

Calerie

Blood Collection Manual of Procedures

General Table of Contents

1. Overview and Background
2. Blood collection
 - 2A. General Overview
 - 2B. Intravenous Blood Collection (BL, 12M, and 24M)
 - 2C. Collection by Venipuncture (3M, 6M, 17M, 18M, AND 23M)
3. CALERIE Visits
 - 3A. Baseline, 12M, and 24M Visits
 - 3B. 3M, 6M, 18M Visits
 - 3C. 17M and 23M Visits (Antibody Response)
 - 3D. Unscheduled Visit
4. Processing Blood Samples
 - 4A. Overview
 - I. General Process
 - II. Materials and Supplies
 - 4B. Visit BL, 12M, and 24M
 - I. Arterialized Venous Blood Collection for Catecholamines
 - II. Fasting Blood Collection
 - III. OGTT Collection
 - IV. Day 2 Collection (for women only)
 - 4C. Off-Cycle Female Sex Hormone Draw
 - I. Day 1 Collection
 - II. Day 2 Collection
 - 4D. Visit 3M, 6M, and 18M
 - 4E. Visit 17M, 23M (AB Response)
 - 4F. Visit Unscheduled
 - 4G. Processing Completion
5. 24 Hr Urine Collection: Visits BL, 12M, and 24M
6. Shipping of Biospecimens

APPENDIX

Section 1: Overview and Background

The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) is a multi-center, parallel group, randomized, controlled trial (RTC) which will study the sustained caloric reduction. The Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont will be responsible for protocol development, performing assays and reporting results for the following study outcome data:

Table 1: Summary of Biomarkers

<i>Inflammation markers</i>	IL1a, IL-8, Tnfa, MCP-1, Insulin, Leptin, IL-6, ICAM-1, and CRP.
<i>Lipids and Lipoproteins</i>	Total cholesterol, HDL-cholesterol, LDL-cholesterol (calculated), and triglycerides
<i>Endocrine Response</i>	Adiponectin, Angiotensin II, Norepinephrine (catecholamines), DHEA-s, Cortisol, TSH, and T3
<i>Growth Hormones</i>	GH, IGF-BP1, TGF-b1
<i>Growth Hormones/Endocrine</i>	IGF-1 IGF-BP3, PDGFAB
<i>Collagen Turnover & Fibrosis:</i>	Serum CTX (also at 6M), PINP (also at 6M)
<i>Glucose Tolerance /Insulin:</i>	Glucose (fasting and OGTT) C-Peptide
<i>Sex Hormones</i>	Progesterone (women only), LH (men & amenorrhea), FSH (men & amenorrhea) Estradiol (amenorrhea), SHBG (men only) Free & Total Testosterone (men only)
<i>Antibody Response</i>	Pneumococcal Vaccine Response, Tetanus Toxoid, IgG Antibody, Hep A antibody

The blood and urine samples collected and processed by the CALERIE technicians are the foundation for many of these tests. The most important step (and potentially the most variable) in these tests is the collection and processing of blood samples. *If the blood sample itself is not correctly drawn and processed, the laboratory results may not be precise or may not be valid.*

Additionally, the Laboratory will create and maintain the study's biorepository. The types of specimens that will be collected and stored are serum, EDTA Plasma, citrated plasma, urine, packed cells for DNA, Paxgene for mRNA, and muscle and adipose tissue.

Volunteers are consented at the first baseline visit and do have the option of declining storage of blood samples in the repository. If this occurs, since the total volume of blood collected is already described in the informed consent, the same guidelines should be

followed in blood collection at the clinical center. The site will need to follow-up with the LCBR, University of Vermont as to the particular participant's ID, etc; so the lab will aliquot their received samples for the outcome labs and then discard any remaining blood, since long term storage in the repository has been refused.

This manual has been prepared to standardize methods for sample collection and handling throughout the study. Some of the tools used to assist in standardization include:

Kits will contain the specific collection tubes and cryovials, transfer tubes for each blood collection visit. The cryovials and transfer tubes will be frozen and shipped monthly to the LCBR for assay work and archive.

Forms will capture information on sample collection, handling, and quality assurance parameters. This information is critical to the interpretation of assay results and maintenance of the sample archive.

Labels will be used on forms, draw tubes, cryovials, transfer tubes and urine collection containers. These Sample ID numbers will be different from the participants CALERIE ID number and care must be taken to correctly identify the CALERIE ID number with the Sample ID number. These unique labels will allow for each sample to be tracked individually throughout the study.

Safety Issues and Precautions for Handling Blood Specimens

In accordance with the Occupational Safety and Health Administration (OSHA) regulations on blood borne pathogens, the LCBR recommends the following laboratory safety protocol for the field center laboratories:

- Use non-permeable lab coats, latex (or nitrile) gloves, and face shields when handling any blood in any situation in which splashes, spray, splatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonable anticipated.
- Use of aerosol containers in all centrifuges is recommended.
- Follow "Standard Precautions" when handling any blood, urine or tissue products.
- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak proof container. Never recap or break needles.
- The Hepatitis B vaccine is offered to all unvaccinated technicians handling blood, and documentation of vaccination, or technician's declining to be vaccinated, should be kept on file at the Clinical Center.

Table 2: Overview of the LCBR Laboratory Sample Collection

Method	Tube	BL (IV)	3M (Venipuncture)	6M (Venipuncture)	12M (IV)	17M / 23M (Venipuncture)	18M (Venipuncture)	24M (IV)	Off-Cycle (BL,12M, 24M) (Venipuncture)	Unscheduled (Venipuncture)
		# tubes	# tubes	# tubes	# tubes	# tubes	# tubes	# tubes	# tubes	# tubes
Hot Box	EDTA*	2 @ 5 mL			2 @ 5 mL			2 @ 5 mL		
Fasting	SERUM	4 @ 10 mL	1 @ 10 mL	1 @ 10 mL	4 @ 10 mL	1 @ 4 mL	1 @ 10 mL	4 @ 10 mL	1 @ 4 mL	1 @ 4 mL
	Citrate	1 @ 3.0 mL			1 @ 3.0 mL			1 @ 3.0 mL		
	EDTA	2 @ 10 mL	1 @ 10 mL	1 @ 10 mL	2 @ 10 mL		1 @ 10 mL	2 @ 10 mL		
	Paxgene	2 @ 2.5 mL			2 @ 2.5 mL			2 @ 2.5 mL		
Glucose Load										
30M	SERUM	1 @ 4 mL			1 @ 4 mL			1 @ 4 mL		
60M	SERUM	1 @ 4 mL			1 @ 4 mL			1 @ 4 mL	1 @ 4 mL	
90M	SERUM	1 @ 4 mL			1 @ 4 mL			1 @ 4 mL		
120M	SERUM	1 @ 4 mL			1 @ 4 mL			1 @ 4 mL		
24H Urine		X			X			X		

*EDTA with 10% sodium metabisulfite added.

Section 2: Blood Collection

2A. General Overview

Daily Preparation

- *Confirmation of the Participant Visit*
- Confirm Participant ID and visit schedule for the day.
- Select appropriate kits and confirm expiration dates.

Preparation of Blood Collection Area

Preparation for specimen collection is done in the following manner. Early morning, prior to arrival of the participant:

- Make sure intravenous/venipuncture supplies are stocked and the tubes are labeled.
- Check that the sample processing station is properly equipped. All items needed are present.
- Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, washcloths, and that the draw tube mixer is functional.
- Approximately 10 minutes before scheduled blood specimen collection, fill ice bucket with crushed ice.

Draw Tube Set-up

To facilitate accurate tracking of collected specimens, we recommend that you set up a blood collection tube rack with the set of pre-labeled draw tubes. The tubes should be in the rack according to the order in which they are to be drawn as specified per visit protocol.

Forms

Make sure the CALERIE ID# is matched to the Sample ID# on the appropriate forms.

Overview of Labeling

Each kit will contain a specific Sample ID# which will be matched to the CALERIE ID#.

The format of the labels will be: XXXX-00-XX ex. 1284-00-03

Labels for forms will be XXXX-XX (sample-visit) – no tube #

Site #	Sample #	Visit	Tube #	comments
X	XXX	00	XX	01 – 17 Collection containers (BL,12,24)
		03		20 – 48 Cryovials, Transfer tubes (BL,12, 24)
		06		
		12		
		17		
		18		
		23		
		24		

		99		Unscheduled
		70		Off-cycle female hormone collection-Baseline
		72		Off-cycle female hormone collection-12 month
		74		Off-cycle female hormone collection-24 month

Supplies and Materials for Blood Collection Area

- Personal Protective Equipment - Lab coats and latex or nitrile gloves
- Forms – Phlebotomy/Processing Forms and Urine Collection Forms*
- Plastic cart with wheels for phlebotomy supplies (or plastic tray with compartments)
- Appropriate Visit Kit* (contents specific to visit)
- Extra (backup) vacutainer tubes*.
- Flush tubes – 4 ml
- Basin (just in case)
- Washcloths/Towels
- Smelling salts
- Tourniquets (Please do not substitute a blood pressure cuff).
- Alcohol prep pads
- Gauze (2x2)
- Surgical tape - paper tape (easier on participants)
- Band-Aids
- Scissors
- Stopwatches or timers
- Pens – for completing forms
- Sharpie markers – in case of need to write on any tubes
- Blood tube rocker (note: tubes may be mixed by hand)
- Blood tube racks
- Biohazardous waste container
- Needle/sharps container
- Ice bucket and crushed ice - filled 10 min before draw
- Lab mats and wipes
- 10% bleach solution or approved biohazard disinfectant
- Blood spill kit or equivalent
- Materials for urine collection and bulk storage
- Boric Acid for urine acidification*

* These items are provided by LCBR either in kit format or bulk supplies.

Table 3: Summary of kit contents

Kit I.	CALERIE		Collection for Baseline, 12 Month, and 24 Month		
	# tubes	Type	Tube Size(mL)	# cryovials	# Transfer Tubes
	2	EDTA/Catecholamines <i>(sent separately)</i>	5	0	2
	4	Serum	10	3	4
	1	Citrate	3	2	0
	2	EDTA	10	3	2
	2	Paxgene	2.5	0	2 primary containers
	4	Serum OGTT	4	0	4
	1	Day 2 Serum(F)	4	0	1
	6	Discard Tubes	4	0	0

Kit II.	CALERIE		Collection for 3 Month, 6 Month,18 Month		
	# tubes	Type	Tube Size (mL)	# cryovials	# Transfer Tubes
	1	Serum	10	4	0
	1	EDTA	10	4	0

Kit III.	CALERIE		Collection for 17 and 23 Month for AB response		
	# tubes	Type	Tube Size(mL)	# cryovials	# Transfer Tubes
	1	Serum	4	0	1

Kit VI.	CALERIE		Unscheduled Visit (Amenorrhea)		
	# tubes	Type	Tube Size(mL)	# cryovials	# Transfer Tubes
	1	Serum	4	0	1

Kit VII.	CALERIE		Off-Cycle Visit (Female Sex Hormone Draw)		
	# tubes	Type	Tube Size(mL)	# cryovials	# Transfer Tubes
	3	Serum	4mL	0	3

Note: There are three types of tubes used for storing plasma, serum, urine:



Above left to right: OGTT tube, Transfer Tube, Cryovials

Paxgene collection tubes will be placed into a 50 mL conical tube before being stored at -20C (below):



2B. Intravenous Blood Collection (BL, 12M, and 24M)

An intravenous line provides convenient access for drawing samples without the discomfort of multiple venipunctures. Given the frequency of blood sampling for these

study visits, *two* intravenous access points will be established via a standardized protocol. Intravenous access for the Catecholamine collection will be placed in the dorsal vein of the hand contralateral to the site to be used for Fasting and OGTT draws. A second intravenous access for collection of all Fasting and Oral Glucose Tolerance Testing (OGTT) (excluding catecholamines), will be placed antecubitally.

Standardized protocol for IV insertion:

1. Follow Nurse/Subject Safety Guidelines including universal precautions, and hand washing.
2. Assemble equipment including solutions as indicated, intravenous catheter, administration set, IV start kit and clean gloves.
3. Check for allergies to iodine products.
4. Place tourniquet about two to four inches above the selected site. Do not occlude the adjacent artery; a pulse should be felt below the tourniquet
5. Select the site for insertion. NOTE: For the fasting and OGTT, the antecubital vein is specified. For the catecholamines, the dorsal vein of the contralateral hand is used.
6. Put gloves on.
7. Promote the distention of the vein by requesting the client to alternately open and close his hand; lightly tap the skin surface toward the tourniquet from the distant portion of the limb.
8. Prepare the area for injection – prepping; scrub briskly with alcohol and betadine wipes.
9. Hold the subject's arm. With the thumb of one hand, apply traction on the skin to counter-balance the thrust of the needle with the other hand.
10. While holding the needle hub, introduce the point of the needle, bevel up 2/5 – 4/5 inches below the proposed site of the puncture, with the point in a direct line with the course of the vein. The needle should be pointing at a 30-degree angle to the skin in order to penetrate the skin quickly and cleanly. Redirect the needle in an almost parallel path along the line of the vein and introduce the needle into the vein. A flash chamber will confirm vein entry.
11. Carefully advance the entire unit until at least ¼” to assure full catheter entry into vein lumen. Continue to hold traction.
12. Holding the needle stable, slide the catheter into the vein lumen until the desired length has been inserted. DO NOT REINSERT THE NEEDLE INTO THE CATHETER AT ANY TIME. The needle could cut the catheter and result in catheter embolus. After advancing catheter, blood should continue to advance back in the flash chamber.
13. Release the tourniquet, then remove the needle and connect IV tubing to the cannula; open clamp on tubing to allow fluid to drip slowly. Watch for any signs of infiltration.
14. Apply transparent dressing and secure tubing IV site with Corflex tape.
15. Dispose of needle in sharps box and dispose of any blood contaminated supplies in appropriate container.
16. Establish a 3-way stopcock system* and establish saline flow through system at a keep vein open (KVO) rate.
17. Remove gloves
18. Regulate IV flow to a KVO rate. Observe for any signs of infiltration.

19. Follow nurse/subject safety guidelines making sure to have a safe environment including hand washing.
20. Record on the appropriate Case Report Form, the type of solution, rate of flow, type of needle and gauge precise IV size, number of attempts and patient's response.
21. Observe the participant frequently for appropriate rate, any signs of redness, tenderness, pain or swelling. If infiltration occurs, do not irrigate the vein, but discontinue and restart.

* *Optional 2-way stopcock set-up shown on page 12*

Arterialized IV Blood Collection for Catecholamines:

Catecholamine concentrations will be measured in **arterialized venous blood**. This study will be performed as part of the **Baseline, Visit 7, Month 12, Visit 4, and Month 24, Visit 4** lab work. Tubes for this study will be the first of the series drawn. All catecholamine measures will be collected with the participant in the **supine position**.

The specialized collection tubes (5 mL EDTA with added 10% sodium metabisulfite) **MUST** be filled by using a 5 mL filled syringe injected into the tube.

Placement of Retrograde IV for Catecholamine/"Hot Box" Blood Collection

A 20-22 gauge angiocatheter will be inserted retrograde in to the dorsal hand vein contralateral to the arm used for the second IV placement. Patency of the catheter will be maintained with a saline flush.

Standardized materials needed:

20-22 gauge angiocatheter
Flush syringe/discard tube (4.0mL)
3-way stopcock
5-ml syringes
Heating Pad
0.9% Normal Saline
Hot box
Two - 5mL syringes
Three - 3mL discard syringes

Detailed Hot Box IV Collection Procedure

1. Turn on Hot Box 20 minutes prior to IV placement to allow box to reach normal operating temperature. Normal operating temperature is recommended to be 50°C for extended placement of the hand in the box. Extended placement means *continuously in the box*. The box was designed for placement of the hand continuously in the box for periods of time from 2-6 hours, as often occurs during metabolic studies where the hand needs to be warmed prior to and during a euglycemic multi-step clamp with insulin infusion.

Optional Step: Prior to placing the angiocatheter into the dorsal hand vein, *wrap* a heating pad around the specified hand and carefully warm for several minutes to assist in engorging the hand veins. This may ease the process of insertion.

2. Insert a 20-22 gauge angiocatheter retrograde in to the dorsal hand vein contralateral to the arm being used for the second IV placement (see p9 for Standard Protocol for IV Insertion). Patency of the catheter will be maintained with a bag of 0.9% Normal Saline (NS) at keep vein open (KVO) rate that hooks into one of the T ports. The other T port will be used for draw-offs and discards.
3. After placement of the dorsal hand vein angiocatheter and collection apparatus is complete, place extremity into a Heated-Hand Box. Normal heated-hand box operating temperature is recommended to be 50°C. Under these conditions, the hand should be placed in the box for 20 minutes prior to sampling.



The photo above shows a box in "action": the box is designed to sit next to the patient on a standard hospital bed. Towels can be placed under the box or patient's arm to accommodate any bed or patient position.

4. After the hand is in the hot box for 20 minutes and the skin temperature has reached 41 to 43°C, the first tube (#01) can be collected as follows:

Collecting the Catecholamine Samples:

1. Turn T to the syringe, draw blood back, filling the syringe until you are sure that there is undiluted (with saline) blood at the top of the T (e.g. if the volume of the extension tube from the catheter to the T is 1 ml, then you would need to draw at least 2 ml up into the syringe – for this reason a 3 or 4 ml syringe is commonly used)

2. Close the T (all sides closed; i.e. T top turned diagonally) & remove and discard the syringe.
3. Put the blood drawing syringe on the T; turn T to the syringe and draw the blood into a 5 mL syringe. Immediately inject the catecholamine collection tube #01. Mix gently by inversion. Start timer for 5 minutes.
4. Turn the T back to infuse saline; remove blood-draw syringe and replace with a clean 3-ml discard syringe.
5. At the end of 5 minutes, repeat the above process filling catecholamine collection tube #02. Mix gently by inversion.
6. The filled catecholamine tubes must immediately be brought to the laboratory for centrifugation.
7. Upon completion of the blood collection, the hand is removed from the hot box. Allow the hand to equilibrate at room temperature for 10 minutes, then remove the IV.

Key Points for Catecholamine Collection:

- The 5 mL tubes containing 10% sodium metabisulfite must be filled by syringe.
- IV must be flushed before each tube collection.
- The filled catecholamine collection tubes (#01 and #02) must be centrifuged within 10 minutes of collection.

Fasting and OGTT Blood Collection:

These studies will be performed as part of the **Baseline**, *Visit 7*, **Month 12**, *Visit 4*, and **Month 24**, *Visit 4* lab work. These will be the second and third blood studies drawn as part of a lab blood draw.

Placement of Second IV access:

A 20-22 gauge angiocatheter will be inserted antecubitally. Patency of the catheter will be maintained with a 250 cc bag of 0.9% Normal Saline (NS) solution at a KVO rate.

Standardized Materials needed:

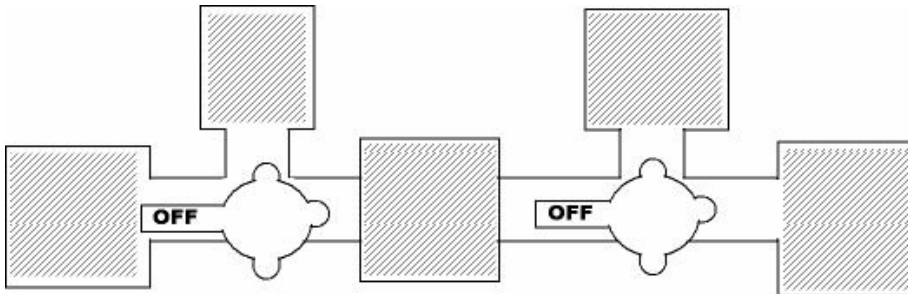
20 - 22-gauge angiocatheter catheter
0.9% Normal Saline (NS) for infusion
IV solution administration kit set (60 gtts./ml)
3-way stop cock*
Small bore extension set (6 inch length with priming volume approximately 0.2 ml)
Tubing with a vacutainer sleeve with a multiple-draw adapter (Collection tubes are NOT filled by syringe)

Detailed procedure for setting up a closed system for multiple blood draws

1. Spike IV Solution Bag with mini-drip solution set
2. Connect 3-way stopcock* to distal end of IV tubing
3. Connect the Extension Set for the multi-draw adapter to distal end of 3-way stopcock*

4. Connect vacutainer sleeve with multi draw adapter to side arm of 3-way stopcock*
5. Turn stopcock "OFF" to sidearm with vacutainer
6. Flush system with NS solution
7. Start indwelling intravenous catheter and attach flushed IV setup
8. Turn stopcock "OFF" to IV setup
9. Draw 4 ml discard tube via sidearm of stopcock using tubing with vacutainer sleeve and multi-draw adapter
10. Draw sample tubes via sidearm of stopcock using tubing with vacutainer sleeve and multi-draw adapter
11. Turn stopcock "OFF" to sidearm with vacutainer
12. Flush system with enough NS solution to clear line
13. Turn stopcock "OFF" to subject
14. Draw 4 ml NS solution from IV solution set to clear blood from stopcock
15. Turn stopcock "OFF" to sidearm with vacutainer
16. Reset IV fluids to KVO rate
17. Fill each draw tube for the Fasting and OGTT Blood Collections in the correct order. (see order listed below under *Key Points for Fasting and OGTT Blood Collection*)

* Optional 2-way stopcock setup is diagrammed below:



Key Points for Fasting and OGTT Blood Collection:

- Collection tubes are filled using a multi-draw adapter – NOT by syringe.
- Collection tubes need to be drawn in the order number on their labels and shown on the forms. The order of tubes is:
 - Tube #03 Serum 10 mL
 - Tube #04 Serum 10 mL
 - Tube #05 Serum 10 mL
 - Tube #06 Serum 10 mL
 - Tube #07 Citrate 3 mL
 - Tube #08 EDTA 10 mL
 - Tube #09 EDTA 10 mL
 - Tube #10 Paxgene 2 mL
 - Tube #11 Paxgene 2 mL
- Serum 10 mL must always be the second tube collected.
- These PAXgene tubes contain a preservative that is toxic; great care must be taken to AVOID any retrograde flow back toward the vein. Tubes are to be held below the level of the participant's arm while filling.

2C. Collection by Venipuncture (3M, 6M, 17M, 18M, 23M, Unscheduled and Off-cycle Visits)

Standard venipuncture technique (antecubital) is used for these visits. A 21-gauge needle is recommended.

ALWAYS WEAR LATEX OR NITRILE GLOVES AND LAB COAT

1. Arrange draw tubes in order of draw.
2. Apply tourniquet (quick-release tourniquet is recommended; please do not use a blood pressure cuff).
3. Examine participant's arms for the best site for venipuncture. Release tourniquet.
4. Cleanse venipuncture site by wiping with alcohol prep pad in a circular motion from center to periphery. Allow area to dry.
5. Re-apply tourniquet and start timer. Document start time.
6. During blood collection, if the collection tube does not fill, try another tube of the same type. **Partially-filled plasma tubes are not acceptable if less than ½ full.** (Partially-filled serum tubes are okay but will result in a reduced number of aliquots. If a tube is not completely filled, clearly note on the Phlebotomy Form.)
7. Clean up the venipuncture area (if necessary). Dispose of needle and tubing in the appropriate biohazard needle sharps containers. Complete the Phlebotomy Form

Section 3: CALERIE Visits

3A. Baseline, 12M, and 24M Visits

Overview

The Baseline (BL), 12 month (12M), and 24 month (24M) blood collections are the most extensive. Blood will be collected in a 'Hot Box' for Norepinephrine. An angiocatheter will be inserted retrograde in to the dorsal hand vein contralateral to the arm used for the second IV placement. Patency of the catheter will be maintained with a saline. After a 15 minute recovery time the fasting blood collection will begin. This will be collected through an antecubital IV. After the fasting blood collection is complete, a glucose load will be administered to the participant and blood will continue to be collected through the IV at 30 minutes, 60 minutes, 90 minutes, and 2 hours. Using the IV requires a flush volume of 4.0 ml and discard tube (4.0 ml) initially and prior to collecting at each time interval. At these visits, female participants will also have another serum tube collected on the following day (Day 2) for progesterone measurements.

A 24 hour urine collection is also obtained from the participants at these visits.

Table 4: CALERIE: Collection for BL, 12M, and 24M

Order #	Tube Type	Flush Vol	Collection Vol	Collection	Collection Method	Comments
1	EDTA*	4.0	5.0	Nor Epi 1	Retrograde IV into dorsal hand vein	Mix 30 sec. Ice. Centrifuge Immediately.
2	EDTA*	4.0	5.0	Nor Epi 2	Retrograde IV into dorsal hand vein	Mix 30 sec. Ice. Centrifuge Immediately.

*with 10% sodium metabisulfite

15 minute Recovery Time Needed before starting Fasting Collection

3	Serum	4.0	10.0	Fasting	Antecubital IV	Room Temp 40 mins
4	Serum		10.0	Fasting	Antecubital IV	Room Temp 40 mins
5	Serum		10.0	Fasting	Antecubital IV	Room Temp 40 mins
6	Serum		10.0	Fasting	Antecubital IV	Room Temp 40 mins
7	Citrate		3.0	Fasting	Antecubital IV	Mix 30 sec. Ice till centrifuging.
8	EDTA		10.0	Fasting	Antecubital IV	Mix 30 sec. Ice till centrifuging
9	EDTA		10.0	Fasting	Antecubital IV	Mix 30 sec. Ice till centrifuging
10	Paxgene		2.5	Fasting	Antecubital IV	Invert 8-10 times. Room Temp. >2 hrs but < 72 hrs.
11	Paxgene		2.5	Fasting	Antecubital IV	Invert 8-10 times. Room Temp. >2 hrs but < 72 hrs.

Administer Glucose Load; Start Timer

12	Serum 30M	4.0	4.0	Glucose	Antecubital IV	Room Temp 40 mins then Centrifuge Immediately.
13	Serum 60 M	4.0	4.0	Glucose/ Progester one 2	Antecubital IV	Room Temp 40 mins then Centrifuge Immediately.
14	Serum 90M	4.0	4.0	Glucose	Antecubital IV	Room Temp 40 mins then Centrifuge Immediately.
15	Serum 120M	4.0	4.0	Glucose	Antecubital IV	Room Temp 40 mins then Centrifuge Immediately.

DAY 2

16	Serum @ Day 2	(4.0)	4.0	Progester one 3 (women only)	Veni-puncture	Room Temp 40 mins
----	---------------	-------	-----	------------------------------	---------------	-------------------

Comments: Use 4.0 mL to flush IV lines

Description of collection tubes

Tubes #1, #2 are 5 ml purple-topped EDTA tubes (Becton Dickinson 367863) with 10% sodium metabisulfite). After centrifugation, plasma from these tubes is

aliquotted into the designated tubes, frozen, and then shipped monthly to LCBR where it will be used for measuring endocrine response markers. Tubes #8 and #9 are 10 ml purple-topped EDTA tubes (Becton Dickinson 366643). After centrifugation, pipet 1.0 ml of plasma into each of the 3 designated cryovials and freeze. The remaining plasma is transferred to the appropriate transfer tubes then frozen. The packed cells from the two draw tubes are transferred to two separate 10 ml tubes and frozen. All frozen samples are shipped monthly to LCBR where some will be used for measuring inflammation markers and the remainder stored in the study bio-repository.

Tubes #3, #4, #5, and #6 are 10mL red-topped Serum tubes Becton Dickinson 367820). After filling, let these tubes stand at room temperature for a minimum of 40 minutes, but a maximum of 120 minutes, to allow the blood to clot. The tubes are then centrifuged. Three 1.0 ml aliquots of serum will be made, and the remaining serum is transferred into designated transfer tubes. After aliquotting all serum is frozen. Samples are shipped monthly to LCBR where some of the serum will be used to measure a wide array of biomarkers. The remaining serum will be stored in the study bio-repository.

Tube #7 is a 3.0 ml blue-topped citrate tube (Becton Dickinson 363083) This tube contains 0.5ml of 3.2% sodium citrate. After centrifugation, plasma is transferred into a 2 x 1.0 ml cryovias, frozen, and shipped to LCBR monthly. The plasma will be banked in the bio-repository for future testing.

Tubes #10 and #11 are 2.5 ml Paxgene tubes (Becton Dickinson 762165). These tubes are NOT centrifuged. Sample is left in the collection tube, frozen at -20C, and shipped monthly to LCBR. mRNA will be obtained from the samples in these tubes. http://www.prealanalytix.com/RNA_Instr.asp

Tubes #12, #13, #14, #15, and #16 are 4 ml Serum (Becton Dickinson 367812) After filling, let these tubes stand at room temperature for a minimum of 40 minutes, but a maximum of 120 minutes, to allow the blood to clot. The tubes are then immediately centrifuged and the serum transferred to separate 5 ml transfer tubes, frozen and shipped monthly to LCBR where the serum will be used for measuring glucose tolerance and sex hormone levels in females.

Kit Contents

The following items will be supplied in the kits labeled for the BL, 12M, and 24M visits:

Labels with Sample ID#s

EDTA draw tubes – 2 x 5 ml (special catecholamine tubes)

EDTA draw tubes – 2 x 10 ml

Serum draw tubes – 4 x 10 ml

Serum draw tubes – 5 x 4 ml

Citrate draw tubes – 1 x 3 ml

Paxgene draw tubes – 2 x 2.5 ml

Cryovials, Transfer tubes, and OGTT tubes – for all plasma, serum, packed cells, and urine aliquots

Transfer pipets

4mL discard tubes will be provided in bulk by the CBAL as needed by the sites

Also, note that Kits for the BL, 12M, and 24M visit will be gender specific

Preparing the Kit for collection

Select the appropriate Kit I based on gender of the participant.

Phlebotomy/Processing Form

The purpose of the Phlebotomy/Processing Forms (P/P Forms) is to facilitate the efficient collection of plasma and serum samples from the participants, with maximum protection for the participant and the technician. In addition, the P/P Forms facilitate the monitoring of sample collection and other quality assurance parameters and provide information critical to the interpretation of the assay results and maintenance of the sample repository.

The completed Phlebotomy/Processing Forms will be included in the sample shipments to LCBR.

Blood Collection

'Hot Box' for Norepinephrine

1. Make sure the Phlebotomy / Processing Form has the CALERIE ID matched to the Sample ID label.
2. Record staff ID#, date, start time, IV size/type
3. Follow protocol for setting up the 'hot box' and retrograde IV lines. Participant should sit recumbent with hand in hot box for 20 minutes prior to draw.
4. Flush lock with 4 mL saline then draw a 4 mL 'discard' tube (serum)
5. Blood is drawn by syringe.
6. Fill special EDTA tube #01 by injecting the filled 6 mL syringe. Gently invert the filled tube for 30 seconds or place on a tube rocker
7. Set timer for 5 minutes
8. At the end of 5 minutes, flush lock with 4 mL saline then draw a 4 mL 'discard' tube (serum)
9. Blood is drawn by syringe.
10. Fill special EDTA tube #02 by injecting the filled 6 mL syringe. Gently invert the filled tube for 30 seconds or place on a tube rocker.
11. Remove participants arm from 'hot box' and allow hand to cool to normal temperature for 10 minutes.
12. Remove IV
13. Complete Phlebotomy/Processing Form section on 'Hot Box' collection.

Fasting Collection

14. After a minimum 15 minute rest period from the 'hot box' collection, begin collection of the fasting bloods from the antecubital IV line. Follow protocol for setting up a closed system for multiple IV blood draws (Section 2A).
15. Record the start time, IV line on the Phlebotomy/Processing Form

16. Use a luer adapter with 12 inches of tubing attached to the IV line.
17. Flush lock with 4 mL saline then draw a 4 mL 'discard' tube (serum)

Fill draw tubes in the following order:

Tube #	Type of Tube	After filling
03	Serum 10 mL	Into rack at room temperature
04	Serum 10 mL	Into rack at room temperature
05	Serum 10 mL	Into rack at room temperature
06	Serum 10 mL	Into rack at room temperature
07	Citrate 3.0 mL	Mix 30 seconds
08	EDTA 10 mL	Mix 30 seconds
09	EDTA 10 mL	Mix 30 seconds
10	Paxgene 2.5 mL*	Into rack at room temperature
11	Paxgene 2.5 mL*	Into rack at room temperature

*Hold the Paxgene tubes below the level of the arm while filling.

After all tubes are filled:

EDTA (#08, #09) and Citrate (#07) are placed on crushed ice.

Serum (#03, #04, #05.#06) remain at room temperature for 40 minutes.

Paxgene (#10, #11) remain at room temperature for 2 hours before placing at -20C.

18. Complete Phlebotomy/Processing Form section on Fasting Collection
19. Administer Glucose load according to protocol. Record time and start timer

OGTT at 30 minutes, 60 minutes, 90 minutes and 120 minutes

Serum will be drawn at 30M (serum #12), 60M (serum #13), 90M (serum #14),120M (serum #15) after completion of the glucose load.

At each collection:

20. Flush lock with 4 mL saline then draw a 4 mL 'discard' tube (serum)
21. Attach appropriate serum tube 4.0 mL and fill. Set in a rack
22. Keep serum tubes in a rack at room temperature for 40 minutes then centrifuge immediately.
23. Complete Phlebotomy/Processing Form section on OGTT Collection

This is the end of Fasting Collection on day 1. The IV may be removed at this time.

Day 2 Sex Hormones

On the following day, an additional serum tube will be collected in the AM on female participants only. This tube may be collected using standard **venipuncture**

technique. Make sure the Phlebotomy / Processing Form is filled out for date, time, etc.

24. Collect Serum tube #16. Place in a rack at room temperature for 40 minutes.

25. Complete Phlebotomy/Processing Form section on Day 2 Collection.

3B. 3M, 6M, and 18M Visits

Overview

At these visits, blood will be collected by standard venipuncture technique. A total of 20 mLs of blood will be collected in one 10 mL EDTA tube and one 10 mL Serum tube. Blood collection should be standardized to a fasting AM collection. These samples will be used for bone markers at the 6M collection and for AB response at the 18 M collection. All samples will be stored for archive.

Kits for this collection period will include:

- EDTA draw tube 1 x 10 mL
- Serum draw tube 1 x 10 mL
- 4 cryovials (1.5 mL size) for EDTA plasma
- 4 cryovials (1.5 mL size) for Serum

Blood Collection

1. Make sure the Phlebotomy / Processing Form has the CALERIE ID matched to the Sample ID label.
2. Record staff ID#, date, start time, needle size (21G)
3. Begin venipuncture following standard protocol
4. Attach Serum tube #01 and fill. Place in a rack at room temperature for 40 minutes.
5. Attach EDTA tube #02 and fill. Gently invert the filled tube for 30 seconds or place on a tube rocker.
6. Complete Phlebotomy/Processing Form

3C. 17M and 23M Visits (Antibody Response)

Overview

At these visits, blood will be collected by standard venipuncture technique. A total of 4 mLs of blood will be collected in one 4.0 mL Serum tube. Blood collection should be standardized to a fasting AM collection. This sample will be used for AB response measurements.

Kits for this collection period will include:

- Serum draw tube 1 x 4 mL
- 1 transfer tube (5 mL size) for Serum

Blood Collection

1. Make sure the Phlebotomy / Processing Form has the CALERIE ID matched to the Sample ID label.
2. Record staff ID#, date, start time, needle size (21G)
3. Begin venipuncture following standard protocol

4. Attach Serum tube #01 and fill. Place in a rack at room temperature for a minimum of 40 minutes.
5. Complete Phlebotomy/Processing Form

3D. Unscheduled Visit

Overview

Blood will be collected for hormone measurements in the case of amenorrhea in women. At these visits, blood will be collected by standard venipuncture technique. A total of 4 mLs of blood will be collected in one 4.0 mL Serum tube. Blood collection should be standardized to a fasting AM collection.

Kits for this collection period will include:

- Serum draw tube 1 x 4 mL
- 1 transfer tube (5 mL size) for Serum

Blood Collection

1. Make sure the Phlebotomy / Processing Form has the CALERIE ID matched to the Sample ID label.
2. Record staff ID#, date, start time, needle size (21G)
3. Begin venipuncture following standard protocol
4. Attach Serum tube #01 and fill. Place in a rack at room temperature for 40 minutes.
5. Complete Phlebotomy/Processing Form

3E. Off-Cycle Female Sex Hormone Visit

Overview

Blood will be collected from CALERIE female participants for measuring Progesterone levels at the Baseline, 12 Month, and 24 Month visits. The blood collected for this testing has to be collected during the mid-luteal phase (days 19-21) of the menstrual cycle, day one being the start of menses. In some instances the mid-luteal phase will not coincide with the date of the Baseline, 12 month and/or 24 month blood collections. For this situation an Off-cycle female hormone collection blood draw may need to occur prior to these visits.

Day 1 Blood Collection

1. Make sure the Phlebotomy / Processing Form has the CALERIE ID matched to the sample ID label
2. Record Staff ID#, date, start time, needle size (21G)
3. Begin venipuncture following standard protocol
4. Attach serum tube #03 and fill. Place tube in rack at room temperature.
5. Attach serum #13 and fill. Place tube in rack at room temperature. Tubes need to clot at room temperature for 40 minutes

Day 2 Blood Collection

1. Make sure Phlebotomy/Processing Form has the CALERIE ID matched to the sample ID label
2. Record Staff ID#, date, start time, needle Size (21G)

3. Begin venipuncture following standard protocol
4. Attach Serum tube #16 and fill. Place in a rack at room temp. for at least 40 minutes, but < 90 minutes prior to centrifuging.
5. Complete Phlebotomy/Processing form

Section 4: Processing Blood Samples

4A. Overview

Processing should be initiated as soon as possible (< 30 minutes) following collection. The red-topped serum tubes must stand at room temperature for at least 40 minutes before centrifugation. If centrifugation of the other tubes is not immediate, the EDTA and Citrate tubes should remain on ice. Personal protective equipment (non-permeable lab coats, double-gloves with at least one latex or nitrile pair, face shields) is required for processing.

I. General Process

After blood collection, the following outlines time limits before the centrifugation step:

EDTA and Citrate - store on ice; preferably < 15 minutes (maximum < 30 minutes) before centrifuging.

Serum - store at room temp for at least 40 minutes, but < 90 minutes prior to centrifuging (except the glucose serum which must be centrifuged immediately after 40 minutes).

Paxene - must be kept at room temperature for a minimum of 2 hours, but not to exceed 72 hours. Do not centrifuge or aliquot these two tubes.

Centrifugation will be standardized to a 30,000 g-minute spin at 4°C. This setting is based on RCF not RPM settings. The LCBR will work with each site to determine the best setting for their centrifuges.

Centrifugation of Plasma Tubes (EDTA, citrate): If centrifugation is not immediate, tubes are stored upright on wet ice for up to 30 minutes. Maximum time elapsed before centrifugation is 30 minutes from time of collection. Please note all centrifugation start times on the Phlebotomy/Processing Form. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

Centrifugation of Serum Tubes: Allow the Serum tubes to clot for 40 minutes at room temperature. After centrifugation is complete, place on ice.

Aliquoting consists of removing the serum or plasma in small amounts (e.g.: 1.0 mL) by pipette and placing it into the appropriate transfer tube (as outlined in tube specifications and the aliquoting schemes).

This process must be done while the centrifuged draw tubes and cryovials and transfer tubes are on ice.

When aliquoting serum and plasma from the centrifuged collection tube, be careful not to disturb the top of the cell layer with the pipette tip, as this will result in platelet, white cell and red cell contamination.

Use a new pipette tip for each draw tube.

Once the sample is aliquoted, transfer tubes should be immediately (within 10 minutes) frozen in an upright position at -80°C.

Every effort should be made to freeze serum and plasma cryovials and the tubes of packed cells at -80°C or below as soon as possible (within 10 minutes) after aliquoting. All samples should be frozen in an upright position. If specimens cannot be placed immediately at -80°C or below, they may be temporarily (< 2 hours) stored at -20°C or placed on dry ice until transfer to -80°C or below. Dry ice is the preferred solution.

If any tubes are accidentally mixed during pipetting so that plasma is contaminated with red cells, they may be recentrifuged.

II. Materials and Supplies

The following items should be on hand before beginning processing:

- Lab coats, gloves, splash shields, and other Personal Protective Equipment as needed.
- Phlebotomy/Processing Forms*
- Labels (included in kit)*
- Cryovials and Transfer tubes (included in kit)*
- Refrigerated Centrifuge: 2,000 g-force minimum, 4 °C, Swinging bucket.
 - 10% bleach solution (or approved biohazard disinfectant)
- Test tube holders (adapters) for centrifuges
- Freezer (-80° C or colder)
- Emergency eye wash station
 - Biohazard trash can, with large and small biohazard bags (biohazardous waste puncture- proof containers)
- Tube racks
 - Fixed volume pipettes with tips (MLA) or adjustable pipettes (Rainin, Finn, etc) with tips. Volumes needed to pipette: 1.0 mL, 2.0 mL, 2.5 mL, 3.0 mL, 5.0 mL and 9.0 mL
 - Revco Freezer Boxes 5x5x2" and 5x5x3" with dividers*
- Styrofoam/insulated shipping boxes*
- Refrigerator – not with automatic defrost.
- Sharpie pens

*provided by LCBR

Aliquot racks will be set up to correspond to each blood collection tube rack. Rack setup is completed prior to the participant's visit. All tubes are labeled with the sample ID labels and arranged in the appropriate working order.

There are three types and sizes of aliquots being used for this study:

- OGTT tubes
- Transfer tubes

Cryovials

Also 50 mL conical tubes as a primary container for the Paxgene tubes.

4B. Visit BL, 12M, 24M

Table 5: Aliquoting Scheme for BL, 12M, and 24M

CALERIE: Aliquoting Scheme for BL, 12M, 24M					
<u>Collection Tube</u>	<u>Collection Tube#</u>	<u>Cryo #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
EDTA*	01	20	Tube	2.5	Nor Epi 1
EDTA*	02	21	Tube	2.5	Nor Epi 2
Serum	03	22	Cryovial	1.0	Repository
Serum	03	23	Cryovial	1.0	Repository
Serum	03	24	Cryovial	1.0	Repository
Serum	03	25	Tube	2.0	LCBR to Aliquot
Serum	04	26	Tube	5.0	LCBR to Aliquot
Serum	05	27	Tube	5.0	LCBR to Aliquot
Serum	06	28	Tube	5.0	LCBR to Aliquot
Citrate	07	29	Cryovial	0.5	Repository
Citrate	07	30	Cryovial	0.5	Repository
EDTA	08	31	Cryovial	1.0	Repository
EDTA	08	32	Cryovial	1.0	Repository
EDTA	08	33	Cryovial	1.0	Repository
EDTA	08	34	Tube	2.0	LCBR to Aliquot
EDTA Packed Cells	08	35	Tube	5.0	DNA Extraction
EDTA	09	36	Tube	5.0	LCBR to Aliquot
EDTA Packed Cells	09	37	Tube	5.0	DNA Extraction
Paxgene	10	None			Leave in tube at RT for 2 Hrs
Paxgene	11	None			Leave in tube at RT for 2 Hrs
Serum 30M	12	38	Tube	2.0	Glucose
Serum 60M	13	39	Tube	2.0	Glucose/Progesterone
Serum 90M	14	40	Tube	2.0	Glucose
Serum 120M	15	41	Tube	2.0	Glucose
Serum Day 2	16	42	Tube	2.0	Progesterone (Females only)
Urine	17	43	Tube	9.0	Repository
Urine	17	44	Tube	9.0	Repository
Urine	17	45	Tube	9.0	Repository
Urine boric acid	17	46	Tube	9.0	Repository
Urine boric acid	17	47	Tube	9.0	Repository
Urine boric acid	17	48	Tube	9.0	Repository

I. Arterialized Venous Blood Collection for Catecholamines

Table 6: Catecholamine (NorEpi) collection via Arterialized venous blood

Draw Tube	Processing	Transfer to	Volume After Centrifugation	Local storage Temperature
# 01 EDTA* 5 mL	Centrifuge 4C	Transfer Tube #20	3.0 mL	-80C
# 02 EDTA* 5 mL	Centrifuge 4C	Transfer Tube #21	3.0 mL	-80C

* EDTA with 10% sodium metabisulfite

Description of process

The special EDTA tubes (#01, 02) should be immediately centrifuged at 4°C at a minimum of 2,000 x g for 15 minutes or 3,000 x g for 10 minutes (for a total of 30,000 g-minutes). Maximum time elapsed before centrifugation is 30 minutes from time of collection. Please note all start times on the Blood Processing Form. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

After centrifugation, the two special EDTA tubes are aliquoted as follows:

EDTA tube #01: Aliquot entire plasma (approximately 3.0 mL) to transfer tube #20.

EDTA tube #02: Aliquot entire plasma (approximately 3.0 mL) to transfer tube #21.

Once the samples are aliquoted, the transfer tubes can be frozen in an upright position at -80C.

Complete the Processing Form.

II. Fasting Blood Collection

Table 7: Fasting Draw for BL, 12M, and 24M

Draw Tube	Processing	Transfer to	Volume After Centrifugation	Local storage Temperature
#8, #9 (2 x EDTA 10 mL)	Centrifuge 4C	3 cryos @ 1.0 mL, remaining plasma into two 5 mL tube plus packed cells into two tubes	9 to 10 mL	-80C
#3, 4,5,6 Serum (4 x Serum 10 mL)	Allow to clot for 40 min then Centrifuge 4C	3 cryos @ 1.0 mL, remaining serum into four 5mL tubes.	20 mL	-80C
# 7 Citrate 3.0 mL	Centrifuge 4C	2 cryos at 0.5 mL	1.0 to 1.5 mL	-80C
#10, #11 Paxgene 2.5 mL	Do NOT centrifuge	Leave in Collection tube. Place entire tube into one 50 mL conical.	N/A N/A	-20C -20C
#12 – 15 Serum (4 x 4 mL)	Allow to clot for 40 min then Centrifuge at 4C	5 mL tube	2 mL	-80C
# 16 Serum 4 mL DAY 2	Allow to clot for 40 min then Centrifuge at 4C	5 mL tube	2 mL	-80C

Forms (see Appendix)

Label Design (Example of 24 Month Visit)

Extra labels, not shown, will be provided for forms and freezer box to VT.

<p>Calerie Baseline EDTA Nor Epi Draw Tube</p>  <p>1234 00 01</p>	<p>Calerie Baseline EDTA Nor Epi Draw Tube</p>  <p>1234 00 02</p>	<p>Calerie Baseline Serum Draw Tube</p>  <p>1234 00 03</p>	<p>Calerie Baseline Serum Draw Tube</p>  <p>1234 00 04</p>	<p>Calerie Baseline Serum Draw Tube</p>  <p>1234 00 05</p>
<p>Calerie Baseline Serum Draw Tube</p>  <p>1234 00 06</p>	<p>Calerie Baseline Citrate Draw Tube</p>  <p>1234 00 07</p>	<p>Calerie Baseline EDTA Draw Tube</p>  <p>1234 00 08</p>	<p>Calerie Baseline EDTA Draw Tube</p>  <p>1234 00 09</p>	<p>Calerie Baseline Paxgene RNA</p>  <p>1234 00 10</p>
<p>Calerie Baseline Paxgene RNA</p>  <p>1234 00 11</p>	<p>Calerie Baseline Serum 30min Draw Tube</p>  <p>1234 00 12</p>	<p>Calerie Baseline Serum 60min Draw Tube</p>  <p>1234 00 13</p>	<p>Calerie Baseline Serum 90min Draw Tube</p>  <p>1234 00 14</p>	<p>Calerie Baseline Serum 120min Draw Tube</p>  <p>1234 00 15</p>
<p>Calerie Baseline Serum Day2 Draw Tube</p>  <p>1234 00 16</p>	<p>Calerie Baseline Urine Collection Flask</p>  <p>1234 00 17</p>	<p>Calerie Baseline EDTA Nor Epi Assay FAHC</p>  <p>1234 00 20</p>	<p>Calerie Baseline EDTA Nor Epi Assay FAHC</p>  <p>1234 00 21</p>	<p>Calerie Baseline Serum Repository A</p>  <p>1234 00 22</p>
<p>Calerie Baseline Serum Repository A</p>  <p>1234 00 23</p>	<p>Calerie Baseline Serum Repository A</p>  <p>1234 00 24</p>	<p>Calerie Baseline Serum Assay LCBR</p>  <p>1234 00 25</p>	<p>Calerie Baseline Serum Assay FAHC</p>  <p>1234 00 26</p>	<p>Calerie Baseline Serum Assay LCBR</p>  <p>1234 00 27</p>

<p>Calerie Baseline Serum Assay LCBR</p>  <p>1234 00 28</p>	<p>Calerie Baseline Citrate Repository</p>  <p>1234 00 29</p>	<p>Calerie Baseline Citrate Repository</p>  <p>1234 00 30</p>	<p>Calerie Baseline EDTA Repository A</p>  <p>1234 00 31</p>	<p>Calerie Baseline EDTA Repository A</p>  <p>1234 00 32</p>
<p>Calerie Baseline EDTA Repository A</p>  <p>1234 00 33</p>	<p>Calerie Baseline EDTA Repository B</p>  <p>1234 00 34</p>	<p>Calerie Baseline EDTA Packed Cells DNA Extraction</p>  <p>1234 00 35</p>	<p>Calerie Baseline EDTA Assay LCBR</p>  <p>1234 00 36</p>	<p>Calerie Baseline EDTA Packed Cells DNA Extraction</p>  <p>1234 00 37</p>
<p>Calerie Baseline Serum 30min Glucose</p>  <p>1234 00 38</p>	<p>Calerie Baseline Serum 60min Glucose/Progesterone</p>  <p>1234 00 39</p>	<p>Calerie Baseline Serum 90min Glucose</p>  <p>1234 00 40</p>	<p>Calerie Baseline Serum 120min Glucose</p>  <p>1234 00 41</p>	<p>Calerie Baseline Serum Day2 Progesterone (Female)</p>  <p>1234 00 42</p>
<p>Calerie Baseline Urine Repository</p>  <p>1234 00 43</p>	<p>Calerie Baseline Urine Repository</p>  <p>1234 00 44</p>	<p>Calerie Baseline Urine Repository</p>  <p>1234 00 45</p>	<p>Calerie Baseline Urine & Acid Repository</p>  <p>1234 00 46</p>	<p>Calerie Baseline Urine & Acid Repository</p>  <p>1234 00 47</p>
<p>Calerie Baseline Urine & Acid Repository</p>  <p>1234 00 48</p>				

Description of process

The EDTA tubes (#08, 09) and the Citrate tube (#07) are processed first. Allow the Serum tubes (# 03, 04, 05, 06) to clot for 40 minutes at room temperature. The Paxgene tubes (#10, 11) are set aside at room temperature until all other samples are processed.

EDTA Tubes:

If centrifugation of the EDTA and citrate tubes is not immediate, tubes are stored upright on wet ice for up to 30 minutes. Tubes are centrifuged at 4°C at a minimum of 2,000 x g for 15 minutes or 3,000 x g for 10 minutes (for a total of 30,000 g-minutes). Maximum time elapsed before centrifugation is 30 minutes from time of collection. Please note all start times on the Blood Processing Form. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

After centrifugation, the two EDTA tubes are aliquoted as follows:

- | | |
|----------------|--|
| EDTA tube #08: | Aliquot exactly 1.0 mL into three purple-top cryovials (#31,32,33)
Aliquot remaining plasma (approximately 2.0 mL) to transfer tube #34.
Carefully pour packed cells into transfer tube # 35 |
| EDTA tube #09: | Aliquot the entire plasma (approximately 5.0 mL) to transfer tube # 36
Carefully pour packed cells into transfer tube # 37. |

Citrate Tube:

After centrifugation, the Citrate tube is aliquoted as follows:

- | | |
|--------------------|---|
| Citrate tube # 07: | Aliquot exactly 1.0 mL into two blue-topped cryovials (# 29, 30). Note: if extra plasma is available, carefully add the volume to cryovial #30.
Dispose of remaining packed cells. |
|--------------------|---|

Once the samples are aliquoted, the EDTA and Citrate cryovials and transfer tubes can be frozen in an upright position at -80C.

Serum Tubes

Allow the Serum tubes to clot for 40 minutes at room temperature. These tubes are centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

After centrifugation, the Serum tubes are aliquoted as follows:

- | | |
|-----------------|---|
| Serum tube #03: | Aliquot exactly 1.0 mL into three red-capped cryovials (# 22,23,and 24)
Aliquot the remaining serum (approximately 2.0 mL) to transfer tube #25. |
| Serum tube #04: | Aliquot the entire serum (approximately 5.0 mL) to transfer tube #26. |

Serum tube #05: Aliquot the entire serum (approximately 5.0 mL) to transfer tube #27.

Serum tube #06: Aliquot the entire serum (approximately 5.0 mL) to transfer tube #28.

Once the samples are aliquoted, the Serum cryovials and transfer tubes can be frozen in an upright position at -80C.

Low sample volume

If there is insufficient sample to make the full set of aliquots for a tube type, fill as many cryovials as possible and make a note on the Blood Processing Form.

Any partially-filled cryovial (less than the specified volume) should be marked with a dot on the cap and a "P" in the comment field on the Blood Processing Form next to that cryovial number.

Paxgene tubes (#10, 11) are kept at room temperature for a minimum of 2 hours. These tubes are NOT centrifuged. Place the entire collection tube as is into a 50 mL conical centrifuge tube. These conical centrifuge tubes act as a primary container in case of any tube breakage. Place these at -20C.

III. OGTT Collection

Serum tubes #12, 13, 14, and 15 sit at room temperature for 40 minutes. Then these tubes are immediately centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

After centrifugation, the Serum tubes are aliquoted as follows:

Serum # 12 Aliquot the entire serum (approximately 2.5 mL) to OGTT tube #38.

Serum #13 Aliquot the entire serum (approximately 2.5 mL) to OGTT tube #39.

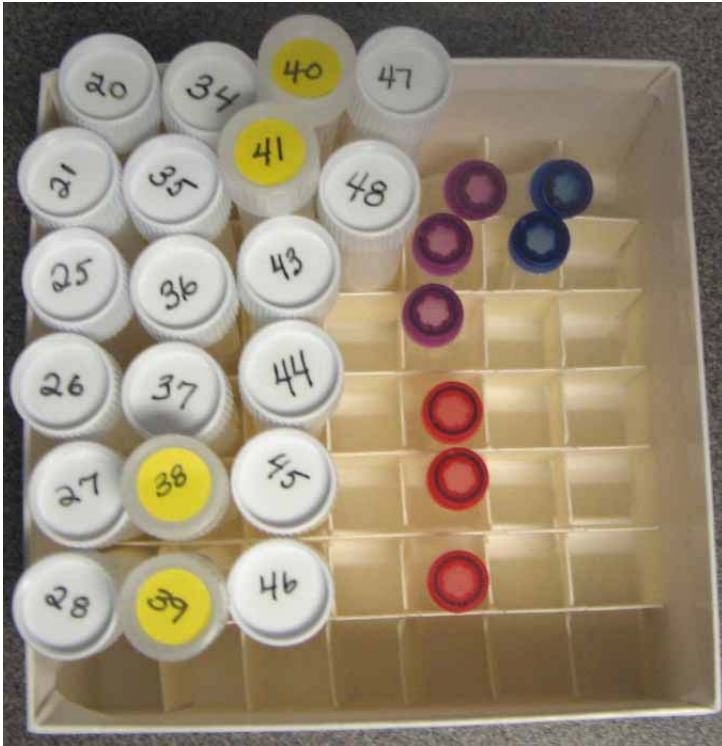
Serum #14 Aliquot the entire serum (approximately 2.5 mL) to OGTT tube #40.

Serum #15 Aliquot the entire serum (approximately 2.5 mL) to OGTT tube #41.

Once the samples are aliquoted, the Serum cryovials and transfer tubes can be frozen in an upright position at -80C.

Box maps (For local storage of samples)

All samples from one participant will be stored in one freezer box with a 7 x 7 grid



IV. Day 2 collection (for women only)

Standard venipuncture technique

Description of aliquots, color codes

Table 8: Aliquoting Scheme for Day 2

<u>Tube/cryo</u>	<u>Collection Tube#</u>	<u>Transfer Tube #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
Serum	16	42	Tube	2.0	AB response

Description of process

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #16: Aliquot the entire serum (approximately 2.0 mL) into transfer tube # 42.

Once the samples are aliquoted, the Serum transfer tube can be frozen in an upright position at -80C

4C. Off-Cycle Female Hormone Draw Visit

4C: Off-Cycle Female Sex Hormone Collection

Table 9: Aliquoting Scheme for Off-cycle Female Hormone Draw Samples

<u>Collection Tube</u>	<u>Collection Tube#</u>	<u>Cryo #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
Serum	03	25	Transfer	2.0	Baseline Progesterone levels
Serum	13	39	Transfer	2.0	60 Min Progesterone levels
Serum	16	42	Transfer	2.0	Day 2 Progesterone levels

Forms (see Appendix)

Label Design (example of baseline off-cycle visit)



I. Day 1 Off-cycle Hormone Draw (for women only)

Standard venipuncture technique

Description of process

Serum Tubes (#03 and #13)

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #03: Aliquot the entire serum (approximately 2.0 mL) to transfer tube #25.

Serum tube #13: Aliquot the entire serum (approximately 2.0 mL) to transfer tube #39.
Once the samples are aliquoted, the Serum cryovials can be frozen in an upright position at -80C

II. Day 2 Off-cycle Hormone Draw (for women only)

Standard venipuncture technique

Description of process

Serum Tube (#16)

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #16: Aliquot the entire serum (approximately 2.0 mL) to transfer tube #42.

Once the sample is aliquoted, the Serum tube can be frozen in an upright position at -80C

Box maps (for local storage of samples)

Sample from multiple participants' will be placed in one freezer box with a 7 x 7 grid. Each participant will have 3-10mL transfer tubes per Baseline, 12M, or 24M. This illustration shows samples from 12 participants. Samples from different visits (Baseline, 12M, or 24M) may be placed in this box for monthly shipment. Label out side of Box "Off-Cycle Hormone Visit" with permanent marker.



4D: Visit 3M, 6M, and 18M

Description of aliquots, color codes

Table 10: Aliquoting Scheme for 3M, 6M, and 18M Samples

<u>Collection Tube</u>	<u>Collection Tube#</u>	<u>Cryo #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
EDTA	02	03	Cryovial	1.0	Repos
EDTA	02	04	Cryovial	1.0	Repos
EDTA	02	05	Cryovial	1.0	Repos
EDTA	02	06	Cryovial	1.0+	Repos
Serum	01	07	Cryovial	1.0	Use at 6M for bone markers; 18M for AB response
Serum	01	08	Cryovial	1.0	Repos
Serum	01	09	Cryovial	1.0	Repos
Serum	01	10	Cryovial	1.0+	Repos

Forms (see Appendix)

Label Design (example of 3 month visit)



Description of process

The EDTA tube (#02) is processed first. Allow the Serum tube (#01) to clot for 40 minutes at room temperature.

EDTA Tubes

If centrifugation of the EDTA tube is not immediate, tubes are stored upright on wet ice for up to 30 minutes. Tubes are centrifuged at 4°C at a minimum of 2,000 x g for 15 minutes or 3,000 x g for 10 minutes (for a total of 30,000 g-minutes). Maximum time elapsed before centrifugation is 30 minutes from time of collection. Please note all start times on the Blood Processing Form. Once centrifugation is complete, tubes are carefully placed on ice and are ready to aliquot.

After centrifugation, the EDTA tube aliquoted as follows:

EDTA tube #02: Aliquot exactly 1.0 mL into four purple-top cryovials (#03, 04, 05, 06). Note: if extra plasma is available, carefully add the volume to cryovial #06.

Once the samples are aliquoted, the EDTA cryovials can be frozen in an upright position at -80C.

Serum Tubes

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

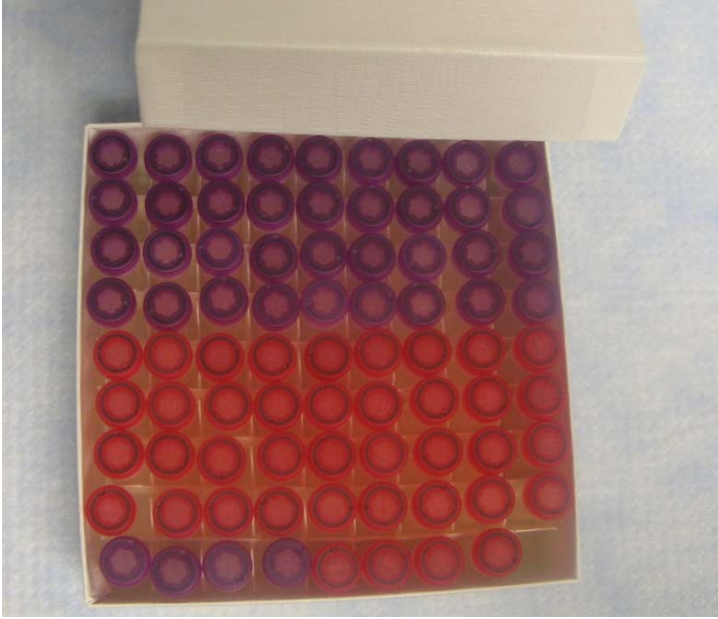
After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #01: Aliquot exactly 1.0 mL into four red-top cryovials (#07, 08, 09, 10). Note: if extra plasma is available, carefully add the volume to cryovial #10.

Once the samples are aliquoted, the Serum cryovials can be frozen in an upright position at -80C

Box maps (for local storage of samples)

Sample from multiple participants' will be placed in one freezer box with a 9 x 9 grid. Each participant will have 4 purple-top cryovials and 4 red-top cryovials per 3M, 6M, or 18M. This illustration shows samples from 10 participants. Samples from different visits (3M, 6M, or 18M) may be placed in this box for monthly shipment.



4E. Visit 17M, 23M (AB Response)

Description of aliquots, color codes

Table 11: Aliquoting Scheme for 17M and 23M visits

<u>Tube/cryo</u>	<u>Collection Tube#</u>	<u>Transfer Tube #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
Serum	01	02	Tube	2.0	AB response

Forms (see Appendix)

Label Design (example of 17M visit)



Description of process

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes.

After centrifugation is complete, place on ice.

After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #01: Aliquot the entire serum (approximately 2.0 mL) into transfer tube # 02.

Once the samples are aliquoted, the Serum transfer tube can be frozen in an upright position at -80C

4F. Visit Unscheduled

Description of aliquots, color codes

Table 12: Aliquoting Scheme for Unscheduled Visit

<u>Tube/cryo</u>	<u>Collection Tube#</u>	<u>Transfer Tube #</u>	<u>Tube Type</u>	<u>Vol (mL)</u>	<u>Comments</u>
Serum	01	02	Tube	2.0	AB response

Forms (see Appendix)

Label Design

Labels are the same design as the 17M and 23M visits except the visit code is 99.

Description of process

Allow the Serum tube to clot for 40 minutes at room temperature. This tube is centrifuged at 4° C at 2,000 x g for 15 minutes or 3,000 x g for 10 minutes for a total of 30,000 g-minutes. After centrifugation is complete, place on ice.

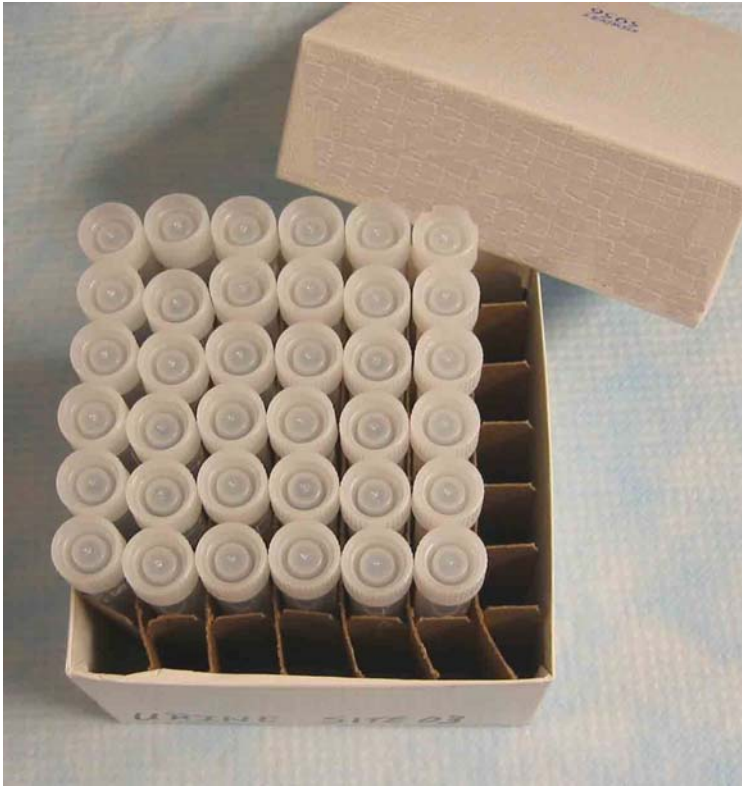
After centrifugation, the Serum tube is aliquoted as follows:

Serum tube #01: Aliquot the entire serum (approximately 2.0 mL) into transfer tube # 02.

Once the samples are aliquoted, the Serum transfer tube can be frozen in an upright position at -80C

Box maps (for local storage of samples at 17M, 23M, and unscheduled)

Sample from multiple participants' will be placed in one freezer box with 7 x 7 grid. Each participant will have one transfer tube per 17M, 23M, or unscheduled visit. Samples from different visits (17M, 23M, or unscheduled) can be placed in this box for monthly shipment.



4G. Processing Completion

Enclose the completed Phlebotomy/Processing Forms with each shipment of samples to the LCBR. (Be sure the Phlebotomy/Processing Forms are completely filled out.)

After a minimum of 2 hours at room temperature, make sure the Paxgene tubes are placed inside a 50 mL conical tube and put in a upright position at -20C.

Wipe down all work areas with 10% bleach solution (or approved biohazard disinfectant)

Section 5: 24 Hr Urine Collection: Visits BL, 12M, and 24M

Materials Needed

Individual void containers

Bulk containers (1 to 2 L size) preferably with covers

The transfer tubes for final aliquoting will be provided in the BL, 12M, and 24M kits.

Preparation and storage of boric acid

Boric Acid will be provided in bulk by the LCBR and shipped to sites prior to start of study. Boric Acid can be stored at room temperature with other non-flammable dry laboratory chemicals.

Forms

The purpose of the Urine Form is to facilitate the efficient collection of the 24 Hr urine samples from the participants, with maximum protection for the participant and the technician. In addition, the Urine Form facilitate the monitoring of collection and other quality assurance parameters and provide information critical to the interpretation of the assay results and maintenance of the sample repository. The completed Urine Collection/Processing Forms will be included in the sample shipments to LCBR.

Collection Scheme

Each time the patient voids during the overnight urine collection, the urine is poured into one large container (1 – 2 L) and refrigerated. The bulk container should be labeled with the sample ID number. The urine voids should be refrigerated (4° C) promptly (within 15 minutes) after removal from the patient's room.

At the completion of the 24 Hour period, the total volume of the urine is measured and recorded on the Urine Form.

The urine can now be processed for aliquoting.

Urine Processing

1. Be sure to mix the urine in the bulk container by gentle swirling before removing any urine for processing.
2. Remove 30 mL urine from the total volume and carefully add 5 mg Boric Acid (powder). Swirl gently to mix the urine and acid. This acidified urine will be aliquoted into three 10 mL transfer tubes. Pipet 9 mL of acidified urine into one 10 mL transfer tube then repeat this process for the next two tubes.

Table 13: Urine Aliquots

<u>Collection Flask</u>	<u>Collection Flask #</u>	<u>Transfer Tube #</u>	<u>Vol (mL)</u>	<u>Comments</u>
Urine	17	43	9.0	Repository
Urine	17	44	9.0	Repository
Urine	17	45	9.0	Repository
Urine Boric Acid	17	46	9.0	Repository
Urine Boric Acid	17	47	9.0	Repository
Urine Boric Acid	17	48	9.0	Repository

3. Aliquot three 9.0 mL samples of urine from container 17 into transfer tubes # 43, 44, and 45 and place on ice.
4. Aliquot three 9.0 mL samples of urine with boric acid into transfer tubes # 46, 47 and 48 and place on ice.
5. Freeze the samples in an upright position at -80° C or on dry ice within 10 minutes of aliquot preparation.
6. Discard left-over urine.
7. Complete processing form.

Box maps (local storage of transfer tubes at the sites)

The urine transfer tubes are stored with the blood and serum tubes collected from this same participant.

Section 6: Shipping of Biospecimens

Overview of Regulations

This shipping protocol follows the procedures mandated by the International Air Transport Association’s Dangerous Goods Regulations-Packaging Instructions 650 and 904.

Schedule for Clinics

Frozen samples are shipped monthly to LCBR by Federal Express priority overnight delivery. Samples are shipped on the following schedule.

Site	Ship Date
Pennington Biomedical	First Monday of each month
Tufts	Second Monday of each month
Wash U	Third Monday of each month

Shipping schedule changes may occur due to public holidays

Materials Needed

- Styrofoam shipping container*
- Rubber bands for freezer boxes
- Ziplock plastic bags for freezer boxes
- Absorbent material (i.e. paper towels, newspaper)
- Packaging tape
- Dry ice (~10 to 20 lbs per mailing container)
- Federal Express Labels*
- Biological Specimens Category B UN3373 labels*
- Dry Ice Labels (class 9, UN1845)*
- “Keep Frozen” labels*
- Labeled freezer boxes with participant samples*

Completed Phlebotomy/Processing Forms*
Completed Urine Collection/Processing Forms *
Completed Shipping Forms (to be included in shipment and faxed)*

*Provided by LCBR

Shipping Procedure

For frozen shipments to LCBR, University of Vermont:

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 Phlebotomy/Processing Forms and Urine Collection Forms 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, Dry Ice Class 9 UN1845 label, and Keep Frozen label) to outside of shipping container.
13. Add extra shipping tape over the labels to ensure they will not fall off in transit.

Packaging and Labeling (see illustrations in Appendix)

Mailing Address

University of Vermont
Department of Pathology
Colchester Research Facility, Room T205
208 South Park Drive, Suite 2
Colchester, VT 05446
Attn: Elaine Cornell
(802) 656-8963
(802) 656-8965 Fax

Notification of shipment

Fill out the Shipping Form including the Fedex airbill #s and fax to LCBR at the University of Vermont at (802) 656-8965. The Shipping Form is to be faxed the same day the samples are packaged and shipped. The Shipping Form lists all the participant sample sets contained in the shipment, by both their CALERIE ID# and the Sample ID#, and lists the Fed Ex tracking number for the shipment.

Appendix

Processing Guides
Phlebotomy and Processing Forms
Shipping Form
Packaging and Labeling Illustrations

Calerie – Blood and Urine Shipping Form

Date of shipment: ___/___/___ Center: _____ Prepared by:

FAX TO LCBR @: 802-656-8965 Number of Pages: _____

FedEx Air Bill#: _____

Sample Collection Date	Calerie ID	Sample ID
		(place label here)
		(place label here)
		(place label here)
		(place label here)
		(place label here)
		(place label here)

		(place label here)
--	--	--------------------

Date Received at LCBR: _____

Frozen: Y/N

APPENDIX: Forms

Calerie

PHLEBOTOMY / PROCESSING FORM - Arterialized Venous Collection For Catecholamines

CHECK VISIT:

BaseLine

12 Month

24 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

D	D	/	M	M	M	/	Y	Y	Y	Y

Blood Collection Date:

D	D	/	M	M	M	/	Y	Y	Y	Y

Phlebotomist Tech Initials:

First	M	Last

IV Needle size: gauge

IV Location: dorsal hand vein. If other, specify: _____

Is participant in the supine position? Yes No. If no, specify position: _____

BLOOD COLLECTION PROCEDURE

(EDTA Tubes contain 10% sodium metabisulfite-confirm expiration date)

1. Flush IV line with 4.0 mLs saline solution.

2. Collect 4.0 mL discard tube (serum).

3. Record Start Time of Collection (EDTA# 01): : AM
Hours Minutes

4. Was any blood collected? Yes No

5. Blood Volume in Tube: Filled
Yes No Partial Other (specify volume) Tube Handling
 Tube# 01 EDTA 5 mL _____ Mix 30 sec; Ice <30 mins

6. Start Timer – Collect EDTA# 02 in 5 minutes.

7. Flush IV line with 4.0 mLs saline solution.

8. Collect 4.0 mL discard tube (serum).

9. Record Start Time of Collection (EDTA# 02): : AM
Hours Minutes

10. Was any blood collected? Yes No

12. Blood Volume in Tube: Filled
Yes No Partial Other (specify volume) Tube Handling
 Tube# 02 EDTA 5 mL _____ Mix 30 sec. Ice <30 mins.

PROCESSING (Immediately!)

Centrifuge Start Time: : AM
(EDTA Tubes 01 and 02) Hours Minutes

Processor Tech Initials:
First M Last

Draw Tube#	Draw Tube	Sample Type	Purpose	Transfer tube size	Transfer Tube #	Sample Vol. mL	<input checked="" type="checkbox"/> If Hemolysis	<input checked="" type="checkbox"/> If Done
01	EDTA	Plasma	Nor Epi 1	10.0 mL	20	~2.5		
02	EDTA	Plasma	Nor Epi 2	10.0 mL	21	~2.5		

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ____ Yes ____ No

Calerie

PHLEBOTOMY FORM - Fasting Blood Collection

CHECK VISIT:

BaseLine

12 Month

24 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

		/				/				
D	D		M	M	M		Y	Y	Y	Y

Blood Collection Date:

		/				/				
D	D		M	M	M		Y	Y	Y	Y

MALE

FEMALE

If FEMALE, did an off-cycle hormone draw occur? Yes No

Phlebotomist Tech Initials:

--	--	--

First M Last

IV Needle size:

--	--

gauge.

IV Location: Antecubital vein.

If other, specify: _____

FASTING STATUS

Date of Last Meal:

		/				/				
D	D		M	M	M		Y	Y	Y	Y

Time of Last Meal:

		:			AM or PM
Hours			Minutes		

BLOOD COLLECTION

1. Flush IV line with 4.0 mLs saline
2. Collect 4.0 mL discard tube (serum)

3. Record Start Time (start of filling Serum# 03):

		:			AM
Hours			Minutes		

4. Record End Time (finish filling of Paxgene# 11):

		:			AM
Hours			Minutes		

5. Was any blood collected? Yes No

6. Blood Volume per Tube:

Fasting draw:

Tube# 03 Serum 10 mL

Tube# 04 Serum 10 mL

Tube# 05 Serum 10 mL

Tube# 06 Serum 10 mL

Tube# 07 Citrate 3.0 mL

Tube# 08 EDTA 10 mL

Tube# 09 EDTA 10 mL

Tube# 10 Paxgene 2.5 mL

Tube# 11 Paxgene 2.5 mL

Filled

Yes

No

Partial

Other

(specify volume)

Tube

Handling

Room Temp 40 mins

Room Temp 40 mins

Room Temp 40 mins

Room Temp 40 mins

Mix 30 sec; Ice < 15 mins, 30 min max

Mix 30 sec; Ice < 15 mins, 30 min max

Mix 30 sec; Ice < 15 mins, 30 min max

Invert 8-10 times; RT >2 hrs

Invert 8-10 times; RT >2 hrs

Comments:

Calerie

PROCESSING FORM - Fasting Blood Collection

CHECK VISIT:

BaseLine

12 Month

24 Month

Calerie ID#:

Sample ID#:

(Affix SampleID label here)

Processing Date:

		D	D	/				M	M	M	/				Y	Y	Y	Y
		D D						M M M							Y Y Y Y			

PROCESSING

1. Centrifuge Start Time 1: : AM
(EDTA and Citrate) Hours Minutes

1. Processor Tech Initials:
First M Last

#	Draw Tube	Purpose	Cryo/Transfer tube size (mL)	Cryo/Tube #	Sample Vol. mL	Color Code	✓ If P*	✓ If H*	✓ If Done
07	Citrate	Citrate plasma cryo to Repos	1.5	29	0.5	Blue			
		Citrate plasma cryo to Repos	1.5	30	0.5	Blue			
08	EDTA	EDTA plasma cryo to Repos	1.5	31	1.0	Purple			
		EDTA plasma cryo to Repos	1.5	32	1.0	Purple			
		EDTA plasma cryo to Repos	1.5	33	1.0	Purple			
		Remaining EDTA plasma	10.0	34	~ 2.0	White	N/A		
		Packed Cells	10.0	35	~ 5.0	White	N/A		
09	EDTA	All EDTA plasma	10.0	36	~ 5.0	White	N/A		
		Packed Cells	10.0	37	~ 5.0	White	N/A		

** P = partial volume in cryovial; *H = hemolysis*

2. Centrifuge Start Time 2: : AM or PM
(Serum tubes) Hours Minutes

2. Processor Tech Initials:
First M Last

#	Draw Tube	Purpose	Cryo/Transfer tube size (mL)	Cryo/Tube #	Sample Vol. mL	Color Code	✓ If P*	✓ If H*	✓ If Done
03	Serum	Serum cryo to Repos	1.5	22	1.0	Red			
		Serum cryo to Repos	1.5	23	1.0	Red			
		Serum cryo to Repos	1.5	24	1.0	Red			
		Remaining serum	10.0	25	~ 2.0	Clear	N/A		
04	Serum	All serum	10.0	26	~ 5.0	Clear	N/A		
05	Serum	All serum	10.0	27	~ 5.0	Clear	N/A		
06	Serum	All serum	10.0	28	~ 5.0	Clear	N/A		

** P = partial volume in cryovial; *H =*

hemolysis

10	Paxgene	Leave in collection tube. Do not centrifuge. RT >2 hrs but <72 hrs before freezing @ -20	
11	Paxgene	Leave in collection tube. Do not centrifuge. RT >2 hrs but <72 hrs before freezing @ -20	

Comments:

For LCBR use only:

Date Samples Received: ___/___/___

Samples Received Frozen: ___ Yes ___ No

Calerie

PHLEBOTOMY / PROCESSING FORM – OGTT Collections

CHECK VISIT:

BaseLine

12 Month

24 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

/ /

Blood Collection Date:

/ /

Glucola Completion Time:

: AM
Hours Minutes

Phlebotomist Tech Initials:

First M Last

30 Minute OGTT Collection

1. Flush IV line with 4.0 mLs
2. Collect 4.0 mL discard tube (serum).
3. Record Start Time of Serum Tube# 12 Blood Collection: : AM
Hours Minutes
4. Was any blood collected? Yes No
5. Blood Volume in Tube: Filled Yes No Partial (specify volume)
Tube #12. Serum 4 mL _____

Tube Handling
Room Temp 40 minutes MAX

60 Minute OGTT Collection

1. Flush IV line with 4.0 mLs
2. Collect 4.0 mL discard tube (serum).
3. Record Start Time of Serum Tube# 13 Blood Collection: : AM
Hours Minutes
4. Was any blood collected? Yes No
5. Blood Volume in Tube: Filled Yes No Partial (specify vol.)
Tube #13 Serum 4 mL _____

Tube Handling
Room Temp 40 minutes MAX

90 Minute OGTT Collection

1. Flush IV line with 4.0 mLs
2. Collect 4.0 mL discard tube (serum).
3. Record Start Time of Serum Tube# 14 Blood Collection: : AM
Hours Minutes
4. Was any blood collected? Yes No
5. Blood Volume in Tube: Filled Yes No Partial (specify volume)
Tube #14 Serum 4 mL _____

Tube Handling
Room Temp 40 minutes MAX

120 Minute OGTT Collection

1. Flush IV line with 4.0 mLs
2. Collect 4.0 mL discard tube (serum).
3. Record Start Time of Serum Tube# 15 Blood Collection: : AM
Hours Minutes
4. Was any blood collected? Yes No
5. Blood Volume in Tube: Filled Yes No Partial (specify volume)
Tube #15 Serum 4 mL _____

Tube Handling
Room Temp 40 minutes MAX.

PROCESSING

1. Processor Tech Initials:
First M Last

2. Processor Tech Initials:
First M Last

#	Draw Tube	Centrifuge Start Time Hours: Minutes	Purpose	OGTT Tube #	Sample Vol. mL	✓ If H*	✓ If Done
12	Serum	____ : ____ AM or PM	30 min OGTT	38	~2.0		
13	Serum	____ : ____ AM or PM	60 min OGTT	39	~2.0		
14	Serum	____ : ____ AM or PM	90 min OGTT	40	~2.0		
15	Serum	____ : ____ AM or PM	120 min OGTT	41	~2.0		

* H = hemolysis

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ____ Yes ____ No

Calerie

PHLEBOTOMY / PROCESSING FORM – Day 2 Collection

Calerie

PHLEBOTOMY / PROCESSING FORM

CHECK VISIT:

3 Month

6 Month

18 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

/ /
D D M M M Y Y Y Y

Blood Collection Date:

/ /
D D M M M Y Y Y Y

Phlebotomist Tech Initials:

First M Last

BLOOD COLLECTION (venipuncture)

1. Venipuncture needle size: gauge

2. How long ago did you last eat or drink anything other than water? Hours.

3. Record Start Time of Serum Tube# 01 Collection: : AM or PM
Hours Minutes

4. Was any blood collected? Yes No

5. Blood Volume per Tube:

Fasting draw:	<u>Yes</u>	<u>No</u>	<u>Filled</u>	<u>Other</u>	<u>Tube</u>
Tube# 01 Serum 10.0 mL	___	___	<u>Partial</u>	<u>(specify volume)</u>	<u>Handling</u>
Tube# 02 EDTA 10.0 mL	___	___	___	___	Room Temp 40 mins.
					Mix 30 sec; Ice <30 mins.

PROCESSING

Centrifuge Start Time: : AM or PM
(EDTA# 02) Hours Minutes

Processor Tech Initials:

First M Last

#	Draw Tube	Purpose	Cryo size (mL)	Cryo #	Color Code	Sample Vol. mL	✓ If P*	✓ If H*	✓ If Done
02	EDTA	EDTA plasma cryo to Repos	1.5 mL	07	Purple	1.0			
		EDTA plasma cryo to Repos	1.5 mL	08	Purple	1.0			
		EDTA plasma cryo to Repos	1.5 mL	09	Purple	1.0			
		EDTA plasma cryo to Repos	1.5 mL	10	Purple	~1.0 +			

* P = partially filled cryovial; H = hemolysis

Centrifuge Start Time: : AM or PM
(Serum# 01) Hours Minutes

Processor Tech Initials:

First M Last

#	Draw Tube	Purpose	Cryo size (mL)	Cryo #	Color Code	Sample Vol. mL	✓ If P*	✓ If H*	✓ If Done
01	Serum	Serum cryo to Repos	1.5 mL	03	Red	1.0			
		Serum cryo to Repos	1.5 mL	04	Red	1.0			
		Serum cryo to Repos	1.5 mL	05	Red	1.0			
		Serum cryo to Repos	1.5 mL	06	Red	~1.0 +			

* P = partially filled cryovial; H = hemolysis

Comments:

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ____ Yes ____ No

Calerie

PHLEBOTOMY / PROCESSING FORM

CHECK VISIT: 17 Month 23 Month **Unscheduled (Progesterone-women only)**

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

/ /
D D M M M Y Y Y Y

Blood Collection Date:

/ /
D D M M M Y Y Y Y

Phlebotomist Tech Initials:

First M Last

BLOOD COLLECTION (venipuncture)

1. Venipuncture needle size: gauge

2. How long ago did you last eat or drink anything other than water? Hours

4. Record Start Time of Serum Tube# 01 Collection: : AM or PM
Hours Minutes

4. Was any blood collected? Yes No

5. Blood Volume per Tube:

Fasting draw: Yes No Filled Other Tube
Tube# 01 Serum 4.0 mL _____ Partial (specify volume) Handling
Room Temp 40 minutes

PROCESSING

Centrifuge Start Time: : AM or PM
(Serum# 01) Hours Minutes

Processor Tech Initials:

First M Last

#	Draw Tube	Purpose	Transfer tube size (mL)	Transfer Tube #	Sample Vol. mL	✓ If H*	✓ If Done
01	Serum	All serum	10.0	02	~2.0		

*H = hemolysis

Comments:

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ____ Yes ____ No

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ___ Yes ___ No

Calerie

MUSCLE and ADIPOSE TISSUE BIOPSY FORM

CHECK VISIT: BaseLine 12 Month 24 Month

CHECK SITE: Tufts Pennington Washington

Calerie ID:

Sample ID:

(Affix Sample ID label here)

DOB: / /
D D M M M Y Y Y Y

DATE OF BIOPSIES: / /
D D M M M Y Y Y Y

SKELETAL MUSCLE BIOPSY

Tissue samples collected:

(Enter weight of samples in mg)

- 01
- 02 (IHC Mounting sample)
- 03
- 04
- 05
- 06
- 07
- 08

ADIPOSE TISSUE BIOPSY

Tissue samples collected:

(Enter weight of samples in mg)

- 21
- 22
- 23
- 24 (Bouin's Cassette sample)
- 25
- 26
- 27
- 28
- 29
- 30

BIOPSY PERFORMED BY (please print):

Physician/Practitioner: _____ Assistant: _____

Sample Prep by: _____

Date Samples shipped to Vermont: ____/____/____ Date Form Faxed to Vermont: ____/____/____

Comments:

For LCBR use only:

Date Samples Received: ___/___/___

Samples Received Frozen: ___ Yes ___ No

Calerie

PHLEBOTOMY / PROCESSING FORM – Off-Cycle Visit Day 1 Collection Sex Hormone Collection (women only)

CHECK VISIT:

BaseLine

12 Month

4 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

/ /
D D M M M Y Y Y Y

Blood Collection Date:

/ /
D D M M M Y Y Y Y

Phlebotomist Tech Initials:

First M Last

BASELINE HORMONE COLLECTION (Venipuncture)

1. Needle size : gauge

2. Record Start Time of Serum Tube# 03 Collection: : AM or PM
Hours Minutes

3. Was any blood collected? Yes No

4. Blood Volume in Tube: Filled

Yes No Partial Other (specify volume) Tube Handling

Tube# 03 Serum 4 mL ___ ___ ___ ___ Room Temp 40 minutes

60 MINUTE HORMONE COLLECTION (Venipuncture)

1. Needle size : gauge

2. Record Start Time of Serum Tube# 13 Collection: : AM or PM
Hours Minutes

3. Was any blood collected? Yes No

4. Blood Volume in Tube: Filled

Yes No Partial Other (specify volume) Tube Handling

Tube# 13 Serum 4 mL ___ ___ ___ ___ Room Temp 40 minutes

PROCESSING

Processor Tech Initials:

First M Last

#	Draw Tube	Centrifuge Start Time Hours: Minutes	Purpose	Transfer Tube #	Sample Vol. mL	<input checked="" type="checkbox"/> If H*	<input checked="" type="checkbox"/> If Done
03	Serum	___ : ___ AM or PM	Baseline Hormone	25	~2.0		
13	Serum	___ : ___ AM or PM	60 min Hormone	39	~2.0		

* H = hemolysis

Comments:

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ___ Yes ___ No

Calerie

PHLEBOTOMY / PROCESSING FORM – Off-Cycle Visit-Day 2 Collection Sex Hormone Collection (women only)

CHECK VISIT:

BaseLine

2 Month

4 Month

Calerie ID#:

Sample ID#:

(Affix Sample ID label here)

DOB:

/ /

D D M M M Y Y Y Y

Blood Collection Date:

/ /

D D M M M Y Y Y Y

Phlebotomist Tech Initials:

First M Last

BLOOD COLLECTION (Venipuncture)

1. Needle size : gauge

2. Record Start Time of Serum Tube# 16 Collection: : AM or PM

Hours Minutes

3. Was any blood collected? Yes No

4. Blood Volume in Tube: Filled Yes No Partial Other (specify volume) Tube Handling

Tube# 16 Serum 4 mL _____ _____ _____ _____ _____ Room Temp 40 minutes

PROCESSING

Processor Tech Initials:

First M Last

Centrifuge Start Time: : AM or PM

Hours Minutes

#	Draw Tube	Purpose	Transfer tube size (mL)	Transfer Tube #	Sample Vol. mL	✓ If H*	✓ If Done
16	Serum	Day 2 Sex Hormones – all serum	10.0	42	~2.0		

* H = hemolysis

Comments:

For LCBR use only:

Date Samples Received: ____/____/____

Samples Received Frozen: ____ Yes ____ No