Manual of Operations Number 7:

Blood – Urine Collection and Processing

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TABLE OF CONTENTS

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 Genetics Laboratory at the University of Texas Health Science Center Houston, School of Public Health; 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), cystatin C, cardiac performance (NT-proBNP and troponin T), and hematological parameters (CBC). The Clinical Chemistry Laboratory performs assays related to renal function and oxidative stress (creatinine, urine albumin, urine creatinine, and serum uric acid), hemoglobin A1C, and a set of analytes in a subset (thyroid stimulating hormone, B12, D-dimer, plasminogen, vWF antigen). All ARIC Visit 5 and Neurocognitive Study (NCS) Stage 1 examinees will have these tests measured with the exception of glucose and insulin. 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.

These tests will be assayed as received, with clinically relevant reports back to participants as specified in Manual 2. All examinees selected for Stage II, because of potential cognitive impairment, whether from clinic or home, will also have the following assays:

- Thyroid Stimulating Hormone (TSH) and B12

TSH and B12 will be assayed as received, with clinically relevant reports back to participants as specified in Manual 2. The University of Minnesota (UMN) Laboratory will access the DMS to determine the participants who qualify for testing.

Notification of the laboratory is described in section 8 of this manual.

All examinees selected for Stage II from clinic (but not home or long-term care facility) will have the following assays:

- β -amyloid 1-40 and β-amyloid 1-42 (from both the current visit and from ARIC visit 3)
- D-dimer, Plasminogen, and vWF antigen (from ARIC visit 3)

These tests will be analyzed in batches with no expected participant reports. Notifications will be made at the completion of testing.

A complete list of the tests performed for the home, long-term care facilities, and clinic sites is located in Appendix 1.

The procedures for the collection, processing, and shipment of blood and urine samples for all collection sites 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 Visit 5/NCS Study collects approximately 80 mL of blood from each participant. A total of 11 tubes of blood are collected. Reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about two ounces, although it may look like more to them.

All sections described in this manual are the same for clinic visits, home visits and long-care facilities unless noted in the description.

2.2 Staff Certification Requirements

The blood collection and processing is performed by ARIC-certified technicians 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. Re-certification takes place annually and is authorized by the supervisory personnel. Monthly performances of certified component tasks are required for training maintenance.

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. Special tube handling (CPT)
    i. Centrifugation
    j. Urine collection
    k. Safety (bloodborne 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 from tube #4
    e. Centrifuging (special emphasis for handling CPT tube)
    f. Urine pH adjustment
    g. Aliquoting
        - Color of caps from which tubes
        - Adding BHT to aliquots from tube #6
        - Urine aliquotting
    h. Safety

3. Shipping Includes:

    a. Sorting aliquots per intended destiniation (ACRL or UMN)
    b. Bagging blood and urine aliquots
    c. Double bagging
    d. Local and FedEx guidelines and regulations
    e. Daily shipments packaging
    f. Weekly shipments packaging
    g. When to ship QC samples
    h. FedEx notifications
    i. Addresses to ship to
    j. 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 three trays: one to hold the blood collection tubes, the other two to hold the plastic vials into which the final packed cells, serum, plasma and urine aliquots are frozen and ultimately transferred to the Central Laboratories for analysis. A list of equipment, supplies, and vendors is provided in **Appendix II**.

2.3.1 Blood Collection Tray

The blood collection tray is made of hard unbreakable plastic that can be easily cleaned. The tray has individual compartments that are filled with the following supplies:

- test tube rack that holds up to 11 blood collection tubes
- sterile, disposable 21 gauge butterfly needles
- plastic vacutainer tube guides
- vacutainer Luer adapters
- sterile alcohol swabs
- gauze sponges
- tourniquet
- bandages ("Band Aids")
- biohazard sharps container
- ice water bath for clinic (combine ice and water in container 10 minutes before blood draw). Ice in bucket for home visit.

Smelling salts, ice packs, and wash cloths should be readily available in the blood collection area for participants who become faint during the blood collection, home and clinic.

Figure 1: Drawing of Clinic and Home Visit Blood Collection Tray Filled With List Above

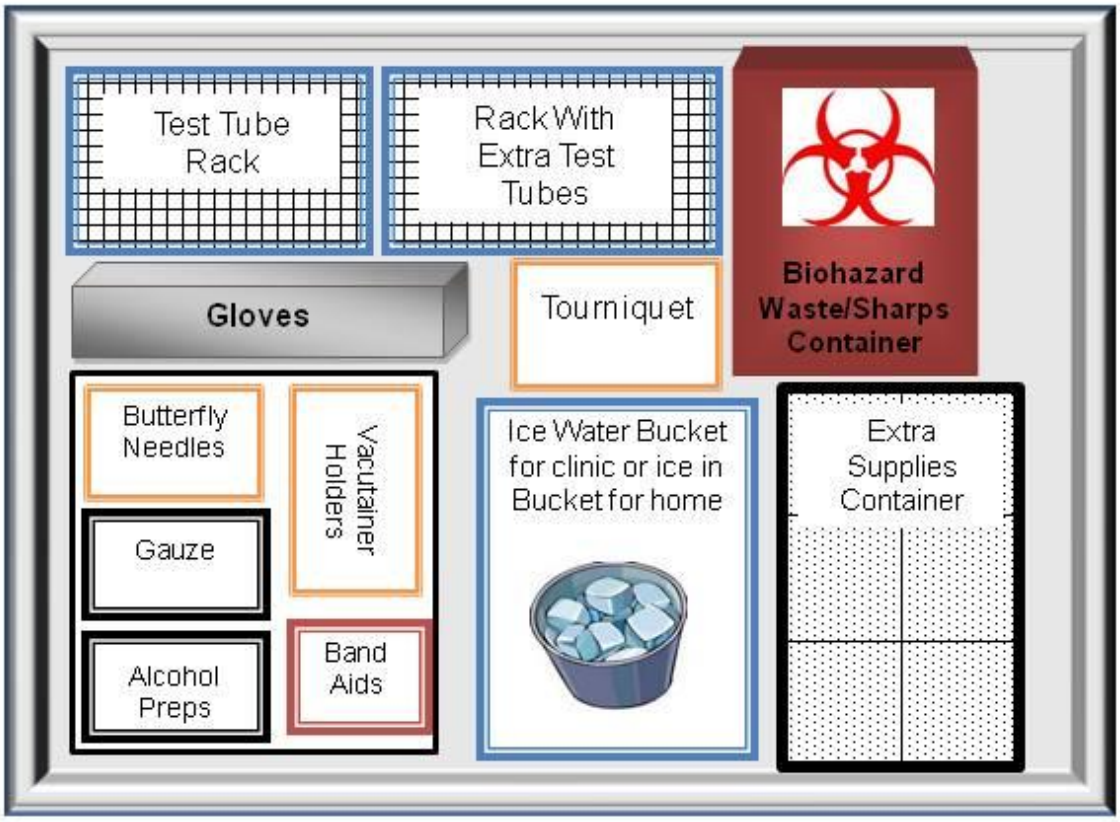


Figure 2: Picture of a Tray With Cover

2.3.2 Blood Collection Tubes: Labeling and Set-up

Technicians must be familiar with the following: 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.

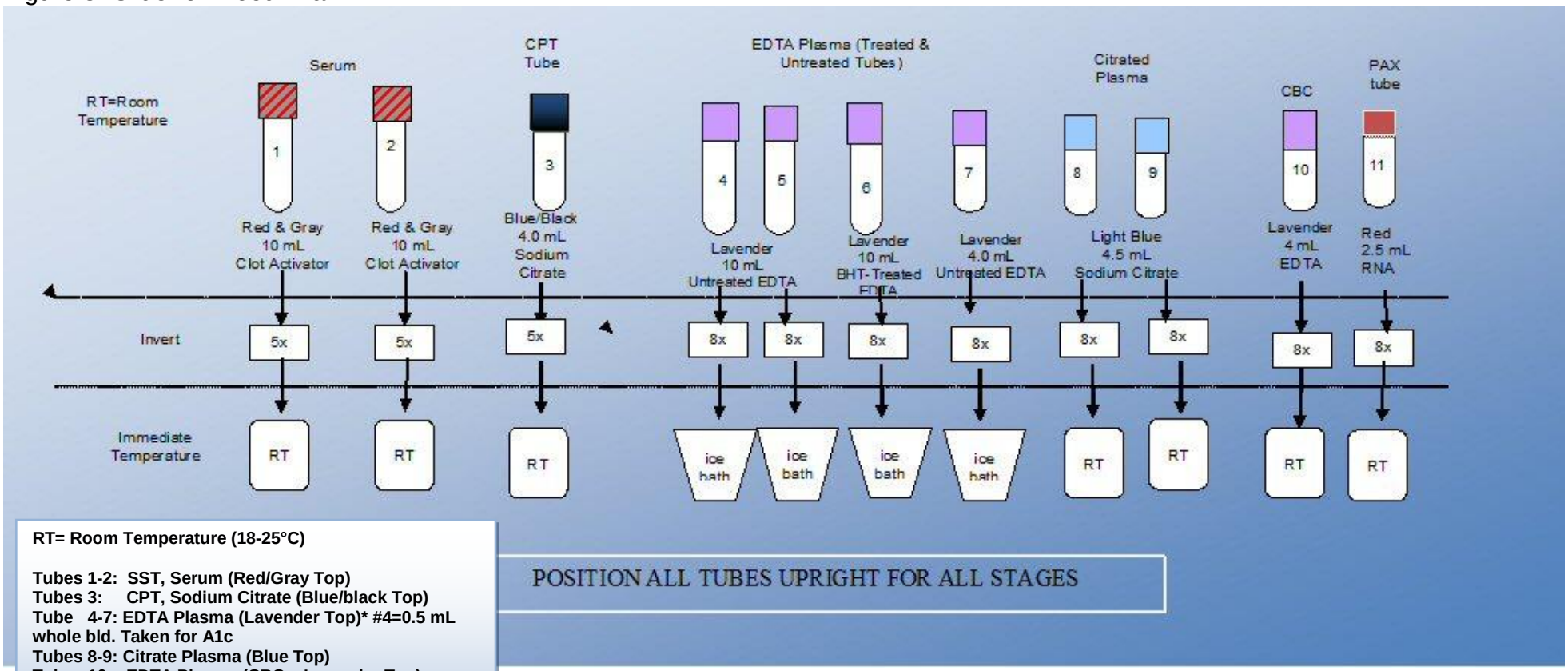
Guidelines:

1. Apply bar-coded ARIC ID labels to the blood collection tubes. Handle only one participant's specimens at a time so the chance of mislabeling is minimized.

2. Fill out the Laboratory Log. Each center must keep a permanent record of the ARIC participant ID number in a Laboratory Log (not provided).

3. Arrange the blood collection tubes in the test tube rack in the same order in which they are to be collected. The order of collection is as follows:

Figure 3: Order of Blood Draw



RT= Room Temperature (18-25°C)

Tubes 1-2: SST, Serum (Red/Gray Top)
Tubes 3: CPT, Sodium Citrate (Blue/black Top)
Tube 4-7: EDTA Plasma (Lavender Top)* #4=0.5 mL whole bld. Taken for A1c
Tubes 8-9: Citrate Plasma (Blue Top)
Tube 10: EDTA Plasma (CBC – Lavender Top)
Tube 11: PAXgene Speciality (Red Top)

TOTAL DRAW= 73.5 mL
[MAX DRAW VOL.= 90 mL]

Tube #1: 10 mL red and gray-stoppered tube containing no anticoagulant.

Tube #2: 10 mL red and gray-stoppered tube containing no anticoagulant

Tube #3: 4.0 mL blue and black stoppered tube (CPT) contains sodium citrate

Tube #4: 10 mL lavender-stoppered tube contains EDTA anticoagulant, 0.5 mL wholeblood removed for A1c, buffy coat will be removed after spin

Tube #5: 10 mL lavender-stoppered tube contains EDTA anticoagulant, buffy coat will be removed after spin

Tube #6: 10 mL lavender-stoppered tube, BHT will be added to aliquots from this tube

Tube #7: 4 mL lavender-stoppered tube contains EDTA

Tubes #8 and #9: 4.5 mL blue-stoppered tube contains sodium citrate anticoagulant

Tube 10: 4 mL lavender-stoppered tube contains EDTA for CBC

Tube #11: 2.5 mL red-stoppered tube (PAX) for RNA

4. Double check that the ARIC ID on the tube matches that on the Laboratory Log.

A number of ARIC participants will be asked to donate one additional tube of blood for quality control purposes. The duplicate sample will be assigned a different ARIC ID number, called a Phantom ID, and shipped to the appropriate Laboratories. This quality control procedure is described more completely in Section 6. **DO NOT SEND THE PHANTOM FORM WITH SHIPMENTS.**
The sample aliquot trays should have individual compartments capable of holding up to 50 vials 10-16 mm in diameter. These trays will hold polypropylene plastic vials that contain the final processed samples (i.e., serum, plasma, urine) for each participant. Each type of storage vial has a corresponding color-coded screw cap that fits onto it. The technicians will need the following supplies for each sample tray:

- 1.5 mL polypropylene microsample vials (Qty=52)
    - 19 lavender screw caps
    - 8 green screw caps
    - 16 red screw caps
    - 2 brown screw caps
    - 6 blue screw caps
    - 1 black screw cap
- white 5 mL polypropylene vials (urine) (Qty=6)
    - 3 yellow screw caps
    - 3 green screw caps
- plastic transfer pipettes (Qty=6)

2.3.4. Sample Aliquot Vials: Labeling and Set-up (Per Participant)

1. Apply a barcode ARIC ID label to the sample vials.

2. Apply a barcode ARIC ID label to Biospecimen Collection Forms.

3. Set up two trays adjacently. Tray 1 has 5 rows and 10 columns. The columns are numbered 1-10 from left to right. The rows are lettered A-E from top to bottom. Arrange the sample vials in the Sample Aliquot Tray 1 in the following order: (See Figure 4a)

Col 1: 1.5 mL vials **Green Caps** (rows A-D); **Brown Cap** (row E)
Col 2: 1.5 mL microsample vials **Green Caps** (rows A-D); **Brown Cap** (row E)
Col 3: 1.5 mL microsample vials **Lavender Caps** (rows A-E)
Col 4: 1.5 mL microsample vials **Lavender Caps** (rows A-E)
Col 5: 1.5 mL microsample vials **Lavender Caps** (rows A-E)
Col 6: 1.5 mL microsample vials **Lavender Caps** (rows A-D); **Black Cap** (row E)
Col 7: 1.5 mL microsample vials **Blue Caps** (rows A-E)
Col 8: 1.5 mL microsample vials **Blue Caps** (rows A); **Red Caps** (rows B-E)
Col. 9: 1.5 mL microsample vials **Red Caps** (rows A-E)
Col 10 1.5 mL microsample vials **Red Caps** (rows A-E)

Figure 4a: Aliquot Tray 1 Layout (Per Participant)

(Stages 1 - 3 Processing)

Col Row	1	2	3	4	5	6	7	8	9	10
A	0.5 mL plasma, Tube #6 BHT	0.5 mL plasma, Tube #6 BHT	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL plasma, Tubes #4,5,7	..1.0 mL citrate plasma, Tubes #8,9	1.0 mL citrate plasma, Tubes #8,9	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2
B	0.5 mL plasma, Tube #6 BHT	0.5 mL plasma, Tube #6 BHT	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5.0 mL plasma, Tubes #4,5,7	1.0 mL citrate plasma, Tubes #8,9	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2
C	0.5 mL plasma, Tube #6 BHT	0.5 mL plasma, Tube #6 BHT	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL plasma, Tubes #4,5,7	1.0 mL citrate plasma, Tubes #8,9	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2
D	0.5 mL plasma, Tube #6 BHT	0.5 mL plasma, Tube #6 BHT	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL plasma, Tubes #4,5,7	1.0 mL citrate plasma, Tubes #8,9	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2
E	Buffy Coat, Tube # 4	Buffy Coat, Tube # 5	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	0.5 mL EDTA plasma, Tubes #4,5,7	HbA1c, Tube #4 (before spin)	1.0 mL citrate plasma, Tubes #8,9	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2	0.5 mL serum, Tubes #1,2

Figure 4b: Aliquot Tray 2 Layout (Per Particiant)

Col 1: 1.5 mL microsample vials **Red Caps** (rows A-B)
Col 2: Empty
Col 3: Empty
Col 4: 7 mL vials **Yellow Caps** (rows A-C) urines, no pH adjustment
Col 5: 7 mL vials **Green Caps** (rows A-C) urines, pH adjusted to 7 (Stage 3 continued and Stage 4 Urines)

Col Row	1	2	3	4	5	6	7	8	9	10
A	0.5 mL serum, Tubes #1,2	Empty	Empty	5 mL urine, no pH adjustment	5 mL urine, pH to 7	Empty	Empty	Empty	Empty	Empty
B	0.5 mL serum, Tubes #1,2	Empty	Empty	5 mL urine, no pH adjustment	5 mL urine, pH to 7	Empty	Empty	Empty	Empty	Empty
C	Empty	Empty	Empty	5 mL urine, no pH adjustment	5 mL urine, pH to 7	Empty	Empty	Empty	Empty	Empty
D	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
E	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

2.4. 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. Place the prepared tray in the refrigerator for day two processing.

4. Check that each storage vial is labeled with the correct ARIC barcode ID label per participant.

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 ± 10°C).

8. Perform and record QC check on room temperature.

9. Set refrigerated centrifuge to 4°C and power on.

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).

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2. (Clinic visit) Place cold water into ice bath.

***At participant's arrival*: DAY 2**

1. Confirm the match between the participant name and the ARIC ID number on the blood collection tubes, urine specimen, aliquot vials and the Biospecimen Collection Form.

2. Check that duplicate Quality Control tubes are prepared and labeled (affix only the QC Phantom label, *do not* place the donor participant's label on the tube or the form), if needed.

2.5. Biospecimen Collection Form

At the completion of specimen collection and processing, the Biospecimen Collection Form (**Appendix III**) is sent to the Atherosclerosis (ACRL) Laboratory. If there are any deviations from the routine collection or processing protocol, record on the biospecimen collection processing form (**Appendix III**). A copy is sent to the ACRL Laboratory and the University of Minnesota (MN) Laboratory with the weekly sample shipment This form is entered on paper first and then entered into the DMS using the participant ID. File and maintain the paper form for a period to be determined.

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 III**). The subject is asked whether he/she has a bleeding disorder before the blood is drawn. If such a disorder is present, ask the subject whether he/she has had blood drawn previously and if so, whether he/she had any problems with excessive bleeding or bruising at the venipuncture site. If the participant has a history of venipuncture problems, the participant's blood should be drawn only if approved. 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. 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 III**).

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.

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

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 hand. If this happens, cleanse the site again with alcohol.

Perform venipuncture.

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

2. With the needle bevel upward, enter the vein in a smooth continuous motion.

3. 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 through #3) five times and (tube# 4 through tube #10) eight times immediately following removal of the tube from the adapter then place them at room temperature except for tubes #4, #5, #6, and #7 which are placed into the ice water bath.

9. When collecting tube #11, 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 8 times and store at room temperature for a minimum of 2 hours.

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, <u>lightly</u> place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent bruising.

- If the blood flow stops before all 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 3.7 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. If necessary, have him/her lie on the floor with their legs elevated. Use of a transfer belt may be indicated in this situation.

2. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds.

3. Provide the person with a basin if he/she feels nauseous.

4. Have the person stay seated until the color returns and he/she feels better.

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

6. Place a cold wet cloth on the back of the person's neck or on their forehead.

7. 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.

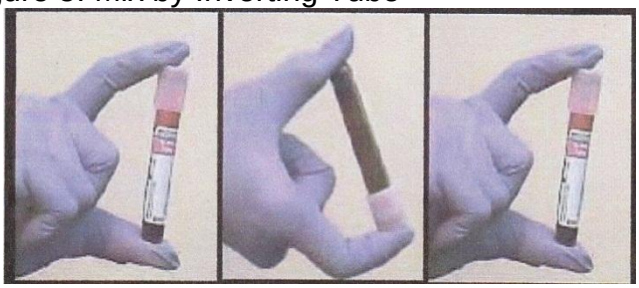
8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.5. Blood Mixing During Venipuncture

All tubes with a clot activator (tubes 1 and 2) and all tubes with an anticoagulant (tubes 3 – 11) must be mixed. Begin by holding the tube upright. Slowly tip the stopper end down while watching the air

bubble rise to the butt. Now, lower the butt end slightly while watching the bubble float to the stopper (one inversion). Invert tubes #1 - #3 five times and tubes #4 - #11 eight times.

Figure 5: Mix by Inverting Tube



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 weekly shipment to the Atherosclerosis Laboratory.

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 weekly shipment to the Atherosclerosis Laboratory.

Tube #3: 4.0 mL blue and black-stoppered tube contains sodium citrate anticoagulant.

Invert gently (do not shake) 5 times immediately after collection to ensure complete mixing with the anticoagulant. Place the tube at room temperature. Within 2 hours of collection (or can be immediately), centrifuge, gently resuspend the cells in the plasma and store at room temperature in an upright postion. Ship daily to Atherosclerosis Laboratory for the UT Genetics Laboratory using refrigerant packs at room (ambient) temperature. (see section 5.3).

Tube #4: 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 #4 and place in microsample tube with black lid. 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.

Invert gently eight times, place in ice water bath until centrifugation. The plasma from tube #5 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 #6: 10 mL lavender-stoppered tube contains EDTA anticoagulant plus BHT added.

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

Tube #7: 4 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 and #9: 4.5 mL blue-stoppered tubes contain sodium citrate.

Invert gently 8 times immediately after collection. Place at room temp until centrifugation, remove plasma, store and freeze.

Tube #10: 4.0 mL lavender-stoppered tube contains EDTA anticoagulant. This tube is for a CBC.

Invert gently eight times. Place at room temperature, ship daily to Atherosclerosis Laboratory, *use ambient packs* (see section 5.3).

Tube #11: 2.5 mL red-stoppered Paxgene (PAX) tube for RNA preservation.

Invert gently eight times. Store upright at room temperature. Ship daily to Atherosclerosis Laboratory for the UT Genetics Laboratory *using ambient packs* (see section 5.3).

3.6 Urine Collection

A urine sample is collected from each participant (preferably) at the beginning of the clinical/home exam. After participants complete the Reception work 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.

Provide a copy of urine collection Instructions to give to the patient prior to collection. When the patient is ready to collect the sample, read through the list with them making sure that they understand how to collect the specimen.

A specimen cup (labeled with the participant's ID), cup lid, and a TIME VOIDED LABEL are provided by the staff member working with the participant at that time. The participant is instructed for urine collection (**Appendix XI**).

How to Collect Urine Sample

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

Female Cleansing Instructions

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.

Male Cleansing Instructions

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.

3.7 Partial Biospecimen Collection Procedures

3.7.1 Participant Sample Set Incomplete (Clinic and Home Visits)

If a full set of biospecimen blood tubes cannot be obtained after 2 venipuncture attempts by each phlebotomist, (this is the maximum allowance, however, use professional judgement) follow the procedures below. A complete blood draw set by protocol, consists of 11 filled tubes.

1. **The participant is willing to schedule a fasting re-collection appointment**

    a) Insert a comment on line item #12 of the Biospecimen Collection Form stating that the participant will come back for a re-collection at another date.

    b) If tube #3 was collected, process according to protocol and ship even though the set is incomplete.

c) Process all tubes and urine according to protocol and hold the incomplete biospecimens in the freezer.

d) Request extra labels a minimum of 5 days before scheduled recollection using the same participant ID.as in the initial blood draw.

e) Collect only the missing tubes, process with the previously collected tubes to complete the set and ship (include the previously collected urine in the shipment).

f) Mark the redraw labels with the letter "R" and highlight alerting that the sample is from a redraw. Use a permanent black marker that will not smear.

g) Enter in the DMS (item 21) comments that this is a redraw,

h) For the Field Center records, save the Biospecimen Collection Form from the first visit and insert a comment on line item #12 that the participant was re-collected

i) The time limit for re-collection appointments is one month. If the participant can not be re-collected within one month, then send the first set of incomplete biospecimens to the Atherosclerosis Laboratory. Indicate on the Frozen Contents Sheet that this biospecimen set is incomplete and no other specimens will be obtained.

j) Once a set of frozen biospecimens from a participant is sent to the Atherosclerosis Laboratory, no other biospecimens from this participant should be sent on a different date.

k) Contact the Atherosclerosis Laboratory if any unusual circumstances or questions arise with any biospecimen collection.

2. The participant is unwilling to schedule a fasting re-collection appointment.

l) Insert a comment on line item #12 of the Biospecimen Collection Form that the participant is unwilling to reschedule and that the biospecimen set for this participant is a partial collection and no other specimens will be obtained.

3. Short Draw Set

a) A "short draw set" is collected as a last resort to obtain sufficient blood from a participant. The "short draw set" should only be attempted ***after*** the first phlebotomy attempt fails and the participant has expressed ***an unwillingness to re-schedule.***

b) If a patient ***consents*** to coming in for a second phlebotomy visit, the aim should be to collect a "complete draw set". If a site search for the patient's veins yields little possibility **or** a first draw attempt fails, the "short draw" option should be utilized.

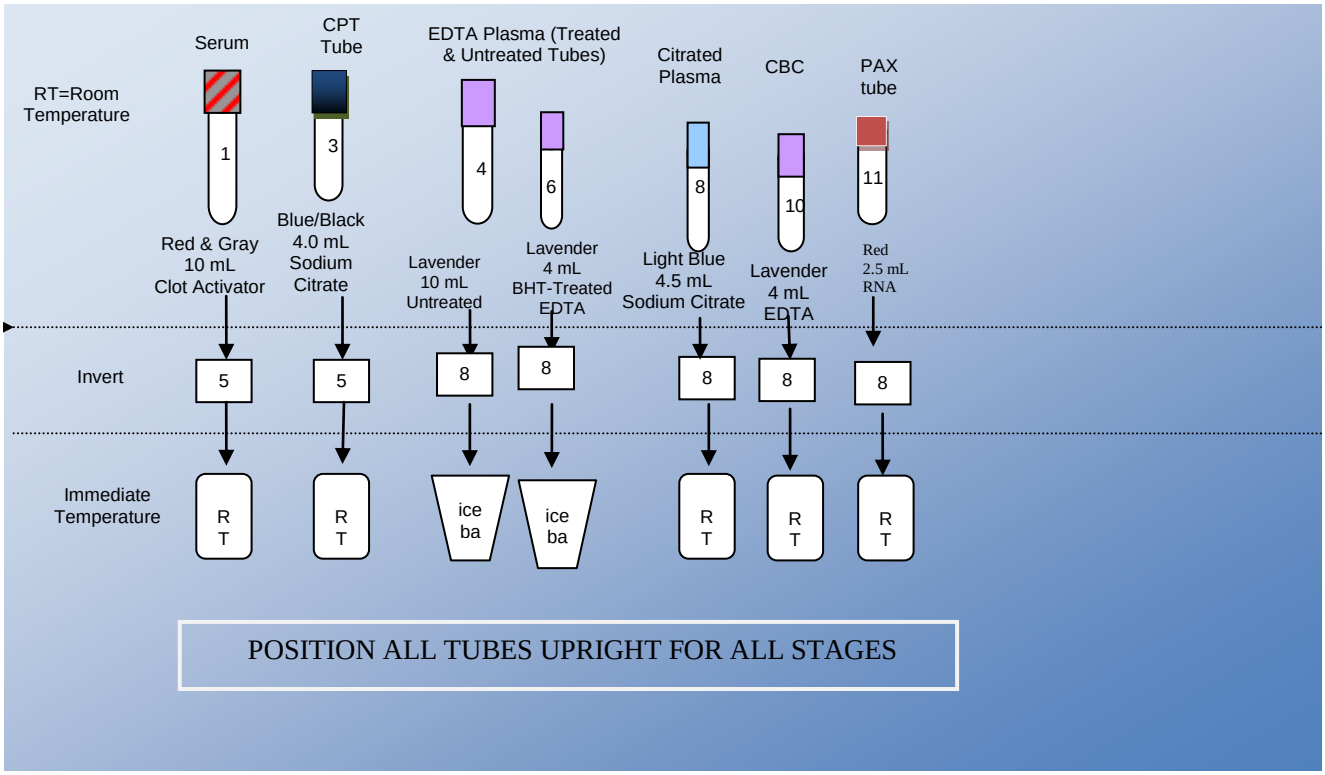
c) The order of draw and number of tubes to draw are as described below:

Order #	Illustration Tube #	Tube Type	Description
1	1	Serum	(1) 10 mL serum tube instead of two
2	3	CPT	(1) 4 mL CPT tube
3	4	EDTA plas	(1) 10 mL EDTA plasma tube instead of tubes #4, #5 & #7. A1c is taken from this tube prior to spin and one buffy coat is removed.
4	6	EDTA plas	(1) 4 mL EDTA plasma for BHT treatment & 2nd buffy coat is removed.
5	8	Citrate plas	(1) 4.5 mL citrate plasma tube instead of 2
6	10	EDTA plas	(1) 4 mL EDTA plasma tube for CBC
7	11	PAXgene	(1) 2.5 mL PAXgene tube

These 7 tubes constitute a short draw set. The numbering on the illustration is relative to the first tube# of the standard complete draw set.

Figure 6. Short Draw Diagram

Order of Short Draw Blood Collection ARIC Visit 5 & NCS



RT= Room Temperature (18-25°C)

Tube 1: **SST, Serum (Red/Gray Top)** **(represents tubes 1&2)**
Tube 3: **CPT, Sodium Citrate (Blue/black Top)** **(represents tube 3)**
Tube 4: **EDTA Plasma (Lavender Top)* #4=0.5 mL whole bld. Taken for A1c** **(represents tubes 4, 5 & 7)**
Tube 6: **EDTA Plasma (Lavender Top)** **(represents tube 6)**
Tube 8: **Citrate Plasma (Blue Top)** **(represents tubes 8 & 9)**
Tube 10: **EDTA Plasma (CBC – Lavender Top** **(represents tube 10)**
Tube 11: **PAXgene Speciality (Red Top)** **(represents tube 11)**

TOTAL DRAW= 40.0 mL
[MAX DRAW VOL.= 90 mL]

3.8 Re-collections Due to Lost or Delayed Shipments

If a daily shipment is delayed or lost the participant, if willing, should be re-scheduled. Redraw, process and ship with notice that these specimens were redrawn.

If a weekly shipment is delayed to the point where upon delivery the samples are warm or if the shipment is lost and delivery does not occur, the participant should be re-scheduled. Explain the reason for the second blood draw to the participant. A complete set minus the daily tubes should be attempted.

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

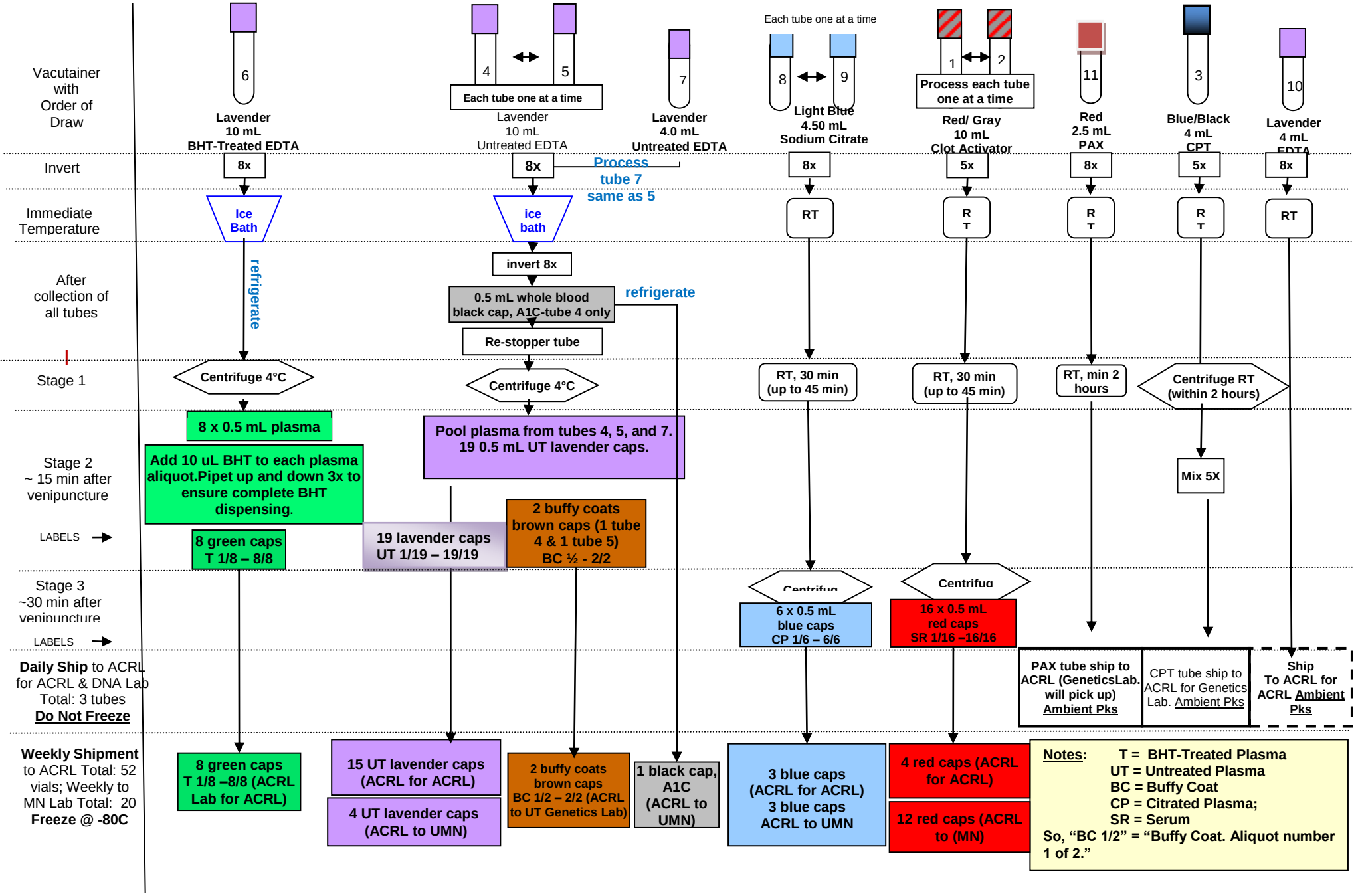
Once the samples are collected at the home visit, the room temperature samples will be transported in a locked cooler and the other samples will be transported in the locked cooler containing the ice water. All needles will be disposed of in a locked needle disposal tank.

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

Processing of the various blood samples is divided into 3 stages. Make note of the conditions at which the collection tubes are kept prior to centrifugation. Urines are processed in stage 4.

Figure 7: Blood Processing


Vacutainer with Order of Draw
Invert
Immediate Temperature
After collection of all tubes
Stage 1
Stage 2 ~ 15 min after venipuncture
LABELS
Stage 3 ~30 min after venipuncture
LABELS
Daily Ship to ACRL for ACRL & DNA Lab Total: 3 tubes Do Not Freeze
Weekly Shipment to ACRL Total: 52 vials; Weekly to MN Lab Total: 20 Freeze @ -80C
6
Lavender 10 mL BHT-Treated EDTA
8x
Ice Bath
refrigerate
Centrifuge 4°C
8 x 0.5 mL plasma
Add 10 uL BHT to each plasma aliquot.Pipet up and down 3x to ensure complete BHT dispensing.
8 green caps T 1/8 – 8/8
8 green caps T 1/8 –8/8 (ACRL Lab for ACRL)
4
5
Each tube one at a time
Lavender 10 mL Untreated EDTA
8x
ice bath
invert 8x
0.5 mL whole blood black cap, A1C-tube 4 only
Re-stopper tube
Centrifuge 4°C
Pool plasma from tubes 4, 5, and 7. 19 0.5 mL UT lavender caps.
19 lavender caps UT 1/19 – 19/19
2 buffy coats brown caps (1 tube 4 & 1 tube 5) BC ½ - 2/2
15 UT lavender caps (ACRL for ACRL)
4 UT lavender caps (ACRL to UMN)
2 buffy coats brown caps BC 1/2 – 2/2 (ACRL to UT Genetics Lab)
7
Lavender 4.0 mL Untreated EDTA
Process tube 7 same as 5
refrigerate
1 black cap, A1C (ACRL to UMN)
Each tube one at a time
8
9
Light Blue 4.50 mL Sodium Citrate
8x
RT
RT, 30 min (up to 45 min)
6 x 0.5 mL blue caps CP 1/6 – 6/6
3 blue caps (ACRL for ACRL) 3 blue caps ACRL to UMN
1
2
Process each tube one at a time
Red/ Gray 10 mL Clot Activator
5x
RT
RT, 30 min (up to 45 min)
16 x 0.5 mL red caps SR 1/16 –16/16
4 red caps (ACRL for ACRL)
12 red caps (ACRL to (MN)
11
Red 2.5 mL PAX
8x
RT
RT, min 2 hours
PAX tube ship to ACRL (GeneticsLab. will pick up) Ambient Pks
3
Blue/Black 4 mL CPT
5x
RT
Centrifuge RT (within 2 hours)
Mix 5X
CPT tube ship to ACRL for Genetics Lab. Ambient Pks
10
Lavender 4 mL EDTA
8x
RT
Ship To ACRL for ACRL Ambient Pks
Notes: T = BHT-Treated Plasma
UT = Untreated Plasma
BC = Buffy Coat
CP = Citrated Plasma;
SR = Serum
So, "BC 1/2" = "Buffy Coat. Aliquot number 1 of 2."

4.1. Stage One: Immediate Processing

After completion of venipuncture: (for clinic visit) blood specimens should be processed within 90 minutes. Blood specimens should be processed within 8 hours for home and long-term care facility collections. The procedure below is the same for both clinic and home visits with the exception of timing.

Procedure Steps:

1. Start the timer (for 90 minutes, home and clinic)

2. Tube #1 and #2 remain incubating at room temperature for 30-45 minutes to allow the blood to clot (blood at 4°C clots extremely slow). (Clotting is already complete for samples collected at home visit)

3. Remove tube #4 from the ice water bath and invert gently 8 times. Aspirate 0.5 mL whole blood from tube #4 and transfer into a micro sample vial located in Aliquot Tray 1, column 6 row E. Screw black lid on and store in the refrigerator until the remainder of the aliquots are to be frozen. Ship weekly to the Atherosclerosis Laboratory. Re-cap tube #4 and return to the ice water bath.

4. Remove tubes #4, #5, #6 and #7 from the ice water bath or ice bucket and place them in the centrifuge holders. Balance for centrifuging, then 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.

5. Wait for the centrifuge to come to a complete stop. Proceed to stage 2 processing.

(Note: home visit only) Depending on the time the home visit is completed and for the sake of time, all samples may be centrifuged as clotting is not a concern, but only if two centrifuges are present for temperature control.

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 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 x *g* force, increase the centrifugation time until the *g*-minutes total 3,000. If, for example, the maximum force is 2000 x *g*, then increase the time from 10 to 15 minutes.

4.2. Stage Two: Remove Plasma with Disposable Pasteur Pipette

Stage two begins approximately 15 minutes after venipuncture for clinic visit, after centrifugation for home visit.

NOTE:
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. This is especially true in tubes #4 and #5 because the platelets from the buffy coat

contain some of the analytes which are to be measured. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube under the initial processing conditions. Indicate on Question #13 of the Biospecimen Collection Form (**Appendix III**) 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 between the buffy coat and the upper lipid layer be aspirated when preparing these sample aliquots.

STEPS:

1. Remove tubes #4,# 5, #6 and #7, from the centrifuge and place in the ice water bath. Remove the lavender-stoppered tubes # 4, #5 and #7 from ice bath and place in the test tube rack.

2. Remove the stoppers. Using a transfer pipette and being careful not to disturb the red or white blood cell layers, remove the supernate (plasma) from tubes #4, 5 and #7, place in holding tube in rack for aliquoting.

3. Using a 1.0 mL pipet set to 0.5 mL, transfer 0.5 mL of plasma from tube #6 into each of the (8) 1.5 mL vials in column 1 and 2 of the sample aliquot tray. Using a 10 µL pipettor add 10 µL of BHT additive to each of the (8) aliquots. With each addition, wash by aspirating and dispensing 3x to mix (you may have to change tips occasionally due to air bubbles generated with the mixing).

4. Using the automatic pipettor aliquot 0.5 mL of the plasma from the holding tube into each of the (16) 1.5 mL vials in columns 3, 4, 5. and row A column 6 of the sample aliquot tray-1. Pipette 1.0 mL into the last (3) vials rows B – D. Do not discard the packed red blood cells.

5. Fasten the lavender screw caps onto the vials in columns 3, 4, 5 and 6 and green screw caps onto the vials in columns 1 and 2.

6. Using the same plastic transfer pipette, remove the buffy coat cells from tube #4 and #5 and transfer them equally into (2) 1.0 mL vials in column 1, row E and column 2, row E. Buffy coats should be aspirated in a single, sweeping, circular motion. Be careful not to aspirate the buffy coat layer into the bulb of the transfer pipette as it decreases the amount of white cells ascertained. Fasten the brown screw caps onto these vials.

7. Re-stopper tubes #4, #5, #6 and #7 and discard them in a biohazard waste bag.

8. Place the sample aliquot tray with all of the aliquot vials into the 4°C refrigerator.

Proceed to Stage 3 processing.

4.3. Stage Three: Processing Serum and Citrate Plasma

Stage three begins approximately 30 minutes after venipuncture for clinic visit and immediately after stage two is completed for home visit.

1. As close to 30 minutes after venipuncture as possible and no longer than 45 minutes after venipuncture, spin the red and gray stoppered tube #1 and #2 and the blue stoppered tubes, #8,

and #9 at 3,000 x *g* for 10 minutes at room temperature. Record the time when centrifugation begins on the Biospecimen Collection Form.

2. When the centrifuge has come to a complete stop, remove the sample aliquot tray from the refrigerator.

3. Remove the blue-stoppered tubes #8 and #9 from the centrifuge. Remove the red and gray stoppered tubes #1 and #2 from the centrifuge and place in the test tube rack.

4. Within a maximum of 2 hours after collection, remove tube #3 from the tube rack stored at room temperature (18°C - 25°C). Invert each CPT vacutainer 8 times, and place in the centrifuge buckets. Balance appropriately and centrifuge at 1,800 x *g* for 20 minutes at room temperature. Record on the Biospecimen Collection Form (item #18) the time at which these tubes began to spin.

5. Remove the supernate (plasma) from tubes #8, and #9 and place in a holding tube in the rack. Using the automatic pipettor, transfer 1.0 mL of plasma into each of the (6) vials in columns 7 and row A of column 8. Fasten the blue screw caps onto these vials in the aliquot tray #1.

6. Re-stopper tubes #8 and #9 and discard in a biohazard bag.

7. Remove the supernate (serum) from tubes #1 and #2 and place in a holding tube in a rack. Using the automatic pipettor, transfer 0.5 mL into each of the (14) vials in columns 8, 9, and 10 of Aliquot Tray #1. Move to Aliquot Tray #2, column 1 to complete the 0.5 serum aliquots.

8. Re-stopper tubes #1 and #2 discard them in a biohazard waste bag.

9. Place processed aliquot trays back into the refrigerator.

10. After centrifugation of tube #3 is complete (from step 4), mix 8x's and store at room temperature for daily shipment to the Atherosclerosis Laboratory.

4.3.1 Stage 4: Urine Processing for Home and Clinic Visits

1. Remove urine from refrigerator or cooler and mix by inverting 3 xs.

2. Pour 30 mL from the urine specimen cup into a 50 mL graduated cylinder or 50 mL conical centrifuge tube pre-labeled with the participant's I.D.

3. Using an eppendorf repeating pipetor or any pipette capable of delivering 5 mL, remove (3) 5 mL aliquots into pre-labeled white vials with yellow screw caps. These vials are in Aliquot Tray #2, column 4

4. Using a urine dip stick (use as instructed by manufacturer), read the pH of the remaining urine in the cylinder or conical centrifuge tube (record the reading in your urine specimen log **Appendix X**).

5. If the urine has a pH reading above 7 (alkaline) use 3.0N HCL solution to adjust the pH to 7.
6. If the urine has a pH reading below 7 (acidic) use 3.0N NaOH solution to adjust the pH to 7.
7. Adjust the urine by adding one drop of the appropriate acid or base solution at a time mixing by swirling and repeating the dip stick reading.
8. Repeat step 7 until the desired pH reading is obtained.
9. Using an Eppendorf repeating pipetor or any pipette capable of delivering 5 mL, pipet (3) 5 mL aliquots into pre-labeled white vials with green screw caps. These vials are in Aliquot Tray #2, column 5

Discard any remaining urines using CDC and OSHA recommended precautions..

4.3.2 Processing Urines with < 30 mL Volume

If collected urine volume is between 30 mL and 15 mL (< 30 mL), **DO NOT pH ADJUST**. Aliquot the non- pH adjusted urines the same as in step 3 above.

1. Discard any remaining urines.
2. If collected urine volume is less than 15 mL, discard and collect at a different date. (Record on the Biospecimen Form, **Appendix III**).

4.4 Freezing

Place the completed sample aliquot trays upright in the -80°C freezer for a minimum of 30 minutes. Specimens must be placed in the freezer within 90 minutes from venipuncture time. They must be thoroughly frozen before packaging them for storage and shipping. Record the time that the specimens are placed in the freezer on the Biospecimen Collection Form.

5. STORAGE AND SHIPPING (FOR FROZEN SPECIMENS)

Bagging Blood Samples:

1. Remove the sample aliquot tray from the -80°C freezer. Package quickly after this point to avoid thawing of the specimens. (Process using a tray-bed of dry ice chips)
2. On DAY 1 the following bags were labeled: three 6x6 bags, and two 3x6 bags with the participant ID"; one 6x6 bag and three 3x6 bags with the participant ID, two 11x15 freezer bags with the appropriate ARIC participant ID label. Use these bags in the following steps.
3. Place (4) red cap vials in a 6x6 bag for ACRL. Place (12) red cap vials in a 6x6 bag for MN. Again, verify that the bags and vials are labeled correctly. Press the air out of the bag and seal.

4. Place (12) 0.5 mL lavender cap vials and the (3) 0.5 lavender cap vials into a 6x6 bag for ACRL and (4) 0.5 mL lavender cap vials in a 3x6 bag for MN. Place (2) brown cap vials in a 3x6 bag for ACRL and (1) black cap vial in a 3x6 bag for MN. Press the air out of the bags and seal.

5. Place the (8) green cap vials into a 6x6 ACRL bag. Press the air out of the bag and seal.

6. Place (3) of the blue capped vials into a 3x6 bag for ACRL and (3) blue cap vials in a 3x6 bag for MN. Press the air out of the bags and seal.

7. Place all bags labeled ACRL into an 11x15 zip lock bag with the participant I.D and place all bags labeled MN into the second 11x15 zip lock bag with the participant's I.D. Place these bags into the styrofoam box in the -80°C freezer.

Bagging Urine Samples:

1. Place two yellow top vials and one green top vial into a 6x6 bag labeled with the participant's I.D. and ACRL.

2. Place one yellow top vial and two green top vials into a 6x6 bag labeled with the participant's I.D. and MN.

3. Place each 6x6 bag respectively into two 8x10 bags (to meet required double bagging rules) labeled with the participant's I.D. on each bag.

Shipping

The samples remain at -80°C until they are shipped. Remember that courier regulations require a double barrier for all specimen shipments.

All frozen specimens collected and stored within the last work week are shipped to the ACRL Laboratory on Monday with the exception of the Quality Control specimens, as discussed in the Quality Control section below, by overnight courier. Specimens can be shipped on Tuesday if the Field Center is closed on Monday, but the contact person at the ACRL must be notified that the shipment will arrive one day later than usual. There is no minimum shipping requirement; frozen samples are shipped weekly regardless of the number of specimens that have been frozen and stored within the last collection period. Weigh all packages before shipping, if possible. It is important to record an accurate weight on the Federal Express air bill. Do not over-estimate the package weight.

Table 1: ARIC Visit 5/NCS Collection – Processing – Shipping

COLLECTION		PROCESSING			PREPARATION		SHIPPING		
VENIPUNCTURE TIME	**IMMEDIATE ACTIONS**	**STAGE I**	**STAGE II**	**STAGE III**	**FREEZE**	**PACKAGING**	**Daily Shipping (Mon-Fri)**	**Weekly Shipping**	**Batched Other Shipping**
Tubes 1,2 red/gray (SST) 10 mL RT	Mix ***5 x's*** and sit at Room Temp (RT)	Incubate UPRIGHT at RT 30-45 minutes	***Step 1, STAGE III:*** Centrifuge 10 min, 3000 g at room temperature ***Step 2:*** remove supernate from tube & place UPRIGHT in holding tube for aliquotting.	***Step 3:*** Using a repeater pipetor, transfer 0.5 ml serum from holding tube into (16) pre-labeled white vials with red screw caps	16 red top vials`	Place 4 red top vials into a 6x6 bag *labl'd w ID & to* ACRL (V-5/NCS) (12) 0.5 mL Red top vials into a 6x6 bag *labl'd w ID & to MN (V-5/NCS)*	NONE	Ship to ACRL who will ship aliquots packaged for MN to MN WEEKLY USE DRY ICE	NONE
Tube 3 (4 mL) CPT, Blue/Black -RT	Mix ***5 x's*** and sit UPRIGHT at Room Temp (RT)	UPRIGHT at Room (< 2 hours)	Centrifuge for 20 minutes @ 1800 x g (room temp). Invert 5x after centrifugation. Store at room temperature until shipping.			Place tube upright in shipping tube/rk for ARIC Genetic Lab	Ship daily to ACRL for pick up by ARIC Genetics Lab; directly to Genetics on Fridays. Use Refrigerant Packs at ambient temperature	NONE	NONE
Tubes 4, 5 (10 ml) 7 (4 mL) EDTA ***UNTREATED***	Mix ***8 x's*** and place in Ice Bath (Remove 0.5mL of whole bld. From tube 4-re-stopper). Place whole blood into white vial with black screw lid (tubes should be UPRIGHT)	***Step 1:*** Centrifuge 10 min, 3000 g at 4°C ***Step 2:*** remove supernate w pasteur pipet from ea tube & place UPRIGHT in holding tube (on ice) for aliquotting (KEEP TUBES 4,5 FOR STAGE II)	***Step 1*** : Transfer from tubes 4,5 white cell layers (buffy coat) into (2) pre-labeled white vials with brown screw caps.	Step 2: Using a repeater pipetor, transfer 0.5 mL EDTA plasma from the holding tube into (19) pre-labeled white vials with lavender screw caps. (Process on wet ice)	19 lavender top vials (EDTA plasma), 1 black top vial (A1c) and 2 brown top vials (buffy coats)	(15) lavender top into a 6x6 bag *labl'd w ID & to ACRL (V-5 & NCS)* & (2) brown top vials in a 3x6 bag *labl'd w ID to ACRL to ARIC Genetic Lab* , (3) lavender top vials into (1) 3x6 bag, *labl'd w ID & to MN & (1) lavender top vial into a 3x6 bag labl'd w ID & to Mayo (V-5 & NCS);* (1) black top vial into 3x6 bag *labl'd w ID & to MN (V-5 & NCS)*	**NONE**	Ship to ACRL who will ship aliquots packaged for MN to MN weekly; ARIC Genetic Lab will pick up via courier brown top vials (buffy coats) from ACRL. USE DRY ICE	MN will ship 1 vial EDTA plasma to Mayo
Tube 6 (10 ml) EDTA ***TREATED w BHT***	Mix ***8 x's*** and place UPRIGHT in Ice Bath	***Step 1:*** Centrifuge 10 min, 3000 g at 4°C ***Step 2:*** remove supernate from tube & place UPRIGHT in holding tube (on ice) for aliquotting & adding BHT	No Activity	***Step 1:*** Using a repeater pipetor, transfer 0.5 ml plasma from the holding tube into (8) pre-labeled white vials with green screw caps. ***Step 2:*** Add 10µL of BHT (mix 3x) on ice	8 green top vials	(8) green top vials into a 6x6 bag *labl'd w ID & to ACRL (V-5)*	NONE	Ship to ACRL weekly for ACRL. USE DRY ICE	NONE
Tubes 8,9 (4.5 ml) Blue (Citrate) RT	Mix 8 ***x's*** @ Room Temperature (place UPRIGHT)	Incubate UPRIGHT at RT 30-45 minutes	***Step 1:*** Centrifuge w serum 10 min, room temp. ***Step 2:*** Remove supernate from each tube and place in a holding tube to aliquot (Rm. Temp)	Using a repeater pipetor, transfer 1.0 mL citrated plasma into (6) pre-labeled white vials, with blue screw caps	6 blue top vials	3 Blue top vials each into a 3x6 bag *labl'd w ID & to ACRL (V-5)* (3) blue top vials into a 3x6 bag *labl'd w ID & to MN(V-5 & NCS)*	**NONE**	Ship to ACRL who will ship aliquots packaged for MN to MN weekly USE DRY ICE	NONE
Tube 10, (4 mL) EDTA Hematology -RT for CBC	Mix 8x at RT (place tube UPRIGHT)	UPRIGHT at Room Temperature hold for daily shipping				Place tube in 3x6 bag or holder for ACRL (V-5)	Ship daily to ACRL for ACRL except Fri. ship to Genetics Lab for ACRL. Use Refrigerant Packs at ambient temperature	NONE	NONE

Tube 11, (2.5 mL) PAX (RT) Hold tube vertically below arm for draw & 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.	Mix 8x at RT (place tube UPRIGHT)	UPRIGHT at Room Temperature (must sit at least two hours), until shipped	Place tube upright in shipping tube holder. Put holder in biomailer for ARIC Genetic Lab. Use refrigerant pks @ ambient temp.	Ship daily to ACRL for pick up by ARIC Genetics Lab; directly to Genetics on Fridays. Use Refrigerant Packs at ambient temperature	NONE	NONE

URINE COLLECTION AND PROCESSING FOR ARIC VISIT 5/NCS

COLLECTION		PROCESSING			PREPARATION	SHIPPING
Collect 30 mL Urine	Refrigerate	***Step 1:*** Pour into graduated cylinder or 50 mL centrifuge tube ***Step 2:*** Mix by inversion 2 x	**NON_ADJUSTED pH:** From graduated cylinder or 50 ml centrifuge tube, use a repeater pipet to transfer **5.0 mL into each of 3 vials with yellow tops**	**ADJUSTED pH**: ***Step 1:*** From the graduated cylinder or 50 mL centrifuge tube pour 15 mL into a beaker & adjust pH to 7(see urine ph adjustment procedure) ***Step 2: Using a repeater pipetor transfer 5.0 mL of the adj. urine into*** ea of 3 pre labl'd vials w green tops	Place 2 yellow tops and 1 green top into a 6x6 bag labl'd w ID & for ACRL; place 2 green top & 1 yellow top into 6x6 bag labl'd w ID & for MN. **Store in freezer til shipment**	Ship weekly to ACRL who will ship aliquots packaged for MN to MN weekly. USE DRY ICE

5.1. Packaging and Mailing Instructions for Weekly Shipment of Frozen Specimens

The bags of frozen serum, plasma and urine samples are packed and shipped in Styrofoam boxes. Packaging instructions are as follows:

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

2. Place the large storage bags into the Styrofoam box on top of the dry ice.

3. Layer more dry ice on top of and around the sample bags. Use 5-10 pounds of dry ice per shipment.

4. Place packing material (do not use "packing peanuts") on top of the dry ice to fill the box.

5. Place the paper shipping forms in a plastic bag on top of the packing material. The shipping forms with instructions are shown in Appendix IV.

6. Seal the box tightly with strapping tape. Affix a dry ice Class 9 label, UN3373 Biological Substance Category B label, contents description label and completed Federal Express air bill to the outside of the box. Select FedEx Priority Overnight for delivery by 10:30 am the following day to the address below.

   ARIC Central Laboratory
   Attention: Charlie Rhodes
   Atherosclerosis Laboratory
   Baylor College of Medicine/The Methodist Hospital
   6550 Fannin Street
   Room F740
   Houston, TX 77030
   Phone: 713.798.3406

7. Contact Federal Express (1-800-GO-FEDEX) for pickup, or use your institution's mailing services.

8. If necessary, more than one box may have to be shipped per week.

Figure 8: Frozen Weekly Specimens Packaging And Shipping

Weekly Sample Shipping from Field Centers to ACRL

Step 1. Bagging

a. Sort samples into small (6x6 and 3x6) bags

= ACRL bag

= MN bag

ON DRY ICE

5.0 mL URINE
3 yellow caps and 3 green caps
(MUST DOUBLE BAG)

19 Lavender caps (0.5 mL EDTA)		1 Black cap (0.5 mL WB)	2 Brown caps (Buffy Coats)	8 Green caps (0.5 mL EDTA + BHT)	6 Blue caps (0.5 mL Citrate)		16 Red caps (0.5 mL Serum)			
x 15								x 12		
(6x6 bag)	(3x6 bag)	(3x6 bag)	(3x6 bag)	(6x6 bag)	(3x6 bag)	(3x6 bag)	(6x6 bag)	(6x6 bag)	(6x6 bag)	(6x6 bag)
									(8x10 bag)	(8x10 bag)

b. Place small sample bags into the ACRL and MN large storage bags

ACRL (11x15 bag)

MN (11x15 bag)

*** Store samples at -80°C until they are shipped**

Shipping & Receiving Form

Biospecimen Collection Forms

Step 2. Shipping

a. Add layer of **dry ice to bottom** of biomailer, followed by specimen bags, then another layer of **dry ice on top**. (use 5-10 lbs. dry ice per shipment)

DRY ICE

ACRL MN

DRY ICE

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

c. Seal box with tape, affix dry ice label, biological substance label, contents description label, and completed Federal Express air bill

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

5.2. Packaging Instructions for Daily Shipment of Ambient Temperature Specimens

1. Tubes 3 (CPT), 10 (CBC), and 11 (PAX) must be shipped daily via FedEx First Overnight delivery. Please note special FedEx airbill instructions in Section 5.3 below for Monday through Thursday shipments and Friday shipments only.

2. Inside the Styrofoam shipping container, place 4 U-tek refrigerant packs around the sides. Place 1 U-tek refrigerant pack on the bottom. The U-tek packs should be stored at room temperature before use as they are being used to help maintain room temperature inside the container. DO NOT USE refrigerant packs that were stored in the refrigerator or freezer. There are several U-tek pack sizes available and ordering information from VWR is provided in the table below.

U-tek Refrigerant Gel Packs Ordering Information

Model	Formula	Weight (in oz.)	Dimensions (in inches)	VWR P/N
429	+30°F (-1°C)	8	6 x 4 x 3/4	15715-134
412		12	6-3/4 x 4 x 1	15715-103
596		16	6-1/2 x 6-1/2 x 7/8	15715-150
414		24	7-1/2 x 6-1/2 x 1	73320-198
598		32	9-3/8 x 7-3/4 x 1	15715-154
426		48	10-1/2 x 7-3/4 x 1-3/16	15715-130

3. Place the 3 daily tubes for each ARIC participant in one 3 tube diagnostic shipper Styrofoam container (Fisher Scientific; P/N 03-528; 150/case). Do not seal with tape. See figure below.

4. Place the 3 tube diagnostic shipper Styrofoam container in the cardboard box (Fisher Scientific; P/N 03-528a; 300/case).

5. Label the cardboard box with up arrows in order to easily package them and keep the samples upright during shipment.

6. Place the ARIC bar-code label for that participant on the top of the cardboard box. Since these containers will be recycled, you can place the new ARIC ID bar-code label on top of an old one.

7. Organize the multiple 3 tube diagnostic shippers within the larger shipping container. See pictures below.

8. Place the MonitorMark Temperature Indicators (VWR; P/N 89032) inside the container. Note: Temperature indicators must be conditioned for a minimum of two hours, and maintained below the specified temperature threshold once conditioning is complete.

9. Fill the remaining space within the box with packing material, such as bubble wrap or brown paper. The less air space in the boxes, the more likely the temperature will remain stable. Include any extra labels from that day's collections in the shipment as well.

10. Close the Styrofoam container by placing on the lid. Do not seal with packing tape.

11. Place the completed Daily Biospecimen Shipping forms (**Appendix IV**) on top of the Styrofoam container.

12. Close the outer box flaps.

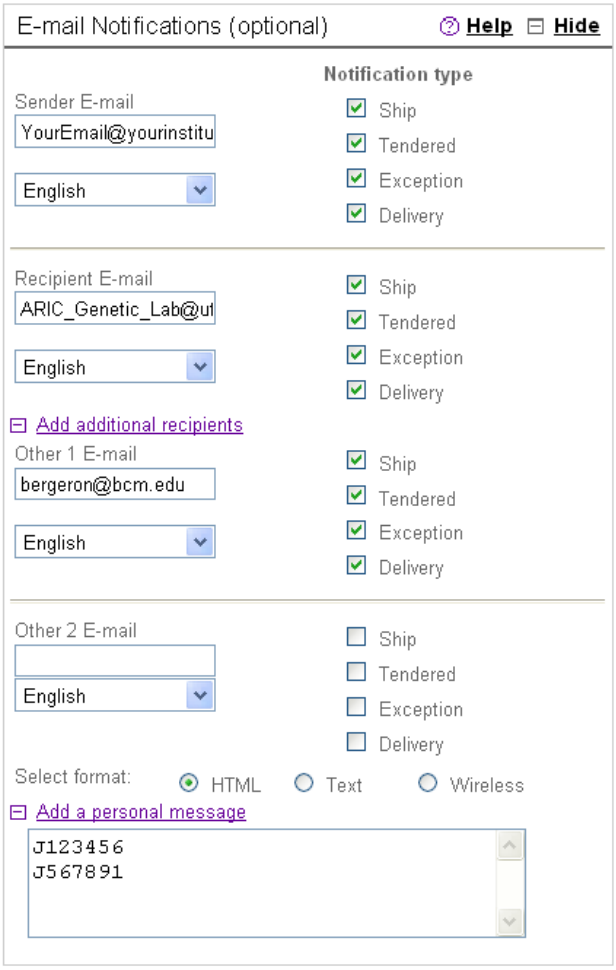
13. Seal the box tightly with strapping tape.

14. Complete a FedEx airbill with the appropriate shipping address as described in section 5.3. Please see important notes regarding the differences between Monday through Thursday shipments and Friday shipments only.

15. Affix a UN3373 Biological Substance Category B label and completed Federal Express air bill to the outside of the box.
16. Contact Federal Express (1-800-GO-FEDEX) for pickup, or use your institution's mailing services.

17. Send an email to ARIC_Genetic_Lab@uth.tmc.edu and ACRL@bcm.edu with the FedEx tracking number and ARIC sample IDs included in the daily shipment.

If you are using FedEx online for scheduling shipping, the ARIC IDs can be scanned, or typed, in the section “Email Notifications”, “Add a personal message”.



5.3. Mailing Instructions for Daily Shipments

All shipping containers are sent to the Atherosclerosis Laboratory by overnight courier (Federal Express) to ensure receipt by 8:00 AM next morning. The empty Styrofoam containers are returned to the Field Centers by USPS. The returned containers may contain information or supplies from the Atherosclerosis Laboratory; open the boxes immediately upon receipt.

For Monday through Thursday (daily) shipments, FedEx airbills should be addressed as follows to the Atherosclerosis Laboratory. The “**FedEx First Overnight**” box in section 4a should be checked so that the deliveries will arrive by 8:00 am the following day.

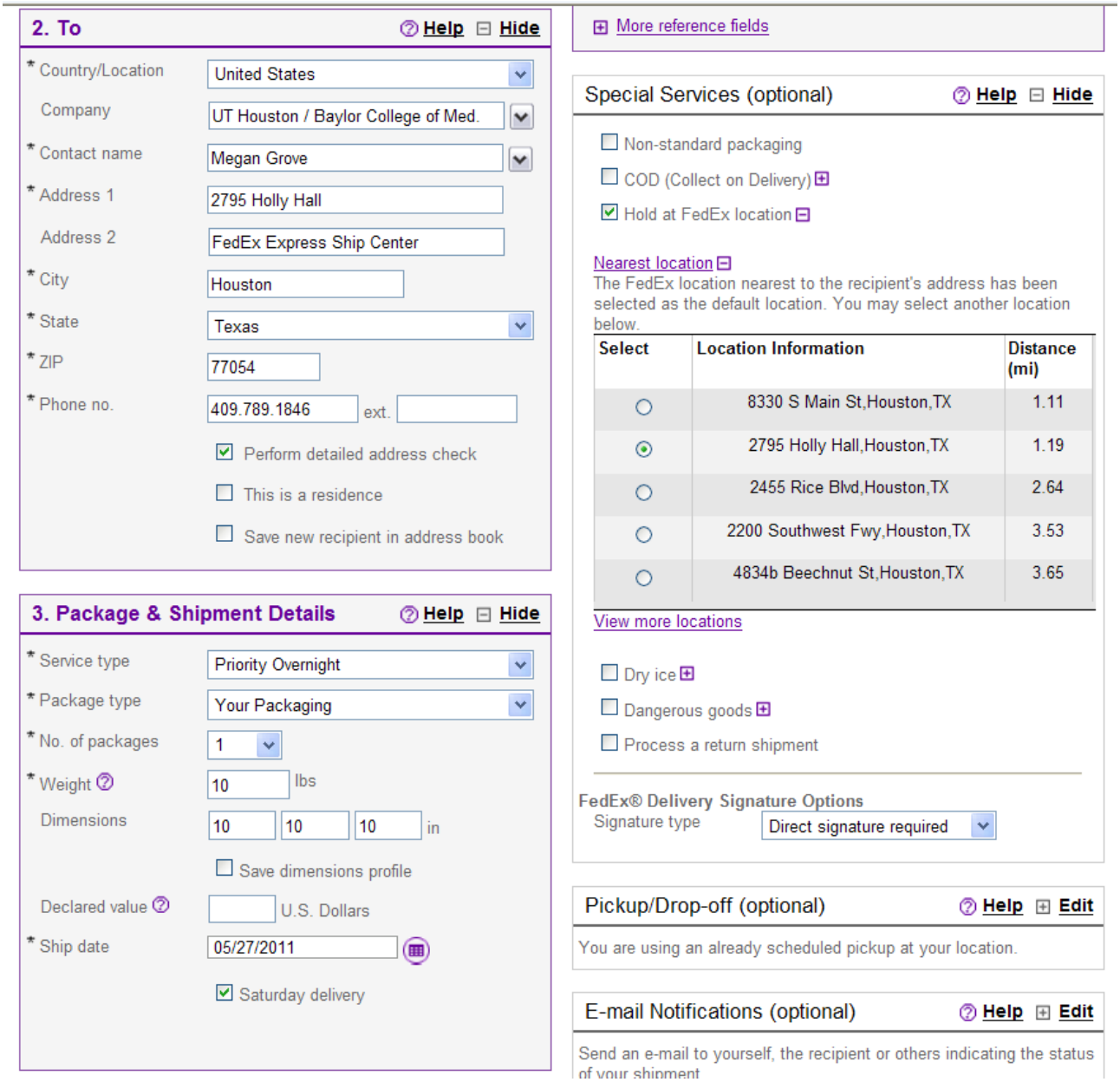
ARIC Central Laboratory
Attention: Charlie Rhodes

Atherosclerosis Laboratory
Baylor College of Medicine/The Methodist Hospital
6550 Fannin Street
Room F740
Houston, TX 77030
Phone: 713.798.3406

For Friday shipments only, FedEx airbills should be addressed as follows to the Houston FedEx holding station as The Methodist Hospital, and the Baylor College of Medicine cannot receive Saturday shipments at this time. Check the "**FedEx Priority Overnight**" box in section 4a, the "**Saturday Delivery**" box in section 6, and the "**HOLD Saturday**" box in section 3 next to the address.

Recipient's Name: Megan Grove
Phone: 409.789.1846
Company: UT Houston / The Methodist Hospital
Address Line 1: FedEx Express Ship Center
Address Line 2: 2795 Holly Hall
City/State/Zip: Houston, TX 770546.

See example screen shot below for those centers using FedEx online.



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 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 (**Appendix III**):

- all or some blood samples not drawn
- tourniquet reapplied
- fist clenching
- needle movement

- incomplete blood collection causing missing tubes
- 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.

Log daily temperatures of all refrigerators, freezers and refrigerated centrifuges (**Appendix V**). In addition, check and record the actual speed of the centrifuge annually with a tachometer. Monthly, the local blood processing certifier completes the Quality Control Checklist (**Appendix VI**), certifying that daily checks have been performed properly and describing problems in this area. The certifier will also enter results of the annual centrifuge check and equipment and supply check.

6.2. Quality Control Duplicate Blood Samples

As part of the overall quality control program for laboratory determinations from blood samples (lipids), duplicate specimens are sent to the laboratory. The QC ID numbers are not distinguishable from other ARIC ID numbers so that this forms a blinded external quality control program monitoring measurement variability.

To reduce the burden on any single participant, extra blood is drawn from several participants and sent out under the same QC ID number. Permission from the participant is acquired prior to the venipuncture. For data analysis, results on each laboratory measurement are matched to the appropriate participant results by the Coordinating Center. The laboratories are blind to which samples are QC.

6.2.1 General Instructions (Appendix IX)

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. This form is entered on paper first as it might take a few days to collect all of the replicate specimens that are assigned to a single phantom ID. After the form is complete, enter the data into the data entry system using the phantom ID, and file the original paper form.

Repeat samples are collected for most, but not all, blood specimens. Repeat blood samples consist of a single tube being drawn per participant for tube #'s 1, 6, and 10. Tubes 3, 4, and 11 are grouped together and collected from a single participant. Tube #'s 8 and 9 are grouped together and collected from a single participant. Repeat samples are also collected for urine. Every participant who undergoes a clinic examination in the first year, June 2011 –May 2012 contributes a second specimen. Starting in June 2012, one participant per day contributes a second specimen. Depending on the yield of QC specimens these dates may be adjusted to meet the goal of 5% QC

repeats for each specimen. The replicate blood samples are collected in sequential order (starting with Tube #1, followed by the group of tubes 3, 4, and 11, tube # 6, etc., up to Tube #10 and cycling back to Tube #1). The replicate urine sample is to be collected from a participant who has also contributed a second blood sample.

6.2.2 Detailed Instructions For Each Item On The Phantom Form

1. Place the phantom ID label in the header portion of the form.
2. The technician who first completes the Phantom Form fills in the date the QC phantom ID was assigned and their code number in Item 1 and 2, respectively.
3. For blood specimens, the technician drawing the blood is the technician ID recorded in the Phantom Form.
4. Eight replicate blood samples and one replicate urine sample are assigned to a single phantom ID. The replicate samples for an assigned phantom ID will come from multiple ARIC participants.

When QC blood is drawn for a tube that is processed for weekly shipment (Tubes 1, 4, 6, and 8) the aliquots are stored at the Field Centers for an extra week and then sent to the Atherosclerosis Laboratory (ACRL) with a regular shipment.

When QC blood is drawn for a tube that is processed for daily shipment (Tubes 3, 10, and 11), the aliquots are sent to the ACRL with the regular daily shipment.

It is important to note that the same subject will be used to collect QC tubes #3, #4 and #11. .

6.2.3.. Phantom QC Sample Set If A Complete Set Cannot Be Obtained

1. If a full Phantom QC sample set can not be obtained, a partial Phantom QC sample set is acceptable. The following guidelines should be observed.

2. A partially filled tube# 1 (50% or greater) is acceptable to provide as much serum as possible.

3. All other tubes must be completely filled (100%) full.

4. The urine aliquots must be completely full since it is simple to select a urine sample with adequate volume.

6.2.4.. Weekly Blood QC Sample Checklist (Appendix VIa)

The venipuncture technicians maintain a weekly checklist posted in their work area of the QC samples to be drawn during the week. As each sample is drawn and processing completed, it is checked off.

6.2.5. Preparation for Drawing and Processing QC Samples

Blood Drawing Tubes: Each morning the blood drawing technicians prepare an extra blood collection tube for the sample to be drawn that day. Each tube is labeled with the QC ID number to be used that week. 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 a separate sample aliquot tray is prepared for the QC blood vials that the technician will process that day. The tray contains all the aliquot vials needed to process the day's **quality control sample**. The tubes in each block are labeled in advance with the **QC ID number** being used that week. Care must be taken not use the donor participant ID label.

Processing and Freezing QC Blood: Process the QC blood samples along with the regular blood samples. At certain points, the QC blood samples must wait for processing until the regular blood samples have completed a particular step. For example, at Stage 2 of processing, QC samples are not taken out of the ice bath until after all of the regular tubes have been aliquotted into sample vials and put in the refrigerator. After processing is completed for each QC blood collection tube, the sample aliquot tubes are put into the -80°C freezer (for a minimum of 30 minutes). After the samples are thoroughly frozen, they are put into a freezer storage bag and put into the freezer box. Keep the QC specimens separate from the other specimens collected during the week so they are not shipped along with them.

Biospecimen Collection Form for QC Blood: No Biospecimen Collection Form is completed.

7. TRAINING PROCEDURES

7.1 Technician Training and Evaluation

The technician must study the ARIC Specimen Collection and Processing Manual and watch several participant specimens being processed. Then the technician may proceed to a mock drawing and mock processing of samples, without performing any actual venipuncture. Mock venipuncture is performed with the butterfly needle and vacutainer system. A piece of latex tubing with a knot in one end leading to a glass of water is used as a target vein. Practice tubes are collected in the correct order, then placed at their proper positions and temperatures. The sample is processed from start to finish exactly as if real blood were being used. Each technician performs a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow the technician to become comfortable with the procedures before proceeding to live participants.

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

written evaluation of their instructor, they may proceed either to drawing blood from the ARIC participants as part of a team, or to do more practice on live volunteers.

In addition to blood collection and processing, urine collection and processing is performed using volunteer samples to practice adjusting the pH of urines.

8. LABORATORY DATA TRANSFER

The Atherosclerosis Laboratory and the University of Minnesota Laboratory have the responsibility for reporting results to the field centers as well as the Coordinating Center. All test results are transmitted to the Coordinating Center in .csv file format. This transmission occurs daily for CBC, weekly for weekly test reports and monthly for monthly batch report (see Appendix 1). 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. Reference ranges and alert values can be found in Appendix 1. Note that CBC, differential, and platelet count results will be available in 4-5 days and all other test results will be available in approximately 3-4 weeks after the sample is collected.

The following table summarizes the reference ranges and ARIC alert ranges for routinely performed ARIC tests

Table 2: ARIC Tests

Analyte	Reference Ranges	Units	Alerts
Cholesterol	<200 mg/dL= Desirable 200 – 239 mg/dL = Borderline High > 240 mg/dL = High	mg/dL	
Triglycerides	< 150 mg/dL = Normal 200 – 199 = Borderline High 200 – 499 = Very High ≥ 500 = Very High	mg/dL	≥ 1000
HDL-Cholesterol	23 – 92 mg/dL	mg/dL	
LDL-Cholesterol calc'd	<100 mg/dL = Optimal 100 – 129 = Near Optimal/Above Normal 131–189 = Very High ≥ 190 Very High	mg/dL	
Fasting Glucose	Adults 70 – 105 mg/dL	mg/dL	≥ 126

Cystatin C	0.61 – 1.17 mg/L	mg/dL	
Hs C-Reactive Protein	0.070 – 1.940 mg/L	mg/dL	
*Troponin T	0.1 – 5 ng/mL	ng/mL	
NTproBNP	≥ 250 pg/mL	pg/mL	
Insulin	5.0 – 10 mIU/mL	mIU/mL	
Creatinine, serum	0.4 – 1.20	mg/dL	
Albumin, urine		mg/L	
Creatinine, urine		mg/dL	
Albumin/Creatinine ratio	0 - 20	mg/g Cr	
Uric Acid	3.4 – 7 mg/L	mg/dL	
Hemoglobin Glycated (HbA1c)	4.3 – 6.0%	%	

(See Appendix # 1 for complete analyte details for ARIC Visit 5/NCS)

APPENDICES

Appendix I: Analyte Table

Analyte	Stable For Home Visit	Lab	Fast	Tube	Amt. blood Sample needed	V3 or V5	Report Frequency	Reporting Variable	Everybody or Subsample	Analyte Pay-ment*	Home Visit?
Cholesterol	Yes	BCM	Y	EDTA	250 µL	V5	Weekly	Values	Everybody	3	**Y**
HDL	Yes	BCM	Y	EDTA	Included	V5	Weekly	Values	Everybody	3	**Y**
LDL-C calc.	Yes	BCM	Y	EDTA	Included.	V5	Weekly	Values	Everybody	3	**Y**
Triglycerides	Yes	BCM	Y	EDTA	Included	V5	Weekly	Alerts ≥1000 mg/dL	Everybody	3	**Y**
Glucose	No	BCM	Y	EDTA	Included	V5	Weekly	Alerts ≥ 126 mg/dL	Everybody	3	**N**
Cystatin C	Yes	BCM	N	EDTA	200 µL	V5	Monthly	Values	Everybody	1	**Y**
Troponin		BCM	N	EDTA	300 µL	V5	Monthly	Values	Everybody	1	**Y**
hsCRP	Yes	BCM	N	EDTA	Included	V5	Monthly	Values	Everybody	3	**Y**
NT-proBNP	Yes	BCM	N	EDTA	Included	V5	Monthly	Values	Everybody	1	**Y**
Insulin	No	BCM	Y	EDTA	Included	V5	Monthly	Values	Everybody	1	**N**
CBCs	Yes	BCM	N	EDTA	2mL	V5	Daily	Values	Everybody	1	**Y**
HbA1C	Yes	UM	N	EDTA	100 uL	V5	Weekly	Values	Everybody	3	**Y**
Creatinine	Yes	UM	N	SST	100 uL†	V5	Weekly	Values	Everybody	3	**Y**
Uric Acid		UM	N	SST	Included	V5	Weekly	Values	Everybody	1	**Y**
Urine Albumin		UM	N	Urine	400 uL†	V5	Weekly	Values	Everybody	1	**Y**
Urine Creatinine		UM	N	Urine	200 uL†	V5	Weekly	Values	Everybody	1	**Y**
Thyroid Stimulating Hormone	Yes	UM	N	SST	500 uL†	V5	Monthly	Values	NCS Subsample (low cognitive score; N~2000)	4	**Y**
B12		UM	N	SST	Included w TSH†	V5	Monthly	Values	NCS Subsample (low cognitive score; N~2000)	4	**Y**

D-dimer		UM	N	Sodium citrate	400 uL	V3	Batch	Values	NCS Subsample (Qualified for Stage 2; N~2600)	4	**Y**
Plasminogen		UM	N	Sodium citrate	50 uL	V3	Batch	Values	NCS Subsample (Qualified for Stage 2; N~2600)	4	**y**
vWF antigen		UM	N	Sodium citrate	Included w plasminogen	V3	Batch	Values	NCS Subsample (Qualified for Stage 2; N~2600)	4	**Y**
β amyloid 1-40		Mayo	N	EDTA	200 uL	Both	Batch	Values	NCS Subsample (Qualified for Stage 2; N~2600)	4	**Y**
β amyloid 1-42		Mayo	N	EDTA	200 uL	Both	Batch	Values	NCS Subsample (Qualified for Stage 2; N~2600)	4	**Y**

Appendix II: Equipment and Supplies

Supplies to be obtained by Field Centers

Supplier	Catalog no.	Description	Usage/ppt
Sarstedt	72.609	1.5 mL Microsample Tubes 500/pk	60
	65.716.005	Green Screw Caps 1000/pk	8
"	65.716.003	Red Screw Caps 1000/pk	24
"		Screw Top Vials w/ Caps (5 mL) 1000/pk	6
Allegiance	B3036-4	Butterfly Needles 21G x 3/4", BD#7250	1
"	B3035-12	Luer Adapters BD #7226 100/pk	1
"	B3062	Alcohol Swabs 2,000/cs	1
"	B3063-5	Gauze Sponges 200/pk	1
"	B3063-70	Band Aids 100/pk	1
"	B3060	Tourniquets	n/a
"	B3035-4	Vacutainer Tube Holders 10/pk	1
"	P5214-12	Transfer Pipettes 500/pk	6
		Freezer Bags 5” x 8”	1
		Freezer Bags 6” x 6”"	1
Freezer Bags 3”x6”			
Allegiance	S9221-1	Sponge Tube Rack	n/a
		Dry Ice (5-10 lbs./shipment)	
"	B3062-40	PDI Ammonia Inhalant	n/a
"	B2970-31	Serum Separator red/gray top, BD367985	1
"	B2994-91	Na Citrate, blue top, BD#6415	1
"	B2991-51	EDTA, lavender top, BD367985	3
Allegiance	B2922-1	Blood Collection Tray	n/a
"	T2050-1	Thermometers -20 C - +110 C	n/a
"	1550SD*RS	Harvard Trip Balance (Ohaus 1550SD)	n/a
Polyfoam Packers	325	Styrofoam shipping box	n/a
"	426	U-TEK +30ºF refrigerant pack	n/a

SAMPLE PROCESSING PRODUCT DESCRIPTION	VENDOR	PART #
1.5 mL Freestanding Cryovials	Phenix	SCS-015TF
Screw Caps w O-ring, lavender	Phenix	SCS-00V
Screw Caps w O-ring, red	Phenix	SCS-R
Screw Caps w O-ring, clear	Phenix	SCS000
Screw Caps w O-ring, blue	Phenix	SCS00B
Screw Caps w O-ring, white	Phenix	SCS00W
Pipetman, 0.5-10 µL	Phenix	P3940-10
Pipetman, 100-1,000 µL	Phenix	P3940-1000
Pipet Tips, 10 µL	Phenix	TX-10EC010
Pipet Tips, 1,000 µL	Phenix	TS-1000BR
Microtube Racks, assort. Colors	Phenix	R-780A

Blood Collection Set-up Tray	Local	
Zip-lock bags, 3x3	Local	
Zip-lock bags, 6x6	Local	
Kimwipes	VWR	21905-026
Ultra Clorox	VWR	37001-056
BHT Additive	Lipid Lab	
Barcoded Labels	CC	
Sterile Disposable Transfer Pipets	VWR	414004-036
7 mL vial w grn cap-urine storage	Evergreen Scientific	240-3007-G60
7 mL vial w yel cap-urine storage	Evergreen Scientific	240-3007-Y60

SHIPPING PRODUCT DESCRIPTION	**VENDOR**
Biomailers, Daily Shipments (BCM & MN)	Local
Biomailers, Weekly Shipments (BCM & MN)	Local
Dry Ice	Local
Packing Material (stuffers)	Local
Address Labels	Local
Class 9 Label (for dry ice)	order from FedEx
UN3373 Biological Substance Category B Label	Local

Equipment purchased and maintained by Field Centers:

PRODUCT DESCRIPTION	**VENDOR**	**PART#**
Refrigerator/Freezer	VWR	35923-350
Ultralow (- 80) Upright Freezer	Local	
Table Top Refrigerated Centrifuge	ThermoFisher	RT6000
Ice Machine or (access to wet ice)	Local	
Table Top Centrifuge (not refriderated)	ThermoFisher	Charlie provide P/N

Appendix III: Biospecimen Collection Form and Instructions

3/31/2014

OMB#: 0925-0281
Exp. 3/31/2014

BIOSPECIMEN COLLECTION FORM

ID NUMBER: ☐☐☐☐☐☐☐ FORM CODE: B I O DATE: 06/01/2011 Version 1.0

ADMINISTRATIVE INFORMATION

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

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

CLINIC VISIT ☐
HOME VISIT ☐

A. URINE SAMPLE

1. Urine sample collected?

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

2. Time/date of urine sample:

   a. Time of urine sample: ..... ☐☐:☐☐ (h h : m m)

   b. AM or PM?

      AM ..... ☐
      PM ..... ☐

   c. Date of urine sample collection: ..... ☐☐/☐☐/☐☐☐☐ (M M / D D / Y Y Y Y)

B. URINE PROCESSING

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

   Yes (≥ 30mL)..... A
   Yes (< 30 mL but at least 15 mL) ..... B
   No (<15 mL, discard)..... C → **Go to Item 6**

4a. Urine pH adjustment made?..... ☐

Yes, ph adjustment made.....A
No, pH adjustment not made.....B→ **Go to Item 6**

Date/time that the pH adjustment is made and technician ID for urine sample

b. Date..... ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

c. Time ..... ☐☐:☐☐
h h m m

d. AM or PM?

AM..... ☐
PM..... ☐

5. Technician ID for urine sample:..... ☐☐☐

C. BLOOD DRAWING

6. 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 7**

a. Please specify the nature of the bleeding disorder:

_____

_____

7. When was the last time you ate or drank anything other than water?

a. Time ..... ☐☐:☐☐
h h m m

b. AM or PM?

AM..... ☐
PM..... ☐

8. Time/date of blood drawing:

a. Time of blood drawing: ..... ☐☐:☐☐
h h m m

b. AM or PM?

AM ..... ☐

PM ..... ☐

c. Date of blood drawing: ..... ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

9. Number of venipuncture attempts: ..... ☐

10. Code number of phlebotomist: ..... ☐☐☐

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

11. Any blood drawing incidents or problems?

Yes ..... ☐

No ..... ☐ → **Go to Item 13**

[*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 12*.]

11

	Tube 1	2	3	4	5	6	7	8	9	10
a. Sample not drawn	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ ☐
b. Partial sample drawn	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ ☐
c. Tourniquet reapplied	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ ☐
d. Fist clenching	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ ☐
e. Needle movement	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ ☐

f. Participant reclining ☐☐☐ ☐ ☐ ☐ ☐ ☐ ☐
☐ ☐

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

_____

_____

_____

D. BLOOD PROCESSING

13. Date/time of processing specimen tubes 4, 5, 6, and 7:

a. Date specimen tubes 4, 5, 6, and 7 were spun: ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

b. Time specimen tubes 4, 5, 6, and 7 were spun: ☐☐:☐☐
h h m m

c. AM or PM?

AM ..... ☐
PM ..... ☐

14. Code number of technician processing blood (tubes 4, 5, 6, 7): ☐☐☐

15. Date/time of processing specimen tubes 1, 2, 8, and 9:

a. Date specimen tubes 1, 2, 8, and 9 were spun: ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

b. Time specimen tubes 1, 2, 8, and 9 were spun: ☐☐:☐☐
h h m m

c. AM or PM?

AM ..... ☐
PM ..... ☐

16. Code number of technician processing blood tubes 1, 2, 8 and 9: ☐☐☐

17. Date/time specimens from tubes 1, 2, 4, 5, 6, 7, 8 and 9 were placed in freezer:

a. Date specimens were placed in freezer: ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

b. Time specimens were placed in freezer: ☐☐:☐☐

h h m m

c. AM or PM?

AM ..... ☐

PM ..... ☐

18. Date/time of processing specimen tube 3:

a. Date specimen tube 3 was spun: ☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

b. Time specimen tube 3 was spun: ☐☐:☐☐
h h m m

c. AM or PM?

AM ..... ☐

PM ..... ☐

d.. Code number of technician processing blood tube 3: ☐☐☐

19. Date/time tubes 3, 10 and 11 were packaged for daily shipment out:

a. Date tubes (3, 10 and 11) were packaged for daily shipment out:

☐☐/☐☐/☐☐☐☐
M M D D Y Y Y Y

b. Time specimens were packaged for daily shipment out: ☐☐:☐☐
h h m m

c. AM or PM?

AM ..... ☐

PM ..... ☐

d. Code number of technician packaging specimens for daily shipment out: ☐☐

☐

20. Any blood processing incidents or problems?

Yes..... ☐ No ☐ FINISHED

[*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 21.*]

11

	Tube 1	2	3	4	5	6	7	8	9	10
a. Broken tube ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
b. Clotted ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
c. Hemolyzed ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
d. Lipemic ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
e. Other ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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

_____

_____

_____

_____

INSTRUCTIONS FOR THE BIOSPECIMEN COLLECTION (BIO) FORM

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.

If not all of the 11 blood sample tubes can be drawn at the initial visit and the participant is willing to return to collect the missing tubes, collect only those tubes not drawn at the initial visit. Go back into the BIO form in the DMS and update it with the information on the newly collected tubes.

If a full set of tubes was lost or destroyed and the participant is willing to return to collect the samples again, call the data coordinating center at least 5 business days in advance of the re-draw visit to get another set of labels. Enter another occurrence of the BIO form for this participant's re-draw (see instructions on completing a second occurrence of a form is located on the ARIC website under 'Training', 'DMS', 'Occurrences Instructions'.

Mark all re-draw tubes with the capital letter, "R" and highlight. Use a black permanent marker that will not bleed.

II. Detailed Instructions for Each Item

Administrative Information

0a. Enter the date the biospecimen samples were collected. The date the urine and blood samples were collected are completely separate in items #2 and 8.

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

0c. Check the appropriate box for Clinic Visit or Home Visit

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, e.g., when the participant changes clothes, 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 examiantion.

1. Indicate whether a urine sample was collected. If NO, urine sample was not collected, go to Item 6; if YES, continue.

2a-c. Record the time and date the urine was collected.

B. URINE PROCESSING

3a-c. Note if urine volume is adequate for processing. Choose either A ≥30 mL (desired). B between 30 mL and 15 mL(do not ph adjust)or C <15 mL (discard and collect at a different date, go to item #6).

4a-d. 4a. Note if a ph adjustment is made. If the answer is no, go to item # 6. 4b.Note the date that the pH adjustment is made. 4c. Note the time the pH adjustment is made. 4d. Enter whether it is morning, a.m. or afternoon, p.m.

5. Enter technician ID for urine sample

C. BLOOD DRAWING

6 – 6a. 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 #7. If the answer is YES, ask that he/she specify the nature of the bleeding disorder and record in 6a. 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.

7a-b. Enter the last time the participant ate or drank anything (other than water or coffee/tea without cream and sugar). If the participant is rescheduled for another day, a new BIO form under a new sequence number should be entered.

8a-c. Record the time and date of venipuncture. This is the time when the vein is punctured and date blood is drawn for specimens.

9. Enter the number of venipuncture attempts.

10 - 10a.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.

11a-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 12. If no incidents or problems occurred while drawing, skip to Item 13.

<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

12. Document any other blood drawing problems not listed in Item11.

D. BLOOD PROCESSING

13a-c. Record the date/time at which the centrifuge containing tubes 4, 5, 6, and7 began to spin.

14. Enter the code number of the technician who began processing blood tubes 4, 5, 6, and 7.

15 a-c. Record the date/time at which the centrifuge containing tubes 1,2, 8 and 9 began to spin.

16. Enter the code number of the technician who began processing blood tubes 1, 2, 8 and 9.

17a-c. Record the date/time at which samples from tubes 1, 2, 4, 5, 6, 7, 8 and 9 were placed in the freezer.

18a-d. Note the date/time tube 3 was spun and the code number of the technician processing tube 3.

19a-d. Note the date/time and code number of technician tubes 3, 10, and 11 were packaged for shipment out.

20a-e. Note any blood processing incidents or problems listed below.

a. Broken tube

b. Clotted

c. Hemolyzed

d. Lipemic

e. Other

Document any other blood processing problems not listed in Item 20. For example, centrifuge or freezer problems.

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

Appendix IV: Weekly and Daily Shipping Forms

Weekly Biospecimen Shipping and Receiving Form

Batch ID Number: | | | | | | | | **Form Code:** | | | | Version: Revised: 05/24/2011

Instructions: Part 1 of this form is to be completed by the field center staff to document the **Weekly** shipping of the biospecimen collec to the ACRL. Part 2 is to be completed by the ACRL staff upon receipt of the shipment. Scan the participant ID to auto-fill the ID numbe 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 1: Shipping (to be completed at the field center)

From:

Forsyth County ☐ **Minneapolis Townships** ☐

Jackson City ☐ **Washington County** ☐

To:

CORE Atherosclerosis Laborat
6565 Fannin Street, Room F-74
Houston, TX 77030

Staff Initials (shipping): | | | | Date Shipped: (MM/DD/YYYY) | | / | | / | | | | |

Number of Pages Attached: | | Time Shipped: | | | : | | | (HH:MM in 24 hr. clock)

Field Center Comments: _____

Example of Complete Sample

Tube #	# of Vials	Cap Color
#1, 2 (Serum)	16 (SR 1/16 – 16/16)	Red
#4, 5,7 (Untreated Plasma)	19 (UT 1/19– 19/19)	Lavender
#4 Red cells	1 (Hgb A1C)	Black
#4, 5 (Buffy Coat)	2 (BC 1/2-2/2)	Brown
# 6 (Treated Plasma)	8 (T 1/8-8/8)	Green
#8, 9 (Plasma)	6 (P 1/6-6/6)	Blue
Urine	6 (Ur 1/6-6/6)	Yellow, Green

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

Staff Initials (receiving): | | | |

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

Comments on condition of total shipment on arrival:

_____

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

Date vials shipped to MN by ACRL lab: (MM/DD/YYYY) | | / | | / | | | | |

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

1. First Participant ID: Affix bar-code label here

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

Plasma (Blue)							
Serum (Red/Gray)							
A1c (Black)							
Urine (Green)							
Urine (Yellow)							

2. Second Participant ID:

Affix bar-code label here

	Shipping			**Receiving**			
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received (ACRL)**	**Condition Code** (ACRL Receiving)	**# Vials Received (UMN)**	**Condition Code** (UMN Receiving)
Plasma (Lavender)							
Buffy (Brown)							
Plasma (Green)							
Plasma (Blue)							
Serum (Red/Gray)							
A1c (Black)							
Urine (Green)							
Urine (Yellow)							

3. Third Participant ID:

Affix bar-code label here

	Shipping			Receiving			
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received (ACRL)**	**Condition Code** (ACRL Receiving)	**# Vials Received (UMN)**	**Condition Code** (UMN Receiving)
Plasma (Lavender)							
Buffy (Brown)							
Plasma (Green)							
Plasma (Blue)							
Serum (Red/Gray)							
A1c (Black)							
Urine (Green)							
Urine (Yellow)							

4. Fourth Participant ID: Affix bar-code label here

	Shipping			Receiving			
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received (ACRL)**	**Condition Code** (ACRL Receiving)	**# Vials Received (UMN)**	**Condition Code** (UMN Receiving)
Plasma (Lavender)							
Buffy (Brown)							
Plasma (Green)							
Plasma (Blue)							
Serum (Red/Gray)							
A1c (Black)							
Urine (Green)							
Urine (Yellow)							

5. Fifth Participant ID: Affix bar-code label here

	Shipping	Receiving

Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials ACRL Received**	**Condition Code** (ACRL Receiving)	**# Vials Received (UMN)**	**Condition Code** (UMN Receiving)
Plasma (Lavender)							
Buffy (Brown)							
Plasma (Green)							
Plasma (Blue)							
Serum (Red/Gray)							
A1c (Black)							
Urine (Green)							
Urine (Yellow)							

DAILY BIOSPECIMEN SHIPPING FORM

BATCH ID NUMBER: ☐☐☐☐☐☐☐ FORM CODE: D S F Date:06/02/2011

Instructions: This form is completed by the field center staff to document the **DAILY** shipping of the CPT, PAX and CBC tubes 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.

(Enter data from this form into the DMS and make a copy for files BEFORE shipping.)

Part 1 – SHIPPING LOG (To be completed at field center) Check below:

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

0c. From Site: Forsyth County ☐

Jackson ☐

Minneapolis ☐

Washington County ☐

For Monday thru Thursday Shipments:	For Friday Shipments Only:
To: CORE Atherosclerosis Laboratory 6565 Fannin Street, Room F-740 Houston, TX 77030	**To: UT Houston / Baylor College of Medicine FedEx Express Ship Center 2795 Holly Hall Houston, TX 77054**

1. Total number of participant IDs enclosed: ☐☐

2. Time batch packed/sealed and date shipped:

   a. Date: ☐☐/☐☐/☐☐☐☐ (MM / DD / YYYY)

   b. Time: ☐☐:☐☐ (HH : MM in 24 hour clock)

(Complete Part 3 Before Shipping)

Part 2 - RECEIVING LOG (To be completed at CORE Atherosclerosis Laboratory)

(*Enter data from this form into the DMS and make a copy for ACRL and ARIC UT Houston GENETICS LABORATORY)*

ACRL

4a. Initial of staff person receiving ☐☐☐ 4b. Total number of specimens received: ☐

4c. Date Received: ☐☐/☐☐/☐☐☐☐ 4d. Time Received: ☐☐:☐☐

MM DD YYYY HH MM (24 hour clock)

4e. 3M MonitorMark Dual Temperature Indicator: ☐☐☐☐ (check box for each index colored blue)

A B C D

ARIC UT GENETICS Lab

5a. Initial of staff person receiving ☐☐☐ 5b. Total number of specimens received: ☐

5c. Date Received: ☐☐/☐☐/☐☐☐☐ 5d. Time Received: ☐☐:☐☐

MM DD YYYY HH MM (24 hour clock)

(*Complete Part 3 Upon Arrival)*

Part 3 – SPECIMEN CONDITION LOG (To be completed by both Field Centers and CORE Atherosclerosis Laboratory)

Specimen Condition Code:

Code	Condition	Code	Condition
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	Clotted
05	Other (specify)		

List of Samples Shipped Daily per Participant:

Tube #	Number Vials	Type of Vials
3	1	CPT
10	1	CBC
11	1	PAX

6. First Participant ID # Affix Bar-code Label Here

Specimen Type	No of Vials (0-1) (Shipped)	Condition Code (Shipped) (00-10)	Field Center Comments	No of Vials (0-1) (Received)	Condition Code (Received) (00-10)	ACRL Comments
a. CPT (Blue/Black)						
b. CBC (Lavender)						
c. PAX (Red)						

7. Second Participant ID # Affix Bar-code Label Here

Specimen Type	No of Vials (0-1) (Shipped)	Condition Code (Shipped) (00-10)	Field Center Comments	No of Vials (0-1) (Received)	Condition Code (Received) (00-10)	ACRL Comments
a. CPT (Blue/Black)						
b. CBC (Lavender)						
c. PAX (Red)						

8. Third Participant ID # Affix Bar-code Label Here

Specimen Type	No of Vials (0-1) (Shipped)	Condition Code (Shipped) (00-10)	Field Center Comments	No of Vials (0-1) (Received)	Condition Code (Received) (00-10)	ACRL Comments
a. CPT (Blue/Black)						
b. CBC (Lavender)						
c. PAX (Red)						

9. Fourth Participant ID #

Affix Bar-code Label Here

Specimen Type	No of Vials (0-1) (Shipped)	Condition Code (Shipped) (00-10)	Field Center Comments	No of Vials (0-1) (Received)	Condition Code (Received) (00-10)	ACRL Comments
a. CPT (Blue/Black)						
b. CBC (Lavender)						
c. PAX (Red)						

10. Fifth Participant ID #

Affix Bar-code Label Here

Specimen Type	No of Vials (0-1) (Shipped)	Condition Code (Shipped) (00-10)	Field Center Comments	No of Vials (0-1) (Received)	Condition Code (Received) (00-10)	ACRL Comments
a. CPT (Blue/Black)						
b. CBC (Lavender)						
c. PAX (Red)						

11. Other remarks concerning shipment contents:

_____
_____

_____
_____

_____
_____

Appendix V: Daily Temperature Log

ARIC DAILY TEMPERATURE RECORD

DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials	DATE Mo/Da/Yr	Freezer	Refrig	Room	Initials

Appendix VI: 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 VI-a: Weekly Blood QC Sample Checklist

Week of:_____

Day	Tubes	Laboratory	Sample collected?
Monday	_____	_____	_____
Tuesday	_____	_____	_____
Wednesday	_____	_____	_____
…			
Thursday	_____	_____	_____
Friday	_____	_____	_____

Daily Blood QC Sample Checklist

Week of:_____

Day	Tubes	Laboratory	Sample collected?
Monday	_____	_____	_____
Tuesday	_____	_____	_____
Wednesday	_____	_____	_____
…			
Thursday	_____	_____	_____
Friday	_____	_____	_____

Appendix VII: Venipuncture and Processing Procedures Certification Checklist

VENIPUNCTURE Satisfactory___Unsatisfactory ____ Comments_____

1. Labels checked	___	_____
2. Participant prepared and procedure explained.	___	_____
3. Biospecimen Collection Form filled.	___	_____
4. Tourniquet application and release	___	_____
5. Venipuncture technique	___	_____
6. Tube collection sequence	___	_____
7. Inversion technique	___	_____
8. Tube incubation location	___	_____
9. Stasis obtained	___	_____
10. Needle disposal	___	_____

PROCESSING

1. Knowledge of centrifuge operation	___	_____
2. Aliquotting supply set-up	___	_____
3. Stage I tube spin	___	_____
4. Stage II aliquotting	___	_____
5. Stage III tube spin	___	_____
6. Vials sealed	___	_____
7. Final processing stage	___	_____
8. Bio-Form completed	___	_____
9. Freezer organization	___	_____
10. Time constraints	___	_____
11. Disposal of contaminated supplies	___	_____

PACKAGING AND SHIPPING

1. Specimens bagged	___	_____
2. Adequate dry ice used in shipping	___	_____
3. Shipping paperwork	___	_____
4. Refrigerant packs used in shipping	___	_____
5. CBC packed without refrigerant pks	___	

MISCELLANEOUS

1. Incident Form	___	_____
2. QC Procedure	___	_____
3. Containers correctly labeled for shipping	___	_____

Appendix VIII: Sample Exams for Certification

PRACTICAL EXAM FOR ARIC BLOOD DRAWING TECHNICIAN

1. Place the following (11) blood collection tubes in the correct set-up order and location for the venipuncture: (2) 10 mL red and gray top; (1) 2 mL lavender top; (2) 4.5 mL blue anticoagulant; (3) 10 mL lavender top; (1) 4 mL lavender top; (1) blue and black top and (1) 2.5 mL red top (PAXgene).

2. Specify which tube(s) go into the ice bath after collection.

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

4. Set up a sponge tray with the correct number and order of specimen storage tubes. Indicate the number and colors of screw caps to be used on these tubes.

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

6. Organize the color-capped sample tubes and prepare them for shipment.

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

SAMPLE WRITTEN EXAM

1. Name the two most significant blood-borne diseases:_____ _____

2. List the three steps to take following an accidental specimen exposure:

   a._____

   b._____

   c._____

3. Give four examples of broken skin other than punctures:

   a._____ c._____

b._____ d._____

4. Give three examples of mucous membranes:

   a._____ c._____

   b._____

5. What three steps should be followed before exiting the laboratory?

   a._____ c._____

   b._____

6. From which tubes are the packed cells used?

   a) #1
   b) #2
   c) #4 and #5
   d) #5 and #6

7. How long should tubes #1and #2 sit at room temperature before centrifugation?

   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required

8. Why is this step (un)necessary?_____

9. Which tube is drawn last?

   a) A 10 mL red and gray-stoppered
   b) A 4.5 blue-stoppered
   c) An 8 mL red stoppered
   d) A 2.5 red-stoppered

10. For what type of tests will the 10-mL lavender-stoppered tubes be used?

    a) Chemistry
    b) Lipid
    c) Coagulation
    d) Special coagulation

11. When is the tourniquet removed?

    a) after tube #1 fills
    b) after tube #2 fills

c) after all tubes fill
d) it does not matter

12. At what temperature are the U-TEK +30ºF refrigerant packs stored for placement with the CPT and PAX tubes that are shipped daily to the Atherosclerosis Laboratory?

13. If you obtain a needle puncture, what should you do?

a) Immediately run water for 3 minutes on wound
b) Fill out an incident form
c) Report to supervisory personnel
d) Seek medical attention
e) A and b
f) All of the above

Appendix IX: Phantom Form and Instructions

PHANTOM FORM

OMB#: 0925-0281
Exp. 3/31/2014

PHANTOM ID NUMBER: | | | | | | | | FORM CODE: | P | H | T | DATE: 06/01/2011

Instructions: *This form should be completed during participants' visit. Enter the matching PARTICIPANT ID for the corresponding QC blood sample or urine specimen.*

1. Date phantom ID assigned: ☐☐/☐☐/☐☐☐☐
   M M D D Y Y Y Y

2. Staff ID assigning phantom ID: ☐☐☐

PROCEDURE	MATCHING PARTICIPANT ID	DATE COLLECTED (MM / DD / YYYY)	TECHNICIA ID
Blood Samples			
3. Tube 1: • 9 mL red-stoppered (serum)			
4. Tube 3, 4, 11: • 4 mL blue/black-stoppered (CPT) • 9 mL lavender-stoppered (untreated EDTA) • 2.5 mL red-stoppered Paxgene			
5. Tube 6: • 9 mL lavender-stoppered (treated EDTA)			
6. Tube 8, 9: • 4.5 mL blue-stoppered (Citrate)			
7. Tube 10: • 4 mL lavender-stoppered (EDTA)			
Urine Specimen			
8. 30 cc Urine			

INSTRUCTIONS FOR THE PHANTOM (PHT) FORM
Version 1

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. This form is entered on paper first as it might take a few days to collect all of the replicate specimens that are assigned to a single phantom ID. After the form is complete, enter the data into the data entry system using the phantom ID, and file the original paper form.

Repeat samples are collected for most, but not all, blood specimens. Repeat blood samples consist of a single tube being drawn per participant for tube #'s 1, 6, and 10. Tubes 3, 4, and 11 are grouped together and collected from a single participant. Tubes 8 and 9 are grouped together and collected from a single participant. Repeat samples are also collected for urine. Every participant who undergoes a clinic examination in the first year, June 2011 –May 2012 contributes a second specimen. Starting in June 2012, one participant per day contributes a second specimen. Depending on the yield of QC specimens these dates may be adjusted to meet the goal of 5% QC repeats for each specimen. The replicate blood samples are collected in sequential order (starting with Tube #1, followed by the group of tubes 3, 4, and 11, tube # 6, etc., up to Tube #10 and cycling back to Tube #1). The replicate urine sample is to be collected from a participant who has also contributed a second blood sample.

II. Detailed Instructions For Each Item

1) Place the phantom ID label in the header portion of the form.

2) The technician who first completes the Phantom Form fills in the date the QC phantom ID was assigned and their code number in Item 1 and 2, respectively.

For blood specimens, the technician drawing the blood is the technician ID recorded in the Phantom Form.

Eight replicate blood samples and one replicate urine sample are assigned to a single phantom ID. The replicate samples for an assigned phantom ID will come from multiple ARIC participants.

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Appendix X: Urine Log

URINE ph LOG					
				(Check One)	
Tech. Code	Date	Participant I.D.	Initial Urine pH	Adj/(3N) HCL	Adj/(3N) NAOH

Appendix XI: Urine Collection Instructions (Hand-out to Patients)

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