Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE)
Laboratory for Clinical Biochemistry Research, University of Vermont
Manual of Operations

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I. STUDY OVERVIEW AND DESCRIPTION

The overall aim of CALERIE Phase 2 is to test the hypothesis that two years of sustained caloric restriction (CR), involving a reduction in energy intake to 75% of baseline (25% CR), in healthy men and women aged 25 to 45, will result in the same adaptive changes that occur in rodents subjected to CR. Particular emphasis on the adaptive responses thought to be involved in slowing the aging process and protecting against age-related disease processes. Primary outcomes include core body temperature and resting metabolic rate. Secondary outcomes include triiodothyronine and catecholamines (as potential mediators of the predicted metabolic adaptation), and plasma concentrations of TNF-α (because inflammation is one of the adaptive responses suggested as a mediator of the salutary effects of CR on the aging process in rodents). An important secondary aim is to identify potential adverse effects of CR in humans. A number of exploratory aims will be assessed to evaluate the effect of CR on body composition, serum hormones, plasma growth factor concentrations, serum lipid and lipoprotein levels, skeletal muscle, adipose tissue and psychological factors. Consistency between the two sexes and across levels of body composition will be explored. In addition, biological samples will be stored in a biosample repository for future analysis.

Basic Study Design: The study will be conducted as a multi-center, parallel-group, randomized, controlled trial (RCT). A sample of 250 participants will be enrolled, and assigned to either the CR intervention or an *ad libitum* (AL) control group. A 2:1 allocation ratio in favor of the CR intervention will be applied in order to maximize the number of subjects receiving the intervention of greater scientific interest. Participants in both treatment arms will be followed over a period of 24 months. A comprehensive set of evaluations will be performed prior to initiating the intervention, with follow-up evaluations at Months 1, 3, 6, 9, 12, 18 and 24 after randomization. It is expected that 10% of study subjects will drop-out in each of the two follow-up years, so that a sample of approximately 200 subjects is expected to complete the study.

The Laboratory for Clinical Biochemistry Research (LCBR) at the University of Vermont is the Central Biochemistry Lab for this project. This role includes:

- Oversee specimen collection at three sites and shipment of samples from the clinical sites to LCBR.
- Create and maintain the CALERIE Phase 2 Biosample Repository.
- Measure markers of inflammation, hormone levels, growth factors, and other analytes as specified.
- Manage shipping of samples (e.g., tissue biopsies) to alternate testing sites as specified.
- Provide QA/QC assurances on the above.

II. SUMMARY OF SAMPLE COLLECTION

Timepoint	Visit#	Summary	Collection method	Comment
BL	7	'Hot Box' for catecholamines (3 collections)	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin (plus Progesterone for Females)	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	
	8	Day 2: Females for Progesterone	IV or venipuncture	Plus tissue kits
Off-Cycle (F only)	BL,12M, 24M	baseline, 1H, and Day 2 Progesterone	venipuncture	No repository
3M	1	Fasting: blood/urine archive	Venipuncture	EDTA/Serum
6M	5	Fasting: blood/urine archive & bone measurements	Venipuncture	EDTA/Serum
12M	4	'Hot Box' for catecholamines 3 collections	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin (plus Progesterone for Females)	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	
	5	Day 2: Females for Progesterone	IV or venipuncture	Plus tissue kits
18M	1	Fasting: blood/urine archive plus AB response	Venipuncture	EDTA/Serum
24M	4	'Hot Box' for catecholamines 3 collections	IV	
		Fasting: All measurements & blood/urine archive	IV	Plus 24H urine
		30 min OGTT, CPEP, Insulin	IV	
		60 min OGTT, CPEP, Insulin (plus Progesterone for Females)	IV	
		90 min OGTT, CPEP, Insulin	IV	
		120 min OGTT, CPEP, Insulin	IV	
	5	Day 2: Females for Progesterone	IV or venipuncture	Plus tissue kits
17M, 23M		Antibody response	Venipuncture	No repository
Unscheduled		Sex hormones for amenorrhea (LH, FSH, Estradiol)	Venipuncture	No repository

III. BIOSPECIMEN KIT PREPARATION

Introduction
The Laboratory for Clinical Biochemistry Research is responsible for supplying the CALERIE study field centers with supplies needed for the blood draw and urine collection sections of participant visits throughout the 24 month exam period. Kits are specific to visits and CALERIE field centers. The Baseline, 12M and 24M visits are also specific to male or female. The following is a protocol for assembly of the CALERIE blood and urine collection kits needed at each of the nine potential visits that occur over the duration of the study.

A. Labels

Tube, cryovial, and form labels
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.

Example:

The format of the labels will be: XYYY-00-ZZ ex. 1234-00-02
Labels for forms will be XYYY-00 (sample-visit) – no tube #

Site #	Sample #	Visit	Tube/cryo #	comments
X	YYY	00	ZZ	
1= Pennington 2= Tufts 3= Wash U		03		
		06		
		12		
		17		
		18		
		23		
		24		
		99		99=Unscheduled Visit
		80		80=Baseline Tissue
		82		82=12M Tissue
		84		84=24M Tissue
		70		70=Baseline Off-cycle hormone
		72		72=Baseline Off-cycle hormone
		74		74=Baseline Off-cycle hormone

Printing a new tube, cryovial, and form label set

The label-printing database is called 'CALERIELabelPrinting.adp' and located in folder \\Med15\Shared\Groups\LCBR\Databases\CALERIE. This will work only on computers having BarTender installed.

1. On the main menu, press the *'Print Vial Labels'* button (at this point, box label printing isn't set up correctly yet, so the 'Print Box Label' button is disabled). On the dialog that pops up, you need to make a number of selections.

2. Select the label printer. You'll need the 5-across labels loaded (These are already set up on the old label printer by Dean's desk, or they may be loaded onto the Zebra printer in T209).

3. Select the Label Group. You'll want to select *Kit labels* here.

4. Select which visit you want labels for.

5. Select which labels you want. By default, the 'Print all labels shown below' option is selected, which will print the full set of labels shown in the Label Selection listbox. But you can clear the checkbox and select individual labels if you wish.

6. Specify the Kit ID Range. Note that there's no site selection since the site is implied by the Kit IDs.

7. Then press the Print button.

Kit component labels

Each kit will be labeled on the outside with the visit, Participant ID, site where the visit will occur, participant gender, and an expiration date of the kit. For the baseline, 12M and 24M visits the individual draw specific Ziplocs that make up the Kit will also have there own labels. These draw specific labels will include the participant ID, site, participant sex, and the specific collection to which the supplies pertain.

Word document templates for these labels are stored in:
\\Med15\Shared\Groups\LCBR\Databases\CALERIE\Labels

Highlight the appropriate kit label info and print labels from Word onto Avery 5162 white mailing labels.

B. Annual (baseline, 12M, and 24M) visit kit preparation

Kits are Male/Female specific - female participants will have a 5th OGTT tube (#16) drawn on day 2 of the Baseline, 12M and 24M visits for progesterone level testing. Items noted with an * asterisk will differ between male and female kits.

Kits are also specific to site and visit. Be sure to use labels with appropriate site and visit ID.

Supplies needed prior to assembly:

- Baseline, 12M or 24M Label Set –next available participant ID* needed at site (see printing label set instructions above)
- Forms – "Hot Box" draw P/P Form (1), Fasting draw P/P forms (2), OGTT draw P/P form (1), Day 2 OGTT draw P/P form* (1), Urine Collection P/P form (1)
- Ziploc bags- 2-Gallon (1), 1-Gallon (5), quart (1), pint (4)
- Draw tubes- 4mL Serum (5*), 10mL Serum (4), 10mL EDTA (2), 2.7mL Citrate (1), and 2.5mL Paxgene (2)
- Transfer Tubes (16) - 10mL white-capped Simport tubes
- OGTT tubes (5*) – 4mL Sarstedt tubes (FAHC tubes)
- 50mL Corning polypropylene tubes
- Cryovials 1.5mL skirted with color-coded caps – red-capped (3), purple-capped (3), blue-capped (2)
- 250mL Absorbent Strips (1)
- Kit Content Labels (6*) – Labels specific to entire kit and 5* draw specific components within kit.

Catecholamine/Hot-Box Draw:

1. Take out supplies needed for hot box draw:
    - 1 - One gallon Ziploc bag
    - 2 - 10mL Transfer tubes
    - "Hot-box" draw P/P form (1)

2. Affix appropriate labels to the transfer tube (20 and 21) and a form label to the P/P form.
3. Affix kit component label to the outside of the 1 gallon Ziploc.
4. Fold P/P form in half and put form and both transfer tubes into the 1 gallon Ziploc.
5. Put sealed Ziploc into the 2 Gallon Ziploc which will hold the entire kit.

Fasting Draw:

1. Take out supplies needed for the fasting draw:
    - 1 - One gallon Ziploc bag
    - 2 - Pint Ziploc bags
    - 1 - Quart Ziploc bag
    - 3 - red-capped cryovials (cryo #22, 23, and 24)
    - 3 - purple-capped cryovials (cryo #31,32, and 33)
    - 2 - blue-capped cryovials (cryo #29 and 30)
    - 8 - 10mL transfer tubes (tube #25, 26, 27, 28, 34, 35, 36, and 37)
    - 4 - 10mL Serum draw tubes (draw tubes #3, 4, 5 and 6)
    - 2 - 10mL EDTA draw tubes (draw tubes #8 and 9)
    - 1 - 2.7mL Citrate draw tubes (draw tubes #7)
    - 2 - 2.5mL PAXgene draw tubes (draw tubes #10 and 11)
    - 2 - 50mL Corning polypropylene tubes
    - 1 - Fasting draw phlebotomy form
    - 1 - Fasting draw processing form
2. Affix appropriate labels to the draw tubes, cryovials, and transfer tubes (see step 1 for cryo and tube #'s) and a form label to the P/P form.
3. Put all blood draw tubes in one pint Ziploc bag. Put cryovials and transfer tubes into the other pint Ziploc bag.
4. Put each labeled PAXgene tube into a 50mL Corning tube. Put both Paxgene tubes into the quart Ziploc bag.
5. Affix kit component label to the outside of the 1 gallon Ziploc.
6. Fold P/P forms in half and put forms, both pint Ziplocs, and the quart Ziploc into the 1 gallon Ziploc.
7. Put sealed Ziploc into the 2 Gallon Ziploc with the "Hot-box" draw supplies.

OGTT Draw:

1. Take out supplies needed for OGTT draw:
    - 1 - One gallon Ziploc bag
    - 4 - 4mL Serum draw tubes (draw tube #'s 12, 13, 14, and 15)
    - 4 - OGTT tubes (tube #'s 38, 39, 40, and 41)
    - 1 - pint Ziploc bag
    - OGTT draw P/P form (1)
2. Affix appropriate labels to the draw tubes, and OGTT tubes (see step 1 for cryo and tube #'s) and a form label to the P/P form.
3. Put all blood draw tubes and OGTT tubes into pint Ziploc bag.

4. Affix kit component label to the outside of the 1 gallon Ziploc.
5. Fold P/P form in half and put form and pint Ziploc into the 1 gallon Ziploc.
6. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with the fasting and hot-box draw supplies.

Day 2 Female Sex Hormone draw* (female participants/kits only):

1. Take out supplies needed for the Day 2 OGTT draw:
    - 1 - One gallon Ziploc bag
    - 1 - 4mL Serum draw tube (#16)
    - 1 - OGTT tube (# 42)
    - Day 2 OGTT draw P/P form (1)
2. Affix appropriate labels to the draw tube (draw tube # 16) and OGTT tube (tube # 42) and a form label to the P/P form.
3. Affix kit component label to the outside of the 1 gallon Ziploc.
4. Fold P/P form in half and put form, OGTT tube #42, and draw tube #16 into the 1 gallon Ziploc.
5. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with the hot-box, fasting, and OGTT draw supplies.

24 Hr Urine Collection:

1. Take out supplies needed for 24 Hr Urine collection:
    - 1 - One gallon Ziploc bag
    - 6 - 10mL transfer tubes (tube #'s 43, 44, 45, 46, 47, and 48)
    - 1 - pint Ziploc bag
    - 24 Hr Urine Collection and Processing form (1)
2. Affix appropriate labels to the transfer tubes (see step 1 for cryo and tube #'s) and a form label to the P/P form.
3. Affix kit component label to the outside of the 1 gallon Ziploc.
4. Fold P/P form in half and put form and transfer tubes into the 1 gallon Ziploc.
5. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with all the other visit supplies.
6. Affix Kit contents label to outside of 2 gallon Ziploc. This label will include participant ID, male/female, visit time-point, expiration date of kit, and field center

Off-Cycle Female Sex Hormone Collection:

1. Take out supplies needed for an Off-Cycle Female Sex Hormone Draw:
    - 1 - Two gallon Ziploc bag
    - 2 - One gallon Ziploc bags
    - 3 - 4mL Serum draw tubes (#03, #13, and #16)
    - 2 - 4mL OGTT tubes (tube #'s 25 and 39)
    - 1 - 10mL transfer tube (tube # 42)

- Off-Cycle Visit Day 1 Phlebotomy and Processing form (1)
- Off-Cycle Visit Day 2 Phlebotomy and Processing form (1)

2. The Off-Cycle kit has two components: 1) Day 1 sex hormone collection and 2) Day 2 sex-hormone collection supplies.
3. Affix appropriate labels to the draw tubes, transfer tubes, OGTT tubes (see step 1 for cryo and tube #'s) and a form label to each P/P form.
4. Affix the appropriate kit component label to the outside of each of the 1 gallon Ziplocs (1 label for Day 1 collection and another label for the Day 2 collection).
5. Fold P/P form in half and put form and transfer tubes into the 1 gallon Ziploc.
6. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with all the other visit supplies.
7. Affix Kit contents label to outside of 2 gallon Ziploc. This label will include female participant ID, visit time-point, expiration date of kit, and field center.

Tissue Biopsy Kits:

Tissue biopsy kits are site specific. The Tufts University field center will be collecting 4 extra muscle tissue biopsy samples that the other two sites will not be collecting.

1. Take out supplies needed for Tissue biopsy kit:
   - 1 - One-gallon Ziploc bag
   - 2 - Pint Ziploc bags
   - 1 - Tissue cassette (sample #24)
   - 1 - 4 oz Corning Snap-Seal container
   - 1 - 5mL vial (sample #02)
   - 12 (16 for Tufts kit) - 2mL Corning cryovials
     - Sample #'s 01, 03, 08, 21, 22, 23, 25-30 (PBRC and Wash. U)
     - Sample #'s 01, 03-08, 21, 22, 23, 25-30 (Tufts)
2. The Tissue biopsy kit has two components: 1) Muscle and 2) Adipose biopsy supplies
3. Use the special pen to write the kit ID directly on the cassette.
4. Affix appropriate labels to the cryovials (see step 1 for sample #'s)
5. Affix kit component label to the outside of each the 1 gallon Ziploc.
6. Fold P/P form in half and put form and transfer tubes into the 1 gallon Ziploc.
7. Put sealed 1 gallon Ziploc into the 2 gallon Ziploc with all the other visit supplies.
8. Affix Kit contents label to outside of 2 gallon Ziploc. This label will include participant ID, visit time-point, and field center. (Note: this kit will not have an expiration, date as there are no components included with an out-date.)

C. 3M, 6M and 18M Kit preparation

The supplies needed for these visits are site and visit specific, but do not differ between male and female.

Supplies needed prior to assembly:

- 3M, 6M, or 18M Label Set –next available participant ID needed at site

- Forms - "3M, 6M, 18M visit" P/P Form (1)
- Ziploc bags - 1 Gallon (1) and quart (1)
- Draw tubes - 10mL Serum (1), 10mL EDTA (1)
- Cryovials 1.5mL skirted w/ color-coded caps - red-capped (4), purple-capped (4)
- 250mL Absorbent Strips (1)
- Kit label (includes site, visit, participant ID, and expiration date of kit)

1. Take out supplies needed for the kit you are preparing (see supplies list above).
2. Affix appropriate labels to the draw tubes, cryos, and a form label to the P/P form.
3. Put labeled draw tubes and cryos into the quart size Ziploc.
4. Fold P/P form in half and put form, quart Ziploc (with tubes and cryos), and an absorbent strip into the 1 gallon Ziploc.
5. Affix Kit contents label to outside of 1 gallon Ziploc. This label will include participant ID, male/female, visit time-point, expiration date of kit, and field center

D. 17M, 23M and Unscheduled Visit Kit Preparation

The supplies needed for these visits are site and visit specific, but do not differ between male and female.

Supplies needed prior to assembly:

- 17M, 23M, or unscheduled visit Label Set –next available participant ID needed at site
- Forms - "17M, 23M, or unscheduled visit" P/P Form (1)
- Ziploc bags - 1-Gallon (1) and quart (1)
- Draw tubes - 10mL Serum (1)
- 10mL Transfer tube (1)
- 250mL Absorbent Strips (1)
- Kit label (includes site, visit, participant ID, and expiration date of kit)

1. Take out supplies needed for the kit you are preparing (see supplies list above).
2. Affix appropriate labels to the draw tube (#01), transfer tube (#02), and a form label to the P/P form.
3. Put labeled draw tube and transfer tube into the quart size Ziploc.
4. Fold P/P form in half and put form, quart Ziploc (with tubes), and an absorbent strip into the 1 gallon Ziploc.
5. Affix Kit contents label to outside of 1 gallon Ziploc. This label will include participant ID, male/female, visit time-point, expiration date of kit, and field center.

E. Preparation of EDTA tubes with 10% Sodium Metabisulfite Additive

Catecholamine concentrations will be measured in ***arterialized venous blood***. This blood draw will be performed as part of the CALERIE Study **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. Blood will be injected via syringe into EDTA tubes containing a 10% sodium metabisulfite additive. The

LCBR will prepare these tubes and ship as requested by the sites. As prepared, the EDTA-Sodium Metabisulfite tubes have an outdate period of 30 days.

Prepare a 10% solution of Sodium metabisulfite as follows:

1. Measure out 500mL of de-ionized water into a 1000mL beaker.
2. Use scale to measure out 50mg of powdered sodium metabisulfite (located on dry chemical shelf behind Mary Ellen's workbench).
3. Add 50mg of sodium metabisulfite to 500mL water.
4. Stir on stir plate with a small stir bar until powder dissolves into solution.
5. Label bottle with date prepared, contents and tech initials (Example: 10% Sodium metabisulfite prepared 3/26/07 by RHB). Please store prepared solution in the basket labeled "CALERIE Hot Box Tube supplies" located on the second shelf above the Phlebotomy bench in T205.
6. Alert Rebekah that solution has been made so she can make labels to affix to draw tubes when they are prepared.

Note: This solution will expire after 30 days.

Inject EDTA tubes with 10% sodium metabisulfite as follows:

At the phlebotomy bench in T205, you'll find a basket labeled "CALERIE Hot Box Tube supplies." In this basket you will find:

- ✓ 1cc Tuberculin syringes
- ✓ 10% sodium metabisulfite solution
- ✓ 6mL EDTA tubes
- ✓ Labels for prepared draw tubes with expiration date (see Rebekah if none are available)

Additional supplies needed:

- ✓ P100 pipette and tips
- ✓ 100µL conical microcentrifuge tubes

* Size of microcentrifuge tubes and pipettes used will vary based on quantity of tubes being prepared at once. Because of the 30 day outdate on these tubes once prepared, plan to send about 10 tubes at a time to the sites or as requested.

1. Set up 6mL EDTA tubes to be filled in a rack
2. Aliquot 30µL of 10% sodium metabisulfite solution into a 100µL microcentrifuge tube.
3. Open a tuberculin syringe from wrapping.
4. Draw up 30µL (.03cc) sodium metabisulfite from microcentrifuge tube into syringe.
5. Inject syringe into rubber stopper of 6mL EDTA vacutainer tube. Vacutainer suction will draw the 30µL of solution from syringe into draw tube.
6. Repeat for the remaining 9 draw tubes. Same syringe can be used for all tubes prepared that day.
7. Dispose of syringe in sharps container.

8. Affix a sodium metabisulfite label to the prepared draw tubes (see Rebekah for labels if none are in the supply basket).

F. Packaging and Shipping of the CALERIE Kits and Supplies to the Field Centers

Upon request from the sites, we will be sending kits and other supplies listed below:

Shipped together from LCBR:

- ✓ Boric Acid
- ✓ 4mL Serum tubes (discard tubes)
- ✓ Transfer pipettes
- ✓ Shipping labels - IATA 650 Category B-Biological Substances, UN1845 dry ice, and "keep frozen."

Shipped separately:

- 6mL EDTA tubes with 10% sodium metabisulfite (prepared on pre-determined schedule)
- Thermosafe Insulated Shippers (6/case, sent directly from manufacturer to sites)

CALERIE Site Supply Shipping Addresses

Attn: Liz Soroe (blood/urine kits) Attn: Stacy Carling (Tissue kits)
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808

Phone number: (225) 763-3047 Email: Elizabeth.Soroe@pbrc.edu
OR Stacy.Carling@pbrc.edu

Attn: Stephanie Leon
Tufts University
USDA Human Nutrition Research Center on Aging
711 Washington St.
Boston, MA 02111
Phone: (617) 556-3143 Email: stephanie.leon@tufts.edu

Attn: Morgan Schram
Washington University School of Medicine
Division of Applied Physiology
660 S. Euclid Ave.
Campus Box 8113
St. Louis, MO 63110
Phone: (314) 747-3182 Email: mschram@im.wustl.edu

IV. PROCEDURES FOR RECEIVING BLOOD SAMPLES

A. Shipping Schedule and Notification/Tracking

The CALERIE samples will arrive in monthly shipments (the first week of each month) from the three study field centers (Tufts University, Washington University, and Pennington Biomedical Research Center).

Prior to shipping, each site will send notification via fax with a list of expected sample IDs and any FedEx tracking numbers. Retrieve all faxed shipping notification forms and check FedEx website for tracking information. Note any missing/late packages and contact the site immediately.

Record all shipments by site and sender on the sample receipt log book (located on a clear clipboard on the shelf above the work bench).

Write the received date on all of the shipping forms.

B. Receipt of the Shipment Containers

Things to Do Before Samples Arrive:
Be sure to have enough sample boxes made and ready for the incoming samples. See Box maps for grid size, tape color, labels.

Clear off the CALERIE Shelves in T115 REVCO number 2.

- Visually inspect condition of shipping containers, labeling.
- Open all boxes and check condition of samples.
- Remove and organize paperwork by visit type.
- Shipping QC Form – record any deviations from shipping protocol. **See Appendix A.**
- Carefully remove sample boxes, confirm against paperwork, and place in temporary storage location.
- Record any sample condition problems or discrepancies.

After everything is verified, put the samples in the sample receipt freezer in a location dedicated for temporary CALERIE samples. If there is not enough space on the CALERIE shelves, there is additional space available on the other shelf just below.

C. Data Entry of Shipping, Phlebotomy, and Processing Forms

1. Separate the forms by visit and site.

2. When applicable, staple all of the loose pages for the same ID and same visit together so that the Phlebotomy form is on top and the Processing form is on bottom. If for any

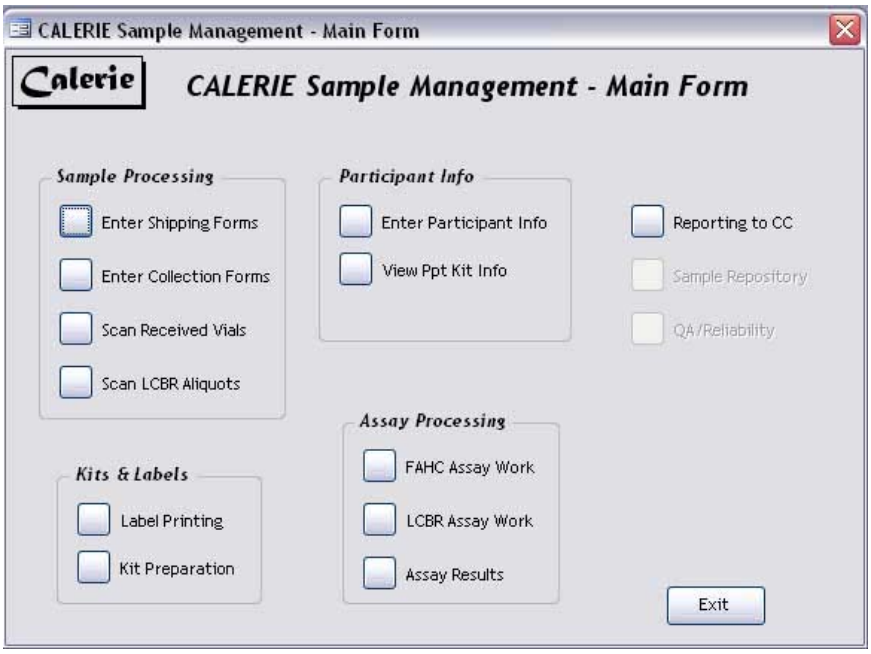
reason any other forms are sent for the same ID, staple them after (behind) the processing form. (Sometimes sites will send extra forms, such as site specific forms.)

3. Record the received date on the processing form and circle Y or N to indicate if they were frozen upon arrival.

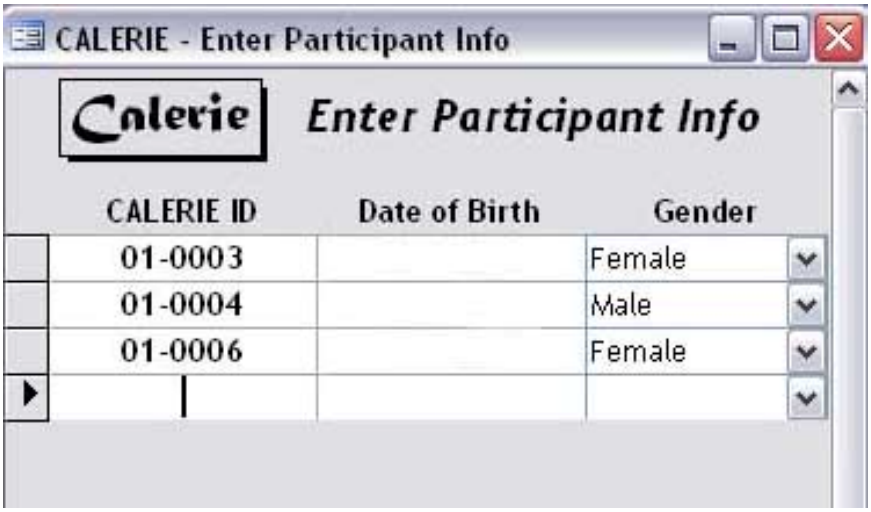
4. Put the stapled pages together so that the participant IDs match in order with the shipping form. Also put the sites together in numerical order, starting with site 1, Pennington.

5. Open the CALERIE Sample Management Database. The CALERIE database can be found at the following location:

   L:\ Groups\LCBR\Databases\CALERIE\ CALERIESampleMgmt_MED27_20080205.adp (or the most current version of the database saved in this location)



6. If this is the baseline visit for a new CALERIE ID, the first step will be entering the CALERIE ID into the database. Under the section heading "***Participant Info"***, click on the option "Enter Participant Info". Enter the requested information for each new CALERIE ID received.



Scanning in Shipping Forms:

1. Next, on the main screen, click on the "Enter Shipping Forms" option to open the data entry screen.

2. Begin scanning in the barcodes from the shipping forms.

3. Each time a new shipping label is scanned a CALERIE ID must be selected from the drop down list.

4. Click on Frozen if samples arrived in an acceptable frozen condition.

5. Print Verification Report and then close out of this screen.

Entering Collection Forms:

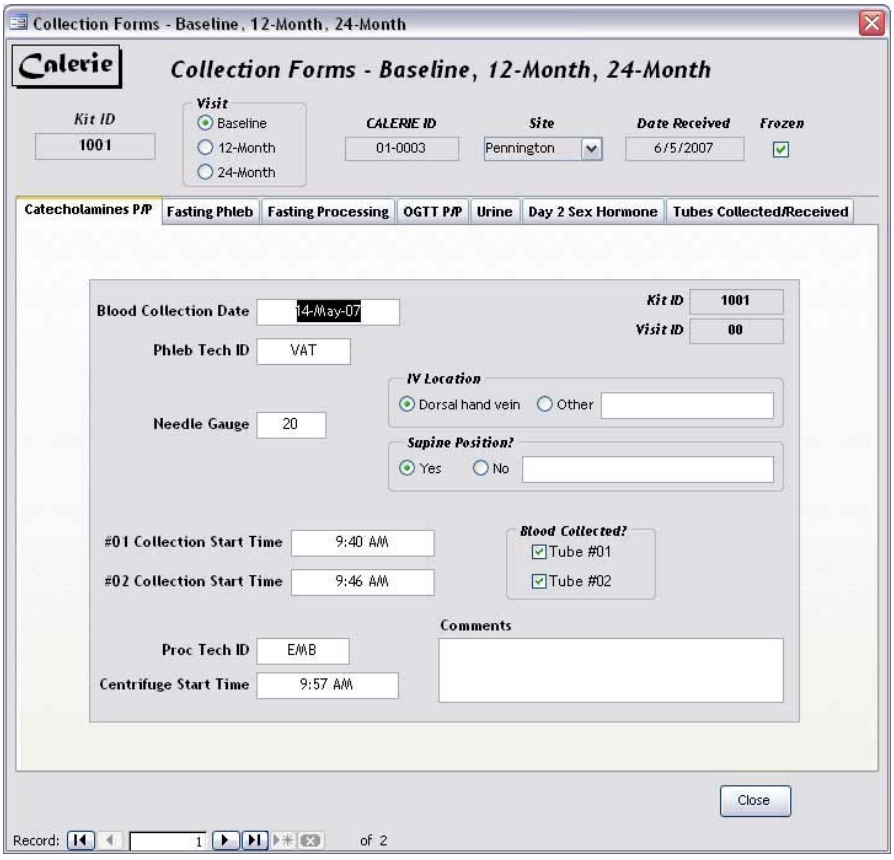
1. Once all of the IDs have been scanned into the database, move onto the collection form entry.

2. Again at the main screen, select "Enter Participant Collection Forms" under the section "Sample Processing."

3. Choose date received and visit type for the paperwork to be entered.

4. A list will appear of all shipping labels that have been scanned in matching the date and visit type.

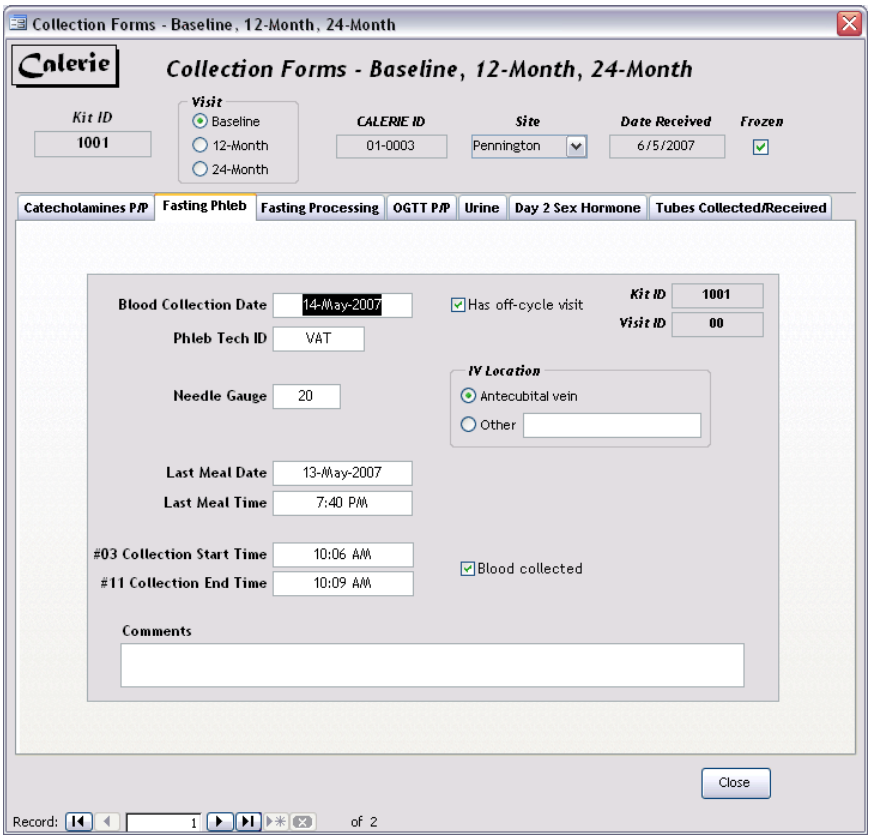
5. Highlight the first kit ID line and select "Enter Participant Collection Forms"

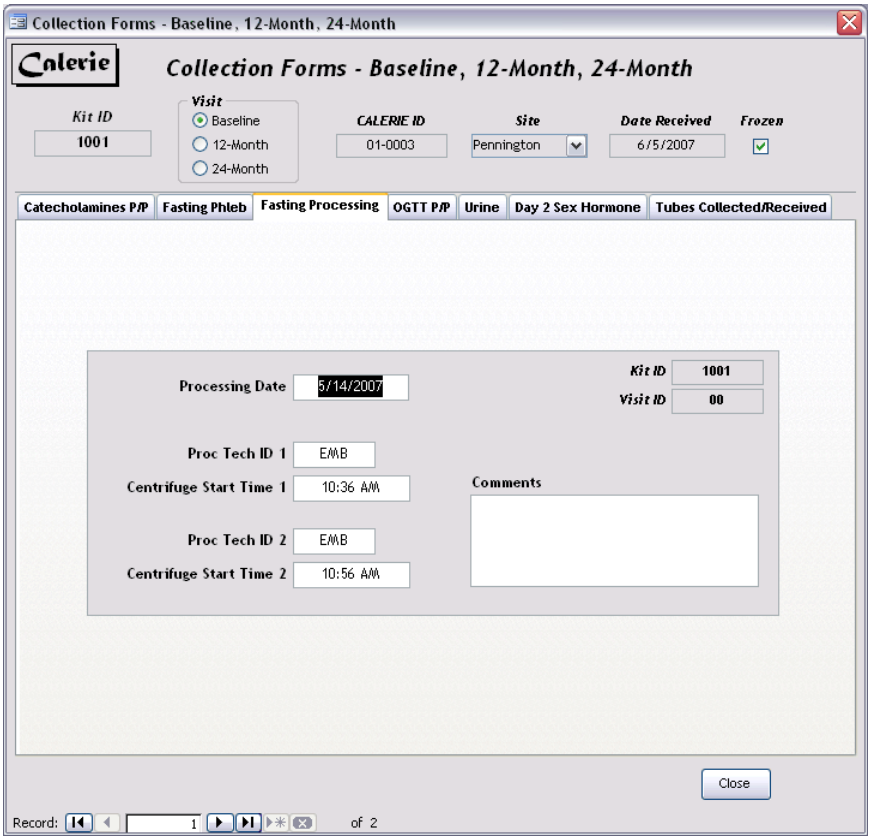


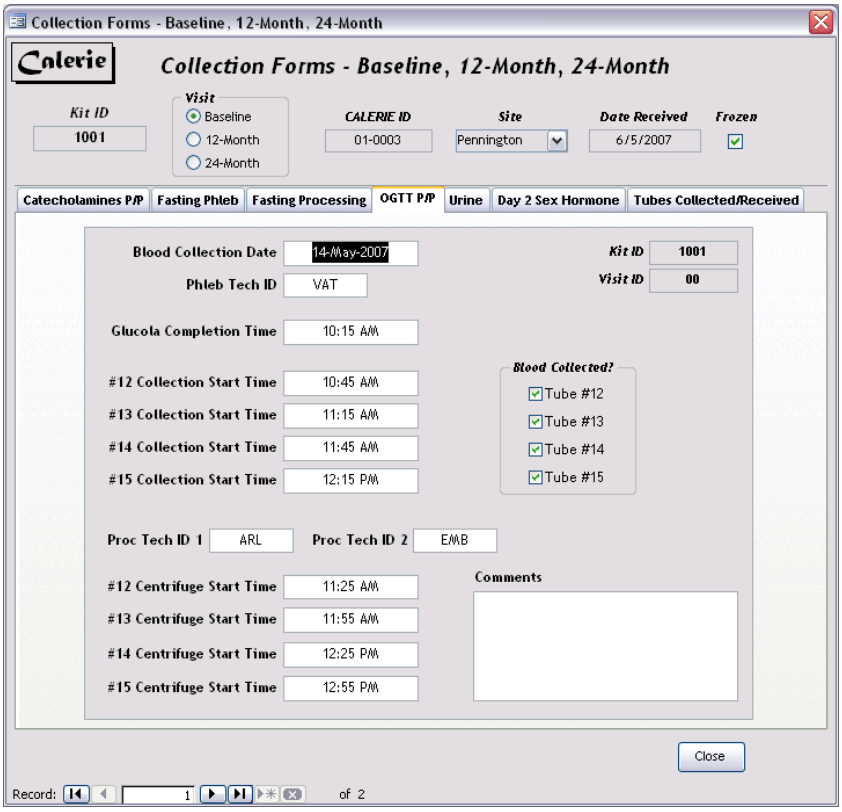
6. The Catecholamine draw phlebotomy form will be the tab that appears. Fill in the information requested from the Catecholamine P/P form for that participant. Next click

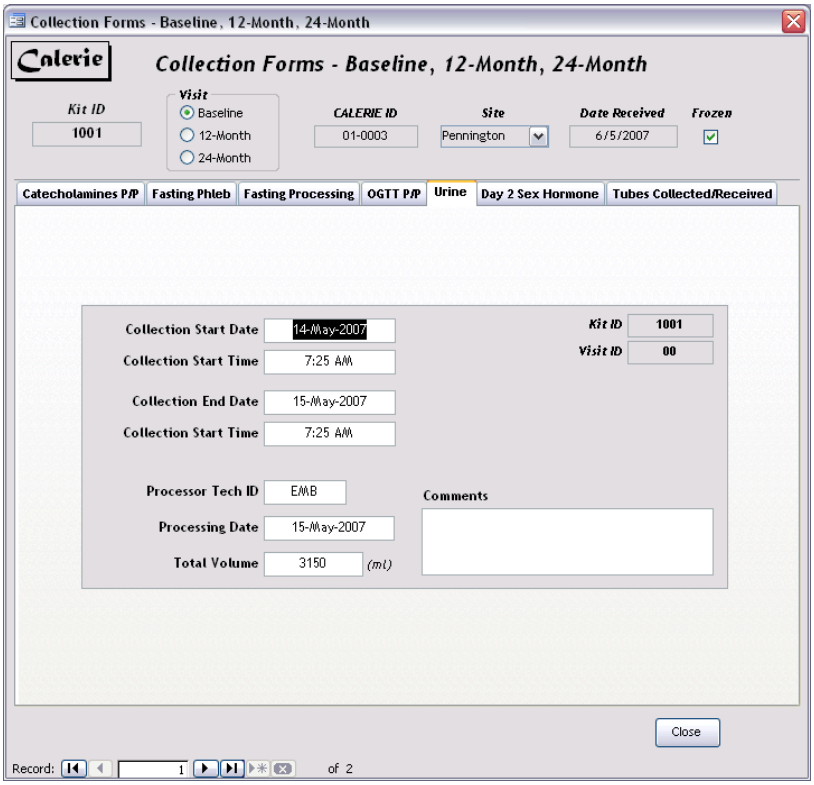
on the last tab “Tubes Collected/Received” and fill in requested information under “Collection Tubes”

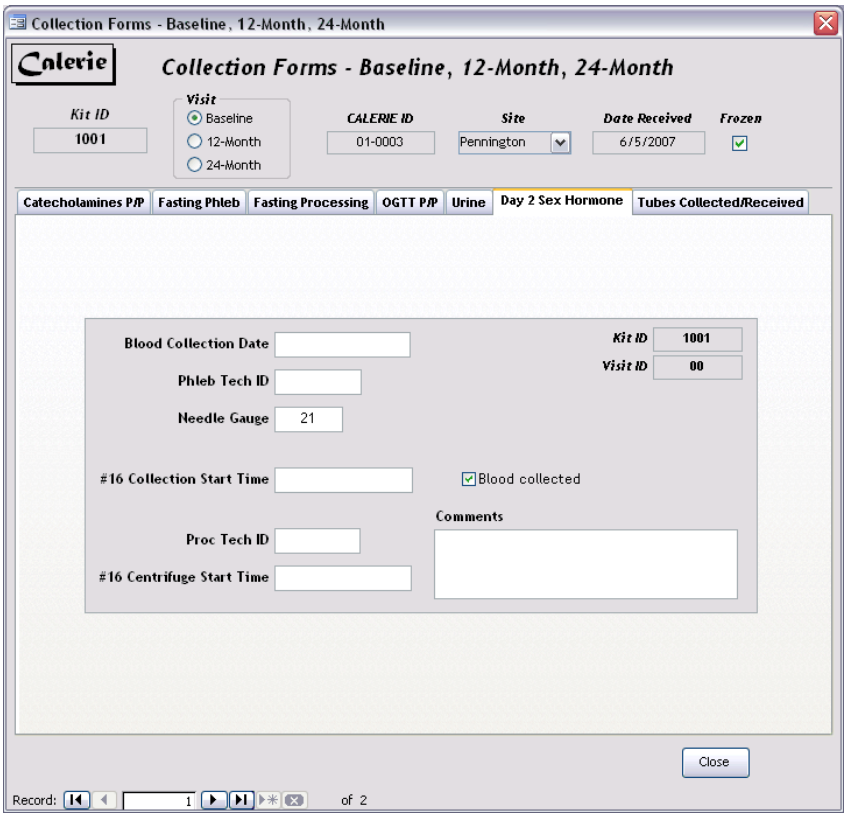
7. Enter this data in the same fashion for the Fasting, OGTT, Urine, and Day 2 Sex Hormone Collections. Enter the Phlebotomy forms first and then the Processing forms.



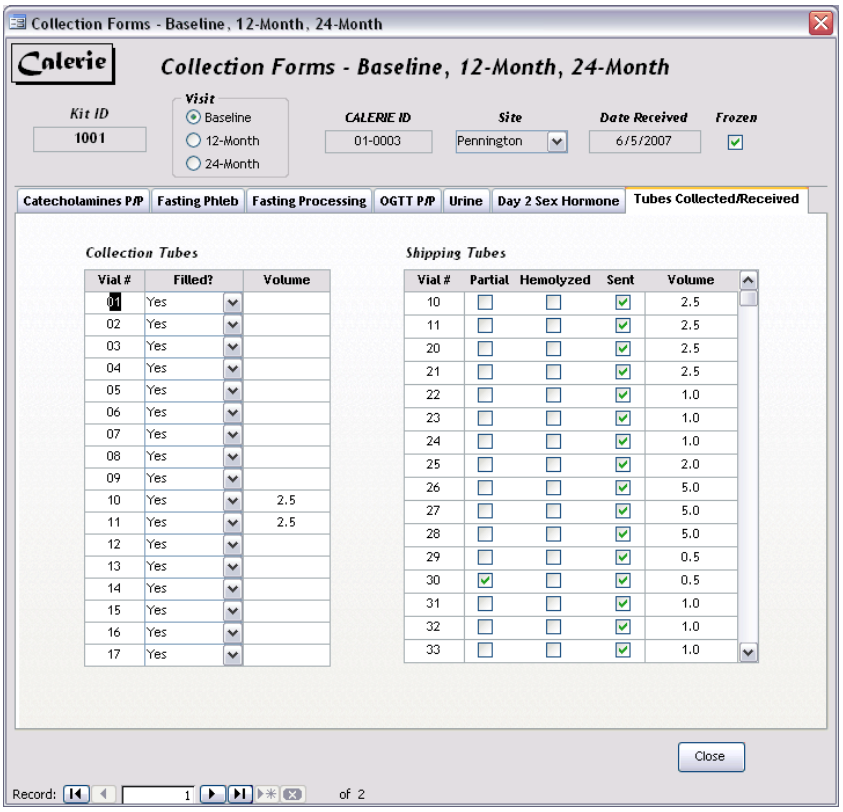

Collection Forms - Baseline, 12-Month, 24-Month
Calerie
Collection Forms - Baseline, 12-Month, 24-Month
Kit ID
1001
Visit
Baseline
12-Month
24-Month
CALERIE ID
01-0003
Site
Pennington
Date Received
6/5/2007
Frozen
Catecholamines P/P
Fasting Phleb
Fasting Processing
OGTT P/P
Urine
Day 2 Sex Hormone
Tubes Collected/Received
Processing Date
5/14/2007
Kit ID
1001
Visit ID
00
Proc Tech ID 1
EMB
Centrifuge Start Time 1
10:36 AM
Comments
Proc Tech ID 2
EMB
Centrifuge Start Time 2
10:56 AM
Close
Record:
1
of 2


Collection Forms - Baseline, 12-Month, 24-Month
Calerie
Collection Forms - Baseline, 12-Month, 24-Month
Kit ID
1001
Visit
Baseline
12-Month
24-Month
CALERIE ID
01-0003
Site
Pennington
Date Received
6/5/2007
Frozen
Catecholamines P/P
Fasting Phleb
Fasting Processing
OGTT P/P
Urine
Day 2 Sex Hormone
Tubes Collected/Received
Blood Collection Date
14-May-2007
Kit ID
1001
Phleb Tech ID
VAT
Visit ID
00
Glucola Completion Time
10:15 AM
Blood Collected?
#12 Collection Start Time
10:45 AM
Tube #12
#13 Collection Start Time
11:15 AM
Tube #13
#14 Collection Start Time
11:45 AM
Tube #14
#15 Collection Start Time
12:15 PM
Tube #15
Proc Tech ID 1
ARL
Proc Tech ID 2
EMB
Comments
#12 Centrifuge Start Time
11:25 AM
#13 Centrifuge Start Time
11:55 AM
#14 Centrifuge Start Time
12:25 PM
#15 Centrifuge Start Time
12:55 PM
Close
Record:
1
of 2


Collection Forms - Baseline, 12-Month, 24-Month
Calerie
Collection Forms - Baseline, 12-Month, 24-Month
Kit ID
1001
Visit
Baseline
12-Month
24-Month
CALERIE ID
01-0003
Site
Pennington
Date Received
6/5/2007
Frozen
Catecholamines P/P
Fasting Phleb
Fasting Processing
OGTT P/P
Urine
Day 2 Sex Hormone
Tubes Collected/Received
Collection Start Date
14-May-2007
Collection Start Time
7:25 AM
Collection End Date
15-May-2007
Collection Start Time
7:25 AM
Kit ID
1001
Visit ID
00
Processor Tech ID
EMB
Processing Date
15-May-2007
Total Volume
3150
(ml)
Comments
Close
Record:
1
of 2


Collection Forms - Baseline, 12-Month, 24-Month
Calerie
Collection Forms - Baseline, 12-Month, 24-Month
Kit ID
1001
Visit
Baseline
12-Month
24-Month
CALERIE ID
01-0003
Site
Pennington
Date Received
6/5/2007
Frozen
Catecholamines P/P
Fasting Phleb
Fasting Processing
OGTT P/P
Urine
Day 2 Sex Hormone
Tubes Collected/Received
Blood Collection Date
Phleb Tech ID
Needle Gauge
21
#16 Collection Start Time
Blood collected
Proc Tech ID
#16 Centrifuge Start Time
Kit ID
1001
Visit ID
00
Comments
Close
Record:
1
of 2

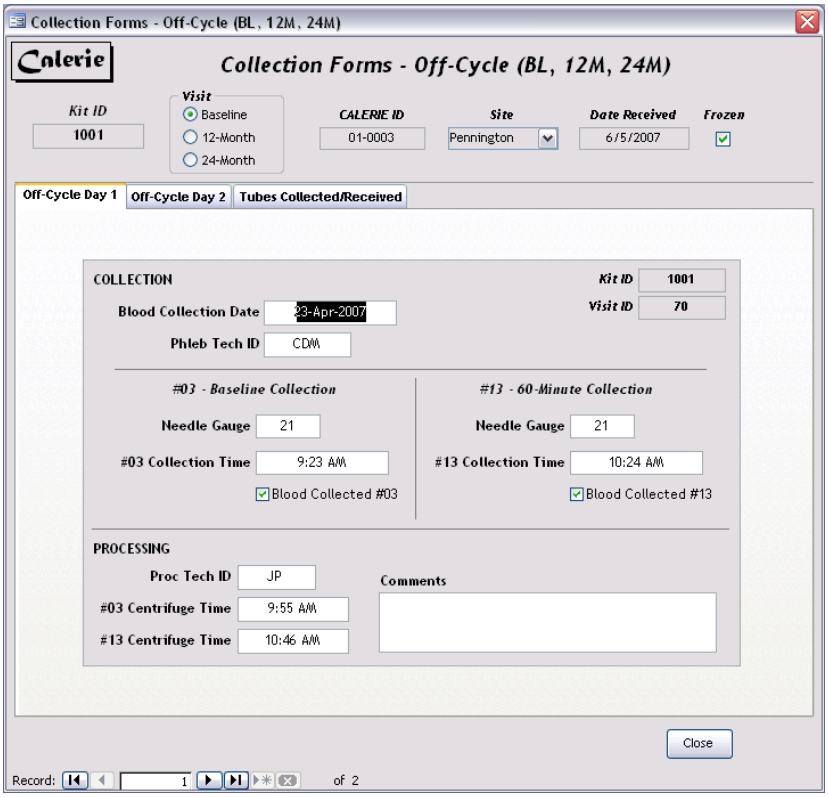
8. When all forms are entered move onto the “Tubes collected/Received” data tab, enter the required data in the same fashion as above.

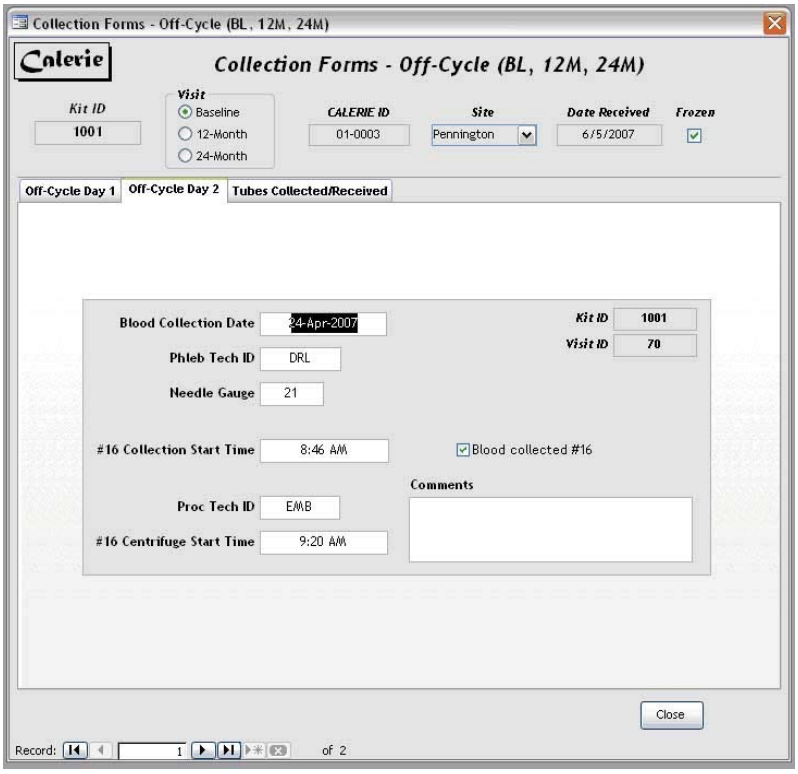


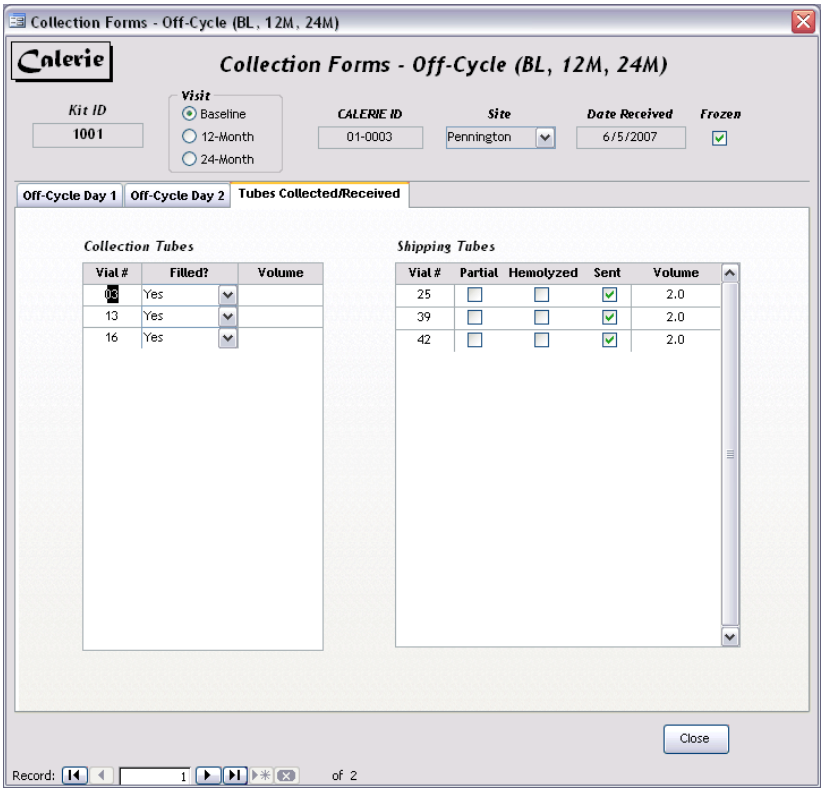
Questions that may arise:

1. If no day 2 sex hormone collection has occurred for a baseline participant, check off the “off-cycle visit is to occur” box on the “Fasting Phlebotomy” tab.
2. If participant is a male or if no day 2 hormone tubes were collected, un-check the blood collected box under the “Day 2 Sex Hormone” tab. Under the ”Tubes Collected/Received” Tab, select “no” for collection tube #16 received and select tube #42 as not received.
3. Note all partial or hemolyzed tube on P/P forms, and check the appropriate boxes under the “Tubes Collected/Received” tab.

9. Once all the Baseline Visit forms and data has been entered move on to the Baseline Off-cycle visit collection forms received. As with the Baseline collection entry above, fill in the information requested from the Off-cycle Day 1 P/P form for that participant. Next click on the last tab "Tubes Collected/Received" and fill in requested information under "Collection Tubes." Continue with the "Off-cycle Day 2" P/P form tab and "Collection Tubes."

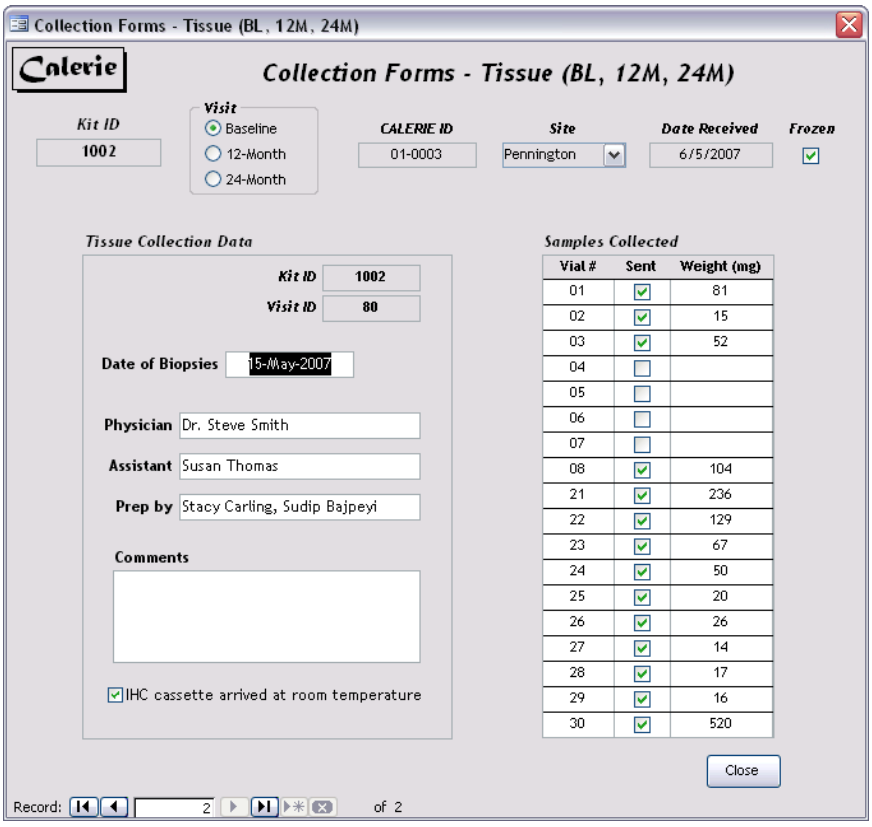






10. Repeat steps 1-8 for all visit types received in shipment. Tissue form entry is completed in the same fashion. Enter all information requested from the biopsy worksheets. Be

sure to correctly record tissue cryovials, sample vials and IHC cassettes sent as they appear on the biopsy worksheet.



11. Once all paperwork is entered for a specific visit type, return to "Kit Receipt Processing" screen and select "View Collection Form Verification Reports."

12. Print all verification files and verify form entry. **ALL OF THE PAPERWORK MUST BE VERIFIED BEFORE SCANNING!**

D. Scanning the Samples

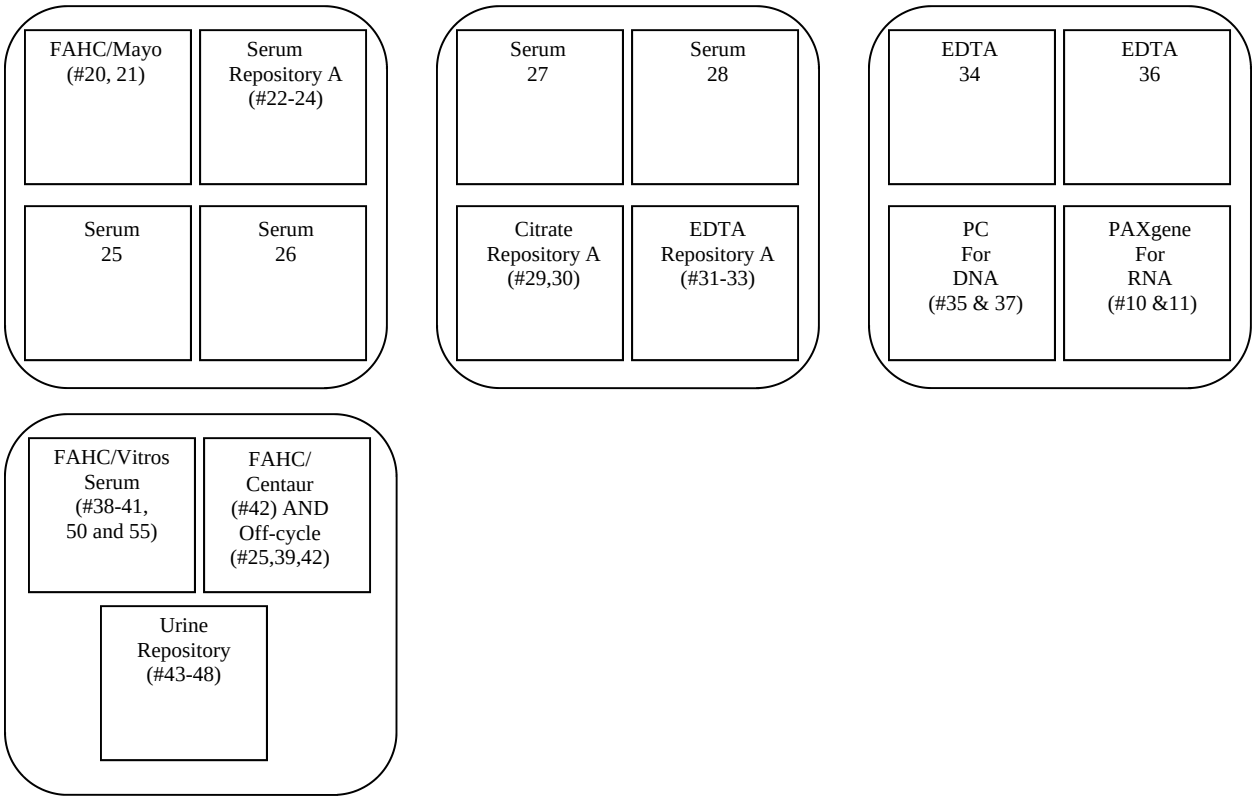
Blood and Urine Samples Scanning

1. After all paperwork is entered and verified for all visit types, the received samples are ready to be scanned.

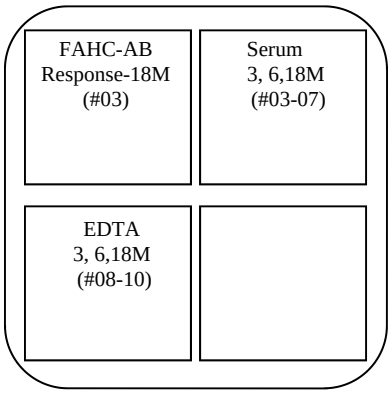
2. Set up baseline visit repository boxes and tubs of dry ice as needed to ensure that the samples remain frozen through the scanning process.

Set up the dry ice bins in the following manner:
Use Box Maps listed in the Appendix B for orientation of cryovials and tubes within boxes.

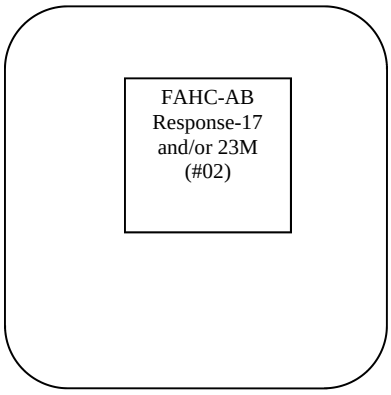
Annual Visit boxes (Baseline, 12M and 24M):



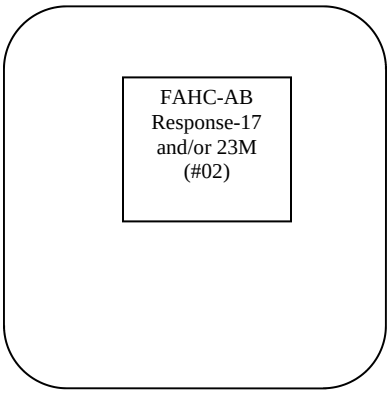
3, 6 and 18 Month Visit boxes Box



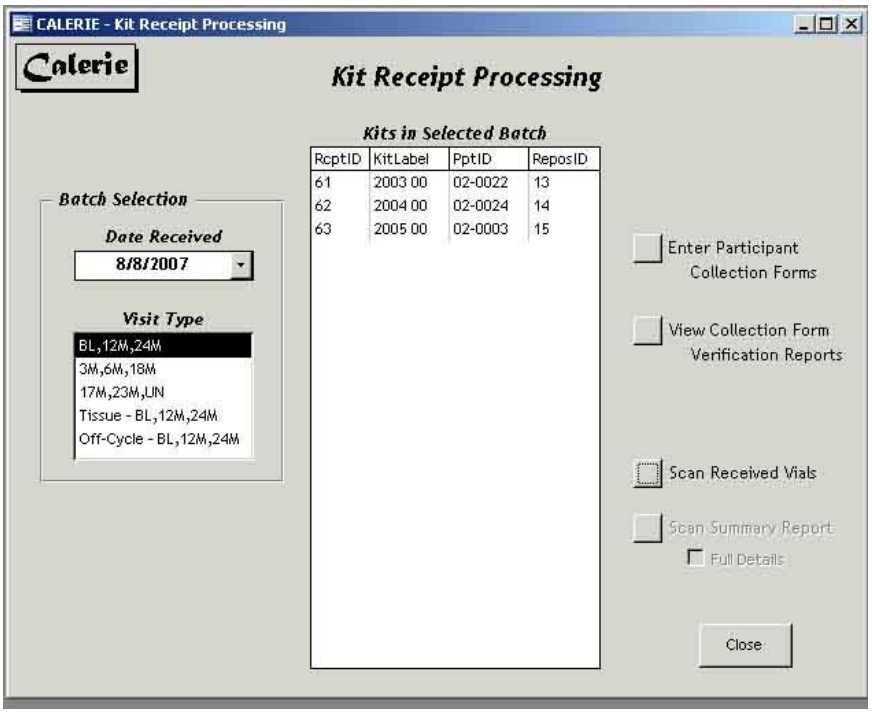
17 and/ or 23 Month Visit box



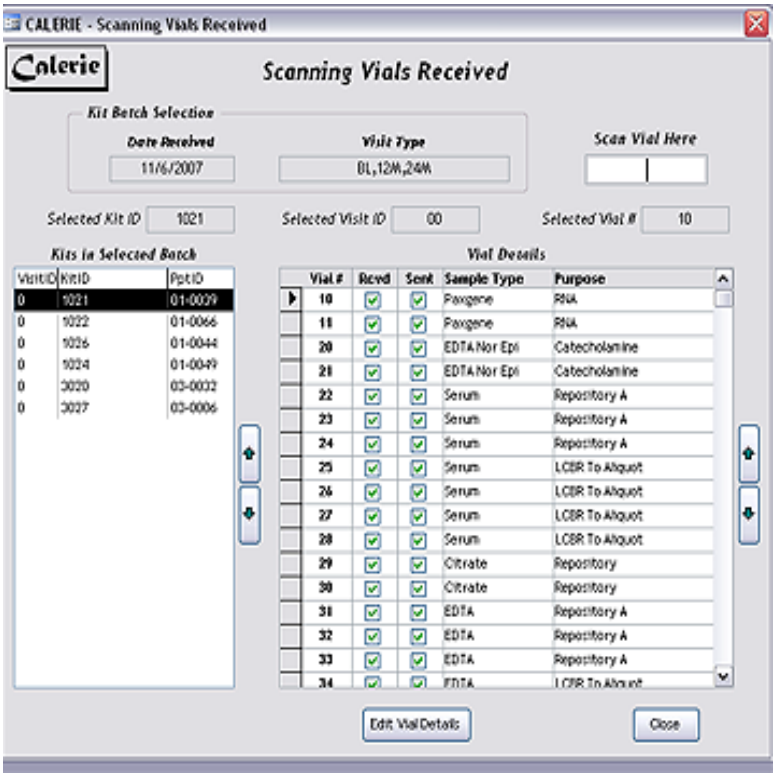
Unscheduled Visit



3. From the main menu, choose “Process Vials Received” and select the date and visit type. Then highlight the first kit on the list and select “Scan Received Vials”.



4. A list will appear of all the vials that should have been received, according to the P/P forms. The left-hand column lists the samples sent and the right-hand column shows the samples received. Verify that vials shown as “sent” were included in the shipment. Any discrepancies should be verified against the P/P forms.



5. Start at the top of the list and scan the received vials in the order shown placing the samples into the appropriate repository box. Annual Visit Box assignments will be designated as follows:

Box	ID extensions that go into it
FAHC/Mayo	EDTA tube _20 and 21
Serum Repository A	Serum cryo _22 thru _24
Serum 25	Serum tubes _25
Serum 26	Serum tubes _26
Serum 27	Serum tubes _27
Serum 28	Serum tubes _28
Citrate Repository A	Citrate cryos _29 and _30
EDTA Repository A	EDTA cryos _31 thru _33
EDTA 34	EDTA tube _34
EDTA 36	EDTA tube _36
PC for DNA	Red Cells tubes _35 and_37
PAXgene for RNA	PAXgene _10 and _11
OGTT Serum	Serum tube_38 thru _41
FAHC/Centaur	Baseline Serum tube_42 AND off-cycle Serum tubes _25,_39 and _42
Urine Repository	Urine tubes _43 thru _48

6. When finished scanning in the first kit ID, verify that all the samples recorded as "received" also appear as "sent."

7. As the boxes fill up, rubber-band them and place them in a separate CALERIE section in the REVCO until the end of the day when scanning is done.

8. Repeat scanning process (steps 1-7) for all kits of each visit type. Empty participant boxes should be returned to each site for future use.

Blood and urine sample box assignments for other visits are as follows:

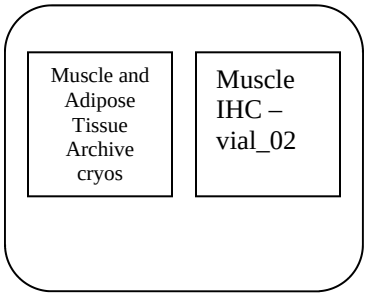
Box	ID extensions that go into it
Serum Repository-3M, 6M, and/or18M	Serum cryo _03 thru_06
EDTA Repository-3M, 6M, and/or 18M	EDTA cryo _07 thru_10
FAHC AB Response-18M	Serum cryo_03
FAHC AB Repsonse-17 and/or 23M	Serum tube_02
FAHC/Centaur-Unscheduled (Women only)	Serum tube_02

When all of the participant blood and urine samples have been scanned, move on to scanning in the annual visit tissue samples.

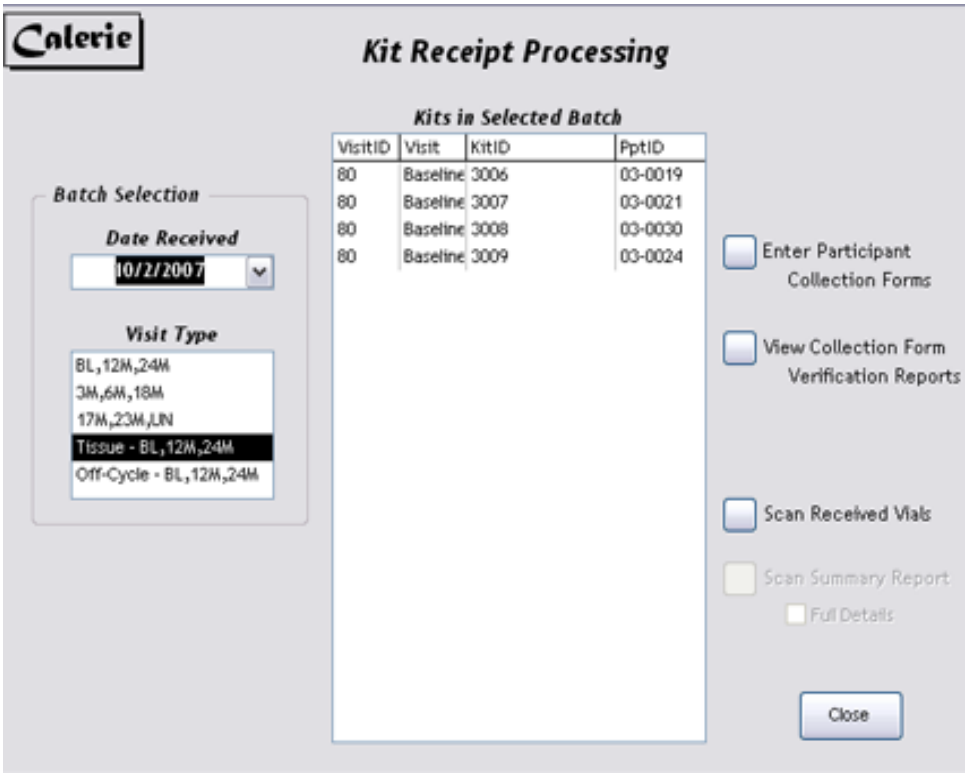
Tissue Sample Scanning

1. After all tissue biopsy worksheets are entered and verified, the received tissue samples are ready to be scanned.
2. Set up tissue repository boxes and tubs of dry ice as needed to ensure that the samples remain frozen through the scanning process. Adipose tissue cassettes (#24) are stored at room temperature.

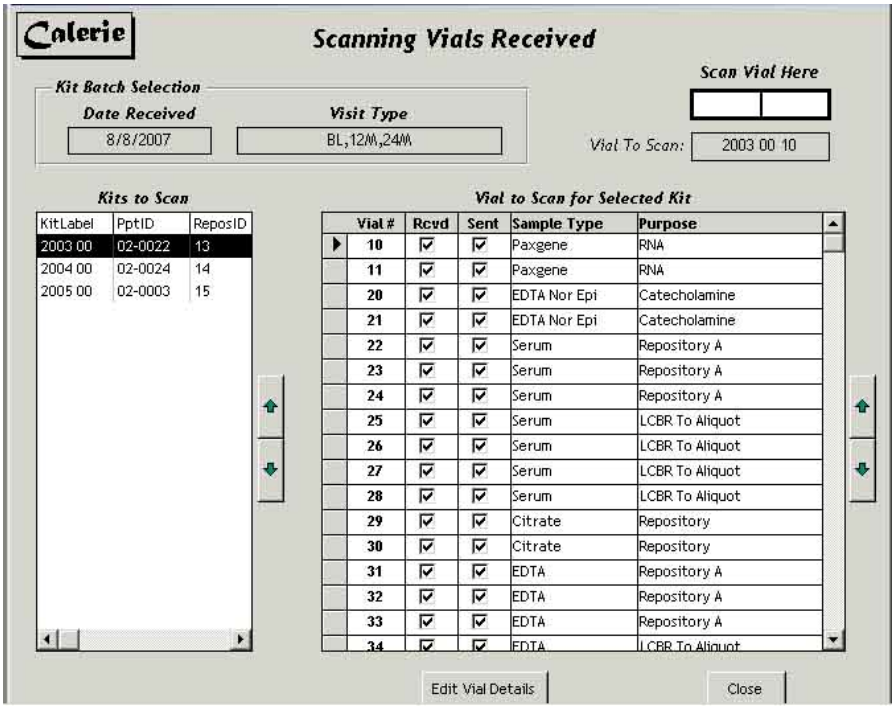
Set up the dry ice bins in the following manner:
Use Box Maps listed in Appendix B for orientation of cryovials and vials within boxes.



3. As with the blood and urine sample scanning, from the main menu, choose "Process Vials Received" and select the Date and Tissue. Then highlight the first kit on the list and select "Scan Received Vials."



4. A list will appear of all the vials that should have been received, according to the biopsy worksheets. The left-hand column lists the samples sent, and the right-hand column shows the samples received. Verify that vials shown as "sent" were included in the shipment. Any discrepancies should be verified against the tissue biopsy worksheets.



5. Start at the top of the list and scan the received vials in the order shown placing the samples into the appropriate repository box. Tissue biopsy assignments will be designated as follows:

6. See box maps for

Box	ID extentions that go into it
Muscle IHC	Vial_02
Adipose IHC (cassettes)	Cassette_24
Tissue RNA and Archive	Cryos_01, _03 thru_08, _21 thru_23, _25 thru_30

This should conclude the scanning for the samples.

Clinic Acknowledgements:

Contact the site immediately if:

- ✓ The expected shipment did not arrive (include any FedEx tracking or correspondence regarding shipment).
- ✓ Any forms or other paperwork is missing (so they can fax it).
- ✓ There are any discrepancies or questions regarding labeling, cryo order, cap color, tube condition, etc., **especially if there are any questions regarding potential participant mix-ups. No question is too small to ask**; correcting minor mistakes now will save us from major headaches later!
- ✓ Any other issues that arise involving any specific site where an immediate response is necessary.
- ✓ Copy Rebekah Boyle on any discrepancies and their resolutions (for the discrepancy log book).

E. Aliquotting of Baseline, 12M, or 24M Serum and EDTA tubes

Of the 31 (30 for male participants) tubes or cryos that are received for each participant's baseline, 12M, or 24M visit, 10 tubes/cryos need to be aliquotted further. The following is a table of the cryo/tube #'s that need to be aliquotted. Samples received from the CALERIE sites at other visits (3M, 6M, 18M, etc) do not require any aliquotting at LCBR.

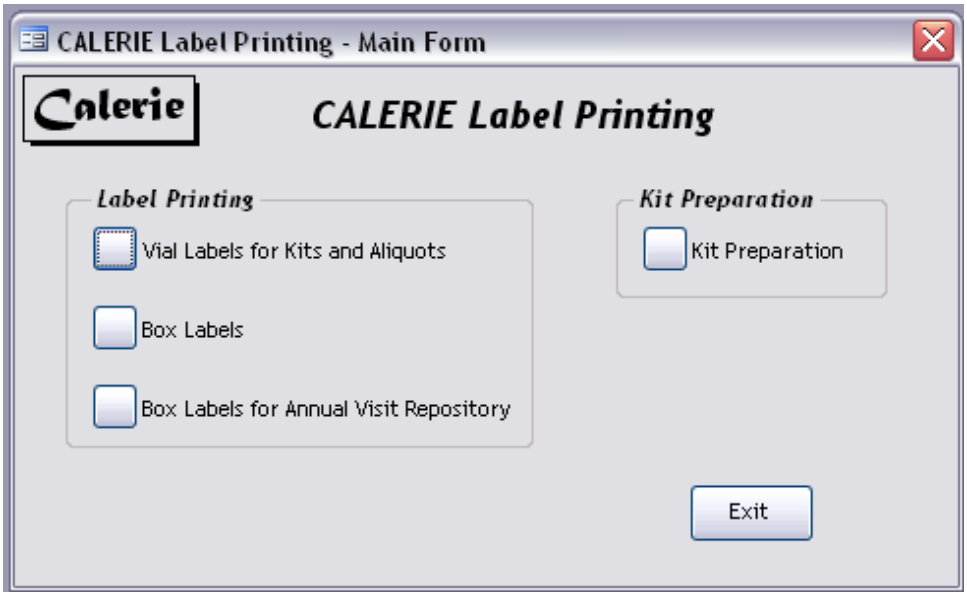
Aliquot Labels

Prepare for aliquotting samples by printing all vial labels needed. Each new participant will need an annual visit repository box. Each kit ID will need a set of LCBR aliquot labels.

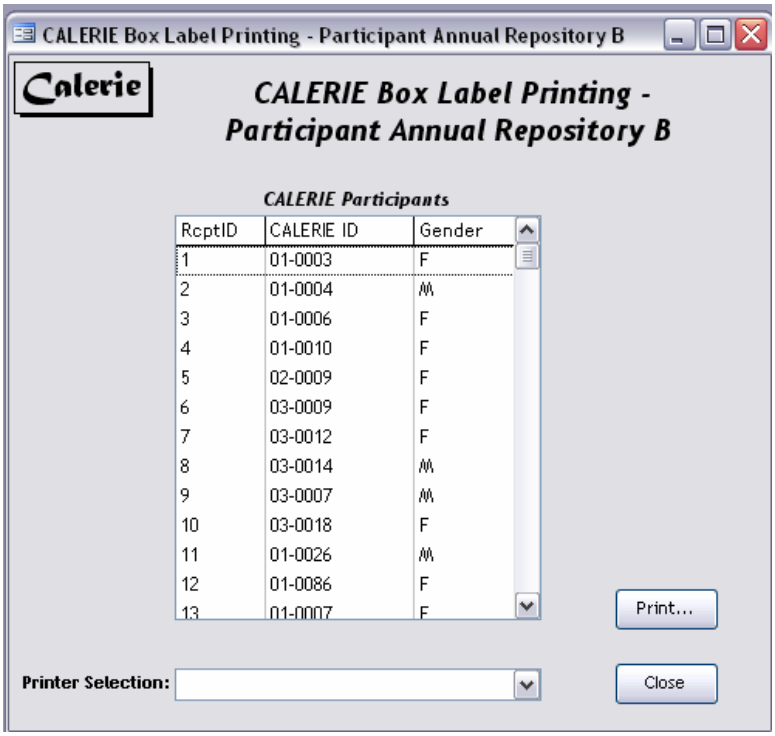
Printing Box labels for Annual Visit Repository:

1. Open the *CALERIE Label Printing* database found in:

   L:\Groups\LCBR\Databases\CALERIELabelPrinting_MED27_20080110.adp



2. At the main screen select *Box Labels for Annual Visit Repository.*



3. Scroll down the list of CALERIE participants and select the ID that you want to print.

4. Click on the printer selection drop down menu and select the printer you are using from the list.

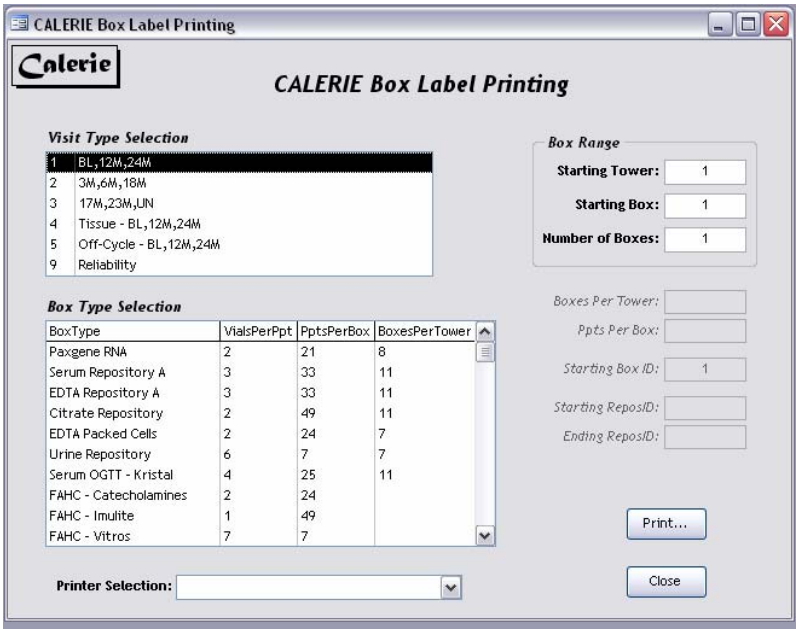
5. Be sure the printer is set up the printer with 4-accross labels. And select *Print.*

Printing Box labels for other boxes:

1. Again go to the CALERIE label printing main screen and select *Box Labels.*

2. Select the appropriate visit and box type from the screens on the left.

3. On the right of the screen, adjust the *Starting Tower*, *Starting Box*, and *Number of Boxes* to reflect the next consecutive set of labels needed.



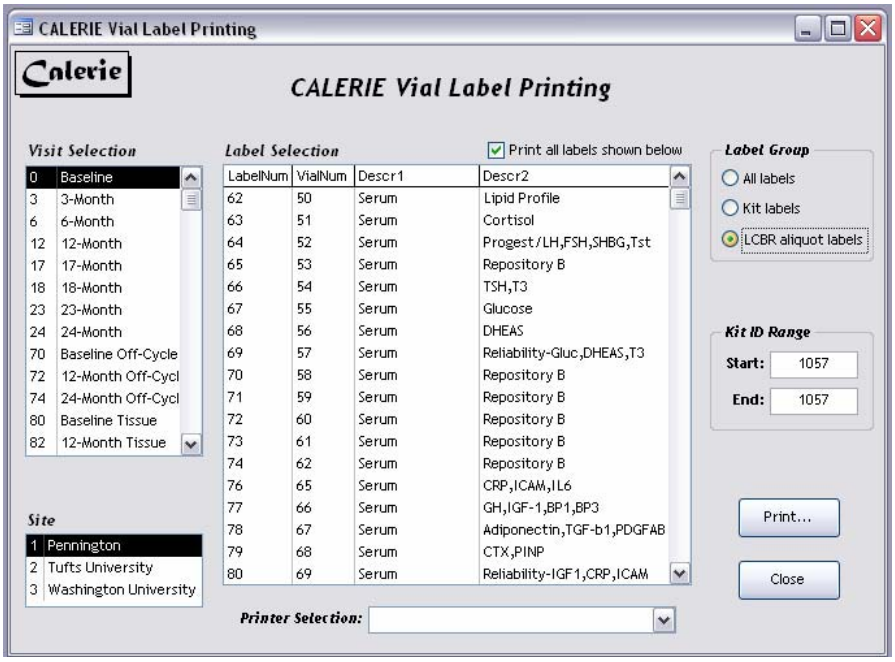
4. Select your printer from the drop-down list at the bottom.

5. Be sure the printer is loaded with 4-across labels and click *Print*.

6. Do steps 1-5 for any other visit type and box type combination that is needed.

Printing Aliquot Labels:

1. Go to the main *CALERIE Label Printing* screen and select *Vials Labels for Kits and Aliquots.*



2. Select the appropriate visit type under *Visit Selection* and *Site*.

3. Select *LCBR Aliquot Labels* from the *Label Group Selection* on the top right.

4. Type in the *Kit ID Range* needed.

5. If you need to print an entire label set, be sure that the check for *print all labels shown below* is selected. Otherwise click on the specific labels you'd like to print (Ctrl click for more than one selection and a time)

6. Select the appropriate printer from the *Printer Selection* menu at the bottom of the screen.

7. Be sure label printer is loaded with 5-across labels and click *Print.*

Serum Transfer Tube (Tubes 25-28) Aliquotting:

1. Label aliquot vials ahead of time to minimize the amount of time the samples remain at room temperature.

2. Label the appropriate tubes (4mL false-bottom, 12x75mm, and 0.5 mL or 1.5mL cryovial) according to the aliquotting guide (See Appendix C).

3. Thaw serum tubes #25, 26, 27 and 28 in a 37°C water bath. Total thaw time should be approximately 5-7 minutes. Invert tubes at least once during thaw.

4. Once tubes have completely thawed, pool all four serum tubes into one 50mL conical tube.

5. Invert pooled serum tube 15 times and aliquot according to aliquotting guide.

6. Put aliquots into -80°C freezer immediately after prepared.

OGTT Tube Aliquotting:

1. Label aliquots (four-red capped 1.5mL aliquots).

2. Thaw all 4 OGTT tubes together in water bath. Invert tubes at least once during thaw.

3. Mix thawed tubes by inverting each tube 15 times prior to aliquotting.

4. Put aliquots into -80°C freezer immediately after prepared.

EDTA Aliquotting:

1. Label aliquot vials ahead of time to minimize the amount of time the samples remain at room temperature.

2. Thaw tube #34 first in a 37°C water bath. Invert tube at least once during thawing.

3. Mix tube #34 by inverting 15 times, then aliquot into 0.5 mL cryovials according to the aliquotting guide. Put aliquotted cryovials immediately into -80°C freezer.

4. Thaw EDTA transfer tube #36 in water bath. Invert at least once during thaw.

5. Mix thawed tube by inverting 15 times.

6. Aliquot into 0.5 mL cryovials according to aliquotting guide.

Scanning LCBR Aliquots:

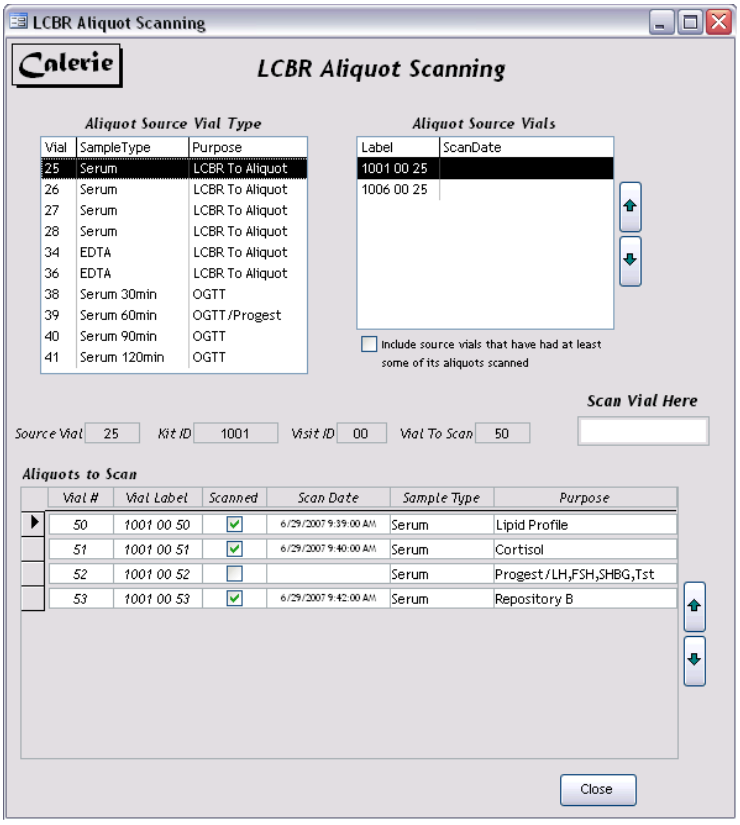
1. All aliquots made at LCBR must be scanned into the database.

2. From the main page of the CALERIE database under “Sample Processing” select “Process LCBR Aliquots.”

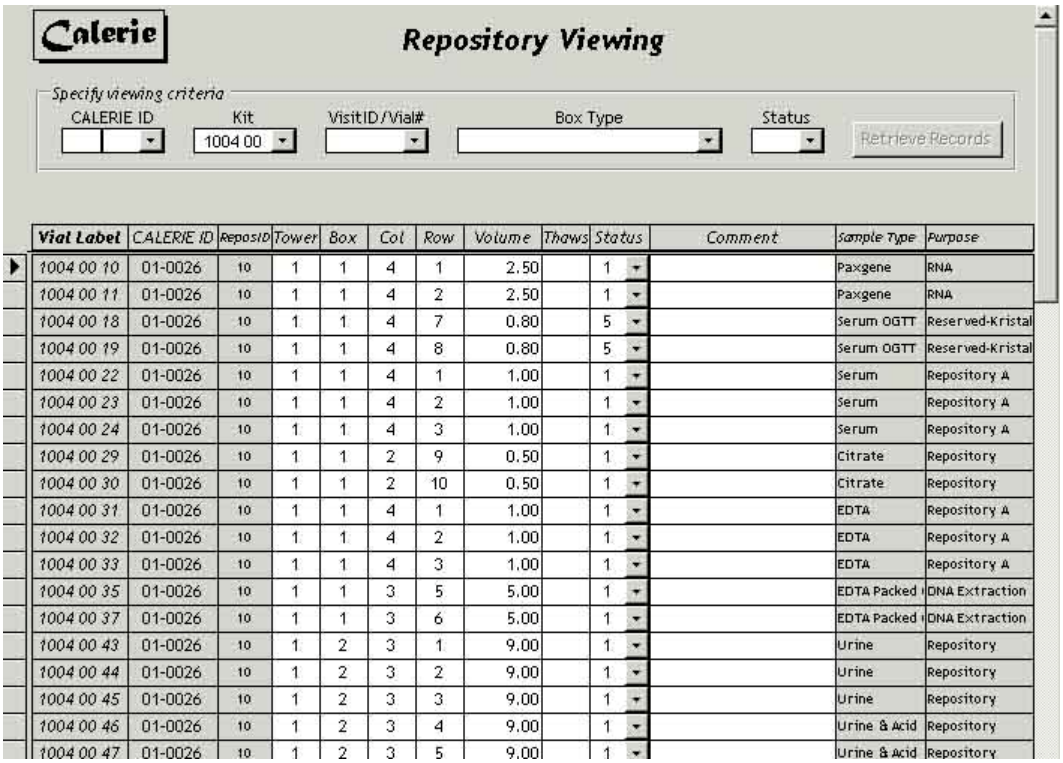
3. On the left of the screen select the appropriate source vial type.

4. A list of Kit IDs that were received that day will appear. Select the first kit ID.

5. A list of aliquots to be scanned will appear on the bottom of the screen. Scan the aliquots prepared in the order they are listed on the screen.



6. Go back to the main screen and under “Repository” select “View Sample Repository”. The aliquots just scanned in will now appear in this list. Select the first kit ID and make any edits to volume, vials not completed, etc., as needed. Complete for other kit IDs as needed.

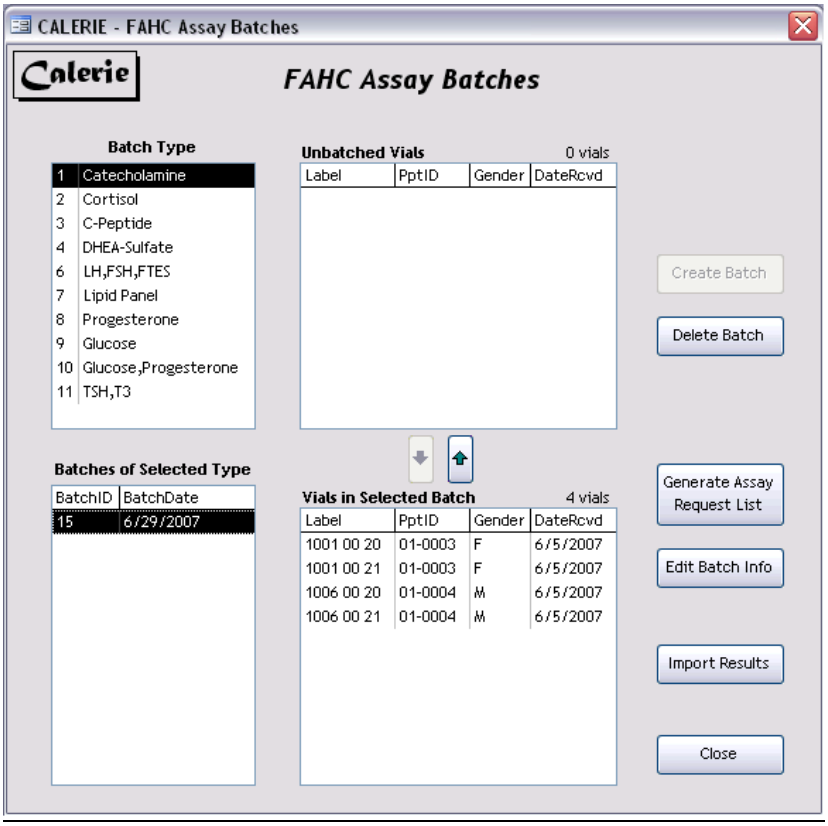


Vial Label	CALERIE ID	ReposID	Tower	Box	Col	Row	Volume	Thaws	Status	Comment	Sample Type	Purpose
1004 00 10	01-0026	10	1	1	4	1	2.50		1		Paxgene	RNA
1004 00 11	01-0026	10	1	1	4	2	2.50		1		Paxgene	RNA
1004 00 18	01-0026	10	1	1	4	7	0.80		5		Serum OGTT	Reserved-Kristal
1004 00 19	01-0026	10	1	1	4	8	0.80		5		Serum OGTT	Reserved-Kristal
1004 00 22	01-0026	10	1	1	4	1	1.00		1		Serum	Repository A
1004 00 23	01-0026	10	1	1	4	2	1.00		1		Serum	Repository A
1004 00 24	01-0026	10	1	1	4	3	1.00		1		Serum	Repository A
1004 00 29	01-0026	10	1	1	2	9	0.50		1		Citrate	Repository
1004 00 30	01-0026	10	1	1	2	10	0.50		1		Citrate	Repository
1004 00 31	01-0026	10	1	1	4	1	1.00		1		EDTA	Repository A
1004 00 32	01-0026	10	1	1	4	2	1.00		1		EDTA	Repository A
1004 00 33	01-0026	10	1	1	4	3	1.00		1		EDTA	Repository A
1004 00 35	01-0026	10	1	1	3	5	5.00		1		EDTA Packed	DNA Extraction
1004 00 37	01-0026	10	1	1	3	6	5.00		1		EDTA Packed	DNA Extraction
1004 00 43	01-0026	10	1	2	3	1	9.00		1		Urine	Repository
1004 00 44	01-0026	10	1	2	3	2	9.00		1		Urine	Repository
1004 00 45	01-0026	10	1	2	3	3	9.00		1		Urine	Repository
1004 00 46	01-0026	10	1	2	3	4	9.00		1		Urine & Acid	Repository
1004 00 47	01-0026	10	1	2	3	5	9.00		1		Urine & Acid	Repository

F. FAHC Laboratory Testing Batches

1. Under “Assay Processing” on the CALERIE main page, select “Manage Assay Batches.”

2. Highlight the first Batch Type desired (*example: Catecholamine)*, and a list will appear containing all vials that are still un-batched.



3. Select each desired vial individually to add to a batch, or select “Create Batch” to automatically add all vials.

4. A new Batch ID will appear with no date listed next to it. Highlight this batch ID and select “Generate Assay Request List.”

5. A date will now be listed next to the batch ID, and an Excel spreadsheet will be created in the ‘Files to FAHC’ folder in the CALERIE database. (L:\ drive location: L:\Groups\LCBR\Databases\CALERIE\Files to FAHC) Each new batch request from this date will create a new worksheet in the workbook of this Excel file.

6. Repeat steps 2 through 4 for all desired Batch Types, then close and return to the ‘Files to FAHC’ folder.

7. Create a new folder with the date the samples are sent to FAHC, and drag the Excel file into this folder.

8. Add any controls as necessary to the individual worksheets.

9. Alert Rebekah that the batches are prepared and the electronic file is saved in the CALERIE data folder. She needs to prepare an electronic file to send to Monica Sullivan prior to samples being brought to the chemistry lab with the courier.

10. Print out hardcopies of all worksheets in this file to be included in the sample boxes along with the vials. Double-check that all vials are present in the samples boxes.

11. Store sample boxes in -80°C until courier is available for transporting samples to FAHC. Courier will pick up samples for delivery to FAHC on Tuesdays and Thursdays.

End of Processing Procedures

- ✓ Put all P/P forms in CALERIE ID order.
- ✓ Put all of the verification forms in order by site.
- ✓ Put all shipping forms in order by site.
- ✓ Put all P/P forms into appropriate drawers of designated filing cabinet. Clearly label all new hanging file folders.
- ✓ Put all verification forms into there appropriate folder separated by site.
- ✓ Put all of the shipping forms into appropriate folder separated by sited and filed by date received.
- ✓ Next remove all completed cryo boxes out of the freezer and put them onto a cart with tubs loaded with dry ice. Update the freezer log book and proceed in putting boxes into there appropriate towers. **Do not allow samples to thaw during this process.**

Housekeeping/Supplies and Mailer Return

- ✓ Replace any shipping boxes that are damaged beyond safe and effective use (torn, leaking etc.).
- ✓ Return the empty sample boxes in the returning shipper.
- ✓ Check with Rebekah to see if any supplies have been ordered by the sites.
- ✓ Return boxes via FedEx Express saver (if no supplies requested); if supplies were requested, use FedEx 2-Day Service.
- ✓ File the supply request form in the appropriate binder and affix the FedEx receipt on back for easy tracking.
- ✓ If supplies are low notify Vicci/Rebekah for restocking.
- ✓ Prepare sample boxes for next month's samples. *(one set of boxes)*

V. OVERVIEW OF REPOSITORY DESIGN AND TRACKING

The main CALERIE protocol calls for samples of blood, urine, and tissue to be procured for repository storage in the following quantities:

Sample type	Visits at baseline, 12 months and 24 months	Visits at 3 months, 6 months, and 18 months
Serum	10 ml	4 ml
Serum (OGTT)	4 ml	
Plasma (EDTA)	9 ml	4 ml
Plasma (citrate)	1 ml	
RNA	from 2 PAXgene tubes	
DNA	from packed cells from 2 EDTA tubes	
Urine (no additive)	18 ml	
Urine (with boric acid)	18 ml	
Skeletal muscle	~45 vials	
Adipose tissue	~30 vials	

Some of the aliquoting of samples will be performed at the field centers; the remainder will occur upon arrival at our laboratory. Regardless of location, personnel entrusted with this responsibility will have been appropriately trained and demonstrated competence in the application of the aliquoting procedure. Most of the aliquots for storage will measure either 0.5 ml or 1.0 ml in volume.

The implementation phase includes bringing the repository database online and procuring the physical resources, i.e., freezers, alarm systems, backup power, and physical space required to accommodate the quantity of samples generated.

Implementation of the CALERIE repository

The three -80ºC ultra-cold chest freezers required for storage of cryovials containing blood and urine will be Revco/Thermo brand Ultima II-9 freezers (model ULT2090-9-D33), which are 20 cubic foot chest units with dual-stage compressors and a voltage (surge and sag) regulator. Each unit has the capacity for monitoring temperature failure, power failure, compressor failure and fuse/battery failure. Each freezer also has a data port for remote alarm connection. Additional storage supplies to be purchased will include freezer racks and boxes.

Skeletal muscle and adipose tissue samples will be stored in one to two Thermo CryoPlus2 liquid nitrogen (LN2) storage units (model 7402), each of which has a 24-inch tank capable of containing 200 liters of LN2. The evaporation rate is 5 liters per day with a static holding time of 40 days. Each unit has an auto-filling system and is capable of monitoring temperature, LN2 levels, and valve malfunctions. Each storage unit also has a data port for remote alarm connection. Additional supplies will include racks, boxes, and liquid nitrogen tanks.

Freezers and liquid nitrogen storage units comprising the CALERIE biosample repository will be installed in accordance the manufacturer's instructions and will reside in a new 14,000 square foot University of Vermont facility located approximately five miles from the main LCBR facility at Flynn Ave. This building features a temperature controlled environment (65ºF) and full generator back-up

capacity (automatic) that will run for 24 hours using 344 gallons of fuel; the generator is maintained and tested on a monthly basis. Access to the building is limited, and it is equipped with an intrusion alarm.

For alarm monitoring, an autodialer (Sensaphone model 1108) will be connected to all freezers and storage units to monitor unit temperature, power failure, and ambient temperature. This instrument is capable of calling a list of phone numbers repeatedly until the alarm is acknowledged by certified LCBR staff. The first number dialed is that of a beeper that is monitored by on-call staff 24 hours per day, seven days per week. The Sensaphone also has a call-in capacity for remote monitoring of conditions by certified staff. Fiber-optic cable is being run to the facility, and once this is completed, we will shift to a web-based primary system with phone dialing backup. We have a certified freezer repair service on call 24 hours per day, seven days per week.

Operation and Maintenance of the CALERIE repository

Operation and maintenance of the repository will be carried out in accordance with the principles outlined in the document titled "NHLBI Biological Samples: Recommendations for Standardized Storage Protocols" Vials to be stored in the repository will be labeled with barcodes to facilitate accurate, efficient data entry, and each vial will be scanned upon entry into the repository. Database fields for each sample will include the participant identifier, the visit number, the aliquot number, sample type, sample volume, and location.

We will generate quarterly reports for the Coordinating Center, the Steering Committee, and other appropriate committees concerning the status of the repository; these reports will include a detailed inventory of the numbers and types of samples received and stored, and any issues related to the physical equipment will also be documented. The data contained in the reports will be sortable, which will permit detailed analysis with respect to fields such as sample ID, CALERIE ID, visit number, or volumes stored, among others. Additional reports will be generated as needed or as requested by the Coordinating Center or by committees such as the Steering Committee.

Monitoring of the repository will follow the following schedule:

Frequency	Type of monitoring
Daily	Temperature and status of freezers and LN2 storage units
	Visual inspection
Monthly	Alarm testing
	Generator testing
Quarterly	Preventive maintenance
Semiannually	HVAC system maintenance

Each of the types of monitoring described above will be documented, as will each occasion on which a sample is placed in or removed from a freezer or storage unit. Protocols for dealing with power outages or freezer/storage unit malfunctions will be posted in the facility, as will names and phone numbers of key personnel to be contacted if such events are noted by security or maintenance personnel. A contract with a maintenance service available 24 hours a day, 7 days a week will be maintained. Access to the repository facility is appropriately limited, and it is equipped with intrusion alarms. Emergency power for the facility is provided by generators fueled sufficiently to provide 24 hours of backup electricity.

VI. **DESCRIPTION OF LABORATORY ASSAY METHODS** *(to be completed)*

CALERIE Core Lab Assay List

LCBR Assays	FAHC Assays
IL-1a (Linco cytokine panel)	Glucose* (fasting and OGTT)
IL-8 (Linco cytokine panel)	C-Peptide (fasting and OGTT)
IL-6 (Elisa)	Norepinephrine
TNFa (Linco cytokine panel)	DHEA(S)*
	Insulin (Fasting and OGTT)
ICAM-1 (Elisa)*	Cortisol
CRP (BNII)*	TSH
MCP-1 (Linco panel B)	T3*
TGF-B1 (Elisa)	Progesterone (mid-Luteal phase: days 19-21) (3x)
Leptin (Linco Panel B)	LH (men)
Adiponectin (total)	FSH (men)
Angiotensin II	LH, FSH, Estradiol (women) (estimate 30 total)
GH	SHBG
IGF-1 (Elisa)*	Free Testosterone
IGF-BP1 (Elisa)	Total Testosterone
IGF-BP3 (Elisa)	AB Response pneumoc vaccines (BL, 17M, 18M, 24M)
PDGFAB (Elisa)	AB response tetanus vaccines (BL, 17M, 18M, 24M)
Serum CTx (plus 6 M visit)	AB response hepatitis B vaccines (BL, 17M, 18M, 23M, 24M)
PINP (plus 6 M visit)	
Extraction Kits for Paxgene tubes (2 per person + 5%)	
Extraction Kits for DNA (+5%)	

* = Reliability Study Assays

	LCBR Tube	Test Requests	FAHC Test Request Codes	Bracket/Ref Code	Detectable Range
Box 1 = Vitros	38	Glucose	SGL	[4902	20-625 mg/dl
	39	Glucose	SGL	[4902	20-625 mg/dl
	39	Progesterone	PROG	[162	0.1-50 ng/mL
	40	Glucose	SGL	[4902	20-625 mg/dl
	41	Glucose	SGL	[4902	20-625 mg/dl
	50	Lipid Profile	LPR	[4957	
	55	Glucose	SGL	[4902	20-625 mg/dl
	74	Hepatitis A antibody	HAAB	[205	
Box 2 = Immulite	56	Insulin	INS	[201	
	56	DHEA-sulfate	DHES	8493	15-1000 ug/dl
Box 3 = Centaur	42	Progesterone	PROG	[162	0.1-50 ng/mL
	51	Cortisol	CORT	[149	1-60 ug/dl
	52	Progesterone	PROG	[162	0.1-50 ng/mL
	51	Estradiol	ESTRA	[167	10-1000 pg/ml
	54	Thyroid Stimulating Hormone	TSH	[226	0.02-125 uIu/ml
	54	T3, Total	T3	[156	20-800 ng/dl
	52	Lutenizing Hormone	LH	[150	0.1-175 mIU/ml
	52	Follicle Stimulating Hormone	FSH	[138	0.3-150 mIu/ml
	52	Free Testosterone	TESFT	8508	
	52	*% Free Testosterone*			
	52	Total Testosterone			
	52	Sex Hormone Binding Globulin			
Box 4 = Mayo	*pending*	*Pneumococcal Vaccine Response*	*PNE*	*80412*	
	pending	*Tetanus Toxoid IgG Antibody*	*TETA*	*80414*	
	20	Catecholamine	CATE	8532	
	21	Catecholamine	CATE	8532	
	75	C-Peptide	CPEP	8804	

LCBR Assay	Category by CALERIE	Manufacturer	Catalog#	Sample type	Vol (uL)
IL1a, IL-8	Inflammation	Linco cytokine panel	HCYTO-60K	EDTA	200
Tnfa,MCP-1, Leptin	Inflammation (Leptin is Endocrine response)	Linco Panel B	HADK2-61K-B	EDTA	200
IL-6	Inflammation	R&D chemiluminescent	Q6000B	Serum	230
ICAM-1	Inflammation	R&D Elisa or Bender Med Systems	*pending*	Serum	25
CRP	Inflammation	BNII Nephelometer	OQ1Y21, OQ1Y13	Serum	200
Adiponectin	Endocrine Response	R&D Elisa	DRP300	Serum	10
TGF-B1	Transforming Growth Factor	R&D Elisa	DB100	Serum	120
Angiotensin II	Endocrine Response	Alpco RIA	01-RK-A22	Serum	500
GH	Growth Hormones	DSL	*active? Ultra-sensitve?*	Serum	100
IGF-1	Growth Hormones/Endocrine	DSL	DSL-10-2800	Serum	20
IGF-BP1	Growth Hormones	DSL	DSL-10-7800	Serum	200
IGF-BP3	Growth Hormones/Endocrine	DSL	DSL-10-6600	Serum	10
PDGFAB	Growth Hormones/Endocrine	R&D Elisa	DHD00B	Serum	10
Serum CTX (also at 6M)	Collagen Turnover & Fibrosis	Orion Diagnostica /UniQRIA	06097	Serum	100
PI NP (also at 6M)	Collagen Turnover & Fibrosis	Orion Diagnostica /UniQRIA	06096	Serum	120

Clinical Chemistry Assays	Category by CALERIE	Method	Analyzer	Sample type	Vol (uL)
Glucose (fasting and OGTT)	Glucose Tolerance/Insulin	Colorimetric Reflectance Spectrophotometry	Vitros	Serum	200
C-Peptide	Glucose Tolerance/Insulin	Chemiluminometric immunoassay		Serum	300
Catecholamines	Endocrine Response	High-Pressure Liquid Chromatography		EDTA	2000
DHEA-s	Endocrine Response	Chemiluminescense Immunoassay	Immulite	Serum	400
Cortisol	Endocrine Response	Chemiluminescense Immunoassay	Centaur	Serum	300
TSH and T3	Endocrine Response	Chemiluminescense Immunoassay	Centaur	Serum	500
Progesterone (women only)	Sex Hormones	Chemiluminescense Immunoassay	Centaur	Serum	500
LH (men & amenorrhea)	Sex Hormones	Chemiluminescense Immunoassay	Centaur	Serum	500
FSH (men & amenorrhea)	Sex Hormones	Chemiluminescense Immunoassay	Centaur	Serum	500
Estradiol (amenorrhea)	Sex Hormones	Chemiluminescense Immunoassay	Centaur	Serum	400
SHBG (men only)	Sex Hormones	Immulite Solid Phase 2-site Chemi Immunoassay	Immulite	Serum	500
Free & Total Testosterone (men only)	Sex Hormones	Equilibrium Dialysis, Chemi Immunoassay	Centaur	Serum	500
Lipid Profile	Serum Lipids and Lipoproteins	Colorimetric Reflectance Spectrophotometry	Vitros	Serum	600
Insulin	Glucose Tolerance/Insulin	Chemiluminescense Immunoassay	Immulite	Serum	
Pneumococcal Vaccine Response	AB response	*method pending*		Serum	
Tetanus Toxoid IgG Antibody	AB response	*method pending*		Serum	
Hep A antibody	AB response	*method pending*		Serum	

VII. PROCEDURES FOR ONGOING QUALITY CONTROL IN THE LABORATORY

A. Laboratory testing and analysis control

- Function checks to verify stability and validity of samples:
  - We believe that sample drift over time in a freezer to be virtually non-existent for most classes of biomarkers including general chemistry assays, and many micro- and macro-nutrients, and all proteins examined so far. We have published our experience with a large variety of protein markers: Lewis MR, et al. Longitudinal stability of coagulation, fibrinolysis, and inflammation factors in stored plasma samples. *Thromb Haemost.* 2001;86:1495-500.
- Control checks:
  - We test control preparations with each run, track control results using Levey-Jennings charts, and apply Westgard Multirule QC to ensure quality assay performance.
  - Our control materials come in lyophilized and frozen forms; as we have published (reference noted above), the stability of these materials over time is excellent.
  - Our procedures ensure that lyophilized controls are reconstituted properly and that frozen controls are thawed properly to ensure optimal performance; for example, making certain that temperatures for control materials for thawing do not exceed lab limits.
- Documentation, including tracking of events:
  - Performance of our control preparations is monitored using Levey-Jennings charts, and Westgard Multirule QC procedures are used to determine whether an analytical test is performing acceptably.
  - All QC data are documented, reviewed in real time, kept on file, and periodically audited. Any corrective action is documented in the appropriate QC files and raw data files.

B. Control of measuring and testing equipment

The laboratory is equipped with smaller equipment such as pH meters, centrifuges, analytical balances, etc. Available major equipment includes:

- Stago STA-R Automated Coagulation analyzer for coagulation and immunoturbidmetric assays
- Dade Behring BNII nephelometer
- BioTek Elx808 Ultra Microplate Reader for fluorescence and absorbance ELISA and Kinetic assays
- Molecular Devices Spectra Max 250 for fluorescence and absorbance ELISA and Kinetic assays
- Bio-Rad BioPlex Protein Array System 100-240V for fluorescent bead-based Luminex technology to simultaneously analyze up to 100 targets in a single microplate well
- Dynex Technologies MLX Microtiter Plate Luminometer for luminescence technology
- Cobra gamma counter for RIA
- MJ Research Programmable thermocyclers (4) for PCR
- ABI Prism 7900 DNA Sequence Detector for TaqMan-based SNP analysis

Certain assays are performed in the Clinical Laboratory of the Department of Pathology at Fletcher Allen Health Care (FAHC) using the following instrumentation:

- Tosoh 2.2 Plus hemoglobin A1C analyzer utilizing automated ion-exchange chromatography
- Ortho Clinical Diagnostics Vitros 950 and 250 chemistry analyzers
- Bayer Advia ACS:Centaur automated chemiluminescence analyzer
- Beckman Immage used for microalbumin analysis
- Diagnostic Products Immulite for insulin measurement

- Calibration is scheduled and documented per our standard procedures based on manufacturer's specifications.
- The LCBR and the FAHC laboratory maintain service contracts to ensure appropriate maintenance of testing instruments and to assure availability of rapid response should issues requiring immediate service attention arise.

C. Preventive maintenance

- Our procedures call for performance of preventive maintenance tasks in accordance with recommendations from instrument manufacturers.
- As noted above, the LCBR and the FAHC laboratory maintain service contracts to ensure appropriate maintenance of testing instruments and to assure availability of rapid response should issues requiring immediate service attention arise.

D. Data validation

- Bar-coding of samples and electronic data transfer from instruments to databases are used whenever possible to avoid opportunities for human error.
- When human involvement in data entry or data transfer is unavoidable, we use a data entry and verification step to minimize the risk of transcription errors. Additional measures taken to ensure data integrity are:
  - Spot checks of data
  - Final reconciling of reported data vs. raw data
- Databases are developed using SQL, Visual Basic, and Microsoft Access to facilitate storage and retrieval of data for our various studies.

E. Nonconformity

- As noted above, for assay QC, Westgard multi-rule control procedures are used to determine whether an analytical test is performing acceptably.
- If non-conforming results are identified, the following steps are taken (Tetrault 2001:154-5):
  - The nature and severity of the problem are assessed:
    - Are there random non-conforming values, or is there evidence of systematic error (bias)?
    - What is the magnitude of the deviation, and for how long (i.e., over how many runs) has the deviation been present?
  - QC data are examined and guide the troubleshooting process:
    - If the non-conforming values appear to reflect random error, factors affecting precision (e.g., sample and reagent factors, external factors) are explored.
    - If there is suspicion of systematic deviation, factors influencing accuracy (e.g., stability of calibration) are assessed.
    - Potential drift and/or reagent lot change affects are monitored by assaying a control set of 20 normal donors over the length of the study.

F. Corrective action

- Corrective action follows the results of the process outlined in item 5. For example if the technologist responsible for the assay, in consultation with supervisory staff, identifies the cause or causes of the deviation, we then go on to identify steps to be taken to avoid similar scenarios in the future and to document the problem identification and resolution information. If additional training is found to be desirable, such training is provided and documented as well.

G. Quality documentation and records control

Documents such as sampling procedures, calibration procedures, analytical and test procedures, data collection and reporting are developed and modified based on individual study requirements. When appropriate, all critical documents are kept in locked, secured locations.

- Documentation of all QA and QC data pertaining to performance of assays in a given study are retained indefinitely (i.e., throughout the length of the study and beyond).
- Documents pertaining to ongoing work are maintained on-site in the LCBR.
- Changes are made only with approval of supervisory staff and are documented appropriately (i.e., time and date of change, name of person authorizing change).
- Changes to procedures are communicated to affected personnel immediately by the project manager and to the entire laboratory through our regular laboratory meeting and/or via e-mail.
- When LCBR staff are not regularly scheduled to be present, access to LCBR facilities is via card access only with documentation of entry based on card identification.

Additional issues for consideration

H. Longitudinal stability of assays

In multi-year studies, it is critical to be certain that assay performance remains stable over long periods of time. To help in this endeavor, we have adopted several tactics in the LCBR.

- As is our practice, in both multiplex and individual ELISA assays, care will be taken to minimize reagent lot changes over this project period.
  - Whenever possible, control materials are purchased in advance in quantities such that they will be available for the duration of the study.
  - Whenever possible, reagents are purchased in lots large enough to be used throughout a study.
  - A serum set of approximately 20 individuals is assayed at the start of the study for all baseline measurements and then reassayed periodically throughout the study and when there is any major reagent or instrument change in order to monitor potential assay drift.
- Lyophilized calibrators are also employed whenever possible to maximize assay stability.
- When appropriate, resampling of a selected subset of the original cohort is performed.

VIII. LABORATORY PERFORMANCE AND QC REPORTS FOR THE QC COMMITTEE

A. Monthly Vermont Core Lab QC Reports

The following reports will be submitted monthly to the CALERIE Coordinating Center.

MONTHLY Summary Report

Lab/Reading Center: VERMONT

Date: September 2007

Site: PBRC

	Timepoints					
Status at the End of this Month:	**BL**	**6M**	**12M**	**18M**	**24M**	**Total**
A. Total # of subjects with new samples/records received in the lab:	5	0	0	0	0	5
B. Total # of subjects whose samples/records have completed processing:	5	0	0	0	0	5
C. Total # of subjects whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of subjects whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Site: Tufts University

	Timepoints					
Status at the End of this Month:	**BL**	**6M**	**12M**	**18M**	**24M**	**Total**
A. Total # of subjects with new samples/records received in the lab:	5	0	0	0	0	5
B. Total # of subjects whose samples/records have completed processing:	5	0	0	0	0	5
C. Total # of subjects whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of subjects whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Site: Washington University

Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of subjects with new samples/records received in the lab:	4	0	0	0	0	4
B. Total # of subjects whose samples/records have completed processing:	4	0	0	0	0	4
C. Total # of subjects whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of subjects whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Grand Total from 3 Sites: Status at the End of this Month:	Timepoints					
	BL	6M	12M	18M	24M	Total
A. Total # of subjects with new samples/records received in the lab:	14	0	0	0	0	14
B. Total # of subjects whose samples/records have completed processing:	14	0	0	0	0	14
C. Total # of subjects whose samples/records are awaiting completion:	0	0	0	0	0	0
D: Total # of subjects whose samples/records have errors, problems, or queries, preventing processing from being completed:	0	0	0	0	0	0

Explanations:

A. This is a count of the number of subjects with new samples / records that have been forwarded from the sites this month.
Do not count replacement samples or revisions of old records in your count.

B. This is a count of subjects whose samples / records have completed processing in the lab this month. Include samples / records received this month as well as any samples / records which had been backlogged from previous months.

C. This is a count of the number of subjects whose samples / records have not completed processing and are backlogged to the next month
Include samples / records received this month as well as any samples / records backlogged from previous months.

D. From the total in C, enter the number of subjects whose processing has not been completed due to errors, problems and queries.
Do not include subjects whose samples / records have not started processing or whose processing is proceeding normally.

Monthly Data						Cumulative Data	
	A. No. subjects with new samples received:	B. No. subjects whose samples completed processing:	C. No. subjects whose samples are backlogged:	D. No. subjects whose samples have errors:		A. Cum. no. subjects with samples received:	B. Cum. no. subjects whose samples completed processing:
May-07	0	0	0	0		0	0
Jun-07	1	1	0	0		1	1
Jul-07	7	7	0	0		8	8
Aug-07	9	9	0	0		17	17
Sep-07	12	12	0	0		29	29
Oct-07	14	14	0	0		43	43
Nov-07							
Dec-07							
Jan-08							
Feb-08							
Mar-08							
Apr-08							
May-08							
Jun-08							
Jul-08							
Aug-08							
Sep-08							
Oct-08							
Nov-08							
Dec-08							

B. Quarterly Vermont Core Lab QC Reports

The following Reports will be submitted for each site quarterly to the CALERIE Coordinating Center:

CALERIE Blood QC Monitoring Summary: Shipping and Packaging

Lab: Vermont Core lab
Review Period: September 1 - November 30, 2007
Site: PBRC

Shipping and Packaging	Current	Cumulative
Notification and delivery not on time	0	0
Shipping container damaged or improperly labeled	0	0
Insufficient dry ice	0	0
Samples thawed	0	0
Not packaged according to IATA regulations	0	0
Aliquots incorrectly organized	0	0

Calerie Blood QC Monitoring Summary: Shipping and Packaging

Lab: Vermont Core lab
Review Period: September 1 - November 30, 2007
Site: PBRC

Shipping and Packaging	Current	Cumulative

Notification and delivery not on time	0	0
Shipping container damaged or improperly labeled	0	0
Insufficient dry ice	0	0
Samples thawed	0	0
Not packaged according to IATA regulations	0	0
Aliquots incorrectly organized	0	0

Calerie Blood QC Monitoring Summary: Off Cycle

Lab: Vermont Core lab
Review Period: September 1 - November 30, 2007
Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

Off-cycle Sex Hormone Collection (women only)	BaseLine	12 Month	24 Month
Number of sample sets received	3	0	0

Phlebotomy and Processing	Female Off-cycle Annual Visit Collections Combined	
	Off-Cycle Visit Day 1	Off-Cycle Visit Day 2
Missing or incomplete forms	1	0
Collection tubes not filled	2	0
Blood collection time exceeds acceptable limits	0	
Number of tubes > 90 min serum processing time	0	0
Missing aliquots or tubes upon receipt at LCBR	2	0
Aliquots not frozen upright	0	0

Calerie Blood QC Monitoring Summary: 3, 6, 18M

Lab: Vermont Core lab
Review Period: September 1 - November 30, 2007
Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

	3 Month	6 Month	18 Month
Number of sample sets received	4	0	0

Phlebotomy and Processing	Counts of Protocol Deviations - 3M, 6M, and 18M Collections Combined
Missing or incomplete forms	0
Collection tubes not filled	0
Number of tubes > 30 min plasma processing time	0
Number of tubes > 90 min serum processing time	0
Missing aliquots or tubes upon receipt at LCBR	0
Aliquots not frozen upright	0

Calerie Blood QC Monitoring Summary: 17, 23, Unscheduled

Lab: Vermont Core lab
Review Period: September 1 - November 30, 2007
Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	3	6
Number of shipments received thawed	0	0

	17 Month	23 Month	Unscheduled (women only)
Number of sample sets received	0	0	0

Phlebotomy and Processing	Counts of Protocol Deviations - 17, 23M and Unscheduled Collections Combined
Missing or incomplete forms	0
Collection tubes not filled	0
Number of tubes > 90 min serum processing time	0
Missing aliquots or tubes upon receipt at LCBR	0
Aliquots not frozen upright	0

Calerie Tissue QC Monitoring Summary: Annual Visits

Lab: Vermont Core lab

Review Period: September 1 - November 30, 2007

Site: PBRC

	Current	Cumulative
Number of shipments received @ LCBR	2	4
Number of shipments received thawed	0	0

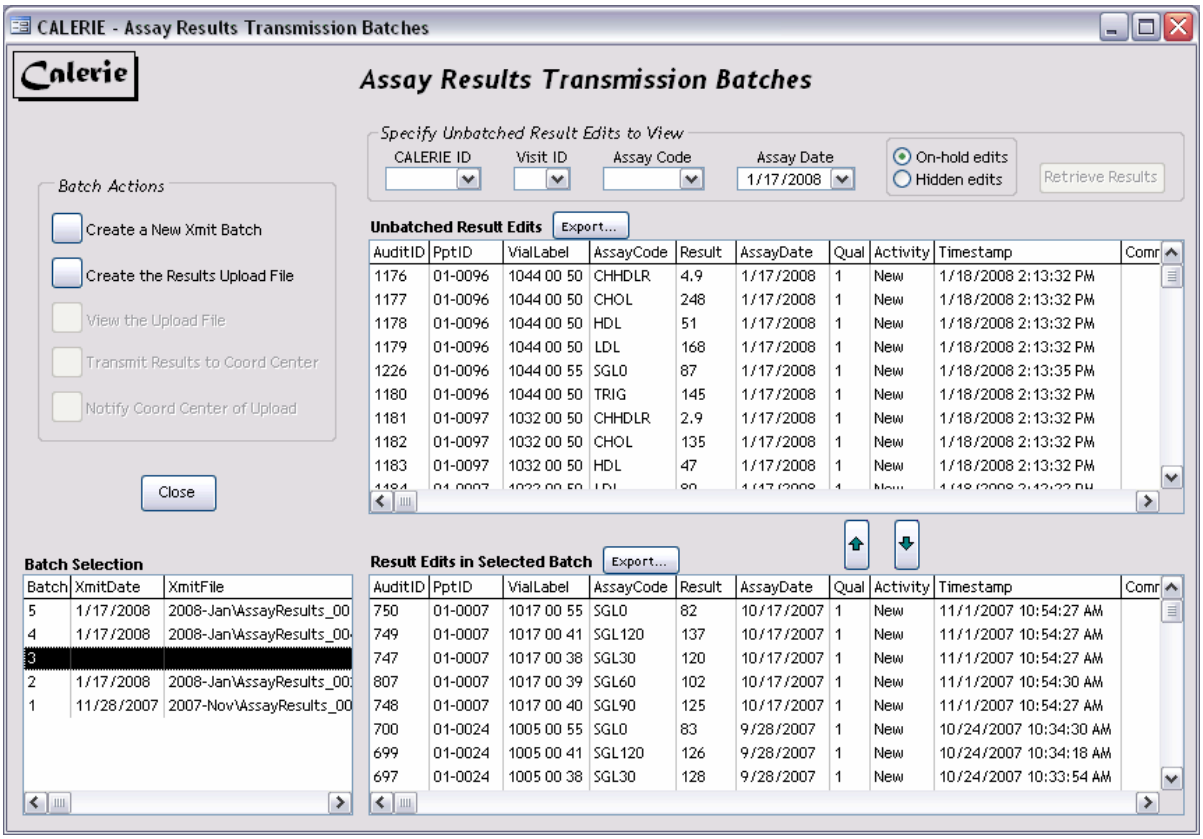
	Baseline	12 Months	24 Months
Number of sample sets received	8	0	0

Phlebotomy and Processing	Counts of Protocol Deviations - Combined
Missing or incomplete forms	0
Biospsy Cryovials not filled	17
Missing cryovials or cassettes upon receipt at LCBR	0
Tissue IHC cassettes not received at 15-30°C	0

IX. ELECTRONIC TRANSFER OF RESULTS TO THE COORDINATING CENTER

A. The Assay Results Transmission Batches Screen

Bring up the Assay Results Transmission Batches form – you can get there from the Main Form or the Assay Results Management screen via the Upload Results to CC button.



The "results" that appear on this screen don't actually come directly from the assay results table; rather, they are drawn from the assay results *audit* table. Thus, on this screen we are actually looking at *audit records* of changes made to the assay results table. The reasoning here is that if an assay result record is altered for any reason, we want the option to transmit that change to the coordinating center.

The *Batch Selection* listbox displays the transmission batches that have been created. When a batch is selected, the results assigned to it appear in the *Result Edits in Selected Batch* listbox.

The *Unbatched Result Edits* listbox is used to display results (or edits thereof) that have not been transmitted to the coordinating center. This is initially empty when the screen is opened. It gets populated by specifying criteria in the *Specify Unbatched Result Edits to View* region and pressing the *Retrieve Results* button.

A note about On-hold edits versus Hidden edits. *On-hold edits* are result audit records that are pending review for transmission. *Hidden edits* are those that are not to be considered for transmission. Normally, when a result is imported or manually edited, that audit record is marked as On-hold (and any previous on-hold edits to that result are automatically flagged as hidden – the assumption being that only the most recent version of a result record should be transmitted). There are some exceptions, the most notable being off-cycle exclusions – these always get marked as hidden.

In either of these result listboxes, double-clicking a row brings up the Assay Result Tracking/Editing screen, which shows the result as currently recorded in the database together with a history of all audit records associated with that result. For unbatched results, this provides a place to manually toggle between On-hold and Hidden status. [This is clunky interface, would like to improve it, but this operation normally isn't needed –the right thing usually happens. --DED]

Results are added to or removed from a batch via the up- and down-arrow buttons. A warning is put up if the batch has an associated XmitDate or XmitFile – the idea being that a change to such a batch will invalidate these data. (Double-clicking an item in the *Batch Selection* listbox brings up screen where these can be edited.)

Preparing and Transmitting a Batch

The procedure of creating and transmitting a batch of assay results to the coordinating center is guided by the Batch Actions section of the Assay Results Transmission Batches screen. A brief summary of the procedure is:

1. Create and prepare a results transmission batch.
2. Create the corresponding results upload file.
3. Transmit the upload file.
4. Notify the coordinating center via email that a results file has been uploaded.

In greater detail, a typical upload session might go as follows. Begin by clicking the Create a New Xmit Batch button. A new batch appears and is selected in the Batch Selection listbox.

Enter criteria specifying which unbatched results you wish to consider and click Retrieve Results. (If you wish to view *all* on-hold edits, just leave these fields blank with On-hold edits selected.) From the Unbatched Result Edits listbox, select which results you wish to include in the new batch and click the down-arrow button to move them.

Once you are satisfied that the new batch contains the results that you want, it is time to create the results text file that actually gets uploaded to the coordinating center. Click *Create the Results Upload File* to accomplish this. This file should be saved somewhere within the folder L:\Groups\LCBR\Databases\CALERIE\FilesToCC. Although you may name this file whatever you want, it is probably best practice to stick with the suggested filename. Once saved, you can click the *View the Upload File* if you wish to look at the file in NotePad.

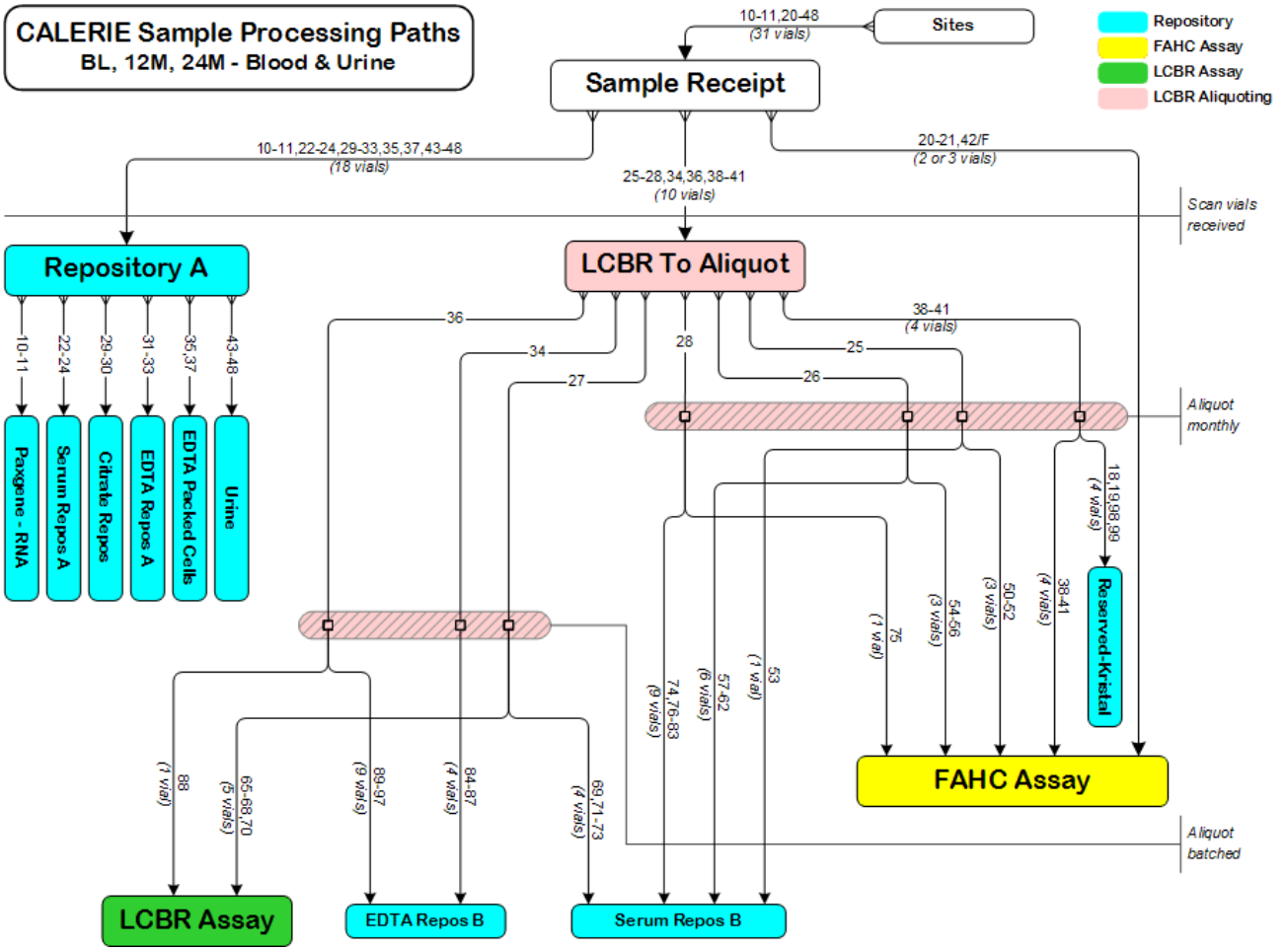
Note: It is suggested to decide on conventions for the naming and location of these upload files. Once saved, it is best to not move them, for that will invalidate the *XmitFile* field in the *Batch*

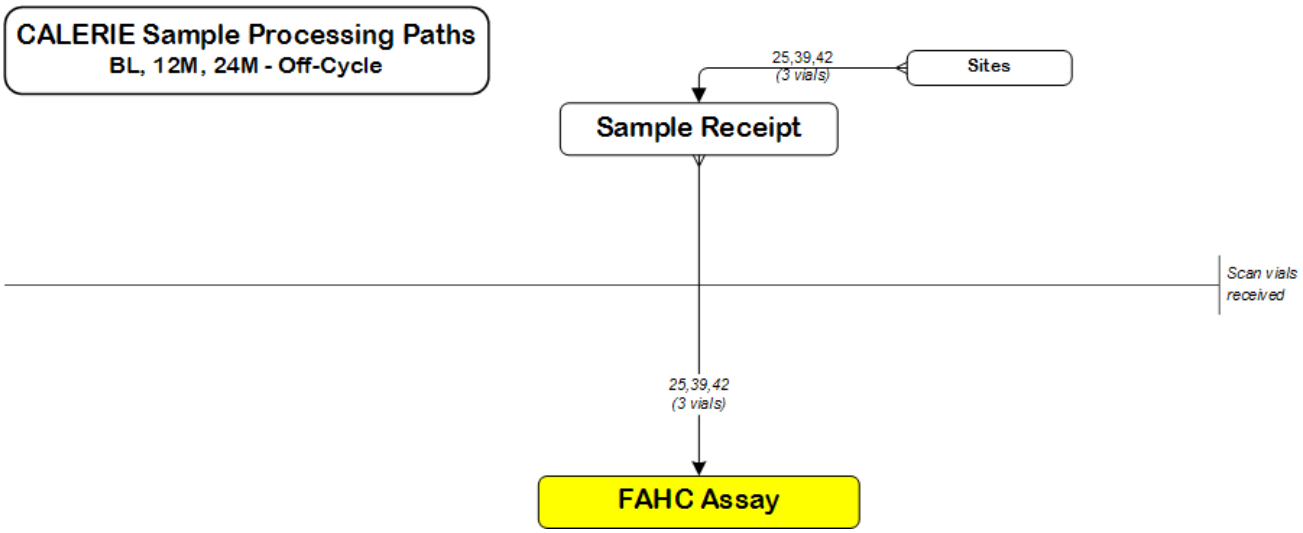
Selection listbox, and the *View the Upload File* button won't work (this link can be reestablished by double-clicking the batch in the Batch Selection listbox).

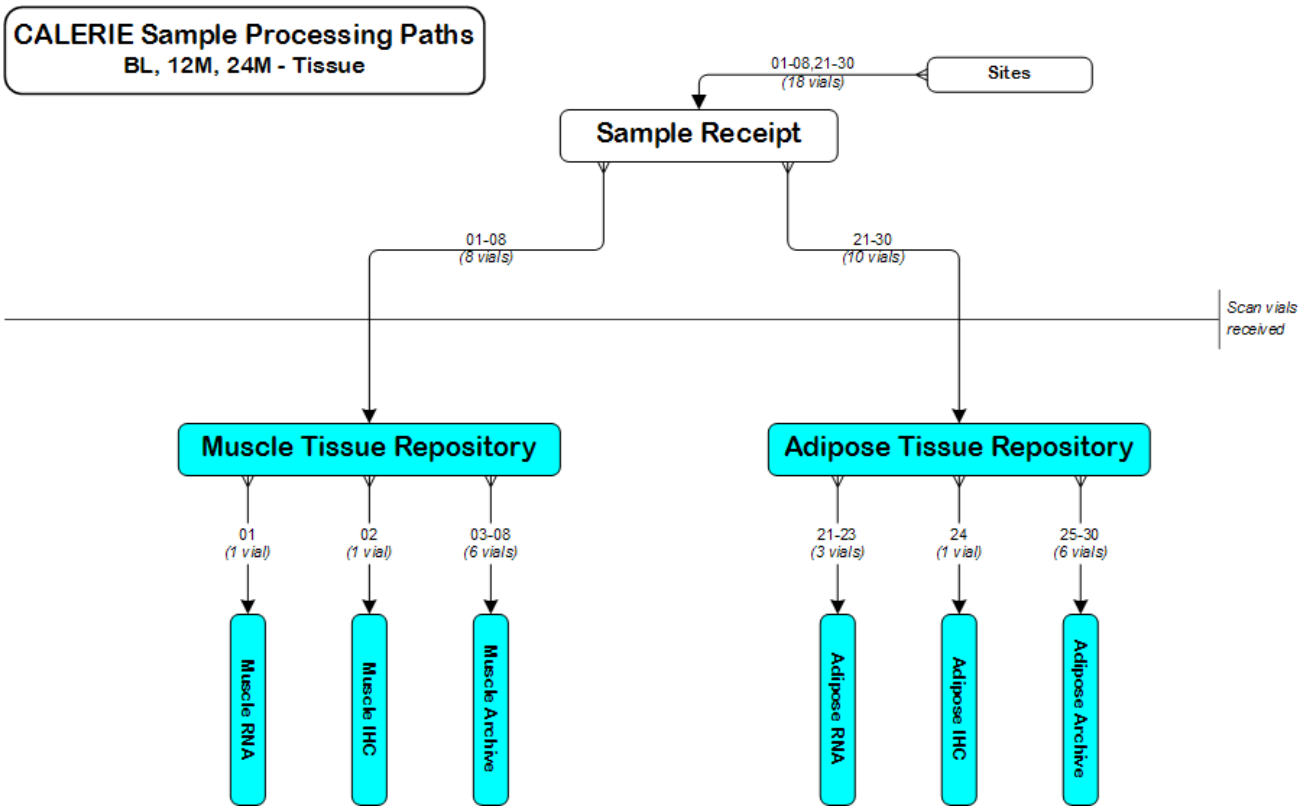
At this point, you are ready to upload the file. Click *Transmit Results to Coord Center*. [NB: The following isn't pretty, but it works. It's surprising how hard it is to make this more friendly. --DED] This will start a command-line script called UploadToDuke.bat that opens a connection to the Duke upload site via the scp2 command (part of the SSH Secure Shell software). You will be prompted for a password. After correctly entering the password [you know it, right? --DED], the file will be uploaded. Wait for the script to finish. It should reply with either Upload succeeded or Uploading terminated with errors. The CALERIE front-end will be asking you whether the file was successfully uploaded – answer appropriately. If it was successful, the batch's XmitDate field will be set to today's date.

Finally, it remains to notify Duke that a file has been uploaded. Click *Notify Coord Center of Upload* – this will bring up an email message formatted appropriately. Feel free to edit or add any comments to the message [although I don't know whether Duke will actually read them - DED]. Click *Send* when you are ready to send the message (or you can cancel the message if you wish; you are not committed to actually sending the message).

APPENDIX A. **Schematics of LCBR Aliquoting Scheme**






CALERIE Sample Processing Paths
BL, 12M, 24M - Tissue
Sites
01-08,21-30
(18 vials)
Sample Receipt
01-08
(8 vials)
21-30
(10 vials)
Scan vials received
Muscle Tissue Repository
Adipose Tissue Repository
01
(1 vial)
02
(1 vial)
03-08
(6 vials)
21-23
(3 vials)
24
(1 vial)
25-30
(6 vials)
Muscle RNA
Muscle IHC
Muscle Archive
Adipose RNA
Adipose IHC
Adipose Archive

APPENDIX B. Testing and Repository Box Maps

B.1 SAMPLE RECEIPT BOXES

FAHC-Mayo Testing (Catecholamines and CPEP)

EDTA Plasma - 10ml Tubes (# 20 & 21) AND Serum 5mL Tube (#75)

PT 1 cryo 20	**PT 4** cryo 21	**PT 8** cryo 20	**PT 11** cryo 21	**X**	**PT 1** cryo 75	**PT 8** cryo 75
PT 1 cryo 21	**PT 5** cryo 20	**PT 8** cryo 21	**PT 12** cryo 20	**X**	**PT 2** cryo 75	**PT 9** cryo 75
PT 2 cryo 20	**PT 5** cryo 21	**PT 9** cryo 20	**PT 12** cryo 21	**X**	**PT 3** cryo 75	**PT 10** cryo 75
PT 2 cryo 21	**PT 6** cryo 20	**PT 9** cryo 21	**PT 13** cryo 20	**X**	**PT 4** cryo 75	**PT 11** cryo 75
PT 3 cryo 20	**PT 6** cryo 21	**PT 10** cryo 20	**PT 13** cryo 21	**X**	**PT 5** cryo 75	**PT 12** cryo 75
PT 3 cryo 21	**PT 7** cryo 20	**PT 10** cryo 21	**PT 14** cryo 20	**X**	**PT 6** cryo 75	**PT 13** cryo 75
PT 4 cryo 20	**PT 7** cryo 21	**PT 11** cryo 20	**PT 14** cryo 21	**X**	**PT 7** cryo 75	**PT 14** cryo 75

CALERIE Annual Visit Pristine Serum Repository Cryovials

Serum- Red-top 1.5mL cryovials (Cryos #22-24)
1.0mL sample volume. 3 Cryos per participant. 33 participants per box.

Pt 1 cryo 22	**Pt 4** cryo 22	**Pt 7** cryo 22	**Pt 10** cryo 22	**Pt 13** cryo 22	**Pt 16** cryo 22	**Pt 19** cryo 22	**Pt 22** cryo 22	**Pt 25** cryo 22	**Pt 28** cryo 22
Pt 1 cryo 23	**Pt 4** cryo 23	**Pt 7** cryo 23	**Pt 10** cryo 23	**Pt 13** cryo 23	**Pt 16** cryo 23	**Pt 19** cryo 23	**Pt 22** cryo 23	**Pt 25** cryo 23	**Pt 28** cryo 23
Pt 1 cryo 24	**Pt 4** cryo 24	**Pt 7** cryo 24	**Pt 10** cryo 24	**Pt 13** cryo 24	**Pt 16** cryo 24	**Pt 19** cryo 24	**Pt 22** cryo 24	**Pt 25** cryo 24	**Pt 28** cryo 24
Pt 2 cryo 22	**Pt 5** cryo 22	**Pt 8** cryo 22	**Pt 11** cryo 22	**Pt 14** cryo 22	**Pt 17** cryo 22	**Pt 20** cryo 22	**Pt 23** cryo 22	**Pt 26** cryo 22	**Pt 29** cryo 22
Pt 2 cryo 23	**Pt 5** cryo 23	**Pt 8** cryo 23	**Pt 11** cryo 23	**Pt 14** cryo 23	**Pt 17** cryo 23	**Pt 20** cryo 23	**Pt 23** cryo 23	**Pt 26** cryo 23	**Pt 29** cryo 23
Pt 2 cryo 24	**Pt 5** cryo 24	**Pt 8** cryo 24	**Pt 11** cryo 24	**Pt 14** cryo 24	**Pt 17** cryo 24	**Pt 20** cryo 24	**Pt 23** cryo 24	**Pt 26** cryo 24	**Pt 29** cryo 24
Pt 3 cryo 22	**Pt 6** cryo 22	**Pt 9** cryo 22	**Pt 12** cryo 22	**Pt 15** cryo 22	**Pt 18** cryo 22	**Pt 21** cryo 22	**Pt 24** cryo 22	**Pt 27** cryo 22	**Pt 30** cryo 22
Pt 3 cryo 23	**Pt 6** cryo 23	**Pt 9** cryo 23	**Pt 12** cryo 23	**Pt 15** cryo 23	**Pt 18** cryo 23	**Pt 21** cryo 23	**Pt 24** cryo 23	**Pt 27** cryo 23	**Pt 30** cryo 23
Pt 3 cryo 24	**Pt 6** cryo 24	**Pt 9** cryo 24	**Pt 12** cryo 24	**Pt 15** cryo 24	**Pt 18** cryo 24	**Pt 21** cryo 24	**Pt 24** cryo 24	**Pt 27** cryo 24	**Pt 30** cryo 24

CALERIE Annual Visit Serum 25 Tubes "LCBR to Aliquot"

Serum-10mL Transfer Tube #25
Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants per box.

PT 1 cryo 25	**PT 8** cryo 25	**PT 15** cryo 25	**PT 22** cryo 25	**PT 29** cryo 25	**PT 36** cryo 25	**PT 43** cryo 25
PT 2 cryo 25	**PT 9** cryo 25	**PT 16** cryo 25	**PT 23** cryo 25	**PT 30** cryo 25	**PT 37** cryo 25	**PT 44** cryo 25
PT 3 cryo 25	**PT 10** cryo 25	**PT 17** cryo 25	**PT 24** cryo 25	**PT 31** cryo 25	**PT 38** cryo 25	**PT 45** cryo 25
PT 4 cryo 25	**PT 11** cryo 25	**PT 18** cryo 25	**PT 25** cryo 25	**PT 32** cryo 25	**PT 39** cryo 25	**PT 46** cryo 25
PT 5 cryo 25	**PT 12** cryo 25	**PT 19** cryo 25	**PT 26** cryo 25	**PT 33** cryo 25	**PT 40** cryo 25	**PT 47** cryo 25
PT 6 cryo 25	**PT 13** cryo 25	**PT 20** cryo 25	**PT 27** cryo 25	**PT 34** cryo 25	**PT 41** cryo 25	**PT 48** cryo 25
PT 7 cryo 25	**PT 14** cryo 25	**PT 21** cryo 25	**PT 28** cryo 25	**PT 35** cryo 25	**PT 42** cryo 25	**X**

CALERIE Annual Visit Serum 26 Tubes "LCBR to Aliquot"

Serum-10mL Transfer Tube #26
Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 26	**PT 8** cryo 26	**PT 15** cryo 26	**PT 22** cryo 26	**PT 29** cryo 26	**PT 36** cryo 26	**PT 43** cryo 26
PT 2 cryo 26	**PT 9** cryo 26	**PT 16** cryo 26	**PT 23** cryo 26	**PT 30** cryo 26	**PT 37** cryo 26	**PT 44** cryo 26
PT 3 cryo 26	**PT 10** cryo 26	**PT 17** cryo 26	**PT 24** cryo 26	**PT 31** cryo 26	**PT 38** cryo 26	**PT 45** cryo 26
PT 4 cryo 26	**PT 11** cryo 26	**PT 18** cryo 26	**PT 25** cryo 26	**PT 32** cryo 26	**PT 39** cryo 26	**PT 46** cryo 26
PT 5 cryo 26	**PT 12** cryo 26	**PT 19** cryo 26	**PT 26** cryo 26	**PT 33** cryo 26	**PT 40** cryo 26	**PT 47** cryo 26
PT 6 cryo 26	**PT 13** cryo 26	**PT 20** cryo 26	**PT 27** cryo 26	**PT 34** cryo 26	**PT 41** cryo 26	**PT 48** cryo 26
PT 7 cryo 26	**PT 14** cryo 26	**PT 21** cryo 26	**PT 28** cryo 26	**PT 35** cryo 26	**PT 42** cryo 26	**X**

CALERIE Annual Visit Serum 27 Tubes "LCBR to Aliquot"

Serum-10mL Transfer Tube #27
Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 27	**PT 8** cryo 27	**PT 15** cryo 27	**PT 22** cryo 27	**PT 29** cryo 27	**PT 36** cryo 27	**PT 43** cryo 27
PT 2 cryo 27	**PT 9** cryo 27	**PT 16** cryo 27	**PT 23** cryo 27	**PT 30** cryo 27	**PT 37** cryo 27	**PT 44** cryo 27
PT 3 cryo 27	**PT 10** cryo 27	**PT 17** cryo 27	**PT 24** cryo 27	**PT 31** cryo 27	**PT 38** cryo 27	**PT 45** cryo 27
PT 4 cryo 27	**PT 11** cryo 27	**PT 18** cryo 27	**PT 25** cryo 27	**PT 32** cryo 27	**PT 39** cryo 27	**PT 46** cryo 27
PT 5 cryo 27	**PT 12** cryo 27	**PT 19** cryo 27	**PT 26** cryo 27	**PT 33** cryo 27	**PT 40** cryo 27	**PT 47** cryo 27
PT 6 cryo 27	**PT 13** cryo 27	**PT 20** cryo 27	**PT 27** cryo 27	**PT 34** cryo 27	**PT 41** cryo 27	**PT 48** cryo 27
PT 7 cryo 27	**PT 14** cryo 27	**PT 21** cryo 27	**PT 28** cryo 27	**PT 35** cryo 27	**PT 42** cryo 27	**X**

CALERIE Annual Visit Serum 28Tubes "LCBR to Aliquot"

Serum-10mL Transfer Tube #28
Approximately 5.0mL sample volume. 1 Tube per participant. 48 participants

PT 1 cryo 28	**PT 8** cryo 28	**PT 15** cryo 28	**PT 22** cryo 28	**PT 29** cryo 28	**PT 36** cryo 28	**PT 43** cryo 28
PT 2 cryo 28	**PT 9** cryo 28	**PT 16** cryo 28	**PT 23** cryo 28	**PT 30** cryo 28	**PT 37** cryo 28	**PT 44** cryo 28
PT 3 cryo 28	**PT 10** cryo 28	**PT 17** cryo 28	**PT 24** cryo 28	**PT 31** cryo 28	**PT 38** cryo 28	**PT 45** cryo 28
PT 4 cryo 28	**PT 11** cryo 28	**PT 18** cryo 28	**PT 25** cryo 28	**PT 32** cryo 28	**PT 39** cryo 28	**PT 46** cryo 28
PT 5 cryo 28	**PT 12** cryo 28	**PT 19** cryo 28	**PT 26** cryo 28	**PT 33** cryo 28	**PT 40** cryo 28	**PT 47** cryo 28
PT 6 cryo 28	**PT 13** cryo 28	**PT 20** cryo 28	**PT 27** cryo 28	**PT 34** cryo 28	**PT 41** cryo 28	**PT 48** cryo 28
PT 7 cryo 28	**PT 14** cryo 28	**PT 21** cryo 28	**PT 28** cryo 28	**PT 35** cryo 28	**PT 42** cryo 28	**X**

CALERIE Pristine Citrate Repository Cryovials

Citrate Plasma-Blue Top 1.5mL Cryovial (#29 &30)
1.0mL sample volume. 2 Cryos per participant, 49 participants per box.

Pt 1 cryo 29	**Pt 6** cryo 29	**Pt 11** cryo 29	**Pt 16** cryo 29	**Pt 21** cryo 29	**Pt 26** cryo 29	**Pt 31** cryo 29	**Pt 36** cryo 29	**Pt 41** cryo 29	**Pt 46** cryo 29
Pt 1 cryo 30	**Pt 6** cryo 30	**Pt 11** cryo 30	**Pt 16** cryo 30	**Pt 21** cryo 30	**Pt 26** cryo 30	**Pt 31** cryo 30	**Pt 36** cryo 30	**Pt 41** cryo 30	**Pt 46** cryo 30
Pt 2 cryo 29	**Pt 7** cryo 29	**Pt 12** cryo 29	**Pt 17** cryo 29	**Pt 22** cryo 29	**Pt 27** cryo 29	**Pt 32** cryo 29	**Pt 37** cryo 29	**Pt 42** cryo 29	**Pt 47** cryo 29
Pt 2 cryo 30	**Pt 7** cryo 30	**Pt 12** cryo 30	**Pt 17** cryo 30	**Pt 22** cryo 30	**Pt 27** cryo 30	**Pt 32** cryo 30	**Pt 37** cryo 30	**Pt 42** cryo 30	**Pt 47** cryo 30
Pt 3 cryo 29	**Pt 8** cryo 29	**Pt 13** cryo 29	**Pt 18** cryo 29	**Pt 23** cryo 29	**Pt 28** cryo 29	**Pt 33** cryo 29	**Pt 38** cryo 29	**Pt 43** cryo 29	**Pt 48** cryo 29
Pt 3 cryo 30	**Pt 8** cryo 30	**Pt 13** cryo 30	**Pt 18** cryo 30	**Pt 23** cryo 30	**Pt 28** cryo 30	**Pt 33** cryo 30	**Pt 38** cryo 30	**Pt 43** cryo 30	**Pt 48** cryo 30
Pt 4 cryo 29	**Pt 9** cryo 29	**Pt 14** cryo 29	**Pt 19** cryo 29	**Pt 24** cryo 29	**Pt 29** cryo 29	**Pt 34** cryo 29	**Pt 39** cryo 29	**Pt 44** cryo 29	**Pt 49** cryo 29
Pt 4 cryo 30	**Pt 9** cryo 30	**Pt 14** cryo 30	**Pt 19** cryo 30	**Pt 24** cryo 30	**Pt 29** cryo 30	**Pt 34** cryo 30	**Pt 39** cryo 30	**Pt 44** cryo 30	**Pt 49** cryo 30
Pt 5 cryo 29	**Pt 10** cryo 29	**Pt 15** cryo 29	**Pt 20** cryo 29	**Pt 25** cryo 29	**Pt 30** cryo 29	**Pt 35** cryo 29	**Pt 40** cryo 29	**Pt 45** cryo 29	**X**
Pt 5 cryo 30	**Pt 10** cryo 30	**Pt 15** cryo 30	**Pt 20** cryo 30	**Pt 25** cryo 30	**Pt 30** cryo 30	**Pt 35** cryo 30	**Pt 40** cryo 30	**Pt 45** cryo 30	**X**

CALERIE Annual Visit Pristine EDTA Repository Cryovials

EDTA-Purple Top 1.5mL Cryovials (#31-33)
1.0mL sample volume. 3 Cryos per participant. 33 participants per box.

Pt	**1**	**Pt**	**4**	**Pt**	**7**	**Pt**	**10**	**Pt**	**13**	**Pt**	**16**	**Pt**	**19**	**Pt**	**22**	**Pt**	**25**	**Pt**	**28**
cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31	
Pt	**1**	**Pt**	**4**	**Pt**	**7**	**Pt**	**10**	**Pt**	**13**	**Pt**	**16**	**Pt**	**19**	**Pt**	**22**	**Pt**	**25**	**Pt**	**28**
cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32	
Pt	**1**	**Pt**	**4**	**Pt**	**7**	**Pt**	**10**	**Pt**	**13**	**Pt**	**16**	**Pt**	**19**	**Pt**	**22**	**Pt**	**25**	**Pt**	**28**
cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33	
Pt	**2**	**Pt**	**5**	**Pt**	**8**	**Pt**	**11**	**Pt**	**14**	**Pt**	**17**	**Pt**	**20**	**Pt**	**23**	**Pt**	**26**	**Pt**	**29**
cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31	
Pt	**2**	**Pt**	**5**	**Pt**	**8**	**Pt**	**11**	**Pt**	**14**	**Pt**	**17**	**Pt**	**20**	**Pt**	**23**	**Pt**	**26**	**Pt**	**29**
cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32	
Pt	**2**	**Pt**	**5**	**Pt**	**8**	**Pt**	**11**	**Pt**	**14**	**Pt**	**17**	**Pt**	**20**	**Pt**	**23**	**Pt**	**26**	**Pt**	**29**
cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33	
Pt	**3**	**Pt**	**6**	**Pt**	**9**	**Pt**	**12**	**Pt**	**15**	**Pt**	**18**	**Pt**	**21**	**Pt**	**24**	**Pt**	**27**	**Pt**	**30**
cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31		cryo 31	
Pt	**3**	**Pt**	**6**	**Pt**	**9**	**Pt**	**12**	**Pt**	**15**	**Pt**	**18**	**Pt**	**21**	**Pt**	**24**	**Pt**	**27**	**Pt**	**30**
cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32		cryo 32	
Pt	**3**	**Pt**	**6**	**Pt**	**9**	**Pt**	**12**	**Pt**	**15**	**Pt**	**18**	**Pt**	**21**	**Pt**	**24**	**Pt**	**27**	**Pt**	**30**
cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33		cryo 33	
Pt	**31**	**Pt**	**31**	**Pt**	**31**	**Pt**	**32**	**Pt**	**32**	**Pt**	**32**	**Pt**	**33**	**Pt**	**33**	**Pt**	**33**	**X**	
cryo 31		cryo 32		cryo 33		cryo 31		cryo 32		cryo 33		cryo 31		cryo 32		cryo 33			

CALERIE EDTA 34 Tubes "LCBR to Aliquot"

EDTA 10mL Transfer Tube #34
Approximately 5.0mL sample volume. 1 tube per participant. 48 participants per box.

PT 1 cryo 34	**PT 8** cryo 34	**PT 15** cryo 34	**PT 22** cryo 34	**PT 29** cryo 34	**PT 36** cryo 34	**PT 43** cryo 34
PT 2 cryo 34	**PT 9** cryo 34	**PT 16** cryo 34	**PT 23** cryo 34	**PT 30** cryo 34	**PT 37** cryo 34	**PT 44** cryo 34
PT 3 cryo 34	**PT 10** cryo 34	**PT 17** cryo 34	**PT 24** cryo 34	**PT 31** cryo 34	**PT 38** cryo 34	**PT 45** cryo 34
PT 4 cryo 34	**PT 11** cryo 34	**PT 18** cryo 34	**PT 25** cryo 34	**PT 32** cryo 34	**PT 39** cryo 34	**PT 46** cryo 34
PT 5 cryo 34	**PT 12** cryo 34	**PT 19** cryo 34	**PT 26** cryo 34	**PT 33** cryo 34	**PT 40** cryo 34	**PT 47** cryo 34
PT 6 cryo 34	**PT 13** cryo 34	**PT 20** cryo 34	**PT 27** cryo 34	**PT 34** cryo 34	**PT 41** cryo 34	**PT 48** cryo 34
PT 7 cryo 34	**PT 14** cryo 34	**PT 21** cryo 34	**PT 28** cryo 34	**PT 35** cryo 34	**PT 42** cryo 34	**X**

CALERIE Packed Red Cell Tubes for DNA Extraction

Packed Cells in 10mL Transfer Tubes (#35 &37)
Approximately 5.0mL sample volume. 2 Tubes per participant. 24 participants per box.

PT 1 cryo 35	**PT 4** cryo 37	**PT 8** cryo 35	**PT 11** cryo 37	**PT 15** cryo 35	**PT 18** cryo 37	**PT 22** cryo 35
PT 1 cryo 37	**PT 5** cryo 35	**PT 8** cryo 37	**PT 12** cryo 35	**PT 15** cryo 37	**PT 19** cryo 35	**PT 22** cryo 37
PT 2 cryo 35	**PT 5** cryo 37	**PT 9** cryo 35	**PT 12** cryo 37	**PT 16** cryo 35	**PT 19** cryo 37	**PT 23** cryo 35
PT 2 cryo 37	**PT 6** cryo 35	**PT 9** cryo 37	**PT 13** cryo 35	**PT 16** cryo 37	**PT 20** cryo 35	**PT 23** cryo 37
PT 3 cryo 35	**PT 6** cryo 37	**PT 10** cryo 35	**PT 13** cryo 37	**PT 17** cryo 35	**PT 20** cryo 37	**PT 24** cryo 35
PT 3 cryo 37	**PT 7** cryo 35	**PT 10** cryo 37	**PT 14** cryo 35	**PT 17** cryo 37	**PT 21** cryo 35	**PT 24** cryo 37
PT 4 cryo 35	**PT 7** cryo 37	**PT 11** cryo 35	**PT 14** cryo 37	**PT 18** cryo 35	**PT 21** cryo 37	**X**

CALERIE EDTA Tube 36 "LCBR to Aliquot"

EDTA 10mL EDTA Transfer Tube #36
Approximately 5.0mL sample volume; 1 tube per participant; 48 participants per box.

PT 1 cryo 36	**PT 8** cryo 36	**PT 15** cryo 36	**PT 22** cryo 36	**PT 29** cryo 36	**PT 36** cryo 36	**PT 43** cryo 36
PT 2 cryo 36	**PT 9** cryo 36	**PT 16** cryo 36	**PT 23** cryo 36	**PT 30** cryo 36	**PT 37** cryo 36	**PT 44** cryo 36
PT 3 cryo 36	**PT 10** cryo 36	**PT 17** cryo 36	**PT 24** cryo 36	**PT 31** cryo 36	**PT 38** cryo 36	**PT 45** cryo 36
PT 4 cryo 36	**PT 11** cryo 36	**PT 18** cryo 36	**PT 25** cryo 36	**PT 32** cryo 36	**PT 39** cryo 36	**PT 46** cryo 36
PT 5 cryo 36	**PT 12** cryo 36	**PT 19** cryo 36	**PT 26** cryo 36	**PT 33** cryo 36	**PT 40** cryo 36	**PT 47** cryo 36
PT 6 cryo 36	**PT 13** cryo 36	**PT 20** cryo 36	**PT 27** cryo 36	**PT 34** cryo 36	**PT 41** cryo 36	**PT 48** cryo 36
PT 7 cryo 36	**PT 14** cryo 36	**PT 21** cryo 36	**PT 28** cryo 36	**PT 35** cryo 36	**PT 42** cryo 36	**X**

CALERIE PAXgene Tubes for RNA Isolation

PAXgene 2.5mL Collection Tubes (Tube #10 and 11)
2 Tubes per participant. 24 participants per box

PT 1 Tube 10	**PT 4** Tube 11	**PT 8** Tube 10	**PT 11** Tube 11	**PT 15** Tube 10	**PT 18** Tube 11	**PT 22** Tube 10
PT 1 Tube 11	**PT 5** Tube 10	**PT 8** Tube 11	**PT 12** Tube 10	**PT 15** Tube 11	**PT 19** Tube 10	**PT 22** Tube 11
PT 2 Tube 10	**PT 5** Tube 11	**PT 9** Tube 10	**PT 12** Tube 11	**PT 16** Tube 10	**PT 19** Tube 11	**PT 23** Tube 10
PT 2 Tube 11	**PT 6** Tube 10	**PT 9** Tube 11	**PT 13** Tube 10	**PT 16** Tube 11	**PT 20** Tube 10	**PT 23** Tube 11
PT 3 Tube 10	**PT 6** Tube 11	**PT 10** Tube 10	**PT 13** Tube 11	**PT 17** Tube 10	**PT 20** Tube 11	**PT 24** Tube 10
PT 3 Tube 11	**PT 7** Tube 10	**PT 10** Tube 11	**PT 14** Tube 10	**PT 17** Tube 11	**PT 21** Tube 10	**PT 24** Tube 11
PT 4 Tube 10	**PT 7** Tube 11	**PT 11** Tube 10	**PT 14** Tube 11	**PT 18** Tube 10	**PT 21** Tube 11	**X**

CALERIE FAHC-Vitros Testing

Box Map for Tubes to FAHC: Serum - 4mL OGTT Tubes (Tubes# 38-41, 50 and 55) and 5mL transfer Tube (Tube #74)

Cryos created during aliquotting

PT 1 cryo 39	**PT 1** cryo 38	**PT 3** cryo 40	**PT 5** cryo 41	**PT 1** cryo 50	**PT 1** cryo 55	**PT 1** cryo 74
PT 2 cryo 39	**PT 1** cryo 40	**PT 3** cryo 41	**PT 6** cryo 38	**PT 2** cryo 50	**PT 2** cryo 55	**PT 2** cryo 74
PT 3 cryo 39	**PT 1** cryo 41	**PT 4** cryo 38	**PT 6** cryo 40	**PT 3** cryo 50	**PT 3** cryo 55	**PT 3** cryo 74
PT 4 cryo 39	**PT 2** cryo 38	**PT 4** cryo 40	**PT 6** cryo 41	**PT 4** cryo 50	**PT 4** cryo 55	**PT 4** cryo 74
PT 5 cryo 39	**PT 2** cryo 40	**PT 4** cryo 41	**PT 7** cryo 38	**PT 5** cryo 50	**PT 5** cryo 55	**PT 5** cryo 74
PT 6 cryo 39	**PT 2** cryo 41	**PT 5** cryo 38	**PT 7** cryo 40	**PT 6** cryo 50	**PT 6** cryo 55	**PT 6** cryo 74
PT 7 cryo 39	**PT 3** cryo 38	**PT 5** cryo 40	**PT 7** cryo 41	**PT 7** cryo 50	**PT 7** cryo 55	**PT 7** cryo 74

CALERIE Urine Repository Tubes

Plain Urine-10mL Transfer Tubes (#43-45) AND Boric Acid Urine 10mL Transfer Tubes (#46-48) 9.0mL sample volume. 6 tubes per participant. 8 participants per box.

PT 1 cryo 43	**PT 2** cryo 43	**PT 3** cryo 43	**PT 4** cryo 43	**PT 5** cryo 43	**PT 6** cryo 43	**PT 7** cryo 43
PT 1 cryo 44	**PT 2** cryo 44	**PT 3** cryo 44	**PT 4** cryo 44	**PT 5** cryo 44	**PT 6** cryo 44	**PT 7** cryo 44
PT 1 cryo 45	**PT 2** cryo 45	**PT 3** cryo 45	**PT 4** cryo 45	**PT 5** cryo 45	**PT 6** cryo 45	**PT 7** cryo 45
PT 1 cryo 46	**PT 2** cryo 46	**PT 3** cryo 46	**PT 4** cryo 46	**PT 5** cryo 46	**PT 6** cryo 46	**PT 7** cryo 46
PT 1 cryo 47	**PT 2** cryo 47	**PT 3** cryo 47	**PT 4** cryo 47	**PT 5** cryo 47	**PT 6** cryo 47	**PT 7** cryo 47
PT 1 cryo 48	**PT 2** cryo 48	**PT 3** cryo 48	**PT 4** cryo 48	**PT 5** cryo 48	**PT 6** cryo 48	**PT 7** cryo 48
PT 8 cryo 43	**PT 8** cryo 44	**PT 8** cryo 45	**PT 8** cryo 46	**PT 8** cryo 47	**PT 8** cryo 48	**X**

CALERIE FAHC-Centaur Testing

Serum 4.0mL OGTT Tubes (Off-cycle visit tubes #25 and 39 AND Day 2 Tube #42)

LCBR prepared aliquots #51, 52, and 54
Use Box map as a guideline; will vary based on samples received.

PT 1 cryo 42	**PT 2** **cryo 25**	**PT 3** **cryo 42**	X	**PT 1** cryo 51	**PT 1** cryo 54	**PT 1** male cryo 52
PT 1 female cryo 52	**PT 2** **cryo 39**	**PT 4** cryo 42	X	**PT 2** cryo 51	**PT 2** cryo 54	**PT 2** male cryo 52
PT 1 **cryo 25**	**PT 2** **cryo 42**	**PT 4** female cryo 52	X	**PT 3** cryo 51	**PT 3** cryo 54	**PT 3** male cryo 52
PT 1 **cryo 39**	**PT 3** cryo 42	**PT 4** **cryo 25**	X	**PT 4** cryo 51	**PT 4** cryo 54	**PT 4** male cryo 52
PT 1 **cryo 42**	**PT 3** female cryo 52	**PT 4** **cryo 39**	X	**PT 5** cryo 51	**PT 5** cryo 54	**PT 5** male cryo 52
PT 2 cryo 42	**PT 3** **cryo 25**	**PT 4** **cryo 42**	X	**PT 6** cryo 51	**PT 6** cryo 54	**PT 6** male cryo 52
PT 2 female cryo 52	**PT 3** **cryo 39**	X	X	**PT 7** cryo 51	**PT 7** cryo 54	**PT 7** male cryo 52

CALERIE- 3M, 6M or 18M Serum Repository Cryovials

Serum - Red Top 1.5ml Cryo (Cryos# 03-06)
1.0ml sample volume. 4 cryos per participant. 25 participants per box.

Pt 1 cryo 03	**Pt 3** cryo 05	**Pt 6** cryo 03	**Pt 8** cryo 05	**Pt 11** cryo 03	**Pt 13** cryo 05	**Pt 16** cryo 03	**Pt 18** cryo 05	**Pt 21** cryo 03	**Pt 23** cryo 05
Pt 1 cryo 04	**Pt 3** cryo 06	**Pt 6** cryo 04	**Pt 8** cryo 06	**Pt 11** cryo 04	**Pt 13** cryo 06	**Pt 16** cryo 04	**Pt 18** cryo 06	**Pt 21** cryo 04	**Pt 23** cryo 06
Pt 1 cryo 05	**Pt 4** cryo 03	**Pt 6** cryo 05	**Pt 9** cryo 03	**Pt 11** cryo 05	**Pt 14** cryo 03	**Pt 16** cryo 05	**Pt 19** cryo 03	**Pt 21** cryo 05	**Pt 24** cryo 03
Pt 1 cryo 06	**Pt 4** cryo 04	**Pt 6** cryo 06	**Pt 9** cryo 04	**Pt 11** cryo 06	**Pt 14** cryo 04	**Pt 16** cryo 06	**Pt 19** cryo 04	**Pt 21** cryo 06	**Pt 24** cryo 04
Pt 2 cryo 03	**Pt 4** cryo 05	**Pt 7** cryo 03	**Pt 9** cryo 05	**Pt 12** cryo 03	**Pt 14** cryo 05	**Pt 17** cryo 03	**Pt 19** cryo 05	**Pt 22** cryo 03	**Pt 24** cryo 05
Pt 2 cryo 04	**Pt 4** cryo 06	**Pt 7** cryo 04	**Pt 9** cryo 06	**Pt 12** cryo 04	**Pt 14** cryo 06	**Pt 17** cryo 04	**Pt 19** cryo 06	**Pt 22** cryo 04	**Pt 24** cryo 06
Pt 2 cryo 05	**Pt 5** cryo 03	**Pt 7** cryo 05	**Pt 10** cryo 03	**Pt 12** cryo 05	**Pt 15** cryo 03	**Pt 17** cryo 05	**Pt 20** cryo 03	**Pt 22** cryo 05	**Pt 25** cryo 03
Pt 2 cryo 06	**Pt 5** cryo 04	**Pt 7** cryo 06	**Pt 10** cryo 04	**Pt 12** cryo 06	**Pt 15** cryo 04	**Pt 17** cryo 06	**Pt 20** cryo 04	**Pt 22** cryo 06	**Pt 25** cryo 04
Pt 3 cryo 03	**Pt 5** cryo 05	**Pt 8** cryo 03	**Pt 10** cryo 05	**Pt 13** cryo 03	**Pt 15** cryo 05	**Pt 18** cryo 03	**Pt 20** cryo 05	**Pt 23** cryo 03	**Pt 25** cryo 05
Pt 3 cryo 04	**Pt 5** cryo 06	**Pt 8** cryo 04	**Pt 10** cryo 06	**Pt 13** cryo 04	**Pt 15** cryo 06	**Pt 18** cryo 04	**Pt 20** cryo 06	**Pt 23** cryo 04	**Pt 25** cryo 06

CALERIE- 18 M Serum *FAHC* Cryo-Ab Response Testing

Serum - Red Top 1.5ml Cryo #03
1.0ml sample volume. 1 cryo per participant. 99 participants per box.

Pt 1 cryo 03	**Pt 11** cryo 03	**Pt 21** cryo 03	**Pt 31** cryo 03	**Pt 41** cryo 03	**Pt 51** cryo 03	**Pt 61** cryo 03	**Pt 71** cryo 03	**Pt 81** cryo 03	**Pt 91** cryo 03
Pt 2 cryo 03	**Pt 12** cryo 03	**Pt 22** cryo 03	**Pt 32** cryo 03	**Pt 42** cryo 03	**Pt 52** cryo 03	**Pt 62** cryo 03	**Pt 72** cryo 03	**Pt 82** cryo 03	**Pt 92** cryo 03
Pt 3 cryo 03	**Pt 13** cryo 03	**Pt 23** cryo 03	**Pt 33** cryo 03	**Pt 43** cryo 03	**Pt 53** cryo 03	**Pt 63** cryo 03	**Pt 73** cryo 03	**Pt 83** cryo 03	**Pt 93** cryo 03
Pt 4 cryo 03	**Pt 14** cryo 03	**Pt 24** cryo 03	**Pt 34** cryo 03	**Pt 44** cryo 03	**Pt 54** cryo 03	**Pt 64** cryo 03	**Pt 74** cryo 03	**Pt 84** cryo 03	**Pt 94** cryo 03
Pt 5 cryo 03	**Pt 15** cryo 03	**Pt 25** cryo 03	**Pt 35** cryo 03	**Pt 45** cryo 03	**Pt 55** cryo 03	**Pt 65** cryo 03	**Pt 75** cryo 03	**Pt 85** cryo 03	**Pt 95** cryo 03
Pt 6 cryo 03	**Pt 16** cryo 03	**Pt 26** cryo 03	**Pt 36** cryo 03	**Pt 46** cryo 03	**Pt 56** cryo 03	**Pt 66** cryo 03	**Pt 76** cryo 03	**Pt 86** cryo 03	**Pt 96** cryo 03
Pt 7 cryo 03	**Pt 17** cryo 03	**Pt 27** cryo 03	**Pt 37** cryo 03	**Pt 47** cryo 03	**Pt 57** cryo 03	**Pt 67** cryo 03	**Pt 77** cryo 03	**Pt 87** cryo 03	**Pt 97** cryo 03
Pt 8 cryo 03	**Pt 18** cryo 03	**Pt 28** cryo 03	**Pt 38** cryo 03	**Pt 48** cryo 03	**Pt 58** cryo 03	**Pt 68** cryo 03	**Pt 78** cryo 03	**Pt 88** cryo 03	**Pt 98** cryo 03
Pt 9 cryo 03	**Pt 19** cryo 03	**Pt 29** cryo 03	**Pt 39** cryo 03	**Pt 49** cryo 03	**Pt 59** cryo 03	**Pt 69** cryo 03	**Pt 79** cryo 03	**Pt 89** cryo 03	**Pt 99** cryo 03
Pt 10 cryo 03	**Pt 20** cryo 03	**Pt 30** cryo 03	**Pt 40** cryo 03	**Pt 50** cryo 03	**Pt 60** cryo 03	**Pt 70** cryo 03	**Pt 80** cryo 03	**Pt 90** cryo 03	**X**

CALERIE 17M, 23M, OR Unscheduled Visits Serum Tubes

Serum-10mL Transfer Tube #02 rec'd for 17M, 23M, or Unscheduled CALERIE Visits

Approx. 2.0ml sample volume. 1 tubes per ppt. 48 ppts per box.

PT 1 tube 02	**PT 8** tube 02	**PT 15** tube 02	**PT 22** tube 02	**PT 29** tube 02	**PT 36** tube 02	**PT 43** tube 02
PT 2 tube 02	**PT 9** tube 02	**PT 16** tube 02	**PT 23** tube 02	**PT 30** tube 02	**PT 37** tube 02	**PT 44** tube 02
PT 3 tube 02	**PT 10** tube 02	**PT 17** tube 02	**PT 24** tube 02	**PT 31** tube 02	**PT 38** tube 02	**PT 45** tube 02
PT 4 tube 02	**PT 11** tube 02	**PT 18** tube 02	**PT 25** tube 02	**PT 32** tube 02	**PT 39** tube 02	**PT 46** tube 02
PT 5 tube 02	**PT 12** tube 02	**PT 19** tube 02	**PT 26** tube 02	**PT 33** tube 02	**PT 40** tube 02	**PT 47** tube 02
PT 6 tube 02	**PT 13** tube 02	**PT 20** tube 02	**PT 27** tube 02	**PT 34** tube 02	**PT 41** tube 02	**PT 48** tube 02
PT 7 tube 02	**PT 14** tube 02	**PT 21** tube 02	**PT 28** tube 02	**PT 35** tube 02	**PT 42** tube 02	**X**

CALERIE Muscle IHC TISSUE (tissue cryo #02)

5.0mL vial with muscle tissue IHC samples
1 vial per ppt and 25 ppts per box in 5x5

PPT 1	**PPT 6**	**PPT 11**	**PPT 16**	**PPT 21**
PPT 2	**PPT 7**	**PPT 12**	**PPT 17**	**PPT 22**
PPT 3	**PPT 8**	**PPT 13**	**PPT 18**	**PPT 23**
PPT 4	**PPT 9**	**PPT 14**	**PPT 19**	**PPT 24**
PPT 5	**PPT 10**	**PPT 15**	**PPT 20**	**PPT 25**

1 vial per ppt expected from all sites: PBRC,Tufts and Wash U

Adipose Tissue Cassette Biopsies

Bouin's Cassette sample #24 received from all 3 sites

n = 280 participants per storage box

40	80	120	160	200	240	280
39	79	119	159	199	239	279
38	78	118	158	198	238	278
37	77	117	157	197	237	277
36	76	116	156	196	236	276
35	75	115	155	195	235	275
34	74	114	154	194	234	274
33	73	113	153	193	233	273
32	72	112	152	192	232	272
31	71	111	151	191	231	271
30	70	110	150	190	230	270
29	69	109	149	189	229	269
28	68	108	148	188	228	268
27	67	107	147	187	227	267
26	66	106	146	186	226	266
25	65	105	145	185	225	265
24	64	104	144	184	224	264
23	63	103	143	183	223	263
22	62	102	142	182	222	262
21	61	101	141	181	221	261
20	60	100	140	180	220	260
19	59	99	139	179	219	259
18	58	98	138	178	218	258
17	57	97	137	177	217	257
16	56	96	136	176	216	256
15	55	95	135	175	215	255
14	54	94	134	174	214	254
13	53	93	133	173	213	253
12	52	92	132	172	212	252
11	51	91	131	171	211	251
10	50	90	130	170	210	250
9	49	89	129	169	209	249
8	48	88	128	168	208	248
7	47	87	127	167	207	247
6	46	86	126	166	206	246
5	45	85	125	165	205	245
4	44	84	124	164	204	244
3	43	83	123	163	203	243
2	42	82	122	162	202	242
1	41	81	121	161	201	241

CALERIE Muscle or Adipose TISSUE cryovials

2.0mL Corning cryovial with muscle or adipose tissue biopsies
9 x 9 grid 5 ppt max

Pt 1 cryo 01	**Pt 1** cryo 23	**Pt 2** cryo 04	**Pt 2** cryo 26	**Pt 3** cryo 06	**Pt 3** cryo 28	**Pt 4** cryo 08	**Pt 4** cryo 30	**Pt 5** cryo 22
Pt 1 cryo 03	**Pt 1** cryo 25	**Pt 2** cryo 05	**Pt 2** cryo 27	**Pt 3** cryo 07	**Pt 3** cryo 29	**Pt 4** cryo 21	**Pt 5** cryo 01	**Pt 5** cryo 23
Pt 1 cryo 04	**Pt 1** cryo 26	**Pt 2** cryo 06	**Pt 2** cryo 28	**Pt 3** cryo 08	**Pt 3** cryo 30	**Pt 4** cryo 22	**Pt 5** cryo 03	**Pt 5** cryo 25
Pt 1 cryo 05	**Pt 1** cryo 27	**Pt 2** cryo 07	**Pt 2** cryo 29	**Pt 3** cryo 21	**Pt 4** cryo 01	**Pt 4** cryo 23	**Pt 5** cryo 04	**Pt 5** cryo 26
Pt 1 cryo 06	**Pt 1** cryo 28	**Pt 2** cryo 08	**Pt 2** cryo 30	**Pt 3** cryo 22	**Pt 4** cryo 03	**Pt 4** cryo 25	**Pt 5** cryo 05	**Pt 5** cryo 27
Pt 1 cryo 07	**Pt 1** cryo 29	**Pt 1** cryo 21	**Pt 3** cryo 01	**Pt 3** cryo 23	**Pt 4** cryo 04	**Pt 4** cryo 26	**Pt 5** cryo 06	**Pt 5** cryo 28
Pt 1 cryo 08	**Pt 1** cryo 30	**Pt 1** cryo 22	**Pt 3** cryo 03	**Pt 3** cryo 25	**Pt 4** cryo 05	**Pt 4** cryo 27	**Pt 5** cryo 07	**Pt 5** cryo 29
Pt 1 cryo 21	**Pt 2** cryo 01	**Pt 2** cryo 23	**Pt 3** cryo 04	**Pt 3** cryo 26	**Pt 4** cryo 06	**Pt 4** cryo 28	**Pt 5** cryo 08	**Pt 5** cryo 30
Pt 1 cryo 22	**Pt 2** cryo 03	**Pt 2** cryo 25	**Pt 3** cryo 05	**Pt 3** cryo 27	**Pt 4** cryo 07	**Pt 4** cryo 29	**Pt 5** cryo 21	**x**

2.0mL Biospy Cryos Expected

Adipose tissue
9 x 2.0ml Corning cryovials (#21-23 and #25-30) — ***All 3 sites***

Muscle Tissue
3 x 2.0mL Corning cryovials #01,03,08 — ***PBRC and Wash U***

7 x 2.0mL Corning cryovials #01-08 — ***Tufts***

B.2 SAMPLE RECEIPT BOXES

CALERIE LCBR Serum Assay Cryovials

Serum Red top 0.5mL or 1.5mL Cryovials (#65-70)
5 cryos per participant. 20participants per box.

Pt 1 cryo 65	**Pt 3** yo 65	**Pt 5** cryo 65	**Pt 7** cryo 65	**Pt 8** cryo 71	**Pt 11** cryo 65	**Pt 13** cryo 65	**Pt 15** cryo 65	**Pt 17** cryo 65	**Pt 19** cryo 65
Pt 1 cryo 66	**Pt 3** yo 66	**Pt 5** cryo 66	**Pt 7** cryo 66	**Pt 9** cryo 65	**Pt 11** cryo 66	**Pt 13** cryo 66	**Pt 15** cryo 66	**Pt 17** cryo 66	**Pt 19** cryo 66
Pt 1 cryo 67	**Pt 3** yo 67	**Pt 5** cryo 67	**Pt 7** cryo 67	**Pt 9** cryo 66	**Pt 11** cryo 67	**Pt 13** cryo 67	**Pt 15** cryo 67	**Pt 17** cryo 67	**Pt 19** cryo 67
Pt 1 cryo 68	**Pt 3** cryo 68	**Pt 5** cryo 68	**Pt 7** cryo 68	**Pt 9** cryo 67	**Pt 11** cryo 68	**Pt 13** cryo 68	**Pt 15** cryo 68	**Pt 17** cryo 68	**Pt 19** cryo 68
Pt 1 cryo 70	**Pt 3** cryo 70	**Pt 5** cryo 70	**Pt 7** cryo 70	**Pt 9** cryo 68	**Pt 11** cryo 70	**Pt 13** cryo 70	**Pt 15** cryo 70	**Pt 17** cryo 70	**Pt 19** cryo 70
Pt 2 cryo 65	**Pt 4** cryo 65	**Pt 6** cryo 65	**Pt 8** cryo 65	**Pt 9** cryo 70	**Pt 12** cryo 65	**Pt 14** cryo 65	**Pt 16** cryo 65	**Pt 18** cryo 65	**Pt 20** cryo 65
Pt 2 cryo 66	**Pt 4** cryo 66	**Pt 6** cryo 66	**Pt 8** cryo 66	**Pt 10** cryo 65	**Pt 12** cryo 66	**Pt 14** cryo 66	**Pt 16** cryo 66	**Pt 18** cryo 66	**Pt 20** cryo 66
Pt 2 cryo 67	**Pt 4** cryo 67	**Pt 6** cryo 67	**Pt 8** cryo 67	**Pt 10** cryo 66	**Pt 12** cryo 67	**Pt 14** cryo 67	**Pt 16** cryo 67	**Pt 18** cryo 67	**Pt 20** cryo 67
Pt 2 cryo 68	**Pt 4** cryo 68	**Pt 6** cryo 68	**Pt 8** cryo 68	**Pt 10** cryo 67	**Pt 12** cryo 68	**Pt 14** cryo 68	**Pt 16** cryo 68	**Pt 18** cryo 68	**Pt 20** cryo 68
Pt 2 cryo 70	**Pt 4** cryo 70	**Pt 6** cryo 70	**Pt 8** cryo 70	**Pt 10** cryo 68	**Pt 12** cryo 70	**Pt 14** cryo 70	**Pt 16** cryo 70	**Pt 18** cryo 70	**Pt 20** cryo 70

CALERIE LCBR EDTA Assay Cryovials

EDTA Purple Top 1.5mL Cryos (#88)
0.5mL sample volume 1 cryo per participant. 99 participants per box

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

CALERIE LCBR Repository B- Annual Visit Repository for 1 Participant

Serum Red top 0.5mL or 1.5mL Cryovials (#53, 57-62,69,71-74,76-83)
EDTA Purple Top 0.5mL Cryovials (#84-87 and 89-97)

BL cryo 53	BL cryo 73	BL cryo 84	BL cryo 95	12M cryo 69	12M cryo 81	12M cryo 92	24M cryo 60	24M cryo 78
BL cryo 57	BL cryo 74	BL cryo 85	BL cryo 96	12M cryo 71	12M cryo 82	12M cryo 93	24M cryo 61	24M cryo 79
BL cryo 58	BL cryo 76	BL cryo 86	BL cryo 97	12M cryo 72	12M cryo 83	12M cryo 94	24M cryo 62	24M cryo 80
BL cryo 59	BL cryo 77	BL cryo 87	12M cryo 53	12M cryo 73	12M cryo 84	12M cryo 95	24M cryo 69	24M cryo 81
BL cryo 60	BL cryo 78	BL cryo 89	12M cryo 57	12M cryo 74	12M cryo 85	12M cryo 96	24M cryo 71	24M cryo 82
BL cryo 61	BL cryo 79	BL cryo 90	12M cryo 58	12M cryo 76	12M cryo 86	12M cryo 97	24M cryo72	24M cryo 83
BL cryo 62	BL cryo 80	BL cryo 91	12M cryo 59	12M cryo 77	12M cryo 87	24M cryo 53	24M cryo 73	24M cryo 84
BL cryo 69	BL cryo 81	BL cryo 92	12M cryo 60	12M cryo 78	12M cryo 89	24M cryo 57	24M cryo 74	24M cryo 85
BL cryo 71	BL cryo 82	BL cryo 93	12M cryo 61	12M cryo 79	12M cryo 90	24M cryo 58	24M cryo 76	24M cryo 86
BL cryo72	BL cryo 83	BL cryo 94	12M cryo 62	12M cryo 80	12M cryo 91	24M cryo 59	24M cryo 77	24M cryo 87

CALERIE OGTT Serum Cryovials for B. Kristal Ancillary

Serum Red Top 1.5mL Cryos (#12-15);
1.0ml sample volume. 4 cryos per participants. 25 participants per box.

Pt 1 cryo 12	**Pt 3** cryo 14	**Pt 6** cryo 12	**Pt 8** cryo 14	**Pt 11** cryo 12	**Pt 13** cryo 14	**Pt 16** cryo 12	**Pt 18** cryo 14	**Pt 21** cryo 12	**Pt 23** cryo 14
Pt 1 cryo 13	**Pt 3** cryo 15	**Pt 6** cryo 13	**Pt 8** cryo 15	**Pt 11** cryo 13	**Pt 13** cryo 15	**Pt 16** cryo 13	**Pt 18** cryo 15	**Pt 21** cryo 13	**Pt 23** cryo 15
Pt 1 cryo 14	**Pt 4** cryo 12	**Pt 6** cryo 14	**Pt 9** cryo 12	**Pt 11** cryo 14	**Pt 14** cryo 12	**Pt 16** cryo 14	**Pt 19** cryo 12	**Pt 21** cryo 14	**Pt 24** cryo 12
Pt 1 cryo 15	**Pt 4** cryo 13	**Pt 6** cryo 15	**Pt 9** cryo 13	**Pt 11** cryo 15	**Pt 14** cryo 13	**Pt 16** cryo 15	**Pt 19** cryo 13	**Pt 21** cryo 15	**Pt 24** cryo 13
Pt 2 cryo 12	**Pt 4** cryo 14	**Pt 7** cryo 12	**Pt 9** cryo 14	**Pt 12** cryo 12	**Pt 14** cryo 14	**Pt 17** cryo 12	**Pt 19** cryo 14	**Pt 22** cryo 12	**Pt 24** cryo 14
Pt 2 cryo 13	**Pt 4** cryo 15	**Pt 7** cryo 13	**Pt 9** cryo 15	**Pt 12** cryo 13	**Pt 14** cryo 15	**Pt 17** cryo 13	**Pt 19** cryo 15	**Pt 22** cryo 13	**Pt 24** cryo 15
Pt 2 cryo 14	**Pt 5** cryo 12	**Pt 7** cryo 14	**Pt 10** cryo 12	**Pt 12** cryo 14	**Pt 15** cryo 12	**Pt 17** cryo 14	**Pt 20** cryo 12	**Pt 22** cryo 14	**Pt 25** cryo 12
Pt 2 cryo 15	**Pt 5** cryo 13	**Pt 7** cryo 15	**Pt 10** cryo 13	**Pt 12** cryo 15	**Pt 15** cryo 13	**Pt 17** cryo 15	**Pt 20** cryo 13	**Pt 22** cryo 15	**Pt 25** cryo 13
Pt 3 cryo 12	**Pt 5** cryo 14	**Pt 8** cryo 12	**Pt 10** cryo 14	**Pt 13** cryo 12	**Pt 15** cryo 14	**Pt 18** cryo 12	**Pt 20** cryo 14	**Pt 23** cryo 12	**Pt 25** cryo 14
Pt 3 cryo 13	**Pt 5** cryo 15	**Pt 8** cryo 13	**Pt 10** cryo 15	**Pt 13** cryo 13	**Pt 15** cryo 15	**Pt 18** cryo 13	**Pt 20** cryo 15	**Pt 23** cryo 13	**Pt 25** cryo 15

CALERIE FAHC-Immulite Testing

Serum 5mL 12 x 75mm Tube (356)
1 Tube per participant, 49 participants per box.

PT 1 cryo 56	**PT 8** cryo 56	**PT 15** cryo 56	**PT 22** cryo 56	**PT 29** cryo 56	**PT 36** cryo 56	**PT 43** cryo 56
PT 2 cryo 56	**PT 9** cryo 56	**PT 16** cryo 56	**PT 23** cryo 56	**PT 30** cryo 56	**PT 37** cryo 56	**PT 44** cryo 56
PT 3 cryo 56	**PT 10** cryo 56	**PT 17** cryo 56	**PT 24** cryo 56	**PT 31** cryo 56	**PT 38** cryo 56	**PT 45** cryo 56
PT 4 cryo 56	**PT 11** cryo 56	**PT 18** cryo 56	**PT 25** cryo 56	**PT 32** cryo 56	**PT 39** cryo 56	**PT 46** cryo 56
PT 5 cryo 56	**PT 12** cryo 56	**PT 19** cryo 56	**PT 26** cryo 56	**PT 33** cryo 56	**PT 40** cryo 56	**PT 47** cryo 56
PT 6 cryo 56	**PT 13** cryo 56	**PT 20** cryo 56	**PT 27** cryo 56	**PT 34** cryo 56	**PT 41** cryo 56	**PT 48** cryo 56
PT 7 cryo 56	**PT 14** cryo 56	**PT 21** cryo 56	**PT 28** cryo 56	**PT 35** cryo 56	**PT 42** cryo 56	**PT 49** cryo 56

*Box Maps for **FAHC –Vitros Testing** and **FAHC –Centaur Testing** are used again at aliquotting. See appendix B.1 for box maps.*

APPENDIX C. CALERIE LCBR ALIQUOTTING GUIDE

CALERIE ALIQUOTTING SCHEME BL,12M, 24M Visits

updated 1/28/08 RHB

***NOTE: ALLOW all Serum and EDTA transfer tubes to completely thaw to room temperature in 37C water bath prior to aliquotting; Approx 5-7 minutes total per rack**

SERUM

1. Thaw Transfer Tubes 25, 26, 27 & 28 to Room Temp. *

2. Pool Transfer Tubes 25, 26, 27 & 28 into a 50mL conical tube

3. Gently INVERT Pooled Serum **15** times

4. Aliquot FAHC Testing Cryovials First

Cryo/Tube#	Aliquot Volume	Tube/Cryo size	FAHC Box
50	600	false bottom (4mL)	FAHC/vitros
51	300	12 x 75 (5mL)	FAHC/Centaur
52	500	12 x 75 (5mL)	FAHC/Centaur
54	500	12 x 75 (5mL)	FAHC/Centaur
55	200	false bottom (4mL)	FAHC/Vitros
56	500	12 x 75 (5mL)	FAHC/Immulite
75	300	12 x 75 (5mL)	FAHC/Mayo

5. Aliquot LCBR Testing Cryovials Second

Cryo/Tube#	Aliquot Volume	Tube/Cryo size	FAHC Box
65	500	0.5mL cryo (red)	LCBR Serum Assay
66	500	0.5mL cryo (red)	LCBR Serum Assay
67	500	0.5mL cryo (red)	LCBR Serum Assay
68	500	0.5mL cryo (red)	LCBR Serum Assay
70	1000	1.5mL cryo (red)	LCBR Serum Assay

6. Aliquot Repository Cryovials Last

Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
53	500	0.5mL cryo (red)	LCBR Repos B
57	1200	1.5mL cryo (red)	LCBR Repos B
58	500	0.5mL cryo (red)	LCBR Repos B
59	500	0.5mL cryo (red)	LCBR Repos B
60	500	0.5mL cryo (red)	LCBR Repos B
61	500	0.5mL cryo (red)	LCBR Repos B
62	500	0.5mL cryo (red)	LCBR Repos B
69	500	0.5mL cryo (red)	LCBR Repos B
71	500	0.5mL cryo (red)	LCBR Repos B
72	500	0.5mL cryo (red)	LCBR Repos B
73	500	0.5mL cryo (red)	LCBR Repos B
74	200	0.5mL cryo (red)	LCBR Repos B
76	500	0.5mL cryo (red)	LCBR Repos B
77	500	0.5mL cryo (red)	LCBR Repos B
78	500	0.5mL cryo (red)	LCBR Repos B
79	500	0.5mL cryo (red)	LCBR Repos B
80	500	0.5mL cryo (red)	LCBR Repos B
81	500	0.5mL cryo (red)	LCBR Repos B
82	500	0.5mL cryo (red)	LCBR Repos B
83	500	0.5mL cryo (red)	LCBR Repos B

7. Thaw OGTT Tubes 38-41 to Room Temp*

8. **Gently INVERT Each** OGTT tube **15** times

9. Aliquot cryos 18, 19, 98, and 99 as follows:
(remember to change tips between OGTT tubes!)

OGTT Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
38	18	500	12 x 75 (5mL)	FAHC/Mayo-CPEP
	38	600	12 x 75 (5mL)	FAHC/Immulite SGL/INS
39	19	500	12 x 75 (5mL)	FAHC/Mayo-CPEP
	39	600	12 x 75 (5mL)	FAHC/Immulite SGL/INS
40	98	500	12 x 75 (5mL)	FAHC/Mayo-CPEP
	40	600	12 x 75 (5mL)	FAHC/Immulite SGL/INS
41	99	500	12 x 75 (5mL)	FAHC/Mayo-CPEP
	41	600	12 x 75 (5mL)	FAHC/Immulite SGL/INS

EDTA

1. Thoroughly thaw EDTA transfer tube 34 to Room Temp.*

2. **Gently INVERT** EDTA Tube # 34 **15** times

3. Aliquot cryovials 84-87

Transfer Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
34	84	500	1.5mL (purple cap)	LCBR Repos B
	85	500	1.5mL (purple cap)	LCBR Repos B
	86	500	1.5mL (purple cap)	LCBR Repos B
	87	500	1.5mL (purple cap)	LCBR Repos B

4. Thoroughly thaw EDTA transfer tube # 36 to Room Temp.

5. **Gently INVERT** EDTA Tube # 36 **15** times

6. Aliquot cryovials 88-97 (remember to change pipette tips!)

Transfer Tube #	Cryo/Tube #	Aliquot Volume	Tube/Cryo size	Repository Box
36	88	500	0.5mL (purple cap)	LCBR EDTA Assay
	89	500	0.5mL (purple cap)	LCBR Repos B
	90	500	0.5mL (purple cap)	LCBR Repos B
	91	500	0.5mL (purple cap)	LCBR Repos B
	92	500	0.5mL (purple cap)	LCBR Repos B
	93	500	0.5mL (purple cap)	LCBR Repos B
	94	500	0.5mL (purple cap)	LCBR Repos B
	95	500	0.5mL (purple cap)	LCBR Repos B
	96	500	0.5mL (purple cap)	LCBR Repos B
	97	500	1.5mL (purple cap)	LCBR Repos B