Youth

Hispanic Community Children's Health Study of Latino Youth (SOL Youth) Manual 2

Biospecimen Collection and Processing

October 9, 2012- Version 3.1

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

Biospecimen Collection and Processing
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1. BIOSPECIMEN COLLECTION AND PROCESSING

The Hispanic Community Children's Health Study – Study of Latino Youth 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). In recent years, the prevalence of overweight and obesity has increased among children and adolescents. The incidence of metabolic syndrome and type 2 diabetes has risen concurrently with obesity among youth. The goal of the SOL Youth Study is to evaluate the prevalence of increased obesity in Hispanic youth and how parental and youth situational, environmental, and personal factors increase the risk for obesity and its related cardiometabolic health complications. Blood samples are collected to study these factors through specialized, state-of-the-art laboratory assays.

The target population of 1600 boys and girls of Hispanic origin, specifically Cuban, Puerto Rican, Mexican, and Central American, aged 8-14 years old, who live in the homes of HCHS/SOL cohort participants.

[**Note:** Protocol revision for increasing the eligible age range from 8-14 to 8-16 years old for SOL Youth was unanimously approved by the HCHS/SOL Ancillary Study Subcommittee and HCHS/SOL Steering Committee on August 8, 2012. All subsequent reference to the eligible age range in the remainder of this MOP will indicate the approved protocol change to 8-16 years old.].

The participants will 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 specimens donated by the study participants who have been asked to fast for 12 hours. DNA is prepared from the packed cells of EDTA blood, and aliquots of serum, Na Citrate plasma, and EDTA plasma are prepared at the field centers and 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 SOL Youth 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 SOL Youth study collects approximately 25 mL of blood from each participant. Five tubes of blood are collected. The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 2 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 SOL Youth 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 SOL Youth 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 final packed cells, serum, and plasma aliquots until they are frozen and ultimately transferred to the Central Laboratory 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 to 10 blood collection tubes (described in the next section)
- sterile, disposable 23 or 21 gauge butterfly needles (23 G is usually the size of needle that is used with a pediatric age group but a 21 G needle may be used on an older child; 25 G needles available for smaller veins; only use for extremely difficult draws, as the smaller gauge needle may cause samples to become hemolyzed).
- 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 5 mL red stoppered tubes. Although these tubes do not contain anticoagulant, they do have a clot activator and therefore require mixing following collection. The serum from these tubes will be used for testing lipids (fats) and other biochemical markers.

Tube #3 is a 4.5 mL blue-stoppered tube containing Sodium Citrate anticoagulant. The plasma from these tubes is used for coagulation studies. These tubes must be filled completely in order to standardize the blood to liquid anticoagulant ratio. Partially filled tubes will result in erroneous test results.

Tubes #4 and #5 are 5 mL lavender-stoppered tubes containing EDTA anticoagulant. The plasma from these tubes is used for several analytical tests including glucose (sugar). The cells will be used to isolate DNA.

Note: Tube Priority for a difficult draw: #1, #4, #3: These tubes will provide a sample type of each kind (serum, EDTA plasma, and Citrate) and will provide enough sample for immediate testing. In a difficult draw situation draw tubes in this specific order: #1, #4, and #3. Immediate testing is done on tubes #1 and #4.

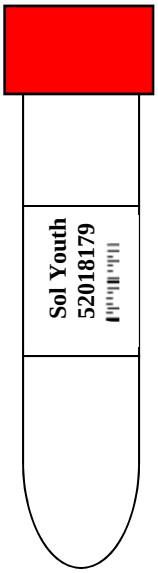
2.2.3 Blood Collection Tubes: Labeling and Set-Up

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 five tubes are collected in the following order:

   Tube #1: 5 mL red stoppered tube (Serum)
   Tube #2: 5 mL red stoppered tube (Serum)
   Tube #3: 4.5 mL blue stoppered tube (Citrate)
   Tube #4: 5 mL lavender stoppered tube (EDTA)
   Tube #5: 5 mL lavender stoppered tube (EDTA)



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

A number of SOL Youth participants will be asked to donate one additional tube 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. 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.

8 – 2 mL polypropylene tubes (purple top)
1 – 2 mL amber polypropylene tube (purple top)
1 – 5 mL polypropylene tube (clear top)
1 – 5 mL polypropylene tube (blue top)
4 – 2 mL polypropylene tubes (blue top)
8 – 2 mL polypropylene tubes (red top)
1 – 2 mL amber polypropylene tube (red top)

2.2.6 Labeling

Vertically label (in the same manner as the collection tubes) 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 – E
Col 3:	5 mL clear top clear tube; row A
Col 4:	5 mL blue top clear tube; row A
Col 5:	Empty
Col 6:	2 mL blue top clear tubes; rows A – D
Col 7:	Empty
Col 8:	2 mL red top *amber* tube; row B
Col 8:	2 mL red top clear tubes; rows A, C, D
Col 9:	2 mL red top clear tubes; rows A - E
Col 10:	Empty

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	0.250 mL plasma, Tube #4	minimum 0.5 mL plasma, Tube #5	Whole Blood Tube #4 1 mL	packed cells, Tubes #4 & #5 4-5 mL	Empty	minimum 0.5 mL plasma, Tube #3	Empty	0.250 mL serum, Tube #1	minimum 0.500 mL serum, Tube #2	Empty
B	minimum 0.5 mL plasma, amber vial, Tube #4	minimum 0.5 mL plasma, Tube #5	Empty	Empty	Empty	minimum 0.5 mL plasma, Tube #3	Empty	minimum 0.500 mL serum, amber vial, Tube #1	minimum 0.500 mL serum, Tube #2	Empty
C	minimum 0.5 mL plasma, Tube #4	minimum 0.5 mL plasma, Tube #5	Empty	Empty	Empty	minimum 0.5 mL plasma, Tube #3	Empty	minimum 0.500 mL serum, Tube #1	minimum 0.500 mL serum, Tube #2	Empty
D	minimum 0.5 mL plasma, Tube #4	minimum 0.5 mL plasma, Tube #5	Empty	Empty	Empty	minimum 0.5 mL plasma, Tube #3	Empty	minimum 0.500 mL serum, Tube #1	minimum 0.500 mL serum, Tube #2	Empty
E	Empty	minimum 0.5 mL plasma, Tube #5	Empty	Empty	Empty	Empty	Empty	Empty	minimum 0.500 mL serum, Tube #2	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 $\pm$ 2°C).
6. Perform and record QC check on refrigerator temperature (4°C $\pm$ 2°C).
7. Perform and record QC check on freezer temperature (-70°C $\pm$ 5°C) and (-20°C $\pm$ 2°C).
8. Perform and record QC check on room temperature.

2.2.8 At Participant Arrival

1. Check that the participant's SOL Youth 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 SOL YOUTH 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 – A3) and the fasting question (section B) of the Biospecimen Collection form (see Appendix 3). **Note: The participant should be fasting for preferably12 hours, a minimum of 8 hours and a maximum of 16 hours. If the participant has been fasting for less than 8 hours or more than 16 hours, then a fasting blood collection should be re-scheduled for a different day.**

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 SOL YOUTH 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, or serum. 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, or serum.

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.

Pediatric Phlebotomy Techniques

See detailed techniques in Appendix 9

Technical Tips for Phlebotomy on Children

1. Use 2 smaller sized tubes versus 1 larger one, e.g. two 5 mL serum rather than one 10 mL serum collection tube.
2. Always have a 2nd person to hold the child's arm steady.

3. Use a 23 or 21 gauge needle (start with a 23G but 21G can be used on an older child with good veins) and have 25 gauge needle available for smaller veins (*only use 25 gauge needle for extremely difficult draws*)
4. Always use a butterfly type of collection set-up.
5. Keep hot packs available to help bring out the vein.
6. Make sure the child is well hydrated.
7. LMX4 (4% lidocaine topical anesthetic cream): LMX4 is an optional OTC numbing medicine that can be used to ease the pain of the blood draw.

LMX4 (4% lidocaine topical anesthetic cream) Instructions: *Optional OTC numbing medicine; use if participant appears apprehensive about phlebotomy during the informed consent process.*

1. LMX4 cream is placed on the participant preferably by a medical caregiver (nurse, etc…) **30-45 minutes before phlebotomy**. If a medical caregiver is not available, then a certified SOL Youth staff member may apply the LMX4 cream on the participant.
2. The SOL Youth staff member will inform the participant/family of the purpose of local anesthetic prior to venipuncture and possible side effects
3. Note: The LMX4 cream can cause blood vessels to constrict in some cases. This could slightly prolong the needle procedure itself. **In most cases, the cream does not constrict the vessels enough to cause difficulty with the blood draw and eases the pain of the needle stick. Many pediatric clinics routinely use LMX4 cream on their patients.**
4. The SOL Youth staff member will verify that the participant has no allergy to lidocaine or LMX4 cream (no allergic reaction to any "amide-type" anesthetics such as Novacain)
5. Make sure participant has intact skin (Do NOT apply to a site that has an open wound such as a cut or scratch)
6. If child weighs 22 pounds or more, place a quarter sized spot (approximately 1 mL) on each anticubital fossa and cover with a clear Tegaderm bandage (do NOT use more cream than directed)
7. Leave the LMX4 cream in place 30-45 minutes prior to venipuncture
8. The phlebotomist will remove the LMX4 cream before the venipuncture procedure
9. The phlebotomist will document the use of LMX4 cream on the Biospecimen Collection form

Expected Side effects of LMX4 cream:

1) Blanching (whitening) of the skin
2) Tightening of blood vessels (possible, but rarely causes enough vasoconstriction to interfere with the blood draw)

If localized reaction to cream (mild irritation, redness, or swelling at application site) follow these steps:

1) Remove LMX4 cream with soap and water
2) Apply cool compress if application site is red and swollen

Rare Side Effects of LMX4 cream: (If too much cream is used)

1) Nervousness
2) Dizziness, lightheadedness
3) Confusion

4) Tremors
5) Slow Breathing
6) Methemoglobinemia (lower blood oxygen capacity)

Important: If any rare side effects listed above are experienced or signs of allergic reaction (fever or chills, rash or hives, wheezing, or shortness of breath) occur from the LMX4 cream; a SOL/ Youth staff member must call 911 and the participant needs to be transported immediately to an emergency room.

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 2 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, 1-3):

1. …if they have had any 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.

If blood is to be drawn, complete the fasting blood collection information with the participant (section B, 4-5). Fill in date and time of blood collection (section C, 6-7).

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.

**If LMX4 anesthetic cream has been placed on participant, remove the clear Tegaderm bandage and wipe off cream with a paper towel prior to venipuncture. Note the use of LMX4 cream in item 15 of the Biospecimen Collection form.*

Perform venipuncture with a 23 or 21 gauge butterfly needle (25-gauge if smaller vein) and 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small thin-walled needle that minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. Give the participant enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for participants will be the contact with the technicians who draw the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure, e.g., "I am going to be drawing about 2 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 Hispanic youth." 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 10).

3.3. Venipuncture

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). ***If LMX4 anesthetic cream has been placed on participant, remove the clear Tegaderm bandage and wipe off cream with a paper towel prior to venipuncture. Note the use of LMX4 cream in item 11 of the Biospecimen Collection form.*** 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.

Note: If LMX4 anesthetic cream has been placed on participant, remove the clear Tegaderm bandage and wipe off cream with a paper towel prior to venipuncture. Note

the use of LMX4 cream in item 11 of the Biospecimen Collection form. **Proceed with phlebotomy procedure.**

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 (5 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 #3 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 #3 (Citrate tube) collect 2mL of blood into a red SST tube before collecting tube #3. (The SST tube blood is discarded.)

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

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

G. Bandaging the arm.
1. Under normal conditions:
   a. Slip the gauze pad down over the site, continuing mild pressure.
   b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
2. If the participant continues to bleed:
   a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
   b. Wrap a gauze bandage tightly around the arm over the pad.
   c. Tell the participant to leave the bandage on for at least 15 minutes.

H. PRECAUTIONS - When a Participant Feels/Looks Faint Following the Blood Drawing:
1. Have the person remain in the chair. If necessary, have him/her lie on the floor with legs elevated. Use of a transfer belt may be indicated in this situation.
2. Take an ampule of smelling salts, crush it, and wave it under the person's nose for a few seconds.
3. Provide the person with a basin if he/she feels nauseous.
4. Have the person stay seated until the color returns and he/she feels better.

5. Have someone stay with the person to prevent them from falling and injuring themselves if he/she should faint.
6. Place a cold wet cloth on the back of the person's neck or on their forehead.
7. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their pre-clinic visit fast.
8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.4. Blood Tube Mixing and Storage During Venipuncture

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

Tube #1 and #2: 5 mL red stoppered tube containing no anticoagulant. Invert tube gently 8 times immediately after collection. Place tubes in room temperature rack and allow the blood to clot for 30 minutes after collection. Protect tubes from light by placing a box over the rack until centrifugation.

Tube #3: 4.5 mL blue-stoppered tube contains sodium citrate anticoagulant. 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 #4 and #5: 5 mL lavendar-stoppered tube contains EDTA anticoagulant. Invert gently 8 times immediately after collection. Place the tubes #4 and #5 in a cup with ice slush and protect them from light by placing a box over the cup of ice slush until centrifugation.

4. BLOOD PROCESSING

4.1 Stage One: Immediate Processing

After completion of venipuncture:

1. Tubes #1 and #2 remain at room temperature for 30-45 minutes to allow the blood to clot (blood at 4°C clots extremely slowly). 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. **Before centrifuging tube #4**, remove purple stopper and using a plastic pipette, transfer 1 mL of whole blood into a labeled 5-mL vial with a clear cap (Position A3 in aliquot tray). Place the 5-mL vial of whole blood into position A3 of the aliquot tray. **Replace the purple stopper tightly into tube #4 before centrifugation.**

3. Within 15 minutes of collection, place tubes, #3, #4, and #5 in the centrifuge trunions. Place tubes in the centrifuge buckets in a balanced manner (see description of balancing the centrifuge in 4.1 “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.

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.

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 #3 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 18 of the Biospecimen Collection form (Appendix 3) 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 19 of the Biospecimen Collection form (Appendix 3) "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 #3, #4, and #5 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 # 4, and #5: Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear plasma supernatant from tube #4. 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 0.250 mL of plasma into the first clear 2 mL vial in position A1 of the sample aliquot tray 1. (Use the "0.250 mL template" vial and 0.250 inidicator notch on the plastic pipette as a

guide for an approximate 0.250 mL volume.) Distribute the remaining plasma equally (approximate 0.5 mL volume) into one amber vial in position B1 and two clear 2 mL vials in positions C1 through D1. Process tube #5 similarly, distributing the plasma equally (approximate 0.5mL volume) into five 2 mL clear vials in positions A2 through E2. (Use "0.5 mL template vial" and the 0.5 indicator notch on the plastic pipette as a guide for 0.5 mL volume).

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

4. Using the same plastic transfer pipette, slowly aspirate the remaining plasma, buffy coat layer, and some of the red cells from tube #4. (Do not let the buffy coat aspirate into the bulb of the disposable pipette.) Transfer this to the 5 mL vial in position A4. Now transfer the remaining red blood cells from tube #4 to this same vial. (This ensures that the buffy coat is adequately rinsed from the transfer pipet.) Repeat these steps for tube #5 and place the liquid into the same 5 mL vial in position A4. Fasten the blue screw cap onto this vial.

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

6. Tube #3: 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 #3. 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 four 2 mL vials in positions A6 through D6 of the sample aliquot tray. (Use the "0.5 mL template" vial and the 0.5 mL indicator notch on the plastic pipette as a guide for an approximate 0.5 mL volume.) Place a blue screw cap on each vial. Replace the blue stopper on both of the collection tubes and discard them 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 and #2 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. Remove the stopper.

3. Using a plastic transfer pipet and being careful not to disturb the red or white blood cell layers, remove the clear serum supernatant from tube #1. Aspirate slowly starting at the top of the serum (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 serum above the buffy coat/red blood cells. Place approximately 0.250 mL of serum into the 2 mL vial in position A8 of the sample aliquot tray. (Use the "0.250 mL template" vial and 0.250 inidicator notch on the plastic pipette as a guide for an approximate 0.250 mL volume). Distribute the remaining serum equally into one amber vial in position B8 and

two 2 mL clear vials in positions C8 and D8 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 five 2 mL vials in positions A9, B9, C9, D9, and E9 of the sample aliquot tray. (Use the "0.5 mL template" vial and the 0.5 mL indicator notch on the plastic pipette as a guide for an approximate 0.5 mL volume. Fasten the red screw caps onto these vials.

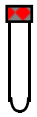
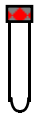
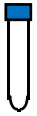
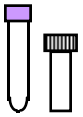
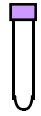
4. Re-stopper tubes #1 and #2 and discard them in a biohazard waste container.

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 and 2 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.

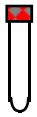
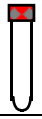
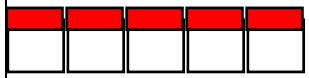
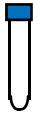
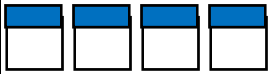
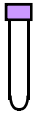
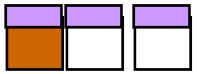
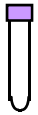
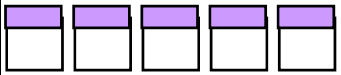
4.4. Overview of Specimen Collection

A summary overview of the protocol steps for the collection and processing of blood specimens is presented in Figure 2 and 3. (Specimen Processing Charts)

SOL Youth Blood Collection/Centrifuging Chart (Stage 1 Immediate Processing) Figure 2

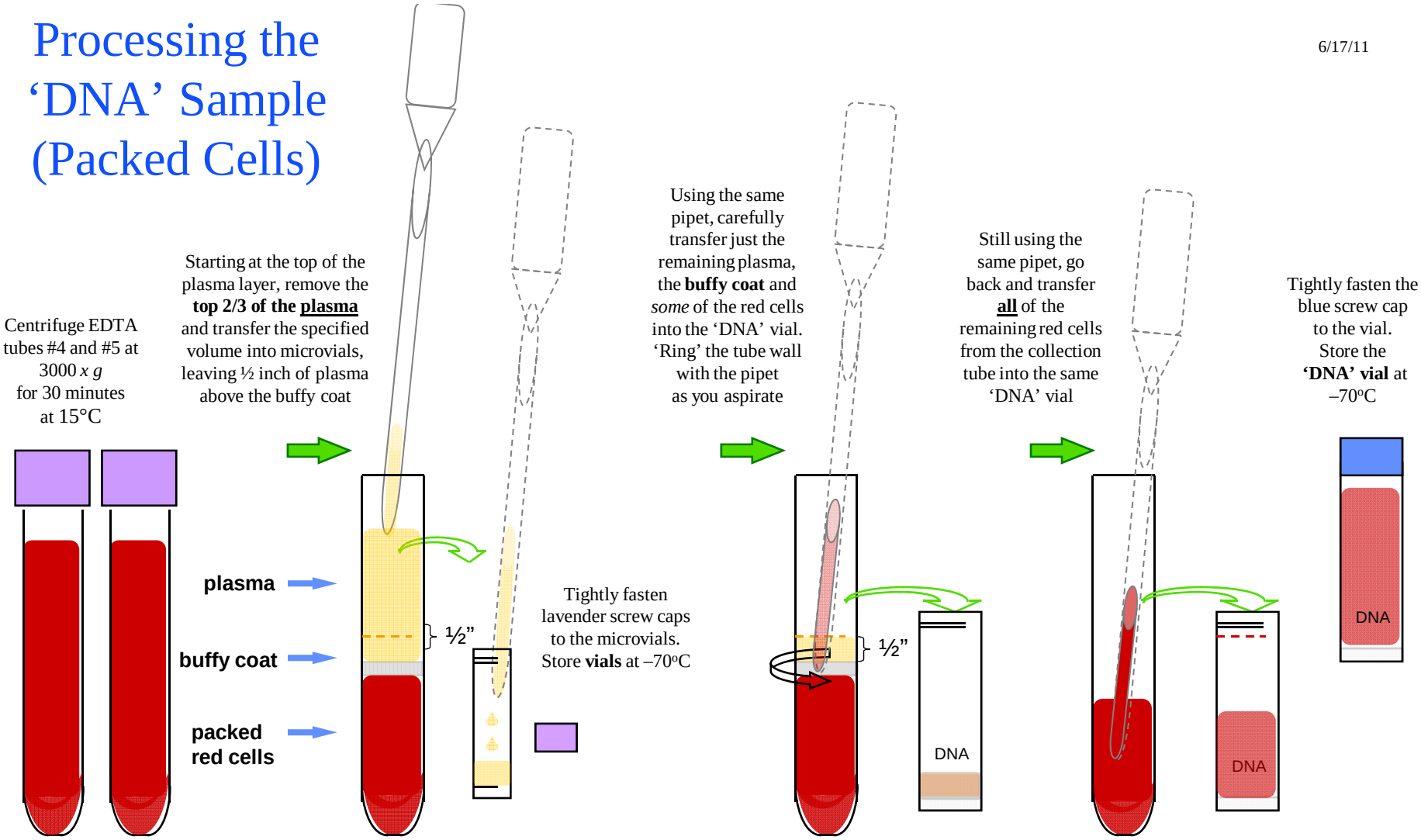
Test	Tube Name	Fasting Condition	Blood Collection Tube	Visual Reference	Collection/Centrifuging
Lipid Profile hsCRP	**Red Top Tube #1**	Fasting	5.0 mL red-top	Label Tube 	• Fill labeled tube completely with blood • Invert tube 8 times/**Cover with Box** • Tube must sit **30-45 min at Room Temp** • Blood will clot • Centrifuge 15°C for 10 min at 3000 x g (Tubes #1 & #2) • Remove collection tube from centrifuge • Proceed to Specimen Aliquot Procedure
Adipocytokines Serum Storage	**Red Top Tube #2**	Fasting	5.0 mL red-top	Label Tube 	• Fill labeled tube completely with blood • Invert tube 8 times/**Cover with Box** • Tube must sit for **30-45 min at Room Temp** • Blood will clot • Centrifuge 15°C for 10 min at 3000 x g (Tubes #1 & #2) • Remove collection tube from centrifuge • Proceed to Specimen Aliquot Procedure
vWF & PAI-1 Na Citrate Plasma Storage	**Blue Top Tube #3**	Fasting	4.5 mL blue top (Na Citrate)	Label Tube 	• Fill labeled tube completely with blood • Invert tube 8 times. • Centrifuge **Immediately** at 15°C for 30 min at 3000 x g • *Centrifuge tubes #3, #4, and #5 at same time* • *Will need to balance tube #3 with a water balance tube* • Remove collection tube from centrifuge • Proceed to Specimen Aliquot Procedure
Hemoglobin A1c Glucose Insulin	**Purple Top Tube #4**	Fasting	5.0 mL purple-top (EDTA)	Label Tubes 	• Fill labeled tube completely with blood • Invert tube 8 times/**Place in Ice Water Bath/Cover with Box** • Remove collection tube stopper • Transfer 1.0 mL of whole blood into clear capped 5.0 mL vial • Place labeled 5.0 mL vial of whole blood in -70°C freezer • Re-stopper collection tube tightly • Centrifuge **Immediately** at 15°C for 30 min at 3000 x g • *Centrifuge tubes #3, #4, and #5 at same time* • Remove collection tube from centrifuge • Proceed to Specimen Aliquot Procedure
EDTA Plasma Storage DNA	**Purple Top Tube #5**	Fasting	5.0 mL purple-top (EDTA)	Label Tube 	• Fill labeled tube completely with blood • Invert tube 8 times/**Place tube in Ice Water Bath/Cover with Box** • Centrifuge **immediately** at 15°C for 30 min at 3000 x g • *Centrifuge tubes #3, #4, #5 at same time* • Remove collection tube from centrifuge • Proceed to Specimen Aliquot Procedure

SOL Youth Specimen Aliquot Chart (Stage 2 Plasma Processing and Stage 3 Serum Processing)

Aliquot Instructions	Tube Name	Blood Collection Tube	Visual Reference	Aliquot Diagram
• From #1 transfer 0.250 mL into one clear 2-mL vial with **red** cap • Distribute remaining serum equally into one **amber** and two clear 2-mL vials with **red** caps	**Red Top** **Tube #1**	5.0 mL red-top	Centrifuged Tube 	**Room Temp/Cover with Box until Freezer Placement**  0.250 mL  Distribute remaining serum equally (0.500 mL minimum volume)
• From #2 distribute serum equally into five clear 2-mL vials with **red** caps • Keep all vials covered with Box • Place all **nine serum vials** into -70 ° C freezer until shipment	**Red Top** **Tube #2**	5.0 mL red-top	Centrifuged Tube 	***Room Temp/Cover with Box until Freezer Placement**  Distribute remaining serum equally (0.500 minimum volume)
• From #3 distribute plasma equally into four clear 2-mL vials with **blue** caps • Place all **four Na Citrate plasma vials** into -70 ° C freezer until shipment	**Blue Top** **Tube #3**	4.5 mL blue top (Na Citrate)	Centrifuged Tube 	***Room Temp until Freezer Placement**  Distribute plasma equally (0.500 mL minimum volume)
• From #4 transfer 0.250 mL into one clear 2-mL vial with **purple** cap • Distribute remaining EDTA plasma equally into one **amber** and two clear 2-mL vials with **purple** caps	**Purple Top** **Tube #4**	5.0 mL purple-top (EDTA)	Centrifuged Tube 	***Ice Water Bath/Cover with Box until Freezer Placement**  0.250 mL  Distribute remaining plasma equally (0.500 mL minimum volume)
• From #5 distribute EDTA plasma equally into five clear 2-mL vials with **purple** caps • Keep all vials covered with box • Keep all vials in ice water bath • Place all **nine plasma vials** into -70 ° C freezer until shipment • Transfer Packed Cells from tubes #4 and #5 into one 5-mL vial	**Purple Top** **Tube #5**	5.0 mL purple-top (EDTA)	Centrifuged Tube 	***Ice Water Bath/Cover with Box until Freezer Placement**  Distribute remaining plasma equally (0.500 mL minimum volume) ***Packed Cells (From tubes #4 and #5); Room Temp until freezer placement**  *See Lab MOP for specific instructions*

Processing the 'DNA' Sample (Packed Cells)

6/17/11



Processing the EDTA tube collected for DNA isolation: Follow steps for tubes #4 and #5 (*Be sure to process only one participant's samples at a time*)

Centrifuge the EDTA tubes #4 and #5 promptly at 3000 x *g* for 30 minutes at 15°C. From tube #4, being careful not to disturb the buffy coat and packed red cell layers, use a plastic transfer pipet to remove the top 2/3 of the clear plasma supernatant and transfer the specified volume into labeled microvials. Aspirate slowly starting at the top of the plasma. The pipet tip should not get any closer than one-half inch from the buffy coat. It is important to withdraw only the plasma and *none* of the buffy coat (containing white cells and platelets) that forms at the cell-plasma interface following centrifugation. Leave a ½-inch layer of plasma above the buffy coat-red blood cell layers.

Using the same plastic transfer pipet, slowly aspirate the remaining ½" layer of plasma, the buffy coat and *some* of the red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipet! 'Ring' the tube with the pipet by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL vial labeled 'DNA'. Still using the same pipet, go back and transfer all of the remaining packed red cells from the tube #4 into the same 5-mL'DNA' vial. This step will ensure that all of the buffy coat is adequately rinsed from the pipet. **Repeat steps for tube #5, placing packed cells in same DNA vial as tube #4.** Tightly fasten the blue screw cap on the DNA vial and place it in the –70°C freezer in aliquot tray until shipment to the Central Laboratory.

4.4.1 Freezing

When all of the blood 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

5.1. 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 and plasma vials are packaged in freezer storage bags according to their specimen type.

5.1.1 Packaging Frozen Specimens

Place eight of the red-capped serum vials into one 4" x 6" storage bag, eight of the purple-capped plasma vials into another 4" x 6" storage bag, and the four blue-capped plasma vials into a third 4" x 6" storage bag. Place the clear-capped whole blood into a fourth 4" x 6" storage bag and the blue-capped vial of packed cells (5 mL vial) into a fifth 4" x 6" storage bag. Place the 0.250 mL red-capped serum vial and the 0.250 mL purple-capped 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, whole blood, and packed cells 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 SOL Youth 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 1-4 below for shipping diagram.

5.1.2 Shipping

The samples remain in their Styrofoam box at -70° C until they are shipped. All frozen plasma, sera, and packed cells 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. 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. If very few participants were seen in the field center during a week, two or three weeks of specimens can be combined into one shipment. Notify the Central Laboratory if the weekly shipment is being held. 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 SOL Youth 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 SOL Youth 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:

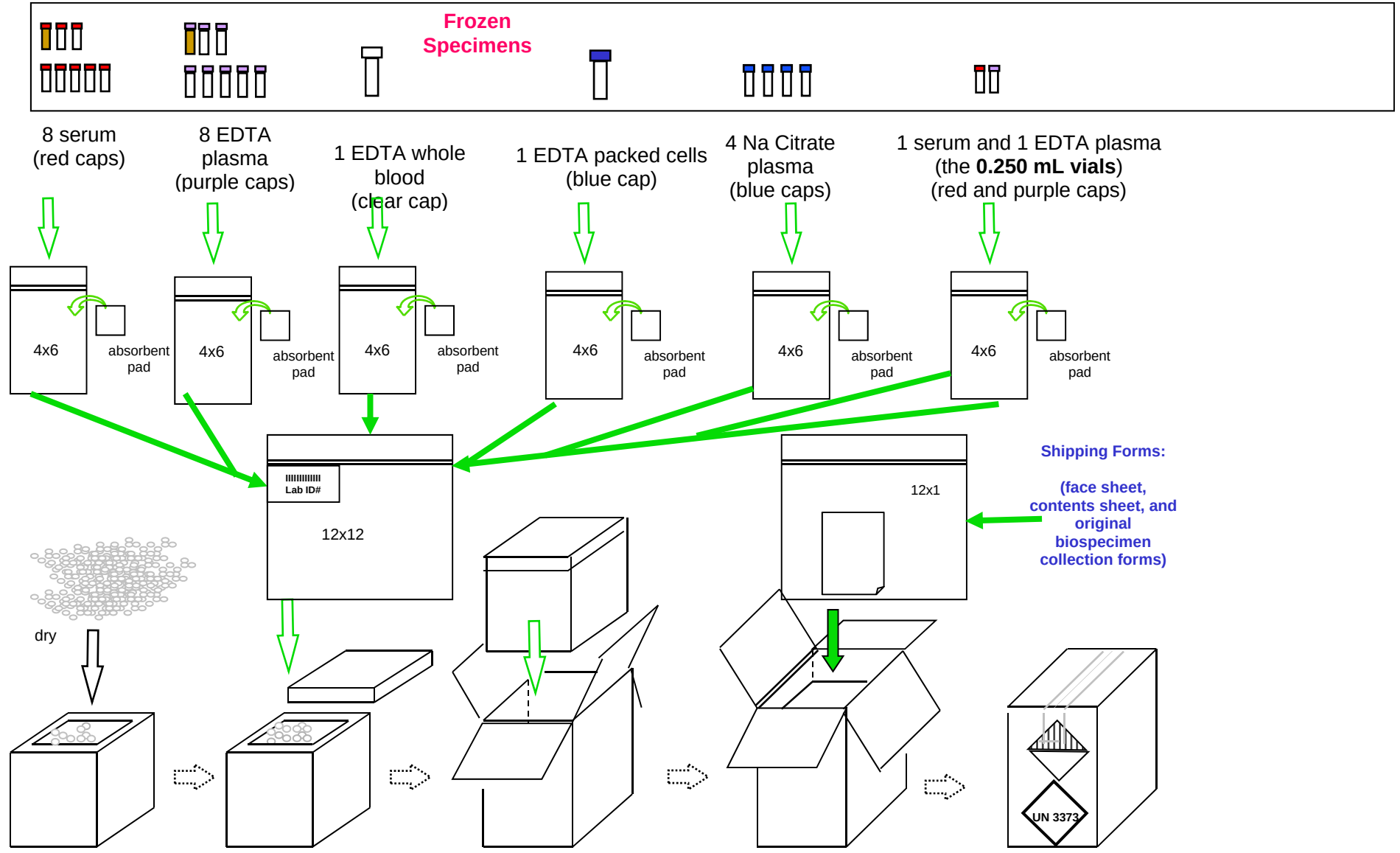
Annie Lukkari/ SOL Youth Central Laboratory
University of Minnesota, Advanced Research and Diagnostic Laboratory (ARDL)
Room L275 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455
Telephone: (612) 273-3645
Fax: (612) 273-3489

SOL Youth Central Laboratory Hours:

Monday-Friday	7:00 am-3:30pm
Saturdays	Open; hours vary
Sundays	Closed

*A holiday schedule will be emailed to all SOL Youth Clinical Sites 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, 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 10, 11, 18, and 19, 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). 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 samples, duplicate specimens are sent to the laboratory, with one half of each specimen pair sent under the participant's regular HCHS-SOL-Youth 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 three 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, 4, and 5), the aliquots are stored at the Field Center for extra week/weeks until the entire QC set is complete and then the complete QC set is sent to the Central Laboratory with a regular shipment. **Two complete QC sample sets per month** should be sent to the Central Lab for the **first six months** of the study and then for the remaining months of the study only **one complete QC sample set per month** should be sent to the Central Lab.

The QC blood samples are collected in sequential order (cycling back to Tube #1 after QC Tube #5 has been collected). Over the entire study, each Field Center will collect QC samples from approximately 7% of all specimens (n=28). Three participants will be needed to provide three QC replicate specimens to form one phantom ID. Thus, one-fifth of participants (n=84=3x28) per field center will contribute to the pool of replicate specimens. Two QC sample sets should be sent to the Central Lab monthly for the first 6 months of the study. One QC sample set should be sent to the Central Lab monthly for the remaining months of the study. Choose older children with a larger body weight as QC sample candidates.

An example plan for collecting the QC samples: **First six months** of the study: Week one of the month; draw tubes #1 and #2 (day 1 of week); draw tube #3 (day 2 of week); draw tubes #4, and #5 (day 3 of the week). Week two of the month; draw tubes #1 and #2 (day 1 of week); draw tube #3 (day 2 of week); draw tubes #4, and #5 (day 3 of the week).Week three of the month can be used to collect QC samples if not enough can be collected in the week one and two of the month. On week four of the month; send the **two completed QC sample sets** to the Central Laboratory. **After the first six months of the study:** Week one of the month; draw tubes #1 and #2. Week two of the month; draw tube #3. Week three of the month draw tubes #4, and #5. On week four of the month; send the **one completed QC sample** set to the Central Laboratory. The plan for collecting QC samples will vary depending on the availability of appropriate candidates for the QC samples.

6.2. Blood 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 QC Sample Checklist
Date:_____

Participant	Tubes	Aliquot Type	Sample collected? (Y/N)
Participant 1	1 & 2	Serum	_____
Participant 2	3	Plasma, sodium citrate	_____
Participant 3	4, & 5	EDTA Whole Blood & Plasma	_____

6.3. Preparation for Drawing and Processing QC Samples

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

Sample Aliquot 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.

6.4. Collecting and Processing QC Blood

Selecting Participants for QC Blood Draw: A QC sample will be collected from a total of six participants each month (two complete QC sample sets) for the first 6 months of the study. After the first 6 months of the study, then only three participants (one complete QC sample set) will be needed. Choose older children with a larger body weight as QC candidates. 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 may 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) After the samples are thoroughly frozen, they are put into a freezer storage bag and put into the freezer box. Keep the QC specimens separate from the other specimens collected during the week so they are not shipped along with them.

Biospecimen Collection form: 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 tubes #1 and #2 is used to complete the form for the QC set.

Logging the Match between QC and Regular SOL Youth 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 SOL Youth Quality Control Phantom Participant Form (see example in Appendix 6), which is used to keep track of the match between the QC and regular SOL Youth 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) or use of a Coordinating Center upload facility that is accessed through the web based DMS. This transmission will occur weekly on Mondays. 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 SOL Youth data management system. The availability report summarizes the receipt of information from the Central Laboratory and reading centers. Tests reported to the participants will be available to the field centers via a report in the DMS called the “End of Study Report”. Any tests included in this report whose results exceed their alert range will be flagged appropriately. In addition, any alert result on a test not normally reported to the participants will be included in a separate upload. Reference ranges and alert values can be found in Appendix 1. Note that test results will be available in approximately 3-4 weeks after the sample is collected.

7. TRAINING PROCEDURES

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

A check list of the venipuncture and processing procedures that SOL Youth technicians must know and be prepared to demonstrate is listed in Appendix 7. The technician must study the SOL Youth 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 SOL Youth participants. Before the technicians draw blood from any SOL Youth participant, they must take and pass the practical and written tests included at the end of this manual (Appendix 8). After passing the test and depending on the written evaluation of their instructor, they may proceed either to drawing blood from the SOL Youth 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. Menus are locally determined.

Appendix 1A. SOL Youth laboratory tests' reference ranges

Test Names	Reference Ranges Males	Females	Age Ranges	Units
hs C-reactive protein	none/NR[a]	none/NR[a]	all	mg/L
e-Selectin	none/NR[a]	none/NR[a]	all	ng/mL
TNF-alpha	none/NR[a]	none/NR[a]	all	pg/mL
IL-6	none/NR[a]	none/NR[a]	all	pg/mL
Adiponectin	none/NR[a]	none/NR[a]	all	ng/mL
VWF (antigen)	none/NR[a]	none/NR[a]	all	%
PAI-1	none/NR[a]	none/NR[a]	all	U/mL
Insulin, fasting	none/NR[a]	none/NR[a]	all	pmol/L
Glucose, fasting[b]	60 – 99	60 – 99	all	mg/dL
Hemoglobin A1c	4.3 – 5.6	4.3 – 5.6	all	%
Total cholesterol[c]	<168	<177	5 – 9 years	mg/dL
	<173	<171	10 – 14 years	mg/dL
	<168	<176	15 – 19 years	mg/dL
Triglycerides[c]	<58	<74	5 – 9 years	mg/dL
	<74	<85	10 – 14 years	mg/dL
	<88	<85	15 – 19 years	mg/dL
LDL-cholesterol[c]	<103	<115	5 – 9 years	mg/dL
	<109	<110	10 – 14 years	mg/dL
	<109	<110	15 – 19 years	mg/dL
HDL-cholesterol[d]	>49	>48	5 – 9 years	mg/dL
	>46	>45	10 – 14 years	mg/dL
	>39	>43	15 – 19 years	mg/dL

[a] Non-reported test result so no reference range is necessary.

[b] Based on American Diabetes Association guidelines as reported in American Diabetes Association: Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2011;34 (Suppl. 1): S11–S61.

[c] Based on 75th percentile for children and adolescents as reported in Daniels SR, Greer, FR et al. Lipid Screening and cardiovascular health in childhood. Pediatrics 2008;122:198-208.

[d] Based on 25th percentile for children and adolescents as reported in Daniels SR, Greer, FR et al. Lipid Screening and cardiovascular health in childhood. Pediatrics 2008;122:198-208.

Appendix 1B. SOL Youth alert values and actions to be taken

Test name	Alert value	Script for result report
hs C-reactive protein	none/NR[a]	
e-Selectin	none/NR[a]	
TNF-alpha	none/NR[a]	
IL-6	none/NR[a]	
Adiponectin	none/NR[a]	
VWF (antigen)	none/NR[a]	
PAI-1	none/NR[a]	
Insulin, fasting	none/NR[a]	
Glucose, fasting	100 – 125 mg/dL	"Your child's glucose is borderline high which is associated with an increased long-term risk of developing diabetes. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
	126 – 299 mg/dL	"Your child's glucose is high which if repeatedly high is diagnostic of diabetes. *You should consult with your child's healthcare provider to discuss these results and options within the next week."*
	≥300 mg/dL	"Your child's glucose is very high which suggests uncontrolled diabetes. *You should consult with your child's healthcare provider to discuss these results and options as soon as possible."*
Hemoglobin A1c	5.7 – 6.4	"Your child's hemoglobin A1c is borderline high which is associated with an increased long-term risk of developing diabetes in the future. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
	≥6.5	"Your child's hemoglobin A1c is very high which suggests your child has

		diabetes. *You should consult with your child's healthcare provider to discuss these results and options as soon as possible."*
Total cholesterol	ULRR[b] - 199 mg/dL	"Your child's total cholesterol is borderline high which is associated with an increased long-term risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
	≥ 200 mg/dL	"Your child's total cholesterol is high which is associated with an increased risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
Triglycerides	ULRR[b] – 199 mg/dL	"Your child's triglycerides are borderline high which is associated with an increased risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
	200 – 499 mg/dL	"Your child's triglycerides are high which is associated with an increased long-term risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next few weeks."*
	≥500	"Your child's triglycerides are very high which is associated with an increased long-term risk of cardiovascular disease and an immediate risk of other serious disorders such as acute pancreatitis. *You should consult with your child's healthcare provider to discuss these results and options as soon as possible."*
LDL-cholesterol	ULRR[b] to 129 mg/dL	"Your child's LDL-cholesterol is borderline high which is associated with an increased long-term risk of

		cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
	≥130 mg/dL	"Your child's LDL-cholesterol is elevated which is associated with a significantly increased long-term risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*
HDL-cholesterol	< LLRR[c] mg/dL	"Your child's HDL-cholesterol is low which is associated with a significantly increased long-term risk of cardiovascular disease. *You should consult with your child's healthcare provider to discuss these results and options within the next month."*

[a] None/NR = no alert value and test result is not reported to study participants.

[b] ULRR = upper limit of gender- and age-specific reference range

[c] LLRR = lower limit of gender- and age-specific reference range

Appendix 2. Equipment and Supplies
Supplies to be supplied by the Central Laboratory:

Description
FedEX pre-printed shipping labels (Airbills)
Dry ice shipping labels
Category B UN 3373 labels

Supplies to be obtained by the Field Center:

Supplier	Catalogue No	Description
Cardinal Health	B3036-15A	Butterfly Needles, 23G x ¾", #367297
Cardinal Health	B3036-14	Butterfly Needles, 21G x ¾", #367296
Cardinal Health	B3036-16A	Butterfly Needles, 25G x ¾", #367298
Cardinal Health	B3035-12	Luer Adapters, #367290
Cardinal Health	40000-110	Alcohol Swabs 2,000/cs
Cardinal Health	KC9132A	Gauze Sponges 200/pk
Cardinal Health	JJ5644	Band Aids 100/pk
Cardinal Health	367203	Tourniquets, Latex free, 50/pk
Cardinal Health	F0882-30	LMX4 (4% lidocaine anesthetic cream); #0882-30
Cardinal Helath	M1634	Tegaderm Clear Dressing (used to cover LMX4)
Cardinal Health	364815	Vacutainer Tube Holders 1000/cs BD #364815
Cardinal Health or	B2953-36 or	Serum, Red top tubes (no gel), 5mL; 100/pk
Fischer Scientific	02-687-94	(BD Manufacturer number: #367814)
Cardinal Health	81-310448 or	EDTA plasma, Purple Top, 5mL; 50/pk
Fischer Scientific	22-310-448	
Cardinal Health	B2994-91 or	Sodium Citrate, Blue Top, 4.5 mL; 100/pk
	B2952-3 or	
Fischer Scientific	02-685-6B	
Cardinal Health	C1300-82	Microvials, clear (2mL) 500/pk
Cardinal Health	C1300-83	Microvials, amber (2 mL) 500/pk
Cardinal Health	C1310-15	Red Screw Caps 1000/pk
Cardinal Health	C1310-20	Purple Screw Caps 1000/pk
Cardinal Health	C1310-13	Blue Screw Caps 1000/pk
Cardinal Health	T1234-3	Screw Top Vials w blue cap (5 mL) 1000/pk
Cardinal Health	T4136-315	Screw Top NUNC Vials w cap (4.5 mL) 1200/pk
Cardinal Health	P5214-12	Transfer Pipettes 500/pk
Cardinal Health	SBE2R46	Ziplock Freezer Bags 4" x 6" 1000/pk, 5000/cs
Cardinal Health	MGRL2P1212	Ziplock Freezer Bags 12" x 12" 5000/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	B3062-40	PDI Ammonia Inhalant
Cardinal Health	B2922-1A	Blood Collection Trays
Cardinal Health	T2941-3 or T2960-4	Thermometers -20 C-+70 C
Cardinal Health	M1050-7	50 mL Absorbent Pads for shipping
Cardinal Health	C6510-1	Timer- 3 channel digital
Cardinal Health	M1066-15A	Styrofoam shipping box w/outer cardboard (Thermosafe Mfr. 355) (11x 11x 11 5/8; Styrofoam shipper) (14 ¼ x 14 ¼ x 14 5/8; outer cardboard) (Fits **5-8** sample sets)

Polyfoam Packers/ samples sets) ThermoSafe	398	Styrofoam shipping box, (Est. **25-30** frozen
Polyfoam Packers/ samples sets) ThermoSafe	352	Styrofoam shipping box, (Est. **28-30** frozen
		Dry Ice (approximately 5-10 lbs. per shipment)
		Packing Material
		3M, Scotch brand 3750 clear packaging tape

Note: Three different sizes of shipping boxes are listed in order to accommodate various recruitment levels.

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 centrifuged serum or plasma (in emergency situations when the samples cannot be aliquoted immediately).

Appendix 3

Youth

BIOSPECIMEN COLLECTION FORM

PARTICIPANT ID NUMBER:									LAB ID#	Place sticker here:
FORM CODE: BIO VERSION: 1 2/1/2012	Contact Occasion	0	1	SEQ #	0	1				

Instructions: *This form should be completed during the participant's visit. Affix the SOL-Youth participant ID label and the Lab ID label above. Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost box. Enter leading zeroes where necessary to fill all boxes.*

A. Safety Questions:

1. Has [CHILD's NAME] ever had a surgery where lymph nodes were removed from his/her armpits?
   ☐[1] Yes ☐[0] No **If Yes, specify in Q11 and follow precautions per QxQ instructions**

2. Does [CHILD's NAME] have any bleeding disorders? ☐[1] Yes ☐[0] No **If Yes, specify in Q11; follow precautions per QxQ instructions**

3. Has [CHILD's NAME] ever had a graft or shunt for kidney dialysis?
   ☐[1] Yes ☐[0] No **If Yes, specify in Q11; follow precautions per QxQ instructions**

B. Fasting Blood Collection Information:

4. On which day did [CHILD's NAME] last eat or drink anything except water: today, yesterday, or the day before yesterday?
   ☐[1] Today.....☐[2] Yesterday ☐[3] Before Yesterday

5. And at what time was that? ☐☐ : ☐☐ A.M / P.M.
   h h : m m **(Circle One)**

C. Blood Collection: ***Note: Remove LMX4 cream if used prior to blood collection; indicate LMX4 use in item 11.***

6. Date of blood collection: ☐☐/☐☐/☐☐☐☐ 7.Collection time: ☐☐ : ☐☐ A.M / P.M.
   m m/ d d / y y y y h h : m m **(Circle One)**

8. Number of venipuncture attempts: ☐

9. Any blood drawing incidents or problems?..... ☐[1] Yes ☐[0] No **If Yes, specify in Q10 and/or Q11**

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

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

11. If any other blood drawing problems/comments not listed above (e.g., fasting status, etc.), describe incident or problem here: (*also list LMX4 cream use here)

_____

_____

_____

12. Phlebotomist's code number: ☐☐☐

D. Blood Processing:

13. Time at which tubes 3 - 5 were centrifuged: ☐☐ : ☐☐ A.M / P.M.
h h : m m **(Circle One)**

14. Time at which tubes 1-2 were centrifuged: ☐☐ : ☐☐ A.M / P.M.
h h : m m **(Circle One)**

15. Time at which aliquot tray 1 vials were placed in freezer: ☐☐ : ☐☐ A.M / P.M.
h h : m m **(Circle One)**

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

17. Any blood processing incidents or problems? ☐[1] Yes ☐[0] No **If yes, specify in Q18 and/or Q19**

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

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

19. Comments on blood processing:

_____

_____

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. The **original** Biospecimen Collection form from each participant is also sent with every frozen shipment; keep a copy of the Biospecimen Collection forms at the field center.

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 original form is sent to the Central Laboratory with the specimen shipment, and a copy is filed at the Field Center.

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
SOL Youth SHIPPING FORM
PART ONE (To be completed at Field Center)

TO: SOL Youth Central Laboratory
University of Minnesota Advanced Research and Diagnostic Laboratory
Room L275 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455

FROM: Name and address of Field Center 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 SOL YOUTH SHIPPING FORM Page ____ of _____

SOL Youth Central Laboratory
University of Minnesota Advanced Research and Diagnostic Laboratory
Room L275 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455

Complete frozen sample for each participant includes 6 bags containing:

4-blue top microvials	8-red top microvials
8-purple top microvials	1- 5 mL clear top (EDTA whole blood)
1-5 mL blue top (packed cells)	2- (1) 0.250 mL red top and (1) 0.250 mL purple top

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

[Place Lab ID label here]

Appendix 5. Daily Temperature Logs SPRINT DAILY TEMPERATURE RECORD

DATE Mo/Da/Yr	-70 Frzr	Refrig	Room	Initials	DATE Mo/Da/Yr	-70 Frzr	Refrig	Room	Initials

Appendix 6. SOL Youth Quality Control Phantom Participant ID Form

SOL Youth Phantom Form

PHANTOM ID NUMBER:									Contact Occasion:	01	SEQ #	00	FORM CODE: PHT VERSION: A 8/19/2011

Instructions: *This form should be completed during participants' visit. Affix the PHANTOM SOL-Youth ID label above. Affix the matching PARTICIPANT SOL-Youth ID labels for the corresponding QC blood sample below. Note: Lab IDs will be linked through the corresponding Laboratory Collection form for each Participant ID, including the Phantom ID.*

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

1a. LAB ID#: ***Affix Spec.Label***

2. Code number of person assigning phantom ID:..... ☐☐☐

PROCEDURE	MATCHING PARTICIPANT ID#	DATE COLLECTED (MM / DD / YYYY)	TECHNICIAN ID
Blood Samples 1. Tubes 1 & 2 - 5 mL red-stoppered (serum)			
2. Tube 3 - 4.5 mL blue-stoppered (Citrate)			
3. Tubes 4 & 5 - 5 mL lavender-stoppered (EDTA)			

Appendix 7. 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. Stage 1 tube spin	___	_____
4. Stage 2 aliquotting	___	_____
5. Stage 3 tube spin and processing	___	_____
6. Vials sealed	___	_____
7. Freezer organization	___	_____
8. Time constraints	___	_____
9. Disposal of contaminated supplies	___	_____
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 8. Sample Exams for Certification

PRACTICAL EXAM FOR SOL YOUTH BLOOD DRAWING TECHNICIAN

1. Place the following blood collection tubes in the correct set-up order and location for the venipuncture: 2-5 mL red top, 1-4.5 mL blue top, and 2-5 mL lavendar top tubes.

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

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

4. Set up a sponge tray with the appropriate number and order of specimen storage 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.

WRITTEN EXAM FOR SOL YOUTH 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. For the first six months of the study, how many HCHS-Youth participants at each field center will be asked to donate additional blood specimens collected to be used as part of the phantom duplicate?
   a) Six per month
   b) Six 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) #3
   d) none of the above

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

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

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

7. 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

8. 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

9. 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

10. In what manner is the buffy coat from tubes #4 and #5 initially pipetted?
   a) Using slow aspiration avoiding plasma and red cells.
   b) Using slow aspiration and include the remaining plasma and some red cells.
   c) Using quick aspiration avoiding plasma and red cells.
   d) Using quick aspiration and include the remaining plasma and some red cells.

11. 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) Original Shipping Form Face Sheet, Original Contents Sheet(s), and Original of all Biospecimen Collection forms

12. The LMX4 anesthetic cream needs to be applied 30-45 minutes prior to the blood collection to adequately numb the skin?
   a) True
   b) False

13. The inner Styrofoam shipper needs to be taped shut?
   a) True
   b) False

14. During the phlebotomy procedure it is important to always maintain a soft, quiet voice with the child?
   a) True
   b) False

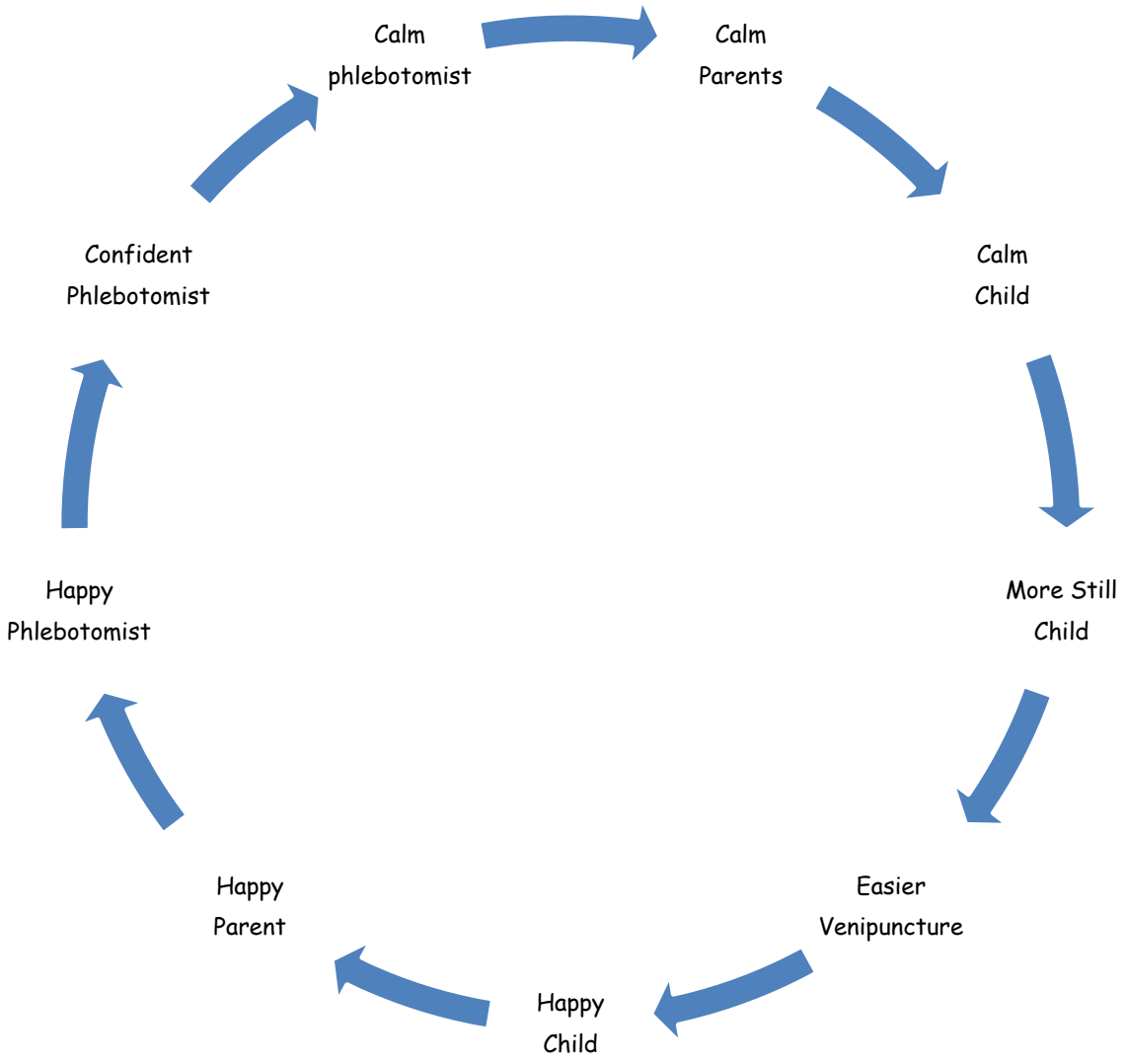
15. What choice below includes positive reinforcement techniques for a “happy child” following the phlebotomy procedure?
   a) Use general positives such as “good girl” or “good boy”
   b) Positive reinforcement is not needed after the phlebotomy procedure
   c) Say specific positive things that the child did; holding still, being brave
   d) None of the above

Appendix 9. Pediatric Phlebotomy Techniques

Pediatric Phlebotomy Techniques:

Information provided by Jina Forys, MT ASCP, Pediatric Supervisor, Acute Care Lab, University of MN Medical Center- Fairview; Minneapolis, MN

Follow the Circle of Calm & Happiness



Calm Phlebotomist

Know Participant Background:

1) Each child has a variation of experiences that contribute to how they will handle this draw
2) Consider age, patient's reaction, parent reaction
3) Ask parent about previous draws and tips on what helps this participant best: "Has your child had a blood draw before?" "Is there anything specific that would help to make this easier?"
4) First time experience? Introduce new ideas to participant/parents: "Would you like me to explain what I am doing?"
5) PREPARE a plan of action for this unique child

Preparation=Confidence:

1) #1 Rule: Be confident that you can assess the situation and look for a vein
2) The more time you spend preparing out of the child's view, the less time they have to get anxious
3) Get equipment ready outside of the participant's view (needles, tubes)
4) Know your minimum volumes (tube priority if it is a difficult draw)
5) Wish for the best, plan for the worst- when you run into problems you will be glad you had everything ready to go

Extra Safety:

1) Pediatric guidelines suggest the first phlebotomist try twice, the second once, then contact the nurse or physician for further instructions

Calm Parent/Child

Phlebotomist Rapport:

1) A child left to their own thoughts can easily become scared and anxious
2) Your attitude can decrease stress
3) Introduce yourself: "My name is Jina, and I will be checking your blood today."
4) Ask questions about their day or things that they may have with them
5) Both participant and parent may see you as a real person and feel more comfortable
6) Parents appreciate common courtesy
7) Simple manners and a friendly tone can ease a parent's worry: "Thank you for coming to the lab today."

One Voice:

1) Be aware of lab environment; too many people talking at the same time can be over-stimulating, confusing, and lead to high anxiety for the child.
2) As the phlebotomist you can: If you notice the parent is talking to the child during the draw, be quiet to let the parent provide comfort.
3) As the phlebotomist you can: Allow the participant to engage in distraction activity without interrupting.
4) As the phlebotomist you can: Be aware that when a child's voice gets louder, adults often talk louder too. Always maintain a soft, quiet voice with the child.

Explanations:

1) Children can have misconceptions or fears about what is going to happen
2) Quite a bit of detail may be necessary
3) Do NOT tell children things that you don't know such as "this won't hurt" or "this is the last time"
4) If a child asks if it will hurt, be honest and say "some people think it feels like a bug bite or a little pinch"

5) Sometimes it may be necessary to give just as much explanation to parents as young children

Show Comfort and Concern for the Participant:

1) Wrap tourniquet over their sleeve so it doesn't pinch their arm (explain you do not want to hurt them)
2) Kindly ask to move things in your way
3) Let the child know it is okay to cry; sometimes it is reassuring to know that pokes are not fun for anyone. Crying is a coping strategy: "I know this is hard. You are doing great." Do NOT say "I'm almost done."

Choices:

1) Children need choices to be comfortable; provides a sense of control
2) Giving the child choices can decrease anxiety
3) Which arm can I look at first?
4) Would you like me to count to three before the poke?
5) What kind of bandage would you like?
6) Would you like to watch the blood draw or look away?
7) Some children may take advantage of choices and continually change their mind as a way to delay a poke
8) If the child cannot make a choice, or uses choices to stall, be confident to move forward with the procedure: "I'm going to look at your arm now."
9) Limit options, as too many could be overwhelming: "Would you like to look at an eye spy book? Or a light wand?"
10) Do NOT give the child options that they don't have: "Can I poke you now?"

Distraction:

1) Offer tools for participants and/or parents when appropriate: "It may help to have something fun to look at while I check your blood. Or it may help to count to 10."
2) Books, TV, stuffed animals, questions about their day
3) Ask parent to be a part of the distraction (reading a book, etc)
4) Giving jobs to take their mind off of the poke: Holding the gauze, counting or singing while the tubes fill, concentrating on staying very still

Comfort Positioning:

1) A child is going to be most trusting and comfortable with a parent or other comfort person/caregiver
2) Ask parent about their level of comfort in participation: "Are you comfortable holding your child's arm still?"
3) Give parent clear instructions on their role
4) Gives a parent a positive way to support their child
5) Having this person help hold the child can be beneficial in certain cases
6) Using comfort holds the child may be more relaxed and resist less, making the collection easier
7) You may still need another staff member to help stabilize the child
8) Seated positions are less scary than lying positions: promotes sense of control

Still Child: Role of Holder

1) Antecubital draw: one hand at wrist and one at elbow to minimize twisting
2) Hand draw: one hand at forearm and one holding fingers in fist

3) Holding too tightly can stop blood flow
4) While drawer is working holder can calm child
5) Never be afraid to ask for extra help from other medical staff or parents to hold child
6) Two or three people in a room are usually enough. Too many people can be scary!

Extra Measures for Comfort:

1) LMX4 anesthetic cream
2) Numbs the surface of the skin
3) Cream must be placed over the site for 30-45 minutes prior to phlebotomy (longer=better)
4) Takes away the initial sting of poke

Happy Child

Reinforcement after the blood collection:

1) Trying to rebuild trust after the poke can be beneficial for the next time
2) Say specific positive things that the child did: holding still, being brave
3) Do NOT use general positives such as “good girl” or “good boy”
4) Small treasures or stickers can also reinforce the experience
5) Leaving them with a positive attitude may bring them back with one at a later time

Appendix 10. 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 11. Partial Biospecimen Collection Procedure

Participant Sample Set

1. If a full set of biospecimen collection tubes cannot be obtained after 2-3 attempts, determine if the SOL Youth participant is willing to return for a (fasting) re-collection appointment. Insert a comment on item #11 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 #11 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. If the participant is scheduled for a re-collection appointment, process all of the collection tubes 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.

3. Assign a new Lab ID to the SOL Youth participant for the re-collection appointment. Attempt to re-collect the entire sequence of biospecimen collection tubes.

4. 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.

5. If the biospecimen set from the first visit is the most complete, then send this set to the Central Laboratory and indicate on the Frozen Contents Sheet that this biospecimen set is incomplete and no other specimens will be obtained. Discard the other incomplete biospecimen set from the re-collection appointment. For the Field Center records, save the Biospecimen Collection Form from the re-collection appointment but insert a comment on item #11 that the biospecimens were discarded and the biospecimens from the first visit were sent to the Central Laboratory.

6. If the biospecimen set from the re-collection appointment is the most complete, then ship this set to the Central Laboratory and send the new Biospecimen Collection Form to the laboratory. Insert a comment on item #11 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 #11 that the participant was re-collected and assigned a new Lab ID number and the first set of biospecimens were discarded.

7. 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.

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

9. 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 #1 or Tube #2 must be completely (100%) full. A partially filled second tube is acceptable to provide as much serum as possible.

3. Tube #3 must be completely (100%) full in order to maintain the proper ratio of blood to liquid anticoagulant.

4. One of Tube #4 or Tube #5 must be completely (100%) full. A partially filled second tube is acceptable to provide as much plasma as possible.