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HCHS/SOL Visit 2
Manual 7
Biospecimen Collection and Processing

December 2, 2016~~August 24, 2015~~ - Version 1.6~~5~~

Study website - http://www.cscc.unc.edu/hchs/

Tracking of Revisions to HCHS/SOL V2 MOP #7

[Previous Manual, Date, Version]	Date(s) of Revisions; source	Approved by, Date	Revisions	Previous Page #s section changed etc.	Distribution Date
Visit 2, version 1.0	8-8-2014	SC	Sample volume blood increased from 59 to 70mL		8-11-2014
version 1.2	10-3-2014	Lab	Fixed several typographical errors		10-15-2014
version 1.2	10-3-2014	Lab	Glucose screening section and Appendix 5 are updated for the new glucometer		10-15-2014
version 1.2	10-3-2014	Lab	Changed the name from Annie Lukkari to Marcia Aaby on the shipping address		10-15-2014
version 1.2	10-3-2014	Lab	Clarified results reporting file transfer information		10-15-2014
version 1.2	10-3-2014	Lab	Updated Appendix 3 reference ranges		10-15-2014
version 1.2	10-3-2014	Lab	Added Appendix 8 - Certification checklist		10-15-2014
version 1.2	10-3-2014	Lab	Added Appendix 9 - Practical and Written exams		10-15-2014
version 1.2	10-15-2014	Lab	Reference to figure 1 in Section 2.2.4		NA
Version 1.2	10-28-2014	Lab	Formatting Edits and Shipping Instructions		10-31-2014
Version 1.3	12/18/2014	Lab	Figure 1. Seq# added to vials – page 9		1-15-2015
Version 1.3	12/18/2014	Lab	Section 3.5 Glucose Screening: updated to agree with or refer to Glucose monitoring Safety Protocol section 15.3 of HCHS SOL Manual 2 – pages 16-17		1-15-2015
Version 1.3	12/18/2014	Lab	Figure 2. correction made to tube #4 for daily shipment		1-15-2015
Version 1.3	12-18-2014	Lab	Change in page 29- Daily Shipping instructions		1-15-2015
Version 1.4	06-30-2015	Lab	Glucose cut point for OGTT is less than 150mg/dL	16,17	7-08-2015
Version 1.5	08-24-2015	CC	Fasting time specified by protocol is at least 8 hours	4	9-03-2015
Version 1.6	12-2-2016	Lab	Added Appendix 10- Biospecimen Collection During the Home Visit	59	12-07-2016
Version 1.6	12-2-2016	Lab	Updated 2- Equipment and Supplies- to include supplies needed for biospecimen collections during the home visit	43	12-07-2016

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Biospecimen Collection and Processing
Table of Contents

1. BIOSPECIMEN COLLECTION AND PROCESSING

The Hispanic Community Health Study – Study of Latinos Visit 2 (HCHS/SOL V2) renewal 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 six 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 14,850 persons of Hispanic origin from the visit 1 cohort, specifically Cuban, Puerto Rican, Mexican, and Central American, to be recruited through four Field Centers affiliated with San Diego State University, Northwestern University in Chicago, Einstein College of Medicine in New York, and the University of Miami. Additional academic centers will serve as scientific and logistical support centers.

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 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 V2 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 V2 study collects approximately 55-70 mL of blood from each participant. Eight tubes of blood are collected; the 8th tube as described below is for the 2 hr post glucose load collection. The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 4.5 tablespoons, although it may look like more to them. The technician may also assure participants that they donate almost 10 times as much blood (450 mL) when they donate a pint of blood.

2.1. Staff Certification Requirements

Blood drawing and processing are performed by a certified HCHS/SOL V2 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 V2 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 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 9 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.

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 used for testing lipids (fats) and other biochemical markers.

Tube #4 is a 4 mL lavender-stoppered tube containing EDTA anticoagulant. This tube will be used for CBC, differential and platelet count, and glycosylated hemoglobin.

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).

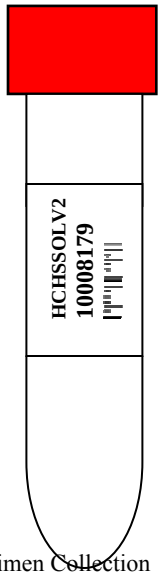
Tube #7 is a 4.5 mL blue-stoppered tube containing liquid sodium citrate anticoagulant. The plasma from this tube is used for coagulation studies. This tube must be filled completely in order to standardize the blood to liquid anticoagulant ratio. A partially filled tube will result in erroneous test results.

Tube #8 is a 4 mL lavender-stoppered tube containing EDTA anticoagulant. This tube will only be collected from participants who have the oral glucose tolerance test (OGTT). The plasma from this tube will be used for post OGTT glucose test and other analytical tests.

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 eight tubes are collected in the following order:

Tube #1:	9 mL red stoppered tube (Serum)
Tube #2:	9 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:	4.5 mL blue stoppered tube (Citrate)
Tube #8:	4 mL lavender stoppered tube (EDTA) (Collected 2 hr after glucola administration.)

3. Additional laboratory ID number labels will be used when the participant arrives to provide a documented match between their HCHS/SOL V2 participant ID number and the laboratory specimen ID number on the Biospecimen Collection form (BIO).

A number of HCHS/SOL V2 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 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 aliquotting 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 11 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.

13 – 2 mL polypropylene tubes (purple top)
1 – 2 mL amber polypropylene tube (purple top)
2 – 2 mL polypropylene tubes (blue top)
14 – 2 mL polypropylene tubes (red top)
1 – 2 mL amber polypropylene tube (red top)
3 – 2 mL polypropylene tubes (clear top)
3 – 2 mL polypropylene tubes (yellow top)
1 – 2 mL polypropylene tubes (green top)
1 – 2 mL polypropylene tubes (orange top)

2.2.6 Labeling

Vertically label the plastic sample aliquot tubes 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:	2 mL purple top *amber* tube; row B
Col 1:	2 mL purple top clear tubes; rows A, C, D
Col 2:	2 mL purple top clear tubes; rows A – D
Col 3:	2 mL purple top clear tubes; rows A – C
Col 4:	2 mL purple top clear tubes; rows A - C
Col 5:	EMPTY
Col 6:	2 mL blue top clear tubes; rows A – B
Col 7:	EMPTY
Col 8:	2 mL red top *amber* tube; rows B
Col 8:	2 mL red top clear tubes; rows A, C, D, E
Col 9:	2 mL red top clear tubes; rows A - E
Col 10:	2 mL red top clear tubes; rows A – E

Tray 2 (for stage 4 and urine processing):

Col 1:	2 mL clear top, clear tubes, rows A – C
Col 2:	EMPTY
Col 3:	2 mL yellow top clear tubes, row A - C
Col 4:	2 mL green top clear tube, row A
Col 5:	2 mL orange top clear tube, row A
Col 6 – 10:	EMPTY

All extra labels are returned to the Central Laboratory with the daily specimen shipment.

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	0.25 mL plasma, Tube #5 **SEQ. 3**	minimum 0.5 mL plasma, Tube #5 **SEQ. 12**	minimum 0.5 mL plasma, Tube #6 **SEQ. 16**	minimum 0.5 mL plasma, Tube #6 **SEQ. 19**	Empty	minimum 0.5 mL plasma, Tube #7 **SEQ. 7**	Empty	1.0mL serum, Tube #1 **SEQ. 5**	minimum 0.5 mL serum, Tube #1 **SEQ.28**	minimum 0.5 mL serum, Tube #2 **SEQ. 33**
B	plasma, **amber vial,** Tube #5 **SEQ. 9**	minimum 0.5 mL plasma, Tube #5 **SEQ.13**	minimum 0.5 mL plasma, Tube #6 **SEQ. 17**	minimum 0.5 mL plasma, Tube #6 **SEQ. 20**	Empty	minimum 0.5 mL plasma, Tube #7 **SEQ. 8**	Empty	serum, **amber vial,** Tube #1 **SEQ. 24**	minimum 0.5 mL serum, Tube #2 **SEQ. 29**	minimum 0.5 mL serum, Tube #2 **SEQ. 34**
C	minimum 0.5 mL plasma, Tube #5 **SEQ.10**	minimum 0.5 mL plasma, Tube #5 **SEQ.14**	minimum 0.5 mL plasma, Tube #6 **SEQ. 18**	minimum 0.5 mL plasma, Tube #6 **SEQ. 21**	Empty	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**
D	minimum 0.5 mL plasma, Tube #5 **SEQ.11**	minimum 0.5 mL plasma, Tube #5 **SEQ.15**	Empty	Empty	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**
E	Empty	Empty	Empty	Empty	Empty	Empty	Empty	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**

Aliquot Tray 2 Layout (Stage 4 and Urine Processing)

Col Row	1	2	3	4	5	6	7	8	9	10
A	0.25 mL plasma, Tube #8 **SEQ. 4**	Empty	1.0mL urine, neutral **SEQ.6**	1.5 mL urine, acid **SEQ. 40**	1.5 mL urine, alkaline **SEQ. 41**	Empty	Empty	Empty	Empty	Empty
B	minimum 0.5 mL plasma, Tube #8 **SEQ. 22**	Empty	1.5 mL urine, neutral **SEQ. 38**	Empty	Empty	Empty	Empty	Empty	Empty	Empty
C	minimum 0.5 mL plasma, Tube #8 **SEQ. 23**	Empty	1.5 mL urine, neutral **SEQ. 39**	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 properly labeled 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 container 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.
9. Perform and record QC checks for glucometer reagents and controls.

2.2.8 At Participant Arrival

1. Check that the participant's HCHS/SOL V2 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 V2 participant ID number, and the laboratory ID number on the blood collection tubes, aliquot containers, 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 safety questions (section A1 – A6) and the fasting question (section B) 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 V2 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 4-5 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). Before blood is collected, the participant is asked the following safety questions (section A, 1-5):

1. …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.
2. …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 15 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.
3. …if they have ever had a graft or shunt for kidney dialysis. If they have, blood should not be collected from the arm where this has occurred. Also, this participant is excluded from the OGTT.
4. …if they have diabetes. If they have been diagnosed with diabetes, they are excluded from the OGTT.
5. …if they have ever had part of their stomach or intestines removed. If yes, they are excluded from the OGTT.

If blood is to be drawn, complete the fasting blood collection information with the participant (section B, 7-8). Fill in date, time, and if the blood will be collected prior to glucola administration (section C, 9-11).

The participant should be seated during the blood draw. It is difficult to standardize the length of time that a person is in the sitting position prior to venipuncture, but to the extent possible attempt to have the participant be 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 14).

3.3. Venipuncture

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). Use a tourniquet to increase venous filling. This makes the veins more prominent and easier to enter. 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 7.3.5 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 tubes #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.

I. Venipuncture for OGTT (Tube #8): If an OGTT test is administered, a separate venipunture is performed 2 hours after the glucola was administered to collect Tube #8. (See section 3.6 OGTT.)

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: 9 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 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 #4: 4 mL lavendar-stoppered tube contains EDTA anticoagulant. Invert gently 8 times immediately after collection. Store this tube in the refrigerator (2 – 8° C) until same-day shipment to the Central Laboratory on refrigerated gel pack.

Tube #5 and #6: 10 mL lavendar-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: 4.5 mL blue-stoppered tube contains sodium citrate anticoagulant. Invert gently 8 times immediately after collection. Place the tubes in room temperature rack until centrifugation at 15º C. (These tubes can be placed under the box, but do not require protection from light.)

Tube #8: 4 mL lavender-stoppered tube contains EDTA anticoagulant. Immediately after collection gently invert tube 8 times. Place the tube in room temperature rack until centrifugation. (This tube does not require protection from light.)

3.5. Glucose Screening

If the participant is eligible for the OGTT based on answers to safety questions A3-A5 and fasting status B7-B8 on the Biospecimen Collection form, then a glucose screen test must be performed and recorded in A6 in order to insure that the OGTT is administered only to participants with a glucose less than 150 mg/dL. For detailed instructions on use, maintenance, and quality control of the glucometer, see Appendix 5.

1. Insert a test strip into the test strip guide. Meter beeps twice when strip is in the correct position.
2. Lift measurement chamber flap.

3. Use Tube #5 (10 mL EDTA tube) for the glucose screening test. Make sure that tube #5 is mixed well by gently inverting it 12 times; then remove the stopper.

4. Using a disposable transfer pipet, remove a small amount of blood and place a drop onto the yellow target area of the test strip. **To assure accurate measurement, fill entire yellow application area with blood.**

5. Close the measurement chamber flap to start glucose measurement. A long beep sounds when the measurement is complete.

6. Record the glucose reading from the meter on the Biospecimen Collection form under item A.6. If the meter reading is "HI", enter 600 on the Biospecimen Collection form.

7. Remove the strip and discard in the biohazard waste container. Replace the cap onto the collection tube for centrifugation.

8. If the reading is less than 150 mg/dL, and the participant is not diabetic and has not been excluded from the OGTT based on safety questions A4- A5 and fasting status based on item B.8, then glucola can be administered. Skip to section 3.6.

9. If the reading is equal to or greater than 150 mg/dL, the participant will not be allowed to do the OGTT procedure. Explain according to the script and enter result on the form.

10. If the reading is 200-399 mg/dL, proceed as follows:

    a. Ascertain symptoms of hyperglycemia (follow script on thirst, frequent urination, dizziness, active infection, or blurred vision). If symptoms(s) are present, contact the clinic manager to refer participant to the emergency room.
    b. Measure urine for ketones (see Appendix 10 for a detailed procedure). If the urine dipstick is positive for ketones, contact the clinic manager to refer participant to the emergency room. If the urine dipstick is negative for ketones, make a note on the Clinic Check off Sheet so that during the exit interview the participant can be referred to a health care provider or a referral physician to be evaluated within one week.

11. If the reading is equal to or greater than 400 mg/dL, STOP the examination and contact the clinic manager to refer the participant to the emergency room (regardless of the presence of symptoms).

12. If the glucose reading is less than 70 mg/dL, follow the Glucose Monitoring Safety Protocol found in section 15.3 of the HCHS/SOL Manual 2, Field Center Procedures for Visit 2

3.6. OGTT

3.6.1 Glucola Administration

The preferred means of serving the glucola drink to the participant is to remove the cap and serve the bottle with a straw. If requested by the participant, the contents can also be poured into a paper cup and served with or without a straw. Participants are instructed to consume the contents of the container in less than 5 minutes. Most individuals consume the full amount in 3 to 5 minutes quite easily.

The timing for the 2 hour post load venipuncture begins as soon as the participant starts to drink the glucose solution. The time the participant began drinking the glucola is recorded in Item 24 of the Biospecimen Collection form (Appendix 3). The time the participant should have the 2 hour post load venipuncture is then recorded on the Clinic Check List.

Study participants are encouraged to drink the full amount of glucola; otherwise they will not get the full benefit of the test. If the individual does not consume the full amount of glucola, the technician measures the residual amount and records it in Item 23 of the Biospecimen Collection form. The measurement of the residual glucola is not necessary if only a few drops are left. If the residual amount is 145 mL or more, the 2 hour blood draw is NOT performed, and Item 24 of the Biospecimen Collection form is completed accordingly. Based on the experience in many epidemiologic studies in the U.S. and elsewhere, this should be a very uncommon event.

3.6.2 Two Hour Post Glucose Load Venipuncture

The two hour blood sample is obtained for measurement of glucose two hours after the start of the test. The blood sample is drawn as close to the two hour time as possible. The phlebotomist records that the post-load blood glucose sample was drawn, and the actual time it was drawn (items 24 and 25 of the Biospecimen Collection form).

Every scheduling effort is made to allow participants to go to the venipuncture work station for the 2 hour blood sample. The Clinic Check List needs to be checked frequently as a guide to scheduling interviews and procedures, especially towards the end of the examination. In a complex field center examination such as the HCHS/SOL V2, it is likely that some participants will be busy with other parts of the examination. If the participant is available within a 10 minute window of the scheduled 2 hour post-load venipuncture, the overlapping interview or procedure does not need to be rescheduled or interrupted. However if the 2 hour blood sample is due and the participant cannot come to the venipuncture work station within the 10 minute window, the phlebotomist, if possible, goes to the participant to obtain the sample at the examination or interview station.

3.6.3 Snack

OGTT study participants should neither drink nor eat anything in the period between the glucose administration and the 2-hour blood draw. After the post-load venipuncture, participants are given the regular snack. Study participants will have been asked at the reception station whether on advice of their physician they can postpone taking the medications they usually take first

thing in the morning until they have their 2 hour post-load Venipuncture. If that is the case, this will be noted on the Clinic Visit Check List and participants are then assisted in retrieving their medications during the snack break.

3.6.4 Documentation of Side Effects

If participants complain of any problems during the test, they should be reported to the Study Coordinator or the Study Nurse and documented on the field center's Incident Log. Based on many previous studies similar to HCHS, side effects are quite infrequent, and vomiting was reported only on 0.1 percent of tests (diarrhea is not a side effect of the OGTT). However, if vomiting has occurred, the 2-hour blood draw should not be done. In this case, Item 24 of the Biospecimen Collection form is completed accordingly and the reason for the incomplete test recorded in Item 23 of the Biospecimen Collection form.

3.6.5 Readiness for Emergencies

Field centers keep on hand orange juice or equivalent, sugar-containing beverages at all times.

Participants with known or undiagnosed diabetes may develop low blood sugar or an "insulin reaction". If recognized promptly by clinic staff, it should be mild and easily treated with orange juice or a similar sugar containing beverage.

Hypoglycemia, or an abnormally low blood glucose level, occurs when there is an imbalance between the dose of hypoglycemic medications (in the treated diabetic) or the blood sugar level (in any person) and the person's food intake and activity level. However, treated diabetics are excluded from the OGTT. Classic symptoms include anxiety, tremor, palpitations, sweating, faintness, and hunger. If untreated, a further decrease in blood glucose may lead to confusion followed by loss of consciousness. Prolonged hypoglycemia may precipitate angina pectoris or seizures.

It is important to remember that symptoms of hypoglycemia are variable and may be partially masked in older participants.

If a person displays any of these symptoms after ingesting the glucola and is able to take food orally, orange juice containing additional sugar should be given immediately and the clinic physician notified as soon as possible. If orange juice or other forms of sugar has been administered a two-hour blood specimen should not be drawn. This is communicated to the participant and recorded on the itinerary form / checklist, and the reason for the incomplete procedure is recorded in Item 23 of the Biospecimen Collection form and Item 24 is completed accordingly. When a hypoglycemic reaction occurs, the person is evaluated by medical staff prior to leaving the field center.

If an OGTT participant loses consciousness, hypoglycemia should be presumed until ruled out. Severe hypoglycemic reactions are a medical emergency and the person should be transported immediately to an emergency care facility.

4. BLOOD AND URINE PROCESSING

4.1 Stage One: Immediate Processing

After completion of 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 4.2 "Operating the Centrifuge"). Spin these tubes at 3,000 x *g* for 30 minutes at 15° C. Record on the Biospecimen Collection form the time at which these tubes began to spin.

3. Place tube #4 in refrigerator until daily shipment on refrigerated gel pack to the Central Laboratory.

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. All other rules regarding the safe blood specimen handling must also be observed.

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 22 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 23 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 serum or 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. Tubes # 5, and #6: 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 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. Place approximately 0.25 mL of plasma into the first clear 2 mL vial in position A1 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 B1 and six clear 2 mL vials in positions C1 through D1 and A2 through D2 of the sample aliquot tray. Process tube #6 similarly, distributing the plasma equally into six 2 mL clear vials in positions A3 through C3 and A4 through C4.

3. Fasten the lavender screw caps onto the vials in columns 1, 2, 3, and 4 and place them in the cup with ice slush.

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

5. 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 2 mL vials in positions A6 through B6 of the sample aliquot tray 1. (Use the "0.5 mL template" vial as a guide for an approximate 0.5 mL minimum volume.) 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.

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. (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 stopper.

3. Using a plastic transfer pipet, withdraw serum from tube #1. Place approximately 1.0 mL of serum into the 2 mL vial in position A8 of the sample aliquot tray. (Use the "1.0 mL template" vial as a guide.) Distribute the remaining serum equally into one amber vial in position B8 and four 2 mL clear vials in positions C8, D8, E8, and A9 of the sample aliquot tray. Remember to withdraw only the clear serum; if lipids are present on top begin aspirating from below that layer. For tube #2, distribute the serum equally into the six 2 mL vials in positions B9, C9, D9, E9, A10, and B10 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 three 2 mL vials in positions C10, D10, and E10 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.

Remove the purple-capped vials from the cup with ice slush, dry them with a paper towel, and place them into positions in columns 1, 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.

4.4. Stage Four: Processing of OGTT Plasma

Stage four will only be needed for participants who qualify and agree to take an OGTT. In this case, tube #8 is collected 2 hours after glucola was administered.

1. Within 15 minutes of collection, place tube #8 in the centrifuge. Balance the tube as described in 4.3 "Operating the Centrifuge"; then spin this tube at 3,000 x *g* for 10 minutes at 15° C. Remove the tube from the centrifuge and place in position 1A of aliquot tray 2.

2. Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear plasma supernatant from tube #8. 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. Place 0.25 mL of plasma into the 2 mL clear vial in positions A1 and distribute the remaining plasma equally into each of two 2 mL clear vials in positions B1 and C1 of sample aliquot tray 2. Fasten the clear screw caps onto these vials and place them in the freezer in an upright position in aliquot tray 2, row A with the other aliquots from this participant.

3. Re-stopper tube #8 and discard in a biohazard waste container.

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 6 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.

Prior to processing, the laboratory staff records whether a urine sample was obtained and transcribes the collection time of the urine void from the ID label onto each participant's Biospecimen Collection form (Appendix 3, Items 27, 28, and 29).

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

Refrigerated urine samples need to be processed and frozen as soon as possible, and within 12 hours of collection. A comment is placed in Item 23 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 four 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 the date and time of collection, the time of processing, and the processing technician's code on the Biospecimen Collection form (items 28, 29, 30, 31, and 32).

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

    a. Neutral urine aliquots (3): Using the plastic transfer pipet, place 1.0 mL of urine in the vial in position A3 and 1.5 mL in the vials in position B3 through C3 of the second sample aliquot tray. (Use the "1.0 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 column 4 of the second sample aliquot tray. Using the MLA D-Tipper Pipetter, add 30 uL of 6 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 column 5 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 to aliquot tray 2, row A in 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 four 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 two vials and comment in item 23 of the Biospecimen Collection form that the volume was insufficient for acid and alkaline aliquots.

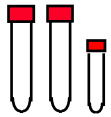
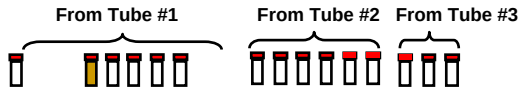
4.5.3 Procedures for Urine Samples Contaminated with Blood

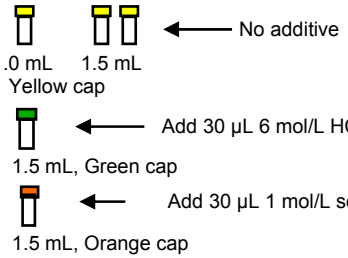
Although urine samples contaminated with blood will affect the measurement of albumin, these specimens should not be thrown out. All urine samples collected from participants that have adequate volume for processing are kept, including those that are (appear to be) contaminated with blood. If a urine 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 23 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 2** 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	STAGE 4 2 hr post OGTT	STORAGE
Tubes #1, #2, & #3  9-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 1.0 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 three 2-mL vials with red caps. Keep covered with box at room temperature until put in freezer.  1.0 mL Equally distribute (0.5 mL minimum)		Store vials at -70°C
Tubes #4  4-mL lavender top tubes (plasma) Invert 8 times	Place in refrigerator (2-8°C) until shipped same day				Store in refrigerator (2-8°C) until shipped same day
Tubes #5 - 6  10-mL lavender top tubes (plasma) Invert 8 times; Place in ice water; Cover with box	**Glucometer:** Mix tube #5 12 times, remove stopper, pipet drop for glucometer strip, restopper; **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.  0.25 mL Equally distribute (0.5 mL minimum)			Store vials at -70°C

BLOOD COLLECTION	STAGE 1 immediate processing	STAGE 2 30 min post-collection	STAGE 3 30 min post-collection	STAGE 4 2 hr post OGTT	STORAGE
Tube **#7**  4.5-mL blue top tube (plasma) Invert 8 times	Room temp rack; centrifuge at 15° C for 30 min at 3000 *x g*	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)			Store vials at -70°C
Tube **#8**  4-mL lavender top tube (plasma) Invert 8 times Draw 2 hr post OGTT				Centrifuge at 15° C for 10 min at 3000 x *g;* Place 0.25 mL plasma in one vial and distribute remaining equally into two 2-mL vials with clear caps.  0.25 mL Equally distribute (0.5 mL minimum)	Store vials at -70°C
Grab urine collection 	Refrigerate within 4 hr; Process within 12 hr; Invert container 8 times; transfer ~ 1.0 mL urine into one vial and 1.5 mL into four 2-mL vials.	 1.0 mL 1.5 mL Yellow cap ← No additive 1.5 mL, Green cap ← Add 30 µL 6 mol/L HCl 1.5 mL, Orange cap ← Add 30 µL 1 mol/L sodium	Freeze within 12 hr of collection		Store vials at -70°C

Shipping to the Central Laboratory: Vials stored at -70°C → weekly on dry ice; Tube # 4 → daily, refrigerated
(Complete HCHS Shipping Forms, Face sheet and Contents sheet, and include in dry ice shipment.)

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 aliquotted 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. Record the time that the aliquots are placed in the freezer on the Biospecimen Collection form.

5. PACKAGING AND SHIPPING

Packaging and shipping instructions for refrigerated tube #4 (4 mL fasting EDTA) shipped daily on the day of collection (See Figure 3.)

5.1. Instructions for Refrigerated Specimens (not frozen)

1. Check to be sure that each tube is properly labeled. Wrap each tube in paper toweling to cushion it and place in a 4" x 6" storage bag. (One to three patients per bag.) Include an absorbent square in each bag.

2. Place bag into a small Styrofoam cooler with a refrigerated gel pack (keep gel packs at 2-8° C in the Styrofoam cooler) to keep the samples cold (not frozen) during shipment. Place bag with sample(s) on top of refrigerated gel pack. Place the Styrofoam cooler into its cardboard sleeve. **Note: One or more refrigerant packs should be stored refrigerated at 2-8° C, preferably inside the open foam box, so they will be ready when needed for shipment. Place the refrigerant pack into the refrigerator at least one day prior to shipping; it must be cold at the time of shipment. DO NOT FREEZE the refrigerant packs.**

3. Shipping boxes received from the Central Laboratory will have a "Biological Substance Category B UN 3373" label affixed to outside of the box. If the shipping box does not have this label on the outside, contact the Central Laboratory.

4. Place shipping box in orange, plastic "FedEx UN 3773 Pak" mailing bag. Note: Two shipping boxes may be placed inside the mailing bag if shipping more than one participant in a day.

5. Insert the original Laboratory Specimen Collection Form for the participant into a 12" x 12" plastic bag and place it inside the orange, plastic "FedEx UN 3773 Pak" mailing bag. (Keep a copy of the Laboratory Specimen Collection Form for your files.)

6. The Central Laboratory will supply the HCHS/SOL V2 clinical sites with pre-printed FedEx billable stamps.

7. Record the clinical site number, address, and telephone number in section 1 of the FedEx Billable Stamp. Peel off the right side of the FedEx Billable Stamp and affix it to the

outside of the orange, plastic "FedEx Clinical Pack" mailing bag. The left side of the form may be kept for your records. Contact Federal Express (1-800-GO-FEDEX) for pickup the same day that the samples are collected. Some clinical sites may have a scheduled FedEx pick up; this will vary from site to site. The packages may also be mailed at FedEx drop-off locations that will accept UN 3373 Pak shipments.

8. 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.

9. The Central Laboratory will check the "FedEx Insight Tracking Log" daily to view what HCHS/SOL V2 packages should be arriving. However, only those packages actually picked up and scanned into the FedEx system will appear on this log.

10. See Figure 3 below for specific visit shipping diagrams.

Note: All shipping containers are sent to the HCHS/SOL V2 Central Laboratory by overnight courier 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:

The CBC tube specimens refrigerated at 2-8° C should be shipped daily except for specimens collected on Sunday. The Sunday specimens can be refrigerated overnight (not frozen) until Monday when they can be sent to the Central Lab for Tuesday delivery. The daily CBC tubes collected on Saturdays must be sent out the same day for Monday delivery shipped on refrigerated gel packs (not dry ice and not frozen packs).

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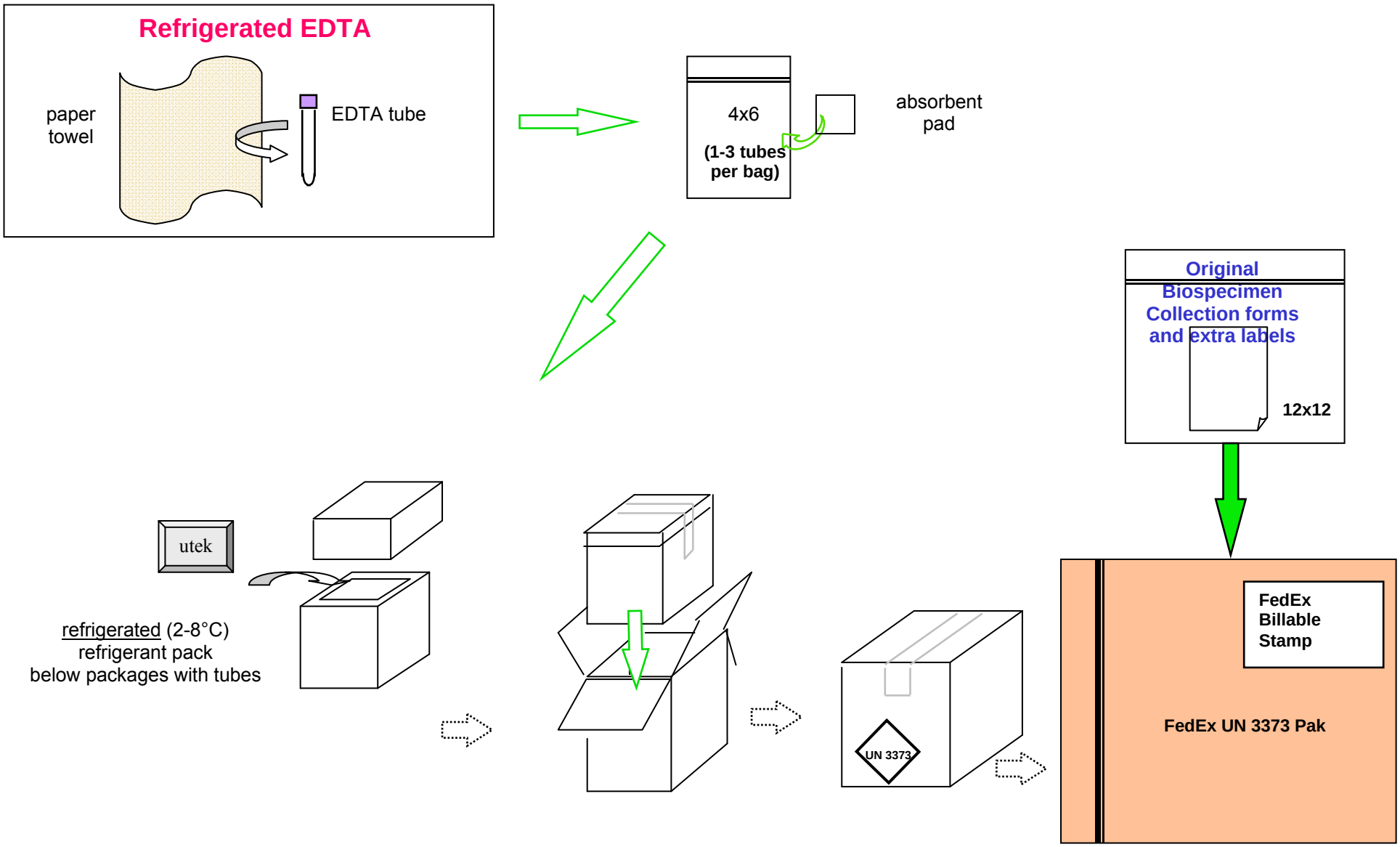
University of Minnesota, Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415
Telephone: (612) 625-5040
Main Fax: (612) 625-4142 (Laboratory)
Alternate Fax: (612) 625-4831 (Business Office)

Figure 3. Refrigerated Sample Packaging



5.2. Storage, Packaging and Shipping (For Frozen Specimens)

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 serum, plasma, and urine samples are packaged in freezer storage bags according to their specimen type.

5.2.1 Packaging Frozen Specimens

Place fourteen of the red-capped serum vials into one 4" x 6" storage bag, thirteen of the purple-capped plasma vials into another 4" x 6" storage bag. Place the 1.0 mL red-capped serum vial, the 0.250 mL purple-capped vial, the 0.250 mL clear capped vial, and the 1.0 mL yellow-capped vial into a third 4" x 6" storage bag. Place the two blue-capped plasma vials into a fourth 4" x 6" storage bag, the two clear-capped OGTT plasma into a fifth 4" x 6" storage bag and the remaining four urine vials into a sixth 4" x 6" storage bag. Check again to make sure all vials/tubes 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 12" x 12" bag. Place the Lab ID# label for that set of aliquots on a piece of paper and insert it into the 12 x 12 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 sera, plasma, and urine are packed and shipped in Styrofoam boxes. Packaging instructions (See Figure 4) are as follows:

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

2. Put one-half of the12"x 12" bags of sample vial/tubes into the Styrofoam box on top of the dry ice.

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

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

5. Layer more dry ice on top of and around the sample bags. The amount of dry ice in the shipping should total at least 5 pounds.

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 12" x 12" 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 V2 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 4 below for shipping diagram.

5.2.2 Shipping Frozen Specimens

The samples remain in their Styrofoam box at -70° C until they are shipped. All frozen 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 V2 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 V2 Central Laboratory by overnight courier 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:

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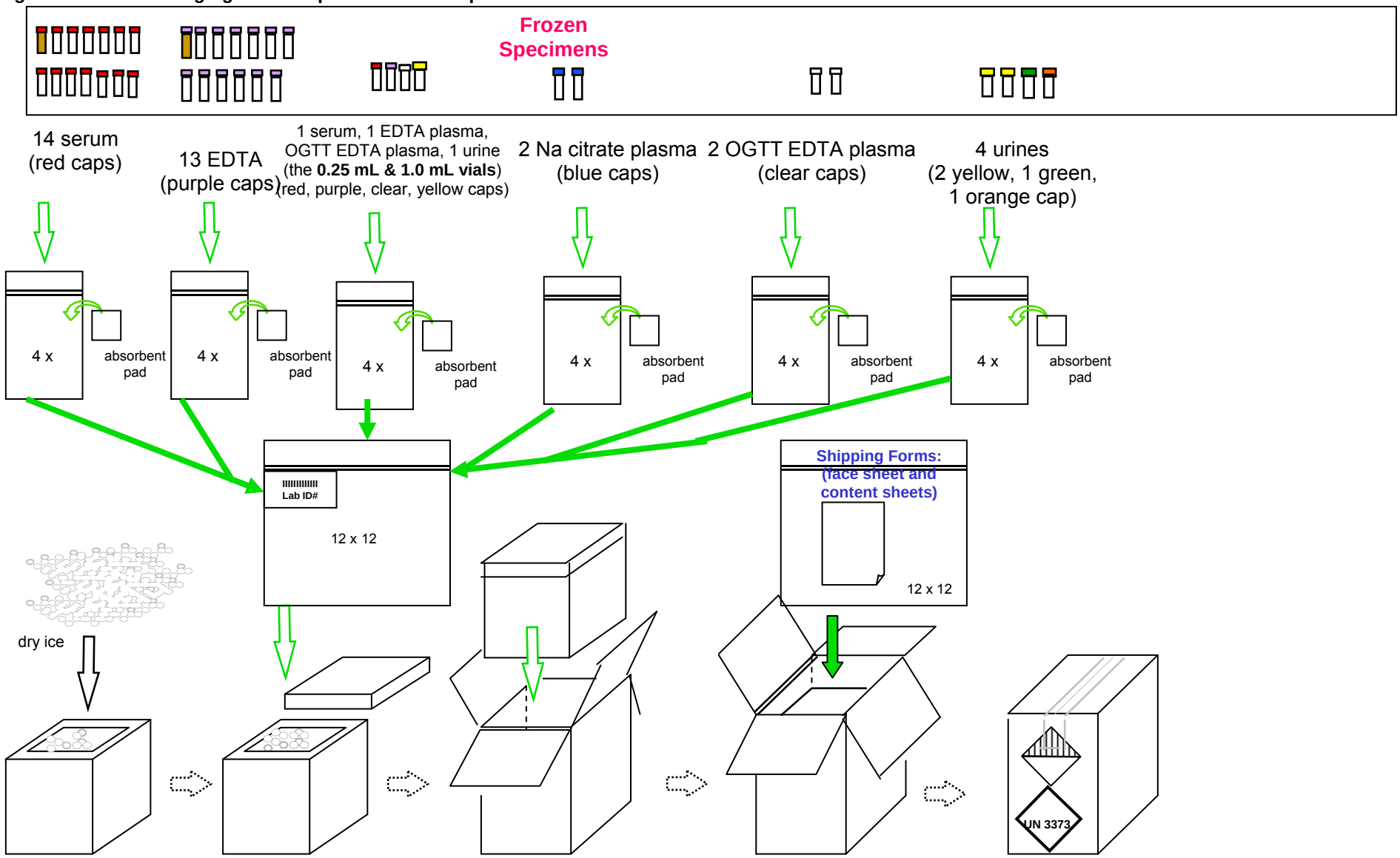
University of Minnesota, Advanced Research and Diagnostic Laboratory (ARDL)
1200 Washington Ave S, Suite 175
Minneapolis, MN 55415
Telephone: (612) 625-5040
Main Fax: (612) 625-4142 (Laboratory)
Alternate Fax: (612) 625-4831 (Business Office)

HCHS SOL V2 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 V2 Clinical Sites or posted on the study website in advance of upcoming holidays.

Figure 4. 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, glucometer, and centrifuge. Daily and monthly measurements (e.g., temperatures) are recorded on a log, as described below. The other aspect of quality control is documentation of problems with blood collection and processing which is part of each participant's record. (See Appendix 3, Items 11, 12, 19, and 20, Biospecimen Collection form.)

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

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

Daily, log the temperatures of the laboratory, all refrigerators, freezers, and refrigerated centrifuges (Appendix 5), and run the glucometer controls (Appendix 9). 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 V2 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 6) that is completed by Field Center technicians.

If extra QC blood is drawn for a tube that is processed for weekly shipment (Tubes #1, 2, 3, 5, 6, 7, or 8), the aliquots are stored at the Field Center for an extra week and then sent to the Central Laboratory with a regular shipment. If extra QC blood is drawn for a tube that is processed for daily shipment (Tube #4), the tube is sent to the Central Laboratory with the regular daily shipment.

The QC blood samples are collected in sequential order (cycling back to Tube #1 after QC Tube #8 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 and #8; 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 & 8	Na Citrate & OGTT plasma	_____
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. However, this should not be done for tube #4 which is sent to the Central Laboratory in the collection tube.

The QC tubes are set in the same rack used to hold the regular blood collection tubes, in a separate row from the other tubes.

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

For the duplicate urine sample, four extra tubes 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 the first 6-7 participants of the day. 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 tubes are put into the -70° C freezer (for a minimum of 30 minutes) with the exception of QC tube #4. QC tube #4 is placed in the refrigerator and shipped the same day with its matched participant pair. 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 four 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 6), 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.

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 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 V2 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 CBC, differential, and platelet count results will be available in 4-5 days and all other test results will be available in approximately 3-4 weeks after the sample is collected.

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 V2 technicians must know and be prepared to demonstrate is listed in Appendix 8. The technician must study the HCHS/SOL V2 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 V2 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 V2 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 only after the 2 hour OGTT blood collection (tube #8) has been collected. Menus are locally determined.

Appendices

Appendix 1 Laboratory Tests, Reference Ranges, and Alert Values

Test Name	Reference Range	Units	Alert Value
*Hemogram (CBC):			
White Blood Count (WBC)	4.0-11.0	x 10^9/L	<2 and >25
Red Blood Count (RBC) – male	4.4-5.9	x 10^{12}/L	
Red Blood Count (RBC) – female	3.8-5.2	x 10^{12}/L	
Hemoglobin - male	13.3-17.7	g/dL	<8 and >20
Hemoglobin - female	11.7-15.7	g/dL	<8 and >20
Hematocrit – male	40.0-53.0	%	
Hematocrit – female	35.0-47.0	%	
Mean Corpuscular Volume (MCV)	78-100	fL	
Mean Corpuscular Hemoglobin (MCH)	26.5-33.0	pg	
Mean Corpuscular Hemoglobin Concentration (MCHC)	31.5-36	g/dL	
Red Cell Distribution Width (RDW)	10.0-15.0	%	
*Platelet Count	150-450	x 10^9/L	<50 and >1000
WBC Differential:			
Neutrophils	40-75	%	
Lymphocytes	20-48	%	
Monocytes	0-12	%	
Eosiniphils	0-6	%	
Basophils	0-2	%	
Immature granulocytes	0 - 0.4	%	
Absolute Neutrophils	1.6-8.3	x 10^9/L	
Absolute Lymphocytes	0.8-5.3	x 10^9/L	
Absolute Monocytes	0-1.3	x 10^9/L	
Absolute Eosiniphils	0-0.7	x 10^9/L	
Absolute Basophils	0-0.2	x 10^9/L	
Absolute Immature Granulocytes	0 – 0.4	x 10^9/L	
*Total cholesterol	<200	mg/dL	>360
*Triglycerides	<150	mg/dL	>1000
*HDL-cholesterol	>40	mg/dL	<20
*LDL-cholesterol, calculated	<129	mg/dL	>260
*Glucose, fasting	60-99	mg/dL	<50 and ≥400
Glucose, post OGTT	50-139	mg/dL	
*Glycosylated Hemoglobin	4.3-6.0	%	
Insulin, fasting	12-150	pmol/L	
Alanine aminotransferase (ALT), male	0-40	U/L	
Alanine aminotransferase (ALT), female	0-31	U/L	
Aspartate aminotransferase (AST), male	0-37	U/L	
Aspartate aminotransferase (AST), female	0-31	U/L	
Gamma-glutamyltransferase (GGT), male	11-51	U/L	
Gamma-glutamyltransferase (GGT), female	7-33	U/L	
Cystatin C	0.51-1.05	mg/L	
*Creatinine, male	0.5-1.2	mg/dL	>2.0
*Creatinine, female	0.4-1.1	mg/dL	>2.0
*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/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
- Vacutainer Tubes 100/pk
  - Serum, red top, 9 mL
  - Serum, red top, 5 mL
  - EDTA, lavender top, 10 mL
  - EDTA, lavender top, 4 mL
  - Sodium citrate blue top, 4.5 mL

FedEx mailing bags
FedEX pre-printed shipping labels
Dry ice shipping labels
Category B UN 3373 label
Glucose strips and controls for screening
Glucose Meters

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
Cardinal Health	KC6818	Alcohol Swabs 200/pk; 4,000/cs
Cardinal Health	KC9132A	Gauze Sponges 200/pk
Cardinal Health	JJ5644	Band Aids 100/pk
Cardinal Health	367203	Tourniquets, Latex free, 25/pk
Cardinal Health	364815	Vacutainer Tube Holders 1000/cs BD #364815
Cardinal Health	P5214-12	Transfer Pipettes 500/pk
Cardinal Health	SBE2R46A	Ziplock Freezer Bags 4" x 6" 100/pk, 5000/cs
Cardinal Health	MGRL2P1212	Ziplock Freezer Bags 12" x 12" 1000/cs
Heathrow Scientific	HS21645A	Polyester Foam Tube Rack, 50 wells, 6/pk
Cardinal Health	C3521-01	Polyester Foam Tube Rack, 50 wells, 6/pk, #0010
Cardinal Health	CAH1612043	PDI Ammonia Inhalant
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)
Polyfoam Packers/ Fischer Scientific ThermoSafe	601 or 0352831	Styro specimen mailer (6 x 3 x 2.5 in.) (daily refrigerated shipments)
Polyfoam Packers/ Fischer Scientific ThermoSafe	602 or 035256	Cardboard sleeve for specimen mailer
Polyfoam Packers/ Fisher Scientific ThermoSafe	429 or 035286	UTEK gel pack, 8 oz.
Cardinal Health	M1067-6	UTEK gel pack, 8 oz. Dry Ice (approximately 5-10 lbs. per shipment) Packing Material
Cardinal Health	P5065-30 (30uL), #1143C	MLA D-Tipper Pipetter, Fixed Volume pipetter
Cardinal Health	P5064-902	MLA pipette tips, #9025 3M, Scotch brand 3750 clear packaging tape
Fisher Scientific	SA56-500	6.0 N HCL, 500 mL
Fisher Scientific	SS148-1	1.0 N sodium carbonate, 1L
Fisher Scientific	BC00515	Chemstrip K (ketone strip), 100/vial
Fisher Scientific	02-675-275	Diascreen 1k (ketone strip), 50/vial
Fisher Scientific	23-029-375	Sentry Urinalysis Control, 2 levels x 25 mL each
Fisher Scientific	02-675-289	Diascreen Liquid Urine Control, 2 x 12 mL each
Fisher Scientific	14-827-122	Point-of-use sharps container (home visit collections)
Fisher Scientific	22-131-401	Benchtop absorbent mat (home visit collections)
Fisher Scientific	19-120-2484	Paper towels (home visit collections)
		Small plastic container to hold ice (home visit collections)
		Small hand held cooler (home visit collections)

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 and 4 mL EDTA tubes prior to shipping. Refrigerator also used to store gel packs for daily refrigerated shipments.

Appendix 3 Partial Biospecimen Collection Procedure

Participant Sample Set

1. 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 #15 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 #15 of the Biospecimen Collection Form that the biospecimen set on this participant is a partial collection and no other specimens will be obtained and proceed to shipping.

2. Send Tube #4 (daily shipment) to the Central Laboratory whether or not some or all of the other biospecimen collection tubes/urine cannot be obtained. (If tube #4 was not collected, send the Biospecimen Collection Form completed as describe in #1 above in the daily shipment.)

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, including Tube #4.

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. Tube #4 was already sent to the laboratory on the day of the first visit. 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 #15 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 and send Tube #4 and the new Biospecimen Collection Form to the laboratory. Insert a comment on item #15 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 #15 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 50% full (2 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. Tube #8 is acceptable if it is at least 50% full (2 mL).

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

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 is sent with the daily refrigerated samples.)

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 Agency 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 2 SHIPPING FORM
PART ONE (To be completed at Field Center)

TO: HCHS/SOL V2 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 SPECIMENS 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 2 SHIPPING FORM Page ____ of _____

HCHS/SOL V2 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:

2-blue top microvials	4-(1)1.0 mL red, (1)0.25 mL purple, (1)0.25 mL clear, (1)1.0 mL yellow
13-purple top microvials	2-clear top microvials
14-red top microvials	4-urine microvials (2-yellow, 1-green, 1-orange)

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

[Place Lab ID label here]

Appendix 5 Instructions for Use of Glucose Meter

(Refer to Roche Accutrend Plus Owner's Booklet for detailed instructions)

1. Maintenance
   a. Perform once every 24 hours (at end of day) on days when the meter is used.
   b. Always turn the power off before cleaning the meter.
   c. Remove test strip guide. Rinse under warm running water. Dry with a lint-free tissue.
   d. Clean optical measuring system with a lightly moistened cotton swab. Allow to air dry.
   e. Document cleaning on QC record sheet.

2. Perform QC Test
   a. Record test strip lot number, code and expiration date on the QC record sheet. Also record lot number and expiration date of controls.
   b. Analyze controls daily in A.M. before specimens are run. Also analyze controls after changing the battery, when test result conflicts with clinical symptoms, when trouble shooting the system, or when starting strips with a different code number.
   c. Turn meter on by pressing the on/off button.
   d. Check that the code number displayed matches the strip code number. If code is incorrect, change code using the code strip included with each bottle of test strips. See manual for instructions.
   e. Press the M button to make the control bottle icon appear. This flags the result as a control.
   f. With the square yellow pad up, insert test strip all the way until it stops.
   g. Lift the measurement chamber flap.
   h. Gently shake control bottle and apply one large drop to the yellow square of test strip.
   i. Close the measurement chamber flap to start glucose measurement.
   j. Record the control result on the QC sheet. Check that result is in range. If out of range, repeat.
   k. Repeat steps c-j with other control.

3. Perform Patient Test.
   a. Turn meter on by pressing on/off button.
   b. Check that the code number displayed matches the strip code number.
   c. Remove test strip from bottle. Tightly recap test strip bottle to prevent damage and false results.
   d. Insert a test strip into the test strip guide. Meter beeps twice when strip is in the correct position.
   e. Lift measurement chamber flap.
   f. Mix the tube of blood (Tube #5) by inverting 12 times and remove the lavender stopper.
   g. Using a plastic dispo pipette, place a large drop of blood from the stopper onto the yellow square of the test strip. To assure accurate measurement, fill entire yellow application area with blood.
   h. Close the measurement chamber flap to start glucose measurement.
   i. A long beep sounds when the measurement is complete. Record result on the Biospecimen Collection form (Item A4). If glucose is ≥150 mg/dL, exclude this participant from the OGTT.
   j. Meter will display **Hi** if results >600 mg/dL. If this occurs, record 600 on the form.
   k. Meter will turn itself off if not used for 2 minutes. Press and hold on/off button to turn meter off.

4. Reagents/Supplies
   a. Roche Accutrend Plus Blood Glucose Monitoring System and Owner's Booklet.
   b. Roche Accutrend Glucose Test Strips. Store test strips tightly capped in original bottle in a cool, dry place at room temperature (below 30°C). Protect from heat and direct sunlight. Do not refrigerate or freeze. Discard 4 months after opening or after expiration date printed on bottle label.
   c. Roche Accutrend Glucose Control Solutions. Store at room temperature (below 30°C). Do not refrigerate or freeze. Discard 3 months after opening or after expiration date printed on vial label.

Appendix 5 (cont) Glucose Meter QC

Glucometer serial number _____

Roche Accutrend Test Strips
Lot # _____
Code # _____
Expiration date _____
Opened date _____
Discard Date (4 mo after opening)_____

Roche Accutrend Control Solution Low
Lot # _____
Expiration date _____
Opened date _____
Discard Date (3 mo after opening)_____
Acceptable range _____

Roche Accutrend Control Solution High
Lot # _____
Expiration date _____
Opened date _____
Discard Date (3 mo after opening)_____
Acceptable range _____

Date	Battery Check	Strip Code	Control Low	Control High	Daily Cleaning Test Strip Guide	Tech Code

Appendix 6 Urine Ketone Procedure

1. Pour 1 mL of well-mixed, room temperature urine into a tube.

2. Completely immerse the entire reagent area of the strip in the urine aliquot. Remove the reagent strip immediately. While removing, run the edge of the reagent strip against the side of the tube to remove excess urine.

3. Hold strip close to the color block on the strip container.

4. Read the test at 15 sec for the Chemstrip K or at 60 sec for Diascreen 1K strips

5. Perform a normal and abnormal QC strip each day that a ketone test is performed on a participant.

6. Reagents/Supplies:
   a. Ketone strip (either strip may be used):
      1) Chemstrip K (ketone strip), 100/vial from Fisher Scientific, part #BC00515. This strip has a shelf-life of up to 2 years opened or unopened.
      2) Diascreen 1K (ketone strip), 50/vial from Fisher Scientific, part #02-675-275. This strip has a shelf-life of 18 months opened or unopened.

   b. QC material
      1) Sentry Urinalysis Control, 2 levels x 25 mL each from Fisher Scientific, part #23-029-375. This material may be used with either Chemstrips or Diascreen strips and can be used until the expiration date on the package if stored at 2-8° C when not in use.
      2) Diascreen Liquid Urine Control, 2 x 12 mL each from Fisher Scientific, part #02-675-289. This material should only be used with Diascreen strips and expires 90 days after the bottle is opened. Unopened bottles have a typical expiration date of up to 1 year.

Appendix 6 cont…Urine Ketone QC

7. Quality Control:

Ketone Test Strips:
Chemstrip K or Diascreen 1K (circle one)
Lot # _____
Expiration date _____
Opened date _____

Normal Control
Sentry or Diascreen (circle one)
Lot # _____
Expiration date _____
Opened date _____
Discard Date (if Diascreen)_____
Acceptable range_____

Abnormal Control
Sentry or Diascreen (circle one)
Lot # _____
Expiration date _____
Opened date _____
Discard Date (if Diascreen)_____
Acceptable range _____

Date	Normal Control	Abnormal Control	Tech Code
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
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_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

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. Aliquotting supply set-up	___	_____
3. Glucometer set-up and use	___	_____
4. Stage 1 tube spin	___	_____
5. Stage 2 aliquotting	___	_____
6. Stage 3 tube spin and processing	___	_____
7. Stage 4 tube spin and processing	___	_____
8. Urine processing	___	_____
9. Vials sealed	___	_____
10. Freezer organization	___	_____
11. Time constraints	___	_____
12. Disposal of contaminated supplies	___	_____
13. Paxgene tube freezing	___	_____

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

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

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: 2-9 mL red top, 1-5mL red top, 1-mL lavender top, 2-10 mL lavender top, 1-4.5 mL blue top, and 1-4 mL lavender top (OGTT)

2. Specify which tube(s) remain at room temperature after collection, which are put into a cup with ice slush, which are stored in the refrigerator.

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 tubes. Indicate the colors of screw caps and the types of specimen put into these tubes.

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

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

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

8. Describe the steps for freezing the Paxgene tubes.

9. Using the MLA D tipper pipetter, add 30 uL of 6 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?

10. Where on the test strip should you place the drop of blood, and how can you ensure that you have an adequate amount of blood on the strip?

WRITTEN EXAM FOR HCHS-SOL V2 BIOSPECIMEN COLLECTION AND PROCESSING TECHNICIAN

Name: (please print) _____ Field Center: _____ DATE: _____

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) #4 and #5
   c) #6, and #7
   d) packed cells are not being collected for visit 2

4. How long should tubes #1, #2, #3 sit at room temperature before centrifugation?
   a) 5 minutes
   b) 30 minutes
   c) 2 hours
   d) No waiting time required

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

6. Which tube must be held below the arm during collection? (*tube is part of ancillary study)
   a) 10 mL lavender-stoppered
   b) 4.5 mL blue-stoppered
   c) 9 mL red-stoppered
   d) 2.5 mL Paxgene

7. For what type of tests will the 4.5-mL blue-stoppered tubes be used?
   a) Chemistry
   b) Lipid
   c) Coagulation
   d) DNA testing

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. Once you have opened the vial of glucometer test strips, how long can you use them before the strips must be discarded?
   a) 1 month
   b) 2 month
   c) 3 month
   d) until the expiration on the test strip vial

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. What is the cut-off level for the glucose screen test above which the participant is not eligible for the OGTT?
   a) 125 mg/dL
   b) 150 mg/dL
   c) 180 mg/dL
   d) 200 mg/dL

13. Tube #4 (4 mL EDTA) is shipped when and how?
   a) weekly, wrapped in paper toweling, on dry ice
   b) daily, wrapped in Styrofoam sheets, on dry ice
   c) weekly, wrapped in Styrofoam sheets, on frozen refrigerant gel packs
   d) daily, wrapped in paper toweling, on refrigerated gel pack

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 Contents Sheet(s)
   d) Shipping Form Face Sheet and Contents Sheet(s)

15. In addition to the extra labels, what paperwork is completed and sent with each daily refrigerated shipment?
   a) Copy of the Biospecimen Collection form for all participants seen that day.
   b) Original of the Frozen Contents Sheet form for all participants in the shipment.
   c) Original of the Biospecimen Collection form for all participants samples in the shipment.
   d) No shipping form is necessary

Appendix 10. Biospecimen Collection During the Home Visit

1. GENERAL INFORMATION

Blood collection at the participant's place of residence may be offered only if they are located within a distance that allows for arrival of the biospecimen at the field center laboratory within 120 minutes from the blood draw. 90 minutes or less is preferred. Longer delays until centrifugation will result in falsely elevated or decreased lab values (example: falsely decreased glucose values).

2. PREPARATION

2.1 Sample Aliquot Trays

Sample aliquot vials should be labeled and placed in trays at the field center laboratory before the trip to the participant's residence so processing can proceed immediately upon return. See MOP 7 Sections 2.2.4-2.2.6. These trays remain at the field center laboratory.

2.2 Home Biospecimen Collection Kit

Assemble the following:

- Tube #1: 9 mL red stoppered tube (Serum)
- Tube #2: 9 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 4.5 mL blue stoppered tube (Citrate)
- Participant ID number label
- Remainder of laboratory barcode ID labels
- (2) sterile, disposable 21 gauge butterfly needles
- Plastic vacutainer tube guide
- Vacutainer Leur adapter
- Sterile alcohol swab
- Gauze sponge
- Bandage ("Band Aid")
- Urine specimen cup
- Urine specimen cup lid
- TIME VOIDED label
- Large ziplock bag

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

Place remaining labels and all other items inside the ziplock bag and seal.

2.3 Biospecimen Collection Supplies

- Biospecimen Collection Forms
- Pen
- Tourniquet
- Test tube rack
- Paper towels
- Ice filled small container
- Gloves
- Sharps container
- Absorbent pad to lay on table
- Ziplock bag
- Biohazard labeled plastic bag
- (2) Refrigerant Pack- refrigerated (Has been held in refrigerator at least 1 day)
- (2) Refrigerant Pack- room temperature
- Small hand held styrofoam or plastic cooler labeled "Refrigerated" with a biohazard label taped to outside
- Small hand held styrofoam or plastic cooler labeled "Room Temperature" with a biohazard label taped to outside

3. ARRIVAL AT RESIDENCE

3.1 Set Up of Venipuncture Area

Set up venipuncture area in a quiet area with a table and two chairs. An absorbent mat should be placed on the area of the table where blood collection will take place. Tubes should be in a rack on the table, along with supplies needed to collect the blood.

3.2 Biospecimen Collection Form

1. Check that participant's HCHS/SOL V2 Participant ID number on the Biospecimen Collection form is correct. If it hasn't been placed on the form yet, do so now.
2. Place the laboratory barcode ID label that matches the labels on the collection tubes onto the Biospecimen Collection form.
3. Confirm the match between the participant name, the HCHS/SOL V2 participant ID number, and the laboratory barcode ID number on the blood collection tubes and the Biospecimen Collection form.

4. URINE COLLECTION

1. Label urine specimen cup with participant's ID.
2. Instruct the participant to void in the cup, filling it if possible, place the lid securely on top of the container, and record the time of voiding on the label.
3. Laboratory staff records whether a urine sample was obtained and transcribes the collection time of the urine void from the ID label onto the participant's Biospecimen Collection form, Section E.

4. Place a cold refrigerant pack at the bottom of a Ziplock bag and then place the urine cup on top of the refrigerant pack inside the bag. NOTE: Make sure the cover on the urine container is tightly sealed so the urine doesn't leak during transport.
5. Place the urine containing plastic bag in the refrigerated cooler.

5. VENIPUNCTURE

1. Informed consent must be obtained before drawing any blood.
2. Complete the Biospecimen Collection form Section A (safety questions), Section B (fasting information), and Section C (blood collection date/time). The remaining questions can be filled out after the venipuncture.
3. Please note in the Comments section (Question 15) of the Biospecimen Collection form that the blood collection was obtained from a home visit.
4. Perform venipuncture following the protocol in MOP 7, Sections 3.2-3.3. Note that there will be no glucola administration during home visits. Thus, tube #8 (4 mL lavender stoppered)(EDTA)(OGTT) will not be collected.
5. All tubes should be inverted gently 8 times immediately after collection.
6. Tube #4 (4 mL lavender stoppered tube containing EDTA) should be placed immediately in the small container of ice to cool. After venipuncture is finished, it can be placed with the other EDTA tubes in the refrigerated cooler.
7. Tubes #1-3 (red stoppered tubes containing no anticoagulant) and tube 7 (blue stoppered tube containing sodium citrate) should be placed in a rack in the room temperature cooler, along with the two room temperature refrigerant packs.
8. Tubes #4-6 (lavender stoppered tubes containing EDTA anticoagulant) should be wrapped in paper towels, placed in a plastic biohazard bag, and placed on top of a cold refrigerant pack in the refrigerated cooler.
9. Any centrifugation, processing, and shipping procedures will be performed back at the field center laboratory.

6. CLEANUP

All used needles, gloves, and any material contaminated with blood should be placed in the sharps container. The area should be left just as it was found.

7. RETURN TO THE FIELD CENTER

1. Samples should be processed immediately upon return to the field center. See MOP 7 Section 4. Both plasma and serum should be processed immediately.
~~1.~~2. Please record the time lapsed from blood collection to centrifugation start in the blood processing comments (Question 23) of the Biospecimen Collection form.