CHAPTER 13

BLOOD COLLECTION AND PROCESSING

Study Documents referred to in this Chapter:

A) Lab site survey

B) Supply request form

C) Sample sheet of barcode labels

D) Phlebotomy log sheet

E) Shipping form

F) Request for sample destruction form

G) Phlebotomy form

H) Processing form

I) Blood processing chart.

CHAPTER 13
BLOOD COLLECTION AND PROCESSING

13.1 Background and Rationale

Aging is associated with declines in physical function that often lead to onset of physical disability and loss of independence. While there is a common pathway of sarcopenia underlying aging-related loss of function, little is known regarding the biological factors that are fundamental for the progression of this process. To date, regular exercise training is the only therapy known to consistently improve physical function in older adults; however, again, there is a paucity of data about the mechanisms by which exercise results in improved physical function in the elderly.

The overall goal of the LIFE pilot study Biological Specimens Repository is to guarantee the proper collection, processing, shipping, and central storage of both blood and DNA specimens. These samples will be used for the purpose of conducting future ancillary studies designed to examine the effects of exercise on circulating biomarkers and/or to examine how variation in genes modulates responses to the exercise treatment. An important step (and potentially the most variable) in answering these questions is the collection and processing of biological specimens. If the sample itself is not correctly collected, processed, handled, or stored, future assay results may not be valid. The LIFE Biological Specimens Repository committee and staff will oversee training and monitor the quality control of the blood collection and processing at each of the 4 Field Centers. Blood collection consists of drawing 64.5 mls of blood at baseline, 6 months, and 12 months on each LIFE participant who provides written, informed consent for this procedure.

13.2 Contact information

LIFE Biological Specimens Repository:

Operations Personnel:

13.3 Equipment/supply list

Each Field Center should have the following equipment/supplies in the blood collection area:

- Refrigerated centrifuge
- Storage space in a -70°C freezer
- Color or black/white printer for printing study forms
- Tube racks
- Plastic-backed bench covers
- Waterproof pens (Sharpie permanent marker/industrial strength)
- Alcohol wipes
- Ammonia spirits, ampules
- Butterfly needles (21 gauge) with luer adapter (B-D # 7251)
- Disposable gloves
- Paper towels
- Disinfectant Cleaner (Kills HIV and HBV) or Bleach decontaminant- 1 part Clorox to 9 parts water, stored in a labeled squeeze bottle)
- Biohazard waster containers
- Sharps/biohazard containers
- 3” Packing tape for sealing shipping containers
- Dry ice available for shipping

The following supplies will be provided by the Administrative Coordinating Center:

Blood Collection Supplies:

- 10 ml EDTA (K3 EDTA) purple-top vacutainers
- 10 ml red-top vacutainers
- 4.5 ml blue-top vacutainers (3.8% buffered Sodium Citrate)

- 10 ml green-top vacutainers (Sodium heparin)
- Timers
- Coolers for transporting frozen samples
- Band-aids
- 21 gauge safety needles
- Tourniquets
- 2 X 2 gauze pads
- Micropore tape

Blood Processing Supplies:

- Sample tube Kryoracks-holds 6 sample tubes
- Cryovials-2 ml graduated, internally threaded with color-coded caps (purple, red, blue, and green)
- Cryovial Kyroracks-holds 50 cryovials
- Disposable transfer pipettes
- Storage boxes (2") with 10 x 10 dividers
- 15 cc conical tubes for pooling plasma and packed cells for DNA

Shipping Supplies:

- Styrofoam shipping containers with cardboard outer shell
- Pre-printed Federal Express air bills

Providing supplies:

All supplies from the Administrative Coordinating Center will be provided in two ways. The supplies for specimen collection will be shipped directly to the Field Center's laboratory staff from the vendor. Additional supplies can be provided to Field Centers by notifying Karin Murphy at least 10 days before needed.

To order additional supplies, use the ***Supply Request Form*** (Attachment B)
Follow the instructions on the form and, once completed, Fax to:
If you have questions, contact:

13.4 Safety Issues/Universal Precautions for Handling Blood

The Occupational Safety and Health Administration (OSHA) mandated Universal

Precaution standards in December of 1991 for workers involved in dealing with patients and materials and/or samples containing any form of body fluids from patients. In 2001, in response to the Needlestick Safety and Prevention Act, OSHA revised the Bloodborne Pathogens Standard 1910.1030. The revised standard clarifies the need for employers to select safer needle devices and to involve employees in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps.

For a complete understanding of OSHA regulations, refer to the following websites:

- http://www.osha.gov
- http://www.niehs.nih.gov

In accordance with the OSHA regulations on blood borne pathogens, the Biological Specimen Repository recommends the following laboratory safety procedures:

1. Each employee involved in drawing blood and blood processing should be vaccinated with the Hepatitis B vaccine.
2. Employees who come in contact with blood should be trained in proper blood borne pathogen procedures and use of universal precautions. This document is only a summary and should not be considered as training in these areas.
3. Supplies for the LIFE Study are selected to meet the current safety device standards set by OSHA. Each facility should keep a log of injuries from contaminated sharps and follow their local procedure for reporting such injuries.
4. Personal protective equipment (PPE) should be used when dealing with blood and other body fluid samples: gloves, lab coats (non-permeable), face-shield, counter-top safety shields, goggles or masks.

   A. Gloves

   1) Wear gloves for all patient contact when body fluids are involved.
   2) Change gloves between patients and when gloves become soiled or torn.
   3) Wash hands thoroughly after removing gloves. Use an

"evaporating" disinfectant if a sink is not available.

4) Any equipment that is to be used while wearing gloves should be labeled with a biohazard sticker.

B. Lab Coats/Gowns

Wear non-permeable lab coats or plastic disposable aprons when processing blood.

C. Splash Shields

Counter-top splash shields, glasses/goggles or face shields should be used to protect mucous membranes (eyes, nose, mouth) from splashes or aerosols created from de-capping samples during the aliquoting process.

13.5 Specimen ID Labels

The Biological Specimens Repository will use an additional ID number to label specimens that is different from those used for the study's main participant ID numbers. **The Phlebotomy forms and the Blood Processing forms should have labels containing both ID numbers**.

The Repository will supply each Field Center with sheets of Biological Specimen ID barcode labels. These will be used for labeling forms, blood collection tubes, cryovials, and sample boxes. There will be a total of 59 labels: 7 labels for collection tubes (plus 3 extra), 3 labels for the 15-ml conical tubes for packed cells, 1 label for each of the freezer storage boxes (blood and cells for DNA), 30 cryovial labels, 2 processing labels used for tubes to pool plasma and serum, and 3 form labels (plus 1 extra). The extra 8 labels will be printed with the 5-digit biological sample ID number and can be used as a back-up if needed. A sample sheet of barcode labels can be found in Attachment C.

All labels have the same 5-digit Biological Specimen ID number, which is different from the participant study ID (the first digit identifies the clinic: 1=Cooper, 2=Stanford, 3=Pittsburgh and 4= Wake Forest University. The next 3 numbers will be the participant's unique Biological Specimen ID number. Biological Specimen ID numbers will run 001 through 120. The next number on

the label is to identify the visit. 1=Baseline, 2=6 month visit, 3=12 month visit. For example, the Biological Specimen ID number may be 1001-1 (Cooper site, participant 001, baseline visit) or 3050-3 (Pittsburgh site, participant 050, 12 month visit).

Collection tube and cryovial labels will have an additional line of the barcode label which contains alpha characters to denote the sample type: E= EDTA Plasma, R= Serum, H= Heparin Plasma, and C= Citrated Plasma. Cryovial labels will contain an additional number which denotes the chronological number of that particular sample type (E-1 will mean EDTA plasma sample, Cryovial # 1)

Other abbreviations used on the label sheet include: BX= Box, PP= Plasma Pooled, RP= Serum Pooled, PC= Packed Cells (from EDTA tube), CT= Collection Tube, FRM= form labels.

It is essential that the labels be used precisely as described to ensure the participant's specimens are not miscoded. It is recommended to pre-label the collection tubes and cryovials prior to the participant's visit with a careful cross-check of the labels with each participant's study ID # and their Biological Specimen ID #.

13.6 Blood collection/Phlebotomy

13.6.1 Phlebotomy forms

The purpose of the ***Phlebotomy form*** (Attachment G) is to facilitate the efficient collection of blood samples from participants, with maximum protection for the participant and the phlebotomist. The form is also used to facilitate the monitoring of the blood drawing procedure and other quality assurance data critical to the interpretation of the assay results.

There are five questions on the form to ask the participant before starting the venipuncture procedure. The first three questions relate to the venipuncture. If the participant's answer is yes to any of the first three questions, the phlebotomist should take extra care during the phlebotomy. Question #4 has to do with the participant's diabetic health status. Question #5 asks about fasting

status. It is recommended that the participant must have fasted for at least 8 hours (12 hours is preferred) prior to the blood draw. They may have water to drink, but no coffee or other caffeinated or caloric beverage. **Try to reschedule the blood draw if the minimum fasting time is less than 8 hours**. If the participant is not willing to reschedule the blood draw, proceed with the blood draw, but make a note that the participant was not fasted and note the specific number of hours since last food or drink.

Participants should take their medications as usual on mornings of each phlebotomy visit. Participants will be bringing all medications for a medication review at each of the phlebotomy visits. Thus, if a participant needs to take their medication with food, they can take the medication after the blood draw during their snack.

Both the participant study ID label and the Biological Specimen ID label should be affixed to each form. All forms must be completed in ink.

Upon completion, the ID barcodes on each form should be scanned at the Field Center and data from the form should be entered on the web-based LIFE database. A completed photocopy of the Phlebotomy form should be included in the sample shipments.

13.6.2 Informed Consent and Participant refusal for blood draw

Some participants will provide informed consent to participate in the LIFE study without consent for blood collection. Blood should only be drawn from those participants who have agreed to participate in this aspect of the study by checking the blood draw box on the informed consent form. In rare instances, some participants may have checked the box on the consent form, but will later refuse the blood draw during their scheduled appointment. The participant has a right to withdraw consent at any time and blood should not be drawn from these participants.

The study coordinator who is conducting the assessments at the visit should inform the phlebotomist that the person has not agreed to a blood draw. However, the

phlebotomy form should still be filled out (with the 'No, Refused' option marked) for any participant who refuses the blood draw. All forms should be labeled with the Participant ID# and the specimen ID #, regardless if blood will be drawn or not. A copy of a Phlebotomy form should be sent to the Biological Specimens Repository for EVERY participant, regardless if blood was drawn or not.

13.6.3 Preparation/Set-up

Prior to the participant's visit, the phlebotomist should label the collection tube vacutainers and prepare all supplies. Collection tubes should be labeled with BOTH the participant ID number legibly hand-written with a Sharpie marker on each tube, and with the provided WHITE label containing the Specimen ID# labeled with 'CT' (collection tube). For the 6-month and 12-month visits, please check again that the participant study ID matches the Biological Specimen ID number and label the Phlebotomy Form. Complete the ***Phlebotomy Log sheet*** (Attachment D).

The phlebotomist must be sure that informed consent forms have been signed before drawing the participant's blood. Seven tubes of blood of various sizes are collected, each containing about 1-2 teaspoons of blood (5-10 ml). Participants whom are concerned about the amount of blood being drawn can be reassured that they donate 7 times that volume (450 ml) when donating a unit of blood.

The phlebotomy procedure should be standardized from a sitting position. A 21-gauge safety needle will be used routinely. A butterfly may be used if needed to minimize the trauma to the skin and vein. The phlebotomy should be timed and the time the tourniquet was in place should be noted on the form. Do not rush the participant before or after the phlebotomy procedure, but make them as comfortable as possible. Remember, they will remember the attitude and competency of the phlebotomist. Be pleasant and treat them the way you would want to be treated. Never force a participant to have their blood drawn.

13.6.4 Priority of collection tubes

A total of 64.5 ml of blood will be drawn in 7 tubes from each participant. **The order in which the tubes are drawn is very important**. **Blood collection**

must follow the order listed:

1. 2 10 ml Serum red-top tubes
2. 2 10 ml EDTA purple-top tubes
3. 1 4.5 ml Citrate blue-top tube
4. 1 10 ml Heparin green-top tube
5. 1 10 ml EDTA purple-top tube (this tube is drawn last in case the total volume cannot be obtained. Thus, all types of sample will have been obtained)

13.6.5 Phlebotomy Room

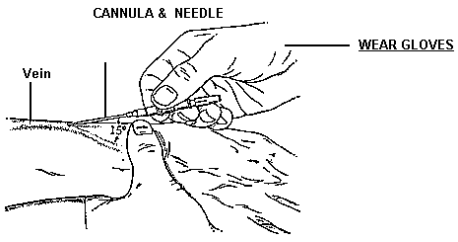
The Phlebotomy Area should include a chair for the participant, a table for blood collection supplies, and a sink for washing hands. A phone/intercom system should be within reach and access to emergency equipment should be available. Accommodations should ensure the participant can sit quietly for 5 minutes prior to the venous blood draw.

13.6.6 Venipuncture Procedure

This section is designed as a brief review of the basics in blood collection techniques. Employees hired for this job must previously be trained in universal precautions and should have successfully completed a phlebotomy course. For additional information on general venipuncture refer to the following websites:
http://phlebotomy.com/links.htm
http://gasbone.herston.uq.edu.au/teach/su602/docs/g44_0ic.html

Universal precautions should be employed during any specimen collection. The following is a suggested method of performing blood specimen collection.

1. Prepare the necessary supplies
2. Confirm the ID of the study participant
3. Tell the participant that you will be obtaining a blood sample
4. Wash your hands
5. Put on non-sterile exam gloves
6. Position patient's arm in comfortable position



7. Select an appropriate vein for venipuncture
8. Place the tourniquet above the selected vein
9. Clean site with alcohol using circular motion from center outward
10. Steady the vein with your thumb 1-2 inches below the site to decrease vein rolling
11. Enter the vein with the vacutainer needle bevel up at a 15 degree angle
12. Fill 2-10 ml red-top tubes, 2-10 ml purple-tops, 1-4.5 ml blue-top, 1-10 ml green-top, and lastly another 10 ml purple-top (if the vein is holding up), **in this precise order**
13. The phlebotomy should be timed and the time recorded on the form.
14. **Avoid under filling the collection tubes**. Purple-top collection tubes containing liquid EDTA must be filled to at least 50% of the fill volume of the tube. If the tube is not filled to at least 50% of fill volume, there will be a dilutional effect from the liquid anticoagulant and the specimen will be unsatisfactory for testing. The Citrated plasma tube (blue top) must be at least 75% full to have the proper ratio of anticoagulant to blood.
15. Mix blood vacutainer tubes several times **immediately** after collection by inverting them **gently and evenly**
16. Remove the needle when the venipuncture is completed. Close the safety device and dispose of the needle into a Sharps Container
17. Apply gauze and tape holding pressure for about 30 seconds to minimize the formation of a hematoma. Ask the participant to apply pressure for another 1-2 minutes
18. Remove the disposable gloves; place them in a biohazard trash container. Wash your hands. Transport the sample to the necessary processing area.

13.6.7 Difficulties or Problems

1. If the participant is apprehensive or tense about the phlebotomy procedure have them drink some water and do some relaxation breathing. This will help the veins to be more accessible.

2. 'Butterfly' collection systems may be used to minimize trauma to the skin and vein. Be aware that clotting may begin in the tubing before blood comes in contact with the anticoagulant in the collection tube. Mix the

tubes thoroughly during collection.

3. If there is difficulty in obtaining the blood you may move the needle slightly to adjust the bevel of the needle. If this is not successful, release the tourniquet and remove the needle. Apply pressure and bandage the site. Ask the participant if you may look at the other arm. Also remember not to apply the tourniquet so tightly so as to obstruct the blood flow.

4. The same phlebotomist should not ever attempt a venipuncture more than two times.

5. Reassure the participant that the inability to obtain a blood sample is not a sign of a medical problem. Reschedule the blood draw visit for another day if you think the participant will be a good candidate for blood collection, but just had a bad day. Make a note on the Phlebotomy form stating that the blood draw will be rescheduled.

6. Make a note on the Phlebotomy form if the venipuncture was unsuccessful.

7. If the participant continues to bleed after the phlebotomy, apply pressure to the site with a gauze pad and keep the arm elevated until the bleeding stops. Tape a gauze bandage securely on the arm and instruct the participant to leave it there at least 1 hr.

13.7 Blood Processing

13.7.1 Blood Processing Forms

The purpose of the ***Blood Processing form*** (posted on the study website) is to facilitate the efficient processing and aliquoting of plasma and serum samples from participants. The form is also used to monitor sample volume and integrity in each cryovial and other quality assurance data.

Both the participant study ID label and the Biological Specimen ID label should be affixed to each form. All forms must be completed in ink.

Complete the form as the aliquoting is conducted. The technician's initial should be listed on the form next to each aliquot along with an indication of any sample that are hemolyzed or are partial volumes. Sequentially number a storage box and record this number on the Blood Processing form. Upon completion of the form, keep it on file in the processing lab. Completed photocopies of each form should be included in the sample shipments.

13.7.2 Participant refusal for DNA

Some participants will provide informed consent to participate in the LIFE study blood draw, but will not want their DNA extracted from this blood for use in genetic testing. The packed cells for extraction of DNA should only be saved from those participants who have agreed to participate in this aspect of the study by checking the DNA genetic testing box on the informed consent form. Make a note on the Blood Processing form that the participant refused the collection of DNA for genetic testing. The packed cells only need to be saved for DNA extraction at the baseline visit, not the 6-month or 12-month visit.

The participant has a right to withdraw consent at any time or to request that their biological specimens be destroyed. If a participant makes this request, complete the ***Request for sample destruction form*** (provided at the end of this chapter). This form should be faxed to the Biological Specimens Repository. After verification of destruction of the samples, the form will be faxed back to the site to be included in the participants research file.

13.7.3 Overview and Description of Aliquots

Processing should be initiated as soon as possible (less than 30 minutes) following the blood draw. The red-top serum tubes must stand at room temperature for at least 30 minutes, but no longer than 60 minutes, before centrifugation. **Plasma tubes should be placed immediately on ice (for no longer than 30 mins) until centrifugation**. Personal protective equipment should be used during processing. All work areas should be wiped down with 10% Bleach solution (or approved biohazard disinfectant).

The number of aliquots for each sample type should follow the protocol listed on the Blood Processing form and the table below:

Sample type	# cryovials	Sample volume Per cryovial	Color
EDTA plasma (pooled from 2 10-ml vacutainers)	12	0.5 ml	Purple
Serum (pooled from 2 10-ml vacutainers)	3	1 ml	Red
	6	0.5 ml	Red
Citrated plasma (from 4.5 ml vacutainer)	3	0.5 ml	Blue
Heparin plasma (from 10-ml vacutainer)	6	0.5 ml	Green

13.7.4 Preparation/Set-up

Collection tubes for phlebotomy, three 15-ml conical tubes for packed cells, one for pooled plasma, one for pooled serum and one for whole blood (for DNA), and all 2 ml cryovials should be pre-labeled and lined up by sample type and by cryovial number in the aliquoting racks before the start of the participant's blood draw visit. **Place specimen labels on tubes so that the barcode runs vertically with the readable portion on the right side**. Kryoracks should be ready and the centrifuge turned on and set at the proper temperature prior to the visit.

13.7.5 Processing/aliquoting

Follow the procedures below precisely for processing and aliquoting of each sample type. **If there is any deviation from the listed procedure, please note it on the Processing form.**

Serum (red-top) samples:

1. Red-top tubes should be allowed to clot by letting them sit at room temperature for at least 30 minutes, but no longer than 60 minutes
2. Centrifuge the red-top tubes at 3000 x g for 15 minutes (45,000 g-min)
3. After centrifugation, pool the serum from both 10 ml red-top vacutainers into a labeled (RP for serum pooled) 15 ml conical tube. Cap the conical tube and mix **gently**. Place the conical tube in the Kryorack to chill the

serum while it is being aliquoted.

4. Aliquot using plastic transfer pipettes into 2-ml prechilled, labeled, plastic, screw-top cryovials with O-ring seals according to the blood storage protocol. The aliquots must be frozen immediately and stored frozen at below -70°C.
5. The remaining cells in the serum tube (and the tubes themselves) can be discarded in the biohazard waste.

EDTA (purple-top) and citrated (blue-top) plasma samples:

1. Purple-top and blue-top tubes should be mixed gently by inversion 4-6 times immediately after drawing and **placed on ice without delay until centrifugation** (must be processed within 30 minutes of blood draw)
2. **Mix gently and slowly,** carefully pour the EDTA whole blood from one of the EDTA vacutainer tube into a 15-ml conical tubes. Use the PC-1 label for this conical tube. Freeze this in an up-right position at -70˚C. Place in a study storage box without dividers until shipment to the repository using dry ice. Centrifuge the other 2 EDTA tubes (or purple-top vacutainers) and the 4.5 ml or 2.7 ml (which ever is appropriate for the participant) blue-top vacutainer at 2000 x g for 15 mins (30,000 g-min) in a refrigerated centrifuge set at 4˚C.
3. After centrifugation, place the tubes in the Kryorack sample tube containers (or an ice bath) to keep them at the proper temperature during aliquoting.
4. Transfer the EDTA **plasma** from the two EDTA vacutainer tubes **into one 15 cc conical tube labeled with PP-1.** Cap the PP conical tube and **gently** mix the pooled plasma with 4-6 inversions. Then aliquot into 12 prechilled and labeled screw-top cryovials with O-ring seals according to the blood storage protocol. The aliquots must be frozen immediately and stored frozen at below -70°C.
5. Aliquot the citrated plasma per the LIFE Blood aliquoting chart.
6. Transfer the Citrated plasma into 2 ml prechilled and labeled screw-top cryovials with O-ring seals according to the blood storage protocol. The aliquots must be frozen immediately and stored frozen at below -70°C.

Heparin (green-top) plasma samples:

1. Green-top tubes should be mixed **gently** by inversion 3-4 times to insure proper anticoagulant mixing.
2. **Store on ice** until centrifugation (must be processed within 30 mins of blood draw)
3. Labeled tubes should be placed in a refrigerated centrifuge (4°C) and spun at 3000 x g for 15 minutes (45,000 g-min) in order to derive platelet-poor plasma
4. Immediately following centrifugation, place the Heparin tube into the Kryorack for blood collection tubes and aliquot the plasma via plastic transfer pipettes into 2-ml plastic, screw-top cryovials with O-ring seals according to the study blood storage protocol. The labeled cryovials should be prechilled in the Kryorack designed specifically for cryovials **before** aliquoting. The aliquots must be frozen immediately and stored frozen at below -70°C until analysis.

DNA samples (Baseline visit only):

At the baseline visit, DNA will be extracted from leukocytes collected in the three EDTA conical tubes that are labeled with the Biological Specimen ID and the designation of PC1, PC2, and PC3 for the EDTA packed cells. After removal of the plasma from the 3 conical tubes the remaining packed cells in the conical tubes labeled PC1, PC2, and PC3 should be placed in a separate storage box (with no dividers) and frozen at -20°C until shipment to the Blood Repository Laboratory.

13.7.6 Freezing and short-term storage

After aliquoting and capping of samples, the cryovials should be placed in a short-term storage box (boxes and box labels are provided by Biological Specimens Repository). The number of the box should be noted on the Blood Processing form. There will be room for storage of 3 participants per box. Ideally, these will be in chronological order (i.e., participants 001, 002, and 003) in the same box. After 6 months, there will be overlap of participants having blood drawn for follow-up and baseline visits. Continue to place the samples in the storage boxes in the order that they are processed.

The PC1, PC2, and PC3 conical tubes containing the packed cells for DNA extraction should be placed in the separate storage box for these 15-ml conical tubes.

Every effort should be made to freeze samples at -70°C or below as soon as possible after aliquoting. If specimens cannot be frozen immediately, they may be temporarily stored (for less than 2 hours) at -20° C or placed on dry ice until transfer to -70°C or below. Dry ice is the preferred solution.

13.7.7 Difficulties or problems

Low sample volume: If there is insufficient sample of a particular sample type to fill the full set of aliquots fill all 0.5 ml aliquots first, then proceed to the 1 ml aliquots. EDTA collection tubes must be greater than ½ full, and Citrate tubes must be greater than ¾ full—if not, discard this blood and do not aliquot. If any aliquot is less than the specified volume, note this as a Partial (P) volume on the Blood Processing form. Also mark the top of the cryovial itself with a P using a black 'Sharpie' marker.

Hemolyzed sample: If any of the serum or plasma is hemolyzed (pinkish or reddish color due to disruption of red blood cells) note this as a hemolyzed (H) sample on the Blood Processing form.

Timing of processing: If, for some unexpected reason, centrifugation of plasma cannot be conducted within 30 minutes of blood collection, try to process specimens as soon as possible. Maintain the plasma tubes on ice until processing. Note the delay in processing on the comments section on the Blood Processing form.

13.8 Specimen shipping

13.8.1 Packaging

Frozen, aliquoted serum, plasma, and packed cells need to be shipped quarterly on dry ice in the supplied shipping containers via **Priority Overnight Federal**

Express courier service. Polyfoam shipping containers are provided by the Biological Specimens Repository at Wake Forest University School of Medicine. **Please place appropriate amount of dry ice in the shipping container for transport**.

13.8.2 Shipping Forms

The ***Specimen Shipping Form*** is posted on the study website. Make multiple copies for your use. These forms are used to indicate the number and types of vials included in the shipment, the identity of the samples, and pertinent clinic and visit information. When a shipment is made, a photocopy of the form(s) should be produced and retained at the clinic. The original(s) are placed in a Ziploc bag and included with the specimens. In addition, a copy of each form should be faxed to the Biological Specimens Repository Laboratory with the FedEx tracking number on the form so that the shipment can be tracked.

13.8.3 Shipping Procedure

1. Put rubber band around each storage box. Place storage boxes in plastic Ziploc bags and surround them with dry ice. There should be enough dry ice in the shipping containers to cover a 2-day shipping time. Once the shipping container has been filled, use 3" clear shipping tape to seal the seam on the Styrofoam box to slow evaporation of the dry ice. Leave 2 inches of the seam open to allow the dry ice to breath.

2. Make a copy of the Shipment Form(s) and retain at the clinic. Place the original(s) in a Ziploc bag, positioning it on top of the sealed shipping container. Close the cardboard exterior shell around the polyfoam container and tape shut. Affix completed air bill to the container, choose Priority Overnight, and call FedEx for pickup.

3. **On the day a shipment is made, send (via FAX:) a copy of each Shipment Form to the Biological Specimens Repository Lab**, to alert them of a shipment's pending arrival. This will allow laboratory personnel to investigate and track packages if there are delays or problems with the courier.

4. Shipping Schedule: Shipments should only be made on a Monday, Tuesday, or Wednesday. **Do not make your quarterly frozen shipment on a Thursday or Friday**, as a delay by the courier will result in the samples arriving thawed. This will compromise the integrity of the specimens.

5. Holiday Schedule: The Biological Specimens Repository Laboratory is officially closed on all US federal holidays and, more importantly, FedEx will NOT deliver on these days. Therefore, avoid shipping on any day preceding a US federal holiday (see calendar).

Due to the length of the Thanksgiving holiday, and possible FedEx delays, we strongly suggest to NOT ship after Monday of Thanksgiving week.

Federal Holiday	2004	2005	2006
New Year's Day	January 1	January 1	January 1
MLK Jr's Birthday	January 19	January 17	January 16
President's Day	February 16	February 21	February 20
Memorial Day	May 31	May 30	May 29
Independence Day	July 4,5	July 4	July 4
Labor Day	September 6	September 5	September 4
Veterans Day	November 11	November 11	November 11
Thanksgiving	November 25,26	November 24,25	November 23,24
Christmas Day	December 24,27	December 26,27	December 25,26

13.8.4 Mailing address

Shipments should be addressed to:

13.9 Quality Assurance

Differences in the procedures used for blood collection or processing could potentially introduce unwanted variance in assays of the samples. Monitoring of the blood collection and processing protocols is critical to identify any deviations from standardized methods described in this Manual of Operations. The LIFE Biological Specimens Repository will monitor the quality of the blood collection and processing at each Field Center via several methods: 1) centralized training and certification of laboratory/phlebotomy staff from each Field Center, 2) oversight of equipment check logs at each Field Center, 3) review of phlebotomy/processing/shipping forms, 4) and, if necessary, oversight visits to each Field Center by Biological Specimens personnel.

Field Center technician Certification: It is strongly recommended that all Field Center technicians drawing and processing blood have prior clinical phlebotomy and blood handling experience/training. In addition, all Field Center technicians should read and understand the LIFE Manual of Operations. Certification in LIFE blood collection and/or processing is required before working with actual study participants' blood samples. Initial certification involves: 1) reading and understanding of the LIFE Manual of Operations, 2) successful completion of training by Biological Specimens Repository personnel or a certified Field Center technician that is qualified to certify other technicians, 3) observation by certified personnel of complete phlebotomy and/or processing procedures on at least one volunteer. Certification/recertification forms (found in Chapter 24 of this manual) must be completed and on file with the Coordinating Center for each Field Center technician.

Field Center equipment records: Each Field Center is responsible for the maintenance of daily and monthly records for equipment performance. Daily temperature checks on freezers and refrigerated centrifuges must be performed. Temperature logs should be kept on file at each Field Center and copies should be sent to the Biological Specimens Repository quarterly. In addition, centrifuge speed should be checked quarterly using a tachometer. This log also should be kept on file and sent periodically to the Biological Specimens Repository. These

equipment records can identify potential problems with sample quality.

13.10 Blood Processing Overview

1. Place specimen labels on tubes so that the barcode runs vertically:
2. Do not share specimen labels between visits or participant sets as they are linked by computer encoding.

3. The phlebotomy should be rescheduled, if the minimum fasting time is less than 8 hours.

4. The Phlebotomy and the Blood Processing forms should have labels containing both ID #s.

5. The order in which the tubes are drawn is very important. Blood collection must follow the specified order.

6. Mix blood vacutainer tubes several times **immediately** after collection by inverting them **gently and evenly** (4-6 gentle inversions).

7. Once all tubes for a participant are filled, place the EDTA, blue-top citrated tubes & the green top Heparin tube into an ice bucket, leaving them no longer than 30 minutes prior to centrifugation.

8. Leave the serum tubes at room temperature for at least 30 minutes but no longer than 60 minutes prior to centrifugation.

9. Centrifuge the tubes as specified. Do **NOT** increase or decrease speed and/or time.

10. Do not use a fixed-angle centrifuge.

11. If you use a non-refrigerated centrifuge, promptly remove tubes as soon as

spinning stops and place them in a Kryorack or ice bath. Make a note of this on the Phlebotomy Processing form comment section that samples were spun in a non-refrigerated centrifuge.

12. Place the tubes in the specimen tube Kryoracks after removing them from the centrifuge.

13. Process all tubes immediately after centrifugation. See processing chart for proper specimen handling.

14. Choose “Priority Overnight” on FedEx airbills.

15. Ship samples on dry ice quarterly on Mon-Wed only.

16. On the day of shipment, FAX the Laboratory with the FedEx tracking Number(s).

13.11 Biological Specimens Repository forms

A. Lab site survey
B. Supply request form
C. Sample sheet of barcode labels
C. Phlebotomy log sheet
D. Shipping form
E. Request for sample destruction form
F. Phlebotomy form
G. Processing form
H. Blood processing chart

LIFE STUDY LAB SITE SURVEY

SITE
NAME:_____/_____
(SITE ASSIGNED #)

LAB CONTACT PERSON:_____
(full name)

PHONE AND FAX NUMBERS :_____/_____

LAB MAILING ADDRESS: _____

_____

_____

LAB INFORMATION

EQUIPMENT SURVEY:

CENTRIFUGE:_____/_____
(Vendor and/or brand) (model #)

CENTRIFUGE ROTOR MEASUREMENT:_____

MAINTENANCE/SERVICE CONTRACT AVAILABLE THROUGH:_____
(Service Supplier & Contract Expiration Date)

FREEZER:_____/_____
(Vendor and/or Brand) (Model #)

FREEZER TOTAL CAPACITY AVAILABLE FOR LIFE STUDY:_____

FREEZER BACK UP SYSTEM:_____/_____/_____
(CO2 BACK-UP) (SENSIPHONE) (OTHER)

MAINTENANCE/SERVICE CONTRACT AVAILABLE THROUGH:_____
(Service Supplier & Contract Expiration date)

BENCHTOP SAFETY SHIELDS AVAILABLE: YES/NO

ALIQUOTING COOLENT SYSTEM IN PLACE:
KRYORACKS: YES / NO
ICE BATHS: YES / NO
DRY ICE: YES / NO

LIFE Study Supply Request Form

Date requested_____

Fax to:

Requested by_____ Site name_____

Item Requested	Quantity requested	Unit (ea, pack, case)	Quantity shipped	Shipping date
10 ml EDTA-Purple top vacutainers				
10 ml -Red top vacutainers				
7 ml CITRATE-Blue top vacutainers				
10 ml SODIUM HEPARIN-Green top vacutainers				
21gauge safety needles				
21 gauge butterfly needles				
Multiple sample luer adapter 100 pk				
Pronto quick release needle holders				
Disposable transfer pipettes				
15 cc conical tubes				
2 ml cryovials (specify cap color)				
cryovial storage boxes				
Cryovial box dividers				
Polyfoam shipping container				
Misc.				

Shipping address: _____

_____

Example of barcode labels

PURPLE	PURPLE	RED	GREEN	BLUE	WHTE
Visit 1 labels					
1001-1-E-1	1001-1-E-2	1001-1-R-1	1001-1-H-1	1001-1-C-1	1001-1-CT
1001-1-E-3	1001-1-E-4	1001-1-R-2	1001-1-H-2	1001-1-C-2	1001-1-CT
1001-1-E-5	1001-1-E-6	1001-1-R-3	1001-1-H-3	1001-1-C-3	1001-1-CT
1001-1-E-7	1001-1-E-8	1001-1-R-4	1001-1-H-4		1001-1-CT
1001-1-E-9	1001-1-E-10	1001-1-R-5	1001-1-H-5	1001-1-BX-B	1001-1-CT
1001-1-E-11	1001-1-E-12	1001-1-R-6	1001-1-H-6	1001-1-BX-D	1001-1-CT
1001-1	1001-1-PC1	1001-1-R-7	1001-1	1001-1-FRM	1001-1-CT
1001-1	1001-1-PC2	1001-1-R-8	1001-1	1001-1-FRM	1001-1-CT
1001-1	1001-1-PC3	1001-1-R-9	1001-1	1001-1-FRM	1001-1-CT
1001-1	1001-1-PP	1001-1-RP	1001-1	1001-1-FRM	1001-1-CT
Visit 2 labels					
1001-2-E-1	1001-2-E-2	1001-2-R-1	1001-2-H-1	1001-2-C-1	1001-2-CT
1001-2-E-3	1001-2-E-4	1001-2-R-2	1001-2-H-2	1001-2-C-2	1001-2-CT
1001-2-E-5	1001-2-E-6	1001-2-R-3	1001-2-H-3	1001-2-C-3	1001-2-CT
1001-2-E-7	1001-2-E-8	1001-2-R-4	1001-2-H-4		1001-2-CT
1001-2-E-9	1001-2-E-10	1001-2-R-5	1001-2-H-5	1001-2-BX-B	1001-2-CT
1001-2-E-11	1001-2-E-12	1001-2-R-6	1001-2-H-6	1001-2-BX-B	1001-2-CT
1001-2	1001-2-PC1	1001-2-R-7	1001-2	1001-2-FRM	1001-2-CT
1001-2	1001-2-PC2	1001-2-R-8	1001-2	1001-2-FRM	1001-2-CT
1001-2	1001-2-PC3	1001-2-R-9	1001-2	1001-2-FRM	1001-2-CT
1001-2	1001-2-PP	1001-2-RP	1001-2	1001-2-FRM	1001-2-CT
Visit 3 labels					
1001-3-E-1	1001-3-E-2	1001-3-R-1	1001-3-H-1	1001-3-C-1	1001-3-CT
1001-3-E-3	1001-3-E-4	1001-3-R-2	1001-3-H-2	1001-3-C-2	1001-3-CT
1001-3-E-5	1001-3-E-6	1001-3-R-3	1001-3-H-3	1001-3-C-3	1001-3-CT
1001-3-E-7	1001-3-E-8	1001-3-R-4	1001-3-H-4		1001-3-CT
1001-3-E-9	1001-3-E-10	1001-3-R-5	1001-3-H-5	1001-3-BX-B	1001-3-CT
1001-3-E-11	1001-3-E-12	1001-3-R-6	1001-3-H-6	1001-3-BX-B	1001-3-CT
1001-3	1001-3-PC1	1001-3-R-7	1001-3	1001-3-FRM	1001-3-CT
1001-3	1001-3-PC2	1001-3-R-8	1001-3	1001-3-FRM	1001-3-CT
1001-3	1001-3-PC3	1001-3-R-9	1001-3	1001-3-FRM	1001-3-CT
1001-3	1001-3-PP	1001-3-RP	1001-3	1001-3-FRM	1001-3-CT

1001-1-E-1

1001-1-E-1 1 (assigned site number)

1001-1-E-1 (next three numbers =participant ID #)

1001-1-E-1 the first dash 1, 2 or 3 determines which visit date,1=SV2,2=FO6,3=F12)

1001-1-E-1 the dash -E is the space for sample type: E=EDTA, R=Serum, H=Heparin, C=Citrate.)

1001-1-E-1 (this is the chronological number of the cryovial)

Color Key:

COLLECTION TUBE	
POOLED SERUM	
FORM LABELS	
DNA TUBES	
POOLED PLASMA	

ABBREVIATION KEY:

E=EDTA PLASMA

PP=POOLED PLASMA

PC=PACKED CELLS

R=SERUM

RP=SERUM POOLED

H=HEPARIN PLASMA

C=CITRATED PLASMA

CT=Collection tube labels-for vacutainer tubes

BX-B=blood cryovial storage box

BX-D=DNA storage boxes(packed cells for 15 CC conical tubes/save from SV2 visit only)

FRM=Form labels

LIFE STUDY—Phlebotomy Log

DATE	VISIT TYPE (BL, 6 mo, 12 mo)	ACROSTIC	Participant ID #	Biological specimen ID #

LIFE STUDY
Request for Sample Destruction

In the event that participants who have given consent for the collection and storage of blood, white cells and DNA decide to withdraw their consent, they have the right to request that these materials be retrieved and destroyed, and they are entitled to confirmation that this has occurred.

Upon receipt of this form, the Blood Repository Laboratory will discard the samples in accordance with standard procedures for decontamination and removal of human specimens.

This form is divided into three sections with section 1 to be completed by the Field Center and signed by the Principal Investigator or Study Coordinator. Once section 1 has been completed, the form should be forwarded to the Laboratory via fax or regular mail, and section 2 is then completed and signed by the log-in supervisor who personally retrieves the sample from storage and notes its removal in the database. Section 2 is also signed by the laboratory technician who personally autoclaves and disposes of the sample. Section 3 is signed by the laboratory director as final confirmation that the request for removal and destruction of the sample has been properly performed. A copy of the form is retained at the Laboratory, and the original is sent back to the clinical site as confirmation that the destruction has been completed.

Section 1: to be completed by the Field Center staff, then forwarded to the lab
Section 2 & 3: to be completed by the blood repository lab, then forwarded back to the Field Center

Section 1

Field Center: _____ Participant ID: _____

I formally request that the vial of white cells and the vial of isolated DNA obtained from the blood of the above study participant be disposed of and not used in any research activities.

Signature: _____ Date: _____
Principal Investigator or Study Coordinator

Section 2

I attest that the samples requested for disposal have been retrieved and provided to the appropriate technician for destruction, and note of this has been made in the database.

Signature: _____ Date: _____
Log-In Supervisor

I attest that the samples provided to me by the log-in supervisor have been destroyed in accordance with standard procedures for decontamination and destruction of human specimens.

Signature: _____ Date: _____
Technician

Section 3

As requested by the Field Center on behalf of the study participant listed above, I affirm that the requested specimens have been completely destroyed.

Signature: _____ Date: _____
Barbara Nicklas, PhD

Life Study Blood Processing Chart

Three- 10 ml Purple top – EDTA tubes 	Mix gently and place on ice immediately after drawing. Mix and transfer 1 **EDTA** tubes into a 15 cc conical tube **Freeze as whole Blood at - 80˚ C..**(use the PC1 label for this conical tube)	**Centrifuge the other 2 EDTA Tubes at 2000 G for 15 minutes.** Remove tubes from centrifuge and place in a rack in an ice bath or prechilled sample Kryorack.	Pool the plasma from these 2 tubes into one 15 cc conical tube (labeled PP), recap &mix gently and aliquot the plasma into prechilled cryovials. →	Aliquot guide **Aliquot 12 cryovials with 0.5 ml plasma**	Freeze plasma aliquots at -70˚ C. The three 15 cc Conical tubes may be frozen at -20˚ C or -70º C
Two 10 ml Red-top serum-tube	Allow to clot at room temperature for 30-60 minutes 	Centrifuge at 3000 G for 15 minutes Remove tubes from centrifuge and place in a rack in an ice bath.	Pool the serum into one 15 cc conical tube labeled PR, recap and mix gently. Aliquot into prechilled cryovials→	Aliquot guide Three **1** ml cryovials and Six **0. 5** ml cryovials Use red caps	Freeze serum aliquots at -70˚ C.
One Citrate-blue top tube	Place on ice immediately after drawing	Centrifuge at 2000 G for 15 minutes	Remove the tube from the centrifuge and place in a rack in an ice bath.	Aliquot Guide Three **0. 5** ml cryovials Use Blue Caps	Freeze plasma aliquots at -70˚ C.
One Heparin plasma-green top	Place on ice immediately after drawing	Centrifuge at 3000 G for 15 minutes Remove tubes from centrifuge and place in a rack in an ice bath.	Aliquot plasma according to study protocol	Aliquot guide Six **0. 5** ml cryovials Use green caps	Freeze plasma aliquots at -70˚C.