Chapter 11
Biological Specimen Collection & Processing

Changes from v2.0 to 3.0 (6/22/17)

- Section 11.7.2.1. Priority of collection tubes
  - Added collection of CPT at Month 12
- Appendix G: QUEST Safety Lab Manual
  - Corrected Quest toll-free phone number

Changes from v1.0 to v2.0 (4/21/2016)

- Section 11.1
  - Changed total volume of blood collected from 56 mL to 39 mL
- Section 11.4 Blood Collection Supplies list
  - Added (or solid red) to description of SST tube used for repository samples
  - Changed BD# from 368016 to 367988 on SST used for Quest samples
- Section 11.7.1 Lab Collection – IL6 & Repository Form
  - Under heading “Current Health Status/Acute Illness” changed “2 weeks” to “a month”
- Section 11.9.6 Blood Processing Overview
  - On #10, changed volume of SST tube used for Quest samples from 5 mL or 4 mL to 8.5 mL
- Appendix G – QUEST Safety Lab Manual
  - Under Completing the Registration form, added bullet, “Requisition forms can also be created online using the Care 360 system.”
  - Under Sample Collection and Processing added, “Either the roll of labels provided by Quest can be used or sites can create these labels on their own,” to final bullet.
  - Under Shipping
    - #1 - replaced “cooler” with “package”
    - #2 – removed “as well as inside with reqs and samples” and added, “Using a brightly colored label is also acceptable.”
    - #9 – added “FedEx Airbills are provided by Quest.”
    - Added “Shipment to the following address is also acceptable: 200 Lewis Drive, Wood Dale, IL, 60191.”

Chapter 11
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11.1 Background and Rationale

Growing evidence shows that low-grade chronic inflammation, characterized by elevations in plasma C-reactive protein, tumor necrosis factor alpha, and particularly Interleukin-6 (IL-6), is an independent risk factor of disability, impaired mobility, and lower walking speed. Low-grade chronic inflammation is a modifiable risk factor. However, it is unknown whether interventions that reduce the levels of inflammatory markers *per se* improve mobility, or avert decline in mobility in older persons. To address this gap in evidence we conduct the randomized clinical trial ENRGISE (ENabling Reduction of low-Grade Inflammation in SEniors) to test the ability of anti-inflammatory interventions for preventing major mobility disability by improving or preserving walking ability. We will test the efficacy vs. placebo of the angiotensin receptor blocker losartan and omega-3 polyunsaturated fatty acids in the form of fish oil, alone and in combination. Both angiotensin receptor blockers and omega-3 polyunsaturated fatty acids have shown to reduce IL-6 in clinical trials and preliminary data suggest that they may improve physical function

The overall goal of the ENRGISE study Biological Specimens Collection and Processing Manual of Procedures is to guarantee the proper collection, processing, shipping, and central storage of blood specimens. 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 ENRGISE Administrative Coordinating Center (ACC) will oversee training and monitor the quality control of the blood collection and processing at the participating Field Centers. Blood collection consists of drawing

blood (4-39 mL per visit) at multiple visits. Samples will be collected only for each ENRGISE participant who provides written, informed consent for these procedures.

11.2 Contact information

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11.3. Quest Diagnostic Testing Laboratory

The ACC has set up an account with Quest, a central diagnostic testing lab, for the following safety test to be performed for each participant:

Test	Test Codes	Scr. Visit 1	Scr. Visit 2	Baseline	3-Mo visit	6-Mo visit	9-Mo visit	12-Mo visit	Safety Visits
Comprehensive Metabolic panel	10231		x						
Vitamin D	17306		x						
CBC (Hg)	6399		x		x	x	x		
Lipid panel only	7600			x	x	x	x		
Basic Metabolic panel only	10165				x	x	x		x

The test results will be sent electronically to Data Management, Analysis, and Quality Control (DMAQC) Center. Results will also be sent to the Field Centers and accessible to investigators.

See Appendix G for Quest Laboratory Blood Collection Instructions.

11.4. 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 (manual defrost)
- Storage space in a -70°C freezer (manual defrost)
- Liquid handling device (pipet) that can dispense 1μl volumes
- Color or black/white printer for printing study forms and labels
- Tube racks
- 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 (EPA-registered tuberculoidal) 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:

- 4mL Gold SST (BD# 367977):IL-6 measurements
- 8.5 mL red/gray (or solid red) SST (BD # 368016):IL-6 measurements and repository
- 10 mL EDTA (BD #366643): Repository (including packed cells)
- 8.5 mL red/gray SST (BD # 367988): Quest Safety Labs (provided by Quest)
- 4mL EDTA Plasma (BD#367862): Quest Safety Labs (provided by Quest)
- 8 mL Cell Preparation Tube (GLASS) with Heparin (BD # 362753): Cells
- Timers
- Band-aids
- 21 gauge safety needles
- Tourniquets
- 2 X 2 gauze pads
- Micropore tape
- Ice bucket/container

Blood Processing Supplies:

- Cryovials- 0.5mL, 2.0mL and 10.0mL graduated, externally threaded with color-coded caps (purple and red) which can accommodate the bar-coded labels (1 ¼" high by 1 7⁄16" wide-circumference)

Cryovials:	Fisher Scientific	# 02-681-343	2.0 ml skirted
Cryovials	Fisher Scientific	# 02-681-337	0.5 mL skirted
Tube:	Fisher Scientific	# 05-679-97	10mL skirted
Caps:	Fisher Scientific	#02-681-361	Red
Caps	Fisher Scientific	#02-681-363	Green
Caps:	Fisher Scientific	#02-781-366	Violet

- Biomerga Cool Cube benchtop coolers (Research Products Inc.) – holds 36 cryovials [RPI # 260105]

- Pipet with disposable tips
- Storage boxes (2”) with 9 x 9 and 7 x 7 dividers
- 15 cc conical tubes for pooling plasma

Cell Preparation Tube Additional Processing Supplies:

- Buffy coat Freezing Media (Millipore cat#s-002-10F) (or use 90% Fetal Bovine Serum/10% DMSO)
- Mr. Frosty (model# 5100-0001)

Shipping Supplies provided by the UVM Biomarker Laboratory:

- Insulated Shippers
- Absorbent Sheets
- 10” x 10” clear, zip style bag w/biohazard symbol and external pouch
- pre-printed Fed Ex airbills

Shipping Supplies NOT provided by the UVM Biomarker Lab:

- Dry ice (10 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 emailing or faxing a Supply Request Form to: Rebekah Boyle at Rebekah.Boyle@uvm.edu or (802) 656-8965 (fax). **Two week notice is required**.

11.5. 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 Blood borne 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 ENRGISE 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.

    1) Gloves

        a. Wear gloves for all patient contact when body fluids are involved.

        b. Change gloves between patients and when gloves become soiled or torn.

        c. Wash hands thoroughly after removing gloves. Use an “evaporating” disinfectant if a sink is not available.

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

2) Lab Coats/Gowns

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

3) Splash Shields

a. 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.

11.6. Specimen ID Labels

Biospecimen 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 Lab Collection & Processing (IL-6 & Repository or Safety) forms, should labeled with both ID numbers**.

The UVM Biomarker Lab will supply each Field Center with sheets of Biological Specimen ID barcode labels. These will be used for labeling forms, blood collection tubes (except those provided by the diagnostic testing lab), tubes, cryovials, and sample boxes. Label Sets for each time point will include: labels for collection tubes (plus extra labels), labels for the freezer storage boxes (blood and packed cells), cryovial labels, processing labels used for tubes to pool plasma and serum, and form labels (plus 1 extra). The extra 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 Appendix C.

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

1. Wake Forest School of Medicine
2. Northwestern University

3. Tufts University
4. University of Pittsburgh
5. University of Florida
6. (if needed) University of Florida sub-site

The next 3 numbers will be the participant's unique Biological Specimen ID number. Biological Specimen ID numbers can run from 001 through 999. The next 2 digits on the label is to identify the visit. 00 = baseline, 03 = 3 month visit, 06= 6 month visit, 09= 9 month visit, 12= 12 Month visit. For example, the Biological Specimen ID number may be 3 001 03 (Tufts site, participant 001, 3 Month visit). Screening Visits will not have a 2 digit visit id. For example the Biological Specimen ID for a screening visit may be 3 200 with text to indicate SV1 or SV2.

Cryovial labels will contain an additional number which denotes the chronological number of that particular sample type (Cryovial # 01-21).

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. The label must be adhered to itself on the cryovial. The proper orientation of labels on cryovials and tubes is shown in Appendix B.

Field sites should request labels by emailing or faxing a Supply Request Form to: Rebekah Boyle at Rebekah.Boyle@uvm.edu or (802)656-8965 (fax)**. Two week notice is required**.

11.7. Blood Collection

Instructions for blood collection and processing for safety testing to be performed by Quest Diagnostics are included in Appendix G.

11.7.1. Lab Collection – IL-6 & Repository Form

The purpose of the Lab Collection – IL-6 & Repository Form 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.

The participant study ID and barcode should be PRINTED on the form and the Biological Specimen ID label should be affixed to the form. All forms must be completed in ink.

Section 1: Consent & Pre-Check

In Section 1 there are eleven questions best completed by the coordinator (rather than the phlebotomist) pertaining to information needed to determine whether a participant's blood should be drawn or rescheduled for a later time point.

Some participants will provide informed consent to participate in the ENRGISE study without consent for blood storage. Participants whom do not provide consent to blood draw are not eligible for participation in the study.

The first two questions indicate the participant's CURRENT consent status for blood collection and storage and DNA collection. If the participant has not consented to a blood storage, ('no' to Q1), only the sample for IL-6 analysis (4 ml SST) should be collected and processed. Samples used for banking will not collected. If the participant has consented to blood storage but not consented to DNA collection ('no' to Q2), then the DNA collection/processing should be excluded. The coordinator conducting the assessment visit should be certain to relay this information to the phlebotomist. The Lab Collection – IL-6 & Repository form should still be filled out for any participant who refuses the blood draw (only Q1 should be answered). All forms

have a printed Participant ID# and barcode and the specimen ID # affixed to the form, regardless if blood will be drawn or not. A copy of a Lab Collection form should be retained for EVERY participant, regardless if blood was drawn or not.

Fasting Status

Questions 3 and 4 are related to fasting status. It is recommended that the participant 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 fast time is less than 8 hours.** If you are unable to reschedule or the participant is unwilling to reschedule the blood draw, proceed but indicate this information in Q13.

Participants should take their medications as usual on mornings of each phlebotomy visit. Participants will be bringing all medications for a medication inventory 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

Current Health Status/Acute Illness

Questions 5-11 are related to the current health status of a participant. If a participant answers ‘yes’ to any of these questions, indicating an acute illness in the past month, the IL-6 and repository samples should not be collected. The participant should be brought back a month after their symptoms have resolved. Blood draws for Quest safety can be drawn as scheduled regardless of fasting status or acute illness in the month prior to the visit.

Section 2: Safety

In Section 2 there are five questions to ask the participant before starting the venipuncture procedure. The first three questions (Q14-16) relate to the venipuncture. If the participant’s answer is yes to any of these questions, the phlebotomist should take extra care during the phlebotomy. Question 17 has to do with the participant’s diabetic health status and Q18 relates to the participant taking blood thinners.

Section 3: Procedure

In Section 3 there are 3 questions to provide information related to the blood draw. In Q19 provide the best description of the venipuncture.

Section 4: Samples Collected

Section 4 provides an area to document the samples collected. Phlebotomist can indicate whether they collected the tube or not, whether it was a partial tube or if there is some other volume they need to specify.

Section 5: Comments

Section 5 is provided for the phlebotomist to provide any relevant comments regarding the procedure.

Upon completion, the Lab Collection – IL-6 & Repository form should be entered into the ENRGISE website data entry system and the paper copy kept on file in the participant's chart.

11.7.2. Preparation/Set-up

Prior to the participant's visit, the phlebotomist should label the collection tube vacutainers and prepare all supplies. Lab collection and processing forms should be printed from the ENRGISE website *by the study coordinator* at the time of the visit and will the Participant ID number and barcode. The collection tubes should be labeled with BOTH the participant ID number legibly hand-written with a Sharpie marker on each tube, and with the collection tube label containing the Specimen ID# provided by the UVM Biomarker Lab. For all follow-up visits, please check again that the participant study ID matches the Biological Specimen ID number and label the Lab Collection – IL-6 & Repository Form.

The study coordinator must be sure that informed consent forms have been signed before drawing the participant's blood. One to five 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.

11.7.2.1. Priority of collection tubes

Refer to the table below for selection of tubes needed for each visit. This information is also provided for you on the Lab Collection form.

Order of Priority

- IL-6
- Safety labs
- Repository samples

Tubes Collected	**SV1**	**SV2**	**BLR**	**F03**	**F06**	**F09**	**F12**	**Safety Visits**
One 4mL Serum (IL-6)	X	X		X	X	X		
One 8.5mL SST (Safety)		X	X	X	X	X		X
One 4mL EDTA (Safety)		X		X	X	X		
One 8.5mL SST (Repository)			X		X		X	
One 10mL EDTA (Repository)			X		X		X	
One 8mL CPT (Repository)			X		X		X	

11.7.2.2. 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.

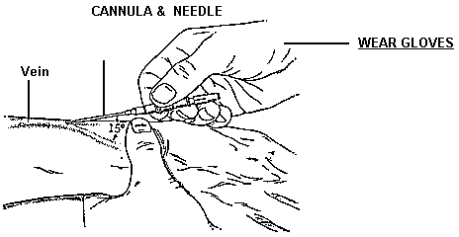
11.7.2.3. Venipuncture Procedure

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. Tourniquest time should be limited to a maximum of 120 seconds.

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 tubes required for the applicable visit. Always collect Serum before EDTA or CPT plasma tubes to avoid additive cross-over from tube to tube.

13. The phlebotomy should be timed and the time recorded on the form.

14. **Avoid under filling the collection tubes**. Cell Prep and EDTA vacutainers 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 additive and the specimen will be unsatisfactory for testing.

15. Mix blood vacutainer tubes several times **immediately** after collection by inverting them **gently and evenly.** This can be accomplished by hand or an automated tube rocker. Remove the needle when the venipuncture is completed. Close the safety device and dispose of the needle into a Sharps Container

16. 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

17. Remove the disposable gloves; place them in a biohazard trash container. Wash your hands. Transport the sample to the necessary processing area.

11.7.2.4. 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 Lab Collection – IL-6 & Repository form stating that the blood draw will be rescheduled.

6. Make a note on the Lab Collection – IL-6 & Repository 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.

11.8. Blood Processing

11.8.1. Lab Processing – IL-6 & Repository Forms

The purpose of the Lab Processing – IL-6 & Repository 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.

The participant study ID and barcode should be PRINTED on the form 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 Lab Processing – IL-6 & Repository form.

Upon completion, the Lab Processing – IL-6 & Repository form should be entered into the ENRGISE 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.

11.8.1.1. Participant refusal for DNA

Some participants will provide informed consent to participate in the ENRGISE study blood draw, but will not want their DNA extracted from this blood for use in genetic testing. The packed cells for future extraction of DNA 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 at the bottom of the Lab Processing – IL-6 & Repository form that the participant refused the collection of DNA for genetic testing.

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*** (Appendix A). This form should be retained by the field center.

11.9. Overview and Description of Aliquots

Processing should be initiated as soon as possible (less than 30 minutes) following the blood draw. Processing delay should be noted at top of the Lab Processing – IL-6 & Repository form. The red-top (all serum tubes) serum tubes must stand at room temperature for at least 30 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 the Lab Processing – IL-6 & Repository form and the table below:

Collection Tube	Centrifuge 4C 30,000 Gmin	Expected volume	Number of Cryovials	Color Code
4mL SST	Allow to clot for 30 Min prior to Centrifugation	2.0mL	1 @ 2.0mL	Red
8.5ml SST	Allow to clot for 30 Min prior to Centrifugation	6.0 mL	6 @ 0.5 mL	Red
10ml EDTA	On ice prior to Centrifugation	8.0 mL	8 @ 0.5 mL	purple
			1 @ ~2.5mL packed cells	white

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

- Whole blood collected in an EDTA (lavender-top) tube (4 mL)
- Serum Separation Tube (SST; 8.5mL)

Please refer to Appendix G for details on labeling and processing of samples going to Quest for analysis.

11.9.1. Preparation/Set-up

Collection tubes for phlebotomy, 15-mL conical tubes, one for pooled plasma and one for pooled serum (only for the Baseline, 3 Month, 6 Month and 12 Month visits) 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 label must be adhered to itself on the cryovial. The labeled cryovials should be pre-chilled 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.

11.9.2. 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 Lab Processing – IL-6 & Repository form. Procedures for processing bloods collected for Quest Diagnostic safety labs are included in Appendix G.

SST samples:

1. Red-top tubes should be allowed to clot by letting them sit at room temperature for at least 30 minutes.
2. Centrifuge the red-top tubes at 2000 x g for 15 minutes 4°C (or equivalent spin of 30,000 g-min), per tube manufacturer's specifications.
3. After centrifugation, place the draw tube on ice to chill the serum while it is being aliquoted.
4. Aliquot using a volumetric pipette into pre-chilled, labeled, plastic, red 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 (10 mL, purple-top) plasma samples:

1. Purple-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. Centrifuge the EDTA tube (purple-top vacutainers) at 2000 x g for 15 minutes 4°C. (or equivalent spin of 30,000 g-min).
3. After centrifugation, place the tubes on ice to keep them at the proper temperature during aliquoting.
4. Aliquot into pre-chilled and labeled purple screw-top cryovials with O-ring seals.
5. Transfer the remaining cells into pre-chilled and labeled white capped 10mL tube. The aliquots must be frozen immediately and stored frozen at below -70°C.

6. The empty EDTA tube can be discarded in the biohazard waste.

SST (8.5mL, red/grey-top) sample for lipid panel and metabolic panel:

1. Follow instructions provided by the diagnostic testing lab.

EDTA (4 mL, lavender-top) whole blood sample for CBC:

1. Follow instructions provided by the diagnostic testing lab.

11.9.3. Freezing and storage

After aliquoting and capping of samples, the cryovials should be placed in a *labeled* and *numbered* storage box (see box maps in Appendix F). The number of the box should be noted on the Lab Processing – IL-6 & Repository form. More than one participant's samples can be placed in the box.

The 10mL EDTA Packed Cells tubes should be placed in the separate storage boxes.

Every effort should be made to freeze plasma/serum samples at -70°C or below in an upright position 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.

11.9.4. Difficulties or problems

Low sample (partial) volume: EDTA and CPT collection tubes must be greater than ½ 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 Lab Processing – IL-6 & Repository 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 Lab Processing – IL-6 & Repository 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 Lab Processing – IL-6 & Repository form.

11.9.5. Cell Prep Tube Processing

11.9.5.1. General Comments on the Collection of Viable Cells

Collection Tube	Centrifuge 4C 30,000 Gmin	Number of Cryovials	Color Code
8 ml CPT	Process within 2 hours	2 @ 0.5 mL	Green

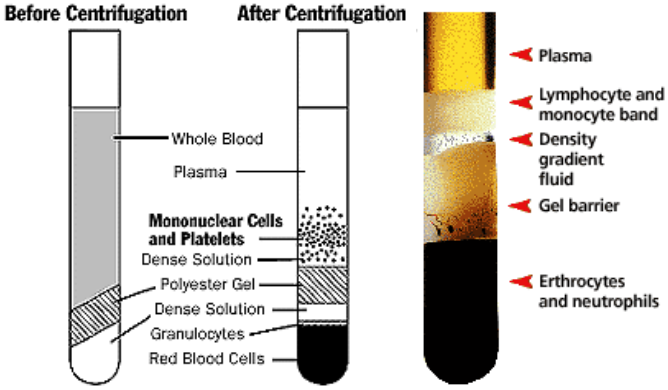
Cells are collected using the special 8 mL cell preparation (Cell Prep Tubes or CPT) 16 x 125 mm glass tubes with green/black (Becton Dickinson #362753 sodium citrate)**.** This tube contains anticoagulant as well as a polyester gel and a density gradient liquid (Ficoll Hypaque) to enhance the separation of white cells. These tubes will be processed to obtain viable white cells (a "buffy coat" layer appears in the density gradient). After filling with blood, these tubes are mixed (either by gentle inversion or on a mixer) and kept at room temperature. Tubes must be processed within two hours of collection.

11.9.5.2. Separation of Cells (Buffy Coat)

The filled Cell Prep Tube may be held at room temperature for up to 2 hours to allow batching of CPT tubes from several participants. If samples from several participants are run at once, it is very important to ensure that the samples do not get mixed up, and that pipet tips and transfer pipets are changed between samples from different participants. Failure to do so could completely invalidate the results.

Step 1: Centrifugation of CPT Tubes. When ready to begin cell separation, the CPT tubes should be centrifuged at 30,000 G-min at room temperature. (2,000 G x 15

minutes; Do NOT exceed 2000 G with these tubes). This requires a temperature controlled centrifuge set at room temperature, approximately 20°C. The centrifuge must NOT be allowed to overheat. Note that these tubes are taller (13cm) than other sample collection tubes, and a different rotor may be necessary. The rotor must be a swinging bucket rotor that has sufficient room for these taller tubes to still swing freely. When centrifugation is complete, several layers will be evident. The top layer is citrated (or heparin) plasma Under this is a whitish cell layer (buffy coat) used for cell collection. Then there is the gradient gel layer that acts as a barrier to prevent contamination by the red cells, which are at the very bottom of the tube.



Step 2: Removing Plasma. Using a transfer pipet, transfer up to 80% of the plasma layer from each tube and either a) discard in biohazard waste or b) save for stored plasma. Be very careful not to disturb the cell layer; this takes some practice. It is okay to leave some extra plasma in the tubes and leaving plasma is preferable to disturbing the cell layer. Any extra plasma left in the cell layer will be separated from the cell layer during the next centrifuge step.

Step 3: Pelleting the Buffy Coat. Next completely remove the cell layer from each CPT tube using a transfer pipet, and transfer it to a separate 15-mL conical centrifuge tube labeled with participant ID#. Fill the centrifuge tube up to the 14 mL mark with PBS. Mix gently and centrifuge at 4500 G-min at room temperature.

Step 4: Resuspension of Buffy Coat Pellets. After centrifugation is complete, a small pellet should be evident in the bottom of the tube. Carefully remove the PBS solution by

pipette, and a) discard in biohazard waste or b) if saving platelets, place in a 15 ml conical and process as below. Do not disturb the pellet. Leave a little PBS in the tube if necessary. Add 1.0 mL of buffy coat Freezing Media (Millipore cat#s-002-10F) (or use 90% Fetal Bovine Serum/10% DMSO) to each tube containing the buffy coat pellet. Resuspend the pellet by gently flushing the pipette tip repeatedly. Be careful to not cause excessive foaming. This may take a little patience and practice. Do not use the same pipette tip for mixing on different participants. Place 0.5ml into each of 2 properly labeled 0.5 ml cryovials and cap. Place the vials in a room temperature Mr. Frosty (model# 5100-0001) for slow freezing of the cells (see below).

11.9.5.3. Freezing Steps for the Cryo-Preserved PBMCs:

The correct method for using this container requires “Mr. Frosty” to be be filled to the line with 100% isopropyl alcohol at room temperature when the cryovials containing cells are put into it. After samples are added to the rack, the entire container is placed in a –70^{o} C or colder freezer for a minimum of 4 hours or preferably overnight. Once the container goes into the freezer, additional cell cryovials should not be added. When “Mr. Frosty” is removed from the freezer the next morning, remove the now frozen cell cryovials and place in their appropriate freezer box at –70^{0}C for short-term storage or -145C for long-term storage. Allow “Mr. Frosty” to warm to room temperature before reusing. Important note: be sure to always use 100% isopropyl alcohol in the “Mr. Frosty”. Less than 100% isopropyl alcohol will result in the cells freezing too quickly. The alcohol in Mr. Frosty should be changed after each fifth freeze. Since it may not always be possible to have all the day’s cell samples ready to freeze at the same time, the centers should have at least three “Mr. Frosty” containers. Use individual “Mr. Frosty” containers per participant if the samples are processed at different times during the day.

11.9.6. Blood Processing Overview

1) Complete phlebotomy log sheet.

2) Place specimen labels on tubes so that the barcode runs vertically (see label orientation diagram in Appendix B)

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 Lab Collection and the Lab Processing – IL-6 & Repository 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 tubes into an ice bucket, leaving them no longer than 30 minutes prior to centrifugation.

9) Leave the SST tubes at room temperature for at least 30 minutes prior to centrifugation.

10) Follow instructions provided by diagnostic testing lab concerning the 8.5 mL, SST and 4mL EDTA tubes.

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

12) Do not use a fixed-angle centrifuge.

13) 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 Lab Processing – IL-6 & Repository form comment section that samples were spun in a non-refrigerated centrifuge.

14) Place the tubes in the specimen tube Biomerga Cool Cube benchtop cooler after removing them from the centrifuge.

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

16) Submit lavender top tube and SST to diagnostic testing lab per their instructions.

17) Input data from both the Lab Collection – IL-6 & Repository form the Lab Processing – IL-6 & Repository form into the ENRGISE web based data entry system.

11.10. 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 ACC will monitor the quality of the sample collection and processing at each Field Center via several methods:

1) centralized training and certification of site coordinators from each Field Center,

2) oversight of equipment check logs at each Field Center,

3) review of collection/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 ENRGISE Manual of Operations. Certification in ENRGISE sample processing is required before working with actual study participants' samples.

Initial certification involves:

1) reading the ENRGISE Manual of Procedures,

2) review of training presentation slides via webinar

3) observation by certified personnel of complete phlebotomy and/or processing procedures on at least one volunteer.

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.

11.11. Shipping of Plasma/Serum Samples

These instructions pertain to EDTA plasma, Serum and Packed cells frozen blood samples shipments to the UVM Biomarker Lab. 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.

Please ship samples weekly on Monday or Tuesday via Fed Ex Priority overnight.

1) Line shipping container with absorbent material (i.e. lab mat, or paper toweling)

2) Place approximately ~5 to 10 lbs of dry ice on the bottom of the shipping container.

3) Place another layer of absorbent material (i.e. lab mat) on top of the dry ice – so it will be between the dry ice and the freezer boxes.

4) Collect the freezer boxes containing samples to be shipped, and check the sample ID numbers against the Shipping Form for that shipment. See Appendix for box diagrams on freezer box organization.

5) Wrap absorbent material around the box and secure with a rubber band around the box.

6) Place each freezer box in a ziplock plastic bag and seal tightly.

7) Place ziplocked freezer boxes in the shipping container. Note: the ziplock bags should NOT be in direct contact with the dry ice.

8) Add another layer of absorbent material on top of the freezer boxes in the shipping container.

9) Add remaining dry ice to the shipping container. Close and tape the Styrofoam lid.

10) Seal Lab Collection and Lab Processing – IL-6 & Repository form in a ziplock bag and place on top of the Styrofoam lid. Include a cover sheet with recipient address and contact information.

11) Close the top of the outer cardboard sleeve of the shipping container with packing tape.

12) Affix shipping labels (Fed Ex label, Biological Specimen Category B UN3373 label and Dry Ice Class 9 UN1845 label) to outside of shipping container.

13) Add extra shipping tape over the labels to ensure they will not fall off in transit.

Fill out the Shipping Log (Appendix D) including the Fedex airbill #s and fax to the University of Vermont at (802) 656-8965 on the day samples are shipped.

NOTE: This shipping protocol follows the procedures mandated by the International Air Transport Association's Dangerous Goods Regulations-Packaging Instructions 650 and 954. Copies of these regulations are included with this MOP (Appendix E).

Shipping Address:

Rebekah Boyle (802) 656-8938

University of Vermont – Pathology

360 South Park Drive, Rm 154A

Colchester, VT 05446

Rebekah.Boyle@uvm.edu

The frozen biomarker samples will be cataloged and stored at -80^{o}C at the UVM Biomarker Lab either until they are analyzed or until the end of the study.

11.12. Appendices

A. Sample Destruction Request Form

B. Label Orientation Diagram

C. Sample Sheet of Specimen ID Barcoded Labels

D. Shipping Log

E. IATA 650 and 954 Regulations

F. Box Maps

G. Quest Safety Laboratory Manual

H. Phlebotomy Log Sheet

I. Dried Blood Spot Pilot Study Collection and Processing Procedure

Appendix A – ENRGISE 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 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.

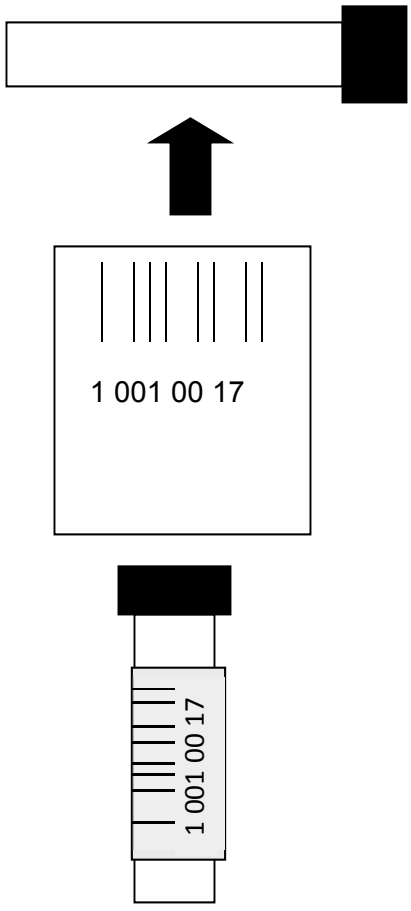
Field Center: _____ Participant ID: _____

I affirm that the vials of white and red cells 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

Appendix B – Label Orientation on Cryovial



Appendix C – Example Set of Barcoded Specimen ID Labels


ENRGISE 6 Mo cryovial #6 serum 3001 06 06
ENRGISE 6 Mo cryovial #7 EDTA 3001 06 07
ENRGISE 6 Mo cryovial #8 EDTA 3001 06 08
ENRGISE 6 Mo cryovial #9 EDTA 3001 06 09
ENRGISE 6 Mo cryovial #10 EDTA 3001 06 10
ENRGISE 6 Mo cryovial #11 EDTA 3001 06 11
ENRGISE 6 Mo cryovial #12 EDTA 3001 06 12
ENRGISE 6 Mo cryovial #13 EDTA 3001 06 13
ENRGISE 6 Mo cryovial #14 EDTA 3001 06 14
ENRGISE 6 Mo Tube #15 Packed Cells 3001 06 15
ENRGISE 6 Mo cryovial #16 PBMCs 3001 06 16
ENRGISE 6 Mo cryovial #17 PBMCs 3001 06 17
ENRGISE 6 Mo cryovial #18 PBMCs 3001 06 18
ENRGISE 6 Mo cryovial #19 PBMCs 3001 06 19
ENRGISE 6 Mo Box 3001 06
ENRGISE 6 Mo Box 3001 06
ENRGISE 6 Mo Box 3001 06
ENRGISE 6 Mo EXTRA 3001 06
ENRGISE 6 Mo EXTRA 3001 06
ENRGISE 9 Mo FORM 3001 09
ENRGISE 9 Mo SST draw tube 3001 09
ENRGISE 9 Mo cryovial serum 3001 09
ENRGISE 9 Mo Box 3001 09
ENRGISE 9 Mo EXTRA 3001 09
ENRGISE 12 Mo FORM 3001 12
ENRGISE 12 Mo FORM 3001 12
ENRGISE 12 Mo SST 1 draw tube 3001 12
ENRGISE 12 Mo SST 2 draw tube 3001 12
ENRGISE 12 Mo EDTA 1 draw tube 3001 12
ENRGISE 12 Mo EDTA 2 draw tube 3001 12
ENRGISE 12 Mo plasma pooling tube 3001 12
ENRGISE 12 Mo serum pooling tube 3001 12
ENRGISE 12 Mo cryovial #1 serum 3001 12 01
ENRGISE 12 Mo cryovial #2 serum 3001 12 02
ENRGISE 12 Mo cryovial #3 serum 3001 12 03
ENRGISE 12 Mo cryovial #4 serum 3001 12 04
ENRGISE 12 Mo cryovial #5 serum 3001 12 05
ENRGISE 12 Mo cryovial #6 serum 3001 12 06
ENRGISE 12 Mo cryovial #7 EDTA 3001 12 07
ENRGISE 12 Mo cryovial #8 EDTA 3001 12 08
ENRGISE 12 Mo cryovial #9 EDTA 3001 12 09
ENRGISE 12 Mo cryovial #10 EDTA 3001 12 10
ENRGISE 12 Mo cryovial #11 EDTA 3001 12 11
ENRGISE 12 Mo cryovial #12 EDTA 3001 12 12
ENRGISE 12 Mo cryovial #13 EDTA 3001 12 13
ENRGISE 12 Mo cryovial #14 EDTA 3001 12 14
ENRGISE 12 Mo Tube #15 Packed Cells 3001 12 15
ENRGISE 12 Mo Box 3001 12 16
ENRGISE 12 Mo Box 3001 12 17
ENRGISE 12 Mo EXTRA 3001 12
ENRGISE 12 Mo EXTRA 3001 12

Appendix D – ENRGISE SHIPPING LOG

Date of shipment: _____

Field Center: _____ Prepared by: _____

FedEx Air Bill#: _____

Specimen ID#	Samples	VISIT	BOX #
Ex. 001, 002, 006	*Packed Cells*	*Baseline*	*1*
Ex. 001, 002, 006	*Plasma & Serum*	*3 mo*	*2*

Appendix E – IATA 650 and 954 Regulations

PACKING INSTRUCTION 650

STATE VARIATIONS: BHG-02, CAG-05, DQG-03, FRG-05, GBG-05, VCG-04

OPERATOR VARIATIONS: AF-02, AM-06/10, AR-02, AS-08, BR-14, BZ-07, CI-01, CO-07, CS-07, FX-09, IJ-06/10, JJ-06, JK-03, KC-08, KE-06, LA-07, LH-05, MN-03, MS-06, MX-06/11, OO-01, OU-12/16, PX-08, SQ-10, SV-12, TN-05, TY-03, UA-14, UU-05

This instruction applies to UN 3373 on passenger and cargo aircraft and Cargo Aircraft Only.

General Requirements

The packagings must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including trans-shipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

The packaging must consist of three components:

(a) a primary receptacle(s);

(b) a secondary packaging; and

(c) a rigid outer packaging.

Primary receptacles must be packed in secondary packagings in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packagings with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

Packages must be prepared as follows:

(a) For liquid substances:

- The primary receptacle(s) must be leakproof and must not contain more than 1 L;
- The secondary packaging must be leakproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F).

Note:
The capability of a packaging to withstand an internal pressure without leakage that produces the specified pressure differential should be determined by testing samples of primary receptacles or secondary packagings. Pressure differential is the difference between the pressure exerted on the inside of the receptacle or packaging and the pressure on the outside. The appropriate test method should be selected based on receptacle or packaging type. Acceptable test methods include any method that produces the required pressure differential between the inside and outside of a primary receptacle or a secondary packaging. The test may be conducted using internal hydraulic or pneumatic pressure (gauge) or external vacuum test methods. Internal hydraulic or pneumatic pressure can be applied in most cases as the required pressure differential can be achieved under most circumstances. An external vacuum test is not acceptable if the specified pressure differential is not achieved and maintained. The external vacuum test is a generally acceptable method for rigid receptacles and packagings but is not normally acceptable for:

- flexible receptacles and flexible packagings;
- receptacles and packagings filled and closed under a absolute atmospheric pressure lower than 95 kPa.
- The outer packaging must not contain more than 4 L. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.

(b) For solid substances:

- The primary receptacle(s) must be siftproof and must not exceed the outer packaging weight limit;
- The secondary packaging must be siftproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;
- If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a packaging suitable for liquids, including absorbent materials, must be used.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm (4 in × 4 in).

The completed package must be capable of successfully passing the drop test described in 6.5.1.1 except that the height of the drop must not be less than 1.2 m. Following the appropriate drop sequence, there must be no leakage from the primary receptacle(s) which must remain protected by absorbent material, when required, in the secondary packaging.

For transport, the mark illustrated below must be displayed on the external surface of the outer packaging on a background of a contrasting colour and must be clearly visible and legible. The mark must be in the form of a square set at an angle of 45° (diamond-shaped) with each side having a length of at least 50 mm (2 in), the width of the line must be at least 2 mm and the letters and numbers must be at least 6 mm high. The proper shipping name "Biological Substance, Category B" in letters at least 6 mm high must be marked on the outer packaging adjacent to the diamond-shaped mark.

Unless all package markings are clearly visible, the following conditions apply when packages are placed in an overpack:

- the overpack must be marked with the word "Overpack"; and
- the package markings must be reproduced on the outside of the overpack.

A Shipper's Declaration for Dangerous Goods is not required.

Alternative packagings for the transport of animal material may be authorized by the competent authority in accordance with the provisions in 5.0.6.7.

Specific Requirements

Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen:

- When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements of these Regulations must be met. When used, ice or dry ice must be placed outside the secondary packagings or in the outer packaging or an overpack. Interior supports must be provided to secure the secondary packagings in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or overpack must be leakproof. If dry ice is used, the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packagings.
- The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures, which could result if refrigeration were to be lost.

Infectious substances assigned to UN 3373 which are packed and marked in accordance with this packing instruction are not subject to any other requirement of these Regulations except for the following:

(a) the name and address of the shipper and of the consignee must be provided on each package;

(b) the name and telephone number of a person responsible must be provided on the air waybill or on the package;

(c) the classification must be in accordance to 3.6.2;

(d) the incident reporting requirements in 9.6.1 must be met; and

(e) the inspection for damage or leakage requirements in 9.4.1 and 9.4.2.

Note:
When the shipper or consignee is also the 'person responsible' as referred to in b) above, the name and address need be marked only once in order to satisfy the name and address marking provisions in both a) and b), above.

Passengers and crew members are prohibited from transporting infectious substances as or in carry-on baggage, checked baggage or on their person.

If an Air Waybill is used, the "Nature and Quantity of Goods" box must show "UN 3373", the text "BIOLOGICAL SUBSTANCE, CATEGORY B" and the number of packages.

Clear instructions on filling and closing such packages must be provided by packaging manufacturers and subsequent distributors to the shipper or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for transport.

Other dangerous goods must not be packed in the same packaging as Division 6.2 Infectious Substances unless they are necessary for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 mL or less of dangerous goods included in Classes 3, 8 or 9 may be packed in each primary receptacle containing infectious substances provided these substances meet the requirements of 2.6. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction, no other requirements in these Regulations need be met.

PACKING INSTRUCTION 954 – for UN1845 (Dry Ice)

This instruction applies to UN 1845, **Carbon dioxide, solid (dry ice)** on passenger aircraft and Cargo Aircraft Only.
The General Packing Requirements of Subsection 5.0.2 must be met.

Additional Packing Requirements

In packages:

(a) must be in packaging designed and constructed to permit the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging;
(b) the shipper must make arrangements with the operator(s) for each shipment, to ensure ventilation safety procedures are followed;
(c) the Shipper's Declaration requirements of Subsections 8.1 and 10.8.1 are only applicable when the Carbon dioxide, solid (dry ice) is used as a refrigerant for dangerous goods that require a Shipper's Declaration;
(d) when a Shipper's Declaration is not required, the following information, as required by 8.2.3 for the Carbon dioxide, solid (dry ice), must be contained in the "Nature and Quantity of Goods" box on the air waybill and should be shown in the following order:
    1) UN 1845;
    2) proper shipping name (Carbon dioxide, solid or Dry ice);
    3) 9 (the word "Class" may be included prior to the number "9");
    4) the number of packages; and
    5) the net weight of dry ice in each package.
(e) the net weight of the Carbon dioxide, solid (dry ice) must be marked on the outside of the package.

Dry ice used as a refrigerant for other than dangerous goods:

(a) may be shipped in a unit load device or other type of pallet prepared by a single shipper provided that the shipper has made prior arrangements with the operator;
(b) the unit load device, or other type of pallet must allow the venting of the carbon dioxide gas to prevent a dangerous build-up of pressure;
(c) the shipper must provide the operator with written documentation stating the total weight of the dry ice contained in the unit load device or other type of pallet.

Notes:

1. *Refer to the relevant airline's loading procedures for Carbon dioxide, solid (dry ice) limitations.*
2. *For Air Waybill requirements see 8.2.3. For loading instructions see 9.3.12.*
3. *For cooling purposes, an overpack may contain Carbon dioxide, solid (dry ice), provided that the overpack meets the requirements of Packing Instruction 904.*

UN Number	Net Quantity per package
UN 1845 **Carbon dioxide, solid (Dry Ice)**	200 kg

Source: IATA Dangerous Goods (HAZMAT) Reformatted Packing Instructions
http://www.iata.org/whatwedo/cargo/dangerous_goods/Pages/icao-packing.aspx 11/10

Appendix F – ENRGISE Box Maps

All samples shipped FROZEN via Priority Overnight on dry ice WEEKLY to UVM
Monday or Tuesday

ENRGISE PILOT STUDY PBMCs SAMPLE BOX MAP

Freezer Box design for FROZEN

Cryo-Preserved PBMCs Cryovials 16-19

Sample sets from participants BL and/or 6 Mo visits included in same box

Set 1 Cryo 16	Set 3 Cryo 16	Set 5 Cryo 16	Set 7 Cryo 16	Set 9 Cryo 16	Set 11 Cryo 16	Set 13 Cryo 16	Set 15 Cryo 16	Set 17 Cryo 16
Set 1 Cryo 17	Set 3 Cryo 17	Set 5 Cryo 17	Set 7 Cryo 17	Set 9 Cryo 17	Set 11 Cryo 17	Set 13 Cryo 17	Set 15 Cryo 17	Set 17 Cryo 17
Set 1 Cryo 18	Set 3 Cryo 18	Set 5 Cryo 18	Set 7 Cryo 18	Set 9 Cryo 18	Set 11 Cryo 18	Set 13 Cryo 18	Set 15 Cryo 18	Set 17 Cryo 18
Set 1 Cryo 19	Set 3 Cryo 19	Set 5 Cryo 19	Set 7 Cryo 19	Set 9 Cryo 19	Set 11 Cryo 19	Set 13 Cryo 19	Set 15 Cryo 19	Set 17 Cryo 19
Set 2 Cryo 16	Set 4 Cryo 16	Set 6 Cryo 16	Set 8 Cryo 16	Set 10 Cryo 16	Set 12 Cryo 16	Set 14 Cryo 16	Set 16 Cryo 16	Set 18 Cryo 16
Set 2 Cryo 17	Set 4 Cryo 17	Set 6 Cryo 17	Set 8 Cryo 17	Set 10 Cryo 17	Set 12 Cryo 17	Set 14 Cryo 17	Set 16 Cryo 17	Set 18 Cryo 17
Set 2 Cryo 18	Set 4 Cryo 18	Set 6 Cryo 18	Set 8 Cryo 18	Set 10 Cryo 18	Set 12 Cryo 18	Set 14 Cryo 18	Set 16 Cryo 18	Set 18 Cryo 18
Set 2 Cryo 19	Set 4 Cryo 19	Set 6 Cryo 19	Set 8 Cryo 19	Set 10 Cryo 19	Set 12 Cryo 19	Set 14 Cryo 19	Set 16 Cryo 19	Set 18 Cryo 19
X	X	X	X	X	X	X	X	X

ENRGISE PILOT STUDY SERUM and PLASMA BLOOD

SAMPLE BOX MAP

Freezer Box design for FROZEN Shipment of EDTA Plasma & Serum

Cryovials 01-14

Sample sets from participants BL, 6Mo, and/or 12Mo visits included in same box

Set 1 Cryo 01	Cryo 10	Cryo 05	Cryo 14	Cryo 09	Cryo 04	Cryo 13	Cryo 08	X
Cryo 02	Cryo 11	Cryo 06	Set 3 Cryo 01	Cryo 10	Cryo 05	Cryo 14	Cryo 09	X
Cryo 03	Cryo 12	Cryo 07	Cryo 02	Cryo 11	Cryo 06	Set 5 Cryo 01	Cryo 10	X
Cryo 04	Cryo 13	Cryo 08	Cryo 03	Cryo 12	Cryo 07	Cryo 02	Cryo 11	X
Cryo 05	Cryo 14	Cryo 09	Cryo 04	Cryo 13	Cryo 08	Cryo 03	Cryo 12	X
Cryo 06	Set 2 Cryo 01	Cryo 10	Cryo 05	Cryo 14	Cryo 09	Cryo 04	Cryo 13	X
Cryo 07	Cryo 02	Cryo 11	Cryo 06	Set 4 Cryo 01	Cryo 10	Cryo 05	Cryo 14	X
Cryo 08	Cryo 03	Cryo 12	Cryo 07	Cryo 02	Cryo 11	Cryo 06	X	X
Cryo 09	Cryo 04	Cryo 13	Cryo 08	Cryo 03	Cryo 12	Cryo 07	X	X

ENRGISE PILOT STUDY Serum for IL-6 SAMPLE BOX MAP

Freezer box design for FROZEN 2.0mL Serum Cryovials for IL-6

2.0mL Serum Samples from up to 80 participants screening visit 1, screening visit 2, 3 Mo and/or 9M included in same box

IL-6 Cryo 1	IL-6 Cryo 10	IL-6 Cryo 19	IL-6 Cryo 28	IL-6 Cryo 37	IL-6 Cryo 46	IL-6 Cryo 55	IL-6 Cryo 64	IL-6 Cryo 73
IL-6 Cryo 2	IL-6 Cryo 11	IL-6 Cryo 20	IL-6 Cryo 29	IL-6 Cryo 38	IL-6 Cryo 47	IL-6 Cryo 56	IL-6 Cryo 65	IL-6 Cryo 74
IL-6 Cryo 3	IL-6 Cryo 12	IL-6 Cryo 21	IL-6 Cryo 30	IL-6 Cryo 39	IL-6 Cryo 48	IL-6 Cryo 57	IL-6 Cryo 66	IL-6 Cryo 75
IL-6 Cryo 4	IL-6 Cryo 13	IL-6 Cryo 22	IL-6 Cryo 31	IL-6 Cryo 40	IL-6 Cryo 49	IL-6 Cryo 58	IL-6 Cryo 67	IL-6 Cryo 76
IL-6 Cryo 5	IL-6 Cryo 14	IL-6 Cryo 23	IL-6 Cryo 32	IL-6 Cryo 41	IL-6 Cryo 50	IL-6 Cryo 59	IL-6 Cryo 68	IL-6 Cryo 77
IL-6 Cryo 6	IL-6 Cryo 15	IL-6 Cryo 24	IL-6 Cryo 33	IL-6 Cryo 42	IL-6 Cryo 51	IL-6 Cryo 60	IL-6 Cryo 69	IL-6 Cryo 78
IL-6 Cryo 7	IL-6 Cryo 16	IL-6 Cryo 25	IL-6 Cryo 34	IL-6 Cryo 43	IL-6 Cryo 52	IL-6 Cryo 61	IL-6 Cryo 70	IL-6 Cryo 79
IL-6 Cryo 8	IL-6 Cryo 17	IL-6 Cryo 26	IL-6 Cryo 35	IL-6 Cryo 44	IL-6 Cryo 53	IL-6 Cryo 62	IL-6 Cryo 71	IL-6 Cryo 80
IL-6 Cryo 9	IL-6 Cryo 18	IL-6 Cryo 27	IL-6 Cryo 36	IL-6 Cryo 45	IL-6 Cryo 54	IL-6 Cryo 63	IL-6 Cryo 72	X

ENRGISE STUDY Packed Cells SAMPLE BOX MAP

Freezer box design for FROZEN Packed Cells **Tube #15**

~2.5 mL Packed Cells in 10mL Tubes from up to 42 participants BL, 6 M, and/or 12M visits included in same box

Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17	Tube #17
X	X	X	X	X	X	X

ENRGISE Study

QUEST DIAGNOSTICS LABORATORY COLLECTION

ENRGISE Study

Completing the Requisition form:

Required HIPAA information that needs to be on the requisition:

- Participant Acrostic will replace the first and last name of the patient. Please place the acrostic in the ID field on research requisition form
- Enter DOB
- Participant ID (PID) should be entered as the Patient ID Number field
- Visit type (SV2, BLR, F03, F06, F09, SAF) can be added in the "Lab Reference" field
- Enter gender
- Date of collection and time
- Enter fasting status
- Check either "order all tests below" or individual tests with the printed menu.
- Handwrite on label DOB and de-identified information replacing patients first and last name. Place the label with two forms of ID on corresponding specimen vial. Participant acrostic and DOB is acceptable.

Requisition forms can also be created online using the Care 360 system.

Sample Collection and Processing:

- The following samples will be collected for the ENRGISE Study:

Test	SV1	SV2	BLR	F03	F06	F09	F12	SAF*	Unscheduled
Comprehensive metabolic panel		X							
CBC		X		X	X	X			
Vitamin D		X							
Lipid panel			X	X**	X**	X**			
Basic metabolic panel				X	X	X		X	

  - *NOTE #1: Safety visits are only required for participants taking part in the Losartan arm of the study.
  - **NOTE #2: Lipid panel test at Months 3, 6, and 9 are only required for participants taking part in the Fish Oil arm of the study.

- All samples will be collected using standard phlebotomy techniques with collection tubes provided by Quest Diagnostics.
- For visits requiring two serum samples (i.e. At SV2 where a comprehensive metabolic panel and vitamin D levels are both performed or F03/F06/F09 where a basic metabolic panel and lipid panel are both performed) only one SST tube needs to be collected. The SST tube will be processed and the serum aliquoted into two separate plastic vials.
- Refer to the following pages of this manual for specific processing instructions for each type of sample collected.
- Be sure that all tubes and/or vials sent to Quest are clearly labeled. Either the roll of labels provided by Quest can be used or sites can create these labels on their own. Include on the label:
    - o Participant acrostic or ID number
    - o Date of birth
    - o Type of sample (i.e. serum)

Shipping (all sites with the exception of Northwestern):

1. Pack samples in a separate package from any other routine samples
2. Attach a flyer on bright-colored paper to the outside of the shipping container: "**PROJECT SAMPLES - ATTN: PROJECT PROCESSOR**". Using a brightly colored label is also acceptable.
3. Place a copy of the same flyer inside the bag with the samples.
4. Include requisition form and flyer with 1) client number, 2) names/IDs to be used for entry of patient name matching samples, 3) test code(s)
5. Mark the paperwork: "**PROJECT SAMPLES - ATTN: PROJECT PROCESSOR**."
6. Send an e-mail to "**sean.w.hansel@questdiagnostics.com**" when the samples are sent, providing any shipping information you have (temp sent, air bill #, expected date/time of receipt in WDL).
7. For questions about lab results please call **1-866-MY-QUEST (1-866-697-8378)** and provide your account.
8. For questions about lab collection or requests for additional supplies, please email ENRGISEACC@aging.ufl.edu.
9. FedEx Airbills are provided by Quest. Ship samples to the following address and attention:

    Quest Diagnostics, LLC.

    1355 Mittel Blvd

    Wood Dale, IL 60191

    Attention: PROJECT SAMPLES - ATTN: PROJECT PROCESSOR

Shipment to the following address is also acceptable: 200 Lewis Drive, Wood Dale, IL 60191.

Shipping (Northwestern University only):

1. Samples will be packed in a biohazard bag.
2. Insert a copy of the requisition form in the bag.
3. Place samples in Quest pickup box and call 1-866-MY-QUEST to schedule a local pickup.
4. For questions about lab results please call **1-866-MY-QUEST (1-866-697-8378)** and provide your account.

5. For questions about lab collection or requests for additional supplies, please email ENRGISEACC@aging.ufl.edu.

CBC (includes Differential and Platelets)

Test Code

6399

Includes

WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count, MPV and Differential (Absolute and Percent - Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils)
If abnormal cells are noted on a manual review of the peripheral blood smear or if the automated differential information meets specific criteria, a full manual differential will be performed.

Preferred Specimen(s)

Whole blood from a full EDTA (lavender-top) tube

Minimum Volume

EDTA (lavender-top) tube must be half full

Collection Instructions

Maintain specimen at room temperature. **Do not refrigerate.** If multiple draw, collect EDTA (lavender-top) tube last. Traumatic draw can introduce thromboplastin and trap WBC and platelets. Refrigeration can precipitate fibrin and trap WBC and platelets.

Transport Container

EDTA (lavender-top) tube

Transport Temperature

Room temperature

Specimen Stability

Room temperature: 48 hours
Refrigerated: 48 hours (may cause platelet clumping)
Frozen: Unstable

Rejected Criteria

Hemolysis, clotted, received frozen

Performing Laboratory

Quest Diagnostics - Great Lakes Region

Comprehensive Metabolic Panel

Test Code

10231

Includes

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

Patient Preparation

Fasting specimen is preferred

Preferred Specimen(s)

2 mL serum

Minimum Volume

1 mL

Collection Instructions

Centrifuge at 3000 RPM for 15-20 minutes within 30-60 minutes following collections and pour off into clear cap tube

Transport Container
Plastic screw-cap vial

Transport Temperature

Room temperature

Specimen Stability

Room temperature: 48 hours
Refrigerated: 72 hours
Frozen: Unacceptable

Reject Criteria

Moderate to gross hemolysis

Performing Laboratory
Quest Diagnostics - Great Lakes Region

Basic Metabolic Panel

Test Code

10165

Includes

BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine with GFR Estimated, Glucose, Potassium, Sodium, Urea Nitrogen (BUN)

Patient Preparation

Fasting specimen is preferred

Preferred Specimen(s)

2 mL serum

Minimum Volume

1 mL

Collection Instructions

Centrifuge at 3000 RPM for 15-20 minutes within 30-60 minutes following collections and pour off into clear cap tube

Transport Container

Plastic screw-cap vial

Transport Temperature

Room temperature

Specimen Stability

Room temperature: 48 hours
Refrigerated: 72 hours
Frozen: 28 days

Reject Criteria

Moderate to gross hemolysis

Performing Laboratory

Quest Diagnostics - Great Lakes Region

Lipid Panel

Test Code

7600

Includes

Total Cholesterol, HDL Cholesterol, Triglycerides, LDL-Cholesterol (calculated), Cholesterol/HDL Ratio (calculated), Non-HDL Cholesterol (calculated)

Patient Preparation

If a cholesterol measurement is to be performed along with triglycerides, the patient should be fasting 9-12 hours prior to collection. The assay manufacturer Beckman Coulter advises: "N-Acetyl Cysteine (NAC), when administered in therapeutic concentrations (for the treatment of acetaminophen overdose), has been. . . determined to interfere with assays for. . . cholesterol, uric acid" where "NAC interference may lead to falsely low results." According to Beckman Coulter, the NAC interference should be insignificant by 12 hours after completion of the initial loading dose of an IV infusion treatment regimen consisting of an initial loading dose of 150 mg/kg administered over 1 hour, a second dose of 50 mg/kg administered over 4 hrs and a third dose of 100 mg/kg administered over 16 hrs.

Preferred Specimen(s)

1 mL serum

Minimum Volume

0.5 mL

Collection Instructions

Centrifuge at 3000 RPM for 15-20 minutes within 30-60 minutes following collections

Transport Container	***Transport Temperature***
Plastic screw-cap vial	Room temperature

Specimen Stability

Serum	***Plasma***
Room temperature: 48 hours	*Room temperature: 48 hours*
Refrigerated: 7 days	*Refrigerated: 48 hours*
Frozen: 15 days	*Frozen: 15 days*

Reject Criteria

Gross hemolysis • Moderate to gross icterus • Anticoagulants other than heparian

Vitamin D, 25-Hydroxy, Total, Immunoassay

Test Code

17306

Patient Preparation

Fasting preferred, but not required

Collection Container

Serum Separator Tube (SST)

Preferred Specimen(s)

0.8 mL serum

Minimum Volume

0.5 mL

Collection Instructions

Centrifuge at 3000 RPM for 15-20 minutes within 30-60 minutes following collections

Transport Container	***Transport Temperature***
Plastic screw-cap vial	Room temperature

Specimen Stability

Room temperature: 14 days
Refrigerated: 14 days
Frozen: 60 days

Reject Criteria

Gross hemolysis • Gross lipemia • Plasma

Performing Laboratory
Quest Diagnostics - Great Lakes Region

Appendix H – Phlebotomy Log Sheet

ENRGISE STUDY – Phlebotomy Log

Date of Visit (MM/DD/YYYY)	Visit Type (BLR, F03, F06, F09, F12, Safety)	ENRGISE Study Participant ID Label	Biological Specimen ID#	Label check

Appendix I – Dried Blood Spot Pilot Study Collection & Preparation Procedure

Appendix I.1 – Required Laboratory Supplies

General lab equipment and supplies

- Class II biological safety cabinet
- Solution of 0.5% sodium hypochlorite in spray bottle (a 1:10 dilution of household bleach)
- Gloves (latex or nitrile) and laboratory coat (cloth or disposable) dedicated for use in each area
- Biohazard waste disposal bin

DBS preparation and storage(provided by UVM)

- Sterile, disposable, Unistick 3 Single-Use Fingerstick Lancet (1.8mm)
- 903 “Protein Saver” filter paper cards* (Whatman)
- Desiccant sachets (1g)
- Humidity indicator (may be combined with desiccant)
- Foil-barrier Resealable Bags
- Zip-locked gas-impermeable plastic bags
- Assorted sterile racks

Appendix I.2 – DBS Collection and Preparation

Dried Blood Spot Collections will be performed on of the first ten participants at each site at their Baseline and 6 Month visits.

Ideal Blood Spot Collection procedure is demonstrated in the following video:
http://www.jove.com/video/50973/dried-blood-spot-collection-health-biomarkers-to-maximize

Preparation of DBS from a finger or heel stick

1. With gloves on, label filter paper with appropriate identification information (patient's identification and collection date). Use only **Whatman 903** protein saver cards.

2. Handle the filter paper by the edges in order to avoid cross-contamination. Do not touch the areas that will be used to collect the specimens.

3. Clean the skin area for puncture with antiseptics. Individually packaged 70% isopropyl alcohol wipes may be used to clean the puncture site. Povidone-iodine swabs may also be used.

4. The puncture must be performed with sufficient force and penetration to sustain a flow of at least several drops of blood. Use a sterile, disposable single-use lancet to puncture the skin to the side of the fingertip. The lancing procedure should yield at least 200 µl whole blood.

5. With the finger extended, allow a large, hanging, drop of free flowing blood to accumulate at the puncture site. To collect the drop, touch the filter paper to the edge of the drop, allowing the blood to be drawn into the card by capillary action. Avoid allowing the finger to touch the card.

6. Then, allow another large drop to form at the puncture site and collect this drop in the next circle. Do not layer successive drops of blood on top of each other.

7. Continue to collect drops in the same manner filling all of the circles completely in the card or until the wound ceases to bleed. If the wound stops flowing before sufficient blood has been collected, the wound may be massaged very gently to encourage blood droplets. Do not squeeze the wound to obtain more blood. If the specimen collection is incomplete and no more blood is being produced from the initial puncture wound, this procedure may be repeated on the adjacent finger.

8. It is important that an adequate sample is collected. To do this you must saturate each circle with blood. For each patient, at least four completely saturated circles must be collected (although five circles are preferable).

9. Supply the participant with a plaster/Band-Aid to cover the puncture site, as required

Appendix I.3 – Drying of Prepared Dried Blood Spot Cards:

- The time needed to dry a DBS will differ according to ambient temperature and humidity conditions.

- Generally, it is recommended to dry all specimens for at least four hours (though preferably overnight) in a suspended horizontal position (on drying rack, if available), or laid flat on a clean paper towel in a biohazard safety cabinet.

- Do not use an external heat source to dry DBS. When dry, the spots will appear a uniform dark brown. The appearance should be similar to that of a dried bloodstain and no areas of red coloration should be seen. (See **Figure 1**.)

Figure 1. Dried DBS on a 903 filter paper card

Appendix I.4 – DBS Packaging:

Ensure that the DBS are completely dry before packing by providing adequate drying time.

1. Filter paper cards should be individually packaged in a single gas-impermeable, sealable plastic bag containing a desiccant packs to remove residual moisture.

2. Ensure that the sample identification and study name are clearly written on both the DBS card and also on the plastic bag.

3. Use of desiccant packs is recommended as free desiccant material should not come into direct contact with the DBS.

4. Place humidity indicator card into the bag in a manner such that the humidity indicator can be read without opening the bag.

5. Gently apply pressure to the partially sealed bag to expel the air before sealing it completely.

6. Place 5-10 of the above small bags into a large plastic bag that also contains a printed manifest with specimen information.

Appendix I.4.1 – Important Considerations for Packaging DBS:

1. In the presence of moisture, the nucleic acids in DBS are extremely sensitive to degradation. This means it is essential to ensure specimens are properly stored in the presence of desiccant packs. Humidity indicator cards and desiccant packs have a color indicator which changes from blue to pink as humidity increases. All desiccant sachets should be immediately replaced if the presence of moisture is indicated.

2. When changing humidity indicator cards and desiccant sachets in specimens that have been stored at 4 degrees or in the freezer, it is important to pre-equilibrate the bag containing the DBS to room temperature. Remember: Opening DBS packs immediately upon transfer from low temperature storage will result in condensation on the DBS specimens and storage bags.

3. Before placing desiccant packs into the plastic bag with the DBS or dried plasma spot (DPS), ensure that the desiccant packs have remained dry during storage. Desiccant packs can become moist after use with DBS, but also after storage in a humid environment.

4. Desiccant packs can be re-used. Moist desiccant packs should be dried in a 65°C oven overnight. Remove from the oven and store in a sealed bag with a humidity indicator.

Note: Plastic or foil bags used for storage must be gas-impermeable. Bags available from grocery stores or other outlets that do not sell scientific supplies are inadequate since they are not humidity proof.

Appendix I.5 – DBS Handling and Storage

Short term storage (14 days from the time of collection)

- DBS should be transferred to -20°C or lower as soon as possible; however, when this is not possible they can be kept and/or transported at ambient temperature up to 14 days after collection.

- As humidity and UV light can damage DBS, always keep them in appropriate storage bags with desiccant, in the dark.

- If direct shipment to the lab is not feasible within 14 days, transport DBS at room temperature to a laboratory with a constant electricity supply and freeze

them in a -70°C freezer or, if not available, in a non-frost-free -20°C (or lower) freezer.

Long term storage (>14 days from the time of collection)

- DBS cannot be kept and/or transported at ambient temperature for longer than 14 days.
- In settings where -70°C freezers are not available, non-frost free -20°C freezers can also be used for long term storage (at least up to two years).
- If DBS have been stored refrigerated or frozen, they should only be taken out of cold storage when they are being tested or when desiccant and humidity indicators are being replaced.

Appendix I.6 – Specimen Transport

- Depending on in-country regulations, specimen cards can be shipped to the testing laboratory as non-hazardous materials using regular mail or courier services.
- Prior to shipment, the quality of the collected specimen should be examined and recorded including: integrity of the packaging, condition of the desiccant, humidity indicator reading, overt signs of specimen cross contamination (i.e. two cards in direct contact with one another) and the quantity of DBS.
- Specimen cards should be maintained in the original gas impermeable plastic bag with desiccant until time of transport.
- DBS cards are shipped to the University of Vermont frozen.

Shipping Address:

Rebekah Boyle (802) 656-8938

University of Vermont – Pathology

360 South Park Drive, Rm 154A

Colchester, VT 05446

Rebekah.Boyle@uvm.edu