Chapter 7

Blood Collection and Processing

Harmonization of laboratory measurements between Visits 1, 2 and 3

Though the laboratory methods used to measure the various analytes (glucose, lipids, creatinine, HbA1C and complete blood counts) will remain identical between Visits 1, 2 and 3, the central laboratory, Advanced Diagnostics and Research Laboratory (ARDL), has upgraded several laboratory instruments after completion of Visit 1 laboratory analyses. Hence prior to start of the Visit 3 examination, the ARDL will conduct a comparability study to ensure that analytical precision and reproducibility of laboratory analytes remains similar between Visits 1, 2 and 3.

To ensure comparability of laboratory values obtained during Visits 1, 2 and 3 of LLFS, we propose the following two procedures:

(a) Since the National Institute of Standards and Technology (NIST) or other agencies provides standard reference materials (SRMs) that enhance the accuracy of the biochemical assays, we propose to first calibrate all the assays where NIST or NIST traceable SRMs are available (cholesterol, triglyceride, HDL, glucose and creatinine) or for hemoglobin A1c the National Glycohemoglobin Standardization Program reference material.

(b) Apply those results to harmonize the various laboratory values across the two examinations by selecting approximately 200 samples from Visit 1 and re-measuring all the analytes mentioned above (except HbA1c as no stored sample is available). These 200 samples will be measured in 4 batches of 50 samples, tested at six-month intervals.

In addition, we will also monitor accuracy by regular participation in external proficiency programs. We will evaluate differences in laboratory values from the values obtained during Visits 1 and 2 to the current values using simple linear regression and, if necessary, recalibrate values from Visits 1 and/or 2 to be comparable to the values obtained from Visit 3.

Once the harmonization is complete change in biomarker concentrations from Visit 1 to Visit 2 to Visit 3 can be estimated using the difference in biomarker concentrations between the 3 Visits.

Harmonization of LLFS laboratory data with the laboratory data from other studies (e.g. the Framingham Heart Study)

Since laboratory analytes are frequently measured using several different methods in different laboratories/studies, laboratory values for the same samples analyzed in different laboratories may not always have similar values. To ensure comparability of laboratory values across different studies, we propose a comparability study in which approximately 175 LLFS samples will be tested by the Central laboratory of an external study. We will utilize samples collected as a part of the Phantom QC process in Visit 2 of LLFS for this purpose.

We will evaluate differences in laboratory values between the two studies using simple linear regression. If there is a high correlation in laboratory values for a particular analyte ($r>0.8$) we will recalibrate values of the individual analytes between the two studies (if needed) to ensure comparability of laboratory values across the two studies. If the correlation in laboratory values for a particular analyte is low ($r<0.8$) this will indicate limited comparability of laboratory values for that particular analyte between the two studies. When a particular analyte cannot be compared across studies, re-measurement of the analyte for all participants across both studies in a single laboratory using a uniform methodology will likely be the only way to ensure comparability across studies.

Similar to Visits 1 and 2, the LLFS Central Laboratory will supply a collection kit to the US field centers that will contain the appropriate collection tubes, processing instructions, shipping label, packaging for IATA approved shipment, gel packs for temperature regulation, and shipping instructions. The Laboratory will supply the Denmark field center with the exact or equivalent supplies to construct their own kits and process their samples. All field centers will supply the items required for venipuncture, labeling of blood collection tubes, centrifugation of the SST tubes, and biohazard containment.

Participants should be strongly encouraged to fast (taking only water) for at least 8 hours prior to venipuncture since non-fasting will impact several of the laboratory test results. If the participant absolutely cannot fast for this amount of time, collect the blood samples anyway. The Venipuncture Form (13) requires recording the time since last food. This will be taken into consideration when testing or evaluating test results from a non-fasting sample.

A protocol for blood collection and processing by non-LLFS, local health care providers at distant locations is included in Chapter 7, Appendix 3.

Training and certification of LLFS field center personnel

The blood collection and processing procedure is performed by study-certified technicians at each field center. Each technician must do the following prior to becoming certified and prior to collecting blood from LLFS participants:

1. Attend an online webinar training
2. Complete and pass a quiz. The quiz is available as a Google form. Go to https://forms.gle/5YtZPE1i5VGUJkjX9 to complete.
3. Collect and ship 1 set of blood samples from a person who is not a LLFS participant
4. Complete the Certification Checklist for the Blood Collection Procedure (see Chapter 20)

Staff at the Central Laboratory will provide feedback on quiz completion and the practice blood collection from the non-LLFS participant and will provide suggestions for improvement as needed.

Ongoing quality control of sample collections

During the course of LLFS Visit 3 blood collections, the Central Laboratory will monitor several aspects of the blood collection and shipping. These include the time from blood collection to receipt and the completeness of sample collections (number of blood tubes collected and number of aliquots prepared from the blood tubes). This information will be monitored on an approximate monthly basis and will be communicated to the field centers on the Field Operations calls. Suggestions for improvement will be provided as needed.

The DMCC will assist with monitoring the amount of time from blood collection until centrifugation and the rate of collection of phantom QCs. These reports will be reviewed by either or both of the Field Operations and Blood Assays committees for adherence to protocol.

Analysis of Phantom QC samples

The DMCC will prepare reports to compare the phantom QC results to the corresponding real LLFS participant results for the testing that is performed in real-time (cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol, glucose, creatinine, HbA1c and CBC+differential). Lipids, glucose and creatinine values will be compared on those participants on whom the SST (serum) phantom QC tube was collected. HbA1c and CBC+differential values will be compared on those participants on whom the lavender-top (EDTA plasma) phantom QC tube was collected. Comparisons will be grouped to include all field centers and will also be broken down by field center to determine if there are any biases or other concerns that may be field center-specific.

An example (from Visit 2 data) is shown below for the type of data that will be generated on the phantom QC samples:

Biomarker	Field Center	QC Pairs					Actual	Phantom	Difference (Actual - Phantom)			
		N	Mean	Within SD	Reliab	CV	Mean	Mean	Mean	95% CI	Prop > 0	pval
Cholesterol (fhsch)	ALL	123	200.09	3.58	0.98	0.018	199.86	200.32	-0.455	(-1.67,0.76)	0.46	0.460
	BU	41	201.77	4.02	0.98	0.020	201.00	202.54	-1.537	(-3.83,0.76)	0.35	0.184
	DK	40	199.84	3.34	0.98	0.017	200.68	199.00	1.675	(-0.33,3.68)	0.66	0.100
	NY	19	210.32	3.80	0.98	0.018	210.05	210.58	-0.526	(-4.09,3.04)	0.41	0.760
	PT	23	189.09	3.01	0.99	0.016	188.00	190.17	-2.174	(-4.64,0.3)	0.33	0.082

Procedures to Be Performed. The goal of the blood collection, as outlined in the LLFS Study Design, is to collect seven (7) tubes of blood from each participant. However, the amount of blood collected will be left to the discretion of the phlebotomist or research assistant, based on the individual's physical condition and tolerance. A protocol for this is outlined in more detail in the Certification Training Module and later in this chapter. Each field center will determine the personnel to perform the phlebotomy procedures, either LLFS staff or Exam One personnel. In the case of a partial blood draw, where fewer than 7 tubes are collected from a participant, the blood draw should not be repeated on the participant at a later time to collect the missing blood tubes. If an Oragene saliva collection container for DNA is initially the only sample collected, a follow-up blood collection by an outside provider is allowed. With the blood samples collected, the LLFS Study Design specifies that the following laboratory tests will be performed in real-time as the specimens are received:

1. Creatinine
2. Glucose
3. Total cholesterol
4. HDL-cholesterol
5. LDL-cholesterol (Calculated LDL if triglycerides are < 400)
6. Triglycerides
7. Glycosylated hemoglobin
8. Complete blood count (CBC), differential and platelet
9. Cryopreservation of lymphocytes
10. Storage of serum, plasma, buffy coat and whole blood for future testing

Additional biomarkers will be measured on Visit 2 and Visit 3 stored samples. The exact tests to be performed are to be determined.

Collection Kit Contents:

***IMPORTANT NOTE*:** When kits are received at the field center, be sure to put a supply of the small Styrofoam shipping box containing a gel pack into the freezer (-20°C) so the gel pack will be frozen and ready when needed.

1 8.0 mL Cell Preservation Tube (CPT)
3 7.5 mL double gel transport serum separator blood collection tubes (SST)
2 10 mL EDTA blood collection tubes
1 PAXgene blood RNA collection tube
1 3.0 mL no additive tube (to be used as a 'discard' tube in venipuncture procedure)
2 Large gel packs for ambient temperature regulation
1 Styrofoam box and cardboard sleeve containing 8oz gel pack for cold temperature regulation. Keep a

supply of these boxes frozen (at -20°C) so they are available on short notice.

1 Tube holder and rubber band for securing tube holder– for ambient temperature tubes
2 Biohazard-marked zippered sealing bags to contain potential leakage/contamination
2 Absorbent wadding squares
1 Paper toweling for tube protection during transport
1 Packaging and shipping instructions
1 FedEx air bill – for US centers

Also provided as needed:

1 Oragene saliva collection system for genetic material (for use only if blood collection fails)

Supplies Provided by Field Center:

BD Safety-Lok Blood Collection Set or equivalent (required for collection of PAXgene tube)
Vacutainer tube holders
Alcohol swab
Tourniquet
Gloves
Bandage or gauze and tape
Biohazard containment system
Strapping tape to seal shipping box
Portable power supply
Centrifuge
Participant ID labels
Timer

Smelling salts, ice packs, and washcloths should be readily available for patients who become faint during the blood draw.

Home Visit Preparation: Before leaving for the blood collection visit, take a small Styrofoam shipping box containing a frozen gel pack and add to the blood collection kit. **BEFORE BLOOD COLLECTION, YOU MUST HAVE A FROZEN SMALL GEL PACK IN THE STYROFOAM SHIPPING CONTAINER.**

VENIPUNCTURE PROCEDURE

Precautions for Handling Blood Specimens:

- Handle all blood specimens as potentially infectious. OSHA rules mandate that technicians must always wear disposable protective gloves when collecting and processing specimens. Use 0.5% sodium hypochlorite (household bleach diluted 10-fold) to clean up any spills of blood, plasma, or serum and to wipe down the entire blood collection and processing work area at the end of each work day. OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers and properly disposed of in biohazard waste (not the routine trash). Place all blood-contaminated products in biohazard bags for proper disposal.

Pre-venipuncture Preparation:

1. Make certain the participant is in a comfortable position, sitting in a chair for a minimum of five minutes.
2. Affix the participant's name code and study ID# label to the Venipuncture Form (13).
3. Affix the participant's study ID# label to the last page of the Venipuncture Form – the Shipping Form. Do NOT use a label that includes the participant's name code on this page. The Central Laboratory should not receive any potentially identifying information.
4. Complete question **Q0a** on page 2 of the Venipuncture Form. If the participant responds "Yes," ask which side of the body was affected. Ask participant which of their arms is usually used for blood collection; if they can definitively respond with a specific (or either) arm, draw from that arm. If the participant doesn't know or is unsure of which arm is used for blood collection:
    a. Draw from left arm if lymph nodes were removed from right side.
    b. Draw from right arm if lymph nodes were removed from left side.
    c. If lymph nodes were removed from both sides, do not collect blood. Collect only saliva using the Oragene saliva collection kit (see appendix 1 of this chapter).
    d. Specify the nature of this issue as briefly as possible in Item 17 of the Venipuncture form.

Record information requested on Venipuncture Form (13) Blood Drawing – Section A:

1. Ask the participant "Do you have any bleeding disorders?" If the participant says they have a bleeding disorder, consult with the designated senior staff member to determine whether venipuncture should be performed or not. If the participant does not know whether they have a bleeding disorder, offer the explanation: "If you have a bleeding disorder, you would have had symptoms like excessive nose bleeds, very easy bruising, or problems with bleeding after a tooth extraction or any type of surgery."
    a. If the participant is still unsure, consult with the designated senior staff member before proceeding.
    b. When the participant reports a bleeding disorder, specify the type of bleeding disorders as briefly as possible in Item 17 of the Venipuncture form. In general, having a bleeding disorder is not a reason for participant deferral because blood is collected from patients with very severe bleeding disorders clinically routinely. A gauze and tape bandage is applied. However, participants who have a bleeding disorder should be instructed to elevate their arm and maintain mild pressure on the venipuncture site for a minimum of 10 minutes following blood collection. Furthermore, closely monitor the participant and venipuncture site during the blood pressure measurements.
2. Ask the participant "On which day did you last eat or drink anything except water?" Check "1" (today) "2" (yesterday) or "3" (before yesterday).
3. Ask the participant "And at what time was that?" Fill in the field using regular 12-hour clock time (12:01-11:59) and select "AM" or "PM".

Venipuncture and Processing:

1. Label each tube with the LLFS participant ID# label. Place this label directly over the tube manufacturer's label. The ID label must be attached so the barcode runs lengthwise on the tube.
    a. **NOTE**: Labels on these tubes cannot contain any other participant identifying information (e.g., participant's name) and should only be labeled with the ID# barcoded label. Do not use

a barcode label that includes the participant's name or name code,

b. **NOTE**: The 3.0 mL no additive tube does not need to be labeled. This tube will not shipped to the laboratory.

2. A black/white topped 3mL no additive tube is provided to use as a discard tube. This tube can be used to evacuate excess air from the blood collection tubing prior to the collection of the 7 tubes described below. Evacuation of excess air may help the 7 blood collection tubes to fill more fully.
   Please **note**: The discard tube does NOT need to be filled. Only a small amount of blood needs to be drawn into the tube to remove excess air from the tubing.
   a. When the tube is used, do NOT ship back to the laboratory. Discard with other biohazard materials.
   b. If the discard tube is NOT used, it can be returned to the laboratory with other blood collection tubes.
3. Fill the seven (7) evacuated blood collection tubes provided in the following order:
   a. Tube 1 – Blue/black topped CPT tube (for cryopreservation of lymphocytes)
   b. Tube 2 – Red/gray topped SST tube (for creatinine, glucose, lipid panel, stored serum for future testing)
   c. Tube 3 – Lavender topped EDTA tube (for CBC/diff/platelet, glycosylated hemoglobin, stored EDTA-plasma, DNA extraction (stored packed cells for DNA--Denmark field center only))
   d. Tube 4 – Lavender topped EDTA tube (for stored EDTA-plasma, stored buffy coat for DNA)
   e. Tube 5 – Red topped PAXgene tube (for RNA expression tests)
   f. Tube 6 – Red/gray topped SST tube (for stored serum for future testing)
   g. Tube 7 – Red/gray topped SST tube (for stored serum for future testing)

   Remove the tourniquet after the first tube fills. Allow all tubes to fill to the capacity determined by the amount of vacuum in each tube (make sure the blood has stopped flowing into the tube before removing the tube from the tube holder). Gently invert each tube eight times immediately after collection.

4. An 'adjustable-clasp' style of tourniquet should be used; this type of tourniquet remains on the participant's arm during the course of the venipuncture, but can be easily re-tightened, while also reducing the chance for hemoconcentration when application of the tourniquet is not needed. (Recommended tourniquet: vendor--Market Lab; catalog # ML6931) This style of tourniquet would be used instead of the traditional latex or nitrile tourniquet. To use,
   a. Apply around the arm and gently pull on the non-looped end of the tourniquet to tighten as needed.
   b. Collect the first blood tube, then release by lifting the clasp to loosen; the tourniquet remains loosely on the arm while the remainder of the tubes are collected.
   c. If blood flow slows to the point that pressure must be reapplied, gently pull on the non-looped end of the tourniquet to re-tighten as needed. However, the tourniquet should be applied for as minimal amount of time possible and re-tightened only to encourage blood flow if needed.
   d. **NOTE**: The phlebotomists should ensure that the time when the tourniquet is applied tightly never exceeds 2 minutes to reduce the effects of hemoconcentration.
5. ***During collection of the PAXgene tube, be sure to hold the tube vertically and keep it <u>below</u> the level of the participant's arm.*** This is important to avoid any possible backflow of the PAXgene tube contents into the participant's vein. Also, make certain that the tube additives do not touch the stopper or the end of the needle during venipuncture. Allow at least ten seconds for a complete blood draw to take place. To ensure a draw volume of 2.5 mL of blood and the correct additive/blood ratio, make sure that the blood has stopped flowing into the tube before removing the tube from the tube holder.
6. Remove the last tube from the collection device before removing the needle from the patient's arm.

Immediately apply pressure to the venipuncture site until bleeding stops. Then apply a bandage to the venipuncture site.

7. Recommendations for difficult blood draws:
    a. Problems may be due to reduced blood flow after removal of tourniquet. See above for recommendation and use of an 'adjustable-clasp' style of tourniquet. Use of this tourniquet and reapplication of pressure during the venipuncture to encourage blood flow is recommended instead of re-sticking the participant.
        i. **NOTE**: The phlebotomists should ensure that the time when the tourniquet is applied tightly never exceeds 2 minutes to reduce the effects of hemoconcentration. Please loosen the tourniquet at least 30 seconds prior to retightening the tourniquet.
    b. If you are having trouble with blood flow and need to reposition the needle, be sure to remove the tube from the collection device before attempting to reposition it. This will prevent loss of vacuum in the tube if the needle is removed from the arm, either intentionally or unintentionally.
    c. If venipuncture attempts fail to yield at least tubes #1 through #3, an Oragene saliva DNA collection must also be performed as an alternate source of DNA. See Appendix 1 for saliva collection instructions using the Oragene collection kit.
8. If it is not possible to collect any of the blood collection tubes, collect saliva for genetic material as described in Appendix 1.
    a. If it is not possible to collect all seven tubes, save any unused blood collection tubes that have not been punctured and return them in the shipping container for re-use. (Some of the tubes are very expensive, and unused tubes in new, unused condition can be re-packaged into another collection kit.)
9. Red/gray topped SST tubes: After drawing and inverting eight times, allow blood to clot at room temperature for a minimum of 30 minutes. As soon as possible after 30 minutes, but not longer than 45 minutes, spin tube at 1200 rcf for 15 minutes at room temperature. This is the maximum speed setting on the portable centrifuge.
    a. See Appendix 2 for complete centrifugation instructions.
    b. **NOTE**: 1200 rcf (or x*g*) does not necessarily equal a setting of 1200 RPM. Consult the centrifuge manual to ensure a correct setting for 1200 rcf.

<u>Record the requested information on the Venipuncture Form – sections A, B and C</u>:

1. Q0a. If "yes" is checked, review the information on page 7-5 and go to question 17.
2. Q1. If "yes" is checked, review the precautions on page 7-5 and then go to question 17.
3. Q4. **Number of venipuncture attempts.** Record the total number of venipuncture attempts made on the participants by ALL technicians.
4. Q5. **Time venipuncture ended?** Record the time of venipuncture on the form. This is the time when the blood collection is completed. Fill in the time field using regular 12-hour clock time (12:01-11:59) and select "AM" or "PM".
5. Q7. **Code number of Phlebotomist.** Record the code number of the phlebotomist who performed the blood drawing procedure. If more than one technician attempts to draw blood, enter the code of the <u>first</u> phlebotomist.
6. Q8. **Is this a non-LLFS local health care provider blood collection?** Blood collections made by LLFS field center personnel should answer "No" to this question. Check "Yes" to this question before placing a Venipuncture Form in a collection kit that will be sent to a local health care provider for blood collection, processing, and shipment to the Central Laboratory.
7. Q9. **Time at which SST1, SST2 and SST3 tubes were spun.** Record the time at which the centrifuge containing these tubes began to spin, using using regular 12-hour clock time (12:01-11:59) and select "AM" or "PM".
8. Q10. **Date specimen tubes were shipped:** Record the date in European format (day/month/year).
9. Q11. **Time specimen tubes were shipped:** Record the time using the standard 12-hour time format showing AM or PM.

10. Q12. **Code number of technician processing the blood.** Record the code number of the technician who centrifuged the blood and shipped the samples.
11. Q13 - Q16. **Blood Drawing Incidents:** Record any of the listed incidents that occurred during venipuncture by placing an "X" in the appropriate box.
12. Q17. **Comments on blood drawing and processing:** Include any clarifications or other information relevant to the assays being performed that are not included on the Incident Report or elsewhere. This information will be keyed into the Venipuncture record. Be as clear and concise as possible. Examples of information that should be provided are: vein collapse, hematoma, vein hard to get, leakage at venipuncture site, excessive duration of draw or information about type of bleeding disorder.

Alternative DNA Collection (Section D): This section of the Venipuncture Form is to be completed only if the Oragene Collection Container is used as an alternate DNA source. The Oragene container should be used only if blood collection attempts have been unsuccessful.

1. Q20. **Date Oragene was shipped:** Record the date in European format (day/month/year).
13. Q21. **Time Oragene was shipped:** Record the time using the standard 12-hour time format showing am or pm.

Record the requested information on the Blood Collection Shipping Form **(final page of Venipuncture Form):**

1. Place a participant ID label on the top of the form in the "Affix Label Here" box. Do not use a label that includes the participant name code. The laboratory must not receive any participant personal identifying information.
2. Record the date the form was completed and circle the field center location in the upper right box. For local health care provider collections, the field center location is the field center shipping the collection kit.
3. In the section Blood Collection, enter the date and time the samples were collected and the technician code of the person performing the blood draw. Enter the date in **day/month/year** format, indicating month with the 3-letter abbreviation for the month; and the time in 12-hour format; circle AM or PM. Enter the Technician code of the LLFS field center personnel shipping the samples.
4. Check the box next to 'Yes' or 'No' to indicate if the participant has fasted for at least 8 hours prior to the blood collection.
5. Place a check in the box next to each blood collection tube to indicate the tubes shipped to the Central Laboratory. Only mark the box with an X if the tube was collected and shipped. If an Oragene collection container was collected because only tubes #1 and 2 were obtained, indicate shipment of that as well.

Record any specimen comments about the venipuncture, centrifugation or shipping (difficult draw, collapsed vein, etc.) at the bottom.

PRECAUTIONS - WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT DURING OR FOLLOWING THE BLOOD DRAWING:

1. Have the person remain in the phlebotomy chair. If necessary, help the participant to the floor and have them lie on the floor with their legs slightly elevated. Use of a patient transfer belt may be useful in this situation.

2. Take an ampoule of smelling salts, crush it, and wave it under the person's nose for a few seconds.

3. Provide the person with a basin if he/she feels nauseous.

4. Have the person stay seated or lying down until they feel better.

5. Have someone stay with the person for at least five minutes after the participant feels better to

prevent them from falling and injuring themselves if they should faint.

6. Place a cold wet cloth on the back of the person's neck or on their forehead.

7. Once the episode has passed, some fruit juice may be given to the participant in order to counteract any possible hypoglycemia due to their fasting status.

8. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member, who should be able to advise you on any further action that needs to be taken.

Collection of quality control phantom participant (blind duplicate) samples

As part of the overall quality control program for laboratory analytes from blood samples, duplicate specimens will be sent to the laboratory. One collection tube will be sent under the participant's regular study ID number, and the other tube under a Quality Control (QC) Phantom Participant study ID number. The QC ID numbers will be indistinguishable from other LLFS ID numbers so that this forms a blinded external quality control program monitoring measurement variability. The proposed blinded duplicate sample collection scheme will allow the laboratory to identify which samples are phantom/blinded duplicate samples. However, the central laboratory will not be able to identify the particular LLFS participants from whom the phantom samples were collected.

Approximately 5% of samples (n=158) collected will be QC samples. The QC samples will be distributed equally across the 4 field centers so that each field center will collect approximately 40 sets of QC samples over the entire duration of specimen collection. A balance of collections across all sites is more important than the total number of QC collections, such that some field centers may be asked to discontinue QC collections while other field centers continue to collect as many complete QC sets as possible. To reduce the burden upon LLFS participants, no one person is asked to contribute sufficient extra blood to make a complete set of duplicate tubes for all tests. Instead, extra blood is drawn from several participants and sent out under the same Phantom ID number to constitute a full set of QC tubes. The QC set will consist of 1 serum (SST) tube, 1 EDTA plasma tube and 1 PAXgene tube. Up to 2 of these phantom tubes may be collected from a single LLFS participant, although the 3 phantom tubes in a complete set could also be collected from 3 different participants.

Each field center will independently follow the QC collection scheme and will collect the specified number of QC samples (n=40). Since the feasibility and logistics to collect blood samples from up to 3 different LLFS participants will vary by field center, each field center will determine quarterly goals for collection of QC sample sets and these goals will be communicated to the Blood Analysis committee prior to start of data collection. The QC samples are collected in the order: one serum tube (Tube 2), EDTA plasma tube (Tube 3) and PAXgene tube (Tube 5). Tube 1 (CPT) is not part of this QC scheme, nor are tubes EDTA2, SST2 and SST3. All duplicate tubes need not be collected from the different participants on the same day. It is acceptable to collect a QC tube on a day when only one participant is seen by any given field center. Duplicate tubes from multiple participants must be shipped using the same shipping container. The DMCC will provide a list of Phantom (QC) ID numbers to the field centers.

Timely progress in collection of QC samples across all field centers will be monitored by the Blood Analysis committee at regular intervals. Any concerns regarding slow accrual of QC samples will be communicated to the Field Operations committee. For data analysis, results on each laboratory measurement are matched to the appropriate participant results at the DMCC from the QC Phantom Participant ID Form (Appendix 4) that is completed by Field Center technicians and entered in the data system. This form is to be maintained at each Field Center and must NOT accompany the QC samples to the laboratory. The DMCC will analyze the reproducibility of the QC samples on an on-going basis

QC Collection procedure

1. Use a new collection kit for collection of the QC blood samples. Tubes that are not collected as QC samples (CPT, EDTA2, SST2 and SST3 tubes) can be returned to the Central Laboratory.
2. Selecting Participants for QC Blood Draw: Care should be taken in the selection of participants who will serve as the donors for the extra QC samples, since the oldest participants with the most fragile veins may not be suitable for donation of extra blood beyond the standard blood collection protocol.
3. Order of QC Tubes in Relation to Regular Blood Collection: Draw the QC tube from the participant after all of the regular collection set tubes have been collected. This procedure is followed to cause the least disruption of the collection of the regular blood samples. If the blood flow falls off at the end of the draw, so that it would be difficult to obtain the extra QC tubes, a different participant should be used to get this blood. DO NOT PERFORM A NEW NEEDLE STICK JUST TO GET MORE BLOOD FOR A QC SPECIMEN. DO NOT REAPPLY THE TOURNIQUET AFTER INITIAL RELEASE.
4. Processing QC Blood: Process the QC blood samples as the regular blood samples are processed; only the serum tube is centrifuged while the EDTA plasma and PAXgene tubes are not. After processing, keep the QC samples separate from the participant's regular collection samples so they are shipped separately. Do NOT ship the QC tubes in the same shipping box as the regular blood collection.
5. Blood Collection Shipping Form: This form is completed for the QC phantom set of samples. However, it is not possible to complete this form truthfully since the set is collected from multiple participants. It is suggested that the information from the participant to donate tube 2 (serum tube) is used to complete the form for the QC set.
6. Logging the Match between QC and Regular LLFS IDs and Reporting to the Coordinating Center: Use the LLFS Quality Control Phantom Participant ID Form (see example in Appendix 4) to keep track of the match between the QC and regular LLFS specimens. On a day when a QC collection is anticipated, prepare the Phantom ID form by recording (or labeling with) an assigned QC phantom ID number; also record collection date. As the QC tubes are collected, record the actual participant ID on the line corresponding to the tube donated. This step must be done immediately after completion of drawing blood for that participant, to minimize the chance of recording the wrong ID number. This form is completed for each QC ID number used. The QC phantom participant form information should be entered into the data system on the same schedule as the entry of regular blood collection form information. Do not send a hardcopy of the LLFS Quality Control Phantom Participant ID Form with the samples to the Central Laboratory because it will unblind the masked QC analysis of the samples. A Blood Collection Shipping form is also completed for the set of QC samples and IS included with the samples.

Specimen Packing and Shipping: The collection samples are shipped to the LLFS Central Laboratory in the dual temperature combined specimen shipping boxes provided. Samples may be collected any day of the week, but Saturday and Sunday collections are discouraged. Specimens must be shipped on the day of collection. Use the FedEx airbill (preprinted FedEx address label) provided with the kit. This airbill will include an automatic Saturday Delivery request whenever shipments are sent on a Friday.

If packaged according to detailed instructions supplied, this package will meet IATA guidelines for transport of diagnostic laboratory samples. Tubes must be maintained during shipment at two different temperatures: near refrigerator temperatures (~4^{o}C) and near typical room temperature (~25^{o}C).

Room Temperature Tubes: Place the black/blue topped CPT tube and the red topped PAXgene tube into the 5-slotted Styrofoam container that also contains an absorbent square (without the frozen gel pack). If there are any unused, unpunctured blood tubes, also place them in this tube-holder. Place the two halves of the 5-slotted Styrofoam container together, then wrap the rubber band once around the short length of the container; carefully insert this box into the 8 x 10" biohazard zippered bag and seal.

Refrigerated Tubes: Wrap the two lavender topped tubes, blue topped tube, and the three centrifuged

red/gray topped tubes in sheets of paper toweling provided for protection from breakage. Place wrapped specimens and absorbent square into 6 x 9" zippered bag and seal. Place the plastic bag into the Styrofoam shipping container with the *frozen* gel pack, and then place the container in the cardboard sleeve.

If there was a partial blood collection, and the Oragene saliva DNA collection system was also used, include this in the bag of refrigerated temperature specimens. If the blood collection was completely unsuccessful, and only the Oragene container was filled, follow the shipping instructions found in Appendix 1.

Shipping Box Assembly: Place the box containing the assembled cardboard box of refrigerated specimens in half of the shipping box. Place a large room-temperature gel pack in the outer shipping container so that it lies next to the refrigerated specimen box. Place the assembled 5-place tube holder and sleeve on top of the gel pack. Place the second room-temperature gel pack on top of the 5-place tube. Close and seal the box with strapping tape.

U.S. Center Shipping Instructions:

1. Peel off the back of the FedEx airbill to expose the adhesive and affix the large, lower portion of the airbill to the outside of the box. The small sticker from the top of the airbill contains a record of the tracking number may be kept for your records.
2. There are two options for getting the assembled shipping box to FedEx: take it to a Staffed FedEx drop-off location or return it to the field center and call FedEx for a package pick-up. In either case be sure the package will be picked up in time for next day delivery to the Central Laboratory. The website queries to identify a FedEx drop-off location nearest the participant's address should be performed before entering the field:
   a. Option 1: Drop the box at the nearest FedEx Service Center or FedEx Office location. The nearest locations of these can be found as follows: Go to the website www.fedex.com. Click on the Locations drop-down and select 'FIND A LOCATION.' Enter the street address, city, state, and zip code of the participant or nearest location. In the Filter, *select only Staffed Location*. A list of FedEx drop-off locations with their hours of operation and last express drop off time is provided. If it is an Office location, it states that fact; otherwise it is a FedEx Service Center. Confirm that the last Express service pickup has not yet occurred (i.e. make sure the package will still be picked up and shipped the day it is dropped off).
   b. Option 2: Contact FedEx (1-800-GO-FEDEX) for pickup at the LLFS field center when you plan to bring the specimens back to the field center in time for the FedEx daily pickup. When calling FedEx, say the word "rep" when the first menu is presented. This will connect you to a representative who will arrange the pickup. The package will then be picked up at the address provided. **Do not leave the package at the participant's residence for FedEx pickup.**

Denmark Shipping Instructions: Some procedures will differ somewhat for the Denmark Field Center; these are briefly summarized below:

- The samples collected by the Denmark Field Center personnel will be shipped to their laboratory via the Danish postal service which provides next day delivery. Samples will be processed by the Denmark Field Center Laboratory using procedures identical to the Central Laboratory. PAXgene tubes, aliquots of serum, plasma, buffy coat, and cryopreserved lymphocytes will be shipped on dry ice to the Central Laboratory in batches via FedEx. It is anticipated that there will be a shipment every month. Aliquots for each participant will be divided into two separate shipments so that, in the rare instance of a lost or delayed shipment, there will still be viable aliquots for every participant.

CENTRAL LABORATORY PROCESSING

- Sample receipt in the laboratory will be confirmed with the shipping Field Center via an automated e-mail sent from the Central Laboratory to the person(s) designated by each Field

Center.

- Upon receipt in the laboratory each tube will be logged and processed and stored as follows per participant collection (assuming collection of seven full tubes):
  - CPT tube: 2 or 3 vials (depending on quantity) of cryopreserved lymphocytes will be processed and lymphocytes cryopreserved in liquid nitrogen (US Central Laboratory in Minneapolis) or -135º C (Denmark Central Laboratory). As a quality control indicator that these cryopreserved cells are viable, two samples per month will be transformed throughout the study.

  - EDTA tube: A small amount of well mixed EDTA-anticoagulated whole blood will be removed for performing CBC/WBC differential/platelet assays, measurement of glycosylated hemoglobin, and for spotting whole blood onto FTA paper for potential confirmation of sample identity at a later time. After centrifugation, up to 16 aliquots (0.25 – 0.5 mL each) of platelet-poor plasma will be stored at -70ºC. One EDTA buffy coat will be prepared and stored at -70°C for potential later DNA isolation. At the central laboratory, the packed red cells will be used for DNA extraction for those samples collected in the US and from the Denmark Field Center. The extracted DNA will be stored at -70°C for future analysis.
  - SST tubes: up to 15 aliquots (0.25 – 0.5 mL each) of serum per participant will be stored at -70ºC; one aliquot of serum will be used for glucose, cholesterol, HDL-cholesterol, triglycerides, and creatinine assays.
  - Sodium citrate tube: up to 4 aliquots (0.25 – 0.5 mL each) of citrate-anticoagulated plasma will be stored at -70ºC; one aliquot of buffy coat will be stored at -70ºC for later DNA isolation.
  - Paxgene tube: This entire unopened tube will be initially stored for 24 hours at -20ºC to allow slow freezing of the contents, prior to transfer and long-term storage at -70ºC.
- Stored aliquots will identified with 'LLFS Visit 3,' the study ID and a container ID. The LLFS subject ID will not be available to the laboratory and the LLFS subject ID will be used by the DMCC to match the phantom IDs appropriately. The aliquot ID will identify sample type and sequence of aliquot and will use numbers in the form 301, 302, 303, etc. This will distinguish Visit 3 samples from both Visit 1 and Visit 2 samples that used different aliquot ID structures. Visit aliquot IDs = 1, 2, 3…37; and Visit 2 aliquot IDs = 201, 202, 203…237.
- A computerized inventory of aliquot ID, aliquot type, approximate volume, and storage location will be maintained by both the Denmark and US central laboratories. The Denmark Field Center will transfer an electronic inventory of this information along with the physical biospecimen transfer.

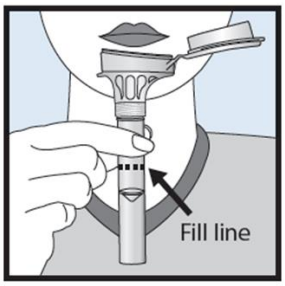
Chapter 7: Appendix 1

Collection of Saliva from Participants Unable or Unwilling to Provide Blood

Materials Required:
Oragene DNA Self-Collection Kit, Catalog no. OGR-500, manufactured by DNA Genotek, Inc.,2 Beaverbrook Road Kanata, Ontario, Canada K2K 1L1 [available from CBL]
Padded mailer, biohazard bag and absorbent square [available from CBL]
Postage for mailing via USPS [to be provided by Field Center]

Procedure:

1. Before providing to the participant, label the Oragene container with the participant's study ID label by placing it horizontally along the length of the container
2. Ask the participant to rinse their mouth with tap water to remove any existing food particles.
3. Wait at least one minute following the rinse before beginning the saliva collection.
4. Spit the saliva into the Oragene container.
5. Keep spitting until the amount of liquid saliva (not counting foam) reaches the top of the blue tab as indicated by the dotted line in the figure. This is approximately 2 mL of saliva. More can be collected if the participant is willing. (The more saliva collected, on average, the more DNA that can be isolated.)



6. Close the lid over the collection funnel. This will release the liquid preservative from the within the lid.
7. Remove the funnel from the tube by unscrewing it from the base. Cap the tube with cap provided in collection kit; make sure the cap is tightly closed to prevent leaking and loss of saliva sample.
8. Gently mix the specimen by inversion several times.
9. Seal the Oragene container inside the plastic biohazard bag and place it inside of the pre-addressed mailing envelope.
10. Add postage to the mailer. Postage price is determined by mailing location; visit local post office to determine postage needed.
11. Mail the Oragene container to the central laboratory via the US postal service
12. Return and complete Section A, as appropriate if any blood tubes were collected, along with Section C and Section D on the Venipuncture Collection Form.

Chapter 7: Appendix 2

Centrifugation of SST Tubes

Field personnel should have a portable centrifuge for their use in the general area where the blood collection is to be performed. The recommended centrifuge is the Fisher Centrific Model 228, if available from Visit 1. This unit operates at one, fixed speed. Other manufacturers and models are acceptable, but the specimens must be centrifuged at a minimum of 1,200 rcf (*g*-force) for 15 minutes. The centrifuge requires an external power source. This source may be the standard electrical wall receptacles within the participant's residence that supply the usual AC voltage and Hertz (in the US: 120 volts AC at 60 cps; in Denmark: 230 volts AC at 50 cps). If the participant is unwilling to allow blood centrifugation in their residence, the electrical power can be provided from a rechargeable battery pack with a DC to AC power inverter appropriate for the centrifuge.

OPTION 1: Using electricity within the participant's residence

Before beginning the venipuncture procedure, ask the participant if he or she will allow the centrifuge to be plugged in and operated within their residence. The power requirements of the centrifuge are very minimal, and pose no threat to their electrical system. If the participant is uncomfortable with having the centrifuge in their kitchen or living area, offer to locate it in a bathroom, storage area or basement. Bring a square piece of plywood or large cutting board to serve as a base for the centrifuge. Bring a grounded (3-prong) extension cord in case the electrical receptacle is some distance from a convenient centrifugation site.

Procedure:

1. Place the centrifuge on a firm surface, plywood, or cutting board.
2. Plug the centrifuge into a wall outlet. Use the grounded extension cord if necessary.
3. Load the SST tubes to be centrifuged into the rotor. The tubes must be balanced for safe centrifuge operation. If only one SST tube has been collected, fill another SST tube with an amount of water equal to the blood volume in the collection tube, and place it opposite the blood-filled SST tube in the rotor. If a full SST tube and a partial SST tube were collected, fill two extra SST tubes with water equal to the two different blood volumes, and place each water-filled tube opposite the corresponding blood-filled tube in the rotor.
4. Close the lid on the centrifuge, and lock the latch. For safety reasons, the centrifuge will not operate unless the lid is closed and locked shut.
5. Turn the timer to 15 minutes. Centrifugation will begin and will stop automatically after 15 minutes.
6. After the centrifuge automatically shuts off and the rotor stops spinning, remove the tubes and continue blood processing for shipment as described in the protocol. NOTE: If good clot and serum separation has not been achieved (serum well separated from the red blood cells by the gel separating material), repeat centrifugation for an additional 15 minutes. **If re-centrifuged, record this on the Blood Collection Shipping Form.

OPTION 2: Using a Portable Power Supply

If the participant will not allow the blood specimens to be centrifuged in their residence, it will be necessary to plug the centrifuge into a portable power pack. The power pack should typically be stored in the trunk of the phlebotomy technician's vehicle with the portable centrifuge, where the centrifugation can be performed if necessary. The recommended power supply is the Powerpack 300, manufactured by Duracell. It may be purchased on the manufacurer’s website, http://www.duracellpower.com, Amazon.com, Newegg.com or other online retailers. The Powerpack 300 contains a rechargeable (from an AC wall receptacle or a vehicle’s DC source) 12-volt DC lead-acid battery, which is similar to a typical car battery, and a DC to AC power inverter. US and European (Danish) centrifuges require different AC voltages and frequencies (Hertz or cps), so the US power pack cannot be used with the Danish centrifuges or vice versa. After the

Chapter 7: Appendix 2

battery is fully charged, the centrifuge may be plugged directly into the grounded outlet on the Powerpack. Read the complete Powerpack 300 Owner's Guide before use. The Powerpack will automatically shut off if excessive surge power is drawn or if the DC battery's voltage falls below 10 volts.

Procedure:

1. Fully charge the battery. Upon receipt, the initial charge may require up to 40 hours while plugged into a standard 120V electrical outlet. The Powerpack 300 has a display feature that indicates the status of battery charge. A fully charged battery is capable of completing at least five 15-minute centrifugation sequences. The Powerpack 300 should be recharged each night to ensure adequate power for the following day's work.
2. After collecting the specimens and allowing the SST tubes to clot, bring the SST tubes to the vehicle for centrifugation.
3. Make sure the centrifuge is in a stable position.
4. Turn the Powerpack 300 AC outlet switch ON.
5. Plug the centrifuge into the grounded outlet on the Powerpack 300 below the switch.
6. Load the SST tubes to be centrifuged into the rotor. The tubes must be balanced for safe centrifuge operation as usual. If only one SST tube has been collected, fill another SST tube with an amount of water equal to the blood volume in the collection tube, and place it opposite the blood tube in the rotor. If a full SST tube and a partial SST tube were collected, fill two extra SST tubes with water equal to these blood volumes, and place these tubes opposite the corresponding blood tube in the rotor.
7. Close the lid on the centrifuge and lock the latch. For safety reasons, the centrifuge will not operate unless the lid is latched is not closed and locked shut.
8. Turn the timer to 15 minutes. Centrifugation will begin automatically.
9. After returning to the residence to continue the exam, set a portable pocket timer for 15 minutes as a reminder to remove the tubes from the centrifuge.
10. After the centrifuge shuts off, turn the AC outlet switch OFF.
11. Remove the tubes and continue processing as described in the standard protocol. NOTE: If good separation has not been achieved (serum well separated from the red blood cells by the gel separating material), repeat centrifugation for an additional 15 minutes. **If re-centrifuged, record this on the Blood Collection Shipping Form.
12. Recharge the battery overnight.

Chapter 7: Appendix 3

Local Health Care Provider Blood Collection

In some circumstances, the LLFS Study proband relative may be located outside of the LLFS field centers' normal catchment area. In these situations, it will be necessary for the LLFS field center either to send a LLFS staffed non-catchment area interview/blood collection team to the participant's residence, or if this is not logistically or economically feasible, to collaborate with local blood collection services and/or health care providers for the collection, processing, and shipment of blood to the LLFS Central Laboratory. The same blood collection kits supplied to the field centers by the LLFS Central Laboratory should be used for these remote site collections by local health care providers except that the blood collection tubes will be prelabeled. Specific detailed written instructions for processing, shipping, and any necessary payment for the blood collection and processing service need to be added by the field center staff prior to sending the collection materials and instructions to the local health care provider. These instructions are intended only for very rare blood collections remote from LLFS field centers within the continental U.S. who cannot be collected by a traveling LLFS non-catchment area interview/blood collection team. A protocol for remote collections outside the continental United States will be determined on a case-by-case basis.

LLFS Field Center Responsibilities:

1. Arrangements for the Local Health Care Provider Blood Collection visit will be made by the LLFS field center staff including arranging a mechanism for payment, if necessary.

2. It is necessary to send the blood collection materials and transport box directly to a local health care provider or a local phlebotomy staff, so that the instructions can be reviewed prior to collecting blood from the participant and to allow a minimum of 24 hours for freezing the gel pack so that appropriate temperature can be maintained during shipping. Complete the following tasks prior to sending the remote kit:

   (a) Print the assigned bar-coded LLFS ID labels and affix them to all blood collection tubes. **NOTE**: this is important. Do not ask or allow the local provider to label the tubes. This is to ensure that the tubes are not received unlabeled by the laboratory.

   (b) Add a Venipuncture Form labeled with the participant's ID# to the kit. Be sure to check "Yes" for QB8, "Is this a Non-study, Local Health Care Provider Blood Collection and Processing?"

   (c) Add a Blood Collection Shipping Form labeled with the participant's ID# to the kit.

   (d) Add a copy of the "Instructions for Local Health Care Provider Blood Collection and Processing" and "Instructions for Shipping Local Health Care Provider Collected Blood" protocols. (These instructions, which are shown below, should be copied onto the front and back of one sheet of paper).

   (e) Add one set of "extra tubes for remote collections" to the kit.

   (f) Include a copy of the signed consent/medical release form from the participant.

   (g) If the specimen will be shipped on a Friday, include Saturday delivery labels for the provider to place on the outside of the blood collection kit.

3. Fax a completed "Local Health Care Provider Blood Shipping Log" form to the Central Laboratory once a remote collection kit is sent to a local provider or lab.

4. If the minimum blood collection of CPT, SST, and EDTA tubes (tubes #1 - #3) is not achieved by the remote collecting site, the participant should be contacted by the field center staff to arrange for a salivary sample using the Oragene Kit.

LLFS Central Laboratory Responsibilities: Upon receipt of the remote site collected samples, the Central Laboratory shall fax a completed "Non-study, Local Health Care Provider Blood Collection and Processing Shipping Log" and the Venipuncture form to the LLFS field center so they know the samples have been received.

Instructions for Local Health Care Provider Blood Collection and Processing

Upon receipt of kits, place the 6 x 3 x 2.5 inch Styrofoam box (containing small gel pack) in the freezer. BEFORE BLOOD COLLECTION, YOU MUST HAVE A FROZEN SMALL GEL PACK for at least 24 hours.

If there are any questions regarding this blood collection, processing, and shipment protocol, please contact the LLFS field center at _____.

1. A black/white topped 3mL no additive tube is provided to use as a discard tube. This tube can be used to evacuate excess air from the blood collection tubing prior to the collection of the 7 tubes described below. Please note:
   - Do NOT fill this tube full of blood. Only a small amount of blood needs to be drawn.
   - When the tube is used, do NOT ship back to the laboratory. Discard with other biohazard materials.
   - If the tube is NOT used (and remains unpunctured), return to the laboratory with other tubes.

2. Collect the pre-labeled blood tubes in the following sequence:

   #1 - blue/black topped CPT tube
   #2 - red/gray topped SST tube
   #3 - lavender topped EDTA tube
   #4 - lavender topped EDTA tube
   #5 - red topped PAXgene tube
   #6 - red/gray topped SST tube
   #7 - red/gray topped SST tube

3. Remove the tourniquet after the first tube fills. Fill all tubes to capacity. Gently invert all tubes 6 to 8 times immediately after collection.

4. During collection of the PAXgene tube, **hold the tube vertically and keep it below the participant's arm level**. This is important to avoid any possible backflow of the PAXgene tube contents into the participant's vein. Also, make certain that the tube additives do not touch the stopper or the end of the needle during venipuncture. Allow at least **10 seconds** for a complete blood draw to take place. To ensure a draw volume of 2.5 mL and the correct additive/blood ratio, make sure that the blood has stopped flowing into the tube before removing the tube from the tube holder.

5. Keep all tubes at room temperature following collection.

6. Allow the three SST tubes to clot at room temperature for between 30 and 45 minutes. Set a timer as a reminder.

7. Centrifuge the three SST tubes for 15 minutes at 1,200 x rcf (*g*-force) and room temperature. If only one SST tube could be collected, use a balance tube (SST tube with water added to equal the blood level in the collected tube) while centrifuging.

8. Complete the Venipuncture Form and Shipment Form.

9. Prepare specimens for shipment as directed on the reverse side of this form.

10. A set of "extra tubes" is included in the collection kit. These may be used if there is a problem with a tube. Please return all unused tubes in the kit.

Instructions for Shipping Local Health Care Provider Collected Blood

1. Room temperature specimens:
   - Place the 8-mL blue/black-topped CPT tube and the red-topped PAXgene tube in the 5-place Styrofoam tube-holder, along with 1 square of absorbent material. If there are any remaining totally unused, unpunctured blood collection tubes, also place them in this tube-holder for possible reuse.
   - Place the two halves of the 5-slotted Styrofoam container together, then wrap the rubber band once around the short length of the container; then place the assembly in the plastic zippered bag and seal it.

2. Refrigerated-temperature specimens:
   - Wrap the three-7.5mL red/gray-topped SST tubes and two-10mL lavender-topped EDTA tubes in paper toweling to protect them from breakage.
   - Place the wrapped tubes and 1 square of absorbent material in the plastic zippered bag and seal it. Place the plastic bag into the Styrofoam container with the **frozen** gel pack, and then place the Styrofoam container in the cardboard sleeve.

3. Box assembly:
   - Place the box containing the assembled Styrofoam/cardboard sleeve of refrigerated specimens at one end of the box.
   - Place a large *room-temperature* gel pack in the other half of the box (the CPT and PaxGene tubes need to remain near room-temperature during shipment).
   - Place the assembled 5-place Styrofoam tube holder on top of the large gel pack.
   - Place the second *room-temperature* gel pack on top of the 5-place Styrofoam tube holder.
   - Place the completed venipuncture form and blood collection shipping form in the box.
   - Close and seal the box with strapping tape.

4. Peel off the back of the FedEx airbill to expose the adhesive and affix the large, lower portion of the airbill to the outside of the shipping box. The small sticker from the top of the airbill contains a record of the tracking number may be kept for your records.

5. There are two options for shipping the box.

   (a) Drop the box at a *FedEx staffed* depot. The locations of these options can be found as follows:
   (i) Go to the website **www.FedEx.com**.
   (ii) In the **Location** drop down menu, click on **Find A Location**.
   (iii) Click the checkbox for 'Drop labeled package at staffed location.'
   (iv) Enter your street address, city, state, and zip code. Click search.
   (vi) A list of nearby Fedex staffed locations will be provided.
   (vii) Be sure to drop the package off at the FedEx location in time the last Express service pickup for the day to ensure next day delivery.

   (b) Alternatively, contact FedEx (1-800-GO-FEDEX) for package pickup. When calling FedEx, say the word "rep" when the first menu is presented, and this will connect you to a representative to arrange the pickup. <u>Do not leave the package at the participant's residence.</u>

Local Health Care Provider Blood Shipping Log

LLFS Central Laboratory Contact Information

Phone: 612-625-5040 FAX: 612-625-3436

The originating field center will fax this completed form to the LLFS Central Laboratory when a collection kit is shipped to a local healthcare provider. The Central Laboratory will send a copy of this form and a copy of the Venipuncture Form by fax to the field center with the receipt information of the blood shipment.

Date form initiated: _____

Field center name: _____

Phone number: _____

FAX number: _____

Date blood collection kit sent to collection site: _____

Expected date of blood shipment to LLFS Central Laboratory: _____

Name and location of blood collecting site: _____

Contact person's name at blood collection site: _____

Contact person's phone number at blood collection site: _____

LLFS label ID# assigned: _____

Fed Ex airbill number: _____

Central Lab Use Only:

Date of receipt of blood: _____

Samples received in shipment, *please circle:* 1 CPT (Blue/black) 1 SST (Red/gray) 1 EDTA(Lavender)

1 EDTA (Lavender) 1 PAXgene (Red) 1 SST (Red/gray) 1 SST (Red/gray)

[ ] This Shipping Log and Venipuncture Form was faxed:

To_____on _____(date/time)

[ ] Unable to contact by fax; notice of receipt phoned:
At ___ _____(date/time)

Tech: _____

Appendix 4. LLFS Quality Control Phantom Participant ID Form

QC/Phantom ID: _____

Date collected: _____

Note: Place the QC ID for the sample set to be collected at the top of the page; record the date of collection. Upon collection of each phantom duplicate tube, place the original Participant ID for the QC sample that is being drawn on the appropriate line next to the sample type. Enter these ID numbers in the data system on the same schedule as entry of the blood collection information. QC samples are shipped on the same schedule as the regular shipments (i.e. the same day the sample is collected), but the QC samples will be shipped in collection kit packaging separate from the participant's regular collection samples. This form is kept by the Field Center (**DO NOT send to Central Laboratory**).

Tube #/sample type **Participant ID**

2 (serum) _____

3 (EDTA plasma) _____

5 (PAXgene) _____

Chapter 7 – Appendix 5

A Collaborative Study, Including:

Boston University Medical Center
Columbia University
University of Pittsburgh
University of Southern Denmark
Washington University School of Medicine

Sponsored by:

National Institute on Aging

Instructions for Long Life Family Study Visit
(Sample Form – Remote Phlebotomy Service Instructions)

Date: _____

Phlebotomy Service Address
Attn: Insert Name
Address
City, State Zip

Re: (Insert Participant Name)'s Blood Draw for the Long Life Family Study

Dear _____:

Thank you for your assistance in completing the venipuncture portion of the Long Life Family Study (LLFS). **_____is planning on coming to your facility on Insert Date.** Enclosed are the following materials that you will need to complete the blood draw:

- All tubes and shipping materials, including an extra set of collection tubes if needed
- Detailed instructions for collecting the LLFS specimen
- Detailed instructions for shipping the LLFS specimen, including Saturday delivery stickers if applicable
- A signed copy of the consent form
- The Blood Collection Form

Insert billing information. If you have any questions regarding this, I may be reached at **[*insert E-Mail address and telephone number*]**. Thank you again for your assistance.

Sincerely,