HCHS/SOL Visit 3
Manual 7
Biospecimen Collection and Processing

February 6, 2023 - Version 3.2

Study website - https://sites.cscc.unc.edu/hchs/

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CHANGES MADE TO MOP

01/03/2023	Tube # 7, sodium citrate (light blue top) tube changed from 4.5 mL to 2.7 mL due to manufacturer discontinuation of the larger tube; pg 3, 4, 12, 19, 32,
01/03/2023	SEQ 8 (sodium citrate) removed from aliquot schema; pg 4, 5, 6, 15, 24, 41
01/03/2023	Tubes # 1 and 2, serum (red top) tube changed from 9 mL to 8.5 mL due to manufacturer shortages of the larger tube; pg 3, 12, 19, 32
01/03/2023	Change in supplier for 1 N sodium carbonate; pg 33
01/03/2023	Added date to Certification Checklist, pg 45
01/03/2023	Added alternative tube collection for difficult blood draws, pg 41

1. BIOSPECIMEN COLLECTION AND PROCESSING

The Hispanic Community Health Study – Study of Latinos Visit 3 (HCHS/SOL V3) third examination is a multi-site, interdisciplinary epidemiologic study in Hispanic populations in the U.S. sponsored by the National Heart, Lung, and Blood Institute (NHLBI) and other institutes, centers, and offices of the National Institutes of Health (NIH). The goals of the renewal include further study of the prevalence and development of disease in Hispanics, the role of acculturation, and to identify risk factors that play protective or harmful roles in Hispanics. Blood and urine samples are collected to study these factors through specialized, state-of-the-art laboratory assays. More routine laboratory tests will also be performed and reported to study participants and their physicians, as described in Section 3.6 of Manual 1. Study Protocol, General Description and Study Management.

The target population of 16,415 persons of Hispanic origin from the visit 1 cohort, specifically Cuban, Puerto Rican, Mexican, and Central American background were seen at baseline and the active participants will be contacted again from 2019-2022 to be re-examined through the four affiliated Field Centers: San Diego State University, University of Illinois at Chicago, Albert Einstein College of Medicine in New York, and the University of Miami. The University of North Carolina at Chapel Hill Collaborative Studies Coordinating Center serves as the scientific and logistical support center for the study.

The Central Laboratory performs the tests on the blood and urine specimens donated by the study participants who have been asked to fast for at least 8 hours. Aliquots of whole blood, serum, plasma, and urine prepared at the field centers will be stored at the Central Laboratory. The Central Laboratory is located at the University of Minnesota Advanced Research and Diagnostic Laboratory in Minneapolis MN. A complete list of the tests performed is located in Appendix 1.

Laboratory tests are performed on specimen samples that are collected and processed by the technicians at each of the four HCHS/SOL V3 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

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 HCHS/SOL Visit 3 study collects approximately 52-54 mL of blood from each participant. Seven tubes of blood are collected. The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 3.5 tablespoons, although it may look like more to them. The technician may also assure participants that they donate over 10 times as much blood (450 mL) when they donate a pint of blood. Participants who are selected to donate additional blood tubes for Quality Control purposes will not exceed 70mL in total collected (about 4.75 tablespoons).

2.1. Staff Certification Requirements

Blood drawing and processing are performed by a certified HCHS/SOL V3 technician(s) at each field center. The technicians complete a training course taught by certified laboratory staff. Each technician must complete the training and pass both written and practical exams before becoming HCHS/SOL V3 certified. Recertification takes place annually and is authorized by the supervisory personnel.

2.2. Blood Collection Trays and Tubes

One day prior to a scheduled participant visit, the technician prepares two trays: one to hold the blood collection tubes, another to hold the plastic vials which will hold the whole blood, serum, plasma, and urine aliquots until they are frozen and ultimately transferred to the Central Laboratories for analysis. Label these sets of tubes with the appropriate code numbers for the participant. A list of equipment, suppliers, and vendors is provided in Appendix 2.

2.2.1 Blood Collection Tray

First, the technician organizes and prepares the 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 at least 10 blood collection tubes (described in the next section)
- sterile, disposable 21 gauge butterfly needles
- plastic vacutainer tube guides
- vacutainer Luer adapters
- sterile alcohol swabs
- gauze sponges
- tourniquet
- bandages ("Band Aids")

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.

2.2.2 Blood Collection Tubes

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

Tubes #1 and #2 are 8.5 mL red stoppered tubes. Although these tubes do not contain anticoagulant, they do have a clot activator and therefore require mixing following collection. The serum from these tubes will be used for testing lipids (fats) and other biochemical markers. Additional aliquots will be stored in the biorepository for future testing.

Tube #3 is a 5 mL red stoppered tube. Although this tube does not contain anticoagulant, it does have a clot activator and therefore requires mixing following collection. The serum from this tube will be stored in the biorepository for future testing.

Tube #4 is a 4 mL lavender-stoppered tube containing EDTA anticoagulant. The whole blood from this tube will be used for testing glycosylated hemoglobin and additional aliquots will be stored in the biorepository for future testing.

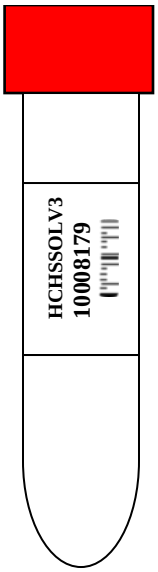
Tubes #5 and #6 are 10 mL lavender-stoppered tubes containing EDTA anticoagulant. (These tubes are labeled as 10 mL tubes, but because they are plastic, a volume as low as 8 - 9 mL of blood is collected.) The plasma from these tubes is used for several analytical tests including glucose (sugar). Additional aliquots will be stored in the biorepository for future testing.

Tube #7 is a 2.7 mL blue-stoppered tube containing liquid sodium citrate anticoagulant. The plasma from this tube will be stored in the biorepository for future testing. This tube must be filled completely in order to standardize the blood to liquid anticoagulant ratio. A partially filled tube will result in erroneous test results.

2.2.3 Blood Collection Tubes: Labeling and Set-Up

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

1. Apply pre-numbered barcode laboratory ID labels to each blood collection tube. Place the labels on the tubes vertically, with the bar-code oriented from the bottom of the tube to the top of the tube. Handle only one participant's specimens at a time so the chance of mislabeling is minimized.



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

Tube #1:	8.5 mL red stoppered tube (Serum)
Tube #2:	8.5 mL red stoppered tube (Serum)
Tube #3:	5 mL red stoppered tube (Serum)
Tube #4:	4 mL lavender stoppered tube (EDTA)
Tube #5:	10 mL lavender stoppered tube (EDTA)
Tube #6:	10 mL lavender stoppered tube (EDTA)
Tube #7:	2.7 mL blue stoppered tube (Citrate)

3. Additional laboratory ID number labels will be used when the participant arrives to provide a documented match between their HCHS/SOL V3 participant ID number and the laboratory specimen ID number on the Biospecimen Collection Form (BIO).

A number of HCHS/SOL V3 participants will be asked to donate one to two additional tube(s) of blood for quality control purposes. The duplicate sample will be assigned a different laboratory ID number, called a Phantom ID, and shipped to the Central Laboratory one week later. This quality control procedure is described more completely below, in Sections 6.1 – 6.4.

2.2.4 Sample Aliquot Trays

The technician prepares a tray of the plastic freezer microvials, which will contain the aliquots to be shipped to the Central Lab for each participant. Each type of whole blood/serum/plasma storage tube has a corresponding color-coded screw cap that fits onto it. The technicians are trained to organize the tray for the sample processing and aliquoting as follows:

The tray itself should be a flexible sponge test tube rack, which will fit tubes from 10-16 mm in diameter. The tray has 5 rows and 10 columns. The columns are numbered 1-10 from left to right. The rows are lettered A-E from top to bottom. See Appendix 7 for cleaning instructions for these trays.

2.2.5 Organization

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

(2) – 2	mL polypropylene tubes (clear top)
(1) – 2	mL amber polypropylene tube (lavender top)
(13) – 2	mL polypropylene tubes (lavender top)
(2) – 2	mL polypropylene tubes (blue top)
(1) – 2	mL amber polypropylene tube (red top)
(15) – 2	mL polypropylene tubes (red top)
(3) – 2	mL polypropylene tubes (yellow top)
(1) – 2	mL polypropylene tube (green top)
(1) – 5	mL polypropylene tube (dark blue top)
(1) – 4.5	mL polypropylene tube (clear top)

2.2.6 Labeling

Vertically label the plastic sample aliquot vials with the laboratory ID number and arrange in the sample aliquot trays in the following order (see Figure 1. Aliquot Tray Layout):

Tray 1(for stages 1 – 3 processing):

Col 1: 4.5 mL clear top, clear vial; row A
Col 1: 2 mL clear tops, clear vials; rows B, C
Col 1: 5 mL dark blue top, clear vial; row E
Col 2: 2 mL lavender top, clear vial; row A
Col 2: 2 mL lavender top, *amber* vial; row B
Col 2: 2 mL lavender tops, clear vials; rows C –E
Col 3: 2 mL lavender tops, clear vials; rows A –E
Col 4: 2 mL lavender tops, clear vials; rows A-D
Col 5: 2 mL blue tops, clear vials; rows A –B
Col 6: EMPTY
Col 7: 2 mL red top, clear vial; row A
Col 7: 2 mL red top, *amber* vial; row B
Col 7: 2 mL red tops, clear vials; rows C –E
Col 8: 2 mL red tops, clear vials; rows A –E
Col 9: 2 mL red tops, clear vials; rows A –E
Col 10: 2 mL red top, clear vial; row A

Tray 2 (urine processing):

Col 1: 2 mL yellow tops, clear vials; rows A –C
Col 2: 2 mL green top, clear vial; row A
Col 3: 2 mL orange top, clear vial; row A
Col 4-10: EMPTY

There is no need to return any extra labels to the Central Laboratory. They can be discarded.

Figure 1. Aliquot Tray Layout

Aliquot Tray 1 Layout (Stages 1 - 3 Processing)

Col Row	1	2	3	4	5	6	7	8	9	10
A	1.5 mL EDTA whole blood, Tube #4 **SEQ. 1**	0.25 mL plasma, Tube #5 **SEQ. 3**	minimum 0.5 mL plasma, Tube #5 **SEQ. 13**	minimum 0.5 mL plasma, Tube #6 **SEQ. 18**	minimum 0.5 mL plasma, Tube #7 **SEQ. 6**	Empty	0.25 mL serum, Tube #1 **SEQ. 2**	minimum 0.5 mL serum, Tube #1 **SEQ. 27**	minimum 0.5 mL serum, Tube #2 **SEQ. 32**	minimum 0.5 mL serum, Tube #3 **SEQ. 37**
B	1.0 mL EDTA whole blood, Tube #4 **SEQ. 4**	plasma, **amber vial** Tube #5 **SEQ. 9**	minimum 0.5 mL plasma, Tube #5 **SEQ .14**	minimum 0.5 mL plasma, Tube #6 **SEQ. 19**	minimum 0.5 mL plasma, Tube #7 **SEQ. 7**	Empty	serum, **amber vial** Tube #1 **SEQ. 23**	minimum 0.5 mL serum, Tube #2 **SEQ. 28**	minimum 0.5 mL serum, Tube #2 **SEQ. 33**	Empty
C	1.0 mL EDTA whole blood, Tube #4 **SEQ. 5**	minimum 0.5 mL plasma, Tube #5 **SEQ. 10**	minimum 0.5 mL plasma, Tube #6 **SEQ. 15**	minimum 0.5 mL plasma, Tube #6 **SEQ. 20**	Empty	Empty	minimum 0.5 mL serum, Tube #1 **SEQ. 24**	minimum 0.5 mL serum, Tube #2 **SEQ. 29**	minimum 0.5 mL serum, Tube #3 **SEQ. 34**	Empty
D	Empty	minimum 0.5 mL plasma, Tube #5 **SEQ. 11**	minimum 0.5 mL plasma, Tube #6 **SEQ. 16**	minimum 0.5 mL plasma, Tube #6 **SEQ. 21**	Empty	Empty	minimum 0.5 mL serum, Tube #1 **SEQ. 25**	minimum 0.5 mL serum, Tube #2 **SEQ. 30**	minimum 0.5 mL serum, Tube #3 **SEQ. 35**	Empty
E	5.0 mL packed cells Tube #5 **SEQ. 22**	minimum 0.5 mL plasma, Tube #5 **SEQ. 12**	minimum 0.5 mL plasma, Tube #6 **SEQ. 17**	Empty	Empty	Empty	minimum 0.5 mL serum, Tube #1 **SEQ. 26**	minimum 0.5 mL serum, Tube #2 **SEQ. 31**	minimum 0.5 mL serum, Tube #3 **SEQ. 36**	Empty

Aliquot Tray 2 Layout (Urine Processing)

Col Row	1	2	3	4	5	6	7	8	9	10
A	1.5 mL urine, neutral **SEQ. 38**	1.5 mL urine, acid **SEQ. 41**	1.5 mL urine, alkaline **SEQ. 42**	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	1.5 mL urine, neutral **SEQ. 39**	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	1.5 mL urine, neutral **SEQ. 40**	Empty	Empty	Empty	Empty	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

2.2.7 Preparation for Specimen Collection

In the morning, prior to drawing blood from the participants:

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.
2. Check that each Vacutainer tube is labeled properly with the correct laboratory barcode ID label.
3. Check that the sample aliquot trays are properly equipped. Every item on the checklist must be ready and in its proper position.
4. Check that each aliquot storage vial is labeled with the correct laboratory barcode ID label.
5. Perform and record quality control (QC) check on centrifuge temperature (15°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 (-70°C ± 5°C) and (-20°C ± 2°C).
8. Perform and record QC check on room temperature.

2.2.8 At Participant Arrival

1. Check that the participant's HCHS/SOL V3 Participant ID number on the Biospecimen Collection Form (BIO) is correct. Place the laboratory ID label that matches the label on the collection tubes and aliquot containers onto the Biospecimen Collection Form (see Appendix 3).
2. Confirm the match between the participant name, the HCHS/SOL V3 participant ID number, and the laboratory ID number on the blood collection tubes, aliquot vials, and the Biospecimen Collection Form.
3. Check that duplicate Quality Control tubes are prepared and labeled, if needed.

2.3. Biospecimen Collection form

Complete the initial SOL VIDA ancillary participation question, (Item 26), safety questions (Items 1-3)) and the fasting questions (Items 4-5) of the Biospecimen Collection Form (see Appendix 3). The remaining sections can be completed after the venipuncture. Any deviations from the routine collection or processing protocol are recorded in the section on venipuncture/processing incidents of the Biospecimen Collection Form.

3. VENIPUNCTURE PROCEDURE

Handle all specimens as potentially infectious for laboratory workers. Blood borne pathogens such as hepatitis B and human immunodeficiency virus (HIV) 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. 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 stick with a contaminated needle, clean the wound thoroughly with disinfectant soap and water, notify a supervisor, and consult the HCHS/SOL V3 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.

Use OSHA-approved cleaning solution to clean up any spills of blood, plasma, serum, or urine. Use this solution to clean all laboratory work surfaces at the completion of work activities. 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, plasma, serum, 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 or under-shelf-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.

3.1. Phlebotomy Room

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 freezers should be nearby.

3.2. Participant Preparation

Informed consent must be obtained before drawing any blood, to ensure that the participants understand the purpose and possible complications of the venipuncture procedure. A standard informed consent has been prepared for this study. The consent statement informs study participants that although there may be some minor discomfort, their blood (about 4 tablespoons) will be drawn by trained technicians. The consent also states that a copy of clinically important test results will be sent to them (and their physician if they authorized it) and that they will be contacted if clinically important tests are abnormal.

Complete the Biospecimen Collection Form (BIO) sections A and B with the participant (Appendix 3) if it hasn't been done so already. Before blood is collected, the participant is asked the following safety questions (Items 1-3):

2. …if they have had a radical mastectomy or other surgery where lymph nodes were removed from their armpits. If they have, blood should not be collected from the arm where this has occurred.
3. …whether he/she has a bleeding disorder. If such a disorder is present, ask the participant 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. When the participant reports a bleeding disorder, specify the type of bleeding disorder(s) as briefly

as possible in Item 12 of the Biospecimen Collection Form. In general, a bleeding disorder is not a reason for participant deferral. A gauze and tape bandage is applied. The participant is instructed to maintain pressure on the venipuncture site for 2 minutes and to keep the bandage on the site for the remainder of the examination visit.

4. …whether he/she has ever had a grant or shunt for kidney dialysis. If they have, follow the precautions in QxQ and note the situation in Item 12 of the Biospecimen Collection Form.

Complete the fasting blood collection information with the participant in Biospecimen Collection Form section B (Items 4-5).

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 if possible try to have the participant sitting for a minimum of five minutes. This allows the participant to relax before the venipuncture takes place.

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 4 tablespoons of blood. This blood will be used in tests for lipids (fats), glucose (sugar), and other biochemistry tests. We hope to be able to use the results of these tests to better understand the health issues of the Hispanic community." 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 extravascular water shift, resulting in a dilutional effect on lipid values. If this option is taken, note it on the Biospecimen Collection Form by placing an “X” in the appropriate boxes. (Appendix 3, Item 13).

3.3 Venipuncture

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the 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. To do 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.

A. Apply tourniquet.
   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.

B. 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.

C. 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.

D. 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.

E. 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 (9 mL red 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 #3 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. If the color of the arm turns red or blue, the tourniquet

is applied too tightly. Loosen it and continue. If the tourniquet is loosened or reapplied, note this on the Biospecimen Collection Form.

6. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.
7. Fill each Vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a Vacutainer tube fills only partially, remove the 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 each tube eight times immediately following removal of the tube from the adapter while the next tube is filling. (See section 3.4 for mixing instructions.)
9. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent bruising.
10. If the blood flow stops before collecting all of the tubes, repeat the venipuncture on the participant beginning with the first unfilled tube. Because of the ratio of anticoagulant to blood, tube #7 must be completely filled in order to perform the analyses. As always, the tourniquet must never be on for longer than two minutes.
11. If phlebotomy is interrupted on tube #7 (Citrate tube) collect 2mL of blood into a red SST tube before collecting tube #7. (The SST tube blood is discarded.)

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

1. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm. 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.
3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

G. 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.

H. PRECAUTIONS - When a Participant Feels/Looks Faint Following the Blood Drawing:
1. Have the person remain in the chair. If necessary, have him/her lie on the floor with 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 he/she 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 pre-clinic visit fast.
8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.4. Blood Tube Mixing and Storage During Venipuncture

All tubes must be mixed with the anticoagulant to prevent clotting. Even tubes #1, #2, and #3 that do not contain an anticoagulant, have a clot activator that needs to be mixed with the blood. Begin by holding the tube horizontal to the floor. Gently tip the stopper end down while watching the air bubble rise to the butt (1st inversion). Now, lower the butt end slightly while watching the bubble float to the stopper (2nd inversion). Lower the stopper end again when the bubble reaches the stopper. This is the third inversion. Invert each tube eight times. Eight inversions should take 6 to 8 seconds.

Tube #1 and #2: 8.5 mL red stoppered tube containing no anticoagulant. Invert tube gently 8 times immediately after collection. Place tubes in room temperature rack and allow the blood to clot for 30 minutes after collection. Protect tubes from light by placing a box over the rack until centrifugation.

Tube #3: 5 mL red stoppered tube containing no anticoagulant. Invert tube gently 8 times immediately after collection. Place tube in a room temperature rack and allow the blood to clot for 30 minutes after collection. Protect tubes from light by placing a box over the rack until centrifugation.

Tube #4: 4 mL lavender-stoppered tube contains EDTA anticoagulant. Invert gently 8 times immediately after collection. Place this tube in a cup with ice slush and protect it from light by placing a box over the cup of ice slush until ready to begin processing.

Tube #5 and #6: 10 mL lavender-stoppered tube contains EDTA anticoagulant. Invert gently 8 times immediately after collection. Place the tubes #5 and #6 in a cup with ice slush and protect them from light by placing a box over the cup of ice slush until centrifugation.

Tube #7: 2.7 mL blue-stoppered tube contains sodium citrate anticoagulant. Invert gently 8 times immediately after collection. Place the tube in a room temperature rack until centrifugation at 15º C. (These tubes can be placed under the box, but do not require protection from light.)

4. BLOOD AND URINE PROCESSING

4.1 Stage One: Immediate Processing

Eye protection, gloves, and lab coat must be used for all blood processing. After venipuncture:

1. Tubes #1, #2, and #3 remain at room temperature for 30-45 minutes to allow the blood to clot (blood at 4°C clots extremely slowly). Keep these covered with a box to protect them from light. Set a timer for 30 minutes as a reminder to centrifuge these tubes.

2. Within 15 minutes of collection, place tubes, #5, #6, and #7 in the centrifuge trunions. Place tubes in the centrifuge buckets in a balanced manner (see description of balancing the centrifuge in 4.1.1 "Operating the Centrifuge"). Spin these tubes at 3,000 x *g* for 30 minutes at 15° C. Record on the Biospecimen Collection Form (Item 14) the time at which these tubes began to spin.

3. Remove tube #4 from the ice cup, remix 8 times, and place it in a wire rack in front of the sample aliquot tray 1. Remove the stopper. Using a plastic transfer pipet, remove the blood from the tube. Aspirate slowly. Place approximately 1.5 mL of EDTA whole blood into the clear 4.5 mL vial in positon A1 of the sample aliquot tray 1. (Use the "1.5 mL template" vial as a guide for an approximate 1.5 mL volume.) Distribute the remaining EDTA whole blood equally into the 2 clear 2.0 mL vials in positions B1 and C1. These two vials should have a minimum volume of 1.0 mL. (Use the "1.0 mL template" vial as a guide for an approximate 1.0 mL volume.) Extra blood may be discarded. Fasten the clear screw caps onto the vials and place them in the cup with ice slush. Re-stopper tube #4 and discard it in a biohazard waste container.

4.1.1 Operating the Centrifuge

Refer to Centrifuge Operating Manual for specific operating and balancing instructions. In order to achieve a 3000 x *g* centrifugal force (rcf) within the centrifuge, the corresponding revolutions per minute (RPM) may vary from centrifuge to centrifuge depending on radius of the centrifuge's rotor. Consult the centrifuge's operating manual for the appropriate RPM for each centrifuge. If the field center's centrifuge is not capable of creating a 3000 rcf, increase the centrifugation time until the rcf-minutes total 90,000. If, for example, the maximum force is 2000 rcf, then increase the time from 30 to 45 minutes. To balance the centrifuge, place tubes of the same size and with equal volume of blood as determined visually in opposite positions in the bucket adaptors. **For tubes of blood that do not have another tube of equivalent blood volume, use a "balance tube" of the same size containing an equivalent volume of water.** Wait for centrifuge to come to a complete stop before opening the lid. Proceed to stage 2 processing. ***Note-If a tube is only partially centrifuged; Do NOT re-centrifuge collection tube, instead transfer the serum or plasma to a secondary plastic vial and re-centrifuge for the same time/speed that was initially indicated.**

4.2. Stage Two: Processing of Plasma

Stage two begins approximately 30 minutes after venipuncture. Eye protection, gloves and lab coat must be used for all blood processing.

When removing the plasma after centrifugation do not disturb the white blood cells layer, also called the buffy coat, which forms a thin layer between the upper plasma layer and the lower

layer of packed red blood cells. This is especially true in tube #7 because the platelets which are found near the top of the buffy coat contain some of the analytes which are to be measured and could cause erroneous result if aspirated with the plasma. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube using the initial processing conditions. Indicate on Item 19 of the Biospecimen Collection Form that the tube was re-centrifuged.

Aspiration of the lipid layer that may float to the surface after centrifugation could also adversely affect the test results. Thus, it is critical that only the clear plasma or serum between the buffy coat and the upper lipid layer be aspirated when preparing these sample aliquots. If lipids floated to the top of the plasma, indicate on Item 25 of the Biospecimen Collection Form "lipids present on top of plasma/serum were not pipetted".

Unless otherwise specified, place at least 0.5 mL and up to, but not more than approximately 1.5 mL of plasma into the 2 mL vials.

1. Remove tubes#5, #6, and #7 from the centrifuge and place them in a wire rack in front of the sample aliquot tray 1. Remove the stoppers. Be careful not to disturb the cell layers.

2. Tube # 5: Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear plasma supernatant from tube #5. Aspirate slowly starting at the top of the plasma (or just below the lipid layer if one is present on the top). The pipette tip does not get any closer than ¼ inch from the cell layer. Leave ¼ to ½ inch layer of plasma above the buffy coat/red blood cells. Place approximately 0.25 mL of plasma into the first clear 2 mL vial in position A2 of the sample aliquot tray 1. (Use the "0.25 mL template" vial as a guide for an approximate 0.25 mL volume.) Distribute the remaining plasma equally into one amber vial in position B2 and five clear 2 mL vials in positions C2, D2, E2, A3 and B3 of the sample aliquot tray. (Use the "0.50 mL template" vial as a guide.) To prepare the packed cell aliquot, use the same plastic transfer pipet to slowly aspirate the remaining plasma, buffy coat layer, and some of the red cells from tube #5. (Do not let the buffy coat aspirate into the bulb of the disposable pipet.) Transfer this to the 5 mL vial in position E1 of the sample aliquot tray 1. Now transfer the remaining red blood cells from tube #5 to this same vial. (This ensures that the buffy coat is adequately rinsed from the transfer pipet.) See Appendix 6.

3. Process tube #6 similarly to tube #5, distributing the plasma equally into seven 2 mL clear vials in positions C3, D3, E3, A4, B4, C4 and D4. There is no need to prepare a packed cell aliquot from tube #6 and the rest of the blood may be discarded. However, if the quantity of blood in tube #5 was insufficient to prepare a packed cell aliquot, Tube #6 may be used instead.

4. Fasten the lavender screw caps onto the vials in columns 2, 3, and 4 and place them in the cup with ice slush. Fasten the dark blue screw cap into the vial in column 1 and keep this vial in the aliquot tray.

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

6. Tube #7: Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear plasma supernatant from tube #7. Aspirate slowly

starting at the top of the plasma (or just below the lipid layer if one is present on the top). The pipet tip does not get any closer than ¼ inch from the cell layer. Leave ¼ to ½ inch layer of plasma above the buffy coat/red blood cells. Distribute the plasma equally into each of the two 2 mL vials in positions A5 and B5 of the sample aliquot tray 1. (Use the "0.5 mL template" vial as a guide.) Place the blue screw cap on each vial. Replace the blue stopper on the collection tube and discard it in a biohazard waste container.

4.3. Stage Three: Processing of Serum

Stage three begins approximately 30 minutes after venipuncture. Eye protection, gloves and lab coat must be used for all blood processing.

1. As close to 30 minutes after venipuncture as possible, and no longer than 45 minutes after venipuncture, spin the red stoppered tubes #1, #2 , and #3 at 3,000 x *g* for 10 minutes. Record the time when centrifugation begins on the Biospecimen Collection Form (Item #15). (Stage 2 processing can be done while these tubes are centrifuging.)

2. When the centrifuge has come to a complete stop, remove tubes and place them in a wire rack in front of the sample aliquot tray 1. Remove the stoppers.

3. Using a plastic transfer pipet, withdraw serum from tube #1. Place approximately 0.25 mL of serum into the 2 mL vial in position A7 of the sample aliquot tray 1. (Use the "0.25 mL template" vial as a guide.) Distribute the remaining serum equally into one amber vial in position B7 and four 2 mL clear vials in positions C7, D7, E7, and A8 of the sample aliquot tray. Remember to withdraw only the clear serum; if lipids are present on top begin aspirating from below that layer. Fasten the red screw caps onto these vials. For tube #2, distribute the serum equally into the six 2 mL vials in positions B8, C8, D8, E8, A9, and B9 of the sample aliquot tray. (Use the "0.5 mL template" vial as a guide) Fasten the red screw caps onto these vials. For tube #3, distribute the serum equally into the four 2 mL vials in positions C9, D9, E9 and A10 of the sample aliquot tray. (Use the "0.5 mL template" vial as a guide.) Fasten the red screw caps onto these vials.

4. Re-stopper tubes #1, #2, and #3 and discard them in a biohazard waste container.

4.4. Stage Four: Final blood processing

Remove the clear-capped vials from the cup with ice slush, dry them with a paper towel, and place them into positons in column 1 of the sample aliquot tray. Remove the lavender-capped vials from the cup with ice slush, dry them with a paper towel, and place them into positions in columns 2, 3, and 4 of the sample aliquot tray. Immediately place the aliquot tray in the -70° C freezer. The aliquots should freeze in an upright position so that the material does not freeze in the cap. Record the time these aliquots are placed in the freezer on the Biospecimen Collection Form (Item 16).

4.5. Urine Collection and Processing

4.5.1 Urine Collection

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

A 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 to:

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

Bathrooms are equipped with a wall clock and pencils for participants to use in recording the time of voiding on the label. The staff member verifies the participant has written the "time voided" on the label, and assesses the adequacy of the sample for processing. At least 8 mL of urine is required for processing. If insufficient, the participant is requested to void again in a clean container prior to leaving the field center. A note is made on the participant's Itinerary Sheet that a second sample is needed by the staff person who observes the placement of the participant's urine specimen in the refrigerator. A note can also be made on the participant's first sample that a second sample is needed. The optimal time for the collection of the second specimen is after the snack when the participant is changing back in to street clothes. The instructions for providing the urine sample are repeated to the participant at that time.

Labeled urine samples should be placed in the designated specimen refrigerator for storage prior to processing and as soon as possible after the specimen has been voided. This can be done either by the participant or a staff member, as determined by local option. However, procedures need to be set up at each field center to verify that urine samples are not inadvertently left out at room temperature. Urine may be left at room temperature for a maximum of 4 hours.

Refrigerated urine samples need to be processed and frozen as soon as possible, and within 12 hours of collection. Item 18 is answered Yes and a comment is placed in Item 25 of the Biospecimen Collection Form if a urine "sample has remained at room temperature for more than 4 hours", or "is not processed and placed in the freezer within 12 hours of collection".

4.5.1 Urine Processing

The technician prepares the work area by laying out a plastic transfer pipet and five 2 mL vials in the second aliquot tray. A Lab ID label is affixed to each specimen vial. ID labels are placed vertically on the vials, as on the blood vials.

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

1. Mix the urine container by inverting eight times.

2. Record whether the urine was collected, date of collection, time of collection (transcribed from the label on the urine cup), the time of processing, and the processing technician's code on the Biospecimen Collection Form (Items 20, 21, 22, 23 and 24).

3. Prepare three different types of urine aliquots (5 total vials) as follows:

   a. Neutral urine aliquots (3): Using the plastic transfer pipet, place 1.5 mL of urine in the vials in position A1, A2, and A3 of the second sample aliquot tray. (Use the "1.5 mL template" vial as a guide.) Fasten the yellow screw caps to these vials.

   b. Acidified urine aliquot (1): Using the plastic transfer pipet, place 1.5 mL of urine in the vial in position A2 of the second sample aliquot tray. Using the MLA D-Tipper Pipetter, add 60 uL of 3 N hydrochloric acid into the vial. Fasten the green screw cap to the vial and gently mix by inverting 8 times. Place it back in the aliquot tray 2.

      CAUTION: HCl is a corrosive poison. WEAR GOGGLES WHEN PIPETTING. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. In case of contact: Immediately flush eyes and skin with water for at least 15 minutes. Remove the contaminated clothing. If inhaled: Remove to fresh air. Assist breathing if needed. If swallowed: Wash out mouth with water. IN ALL CASES GET MEDICAL ATTENTION IMMEDIATELY. Follow your institution's policy for storage and disposal of this chemical.

   c. Alkaline urine aliquot (1): Using the plastic transfer pipet, place 1.5 mL of urine in the vial in position A3 of the second sample aliquot tray. Using the MLA D-Tipper Pipetter, add 30 uL of 1 N sodium carbonate into the vial. Fasten the orange screw cap to the vial and gently mix by inverting 8 times. Place it back in the tray. Some precipitate may form, which is normal.

      CAUTION: Sodium carbonate is a corrosive poison. WEAR GOGGLES WHEN PIPETTING. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. In case of contact: Immediately flush eyes and skin with water for at least 15 minutes. Remove the contaminated clothing. If inhaled: Remove to fresh air. Assist breathing if needed. If swallowed: Wash out mouth with water. IN ALL CASES GET MEDICAL ATTENTION IMMEDIATELY. Follow your institution's policy for storage and disposal of this chemical.

4. Immediately after processing, transfer the five urine aliquot vials in aliquot tray 2 to the -70° C freezer with the other aliquots from this participant.

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

4.5.2 Procedures for Small Urine Samples

If the volume of urine sample is inadequate to process the five sample aliquots, check to see if a second sample was provided. If there is a second sample and it (in and of itself) is adequate for processing, use the second sample (record the time voided on the Biospecimen Collection form based on that sample) and discard the first sample. If neither is adequate, combine the specimens, and transcribe the latest voiding time on the Biospecimen Collection form. If there appears to be adequate urine for the neutral aliquots, split the sample into these three vials and answer Item 18 as Yes and comment in Item 25 of the Biospecimen Collection Form that the volume was insufficient for acid and alkaline aliquots.

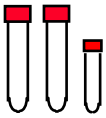
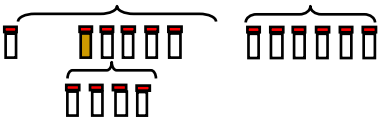
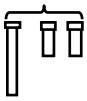
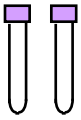
4.5.3 Procedures for Urine Samples Contaminated with Blood

Although urine samples contaminated with blood will affect the measurement of albumin, these specimens should not be thrown out. All urine samples collected from participants that have adequate volume for processing are kept, including those that are (appear to be) contaminated with blood. If a urine sample is contaminated with blood, ask the participant to provide a second urine sample at the end of the examination. Use the second sample if it has adequate volume and is less contaminated. Document urine blood contamination by entering the comment, "sample contaminated with blood" in Item #25 on the Biospecimen Collection Form.

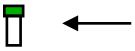
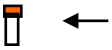
4.6. Overview of Specimen Collection

A summary overview of the protocol steps for the collection and processing of blood and urine **specimens is presented in Figure 2. (Specimen Processing Flow Diagram)**

Figure 2. HCHS – SOL Visit 3 Blood & Urine Processing Work Flow

BLOOD COLLECTION	STAGE 1 immediate processing	STAGE 2 30 - 45 min post-collection	STAGE 3 30 - 45 min post-collection	STORAGE
Tubes #**1, #2, & #3**  8.5-mL red top tubes (serum) 5-mL red top tube (serum) Invert 8 times	Room Temperature rack for 30 - 45 min Cover with a box		Centrifuge at 15º C for 10 min at 3000 *x g*; From #1, place 0.25 mL in one vial and distribute remaining serum equally into one amber and four clear 2-mL vials with red caps. From #2, distribute the serum equally into six 2-mL vials with red caps. From #3, distribute the serum equally into four 2-mL vials with red caps. Keep covered with box at room temperature until put in freezer. **From Tube #1** **From Tube #2** **From Tube #3**  0.25 mL Equally distribute (0.5 mL minimum)	Store vials at -70°C
Tubes **#4**  4-mL lavender top tubes (plasma) Invert 8 times	Remix right before aliquoting. Place 1.5 mL whole blood in the 4.5 mL clear vial and 1 mL whole blood in two clear 2 mL vials with clear caps; place in ice water; cover with box until put in freezer. **From Tube #4** 			Store vials at -70°C
Tubes **#5 – 6**  10-mL lavender top tubes (plasma) Invert 8 times; Place in ice water; Cover with box	**Centrifuge:** at 15º C for 30 min at 3000 *x g*	1. Place 0.25 mL plasma in one vial and distribute remaining equally into one amber and twelve clear 2-mL vials with purple caps; place in ice water; cover with box until put in freezer. In addition, for Tube #5, place packed cell aliquot in a 5 mL vial with a dark-blue cap. Keep covered with box at room temperature until put in freezer. **From Tube #5** **From Tube #6** 	**From Tube #5**  Approximately 5 mL	Store vials at -70°C
Tube #**7**  2.7-mL blue top tube (plasma) Invert 8 times	Room temp rack; centrifuge at 15º C for 30 min at 3000 *x g*	**30 MIN POST-COLLECTION** Equally distribute plasma into two 2-mL vials with blue caps; keep at room temp until put in freezer. **From Tube #7**  Equally distribute (0.5 mL minimum)	**30 MIN POST-COLLECTION**	Store vials at -70°C

Urine Processing Work Flow

Grab Urine Collection	STAGE 1	STAGE 2		STAGE 3	STORAGE
	Refrigerate within 4 hr; Process within 12 hr; Invert container 8 times; transfer ~ 1.5 mL urine into five 2-mL vials.	 1.5 mL, Yellow cap	No additive	Freeze within 12 hr of collection	Store vials at -70°C
		 1.5 mL, Green cap	Add 60 µL 3 mol/L HCl		
		 1.5 mL, Orange cap	Add 30 µL 1 mol/L sodium carbonate		

Shipping to the Central Laboratory: Vials stored at -70°C → weekly on dry ice
(Complete HCHS Shipping Forms, Face sheet and Contents sheet, and include in dry ice shipment, along with Biospecimen Collection Forms.)

Questions regarding this protocol can be answered by contacting the Central Laboratory at 612-625-5040

4.6.1 Freezing

When all of the blood and urine specimens have been aliquoted into their respective vials and the vials have been replaced in the sponge rack, the entire rack is placed upright in the -70° C freezer for a minimum of 30 minutes. Samples must be placed into the freezer within 90 minutes from venipuncture time. Samples must be thoroughly frozen before packaging them for storage and shipping.

5. PACKAGING AND SHIPPING

5.1. Storage, Packaging and Shipping

The frozen samples are shipped once per week on Monday or Tuesday. Remove the sample aliquot tray from the -70° C freezer. Package quickly after this point to avoid thawing of the specimens and exposure to light. Each participant's whole blood, serum, plasma, and urine samples are packaged in freezer storage bags according to their specimen type. See Appendix 4 for shipping forms.

5.1.1 Packaging Frozen Specimens

Place the 4.5 mL clear-capped EDTA whole blood vial (A1, tray1), the 0.25 mL lavender-capped vial (A2, tray 1), the 0.25 mL red capped vial (A7, tray 1) into a 4" x 6" storage bag. These are the immediate testing vials. Place thirteen of the lavender-capped plasma vials into another 4" x 6" storage bag. Place the two blue-capped plasma vials into a third 4" x 6" storage bag. Place fifteen of the red-capped serum vials into a fourth 4" x 6" storage bag, . Place the dark blue-capped packed cell vial and the two clear-capped vials into a fifth 4" x 6" storage bag. Place the five urine vials into a sixth 4" x 6" storage bag. Check again to make sure all vials are labeled as they are placed into the storage bags. Add an absorbent pad to each bag of samples. Press the air out of each bag and seal. Place all six of the sealed 4" x 6" bags into one 10" x 10" bag. Place the Lab ID# label for that set of aliquots on a piece of paper and insert it into the 10 x 10 bag so that it shows through. Expel the air from the bag and seal it. Place this bag in the Central Laboratory Styrofoam box in the -70° C freezer and do not remove it until the time of shipment. Complete the shipping log with appropriate information for these samples.

The bags of frozen EDTA whole blood, sera, plasma, and urine are packed and shipped in Styrofoam boxes. Packaging instructions (See Figure 3) are as follows:

1. Place a layer of dry ice on the bottom of the Styrofoam box.
2. Put one-half of the10"x 10" bags of sample vial/tubes into the Styrofoam box on top of the dry ice.
3. Layer more dry ice on top of and around the sample bags.
4. Put the remaining sample bags into the Styrofoam box on top of the dry ice.
5. Layer more dry ice on top of and around the sample bags. The amount of dry ice in the shipping should total at least 5 pounds. When shippng many sample collections, it will be many times more than this.

6. Place packing material on top of the dry ice to fill the box. Replace the Styrofoam cover. **DO NOT** tape the Styrofoam cover to the Styrofoam container; this damages the shipping containers making them unable to be reused.
7. Insert the paper shipping forms (**original** Face sheet, Contents sheet, and Biospecimen Collection Forms; keep a copy of all forms at field center) into a 10" x 10" bag and place inside the shipping box. The shipping forms with instructions are shown in Appendix 4.
8. Seal the outer cardboard box tightly with strapping tape. Affix "Category B UN 3373" label and a Fed-Ex dry ice label to outside of box. These labels are provided by the Central Laboratory.
9. Affix the FedEx airbill to the outside of the box. Record the site address and telephone number in section 1. (Do NOT use FedEx billable stamps on dry ice shipments.) Contact Federal Express (1-800-GO-FEDEX) for pickup.
10. If necessary, more than one box may have to be shipped per week.
11. It is the clinical site's responsibility to ensure that the package is picked up by FedEx and delivered to the Central Laboratory. Follow these steps to track your package: Go to the FedEx website www.fedex.com/us/, click on <Track> drop-down menu, click on <Track by Tracking Number>, enter tracking number, and click on <Track>. The tracking information will be displayed on the <Summary> screen. If the <Summary Results> state "Not Found"; this means your package has not been picked up and FedEx should be contacted. Check to see if your package has been delivered to the Central Laboratory the morning following shipment using the same tracking procedure.
12. The Central Laboratory will check the "FedEx Insight Tracking Log" daily to view what HCHS SOL V3 packages should be arriving. However, only those packages actually picked up and scanned into the FedEx system will appear on this log.
13. See Figure 3 below for shipping diagram.

5.1.2 Shipping Frozen Specimens

The samples remain in their Styrofoam box at -70° C until they are shipped. All frozen EDTA whole blood, plasma, sera, and urine tubes collected and stored within the last work week are shipped to the Central Laboratory on **Monday** with the exception of Quality Control aliquots, as discussed in the Quality Control section below. Frozen samples can be shipped on Tuesday if the Field Center is closed on Monday, but the contact person at the Central Laboratory must be notified that the shipment will arrive one day later than usual. Weigh all packages before shipping, if possible. It is important to record an accurate weight on the Federal Express Airbill. Do not over-estimate the package weight.

Remember to track your package the day following shipment to ensure that it was picked up. The Central Laboratory will check the "FedEx Insight Tracking Log" daily to view what HCHS SOL V3 packages should be arriving. However, only those packages actually picked up and scanned into the FedEx system will appear on this log.

Note: All shipping containers are sent to the HCHS SOL V3 Central Laboratory by overnight courier (FedEx Priority Overnight) to ensure receipt within 24 hours. The empty Styrofoam

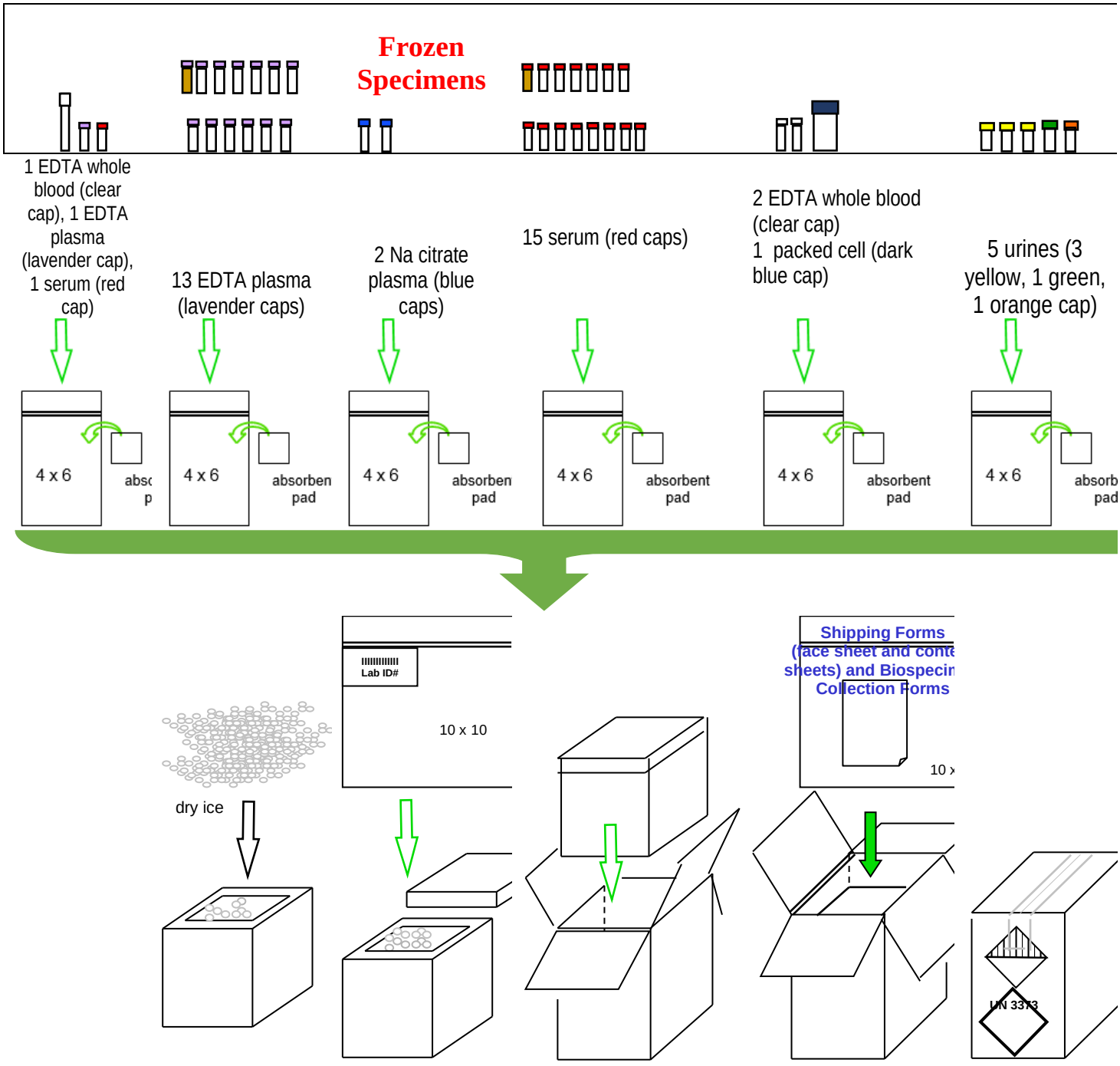
containers are recycled by returning them to the Clinical Centers via FedEx Express Service. Shipping containers to the Central Laboratory are addressed as follows:

Sharon Minnerath/ HCHS/SOL V3 Central Laboratory
University of Minnesota, Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415
Telephone: (612) 625-5040
Main Fax: (612) 625-4142 (Laboratory)
Alternate Fax: (612) 625-4831 (Business Office)

HCHS SOL V3 Central Laboratory Hours:	Monday-Friday	7:00 am-3:30pm
	Saturdays	Open; hours vary
	Sundays	Closed

*A holiday schedule will be emailed to all HCHS SOL V3 Clinical Sites or posted on the study website in advance of upcoming holidays

Figure 3. Packaging Frozen Specimens for Shipment



6. QUALITY CONTROL

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 which is part of each participant's record. (See Appendix 3, Items 11, 12, 19, and 25, Biospecimen Collection Form.)

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

This record provides documentation that blood was drawn in a standardized manner and that the equipment was functioning properly. This quality control documentation is the best evidence that samples in each of the four Field Centers are being drawn and processed identically. Differences in the way the samples are collected or processed could potentially create a significant difference in assay results, which could seriously compromise the laboratory test data. It is very important that the quality control records of the procedures and the equipment be properly maintained.

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

6.1. Quality Control Duplicate Blood Samples

As part of the overall quality control program for laboratory determinations from blood and urine samples, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under the participant's regular HSHC-SOL V3 laboratory ID number, and the other half under a Quality Control Phantom Participant (QC) laboratory ID number. The QC laboratory ID numbers are not distinguishable from other laboratory 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. For data analysis, results on each laboratory measurement are matched to the appropriate participant results at the Coordinating Center from the QC Phantom ID Form (Appendix 10) that is completed by Field Center technicians.

QC blood is stored at the Field Center for an extra week and then sent to the Central Laboratory with a regular shipment.

The QC blood samples are collected in sequential order (cycling back to Tube #1 after QC Tube #7 has been collected). Each Field Center will collect a QC samples from approximately 25% of the participants. QC samples are drawn daily. Initially, we will try to collect a QC sample from every participant to have more QC data available at the start of the study. After a period of time (to be determined by the QC Committee), we will ask each Field Center to collect QC samples on fewer participants.

The plan for collecting the QC samples each day is as follows: From the first participant of the day, draw tubes #1; from the second participant of the day draw tube #2; from the third participant, draw tube #3 and #4; from the fourth participant draw tubes #5; from the fifth participant draw tube #6; from the sixth participant, draw tube #7; use a urine sample with sufficient volume to provide 2 sets of aliquots (one for the QC duplicate) from one participant each day. (This could be urine from a participant who has also volunteered to donate additional blood.)

6.2. Blood and Urine QC Sample Checklist

The venipuncture technicians maintain a daily checklist posted in their work area of the QC samples to be drawn. As each sample is drawn and processing completed, it is checked off. An example of the checklist is given below.

Blood and Urine QC Sample Checklist
Date:_____

Participant	Tubes	Aliquot Type	Sample collected? (Y/N)
Participant 1	1	Serum	_____
Participant 2	2	Serum	_____
Participant 3	3 & 4	Serum & Whole Blood	_____
Participant 4	5	Plasma, EDTA	_____
Participant 5	6	Plasma, EDTA	_____
Participant 6	7	Na Citrate	_____
Participant 7	Urine	Urine	_____

6.3. Preparation for Drawing and Processing QC Samples

Blood Drawing Tubes: Each morning (or the afternoon before) the blood drawing technician(s) prepares the extra blood collection tube(s) for the QC sample(s) to be drawn that day. Each tube is labeled with the QC ID number to be used that day. In addition, the technicians may wish to mark QC blood drawing tubes "QC" in a clearly visible fashion, to reduce the chance that these tubes might be mixed up with the regular blood collection tubes during processing. 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 Vials: Each morning (or the afternoon before) 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 vials in each block are

labeled in advance with the QC ID number being used that day. Care must be taken during processing that the labels on the sample aliquot vials match the label on the QC blood collection tubes.

For the duplicate urine sample, five extra vials for the urine QC duplicates are set out and labeled with the urine QC ID number. A participant's sample with adequate urine volume to provide duplicate aliquots is chosen for the QC sample.

6.4. Collecting and Processing QC Blood and Urine

Selecting Participants for QC Blood Draw: Initially, a QC sample will be collected from all participants for which it is possible. Based upon the size of their veins, the difficulty of drawing the blood, and the apprehension a participant shows about the blood draw, the venipuncture technician may forego the drawing of the QC tube from certain participants. (After a specific number of QC sample sets are collected from each field center as determined by the Coordinating Center, the frequency of participant QC collections will decrease.)

Order of QC Tubes in Relation to Regular Blood Collection: Draw the QC tubes after the other tubes have been collected. This procedure is followed to cause the least disruption of the collection of the regular blood samples. If the blood flow falls off at the end of the draw, so that it would be difficult to obtain the extra QC tubes, a different participant is used to get this blood. DO NOT PERFORM A NEW NEEDLE STICK JUST TO GET MORE BLOOD FOR A QC SPECIMEN. DO NOT REAPPLY THE TOURNIQUET AFTER INITIAL RELEASE.

Processing and Freezing QC Blood: Process the QC blood samples along with the regular blood samples. After processing is completed for each QC blood collection tube, the sample aliquot vials are put into the -70° 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.

The five urine QC samples are placed into the freezer at the same time as their matched participant pair. As with the blood specimens, the urine samples are kept away from the other urines collected during the week so they are not included with that week's shipment.

Biospecimen Collection Form: This form is completed for the QC phantom set of samples. However, it is not possible to complete this form truthfully since the set is collected from multiple participants. It is suggested that the information from the participant to donate tube #4 is used to complete the form for the QC set.

Logging the Match between QC and Regular HCHS/SOL ID's and Reporting these to the Coordinating Center: The QC Phantom Participant's folder is kept in the blood drawing area. In the folder is the HCHS/SOL Quality Control Phantom Participant Form (see example in Appendix 10), which is used to keep track of the match between the QC and regular HCHS/SOL specimens. At the top of the log sheet is a space for the QC Phantom Participant's laboratory ID number. As participants donate blood to make up a QC set, labels with their participant ID numbers (not their Lab ID#) are added to the line corresponding to the tubes donated. This step

must be done immediately after completion of drawing blood for that participant, to minimize the chance of recording the wrong ID number. One such form is recorded for each QC ID number used. As soon as the full set of tubes is completed for each phantom participant, the QC phantom participants' folder with this form is given to the receptionist (or other person designated by the Study Coordinator). The folder is processed like other participants' folders, with the QC phantom participant form transferred to the Coordinating Center by keying the Phantom form (PHT) into the data management system. Do not send a hardcopy of the Phantom form (PHT) to the Central Laboratory because it will unblind the masked QC analysis of the samples. A Biospecimen Collection form is also completed for the phantom duplicate, and that is sent to the Central Laboratory with the biospecimens.

6.5. Internal Laboratory Control

Internal quality control procedures monitor analytical performance of the test relative to medical goals and alert analysts to unsatisfactory analytical performance. Quality control statistics are used to make judgments about the quality of analytical results, whether system correction is necessary, whether patient data should be accepted or rejected, and for estimating performance parameters which can be compared to analytical and medical goals. Testing is monitored by two control samples analyzed daily in each batch of samples. A permanent standard deviation (SD) and coefficient of variation (CV) is determined by analyzing the material on 50 – 100 separate days. The mean for new lots of material is established by analyzing the material on 20 separate days. The SD and CV from the data collected over 20 days is used to monitor the permanently established SD. Quality control results are plotted on Levy-Jennings plots and acceptability (i.e. in statistical control) is determined using three Westgard rules (1-2s, 1-3s, and 2-2s). Documentation is made on the control charts when there is a change in reagent lot numbers, any action is taken due to unacceptable control results, and when other pertinent information is observed.

6.6. Reporting Results

The Central Laboratory has the responsibility for reporting results to the HCHS Coordinating Center. All test results are transmitted to the Coordinating Center through file transfer protocol (FTP). This transmission will occur daily, Monday through Friday. In order to see if the Coordinating Center has received and processed the lab results for a participant, the field center can run the “End of Study Availability Report” using the HCHS/SOL V3 data management system. The availability report summarizes the receipt of information from the Central Laboratory and reading centers. Tests reported to the participants will be available to the field centers via a report in the DMS called the “End of Study Report”. Any tests included in this report whose results exceed their alert range will be flagged appropriately. In addition, any alert result on a test not normally reported to the participants will be included in a separate upload. Reference ranges and alert values can be found in Appendix 1. Note that test results will be available in approximately 3-4 weeks after the sample is collected.

7. TRAINING PROCEDURES

Technicians will be trained in actual procedure of phlebotomy by their respective institutions. The study does not provide phlebotomy training.

A check list of the venipuncture and processing procedures that HCHS/SOL V3 technicians must know and be prepared to demonstrate is listed in Appendix 8. The technician must study the HCHS/SOL V3 Specimen Collection and Processing Manual and watch a few participant samples 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 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, and then placed at their proper positions. The sample is processed from start to finish exactly as if real blood were being used. Each technician performs a minimum of two mock draws from beginning to end. Although the mock draws take time, they provide hands-on experience and allow the technician to become comfortable with the procedures before proceeding to live participants.

At this point the technicians are ready to practice on live volunteers. The technicians practice at least once with just one volunteer at a time and again process the blood entirely by themselves from start to finish. If the technicians do not feel comfortable, they can always go back and repeat the process with dummy tubes. If volunteers are available, it may be beneficial to repeat this several times. Any questions or problems that the technicians have must be solved before the technicians actually proceed to drawing the HCHS/SOL participants. Before the technicians draw blood from any HCHS/SOL V3 participant, they must take and pass the practical and written tests included at the end of this manual (Appendix 9). After passing the test and depending on the written evaluation of their instructor, they may proceed either to drawing blood from the HCHS/SOL V3 participants as part of a team, or do more practice on live volunteers.

8. SNACK

A light snack for the participant is scheduled as soon as possible after venipuncture. Make sure that this is provided after blood collection (tube #7) has been collected. Menus are locally determined.

Appendices

Appendix 1 Laboratory Tests, Reference Ranges, and Alert Values

Test Name – Core Study	Reference Range	Units	Alert Value
*Total cholesterol	<200	mg/dL	>360
*Triglycerides	<150	mg/dL	>1000
*HDL-cholesterol, male	>40	mg/dL	<20
*HDL-cholesterol, female	>50	mg/dL	<20
*LDL-cholesterol, calculated	<129	mg/dL	>260
*Glucose, fasting	60-99	mg/dL	<50 and ≥400
*Glycosylated Hemoglobin	<5.7% normal 5.7-6.4 prediabetes >/= 6.5 diabetes	%	

Test Name – Ancillary Studies funded as of 7/17/19	Reference Range	Units
Insulin, fasting	12-150	pmol/L
Alanine aminotransferase (ALT), male	<50	U/L
Alanine aminotransferase (ALT), female	<35	U/L
Aspartate aminotransferase (AST), male	15-46	U/L
Aspartate aminotransferase (AST), female	15-46	U/L
Cystatin C	0.51-1.05	mg/L
Creatinine, male	0.5-1.2	mg/dL
Creatinine, female	0.4-1.1	mg/dL
CRP(hs)	<5.00	mg/L
eGFR	>60	mL/min/1.73m^2
Creatinine, random urine, male	29 - 226	mg/dL
Creatinine, random urine, female	40 - 278	mg/dL
Albumin, random urine	<20	mg/L
Albumin/creatinine ratio	<30	mg albumin/g creatinine

Reference ranges for these tests are given in the form of a comment accompanying all result reports. National Cholesterol Education Program guidelines suggest that : 1) LDL-cholesterol values less than 100 mg/dL are optimal, 100-129 mg/dL are near or above optimal, 130-159 mg/dL are borderline high, 160-189 mg/dL are high. 190 mg/dL and above are very high; and 2) HDL-cholesterol values below 40 mg/dL are undesirable. (JAMA 2001; 285:2486-2497).
*These tests will be reported to the participants.

Appendix 2 Equipment and Supplies

Supplies to be supplied by the Central Laboratory:

Description

- Microvials, clear (2 mL) 500/pk
- Microvials, amber (2 mL) 500/pk
- Red screw caps 1000/pk
- Yellow screw caps 1000/pk
- Purple screw caps 1000/pk
- Blue screw caps 1000/pk
- Clear screw caps 1000/pk
- Green screw caps 1000/pk
- Orange screw caps 1000/pk
- Microvials (Nunc), clear (4.5 mL) w/clear screw cap 300/pk
- Vial, clear (5 mL) w/dark blue screw cap 1000/cs
- Vacutainer tubes 100/pk
  - Serum, red top, 8.5 mL
  - Serum, red top, 5 mL
  - EDTA, lavender top, 10 mL
  - EDTA, lavender top, 4 mL
  - Sodium citrate blue top, 2.7 mL
- Dry ice shipping labels
- Category B UN 3373 label
- Barcode laboratory ID labels

Supplies to be obtained by the Field Center:

Supplier	Catalogue No	Description
Cardinal Health	B3036-14	Butterfly Needles, 21G x ¾", #367296
Cardinal Health	B3035-12	Luer Adapters, #367290
Henry Schein	1126131	Alcohol Swabs 200/pk;
Henry Schein	1002524	Gauze Sponges 3" x 3" 200/pk
Henry Schein	1271247	Band Aids 1" x 3" 100/box
Cardinal Health	367203	Tourniquets, Latex free, 25/pk
Cardinal Health	364815	Vacutainer Tube Holders 1000/cs BD #364815
Cardinal Health	P5214-12	Transfer Pipets 500/pk
Henry Schein	1069275	Ziplock Freezer Bags 4" x 6" 1000/cs
Henry Schein	3956633	Ziplock Freezer Bags 10" x 10" 1000/cs
Henry Schein	121-9125	Polyester Foam Tube Rack, 50 wells, 30/cs, #0010
Henry Schein	1160710	PDI Ammonia Inhalant 10/pbx
Cardinal Health	C8827-24	Grab Urine Collection Container
Cardinal Health	B2922-1A	Blood Collection Trays
Cardinal Health	CH2212-2 or CH2960-4	Thermometers -20 C-+70 C
Cardinal Health Fischer Scientific	M1050-7 or 19075194	50 mL Absorbent Pads for shipping
Cardinal Health	B1900-18	Harvard Trip Balance (Ohaus 1550SD) (*optional)
Cardinal Health	C6510-1	Timer- 3 channel digital
Polyfoam Packers/ThermoSafe	398	Styrofoam shipping box, (Est. 25-30 frozen samples sets)
Polyfoam Packers/ThermoSafe	352	Styrofoam shipping box, (Est. 28-30 frozen samples sets)

Cardinal Health	P5065-30 (30uL), #1143C	MLA D-Tipper Pipetter, Fixed Volume pipetter
Cardinal Health	P5064-902	MLA pipette tips, #9025
VWR	101226910 OR	3.0 N HCL, 500 mL
RICCA Chemical Co.	R3720000-120A	3.0 N HCL, 120 mL
Greinger	45F542 OR	1.0 N sodium carbonate, 1L
VWR	RC719032 OR	1.0 N sodium carbonate, 1 L
VWR	RCR7190000500A	1.0 N sodium carbonate, 500 mL
		Dry Ice (approximately 5-10 lbs. per shipment)
		Packing Material
		3M, Scotch brand 3750 clear packaging tape

Equipment purchased and maintained by Field Centers:
Table-top centrifuge with swinging buckets, refrigerated, and capable of producing 3,000 x *g*
Freezer capable of maintaining -70˚ C with a minimum of 5 cu ft storage
Refrigerator 4˚ C for storing urine containers.

Appendix 3 Biospecimen Collection Form

HCHS/SOL Biospecimen Collection Form (BIO)

Participant ID #:								

FORM CODE: BIO
VERSION: 2, 12/16/2019

Contact Occasion: 0 3

Occurrence: 0 1

0a. LAB ID#:

Instructions: *This form should be completed during the participant's visit. Affix the participant ID label and the Lab ID label above. Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeroes where necessary to fill all boxes. Use a 24-hour clock for time (e.g. noon=12:00, 1pm=13:00)*

A. Safety Questions:

1. Have you ever had a radical mastectomy or other surgery where lymph nodes were removed from your armpits?

   ¿Ha tenido una mastectomía radical o alguna otra cirugía que le haya removido ganglios linfáticos en sus axilas (debajo de su brazo)?

   ☐ (0=No, 1=Yes) **[If Yes, specify in Q12; follow precautions in QxQ]**

2. Do you have any bleeding disorders? ☐ (0=No, 1=Yes) **[If Yes, specify in Q12; follow precautions in QxQ]**

   ¿Tiene problemas de coagulación de la sangre?

3. Have you ever had a graft or shunt for kidney dialysis?

   ¿Le han hecho algún injerto o shunt arterial como vía para diálisis de los riñones?

   ☐ (0=No, 1=Yes) **[If Yes, specify in Q12; follow precautions in QxQ]**

B. Fasting Blood Collection Information:

4. On which day did you last eat or drink anything except water: today, yesterday, or the day before yesterday?

   ¿Qué día comió o bebió algo excepto agua por última vez: hoy, ayer o anteayer?

   ☐ (1=Today, 2=Yesterday, 3=Day before yesterday)

5. And at what time was that? ☐☐ : ☐☐ hh:mm (24-hour format)

   ¿Y, a qué hora fue eso?

C. Blood Collection:

6. Date of blood collection: ☐☐/☐☐/☐☐☐☐ (mm/dd/yyyy)

7. Collection time: ☐☐ : ☐☐ hh:mm (24-hour format)

8. Was fasting blood collected before the snack? ☐ (0=No, 1=Yes)

9. Number of venipuncture attempts: ☐

10. Any blood drawing incidents or problems? ☐ (0=No, 1=Yes) **[If Yes, specify in Q11, Q12 and/or Q25]**

PARTICIPANT ID #:									FORM CODE: BIO VERSION: 2, 12/16/2019	Contact Occasion	0	3	occurrence	0	1

11. 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.

Tube Number	1	2	3	4	5	6	7
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, problem, or issue here:

_____

_____

13. Phlebotomist's code number: ☐☐☐

D. Blood Processing:

14. Time at which tubes 5 - 7 were centrifuged: ☐☐ : ☐☐ hh:mm (24-hour format)

15. Time at which tubes 1 - 3 were centrifuged: ☐☐ : ☐☐ hh:mm (24-hour format)

16. Time at which aliquot tray 1 vials were placed in freezer: ☐☐ : ☐☐ hh:mm (24-hour format)

17. Blood Processor's code number: ☐☐☐

18. Any blood processing incidents or problems? ☐ (0=No, 1=Yes) **[If Yes, specify in Q19 and/or Q25]**

19. Blood processing incidents: Document problems with the processing of specimens in this table. Place an "X" in box(es) corresponding to tubes in which the processing problem(s) occurred. If a problem other than those listed occurred, use Item 25.

Tube Number	1	2	3	4	5	6	7
a. Broken tube	☐	☐	☐	☐	☐	☐	☐
b. Sample re-centrifuged	☐	☐	☐	☐	☐	☐	☐
c. Clotted	☐	☐	☐	☐	☐	☐	☐
d. Hemolyzed	☐	☐	☐	☐	☐	☐	☐
e. Lipemic	☐	☐	☐	☐	☐	☐	☐

E. Urine Sample

20. Was a urine sample collected? ☐ (0=No, 1=Yes) **[If No, Go to Q25]**

21. Date of urine sample: ☐☐/☐☐/☐☐☐☐ (mm/dd/yyyy)

22. Time urine sample collected: ☐☐ : ☐☐ hh:mm (24-hour format)

23. Time urine sample was processed: ☐☐ : ☐☐ hh:mm (24-hour format)

24. Urine processor's code #: ☐☐☐

25. Comments on blood processing, urine collection/processing:

_____

_____

_____

F. V3 Ancillary Studies

26. Consented to participate in SOL VIDA? ☐ (0=No, 1=Yes)

Appendix 4 Shipping forms

SHIPPING FORMS INSTRUCTIONS

There are two types of shipping forms: (1) the face sheet and (2) the contents sheet. Both forms are included in every frozen shipment. A copy of the Biospecimen Collection Form for each participant is also included.

FACE SHEET

The FACE SHEET is a two part form. Part One, on the top of the page, is completed by the Field Center. Part Two, on the bottom of the page, is completed by the Central Laboratory.

The NAME AND ADDRESS of the SHIPPER (Field Center) and the RECIPIENT (central laboratory) is printed on each shipping form.

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

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

The TOTAL NUMBER OF SPECIMENS ENCLOSED in the shipping container is confirmed by the Field Center technician by counting specimen bags and the total number of specimens within them.

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

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

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

Part Two of the SHIPPING FORM is completed by the receiver (e.g. the Central Laboratory).

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

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

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

CONTENTS SHEET

The contents sheet lists the complete inventory of tubes in a given shipment. The original form is sent to the Central Laboratory with the specimen shipment, and a copy is filed at the Field Center. More than one contents sheet may be used in each shipment, depending on the number of specimens enclosed. The number of pages attached and each page number are filled in at the top of the contents page (e.g. "page 1 of 5"). This form is filled out at the Field Center as the specimens are collected and stored. This form must be checked against the specimens when packed for shipment.

The SPECIMEN ID number is entered in the left hand column of the contents sheet. This is most easily done by attaching one of the adhesive specimen ID number labels in the space provided. This is done at the time of collection. It is suggested that a second person check these IDs against the IDs on the vials to correct any errors.

The tubes comprising a complete sample are listed in the upper left hand corner of the sheet. Under the category SPECIMEN COMPLETE?, YES or NO is marked for each participant to indicate whether the correct number of tubes has been shipped. If there is some deviation from the correct count, "NO" is marked, and a description of the problem follows in the column headed MISSING VIALS. The number of missing tubes and the color of their caps is recorded here.

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

Face Sheet
HCHS/SOL Visit 3 SHIPPING FORM
PART ONE (To be completed at Field Center)

TO: HCHS/SOL V3 Central Laboratory
University of Minnesota, Advanced Research and Diagnostic Laboratory
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415

FROM: Name and Field Center Address printed here.

SHIPMENT PACKED AND SEALED:
TIME: _ _ : _ _ AM PM DATE: _ _ / _ _ / _ _.

REPORTING PERIOD: STARTING DATE: _ _ / _ _ /_ _

ENDING DATE: _ _ / _ _ / _ _

TOTAL NUMBER OF SPECIMEN COLLECTION SET ENCLOSED: _____

NUMBER OF CONTENTS PAGES ATTACHED: ____

COMMENTS CONCERNING SHIPMENT CONTENTS:

_____

_____

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

****

PART TWO (To be completed at Central Laboratory)

SHIPMENT ARRIVED AT CENTRAL LABORATORY:
TIME: _ _ : _ _ AM PM DATE: _ _ / _ _ / _ _.

COMMENTS ON CONDITION OF SHIPMENT ON ARRIVAL:

_____

_____

INITIALS OF PERSON UNPACKING SPECIMENS: _ _ _

Frozen Contents Sheet HCHS/SOL Visit 3 SHIPPING FORM Page ____ of _____

HCHS/SOL V3 Central Laboratory
University of Minnesota, Advanced Research and Diagnostic Laboratory
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415

Complete frozen sample for each participant includes 6 bags containing:

3-(1) 1.5 mL clear (4.5 mL tube), (1) 0.25 mL lavender, (1) 0.25 red
13-lavender top microvials
2-blue top microvials
15-red top microvials
3-(2) 1.0 mL clear, (1) 5.0 mL dark blue (5.0 mL tube)
5-urine microvials (3-yellow, 1-green, 1-orange)

SPECIMEN ID	SAMPLE COMPLETE? YES	NO	MISSING VIALS #	COLOR	COMMENTS _____

[Place Lab ID label here]

Appendix 5 Partial Biospecimen Collection Procedure

Participant Sample Set

1. If the blood draw is very difficult, and it is clear that a full set of biospecimen collection tubes will not be obtained, the phlebotomist can use their discretion and collect an alternate sequence collection (Collection Tube #1,4,5,6 and 7 only). This ensures that all types of samples are collected. If this is done, item #10 of the Biospecimen Collection Form should be answered as Yes and a comment should be put in item #12 that it was a difficult draw. If things go better than expected, it is OK to continue to draw additional skipped tubes, in the regular order (Tube #2,3)

2. If a full set of biospecimen collection tubes/urine cannot be obtained after 2-3 attempts, determine if the HCHS participant is willing to return for a (fasting) re-collection appointment. Insert a comment on item #12 of the Biospecimen Collection Form that the participant will be coming back for a re-collection at another date. If the participant is unwilling to come back for a re-collection then state on item #12 that the biospecimen set on this participant is a partial collection and no other specimens will be obtained and proceed to shipping.

3. If the participant is scheduled for a re-collection appointment, process all of the collection tubes/urine that were obtained as directed in the Biospecimen Collection and Processing Manual and save them in a designated location in the freezer. DO NOT send the incomplete frozen biospecimen set to the Central Laboratory if the participant is coming back for a re-collection appointment.

4. Assign a new Lab ID to the HCHS participant for the re-collection appointment. Attempt to re-collect the entire sequence of biospecimen collection tubes/urine.

5. Choose the most complete biospecimen set; either the biospecimen set that was obtained at the first visit or the biospecimen set that was obtained at the re-collection appointment. DO NOT combine biospecimens from both sets to make a full set.

6. If the biospecimen set from the first visit is the most complete, then send this set to the Central Laboratory and indicate on the Frozen Contents Sheet that this biospecimen set is incomplete and no other specimens will be obtained. Discard the other incomplete biospecimen set from the re-collection appointment. For the Field Center records, save the Biospecimen Collection Form from the re-collection appointment but insert a comment on item #12 that the biospecimens were discarded and the biospecimens from the first visit were sent to the Central Laboratory.

7. If the biospecimen set from the re-collection appointment is the most complete, then ship this set to the Central Laboratory. Insert a comment on item #12 of the Biospecimen Collection Form that this participant had a re-collection. The new Lab ID number will be entered in place of the old Lab ID number from the first incomplete visit into the field center's data management system. Discard the incomplete biospecimen set from the first visit. For the Field Center records, save the Biospecimen Collection Form from the first visit but insert a comment on item #12 that the participant was re-collected and assigned a new Lab ID number and the first set of biospecimens were discarded.

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

9. Once a set of frozen biospecimens from a HCHS participant is sent to the Central Laboratory, no other biospecimens from this participant can be sent on another date.

10. Contact the Central Laboratory if any unusual circumstances or questions arise with any biospecimen collections.

Phantom QC Sample Set

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

2. One of Tube #1or Tube #2 must be completely (100%) full. A partially filled second tube is acceptable to provide as much serum as possible.

3. Tube #3 is acceptable if it is at least 50% full, a partially filled tube is acceptable to provide as much serum as possible.

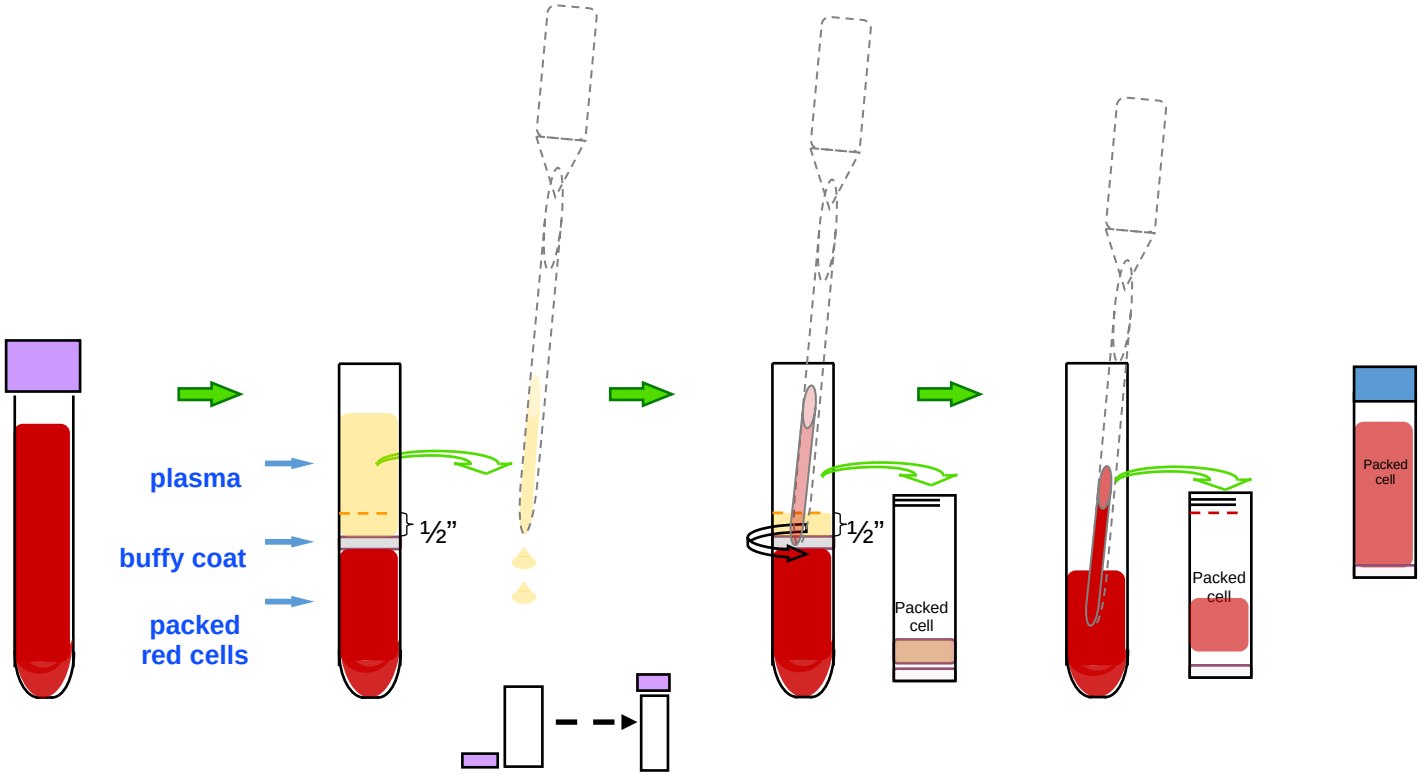
4. Tube #4 is acceptable if it is at least 90% full (3.5 mL).

5. One of Tube #5 or Tube #6 must be completely (100%) full. A partially filled second tube is acceptable to provide as much plasma as possible.

6. Tube #7 must be completely (100%) full in order to maintain the proper ratio of blood to liquid anticoagulant.

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

Appendix 6 Packed Cell Aliquot Processing



Processing the EDTA tube for packed cells and plasma aliquots:

1) Taking care not to disturb the cell layer, remove the clear plasma supernatant and transfer 0.5 mL plasma into each of the 2-mL cryovials. Aspirate slowly starting at the top of the plasma. Leave a ½ inch layer of plasma above the buffy coat-red blood cell layers. It is important to withdraw only the plasma and none of the buffy coat (containing white blood cells and platelets) that forms at the cell-plasma interface following centrifugation. If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube under the initial processing conditions. Fasten purple screw caps tightly onto the cryovials
2) Using the same plastic transfer pipet, slowly aspirate the remaining ½" layer of plasma, the buffy coat and *some* of the remaining red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipet! 'Ring' the tube with the pipet by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL 'packed cell' vial.
3) Still using the same plastic pipet, transfer all of the remaining packed red cells from the tube into the same 5-mL'packed cell' vial. This step will ensure that all of the buffy coat is adequately rinsed from the pipet. Fasten dark-blue screw cap tightly on the vial.

Appendix 7 Aliquot Tray (sponge rack) Cleaning Procedure

NOTE: Wear safety glasses and gloves for this procedure.

1. Perform this procedure weekly or sooner if there is noticeable contamination.

2. Make a solution of 10% bleach by adding 1 part of household bleach to 9 parts of tap water in a bucket. Make this fresh each week.

3. Submerge the racks in the bleach solution and squeeze in and out 5 times.

4. Rinse under running tap water. Squeeze the racks under running tap water 10-20 times.

5. Squeeze out any remaining liquid and air dry overnight.

Appendix 8 Venipuncture and Processing Procedures Certification Checklist

VENIPUNCTURE	Satisfactory/ Unsatisfactory	Comments
1. Labels checked	___	_____
2. Participant prepared and procedure explained	___	_____
3. Venipuncture Form completed.	___	_____
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. Aliquoting supply set-up	___	_____
3. Stage 1: tube spin and whole blood processing	___	_____
4. Stage 2: plasma/packed cell processing	___	_____
5. Stage 3: tube spin and serum processing	___	_____
6. Stage 4: placing in freezer	___	_____
7. Urine processing	___	_____
8. Vials sealed	___	_____
9. Freezer organization	___	_____
10. Time constraints	___	_____
11. Disposal of contaminated supplies	___	_____

PACKAGING AND SHIPPING		
1. Specimens bagged	___	_____
2. Adequate dry ice used in frozen shipping	___	_____
3. Shipping paperwork	___	_____

MISCELLANEOUS		
1. Quality Control temps and documentation	___	_____
2. Phantom QC Procedure	___	_____
3. Containers correctly labeled for shipping	___	_____

Technician Name_____ Technician study ID_____
Trainer Name_____ Trainer Signature_____
Date_____

Appendix 9 Sample Exams for Certification

PRACTICAL EXAM FOR HCHS/SOL BLOOD DRAWING TECHNICIAN

1. Place the following blood collection tubes in the correct set-up order and location for the venipuncture: (2)-8.5 mL red top, (1)-5 mL red top, (1)-4 mL lavender top, (2)-10 mL lavender top, (1)-2.7 mL blue top.

2. Specify which tube(s) remain at room temperature after collection and which are put into a cup with ice slush.

3. Remove the appropriate tubes from the tray and place them in the centrifuge in balanced positions. How long do they spin? At what speed?

4. Set up a sponge tray with the appropriate number and order of specimen storage vials. Indicate the colors of screw caps and the types of specimen put into these vials.

5. Place the collection tubes in front of their respective storage vials. Describe what further processing is required of each collection tube before it is aliquoted into its respective vial.

6. Organize the color-capped storage vials and prepare them for shipment.

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

8. Using the MLA D tipper pipetter, add 60 uL of 3 N HCl into a 1.5 mL aliquot of a urine specimen. What should you do if a drop of acid comes in contact with your skin or clothes?

WRITTEN EXAM FOR HCHS-SOL V3 BIOSPECIMEN COLLECTION AND PROCESSING TECHNICIAN

Name:_____Study ID_____ Field Center:_____DATE: _____

(please print)

1. When handling biological specimens, which of the following protective apparel must **ALWAYS** be worn?
   a) gloves
   b) sterile shoe covers
   c) sterile head covers
   d) lab coat and gloves

2. Initially, how many HCHS-SOL participants at each field center will be asked to donate additional blood specimens collected to be used as part of the phantom duplicate?
   a) One per day
   b) Two per week
   c) Everyone
   d) Eight per week

3. From which tubes are the packed cells used?
   a) #1 and #2
   b) #5
   c) #6, and #7
   d) packed cells are not being collected for Visit 3

4. How long should tubes #1, #2, #3 (red top) sit at room temperature before centrifugation?
   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required; must be centrifuged within 15 minutes

5. How long should tubes #5 and #6 (lavender top) sit on crushed ice before centrifugation?
   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required; must be centrifuged within 15 minutes

6. What storage vial does the packed cell sample go into?
   a) 5 mL vial w/dark blue cap
   b) 2 mL vial w/red cap
   c) 4.5 mL vial w/clear cap
   d) packed cells are not being collected for Visit 3

7. What whole blood storage vials are processed?
   a) (1) 4.5 mL vial w/clear cap and (2) 2 mL vials w/clear cap
   b) 4.5 mL vial w/clear cap
   c) (3) 2 mL vials w/red cap
   d) whole blood is not being stored for V3

8. Which of the following labels must be affixed to the outside of a frozen shipping box?
   a) biohazardous specimens
   b) dry ice
   c) category B UN3373
   d) dry ice and category B UN3373

9. What is the minimum amount of dry ice that must be used for frozen shipments?
   a) 2 lbs
   b) 5 lbs
   c) 10 lbs
   d) 12 lbs

10. For what type of tests will the red-stoppered tubes be used?
   a) They are for biorepository storage only
   b) Lipid
   c) Coagulation
   d) DNA testing

11. When transferring plasma to the microvials, how much plasma is left above the cells in the tubes?
   a) ¼ - ½ inch
   b) ½ - ¾ inch
   c) ¾ - 1 inch
   d) none, all the plasma is removed

12. The orange top (alkaline) urine aliquot vial has what added to it?
   a) 30 uL 1 N sodium carbonate
   b) 60 uL 3 N hydrochloric acid
   c) 30 uL 1 N NaCl
   d) nothing

13. If a full set of blood collection tubes and urine cannot be obtained, a re-collection appointment may be scheduled. What is the time limit for re-collection appointments?
   a) 2 weeks
   b) 1 month
   c) 3 months
   d) 1 year

14. What paperwork is completed and sent with each weekly frozen shipment?
   a) Copy of the Biospecimen Collection Form
   b) Original of the Biospecimen Collection Form
   c) Shipping Form Face Sheet andContents Sheet(s)
   d) Shipping Form Face Sheet and Contents Sheet(s) and Original of the Biospecimen Collection Form

15. What day of the week are frozen shipments sent?
   a) Monday, Tuesday, Wednesday or Thursday
   b) Any day of the week
   c) Monday or Tuesday
   d) Usually Monday, Tuesday if you contact the Central Laboratory and let them know

Appendix 10. QC Phantom ID Form

HCHS /SOL Visit 3 PHANTOM FORM

PHANTOM ID NUMBER:									FORM CODE: PHT VERSION: 1, 7/22/2019	Contact Occasion:	03	SEQ #	01

Instructions: *This form should be completed during participants' visit. Affix the PHANTOM HCHS ID label above. Affix the matching PARTICIPANT HCHS ID labels for the corresponding QC blood sample or urine specimen below. Note: Lab IDs will be linked through the corresponding Laboratory Collection form for each Participant ID, including the Phantom ID. Set CDART* ***Field Status*** *to 'Refused', 'No Response', 'Missing', etc for those questions that do not list these values as possible answer choices.*

1. Date Phantom ID assigned: ☐☐/☐☐/☐☐☐☐ (M M / D D / Y Y Y Y)

2. Staff Code of person assigning Phantom ID: ☐☐☐

1a. LAB ID:

Affix Specimen Label

PROCEDURE	MATCHING PARTICIPANT HCHS ID#	DATE COLLECTED (MM / DD / YYYY)	TECHNICIAN ID
Blood Samples 3. Tube 1 9 mL red-stoppered (serum)			
4. Tube 2 9 mL red-stoppered (serum)			
5. Tubes 3 & 4 5 mL red-stoppered (serum) 4 mL lavender-stoppered (EDTA)			
6. Tube 5 10 mL lavender-stoppered (EDTA)			
7. Tube 6 10 mL lavender-stoppered (EDTA)			
8. Tubes 7 4.5 mL blue-stoppered (Citrate)			
9. **Urine Specimen**			