CHAPTER 13
BIOLOGICAL SAMPLE COLLECTION AND PROCESSING

Study Documents referred to in this Chapter:

A) Lab site survey
B) Supply List
C) Sample sheet of barcode labels
D) Phlebotomy log sheet
E) Request for sample destruction form
F) Phlebotomy form
G) Processing form
H) Blood processing chart.
I) Urine Collection form
J) Urine Processing form
K) Freezer Box Maps
L) University of Florida CTSI Bioprepository SOP

CHAPTER 13
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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 study Biological Specimens Collection and Processing Method of Operation is to guarantee the proper collection, processing, shipping, and central storage of both blood and urine 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 Administrative Coordinating Center will oversee training and monitor the quality control of the blood and urine collection and processing at the participating Field Centers. Blood collection consists of drawing blood at baseline, 6 months[1], 12 months and 24 months (57.5-66.5 mL per visit)[2]. Urine collection consists of

[1] Northwestern – Yes
Pennington – No
Stanford University – No
Tufts University – Yes
University of Florida – No
University of Pittsburgh – Yes
Wake Forest University – Yes
Yale University – No

[2] The diagnostic testing panels are only performed at baseline**;** the DNA testing sample will be collected at 12 months**.** Baseline=SV2; 6 months=F06; 12 months= F12; 24 months=F24 .

obtaining at least 10 mL at baseline, 12-month, and 24-month visits. Samples will be collected only for each LIFE participant who provides written, informed consent for these procedures.

13.2 Contact information

LIFE Biological Specimens Collection and Processing Oversight Committee:

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13.3 Diagnostic Testing Laboratory

The Administrative Coordinating Center will set up an account with a central diagnostic testing lab for the following test to be performed for each participant at the baseline visit.

1. Complete Blood Count (CBC): WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count.
2. Lipid Panel: Triglycerides, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol (calculated), Cholesterol/HDL Ratio (calculated)

3. Comprehensive Metabolic Panel with Glomerular Filtration Rate, Estimated (eGFR): Albumin, Albumin/Globulin Ratio (calculated), Alkaline Phosphatase, ALT, AST, BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine with GFR Estimated, Globulin (calculated), Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Urea Nitrogen

The test results will be sent electronically to DMAQC. Results will also be accessible to investigators.

The diagnostic testing lab will provide all the necessary instructions and supplies to each site.

13.4 Equipment/supply list

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

- Refrigerated centrifuge
- Storage space in a -20°C freezer
- Storage space in a -70°C freezer
- Liquid handling device (pipet) that can dispense 1μl volumes
- Color or black/white printer for printing study forms and labels
- 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 waste containers
- Sharps/biohazard containers
- Ice making machine

Each Field Center should have the following supplies:

Blood Collection Supplies:

- 10 mL EDTA (K2 EDTA) purple-top vacutainers [BD# 366643]
- 10 mL red-top vacutainers [BD# 367820]
- 4.5 mL blue-top vacutainers (3.2% buffered Sodium Citrate) [BD# 369714]
- 10 mL green-top vacutainers (Sodium heparin) [BD# 367874]
- Timers
- Band-aids
- 21 gauge safety needles
- Tourniquets
- 2 X 2 gauze pads
- Micropore tape
- Ice bucket/container

Blood Processing Supplies:

- Cryovials-0.5mL and 1.5 mL graduated, externally threaded with color-coded caps (purple, red, blue, green, and clear) which can accommodate the color-coded labels (1 ¼ " high by 1 $^{7}/_{16}$ " wide-circumference)

Description[3]	USA Scientific cat #
Saf-T Seal Screw Cap 0.5mL Tubes (red cap)	1405-9704
Saf-T Seal Screw Cap 1.5mL Tubes (red cap)	1415-9704
Saf-T Seal Screw Cap 0.5mL Tubes (violet cap)	1405-9705
Saf-T Seal Screw Cap 0.5mL Tubes (green cap)	1405-9702
Saf-T Seal Screw Cap 0.5mL Tubes (blue cap)	1405-9701

- Biomerga Cool Cube benchtop coolers (Research Products Inc) -holds 36 cryovials [RPI # 260105]
- Disposable graduated transfer pipettes or pipet with disposable tips
- Storage boxes (2”) with 10 x 10 dividers

[3] Saf-T-Seal screw cap microcentrifuge tubes are strong enough to withstand centrifugation up to 20,000 x g. Suitable for use in vapor-phase liquid nitrogen storage, -80°C freezers, boiling, and autoclaving. Deep external threads protect against sample loss and seal failure. An interior U channel in the lid keeps the ethylene propylene rubber (EPR) "O"-ring in place as the cap is tightened. RNase, DNase, DNA, and pyrogen free. Autoclavable.

- 15 cc conical tubes for pooling plasma and whole blood for DNA

Urine Collection/Processing:

- Covered specimen container (wide mouth)
- Soap pads or towelettes
- Disposable gloves
- 10-15mL centrifuge tubes
- Cryovials (10 per patient)

Description	USA Scientific #
Saf-T Seal Screw Cap 1.5mL Tubes (natural cap)	1415-9700

- Pipette and disposable pipette tips
- Storage boxes (2") with 10 x 10 dividers

Blood Collection and Shipping supplies for ancillary study conducting gene expression analysis:

Tempus™ Blood RNA Tubes, materials for shipping and courier account number will be provided by Dr. Geoffrey Chupp's laboratory at Yale University. Tubes should be stored @ 4-25^{o}C (40-77^{o}F); please avoid exposure to direct sunlight and do not use tubes beyond expiration date. This tube will be drawn at each of the blood collection visits and labeled with one of the extra collection tube labels (CT). Blood will be stored frozen **at -20 ^{o}C** and shipped on dry ice in batches (50 tubes or 3 months accumulation) to the Chupp Laboratory.

Field sites should request shipment of RNA tubes and shipping materials by contacting ___ **Two week notice is required**.

Providing Antioxidant/Chelator Reagents and Additions:

Additions to Selected Plasma Samples. Butyl Hydroxytoluene (BHT) is an antioxidant that prevents lipid peroxidation and aids in sample preservation during long term storage. Diethylenetriaminepenta- acetic acid (DTPA) is a metal chelator.

- 5 μl of each chemical will be added to three (3) of the twelve (12) aliquots of the EDTA-plasma samples.

Additions to Selected Urine Samples. Diethylenetriaminepenta- acetic acid (DTPA) is a metal chelator. Phenol is an antioxidant and has bacterialcidal properties.

- 5μl of 20μM DTPA in phenol will be added to two (2) of the ten (10) urine aliquots.

Shipments of Compounds

5 mmol BHT in Ethanol, 5mmol DTPA in water, and 20μM DTPA in phenol (antioxidant and bactericidal agent) will be provided by the Administrative Coordinating Center and shipped to each Field Center, in sufficient quantities for the entire study (9-12mLof each solution for blood and 5mL of the solution for urine). Store compounds in standard refrigerator at 4 C to avoid bacterial growth.

Field sites should request shipment of reagents by contacting___

Two week notice is required.

Shipping Supplies provided by the Administrative Coordinating Center:

- Insulated Shippers
- Absorbent Sheets
- 10" x 10" clear, zip style bag w/biohazard symbol and external pouch

Shipping Supplies NOT provided by the Administrative Coordinating Center:

- Dry ice (10-20 lbs per insulated shipper)
- Rubber bands to secure the freezer boxes

- Kitchen-grade zip style bags (for packing list)

Field sites should request shipping supplies by contacting __

Two week notice is required.

13.5 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 Specimens Committee 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.

13.6 Specimen ID Labels

Biological Specimens Collection and Processing 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, the Blood Processing forms, Urine Collection forms, and Urine Processing forms should have labels containing both ID numbers**.

The Administrative Coordinating Center will supply each Field Center with sheets of Biological Specimen ID barcode labels (baseline followed in the future by other visit labels). These will be used for labeling forms, blood collection tubes (except those

provided by the diagnostic testing lab), urine collection container, tubes, cryovials, and sample boxes. The color-coded labels are 1 ¼ " high by 1 $^{7}/_{16}$ " wide-circumference with the bar code running as shown.

There will be a total of ~73 labels: 7 labels for collection tubes (plus 5 extra), 1 label for urine collection container, 2 labels for the 10-15 mL tubes for urine, 2 labels for each of the freezer storage boxes (blood and urine), 40 cryovial labels, 2 processing labels used for tubes to pool plasma and serum, and 4 form labels (plus 1 extra). The extra 6 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. Participant ID labels can be printed using the equipment provided to each site by the DMAQC.

All labels have the same 6-digit Biological Specimen ID number, which is different from the participant study ID (the first two digits identify the clinic):

10 – Northwestern University

20 – Pennington Biomedical Research Center
21 – Baton Rouge General

30 – Stanford University

40 – Tufts University

50 – University of Florida – Gainesville
51 – University of Florida – Jacksonville

60 – University of Pittsburgh

70 – Wake Forest University

80 – Yale University

The next 3 numbers will be the participant's unique Biological Specimen ID number. Biological Specimen ID numbers will run 001 through 110 or 220, depending on the site. The next number on the label is to identify the visit. 1 = Baseline, 2 = 6 month visit, 3 = 12 month visit, 4 = 24 month visit[4]. For example, the Biological Specimen ID number

[4] **Baseline=SV2; 6 months=F06; 12 months= F12; 24 months=F24**

may be 30001-1 (Stanford site, participant 001, baseline visit) or 51050-3 (UF-Jacksonville site, participant 050, 12 month visit). Please note that sites opting out of the 6 month sample collection will not have any labels for that visit.

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, C= Citrated Plasma, U= Urine. 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, 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 (except those provided by the diagnostic testing lab) 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 #; the Phlebotomy Log Sheet will be used to track this additional measure.

To request supplemental Biological Specimen ID barcode labels, if more needed beyond the original sets, contact __ _____
and this will be shipped to you. **Two week notice is required.**

13.7 Blood collection/Phlebotomy

13.7.1 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 9 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 the form. All forms must be completed in ink.

Upon completion, the ***Phlebotomy form*** should be entered into the LIFE website data entry system and the paper copy kept on file in the participant's chart.

13.7.2 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 retained for EVERY participant, regardless if blood was drawn or not.

13.7.3 Preparation/Set-up

Prior to the participant's visit, the phlebotomist should label the collection tube vacutainers and prepare all supplies. Tubes provided by the diagnostic testing lab will be pre-labeled with their own barcode which matches the accompanying form. A label containing the Participant ID and Acrostic will need to be printed from the LIFE website *by the study coordinator* at the time of the visit and attached to the diagnostic testing lab's form and not the tube (these labels are *not provided by the ACC*). The remaining collection tubes (including the Tempus™ Blood RNA Tube) 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). Both the Phlebotomist and another individual must initial the sheet as confirmation that all information is correct.

The phlebotomist must be sure that informed consent forms have been signed before drawing the participant's blood. Six to nine tubes of blood of various sizes are collected, each containing about 1-2 teaspoons of blood (2-10 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.

13.7.4 Priority of collection tubes

A total of 57.5-66.5 mL per visit [5] mL of blood will be drawn in up to nine 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* [6]	*5 mL Serum Separation Tube*	*gold top tube*
3.	2	10 mL EDTA	purple-top tubes
4.	1	4.5 mL Citrate	blue-top tube
5.	1	10 mL Heparin	green-top tube
6.	*1* [7]	*2 mL EDTA lav*	*ender-top tube*
7.	*1* [8]	*10 mL EDTA*	*purple-top tube (this tube is for DNA analyses only at one of the yet to be determined follow up visits)*
8.	1[9]	3 mL Tempus™ Blood RNA Tube *blue*	*-top tube*

13.7.5 Phlebotomy Room

[5] The exact volume may vary slightly once the tubes are specified by the Diagnostic Testing Laboratory.
[6] Baseline visit only: This tube is for the comprehensive metabolic panel and lipid panel tests performed by a centralized diagnostic testing lab per their requirements. See section 13.3.
[7] Baseline visit only: If multiple draw, collect lavender-top tube second to last. Traumatic tap can introduce thromboplastin and trap WBC/platelets. This tube is for the CBC test performed by a centralized diagnostic testing lab per their requirements. See section 13.3.
[8] This extra EDTA tube only needed for the 12 month visits, for the DNA testing.
[9] This tube should be drawn to line indicated on tube; collected at each blood draw visit.

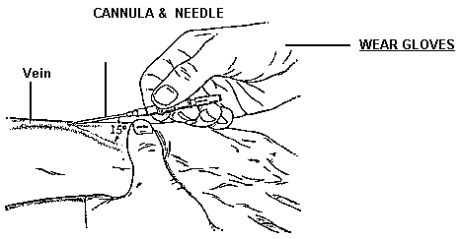
The Phlebotomy Area should include a chair for the participant, a table for blood collection supplies, and a sink for washing hands. A phone/intercom system should be within reach and access to emergency equipment should be available. Accommodations should ensure the participant can sit quietly for 5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

13.7.6 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)-5 mL SST, (2)-10 mL purple-tops, (1)-4.5 mL blue-top, (1)-10 mL green-top, (1)-2 mL lavender-top, another 10 mL purple-top (if the vein is holding up on a follow up visit only for DNA),and lastly (1)-3mL blue top Tempus™ Blood RNA Tube **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 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 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. The Tempus™ Blood RNA Tube must be drawn to the fill line indicated on the tube to have the proper ratio of reagent to blood (do not transfer sample from syringe to a tube).
15. Mix blood vacutainer tubes several times **immediately** after collection by inverting them **gently and evenly. AND the Tempus™ Blood RNA Tube tubes should be mixed vigorously for at least 10 seconds, about 15 shakes, by inverting tube back and forth.**
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.

13.7.7 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.

13.8 Blood Processing

13.8.1 Blood Processing Forms

The purpose of the ***Blood Processing form*** 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 the 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, the ***Blood Processing form*** should be entered into the LIFE web-based data entry system and the original paper copy kept on file in the participant's chart with a photocopy of the completed form kept in the lab.

13.8.2 Participant refusal for DNA/RNA

Some participants will provide informed consent to participate in the LIFE study blood draw, but will not want their DNA/RNA extracted from this blood for use in genetic testing. The whole blood for extraction of DNA and/or Tempus™ Blood RNA Tube should only be saved from those participants who have agreed to participate in this aspect of the study by checking the genetic testing box on the informed consent form. Make a note on the Blood Processing form that the participant refused the collection of DNA and/or RNA for genetic testing. The whole blood only needs to be saved for DNA extraction at the 12-month visit, not the baseline, 6-month visit, or 24-month visit. The Tempus™ Blood RNA Tube needs to be collected at all blood draw visits.

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 retained by the field center.

13.8.3 Overview and Description of Aliquots

Processing should be initiated as soon as possible (less than 30 minutes) following the blood draw. The red-top (all serum tubes) 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 EXCEPT THE 2 mL TUBE FOR THE CBC TEST WHICH MUST REMAIN AT ROOM TEMPERATURE**. The Tempus™ Blood RNA Tube may be kept at room temperature until stored **at -20˚C**. 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[10]	Color
EDTA plasma (pooled from 2 10-mL vacutainers)	9	0.5 mL	Purple
with antioxidants (5μl BHT+5μl DTPA)	3	0.5 mL	Purple
Serum (pooled from 2 10-mL vacutainers)	3	1.0 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

In addition to the above, blood will be collected/processed using materials and following instructions provided by the diagnostic testing at the baseline visit:

- Whole blood collected in an EDTA (lavender-top) tube (2 mL)

[10] These volumes should be dispensed using a graduated pipet or other volumetric liquid dispensing device. Excess serum/plasma is to be discarded.

- Serum Separation Tube (SST;2x 5 mL)

Tubes provided by the diagnostic testing lab will be pre-labeled with their own barcode which matches the accompanying form. A label containing the Participant ID and Acrostic will need to be printed from the LIFE website *by the study coordinator* at the time of the visit and attached to the diagnostic testing lab's form and not the tube (these labels are *not provided by the ACC*).

13.8.4 Preparation/Set-up

Collection tubes for phlebotomy, two (three at 12 month visit) 15-mL conical tubes, one for pooled plasma, one for pooled serum and one (only for the 12 month visit) for whole blood (for DNA), and all 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**. The labeled cryovials should be prechilled in the Biomerga Cool Cube benchtop coolers designed specifically for cryovials **before** aliquoting. The centrifuge should be turned on and set at the proper temperature.

13.8.5 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 1600 x g for 15 minutes 4°C, per tube manufacturer's specifications.
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 on ice to chill the serum while it is being aliquoted.

4. Aliquot using plastic graduated transfer pipettes into 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.

SST (5 mL, gold-top) sample for lipid panel and metabolic panel:

1. Follow instructions provided by the diagnostic testing lab.

EDTA (2 mL, lavender-top) whole blood sample for CBC:

1. Follow instructions provided by the diagnostic testing lab.

EDTA (10 mL, 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. **[ONLY FOR 12 MONTH VISIT-FOR DNA TESTING] Mix gently and slowly,** carefully pour the EDTA whole blood from one of the EDTA vacutainer tubes into a 15-mL conical tube. Use a collection tube label (CT) label for this conical tube. Freeze this in an up-right position at -70˚C. Place in a study storage box without dividers.
3. Centrifuge the remaining (2) EDTA tubes (purple-top vacutainers) and the 4.5 mL blue-top vacutainer at 1600 x g for 15 minutes 4°C.
4. After centrifugation, place the tubes in the on ice to keep them at the proper temperature during aliquoting.
5. Transfer the EDTA **plasma** from the two EDTA vacutainer tubes **into one 15 cc conical tube labeled with PP.** 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 either with or without the antioxidant/chelator (Aliquot 9 cryovials with 0.5 mL plasma; Aliquot 3 cryovials

with 0.5 mL Plasma plus 5 μl DTPA solution, 5μl BHT solution). The aliquots must be frozen immediately and stored frozen at below -70°C.

6. Aliquot the citrated plasma per the LIFE Blood aliquoting chart.
7. Transfer the Citrated plasma into the 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.
8. The remaining cells in the serum tube (and the tubes themselves) can be discarded in the biohazard waste.

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 1600 x g for 15 minutes 4°C, per tube manufacturer's specifications in order to derive platelet-poor plasma
4. Immediately following centrifugation, place the Heparin tube on ice and aliquot the plasma via plastic transfer pipettes into the prechilled, screw-top cryovials with O-ring seals according to the study blood storage protocol. The aliquots must be frozen immediately and stored frozen at below -70°C until analysis.
5. The remaining cells in the serum tube (and the tubes themselves) can be discarded in the biohazard waste.

Tempus™ Blood RNA Tubes (3mL, blue-top):

1. Tempus Blood RNA blue-top tubes should be mixed vigorously for at least 10 seconds, about 15 shakes, by inverting tube back and forth and can remain at room temperature prior to freezing.
2. Freeze this tube in an up-right position **at -20˚C.** Afterwards, place in a labeled study storage box without dividers and store **at -20˚C** until shipped.

13.8.6 Freezing and storage

After aliquoting and capping of samples, the cryovials should be placed in a *labeled* and *numbered* storage box with 2 spaces between participants or collection visits, and avoiding the starting corner (see box maps). The number of the box should be noted on the Blood Processing form. If there is sufficient storage space in a site's freezer, 1 box should be used for 1 participant. If there is a fourth visit, then 4th visits for 3 participants can be placed in one box. If there is not sufficient space in a site's freezer, then more than one participant's samples can be placed in the box.

There will be room for storage of 3 collection visits per participant or 3 participants' samples per box. Ideally, the boxes will be in chronological order (i.e., participants 001, 002, and 003 in boxes 1, 2 and 3). After 6 months, there may 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 Whole Blood tube for DNA extraction should be placed in the separate storage box for these 15-mL conical tubes (only collected at the 12 month visit).

The Tempus RNA tubes for gene expression analysis should be placed in a separate storage box for these tubes and stored **at -20˚C** until shipped.

Every effort should be made to freeze plasma/serum/urine 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.

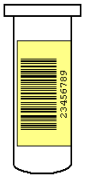
13.8.7 Difficulties or problems

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 **EXCEPT THE 2 mL LAVENDAR-TOP TUBE FOR THE CBC TEST WHICH MUST REMAIN AT ROOM TEMPERATURE**. Note the delay in processing on the comments section on the Blood Processing form.

13.8.8 Blood Processing Overview



1. Complete phlebotomy log sheet.
2. Place specimen labels on tubes so that the barcode runs vertically:
3. Do not share specimen labels between visits or participant sets as they are linked by computer encoding.
4. The phlebotomy should be rescheduled, if the minimum fasting time is less than 9 hours.
5. The Phlebotomy and the Blood Processing forms should have labels containing both ID #s.
6. The order in which the tubes are drawn is very important. Blood collection must follow the specified order.

7. Mix blood vacutainer tubes several times **immediately** after collection by inverting them **gently and evenly** (4-6 gentle inversions).
8. Once all tubes for a participant are filled, place the EDTA, blue-top citrated tubes & the green top Heparin tube into an ice bucket **EXCEPT THE 2 mL TUBE FOR THE CBC TEST WHICH MUST REMAIN AT ROOM TEMPERATURE**, leaving them no longer than 30 minutes prior to centrifugation.
9. Leave the serum tubes at room temperature for at least 30 minutes but no longer than 60 minutes prior to centrifugation.
10. The Tempus RNA tube may remain at room temperature prior to **freezing at -20 ^{o}C.**
11. Follow instructions provided by diagnostic testing lab concerning the 5 mL SST and 2mL EDTA tubes.
12. Centrifuge the tubes as specified. Do **NOT** increase or decrease speed and/or time.
13. Do not use a fixed-angle centrifuge.
14. If you use a non-refrigerated centrifuge, promptly remove tubes as soon as spinning stops and place them in a Biomerga Cool Cube benchtop cooler or ice bath. Make a note of this on the Blood Processing form comment section that samples were spun in a non-refrigerated centrifuge.
15. Place the tubes in the specimen tube Biomerga Cool Cube benchtop cooler after removing them from the centrifuge.
16. Process all tubes immediately after centrifugation. See processing chart for proper specimen handling.
17. Submit lavender top tube and SST to diagnostic testing lab per their instructions.
18. Input data from both the Phlebotomy Form and the Blood Processing Form into the LIFE web based data entry system.

13.9 Urine Specimen Collection

Urine samples for future assessment of biomarkers in ancillary studies are collected at baseline, 12-month, and 24-month visits. The urine is collected via clean-catch

midstream technique. Participants are instructed on the technique and escorted to the restroom where they will provide at least 10 cc of urine into a urine collection cup. Urine samples will be centrifuged at room temperature at 1000 g to remove cellular debris. Supernatants will be aliquoted into 1 mL samples, labeled, and stored at -80^{o} C. Specimens will either be stored locally at each clinical center, or they will be shipped in batches to a central repository.

13.9.1 Informed Consent and Participant refusal for urine collection

Some participants will provide informed consent to participate in the LIFE study without consent for urine collection. Urine should only be collected from those participants who have agreed to participate in this aspect of the study by checking the urine specimen 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 urine collection during their scheduled appointment. The participant has a right to withdraw consent at any time and urine should not be collected from these participants.

The study coordinator who is conducting the assessments at the visit should still complete the Urine Collection form (with the 'No, Refused' option marked) for any participant who refuses the urine collection. 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 Urine Collection form should be retained for EVERY participant, regardless if urine was collected or not.

13.9.2 Urine Collection and Urine Processing Forms

The purpose of the Urine Collection and Urine Processing forms are used to facilitate the monitoring of the urine collection procedure and other quality assurance data critical to the interpretation of the assay results.

13.9.3 Preparation/Collection

Over the phone, prior to the visit, instruct the participant to drink a full glass of water 1 hour prior to the scheduled visit.

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

The collection container should be labeled prior to giving it to the participant.

In person, the following procedures should be explained to the participant.

Females:

1. Wash hands thoroughly with soap and water.
2. Separate the skin folds around the urinary opening. Wash the area with a soap pad or towelette using a front to back motion. Repeat two additional times.
3. Begin urinating into the toilet with skin folds held apart with the fingers.
4. Insert collection container into urine stream without allowing container to touch the skin area.
5. Fill half of the container and remove from the urine stream.
6. Replace the container lid and screw tightly and snap shut (for participants with severe arthritis, the study coordinator can perform this step).
7. Specimen can be stored at room temp for up to 6 hours.

Males:

1. Wash hands thoroughly with soap and water.
2. Wash the head of the penis with the towelette or soap pad.
3. Complete above procedure steps 4-6.

Upon completion, the ***Urine Collection form*** should be entered into the LIFE website data entry system and the paper copy kept on file in the lab.

13.10 Urine Processing

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

The two 10 -15mL centrifuge tubes and ten cryovials should be pre-labeled.

1. Processing Return to laboratory processing area with labeled urine in specimen cup

2. Transfer 10 mL of urine from the specimen cup into large plastic centrifuge tube
3. Cap centrifuge tube
4. Centrifuge at room temperature for 1600 x g for 15 minutes 4°C (use appropriate balancing tubes)
5. While urine is being spun, label (10) 1.5mL clear-cap cryovials with study labels. Labels should run vertically on tube, so that barcode can be read by a scanner.
6. After centrifugation, pour off the supernatant into a 2nd clean centrifugation tube, potentially leaving the "pellet" behind
7. From this tube, pipette 1.0 mL aliquots of urine into 10 screw cap cryovials
8. Into the (2) two cryovials labeled with "ao" add 5μl of 20μM DTPA in phenol.
9. Screw caps onto the cryovials
10. Discard excess urine
11. Place the cryovials into specimen box
12. Can store urine-filled cryovials from 10 patients per specimen box (space for 100 aliquots in each box)[11]
13. Store the box in freezer at -80 degrees C

Upon completion, the ***Urine Processing form*** should be entered into the LIFE website data entry system and the original paper copy kept on file in the participant's chart with a photocopy of the completed form kept in the lab.

13.11 Quality Assurance

Differences in the procedures used for sample collection or processing could potentially introduce unwanted variance in assays of the samples. Monitoring of the sample collection and processing protocols is critical to identify any deviations from standardized methods described in this Manual of Operations. The Administrative Coordinating Center will monitor the quality of the sample 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/collection/processing/shipping forms,

[11] If there is sufficient storage space in a site's freezer, 1 box should be used for 2 participants. If there is not sufficient space in a site's freezer, then more than two participant's samples can be placed in the box.

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 sample collection and/or processing is required before working with actual study participants' samples. Initial certification involves: 1) reading and understanding of the LIFE Manual of Operations, 2) successful completion of training by Administrative Coordinating Center 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. In addition, centrifuge speed should be checked quarterly using a tachometer (Each facility will need to purchase one or borrow one). This log also should be kept on file. These equipment records can identify potential problems with sample quality.

13.12 Shipping of Tempus RNA blood tubesShipping Procedure

Each site must ensure compliance with their own State as well as Federal laws concerned with the packaging and shipment of Biohazardous samples, in accordance with HIPPA, Environmental, Health and Safety and OSHA Laws.

a. Pre- Shipping Approval

i. Before shipping any materials to the Chupp Laboratory, you must have an email confirmation from __ ,or her designate that confirms that it is acceptable to send a shipment on your requested date. Shipments (batches of 50 tubes or 3 months accumulation) will only occur on Mondays (barring holidays). Shipment dates can be coordinated between the LIFE study sites and Chupp Laboratory through _ . However, the final confirmation of approval for a shipment must come directly from the Chupp Laboratory. The email notification acknowledging approval from the Chupp Laboratory will have the following subject line: **Approval of Arrival of Shipment on XX/XX/XXXX from *LIFE study site name* to the Chupp Laboratory**.

b. Shipping Requirements

i. Each box should be labeled with a list of the Patient IDs who have materials contained within that box.

ii. Put rubber band around each storage box and place each full box into a separate zip-lock bag with absorbent pad.

iii. A maximum of 10 boxes of samples should be sent per shipment.

iv. A minimum of 10lbs of dry ice should be used for overnight shipments as it sublimates at the rate of 5-10lbs/24hrs depending on shipping materials and outside temperatures.

v. A packing list of all samples and boxes that are being shipped must be included as well as emailed to Carole Holm (Excel Document which can be generated from the LIFE website). The enclosed list should be placed in a Ziploc bag and placed between the top of the Styrofoam box and the outer cardboard box.
    1. The packing list should include a list of all vials designated by their barcode numbers that are in a particular box.

vi. Ship via FEDEX Priority Overnight using account number provided

vii. The tracking number generated by the courier should be emailed to __ so that the shipment can be tracked.

viii. You will receive an email confirmation from __on the day your shipment arrives and __ will be copied on all correspondence

1. The email will notify you that box was received from the courier, will acknowledge whether or not there were any visual signs of damage to the external shipment package, and will acknowledge any discrepancies or issues between the contents listed in the packing list and those found in the package itself and will report any visual damage or concerns with the contents.

13.12.2 Shipping address

Shipments should be addres

13.13 Shipping of Plasma/Serum/Urine Samples

13.13.1 Shipping Procedure

Each site must ensure compliance with their own State as well as Federal laws concerned with the packaging and shipment of Biohazardous samples, in accordance with HIPPA, Environmental, Health and Safety and OSHA Laws.

13.13.2 Generating the packing list

From the LIFE Website, upon logging in, go to “Reports > Administrative > Laboratory/Repository Reports > Biological Specimens Site Inventory”

The report generated is a site specific inventory in an excel file containing a list of ALL samples indicated as collected by the site from data entered Blood Processing forms. This is an inventory of what samples the site has stored at the clinic. In order to provide the Repository, as specified in the instructions (see section 13.13.3) with an electronic list and hardcopy shipping/packing list each site must download the report and filter the inventory list each time they plan to ship samples so that the inventory list will match precisely what the Repository

will receive. The filtered list then can be copied into a new Excel file and emailed to the Repository and printed to be packed with the samples. If assistance is needed with filtering in Excel please contact

Upon arrival at the Repository, each vial will be scanned to generate an accurate central biological specimen inventory. DMAQC will be provided this information and will use it to update the Biological Specimens Site Inventory report. Specimens indicated as received by the Repository will be removed from the report to assist the site in providing accurate electronic and hardcopy shipping/packing lists. In addition, information from the Repository received by the DMAQC will be added to the LIFE Study database as a listing of the centrally stored biological specimen inventory.

13.13.3 Biorepository Shipping Procedure

All other instructions can be found in the attached document:
BIOR-CSH-001

13.14 Shipping of Whole Blood Samples for DNA Extraction

Samples must be shipped so that DNA extraction can be accomplished prior to 12 months from date of collection.

13.14.1 Shipping Procedure

Each site must ensure compliance with their own State as well as Federal laws concerned with the packaging and shipment of Biohazardous samples, in accordance with HIPPA, Environmental, Health and Safety and OSHA Laws.

13.14.2 Generating the packing list

An accurate packing list of all samples and boxes that are being shipped must be included as well as emailed to the Clinical Research Center (CRC; Excel Document which can be generated from information on the LIFE website).

The report generated is a site specific inventory in an excel file containing a list of ALL tubes indicated as collected by the site from data entered Blood Collection forms. This is an inventory of the tubes the site has stored at the clinic.

Upon arrival at the CRC, each tube will be scanned to generate an accurate central biological specimen inventory of the whole blood samples. Once the DNA has been extracted and aliquoted into labeled cryovials, they will be stored in the Repository and inventoried as is done for the serum, plasma and urine samples...

13.14.3 Whole Blood Shipping Procedure

All other instructions can be found in the attached document:
UF-CRC-Life Study -01

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: BIOMERGA COOL CUBE
BENCHTOP COOLERS: YES / NO

ICE BATHS: YES / NO
DRY ICE: YES / NO

LIFE Study Supply List

	Required	Suggestion	Unit (min)	Qty per Participant per Visit
Blood Collection Supplies:				
10 ml EDTA (K3 EDTA) purple-top vacutainers	BD# 366643	--------	100	2 (3 for DNA visit)
10 ml red-top vacutainers	BD# 367820	--------	100	2
4.5 ml blue-top vacutainers (3.2% buffered Sodium Citrate)	BD# 369714	--------	100	1
10 ml green-top vacutainers (Sodium heparin)	BD# 367874	--------	100	1
Timers	--------	Fisher Sci # 14-649-19	ea	
Band-aids	--------	Fisher Sci # 19-054-502	100/pk	
21 gauge safety needles	--------	BD# 367344	50/pk	1
Tourniquets (non-latex)	--------	Fisher Sci # 22-035-70	100/pk	
2 X 2 gauze pads	--------	Fisher Sci # 19-060407	600/cs	
Micropore tape	--------	Fisher Sci # 19-027-761	12/pk (10 yrd rolls)	
Ice bucket/container	--------	--------		
Blood Processing Supplies: Cryovials-0.5ml and 1.5 ml graduated, externally threaded with color-coded caps (purple, red, blue, green, and clear) which can accommodate the color-coded labels (1 ¼ " high by 1 $^{7}¤_{16}$ " wide-circumference)				

Description[1]	USA Scientific cat #			
Saf-T Seal Screw Cap 0.5ml Tubes (red cap)	1405-9704	--------	500/pack	9
Saf-T Seal Screw Cap 1.5ml Tubes (red cap)	1415-9704	--------	500/pack	3
Saf-T Seal Screw Cap 0.5ml Tubes (violet cap)	1405-9705	--------	500/pack	9
Saf-T Seal Screw Cap 0.5ml Tubes (green cap)	1405-9702	--------	500/pack	6
Saf-T Seal Screw Cap 0.5ml Tubes (blue cap)	1405-9701	--------	500/pack	3
Biomerga Cool Cube benchtop coolers (Research Products Inc) -holds 36 cryovials	RPI # 260105			
Disposable graduated transfer pipettes	--------	Fisher Sci # 13-711-9AM	500/pack	
Pipet	--------	Fisher Sci # 14-386-74		
Disposable tips	--------	Fisher Sci # 02-707-51	1000/pk	
Storage boxes (2") with 10 x 10 dividers	--------	Fisher Sci# 03-395-114	each or 200/cs	
15 cc conical tubes for pooling plasma and whole blood for DNA	--------	BD# 352095	CS of 500	2-3
Urine Collection/Processing:				
Covered specimen container (wide mouth)	--------			
Soap pads or towelettes	--------	Fisher Sci# 13-711-73		
Disposable gloves	--------	--------		
10-15ml centrifuge tubes	--------	BD# 352096		2
Cryovials (10 per patient)				
Description	USA Scientific #			
Saf-T Seal Screw Cap 1.5ml Tubes (natural cap)	1415-9700	--------	500/pack	10
Pipette and disposable pipette tips	--------	(see above under Blood Processing)		
Storage boxes (2") with 10 x 10 dividers	--------	Fisher Sci# 03-395-114	each or 200/cs	

[1] Saf-T-Seal screw cap microcentrifuge tubes are strong enough to withstand centrifugation up to 20,000 x g. Suitable for use in vapor-phase liquid nitrogen storage, -80°C freezers, boiling, and autoclaving. Deep external threads protect against sample loss and seal failure. An interior U channel in the lid keeps the ethylene propylene rubber (EPR) "O"-ring in place as the cap is tightened. RNase, DNase, DNA, and pyrogen free. Autoclavable.

[2] Hydrophobic, biologically inert surface for good cell or protein recovery
Suitable for cell centifugation applications such as pelleting and separation by density gradients; molecular biology applications including concentrating bacteria for DNA isolation, purification and precipitation of nucleic acids; sample storage (ambient temperature to -80°C); and centrifugation of precipitates.
Includes: Blue HPDE 20mm threaded dome seal closure (threaded to fit BD Falcon tubes only). Closure provides positive seal over the full circumference.

Refrigerated centrifuge	--------	Eppendorf Model 5702R	each
Storage space in a -70°C freezer	--------	Forma Model 906 23 cu ft ULT -80	each
Liquid handling device (pipet) that can dispense 1µl volumes	--------	Fisher Sci cat# 14-386-71	each
Tube racks	--------	Fisher Sci cat# 05-541-44 / 05-541-28	5/pack
Plastic-backed bench covers	--------	--------	
Waterproof pens (Sharpie permanent marker/industrial strength)	--------	--------	
Alcohol wipes	--------	Fisher Sci cat# 19-120-316	200/pk
Ammonia spirits, ampules		Fisher Sci cat# 22-100-105	10/pk
Butterfly needles (21 gauge) with luer adapter		BD # 7251	
Disposable gloves	--------	--------	
Paper towels	--------	--------	
Disinfectant Cleaner (Kills HIV and HBV) or Bleach decontaminant-1 part Clorox to 9 parts water, stored		Fisher Sci Bleach Solution Dispenser cat # 23-640-500 / uclean.com: Virex TB Germicidal Clnr/Deod - 12 qt. cat# CF1030	each or 12/pk

in a labeled squeeze bottle)			
Biohazard waste containers	--------	--------	
Sharps/biohazard containers	--------	--------	
Ice making machine	--------	--------	
Tachometer		Fisher Sci cat# 05-028-23	each

Representative Examples of barcode labels

PURPLE	PURPLE	RED	GREEN	BLUE	WHTE
Visit 1 labels					
10001-1-E-1ao	10001-1-E-2 ao	10001-1-R-1	10001-1-H-1	10001-1-C-1	1001-1-CT
10001-1-E-3 ao	10001-1-E-4	10001-1-R-2	10001-1-H-2	10001-1-C-2	10001-1-CT
10001-1-E-5	10001-1-E-6	10001-1-R-3	10001-1-H-3	10001-1-C-3	10001-1-CT
10001-1-E-7	10001-1-E-8	10001-1-R-4	10001-1-H-4		10001-1-CT
10001-1-E-9	10001-1-E-10	10001-1-R-5	10001-1-H-5	10001-1-BX-B	10001-1-CT
10001-1-E-11	10001-1-E-12	10001-1-R-6	10001-1-H-6	10001-1-BX-U	10001-1-CT
10001-1	10001-1	10001-1-R-7	10001-1	10001-1-FRM	10001-1-CT
10001-1	10001-1	10001-1-R-8	10001-1	10001-1-FRM	10001-1-CT
10001-1	10001-1	10001-1-R-9	10001-1	10001-1-FRM	10001-1-CT
10001-1	10001-1-PP	10001-1-RP	10001-1	10001-1-FRM	10001-1-CT
					10001-1-U-1 ao
					10001-1-U-2 ao
					10001-1-U-3
					10001-1-U-4
					10001-1-U-5
					10001-1-U-6
					10001-1-U-7
					10001-1-U-8
					10001-1-U-9

10001-1-E-1	
10001-1-E-1	1 (assigned site number)
10001-1-E-1	(next three numbers =participant ID #)
10001-1-E-1	the first dash 1, 2, 3 or 4 determines which visit date,1=SV2,2=FO6,3=F12, 4=F24, 5=closeout)
10001-1-E-1	the dash -E is the space for sample type: E=EDTA, R=Serum, H=Heparin, C=Citrate.)
10001-1-E-1	(this is the chronological number of the cryovial)
10001-1-E-1ao	(this indicates the presence of antioxidant/chelator)

ABBREVIATION	KEY:	
E=EDTA PLASMA	C=CITRATED PLASMA	
PP=POOLED PLASMA	CT=Collection tube labels-for vacutainer tubes	
	BX-B=blood cryovial storage box	
R=SERUM		
RP=SERUM POOLED		
H=HEPARIN PLASMA		
	FRM=Form labels	

Color Key:

COLLECTION TUBE
POOLED SERUM
FORM LABELS
POOLED PLASMA

LIFE STUDY – Phlebotomy Log

Date of Visit (MM/DD/YYYY)	Visit Type (BL, 6, 12, 24 mo)	LIFE Study Participant ID Label	Biological Specimen ID#	Label check

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/RNA 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 Field Center will discard the samples in accordance with standard procedures for decontamination and removal of human specimens.

This form is to be completed by the Field Center and signed by the Principal Investigator or Study Coordinator. A copy of the form is retained at the clinical site as confirmation that the destruction has been completed.

Field Center: _____ Participant ID: _____

I affirm that the vial of white cells and the vial of isolated DNA/RNA obtained from the blood of the above study participant have been completely destroyed and not used in any research activities.

Signature: _____ Date: _____
Principal Investigator or Study Coordinator

-LIFE STUDY SAMPLE BOX MAP-

(For sites with 3 blood draw visits)

1) Empty	2) Empty	3) 10001-1-E-1ao	4) 10001-1-E-2ao	5) 10001-1-E-3ao	6) 10001-1-E-4	7) 10001-1-E-5	8) 10001-1-E-6	9) 10001-1-E-7	10) 10001-1-E-8
11) 10001-1-E-9	12) 10001-1-E-10	13) 10001-1-E-11	14) 10001-1-E-12	15) 10001-1-R-1	16) 10001-1-R-2	17) 10001-1-R-3	18) 10001-1-R-4	19) 10001-1-R-5	20) 10001-1-R-6
21) 10001-1-R-7	22) 10001-1-R-8	23) 10001-1-R-9	24) 10001-1-C-1	25) 10001-1-C-2	26) 10001-1-C-3	27) 10001-1-H-1	28) 10001-1-H-2	29) 10001-1-H-3	30) 10001-1-H-4
31) 10001-1-H-5	32) 10001-1-H-6	33) Empty	34) Empty	35) 10001-2-E-1 ao	36) 10001-2-E-2 ao	37) 10001-2-E-3 ao	38) 10001-2-E-4	39) 10001-2-E-5	40) 10001-2-E-6
41) 10001-2-E-7	42) 10001-2-E-8	43) 10001-2-E-9	44) 10001-2-E-10	45) 10001-2-E-11	46) 10001-2-E-12	47) 10001-2-R-1	48) 10001-2-R-2	49) 10001-2-R-3	50) 10001-2-R-4
51) 10001-2-R-5	52) 10001-2-R-6	53) 10001-2-R-7	54) 10001-2-R-8	55) 10001-2-R-9	56) 10001-2-C-1	57) 10001-2-C-2	58) 10001-2-C-3	59) 10001-2-H-1	60) 10001-2-H-2
61) 10001-2-H-3	62) 10001-2-H-4	63) 10001-2-H-5	64) 10001-2-H-6	65) Empty	66) Empty	67) 10001-3-E-1 ao	68) 10001-3-E-2 ao	69) 10001-3-E-3 ao	70) 10001-3-E-4
71) 10001-3-E-5	72) 10001-3-E-6	73) 10001-3-E-7	74) 10001-3-E-8	75) 10001-3-E-9	76) 10001-3-E-10	77) 10001-3-E-11	78) 10001-3-E-12	79) 10001-3-R-1	80) 10001-3-R-2
81) 10001-3-R-3	82) 10001-3-R-4	83) 10001-3-R-5	84) 10001-3-R-6	85) 10001-3-R-7	86) 10001-3-R-8	87) 10001-3-R-9	88) 10001-3-C-1	89) 10001-3-C-2	90) 10001-3-C-3
91) 10001-3-H-1	92) 10001-3-H-2	93) 10001-3-H-3	94) 10001-3-H-4	95) 10001-3-H-5	96) 10001-3-H-6	97) Empty	98) Empty	99) Empty	100) Empty

-LIFE STUDY BLOOD SAMPLE BOX MAP-

(For sites with 4th blood draw visits)

1) Empty	2) Empty	3) 100001-4-E-1ao	4) 100001-4-E-2ao	5) 10001-4-E-3ao	6) 10001-4-E-4	7) 10001-4-E-5	8) 10001-4-E-6	9) 10001-4-E-7	10) 10001-4-E-8
11) 10001-4-E-9	12) 10001-4-E-10	13) 10001-4-E-11	14) 10001-4-E-12	15) 10001-4-R-1	16) 10001-4-R-2	17) 10001-4-R-3	18) 10001-4-R-4	19) 10001-4-R-5	20) 10001-4-R-6
21) 10001-4-R-7	22) 10001-4-R-8	23) 10001-4-R-9	24) 10001-4-C-1	25) 10001-4-C-2	26) 10001-4-C-3	27) 10001-4-H-1	28) 10001-4-H-2	29) 10001-4-H-3	30) 10001-4-H-4
31) 10001-4-H-5	32) 10001-4-H-6	33) Empty	34) Empty	35) 10002-4-E-1 ao	36) 10002-4-E-2ao	37) 10002-4-E-3ao	38) 10002-4-E-4	39) 10002-4-E-5	40) 10002-4-E-6
41) 10002-4-E-7	42) 10002-4-E-8	43) 10002-4-E-9	44) 10002-4-E-10	45) 10002-4-E-11	46) 10002-4-E-12	47) 10002-4-R-1	48) 10002-4-R-2	49) 10002-4-R-3	50) 10002-4-R-4
51) 10002-4-R-5	52) 10002-4-R-6	53) 10002-4-R-7	54) 10002-4-R-8	55) 10002-4-R-9	56) 10002-4-C-1	57) 10002-4-C-2	58) 10002-4-C-3	59) 10002-4-H-1	60_ 10002-4-H-2
61) 10002-4-H-3	62) 10002-4-H-4	63) 10002-4-H-5	64) 10002-4-H-6	65) Empty	66) Empty	67) 10003-4-E-1ao	68) 10003-4-E-2ao	69) 10003-4-E-3ao	70) 10003-4-E-4
71) 10003-4-E-5	72) 10003-4-E-6	73) 10003-4-E-7	74) 10003-4-E-8	75) 10003-4-E-9	76) 10003-4-E-10	77) 10003-4-E-11	78) 10003-4-E-12	79) 10003-4-R-1	80) 10003-4-R-2
81) 10003-4-R-3	82) 10003-4-R-4	83) 10003-4-R-5	84) 10003-4-R-6	85) 10003-4-R-7	86) 10003-4-R-8	87) 10003-4-R-9	88) 10003-4-C-1	89) 10003-4-C-2	90) 10003-4-C-3
91) 10003-4-H-1	92) 10003-4-H-2	93) 10003-4-H-3	94) 10003-4-H-4	95) 10003-4-H-5	96) 10003-4-H-6	97) Empty	98) Empty	99) Empty	100) Empty

Box contents: **3 participants, 4th visit**

KMMURPHY 1/12/2010

-LIFE STUDY URINE SAMPLE BOX MAP-

1 **10001-1-U-1 ao**	2 **10001-1-U-2 ao**	3 **10001-1-U-3**	4 **10001-1-U-4**	5 **10001-1-U-5**	6 **10001-1-U-6**	7 **10001-1-U-7**	8 **10001-1-U-8**	9 **10001-1-U-9**	10 **10001-1-U-10**
11 **10001-2-U-1 ao**	12 **10001-2-U-2 ao**	13 **10001-2-U-3**	14 **10001-2-U-4**	15 **10001-2-U-5**	16 **10001-2-U-6**	17 **10001-2-U-7**	18 **10001-2-U-8**	19 **10001-2-U-9**	20 **10001-2-U-10**
21 **10001-3-U-1 ao**	22 **10001-3-U-2 ao**	23 **10001-3-U-3**	24 **10001-3-U-4**	25 **10001-3-U-5**	26 **10001-3-U-6**	27 **10001-3-U-7**	28 **10001-3-U-8**	29 **10001-3-U-9**	30 **10001-3-U-10**
31 **10001-4-U-1 ao**	32 **10001-4-U-2 ao**	33 **10001-4-U-3**	34 **10001-4-U-4**	35 **10001-4-U-5**	36 **10001-4-U-6**	37 **10001-4-U-7**	38 **10001-4-U-8**	39 **10001-4-U-9**	40 **10001-4-U-10**
41 **10002-1-U-1 ao**	42 **10002-1-U-2 ao**	43 **10002-1-U-3**	44 **10002-1-U-4**	45 **10002-1-U-5**	46 **10002-1-U-6**	47 **10002-1-U-7**	48 **10002-1-U-8**	49 **10002-1-U-9**	50 **10002-1-U-10**
51 **10002-2-U-1 ao**	52 **10002-2-U-2 ao**	53 **10002-2-U-3**	54 **10002-2-U-4**	55 **10002-2-U-5**	56 **10002-2-U-6**	57 **10002-2-U-7**	58 **10002-2-U-8**	59 **10002-2-U-9**	60 **10002-2-U-10**
61 **10002-3-U-1 ao**	62 **10002-3-U-2 ao**	63 **10002-3-U-3**	64 **10002-3-U-4**	65 **10002-3-U-5**	66 **10002-3-U-6**	67 **10002-3-U-7**	68 **10002-3-U-8**	69 **10002-3-U-9**	70 **10002-3-U-10**
71 **10002-4-U-1 ao**	72 **10002-4-U-2 ao**	73 **10002-4-U-3**	74 **10002-4-U-4**	75 **10002-4-U-5**	76 **10002-4-U-6**	77 **10002-4-U-7**	78 **10002-4-U-8**	79 **10002-4-U-9**	80 **10002-4-U-10**
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

APPENDIX A:

UNIVERSITY OF FLORIDA CTSI BIOREPOSITORY CONFIDENTIAL – DO NOT DUPLICATE		UF Clinical and Translational Science Institute UNIVERSITY of FLORIDA
SOP No. BIOR-CSH-001		Revision No. 03
Approval – Operating Unit	Date: 6/29/11	Supersedes: 02
Approval – Quality Assurance	Date: 6-29-2011	Issue Date: JUN 29 2011

TITLE

LIFE STUDY – SITE PROTOCOL FOR SHIPMENT OF PLASMA/SERUM AND URINE TO THE CTSI BIOREPOSITORY AT THE UNIVERSITY OF FLORIDA

1 PURPOSE

The purpose of this SOP is to provide general guidelines to the LIFE Study sites regarding shipment of plasma, serum and urine to the CTSI Biorepository at the University of Florida for centralized sample storage.

2 SCOPE

This SOP applies to all of the sites who participate in the LIFE Study and will be shipping biological samples to the CTSI Biorepository.

3 RESPONSIBILITIES

The CTSI Biorepository Director, Assistant Director, and Laboratory staff who will receive shipments from the LIFE study participant sites are responsible for following this SOP. Managers of staff members asked to carry out this activity will assure they are trained on this SOP.

4 SPECIMEN TYPE

This SOP applies to plasma, serum and urine. Whole blood samples for DNA processing will be shipped directly to UF CRC Processing Laboratory at the University of Florida using a separate SOP.

5 SPECIMEN COLLECTION AND PREPARATION

Samples will be shipped as frozen samples post-processing according to approved LIFE study procedures.

6 MATERIALS AND EQUIPMENT

6.1 Standard Cryovial Storage Boxes Used by the LIFE Study (Dimensions 5.5" x 5.5" x 2" with 10 x 10 Grid = max 100 Cryovials)
6.2 Patient labeled cryovials
6.3 Dry Ice
6.4 Cryo & Disposable gloves
6.5 Lab coat and safety glasses
6.6 Zip-Style Re-closable Biohazard Transport Bag
6.7 Small absorbent pads

6.8 Appropriate Bio-safety shipping containers as specified in LIFE Study MOP

6.9 Rubber bands

7 FORMS

7.1 LIFE Study Packing List

7.2 LIFE Study Blood and Urine Processing Forms

7.2 BIOR-QC-FM-003 Assessment of the Integrity of Shipments Received

8 SPECIAL HANDLING

All reagents/specimens are potentially hazardous. Use appropriate safety procedures when handling these materials. Avoid contact with skin and mucous membranes by wearing a laboratory coat and gloves, eye protection, and a mask if needed. Observe OSHA standards when working with bodily fluids and cultures derived from bodily fluids.

9 PROCEDURE

9.1 Pre- Shipping Approval

9.1.1 Before shipping any samples to the CTSI Biorepository, the LIFE study site must submit a request to ship samples to UF on the CTSI Biorepository website at http://biorepository.pathology.ufl.edu/example-2/affiliated-studies/life-study/request-to-ship-samples/ to request approval to ship samples on a particular date. An email confirmation from the CTSI Biorepository (biorepository@pathology.ufl.edu) approving the requested shipment date must be received prior to shipment of the samples. The email notification acknowledging approval from the CTSI Biorepository will have the following subject line: **Approval of Arrival of Shipment on mm/dd/yyyy from *LIFE study site name* to UF CTSI Biorepsitory**. A notification email will be also be sent if the shipment receipt date is not approved.

9.1.1.1 Shipments must only occur on Mondays, Tuesdays and Wednesdays (baring holidays) for receipt by the CTSI Biorepository on Tuesday-Thursday because a delay by the courier with shipments sent on a Thursday or Friday would

result in the samples arriving thawed compromising the integrity of the specimens.

9.2 Shipping Requirements

9.2.1 Samples should remain separated into boxes according to type as follows:

9.2.1.1 Plasma/Serum

9.2.1.2 Urine

9.2.2 The labels on the outside of the storage boxes must correspond directly with the information found on the LIFE Study's Blood Processing and Urine Processing Forms (i.e., biological specimen ID and sample box numbers). If this information is different from the original information recorded on the Forms, the Forms must be updated with the most up to date storage box information.

9.2.3 Put rubber band around each storage box and place each box into a separate Zip-Style Re-closable Biohazard Transport Bag (Two-Pouch) with absorbent pad.

9.2.4 Place a photocopy of the LIFE Study Blood Processing or Urine Processing Form(s) that is specific to that particular box in the outer pouch of the Zip-Style Re-closable Biohazard Transport Bag (originals should be retained at the site).

9.2.4.1 Example: Blood Sample Box # 1 contains Specimen Label(s) 30001-1, 30002-1, 30003-1

9.2.4.1.1 The three corresponding Blood Processing Forms for Specimen Label(s) 30001-1, 30002-1, 30003-1 should be included in the outer pouch of Zip-Style Re-closable Biohazard Transport Bag.

9.2.5 The maximum number of cryovials should be 500 +/-60 rounding down to the nearest full box (i.e. do not remove samples from their original box).

9.2.6 A minimum of 10-20 lbs of dry ice, depending on the size of the shipper, should be used for overnight shipments as it sublimates at the rate of 5-10lbs/24hrs depending on shipping materials and outside temperatures.

9.2.6.1 Sites should use their judgment regarding the size of the shipper based on the number of samples being shipped and need for dry ice to maintain the temperature of the samples.

9.2.6.2 Dry ice should surround the samples with little empty space in the box; this can typically be accomplished by placing half of the dry ice under the sample boxes and half of the dry ice on top of the sample boxes.

9.2.7 In addition, an all-inclusive LIFE Study Packing List should be included in each shipment **and** sent electronically as described below (see LIFE study MOP for instructions on how to generate the Excel Document from the LIFE study website that will act as the Packing List).

9.2.7.1 Hard Copy

9.2.7.1.1 A packing list of all samples and boxes that are being shipped must be included in the shipment to the CTSI Biorepository. The enclosed list should be placed in a standard zip-lock bag and placed between the top of the Styrofoam box and the outer cardboard box.

9.2.7.1.1.1 The packing list should include a list of all cryovials designated by their Biological Specimen ID (this label matches the information in the barcode). The barcodes listed in the packing list should be identical to the physical inventory being shipped.

9.2.7.1.1.1.1 If samples are received by the CTSI Biorepository without labels, the vials and their contents will be destroyed, and the site will be notified which vials have been destroyed.

9.2.7.1.1.1.2 If vials arrive empty, these vials will be disposed of, and this will be reported to the site.

9.2.7.1.1.1.3 The LIFE study sites will be responsible for reconciling any discrepancies between the inventory reports provided by the CTSI Biorepository and the information initially input into the LIFE study database by the site.

9.2.7.2 Electronic Copy

9.2.7.2.1 An electronic copy of the LIFE study packing list must also be sent to the CTSI Biorepository via email to biorepository@pathology.ufl.edu.

9.2.8 Ship via FEDEX Priority Overnight using account number provided by the LIFE Study. The "**Direct Signature**" box must be checked on the FedEx shipping request for all shipments sent to the CTSI Biorepository to ensure that all packages are signed for by someone at the CTSI Biorepository upon receipt.

9.2.8.1 Ship To:

CTSI Biorepository
University of Florida
Dept of Pathology, Room # M621
Gainesville, FL 32610
Tel (352)273-7649, (352)392-0089
Attn: Tony Higgs or Eric Elmadani

9.2.8.2 If the Biorepository Laboratory staff cannot be reached on the day of shipment, please contact the Assistant Director of the Biorepository (contact information can be found on the Biorepository

website at: http://biorepository.pathology.ufl.edu/about/biorepository-staff-2/).

9.2.9 Provide the CTSI Biorepository with the FedEx tracking number using one of the two methods listed below (first method is preferred if feasible)

9.2.9.1 Include the CTSI Biorepository email address (Biorepository@pathology.ufl.edu) as a contact for all communication with FedEx regarding the shipment

9.2.9.2 The tracking number generated by the courier should be emailed to Brian Bouverat (bbouverat@aging.ufl.edu) and the CTSI Biorepository at biorepository@pathology.ufl.edu to allow for tracking of the shipment.

9.2.10 If the shipment does not arrive to the CTSI Biorepository on the expected day of delivery, an email will be sent to Brian Bouverat and to the LIFE study site that sent the package to initiate tracking activities.

9.2.11 An initial email confirmation from the CTSI Biorepository will be sent on the day the shipment arrives to inform the site that the shipment was received. A follow-up email will be sent within 3-5 business days providing additional details about the integrity of the shipment received (BIOR-QC-FM-003), an inventory report listing all of the samples registered in the CTSI Biorepository database from that shipment, and an inventory reconciliation report that details any discrepancies between the packing list and the physical inventory shipped (if required). Brian Bouverat will be copied on all correspondence.

9.2.11.1 The follow-up email will acknowledge whether or not there were any visual signs of damage to the external shipment package. The follow-up email will also identify any discrepancies or issues between the contents listed in the packing list and those found in the package itself and will report any visual damage or concerns with the contents as described in 9.2.11.1.1.

9.2.11.1.1 At minimum, the following will be assessed regarding the integrity of the package and

samples received: box integrity (crushed, open, etc.), adequacy of dry ice upon arrival, loose samples in the box, loose caps, label integrity (cracking, adhesion to tube, clarity of the label/barcode, ability to scan the barcode), and thawed samples. This information will be recorded on BIOR-QC-FM-003 and a scanned copy will be included in the follow-up email to the site.

9.2.11.2 Discrepancies or issues between the contents listed in the packing list and those found in the package itself or any visual damage or concerns with the contents will be documented using form CTSI-QA-FM-009 GLP Deviation Report.

9.2.11.2.1 The completed Deviation Report will be signed by the BIOR Assistant Director (or designee) and CTSI QA and provided to the site and Brian Bouverat (see step 9.2.11.1.1) as soon as it becomes available.

9.2.11.2.2 After review of the Deviation Report, Brian Bouverat (or designee) will sign the report and return the original to BIOR.

9.2.11.2.2.1 Disagreements or clarification required in the Deviation Report will be handled on a case by case basis.

9.2.11.2.3 Finalized Deviation Reports will be transferred to CTSI Quality Assurance.

9.3 The samples will be registered into the CTSI Biorepository inventory system, stored at -80 degrees Celsius, and will only be distributed upon request/approval from one of the LIFE study PIs at the University of Florida (Pahor or Leeuwenburgh).

9.4 Contact Information for the Biorepository Staff can be found on the CTSI Biorepository website at http://biorepository.pathology.ufl.edu/about/biorepository-staff-2/

10 USE OF FORM(S)

10.1 The form listed below is copied from the SOP book.

BIOR-QC-FM-003 Assessment of the Integrity of Shipments Received

10.1.1 The form is completed as needed and filed in the Life Study Chart.

10.2 The forms listed below are provided with the Study shipment.

LIFE Study Packing List
LIFE Study Blood and Urine Processing Forms

10.1.1 These forms are filed in the Life Study Chart.

11 CHANGE HISTORY

Rev. Number 03 Revised

1. Section 6 - materials and equipment updated
2. Section 7- Forms section added
3. Numbering updated to account for addition of Forms and Use of Forms sections
4. Section 9.2.5 - number of specimens per shipment updated for clarity
5. Section 9.2.8 - added request for signature upon shipment receipt
6. Section 9.29 - added secondary option for providing FedEx tracking information to CTSI Biorepository
7. Section 10- Use of Forms section added
8. Issue date of Rev 02 added to Change History

Rev. Number 02 Revised– Issued 3-24-2011

1. Rev. Number 01 issue date added to the Change History.
2. Replaced header.
3. Section 6 – Refer to LIFE Study MOP for appropriate Bio-saftey shipping containers.

4. Section 8.1.1 – Changed dd/mm/yyyy to mm/dd/yyyy and added text saying to send an email notification if shipment receipt dates are not approved.
5. Section 8.1.1.1 – Updated when shipments are to be received by the CTSI Biorepository.
6. Section 8.2.5 – Updated the maximum number of cryovial from 1000 to 560 per shipment.
7. 8.2.6 – Changed the minimum amount of dry ice needed to 10-20lbs depending on the size of the shipment.
8. Section 8.2.6.1 – Section added saying that the shipping sites should use their judgment on how much dry ice would be needed based on shipment size.
9. Section 8.2.6.2 – Section added saying how to place dry ice with the samples
10. Section 8.2.7.1.1.1 – Text added saying to match barcodes in the packing list to the physical inventory.
11. Sections 8.2.7.1.1.1.1-8.2.7.1.1.1.3 added to describe what will happen to vials that arrive unlabeled or empty and to outline the sites role in inventory reconciliation
12. Section 8.2.11 – Text added on what information to provide to the site about the integrity of the shipment received.
13. Section 8.2.11.1 – Removed text for clarity and changed the reference from 8.2.10.1.1 to 8.2.11.1.1.
14. Section 8.2.11.1.1 – Added text on how information will be recorded.
15. Section 8.2.11.2.1 – Section added to clarify who signs the Deviation Reports.
16. Section 8.2.11.2.2 – Section added saying that Brian Bouverat is to sign and return Deviation Report to BIOR.
17. Section 8.2.11.2.2.1 – Section added to clarify how disagreements on Deviation Reports are to be handled.
18. Section 8.2.11.2.3 – Section added saying to transfer finalized Deviation Reports to CTSI QA.

Rev. Number 01 Revised – Issued 12-10-2010

1. Section 6 – Size of cryovial storage boxes changed and reworded.
2. Section 8.1.1 – Updated procedure for requesting shipment of samples.
3. Section 8.2 – Removed section 8.2.1 (information about placing an x on the box).
4. Section 8.2.2 – Previously section 8.2.3; Corrected box label and updated text for clarity.

5. Section 8.2.4 – Added section to include copies of LIFE Study Blood Processing or Urine Processing Form(s).
6. Section 8.2. – Updated text for clarity due to the removal of the need to consolidate which will allow for the shipment of partial boxes.
7. Section 8.2.7 – Previously Section 8.2.6.1 (version (00)) was moved to its own section and updated for clarity.
8. Section 8.2.8.2 – Updated to direct LIFE study sites to Biorepository website for contact information rather than listing staff here.
9. Section 8.4 – Updated to direct LIFE study sites to biorepository website for contact information rather than listing staff here.
10. Issue date of Rev 00 added to Change History

Rev. Number 00 New – Issued 10-25-2010

APPENDIX B:

UNIVERSITY OF FLORIDA AT CLINICAL RESEARCH CENTER	
SOP No: UF-CRC-Life Study -01	Revision No:2.0
Title: SITE PROTOCOL FOR SHIPMENT OF FROZEN WHOLE BLOOD TO THE UF-CRC AT THE UNIVERSITY OF FLORIDA	Page: 1 of 5

Revision history — Revised to indicate shipping in bags versus boxes and other grammatical errors corrected, 10/17/2011
Revised to indicate a maximum number of 24 samples processed/week 11/1/11

1 PURPOSE

The purpose of this SOP is to provide general guidelines to the LIFE Study sites regarding shipment of whole blood to the UF-CRC at the University of Florida for centralized DNA Extraction and Storage

2 SCOPE

This SOP applies to all of the sites who participate in the LIFE Study and will be shipping biological samples to the UF-CRC at the University of Florida

3 RESPONSIBILITIES

The CTSI Biorepository Director, Assistant Director, and Laboratory staff who will receive shipments from the LIFE study participant sites are also responsible for following this SOP. Managers of staff members asked to carry out this activity will assure they are trained on this SOP.

4 SPECIMEN TYPE

Whole blood samples for DNA Extraction

5 SPECIMEN COLLECTION AND PREPARATION

All Biological samples should be treated as potentially infectious.

6 MATERIALS AND EQUIPMENT

(For Study site)

15 ml disposable centrifuge tubes
Dry Ice
Cryo & Disposable gloves
Lab coat and safety glasses
Biohazard and zip-lock bags+
Small absorbent pads
Appropriate Bio-safety shipping containers

7 SPECIAL HANDLING

Each LIFE Study site and the CTSI Biorepository must ensure compliance with their own State as well as Federal laws concerned with the packaging and shipment of Biohazardous samples, in accordance with HIPPA, Environmental, Health and Safety and OSHA Laws and the shipping person should be certified for shipping and transport of biological materials.

8 PROCEDURE

8.1 Pre- Shipping Approval

8.1.1 Before shipping any materials to the UF-CRC, you must have an email confirmation from a member of the staff within the UF-CRC (either Tomy Mathew or Tim Palmer) that confirms that it is acceptable to send a shipment on your requested date. Shipments will only occur on Mondays, Tuesdays and Wednesdays (baring holidays) because if there were to be a delay by the courier with shipments sent on a Thursday or Friday, this would result in the samples arriving thawed compromising the integrity of the specimens.

8.1.2 Shipment dates can be coordinated between the LIFE study sites and UF -CRC through Brian Bouverat of the LIFE study

(University of Florida Institute on Aging; bbouverat@ ufl.edu; 352-273-5730). However, the final confirmation of approval for a shipment must come directly from the UF-CRC. The email notification acknowledging approval from the UF-CRC will have the following subject line: **Approval of Arrival of Shipment on XX/XX/XXXX from *LIFE study site name* to UF-CRC**.

UF-CRC contact information

Tomy Mathew
Sr. Medical Technologist
tomathew@ufl.edu Phone # 352-265-0111 ext.44970

Tim Palmer
Sr. Medical Technologist
timpalmer@ufl.edu Phone # 352-265-0111 ext.44970

Timothy J. Garrett, Ph.D.
Research Assistant Professor
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8.2 Shipping Requirements

Maximum of 24 samples per shipment and one shipment per week for DNA extraction.

8.2.1 A minimum of 10lbs of dry ice should be used for overnight shipments as it sublimates at the rate of 5-10lbs/24hrs depending on shipping materials and outside temperatures.

8.2.1.1 A packing list of all samples that are being shipped must be included as well as emailed to the UF-CRC (Excel Document which can be generated from the LIFE website). The enclosed list should be placed in a Ziploc bag and placed between the top of the Styrofoam box and the outer cardboard box.

8.2.1.1.1 The packing list should include a list of all samples designated by their Biological Specimen ID (this label matches the information in the barcode) and the date the sample was collected

8.2.2 **Frozen specimens must be completely packaged in shipping containers prior to Fed Ex pickup**

8.2.1 Place each frozen specimen in a single biohazard bag with absorbent material.

8.2.2 Place all those samples along with commercially prepared dry ice, into the Styrofoam container.

8.2.3 Place this Styrofoam container into an appropriate cardboard bio- shipping container and place all the shipping labels, orientation and dry ice stickers outside of that container.

8.2.3 Ship via FEDEX Priority Overnight using account number provided by Brian Bouverat, to:

UF-CRC University of Florida
Shands Hospital
1600 SW Archer Rd; Room # 3265
Gainesville, FL 32610
Tel (352)265-0032, (352)265-0111 ext.44970
Attn: Tomy Mathew or Tim Palmer

Please check the "Direct Signature" box on the FedEx Airbill.

8.2.4 If Tomy Mathew or Tim Palmer cannot be reached on the day of shipment, contact Timothy J. Garrett, Ph.D.
Research Assistant Professor
tgarrett@ufl.edu Phone # 352-273-5050

8.2.5 The tracking number generated by the courier should be emailed to UF-CRC so that the shipment can be tracked.

8.2.6 You will receive email confirmations from the UF-CRC on the day your shipment arrives at UF-CRC.

8.3 Rejection Policy

All specimens received here at UF-CRC must follow the acceptability guidelines of proper labeling, correct transport medium, correct specimen type for the specific study and no visible leakage or breakage of the transport container or the specimen tubes. When a specimen does not comply with such criteria, the UF-CRC Laboratory staff shall treat it as unacceptable and will e-mail to the study site and copy the same to Brian Bouverat of the LIFE study (University of Florida Institute on Aging; bbouverat@ ufl.edu and Timothy J. Garrett, Ph.D. Research Assistant Professor tgarrett@ufl.edu.