) black cap cryovials in primary bag #2 for UMN. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
4. Place (4) yellow caps cryovials in primary bag #3 for UMN. Place (2) yellow cap cryovials in primary bag #4 for ACRL. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
5. Place (2) brown cap cryovials into primary bag #5 for ACRL. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
6. Place (1) Paxgene tube wrapped in bubble wrap to cushion it into primary bag #6 for ACRL. Verify that the bags are labeled correctly. Add an absorbent pad, press the air out of the bag and seal.
7. Place primary bags #1-6 for ACRL into the large shipping bag for ACRL. Place primary bags #1-3 for UMN into the large shipping bag for UMN. Press the air out of the bags and seal.
8. Place the large shipping bags in the -80 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 Bi-Weekly Shipment of Specimens

The bags of frozen sera, plasma, whole blood, urine samples, and Paxgene tubes are packed and shipped in Styrofoam boxes. Packaging instructions (See Figure 4) are as follows:

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

2. Put one-half of the12"x 12" bags of sample vial/tubes 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 into a 12" x 12" bag and place on top of the packing material. For the shipment to the ACRL laboratory also include a copy of the BIO form. The shipping forms are shown in Appendix 6.

8. Secure the 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 Fed-Ex 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 Federal Express (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, sera, whole blood, urine, and Paxgene tubes and phantom aliquots collected and stored within the last two work weeks are shipped to the Laboratories on Monday. Samples can be shipped on Tuesday 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 bi-weekly 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 Federal Express 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 Charlie Rhodes (crhodes@bcm.edu), Valerie Arends (aren0085@umn.edu) and Barbara Cochran (barbara.j.cochran@uth.tmc.edu).

All shipping containers are sent to the Laboratories by "Priority Overnight" shipping NOT "First Overnight" since First Overnight is very expensive.r 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:

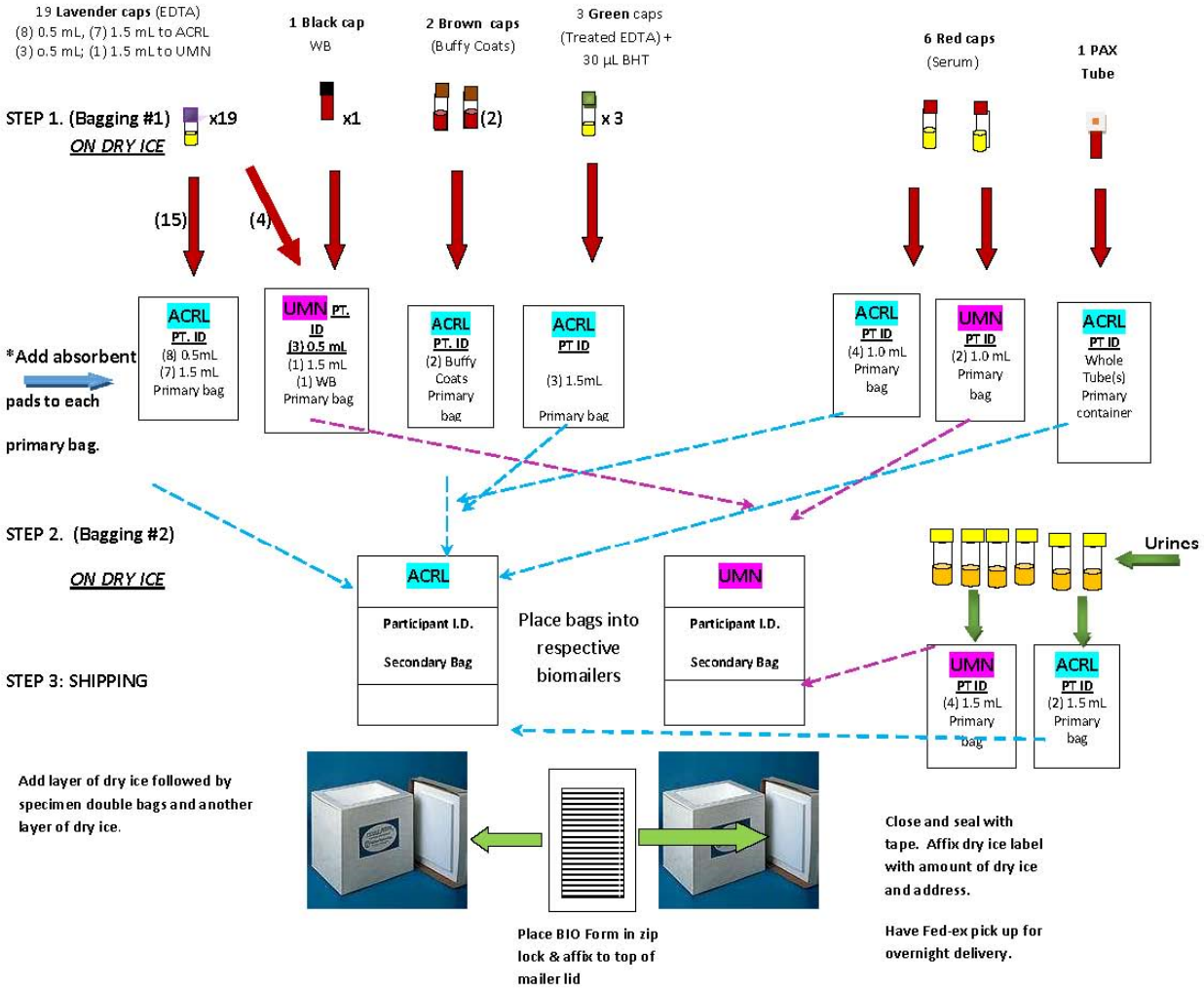
ARIC Central Laboratory

Charlie Rhodes
Atherosclerosis Clinical Research Laboratory (ACRL)
The Methodist Hospital
6565 Fannin Street
Mail Station:F701, Room F740
Houston, TX 77030
Telephone (713) 798-3406
Fax: (713) 798-7400

ARIC Chemistry Laboratory

Valerie Arends/ARIC V6
University of MN/ARDL
1200 Washington Ave S Ste 175
Minneapolis, MN 55415
Telephone: (612) 273-3645
Fax: (612) 273-3489

Figure 6. Shipping Diagram



Add absorbent packs to each bag.

6. QUALITY CONTROL

6.1 Venipuncture and equipment records

There are two different aspects of quality control. One is the daily or monthly record of the performance of the refrigeration equipment, glucometer, 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 Appendix 4, Items 10, 11, 15, and 16, Biospecimen Collection form.)

- 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 (Appendix 5), In addition, check and record the actual speed of the centrifuge annually with a tachometer. (This is usually performed by a biomedical engineer.)

6.2 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.

To reduce the burden on any single participant, only one or two extra tubes of blood is drawn and sent out under different Phantom ID's. For data analysis, results on each laboratory measurement are matched to the appropriate participant results at the Coordinating Center from the QC Phantom ID Form (Appendix 11) that is completed by Field Center technicians.

The QC blood samples are collected in sequential order (cycling back to Tube #1 after QC Tube #7 has been collected). Each Field Center will collect a QC samples from approximately 5% of the specimens. Check the Snapshot Report to see if the participant has been selected for lab QC.

6.3 Blood and Urine QC Sample Checklist

The venipuncture technicians maintain a Phantom Tracking sheet in their work area of the QC samples to be drawn. As each sample is drawn and processing completed, it is filled in. An example of the checklist is given in Appendix12

6.4 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, this should not be done for tube #8 which is sent to the ACRL Laboratory in the collection tube. The 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) a separate sample aliquot tray is 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.

6.5 Collecting and Processing QC Blood and Urine

Selecting Participants for QC Blood Draw: A QC sample will be collected from everyone selected for lab QC but based upon the size of their veins, the difficulty of drawing the blood, and the apprehension a participant shows about the blood draw, the venipuncture technician may forego the drawing of the QC tube from certain participants.

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 falls off at the end of the draw, so that it would be difficult to obtain the extra QC tubes, a different participant is used to get this blood. 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 #8. QC tube #8 is stored identically to the regular Paxgene tubes. 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. As soon as the full set of tubes is completed the Phantom Tracking form information 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. File and maintain the original paper PHT form for a period to be determined

6.6 Internal Laboratory Control

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.

7. TRAINING PROCEDURES

Technicians will be trained in actual procedure of phlebotomy 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 Appendix8. The technicians must study the ARIC Specimen Collection and Processing Manual and pre-training slides providing basic knowledge prior to actual training session.

At training the technician will be walked through various procedures before proceeding to real drawing and processing of samples. Venipuncture is performed and practice tubes are collected in the correct order, and then placed at their proper positions. Technicians will process samples from beginning to end providing hands-on experience and allowing the technicians to become comfortable with the procedures before proceeding to ARIC participants.

Any questions or problems that the technicians have must be solved before the technicians actually proceed to drawing the ARIC participants. Before the technicians draw blood from any ARIC participant, they must take and pass the practical and written tests included at the end of this manual (Appendix 8). After passing the test and depending on the written evaluation of their instructor, they may proceed either to drawing blood from the ARIC participants as part of a team, or do more practice on volunteers. (See Manual 12 for more information)

8. SNACK

A light snack for the participant is scheduled as soon as possible after venipuncture. Menus are locally determined.

9. 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 bi-weekly. A selected group of these tests is reported to the field centers in order 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 DMS. (See Appendix # 1 for complete analyte details for ARIC Visit 6)

10. REPORTING RESULTS

The Laboratories have the responsibility for reporting results to the Coordinating Center. All test results are transmitted to the Coordinating Center through file transfer protocol (FTP) or use of a Coordinating Center upload facility that is accessed through the web based DMS. This transmission will occur 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 “Summary of Results Report” using the ARIC data management system. The Summary of Results report summarizes the receipt 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 the DMS called the “Summary of Results Report”. Any tests included in this report whose results exceed their alert range will be flagged appropriately. In addition, any alert result on a test not normally reported to the participants will be included in a separate upload. Reference ranges can be found in Appendix 2.

11. LOCAL FIELD CENTER ALIQUOTS

Field centers Jackson and Washington will be processing ~~2~~1 extra serum aliquots, 4 extra plasma aliquots and 2 extra urine aliquots. Minneapolis will be processing 1 extra serum and 1 extra plasma aliquots. Each morning (or the afternoon before) a separate sample aliquot tray is prepared for any local aliquots that the technician will process that day. The tray contains all the cryovials needed to process the local samples. The CC will provide the labels for the local cryovial tubes. These labels will have "L" on the label, for local. The local aliquots should be measured out using the leftover serum, plasma and urine AFTER all of the ARIC and QC aliquots have been created. Process the local samples along with the regular blood samples. After aliquoting is completed, the local sample cryovials are put into the -80° C freezer

12. APPENDICES

Appendix 1. Laboratory tests ARIC Visit 6

Sample Type - Visit 6 Test	Suitable for home visit?	Lab	Fasting	Vol. specimen needed	Machine (Method) –	Report to Participant
Whole Blood - HbA1c	**Yes**	**Minn**	**No**	**0.5 mL**	**Tosoh G8 (HPLC)**	**YES**
Urine – Albumin	Yes	Minn	No	1.5 mL	Roche Cobas 6000	**YES**
Urine - Creatinine	Yes	Minn	No		Roche Cobas 6000	**YES**
***Urine – calculated ACR**	Yes	Minn	-	N/A	calculated value	**YES**
Serum – Glucose	**No**	Minn	**YES**	0.5 mL	Roche Cobas 6000	**YES**
Serum - Creatinine	Yes	Minn	No		Roche Cobas 6000	**YES**
Serum – Fructosamine (Roche)	Yes	Minn	No		Roche Cobas 6000	No
Serum - Glycated Albumin (Asahi Kasei)	Yes	Minn	No		Roche Cobas 6000	No
Serum - 1,5-anhydroglucitol (GlycoMark)	Yes	Minn	No		Roche Cobas 6000	No
Plasma – hs-cTnT (Roche)	Yes	Baylor	No	0.5 mL	Roche Cobas e411	No
Plasma – NT-proBNP (Roche)	Yes	Baylor	No		Roche Cobas e411	No
Plasma – hs-cTnI (Abbott)	Yes	Baylor	No	0.5 mL	Architect Plus i2000sr	No
Plasma - Galectin-3 (Abbott)	Yes	Baylor	No		Architect Plus i2000sr	No
Plasma - ST2	Yes	Baylor	No	0.5 mL	ELISA	No
Plasma - hs-CRP	Yes	Baylor	No	0.5 mL	Beckman Coulter AU480	No
Plasma - lipids TC	Yes	Baylor	No		Beckman Coulter AU480	**YES**
Plasma - lipids HDL-C	Yes	Baylor	**YES**		Beckman Coulter AU480	**YES**
Plasma - lipids TG	Yes	Baylor	**YES**		Beckman Coulter AU480	**YES**
***Plasma – lipids calculated LDL-C**	Yes	Baylor	-	N/A	calculated value	**YES**
***Plasma – lipids calculated non-HDL-C**	Yes	Baylor	-	N/A	calculated value	**YES**
Plasma - Cytokine/MMP panel GDF-15	Yes	Baylor	No	0.5 mL	BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel IL-1β	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel IL-6	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel IL-10	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel IL-18	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel TNF-α	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel MMP-1	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel MMP-2	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel MMP-7	Yes	Baylor	No		BioRad Bio-Plex 200	No
Plasma - Cytokine/MMP panel TIMP-1	Yes	Baylor	No		BioRad Bio-Plex 200	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, LDL-cholesterol and non-HDL-cholesterol will be reported to the participants.

Appendix 2. Reference ranges

DESCRIPTION	UNIT	REFERANCE RANGES
Total cholesterol	mg/dL	<200 desirable 200-239 borderline ≥240 high
High Density Lipoprotein-Cholesterol	mg/dL	>55 favorable-M >65 favorable-F
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
High Sensitive C-Reactive Protein	mg/L	<2.5
HbA1c	%	4.3 - 6.0%
Urine ACR	mm/g Cr	<30 mg/g creatinine
Urine Albumin	mg/L	<20 mg/L
Urine Creatinine	mg/dL	40 - 278 mg/dL
Glucose	mg/dL	60 - 99 mg/dL
Creatinine, Serum	mg/dL	females: 0.4 - 1.1 mg/dL; males: 0.5 - 1.2 mg/dL
Fructosamine	µmol/L	205 - 285 µmol/L
Glycated albumin	%	11 - 16%
1,5-anhydroglucitol	µg/mL	females: 6.8-29.3 µg/mL; males: 10.7-32.0 µg/mL

Appendix 3. Equipment and Supplies

Description	Vendor	Required	Cat#	Unit	Notes
Shipping					
Biomailers		X			To be sent to us by the Main Lab
Pax tube mailers	TBD				
3x3 zip lock bags	Uline or local		S-3795	1000/bag	Secondary containment for cryovials--size/vendor is optional
6x6 zip lock bags	Uline or local		S-12263	1000/bag	Secondary containment for cryovials--size/vendor is optional
Dry ice	local				order enough in case delivery of samples is delayed!
IATA approved Dry Ice shipping labels	VWR or local		10029-350	pk 500	If not provided by shipping company
IATA approved UN3373/ Category B labels	VWR or local		10029-360	pk 500	If not provided by shipping company
Absorbent sheeting					Something absorbent is required in case tubes thaw and leak during shipping. What it is up to sites
Phlebotomy supplies					
21 guage Safety-lok bld collection set w holder	VWR	***	BD 89005-534	cs/200	This size works well for most ppts. Can order parts separately
23 guage Safety-lok bld collection set w holder	VWR	***	BD 89005-532	cs/200	This size is better for fragile veins esp in the hand.Can order parts separately
Gauze	VWR or local		82004-740	cs/5000	2"x2" is a good size, but it's your choice
Tourniquets	VWR or local		89511-830	pk/100	no latex please!
Bandaids	local				caution: may tear fragile skin
Or 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 red/gray serum SST/clot activator vacutainer tube	VWR	X	BD 367985	case 1000	reminder: these tubes expire
10 mL lavender EDTA vac. tube	VWR	X	BD366643	pkg 100	
10 mL lavender EDTA vac. tube	VWR	X	BD366643	case 1000	reminder: these tubes expire
2.5 mL red PAXgene vac tube	VWR	X	77776-026	case 100	
Lab Coats					disposable or otherwise
Full Face Shields	Fisher or local		19-181-600A	25/bx	or some sort of protective covering for your eyes and mouth. See your institution's requirements
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				
Surface Guard Liners (home collections)	Market Lab		ML2660-BL	500/pk	a good idea, or something like it, for home draws
*Phlebotomy Wedge Anti-Microbial Coating	Market Lab		ML9623	ea	optional
Processing Supplies					
**Racks for aliquotting	Phenix		R-780A	5/pk	These work well and are very space efficient, but your choice
Set-up trays					Whatever works for your site
Timers (2 or more)	local				we use 1 for each ppt--your choice
10% bleach or other disinfectant	local				Make 10% bleach up daily! It degrades quickly
urine cups	local or VWR		89508-712	cs/100	your choice. Don't need to be sterile
Castile soap towelettes	local or VWR		15648-924	pkg/100	for clean catch urine collection
**Screw-cap vials (2.0 mL)	Phenix	X	SCS-020TF	500/bag	for blood and urine aliquots

**Screw caps -lavender	Phenix	X	SCS-000V	500/bag	For EDTA aliquots, tubes 3,4,5,7
**Screw caps -red	Phenix	X	SCS-000R	500/bag	For SST aliquots, tubes 1,2
**Screw caps -green	Phenix	X	SCS-000G	500/bag	For BHT-treated EDTA aliquots, tube 6
**Screw caps -yellow	Phenix	X	SCS-000Y	500/bag	for urine aliquots
**Screw caps -brown	Phenix	X	SS-000BN	1000/bag	for buffy coats, tubes 3 and 4
**Screw caps -black	Phenix	X	SS-000BK	1000/bag	for A1c aliquot of whole blood, tube 3
**200 µL pipette tips-racks	Phenix		TS-200BR	10rks/96	or whatever works for your pipetter
**200 µL pipette tips-refills	Phenix		TS-200RFL	10rks/96	or whatever works for your pipetter
**1000 µL pipette tips	Phenix		TS-1000BR	10rks/100	or whatever works for your pipetter
**BioPipettor (100 µL)	Phenix		P300-100	each	pick the size that will work best for you
**or: BioPipettor (200 µL)	Phenix		P300-200	each	pick the size that will work best for you
**BioPipettor (1000 µL)	Phenix		P300-1000	each	or can use a repeat pipetter of your choice
disposable transfer pipets 5 mL plastic	VWR or local		414004-001	500/box	good for taking off buffy coats
**15 mL conical tubes in rack, sterile	local or Phenix		SS-2265	500/cs	To pour off serum from tubes 1,2 prior to aliquoting. Don't have to be sterile
**15 mL conical tubes bagged, sterile	local or Phenix		SS-2266	500/cs	save the racks and refill with these. Don't have to be sterile
**50 mL conical tubes in rack, sterile	local or Phenix		SS-2262	500/cs	To collect plasma from tubes 3, 4,5,7 prior to aliquoting. Don't have to be sterile
**50 mL conical tubes bagged, sterile	local or Phenix		SS-2263	500/cs	save the racks and refill with these. Don'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-- You must order this specific item made by this manufacturer. Several suppliers carry the BD blood tubes

* Optional

** Please reference Phenix Quote # 42551 for special ARIC Study pricing

***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.

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 a of 5 cu ft storage
Refrigerator 4˚ C

Appendix 4. Biospecimen Collection Form and Instructions

BIOSPECIMEN COLLECTION FORM

ID NUMBER: | | | | | | | | FORM CODE: B I O DATE: 04/01/2016 Version 2.0

ADMINISTRATIVE INFORMATION

0a. Completion Date: ☐☐/☐☐/☐☐☐☐ (Month / Day / Year) 0b. Staff ID: ☐☐☐

0c. Selected for additional phantom tube? _____

Instructions: *This form should be completed during the participant's clinic or home visit.*

A. URINE SAMPLE

1. Urine sample collected?

   Yes ..... ☐
   No ..... ☐ → **Go to Item 5**

2. Time of urine sample: ☐☐:☐☐ (H H : M M)

B. URINE PROCESSING

3. Volume adequate for processing? ..... ☐

   Yes (≥ 10mL) ..... Y
   Yes (< 10 mL but at least 5 mL)..... B
   No (<5 mL, discard) ..... N → **Go to Item 5**

4. Technician ID for urine sample: ☐☐☐

C. BLOOD DRAWING

5. Do you have any bleeding disorders other than easy bruising which is often caused by medications like aspirin or Plavix?

   Yes ..... ☐
   No ..... ☐ → **Go to Item 6**

   a. Please specify the nature of the bleeding disorder:

   _____

   _____

6. When was the last time you ate or drank anything other than water? ☐☐:☐☐ (H H : M M)

7. Time of blood draw….:…….☐☐:☐☐ (H H : M M)

7a. Fasting at least 8 hours?
Yes ..... ☐
No ..... ☐

8. Number of venipuncture attempts:..... ☐

9. Code number of phlebotomist:..... ☐☐☐

a. Code number of assistant: ..... ☐☐☐

10. Any blood drawing incidents or problems?

Yes..... ☐
No ..... ☐ → **Go to Item 12**

[*Blood drawing incidents: Document problems with venipuncture in this table. Place an "X" in box(es) corresponding to the tubes in which the blood drawing problem(s) occurred. If a problem other than those listed occurred, use Item 11*.]

	Tube 1	2	3	4	5	6	7	8
a. Sample not drawn	☐	☐	☐	☐	☐	☐	☐	☐
b. Partial sample drawn	☐	☐	☐	☐	☐	☐	☐	☐
c. Tourniquet reapplied	☐	☐	☐	☐	☐	☐	☐	☐
d. Fist clenching	☐	☐	☐	☐	☐	☐	☐	☐
e. Needle movement	☐	☐	☐	☐	☐	☐	☐	☐
f. Participant reclining	☐	☐	☐	☐	☐	☐	☐	☐

11. If any other blood drawing problems not listed above (e.g., fasting status, etc.), describe incident or problem here:

_____

_____

_____

D. BLOOD PROCESSING

12. Time specimen tubes 3, 4, 5, 6 and 7 were spun: ..... ☐☐:☐☐ (H H : M M)

13. Time specimen tubes 1, 2 were spun: ..... ☐☐:☐☐ (H H : M M)

14. Time specimens from tubes 1, 2, 3, 4, 5, 6, 7, and 8 were placed in freezer: .... ☐☐:☐☐
H H M M

15. Any blood processing incidents or problems?

Yes..... ☐

No ..... ☐ → **Go to Item 17a**

[*Blood processing incidents: Document problems with the processing of specimens in this table. Place an "X" in box(es) corresponding to the tubes in which the processing problem(s) occurred. If a problem other than those listed occurred, use Item 16*.]

	Tube 1	2	3	4	5	6	7	8
a. Broken tube.....	☐	☐	☐	☐	☐	☐	☐	☐
b. Clotted.....	☐	☐	☐	☐	☐	☐	☐	☐
c. Hemolyzed	☐	☐	☐	☐	☐	☐	☐	☐
d. Lipemic.....	☐	☐	☐	☐	☐	☐	☐	☐
e. Other.....	☐	☐	☐	☐	☐	☐	☐	☐

16. Comments on blood processing or other problems in blood processing: (attach a sheet if needed)

_____

_____

_____

17. a. Technician ID for processing blood specimens: ..... ☐☐☐

b. Technician ID for processing blood specimens: ..... ☐☐☐

c. Technician ID for processing blood specimens: ☐☐☐

INSTRUCTIONS FOR THE BIOSPECIMEN COLLECTION (BIO) FORM Version 2.0

I. General Instructions

The BIOSPECIMEN COLLECTION FORM is completed during the participant's clinic or home visit to record information on the collection and processing of blood and urine samples. Technicians performing venipuncture and processing blood and urine samples must be certified and should have a working knowledge of the relevant Manuals of Operations. Technicians should also be familiar with and understand the document entitled "General Instructions for Completing Paper Forms" prior to completing this form. ID Number, Contact Year, and Code Number of person completing this form (described in the General Instructions) should be completed prior to the arrival of the participant.

Record all times using a 24-hour clock (e.g. 8:00AM is 8:00, 3:00PM is 15:00).

II. Detailed Instructions for Each Item

Administrative Information

0a. Enter the date the biospecimen samples were collected.

0b. Enter the technician code of the person completing this form.

0c. Selected for Additional Phantom Tube. This item is system generated in CDART. If the participant is randomly selected to donate an additional “phantom” tube then you will see a “Yes” in this item. If they were not selected, then you will see “NO”. This information can also be found on the Participant Snapshot Report in the “General Appointment Information” section. If selected to provide a phantom sample, you will use the Phantom Tracking Sheet to determine which additional tube to collect.

.

A. URINE SAMPLE

At the reception station (clinic visits) or upon arrival at the home visit the participant is told that a urine specimen will be collected when it is convenient for the participant. This is best done early during the clinic/home visit, but can be done anytime during the examination sequence if the participant is not able to provide a specimen before blood drawing and the snack. In the latter case, it is useful to encourage the participant to drink one or two glasses with the snack and alert the technician when he/she wishes to empty his/her bladder. If a urine specimen has not been obtained over the course of the examination visit, the technician asks the participant again to provide a specimen at the end of the examination.

1. Indicate whether a urine sample was collected. If NO, urine sample was not collected, go to Item#5; if YES, continue.
2. Record the time the urine was collected using a 24-hour clock.

B. URINE PROCESSING

3. Note if urine volume is adequate for processing. Choose either Y ≥10 mL (desired). B between 10 mL and 5 mL or N <5 mL (discard and go to item #5).
4. Enter technician ID for urine sample.

C. BLOOD DRAWING

5 – 5a. For the clinic and home visits: Ask if the participant has a bleeding disorder that is not related to the use of medications such as aspirin and Plavix. If the participant's answer is NO, check the box indicating the negative answer and proceed to item #6. If the answer is YES, ask that he/she specify the nature of the bleeding disorder and record in 5a. Proceed with caution by executing pressure at the venipuncture site for a prolonged period. You may have the participant assist by elevating the arm and holding the gauze firmly on the venipuncture site. You must check that clotting has occurred and bleeding stopped before applying a band aid and releasing the participant. If the participant does not know whether he/she has a bleeding disorder, offer the explanation, "*If you have a bleeding disorder you would have symptoms like excessive nose bleeds, or very easy bruising, or problems with bleeding after tooth extractions, or any type of surgery* and continue as described above for NO or YES responses.

6. Enter the last time the participant ate or drank anything using a 24-hour clock (other than water or coffee/tea without cream and sugar) using a 24-hour clock. If the participant is rescheduled for another day, a new BIO form under a new sequence number should be entered.

7. Record the time of venipuncture using a 24-hour clock. This is the time when the vein is punctured and blood is drawn for specimens.

7a. Select YES if the time of venipuncture recorded in Item#7 is at least 8 hours after the last time the participant ate or drank anything recorded in Item#6.

8. Enter the number of venipuncture attempts.

9 - 9a. Enter the code number of the technician who performed the venipuncture and the blood drawing assistant. If more than one technician attempts to draw the blood, enter the code of the <u>first</u> technician. The same technician should not attempt a venipuncture more than twice.

10a-f. Note any blood drawing incidents or problems, and document in the table provided. Place an "X" in box (es) corresponding to the tubes in which the blood drawing problem(s) occurred. If an incident/problem is not listed below, document it on Item#11. If no incidents or problems occurred while drawing, skip to Item#12.

<u>Blood drawing incidents or problems:</u>

a. Sample not drawn

b. Partial sample drawn

c. Tourniquet reapplied

d. Fist clenching

e. Needle movement

f. Participant reclining

11. Document any other blood drawing problems not listed in Item#10.

D. BLOOD PROCESSING

12. Record the time using a 24-hour clock at which the centrifuge containing tubes 3,4, 5, 6 and 7 began to spin.

13. Record the time using a 24-hour clock at which the centrifuge containing tubes 1 and 2 began to spin.

14. Record the time using a 24-hour clock at which samples from tubes 1, 2, 3, 4, 5, 6, 7 and 8 were placed in the freezer.

15. Record if there were any specimen processing incidents or problems. If no incidents or problems occurred while processing, skip to Item#17a.

15a-e. Note any specimen processing incidents or problems listed below. Place an "X" in box(es) corresponding to the tubes in which the problem(s) occurred. If an incident/problem is not listed below, document it on Item#16.

a. Broken tube

b. Clotted

c. Hemolyzed

d. Lipemic

e. Other

16. Record comments or other problems in blood processing such as centrifuge or freezer issues and shipping problems such as lost shipments or broken tubes. Attach a sheet if more space is needed for notations.

17a-c. Enter technician(s) ID for processing blood specimens. If there was only one technician then leave items 17b and 17c blank.

Appendix 5. Bi-Weekly ACRL Biospecimen Shipping and Receiving Form

Batch ID Number: | | | | | | | |

Form Code: | W | S | F |

Version:2.0
Revised: 6/1/16

Instructions: Part 1 of this form is to be completed by the field center staff to document the **Bi-Weekly** shipping of the biospecimen collectio to the ACRL. Scan the participant ID to auto-fill the ID number into your Data Entry System. Double-check participant ID # documented on this form with ID # of each sample during the packing of specimens (blood and urine samples inclusive). Part 2 is to be completed by the ACR and UMN staff upon receipt of the shipment.

Part 1: Shipping (to be completed at the field center)

From:

Forsyth County ☐ **Minneapolis** ☐

Jackson City ☐ **Washington County** ☐

To: Charlie Rhodes
Atherosclerosis Laboratory (ACRL)
The Methodist Hospital
6565 Fannin Street, Station F701
Room 740
Houston, TX 77030

Staff Initials (shipping): | | | |

Shipped Date: (MM/DD/YYYY) | | | / | | | / | | | | |

Number of Pages Attached: | |

Time Packed: | | | : | | | (HH:MM in 24 hr. clock)

Field Center Comments: _____

Example of Complete Sample

Tube #	# of Vials	Cap Color
#1, 2 (Serum)	4 (SR)	Red
#3,4, 5,7 (Untreated Plasma)	15 (UT) (7 x 1.5 ml, 8 x 0.5 ml)	Lavender
#4, 5 (Buffy Coat)	2 (BC)	Brown
# 6 (Treated Plasma)	3 (T)	Green
#8 PAX	1 (PAX)	Red
Urine	2 (UR)	Yellow

Part 2: Receiving (to be completed at the ACRL lab)

Staff Initials (receiving): | | | |

Date Received: (MM/DD/YYYY) | | | / | | | / | | | | |

Date Buffy Coat and PAX vials picked up by Genetics Lab: (MM/DD/YYYY) | | / | | | / | | | | |

Scan the participant ID label for each collection of specimens and record the number of vials enclosed and condition code for each category (examples below) **before shipping and upon arrival**. (If more than one code for a specimen, choose "Other" and specify in a notelog).

Sample Condition Codes

00 Good Condition	06 Hemolyzed
01 Thawed	07 Lipemic
02 Warm	08 Short Sample

03 Broken Bag/Vial	09 No Sample
04 Missing Label	10 Other on arrival
05 Other on shipping	

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			**Receiving**		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			**Receiving**		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			**Receiving**		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping	**Receiving**

Type (Cap Color)	# Vials Shipped	Condition Code (Shipping)	Field Center Comments	# Vials Received	Condition Code (Receiving)	Lab Comments (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	# Vials Shipped	Condition Code (Shipping)	Field Center Comments	# Vials Received	Condition Code (Receiving)	Lab Comments (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	# Vials Shipped	Condition Code (Shipping)	Field Center Comments	# Vials Received	Condition Code (Receiving)	Lab Comments (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	# Vials Shipped	Condition Code (Shipping)	Field Center Comments	# Vials Received	Condition Code (Receiving)	Lab Comments (Receiving)
Plasma (Lavender)						
Buffy (Brown)						

Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			**Receiving**		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Participant ID:	Affix bar-code label here

	Shipping			**Receiving**		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Buffy (Brown)						
Plasma (Green)						
Serum (Red/Gray)						
PAX (Red)						
Urine (Yellow)						

Appendix 6. Bi-Weekly UMN Biospecimen Shipping and Receiving Form

Batch ID Number: | | | | | | | |

Form Code: W S F

Version:2.0
Revised: 6/1/16

Instructions: Part 1 of this form is to be completed by the field center staff to document the **Bi-Weekly** shipping of the biospecimen collection to the UMN. Scan the participant ID to auto-fill the ID number into your Data Entry System. Double-check participant ID # documented on this form with ID # of each sample during the packing of specimens (blood and urine samples inclusive). Part 2 is to be completed by the UMN staff upon receipt of the shipment.

Part 1: Shipping (to be completed at the field center)

From:

Forsyth County ☐ **Minneapolis** ☐

Jackson City ☐ **Washington County** ☐

To:

Valerie Arends/ARIC V6
University of MN/ARDL
1200 Washington Ave S Ste 175
Minneapolis, MN 55415

Staff Initials (shipping): | | | |

Shipped Date: (MM/DD/YYYY) | | / | | / | | | | |

Number of Pages Attached: | |

Time Packed: | | : | | (HH:MM in 24 hr. clock)

Field Center Comments: _____

Example of Complete Sample

Tube #	# of Vials	Cap Color
#1, 2 (Serum)	2 (SR)	Red
#3,4, 5,7 (Untreated Plasma)	4 (UT) (1 x 1.5 ml, 3 x 0.5 ml)	Lavender
#4, (Red Cells)	1 (Hgb A1c)	Black
Urine	4 (UR)	Yellow

Part 2: Receiving (to be completed at the UMN lab)

Staff Initials (receiving): | | | |

Date Received: (MM/DD/YYYY) | | / | | / | | | | |

Scan the participant ID label for each collection of specimens and record the number of vials enclosed and condition code for each category (examples below) **before shipping and upon arrival**. (If more than one code for a specimen, choose "Other" and specify in a notelog).

Sample Condition Codes

00 Good Condition	06 Hemolyzed
01 Thawed	07 Lipemic
02 Warm	08 Short Sample
03 Broken Bag/Vial	09 No Sample
04 Missing Label	10 Other on arrival
05 Other on shipping	

Home Visit: Yes No

Participant ID: Affix bar-code label here

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Home Visit: Yes No

Participant ID: Affix bar-code label here

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Home Visit: Yes No

Participant ID: Affix bar-code label here

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Participant ID: Affix bar-code label here

Home Visit: Yes No

Collection Date: (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Time of Blood Draw: (HH:MM in 24 hr. clock) __ __ : __ __

	Shipping			Receiving		
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received**	**Condition Code** (Receiving)	**Lab Comments** (Receiving)
Plasma (Lavender)						
Serum (Red/Gray)						
A1c (Black)						
Urine (Yellow)						

Appendix 7. Daily Centrifuge, Freezer, Refrigerator and Room Temperature Log

Tech ID	Date	Centrifuge	Freezer	Refrigerator	Room
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐
______	___/___/___	☐☐☐	☐☐☐	☐☐☐	☐☐☐

Blood Collection – Processing – Shipping Training Written Examination

(Answer questions completely. Each question is worth 5 points)

1. What does Stage One consist of?

   _____
   _____
   _____
   _____

2. What pH adjustment is made for the urine samples? _____

3. Which centrifuge procedural check points are most critical? (Check all that apply)
   a) Centrifuge temperature _____
   b) Centrifuge speed verification _____
   c) Centrifuge balancing _____
   d) Centrifuge cleaning _____

4. List the tube types to be collected and how many inversions and what temperature each are to be kept.

#	Color/Sample Type	# Inversions	Temperature (RT/Ice)
a) Tube #1	_____	_____	_____
b) Tube #2	_____	_____	_____
c) Tube #3	_____	_____	_____
d) Tube #4	_____	_____	_____
e) Tube #5	_____	_____	_____
f) Tube #6	_____	_____	_____
g) Tube #7	_____	_____	_____
h) Tube #8	_____	_____	_____

5. What is BHT and why is it added to some EDTA plasma aliquots?

   _____
   _____
   _____

6. Which of the list below are personal barriers that may be used to prevent exposure to biohazardous specimens? Check all that apply
   a) Face Shields _____
   b) Gloves _____
   c) Goggles _____
   d) Lab Coats _____
   e) All of the above _____
   f) None of the above _____

7. List what is included in "Day ONE" preparations.

_____
_____
_____
_____

8. A partial biospecimen collection does not have to include which tube? _____

9. Which tube # does not require processing? _____

10. Your protocol require you to centrifuge your samples at 3000 x g for 10 minutes. If your centrifuge is only capable of spinning at a maximum of 2000 x g, how would you adjust your centrifuge protocol?

_____
_____
_____

11. The phlebotomist should always recap the needle before discarding for the safety of waste disposal personnel. True_____ False _____

12. The buffy coat is embedded in the red blood cells requiring the removal of all the red cells. True _____ False _____

13. In the home setting, a patient is bed ridden and cannot sit upright. How do you handle the blood draw?

_____
_____
_____
_____

14. In shipping, it is okay to label only the secondary bag since all samples have the participant's ARIC ID label affixed. True _____ False _____

15. Since a maximum of 90 minutes is allowed for completion of stages one through three for clinic visits, when do you actually start your timer(s)?

_____
_____
_____
_____

16. List how many aliquots you should have next to the sample type listed below:
    a) SST Serum _____
    b) EDTA Plasma _____
    c) PAXgene sample _____

17. The Quality Control (QC) Phantom collection consists of which tubes and how are they processed?

_____
_____
_____
_____
_____

18. During the blood collection process, when are the QC samples drawn?
    a) Before tube #1 _____
    b) After tube # 8 _____
    c) Between tubes #2 and #7

19. All blood samples must be handled as potential _____.

20. When a participant arrive at the clinic or you arrive at the home site, what checks do you make?

    a) Confirm the match between the participant name and the ARIC ID _____
    b) Confirm that labels match on collection tubes _____
    c) Confirm that labels match on urine specimens _____
    d) Confirm that labels match on aliquot vials & Biospecimen Form _____
    e) Check that duplicate QC tubes are prepared & labeled if needed _____
    f) None of the above _____
    g) All of the above _____

Appendix 9. Checklist for Observation of Biospecimen Collection and Processing

Instructions: This checklist documents observation of technicians responsible for biospecimen collection processing and shipping by supervisors. Quarterly checklists and logs are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 1). Copies of this log may be requested by the CC.

TECH ID NUMBER: | | | | SUPERVISOR ID NUMBER: | | | | DATE: | | | / | | | / | | | | |
Month Day Year

Biospecimen Collection	Satisfactory/ Unsatisfactory	Comments
1. Labels checked	___	_____
2. Participant prepared and procedure explained	___	_____
3. Tourniquet application and release	___	_____
4. Venipuncture technique	___	_____
5. Tube collection sequence	___	_____
6. Inversion technique	___	_____
7. Tube incubation location	___	_____
8. Stasis obtained	___	_____
9. Needle disposal	___	_____
10. Biospecimen Collection form completion	___	_____
Biospecimen Processing		
1. Knowledge of centrifuge operation	___	_____
2. Aliquotting supply set-up	___	_____
3. Stage 1 tube spin	___	_____
4. Stage 2 aliquotting	___	_____
5. Stage 3 tube spin and processing	___	_____
6. Stage 4 urine and processing	___	_____
7. Volume correct for each aliquot	___	_____
8. Vials sealed	___	_____
9. Biospecimen Form completed	___	_____
10. Freezer organization	___	_____
11. Time constraints	___	_____
12. Disposal of contaminated supplies	___	_____
Biospecimen packing and shipping		
1. Specimens bagged	___	_____
2. Adequate dry ice used in shipping	___	_____
3. Shipping paperwork	___	_____
Miscellaneous		
1. Incident(s) documented on Biospecimen Form	___	_____
2. QC Procedure	___	_____
3. Containers correctly labeled for shipping	___	_____

Comments: _____

Appendix 10. Monthly Equipment Quality Control Checklist

CENTER_____
DATE_____
CERTIFIER_____
ID NUMBER_____
TECHNICIAN_____
ID NUMBER_____

SET UP:	(S)atisfactory/(U)nsatisfactory	Comments
1. Daily QC records		
refrigerator temperature	___	_____
centrifuge temperature	___	_____
freezer temperature	___	_____
room temperature	___	_____
2. Annual QC records		
centrifuge tachometer check	___	_____
3. Equipment and Supplies		
refrigerated centrifuge	___	_____
non-refrigerated centrifuge	___	_____
refrigerator	___	_____
-80 C freezer	___	_____
ice bath	___	_____
butterfly needles w/ adapter	___	_____
syringe	___	_____
tourniquet	___	_____
Vacutainer tubes	___	_____

Appendix 11. Phantom Form and Instructions

PHANTOM FORM

ID NUMBER: | | | | | | | |

FORM CODE: | P | H | T |

DATE: 04/01/2016
Version 2.0

ADMINISTRATIVE INFORMATION

0a. Completion Date: ☐☐/☐☐/☐☐☐☐ 0b. Staff ID: ☐☐☐

Instructions: *This form should be completed during participants' visit. Enter the PHANTOM ID for the corresponding QC blood sample or urine specimen. Only one box below will be collected for each phantom ID.*

PROCEDURE	PHANTOM ID	DATE COLLECTED (MM / DD / YYYY)	TECHNICIAN ID
Blood and Urine Samples			
1. Tube 1 and 10 cc Urine • 10 mL red-stoppered (serum)			
2. Tube 2 • 10 mL red-stoppered (serum)			
3. Tubes 3 and 8 • 10 ml lavender-stoppered (untreated EDTA) • 2.5 mL red-stoppered Paxgene			
4. Tube 4 • 10 ml lavender-stoppered (untreated EDTA)			
5. Tube 5 • 10 ml lavender-stoppered (BHT-treated EDTA)			
6. Tube 6 • 10 mL lavender-stoppered (untreated EDTA)			
7. Tube 7 • 10 ml lavender-stoppered (untreated EDTA)			

INSTRUCTIONS FOR THE PHANTOM (PHT) FORM
Version 2.0

I. General Instructions

The Phantom Form is used to match the phantom ID to the original ARIC participant ID who is providing a replicate specimen **within** the same visit. After the form is complete, enter the data into the data entry system using the ARIC ID, and file the original paper form.

Repeat samples are collected for all blood specimens as well as urine. Repeat blood samples consist of extra tubes being drawn per participant. A phantom ID will be assigned to every extra tube collected with the exception of Tubes 3 and 8 which are grouped together and collected from a single participant and will be assigned the same phantom ID. The Urine will be collected along with Tube 1 from a single participant and will be assigned the same phantom ID. We will be collecting a total of 5% QC repeats for each specimen tube. To determine if the participant has been randomly selected to donate an additional Phantom tube look at the Participant Snapshot Report in the General Appointment Information section. This information can also be found in the participants BIO form, item 0c which is system generated in CDART.

To track the phantom and matching ARIC ID's as specimens are collected place the ARIC ID and the Phantom ID on the Phantom Tracking Sheet (see below). Collect the phantom tubes in the order they are listed on the Phantom Tracking Sheet. Once all 8 additional tubes and the additional urine have been collected file the completed Phantom Tracking sheet in a confidential location and start a new tracking sheet.

For QC's all of the plasma aliquots be 1.0 mL. If it is not possible to obtain 1.0 mL then collect 0.5 mL

NOTE: Phantom ID numbers are confidential. Do not send a copy of the PHT or the Phantom Tracking sheet to the laboratories.

II. Detailed Instructions for each item

Place the ARIC ID label for the participant who is providing the repeat sample, in the header portion of the form.

0a. Enter the date the phantom specimen is collected.

0b. Enter the technician code of the person drawing the samples.

1. If tube1 and the urine sample are collected from the ARIC participant insert the Phantom ID in this item and enter the date samples collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are able to obtain tube 1 but are unable to obtain the urine sample, make a note of this in a notelog. If you are unable to draw 10 mL of serum or 10 cc urine make a note of the amount that you were able to obtain in a notelog.

   Using tube 1, make the following aliquots for this QC: SR1, SR2, SR3, SR4. Using the Urine collection make the following aliquots, UR1, UR2, UR3, UR4, UR5 and UR6 record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

   The SR1-4 along with two urine aliquots will be shipped to the ACRL laboratory. The remaining 4 urine aliquots will be shipped to the UMN laboratory.

2. If tube 2 is collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of serum make a note of the amount that you were able to obtain in a notelog.

   Using tube 2, make the following aliquots for this QC: SR5-6. These two aliquots will be shipped to the UMN laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

3. If tubes 3 and 8 are collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of untreated EDTA or 2.5 mL red blood for the Paxgene tube, make a note of the amount that you were able to obtain in a notelog.

   Using tube 3, make the following aliquots for this QC: UT1-4 and one Buffy Coat (BT1). These aliquots as well as the Paxgene tube will be shipped to the ACRL laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

4. If tube 4 is collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of untreated EDTA make a note of the amount that you were able to obtain in a notelog.

Using tube 4, make the following aliquots for this QC: UT50-10. These aliquots will be shipped to the ACRL laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

5. If tube 5 is collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of untreated EDTA make a note of the amount that you were able to obtain in a notelog.

   Using tube 5, make the following aliquots for this QC: T1-3. These aliquots will be shipped to the ACRL laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

6. If tube 6 is collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of BHT-treated EDTA make a note of the amount that you were able to obtain in a notelog.

   Using tube 6, make the following aliquots for this QC: UT11-15. These aliquots will be shipped to the ACRL laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

7. If tube 7 is collected from the ARIC participant insert the Phantom ID in this item and enter the date collected. Enter the technician ID for the technician processing the samples, then skip to the end of the form.

   If you are unable to draw 10 mL of untreated EDTA make a note of the amount that you were able to obtain in s notelog.

   Using tube 7, make the following aliquots for this QC: Hgb A1C and UT16-19. These aliquots will be shipped to the UMN laboratory. Record in a notelog which aliquots you were able to create, if you are unable to make all of the aliquots.

Appendix 12. Phantom Tracking Sheet

PHANTOM TRACKING SHEET

DATE: 5/11/2016

0a. Completion Date: ☐☐/☐☐/☐☐☐☐

Instructions: *This form should be completed during participants' visit. When entering into CDART enter each line in the PHT under the ARIC STUDY ID for the corresponding QC sample. Only one line below will be collected for each ARIC STUDY ID and every ARIC STUDY ID will have a unique PHANTOM ID.*

PROCEDURE	ARIC STUDY ID	PHANTOM ID	DATE COLLECTED (MM / DD / YYYY)	TECHNICIAN ID	Data Entered in CDART in PHT
Blood Samples					
1. Tube 1 and 10 cc Urine • 10 mL red-stoppered (serum) **(SR1-4 (1.5 mL each) and UR1-6 (1.5 mL each))** **SR1-4 and UR1-2 to to ACRL, UR3-6 go to UMN**					
2. Tube 2 • 10 mL red/grey-stoppered (serum) **(SR5-6 (1.5 mL each)) All go to UMN**					
3. Tubes 3 and 8 • 10 ml lavender-stoppered (untreated EDTA) • 2.5 mL red-stoppered Paxgene **(UT1-4 (1.0 mL each), BC1 and PAX) All go to ACRL**					
4. Tube 4 • 10 ml lavender-stoppered (untreated EDTA) **(UT 5-10 (1.0 mL each)) All go to ACRL**					
5. Tube 5 • 10 ml lavender-stoppered (BHT-treated EDTA) **(T1-3 (1.5 mL each)) All go to ACRL**					
6. Tube 6 • 10 mL lavender-stoppered (untreated EDTA) **(UT11-15 (1.0 mL each)) All go to ACRL**					
7. Tube 7 • 10 ml lavender-stoppered (untreated EDTA) **(Hgb A1C, UT16-19 (1.0 mL each)) All go to UMN**					