THE STRONG HEART STUDY

Cardiovascular Disease in American Indians
(Phase V)

Operations Manual

Volume Four

Laboratory Manual

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For copies, please contact

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# VOLUME IV

## LABORATORY MANUAL

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STRONG HEART STUDY LABORATORY PROCEDURES

1.0 Safety Precautions, Universal Precautions and Personal Protective Equipment for the Handling of Blood and Working in a Laboratory Setting:

Lab testing in research is important. Your work brings new and important information to the scientific and medical community. The special equipment and skills such as attention to detail, organization and phlebotomy are critical to the success of this project. Your work on this project will probably expose you to a variety of potentially hazardous situations. The following learning modules are designed to help you keep safe on the job.

Each site should have at least one staff member who will be actively involved in this process attend the initial training session. This person, in turn will be responsible for training additional personnel at his/her facility. The training session will cover all procedures related to supplies, equipment, and preparation of log sheets, labeling, collection, processing, storage, packing and shipping of specimens.

Throughout the study, a qualified observer should regularly monitor and evaluate the work of those involved in the collection and processing of blood samples. Specific plans should be made to train new staff members at each facility. Training should include a detailed review of the Strong Heart Study laboratory manual as well as supervised practice in the application of the techniques required by the protocol.

This section will provide knowledge to protect you and others. In addition to these instructions, use commonsense on the job every day.

- Be cautious and follow procedures.
- Take your time and concentrate on what you are doing.
- Avoid contact with hazardous chemicals
- Treat unknown substances as hazardous
- Follow all equipment operating instructions
- Don’t take chances
- Don’t operate anything if you have not been trained on that equipment
- Follow all instructions
- Report any malfunctioning or suspicious-appearing equipment immediately
- Never pipette samples by mouth.
- Avoid direct contact with blood, sera or plasma. Cover any scratches or cuts on fingers and hands very carefully and use gloves in the handling of specimens.
- All tubes, containers and other materials exposed to blood must be disposed of in appropriately labeled waste receptacles for biohazardous materials.
- When removing stoppers from vacutainers, use a splash shield to prevent
droplets from spraying onto your skin or eyes.

- All samples should be stored in sealed containers or tubes.
- All site personnel should be informed of and guided by current OSHA and CDC guidelines particularly those concerning "Universal Blood and Body Fluid Precautions" published by the CDC.
- Immunization against Hepatitis B is highly recommended for persons drawing blood and handling biological specimens.
- Avoid working alone
- Post important phone numbers and emergency numbers prominently
- Know before an accident where to go and what to do
- Know the location of safety showers, eyewashes and fire extinguishers

Module I: Safety Precautions

This module will include the following:

- Provide knowledge to protect you and others.
- Demonstrate common procedures that will be used on the job every day.

Here are some guidelines:

- Be cautious and follow procedures.
- Take your time and concentrate on what you are doing.
- Avoid contact with hazardous chemicals
- Treat unknown substances as hazardous
- Follow all equipment operating instructions
- Don’t take chances
- Don’t operate anything if you have not been trained on that equipment
- Follow all instructions
- Report any malfunctioning or suspicious-appearing equipment immediately
- Never pipette samples by mouth.
- Avoid direct contact with blood, sera or plasma. Cover any scratches or cuts on fingers and hands very carefully and use gloves in the handling of specimens.
- All tubes, containers and other materials exposed to blood must be disposed of in appropriately labeled waste receptacles for biohazardous materials.
- When removing stoppers from vacutainers, use a splash shield to prevent droplets from spraying onto your skin or eyes.
- All samples should be stored in sealed containers or tubes.
- All site personnel should be informed of and guided by current OSHA and CDC guidelines particularly those concerning "Universal Blood and Body Fluid Precautions" published by the CDC.
• Immunization against Hepatitis B is highly recommended for persons drawing blood and handling biological specimens.

**Emergencies can happen, so be prepared:**

• Avoid working alone
• Post important phone numbers and emergency numbers prominently
• Know before an accident where to go and what to do
• Know the location of safety showers, eyewashes and fire extinguishers

**Some of the equipment in the areas you will be working is reviewed below:**

• **Glassware** like vacutainers can break, causing chemical and cut hazards. Some of the chemicals contained in the vacutainers are EDTA and heparin. Although serious hazards are unlikely if exposed, still follow procedures if an accident occurs. To avoid contact, use the right type of glassware for each job, and discard chipped or cracked vacutainers in an approved receptacle. Don’t force anything made of glass.

• **Electrical equipment** always carries the potential of shock or fire. Don’t touch it with wet hands or while standing on a wet floor. Report any shocks, and don’t attempt to do repairs if you haven’t been trained.

• **Centrifuges** and other equipment with moving parts can catch your clothing or open up suddenly, showering you with dangerous material. Keep clothing or long hair away from them. Make sure the load is balanced, the top is locked down, and the equipment has stopped before you open it.

**Module II: Personal Protective Equipment**

This module will include the following:

° Proper use of protective clothing.
° First-aid instructions

Let protective equipment work for you.

For this aspect of the study, always use assigned protective clothing and equipment. Always check that it is in good condition before putting it on. For this study the following are required:

• **Goggles or side shield safety glasses** to protect against splashes or flying objects are required any time you are working with specimens or
performing phlebotomy.

• **Gloves** must be worn to protect against any chemicals or exposure to samples
• **Long sleeves** are required to the length of your wrist and meet the glove.
• **Lab coats** must be full length and fully buttoned down the front.
• **Sturdy closed toed shoes** are required to cover your feet in case of spills or accidents

**If you are exposed to a hazardous substance or samples, take the following actions:**

For first-aid instructions, here are some general instructions. You should check with your supervisor for specific instructions at your institution prior to an accident.

• **Eyes:** Flush with water for 15 minutes.
• **Ingestion:** Follow labels and MSDS instructions. MSDS is an abbreviation for Materials Safety Data Sheet and is available from the manufacturer for every chemical produced.
• **Skin Contact:** If limited to a small area of the body such as the hands, remove any contaminated gloves or clothing and wash with copious amounts of water. If there is greater exposure, stand under emergency shower and remove contaminated clothing immediately.
• **Inhalation:** Get to fresh air and get prompt medical attention.

**Module III:** Preventing Exposure to Blood Borne Pathogens:

This module will include the following:

° Universal precautions
° Work practices, including the use of protective clothing that eliminates or minimizes exposure to staff and subjects
° Housekeeping procedures to ensure cleanliness and possible spread of infection
° Hepatitis B vaccinations for employees at risk
° Exposure evaluation and follow-up for exposure incidents
° Hazardous material container warnings such as biohazard labels
° Confidential, accurate employee medical records

Your chance of being directly exposed to bloodborne pathogens on the job is small. But keeping exposure minimal can only succeed if staff members use the tools to protect themselves on the job.
• **Universal Precautions** are your best protection against any risk to exposure. This means all staff must treat all blood, urine, and other potentially infectious body fluids as if they are infected.

  All specimens should be regarded as potentially hazardous.

**DO:**

- Wash hands and exposed skin with soap and water immediately after exposure to infectious materials or after taking off gloves or other personal protective equipment.
- Use antiseptic or cleansers or towelettes only if washing facilities aren’t available.
- Minimize splashing, spraying, or spattering of blood or other potentially infectious materials.
- Place contaminated sharps in assigned labeled, puncture-resistant, leak-proof containers.

**DON’T:**

- Don’t shear or break contaminated needles or other sharps, and don’t bend, recap, or remove unless specifically instructed.
- Don’t keep food, drink, medication or makeup in work areas with exposure potential.
- Don’t eat, drink, smoke apply cosmetics or lip balm, or handle contact lenses in work areas with exposure potential.
- Don’t pipette or suction anything by mouth.

• **Protective Clothing:**

  **BEFORE you** put on protective clothing, make sure it’s in good condition. Don’t wear anything that’s damaged or does not fit properly.

  **AFTER tasks** in the area are completed, remove all protective clothing before leaving that area. Remove protective clothing in such a manner as to minimize exposure and avoid contamination. Place protective clothing in a specially assigned area or container for decontamination, washing, storage or disposal.

• **Housekeeping:**

  Written procedures and a cleaning schedule help keep the workplace free of infection.
• **Cover** equipment and surfaces with plastic wrap, aluminum foil, or impervious absorbent paper. Remove and replace covering that is, or may be, contaminated.

**Module IV: Proper Labeling**

This module will include the following:

- Correct identification and labeling of containers with biohazardous labels
- Instructions in case of exposure

Proper labeling of containers for regulated waste must be labeled with fluorescent orange or orange-red biohazard warning labels.

Examples in the clinical area or lab are: refrigerators and freezers containing blood and other potentially infectious materials and other containers used to store, transport or ship blood and other potentially infectious materials.

Biohazard labels **ARE** required for the following:

- waste containers used for disposal of contaminated needles
- refrigerator or freezer holding blood or other potentially infectious material
- individual specimen containers for storage or shipment zip-lok biohazard bags

Biohazard labels **ARE NOT** required for the following:

- when red bags or red containers are used
- on individual containers or blood of other potentially infectious materials that are placed in a labeled container during storage, transport, shipment or disposal

The risk of exposure is very small and most encounters with an HIV or HBV carrier pose no risk. AIDS and Hepatitis B can be transmitted through:

- Sexual contact
- Shared needles
- Needlestick injuries from infected needles or sharps
- Direct contact between broken or chaffed skin and infected body fluids.
- Hepatitis B can also be transmitted through dried blood and contaminated surfaces.

Neither AIDS (HIV) nor Hepatitis B are transmitted by:
• Coughing or sneezing
• Touching an infected person
• By using the same equipment, materials, toilets, showers, or water fountains.

**Be safe!!!** Your employer must make available, free of charge or at a reasonable time and place, the hepatitis B vaccine and vaccination series to all employees at risk. Any booster doses recommended by the US Public Health Service also must be provided. You are not required to participate in a prescreening program to receive the vaccine series. Also, the vaccine can be available at a later time if initially declined. If you choose to not receive the vaccine, your facility will ask you to complete and sign a form stating your refusal. This is required by law.

**If you are directly exposed, REPORT IT IMMEDIATELY!!!**

An exposure incident is specific to eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee’s duties. A common example of exposure would be a puncture from a used needle.

If exposed, you should contact your supervisor immediately. This allows for timely medical evaluation and follow up as well as for timely testing of the source. Your facility will provide immediate, confidential assistance and medical evaluation, including a blood test. All information will be treated with the strictest of confidence.

**2.0 Sample Collection Instructions:**

Personnel involved in sample collection should be highly experienced with vacutainer and butterfly blood collections, and be prepared to handle common problems, such as difficult blood collection and situations such as fainting. The phlebotomist should also be familiar with precautions to avoid exposing themselves to blood and be trained in the following:

• Ideally staff will have cardiopulmonary resuscitation (CPR) certification.
• It is suggested that they read "Collection and Handling of Laboratory Specimens: A Practical Guide"\(^1\) or a similar phlebotomy manual.
• Personnel should wear clean white lab coats (with no blood stains) and

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maintain a neat appearance. Lab coats will be full length, with long sleeves. Lab coats will be buttoned closed down the full length of the coat.

- Personnel will wear protective eyewear. Safety glasses are required when performing phlebotomy, processing specimens and preparing samples for storage and/or shipment.
- Staff should wear nametags and introduce themselves (if necessary) before a blood draw.
- Long hair and bangs should be pulled back.
- Phlebotomists and assistants should not chew gum or have any food in their mouths during blood draws. Food and drink must never be brought into areas used for blood drawing or processing.
- Staff will attempt no more than three venipunctures on the same subject. After three failed attempts, another person will be asked to try.

**Module I: Sample Collection Facilities**

This module will include the following:

- Room requirements for sample collection
- Supplies for sample collection

The area in which phlebotomy will occur should be clean and tidy with no evidence of previous blood draws such as used needles, blood stains, etc. A phlebotomy chair should be available for 15-20 minute periods to allow subjects to be seated for 10 minutes prior to a blood draw. If not available within the room, there should be quick access to a bed or examining table and ammonia capsules in case a subject feels faint. Also, there should be easy access to emergency equipment in case of cardiac arrest. Ideally, only the participant and phlebotomist (and assistant when needed) are in the room during the procedure.

The room should be set up in advance with basic supplies for blood collection:

- Vacutainer holders/hub
- Vacutainer needles
- Disposable graduated transfer pipettes
- Alcohol wipes or swabs
- 2x2 sterile gauze pads
- Band aids
- Adhesive tape
- Urine collection cups
- Disposable latex gloves
- Ammonia inhalants
- Paper cups
- Emesis basin
- Tourniquets
- biohazard labels
- biohazard needle disposal boxes
- biohazard bags
- Tube racks or supports
- Waterproof marking pen
- Refrigerator
- Centrifuge
- -70°C Freezer (or lower temperature Freezer than -70°C)

Module II: Sample Collection and Processing

This module will include the following:

- Completion of clinical logs
- Completion of laboratory requisitions
- Demonstration of One-Touch Sure Step Flexx procedure
- Proper labeling of vacutainers and transport tubes
- Venipuncture Instructions
- Posture during blood collection
- Difficult Venipuncture Techniques
- Vacutainers for Sample Collection and Processing Instructions

Sample Collection Logs and Laboratory Requisition Forms

Clinic personnel should carefully review the description of collection requirements to ensure that specimens are collected in the proper order and use the proper technique. Each clinic should set up a blood collection and blood processing notebook or a laboratory logbook in advance. It should be located in the blood collection/processing area. This should be a hardbound notebook from which pages cannot be easily removed. Pages should have columns headed for date, visit number, participant name and ID, barcode labels, redraw labels and room to write "comments" about any problems with blood draws or processing, including hemolysis of samples, etc.

In addition to the logs for the clinical area, it will be necessary to complete a laboratory requisition form for each subject (see example of this PARTICIPANT SAMPLE FORM in Appendix C below). The completed requisition form should include the following:

- Exam ID
- Date of Collection
- Under left column marked “write the number of samples sent” record the actual number of samples sent.

After proper completion of requisition form, affix barcode label to both copies of the form and one label in the laboratory log book.

Redraw
If sample collection is a redraw, indicate “yes” on new requisition form and take the following steps:

- Affix original barcode label to both copies of the requisition form
- Also affix redraw barcode label to both copies of the requisition form
- Place redraw label by the appropriate participant ID in laboratory log book

One-Touch Sure Step Flexx Procedure

1) One-Touch Sure Step Flexx glucose reading from a drop of blood obtained by finger stick. Using the blood from the venipuncture procedure below will not provide comparable results since there is a difference between capillary blood (fingerstick) and venous blood values.

2) See One-Touch Sure Step Flexx procedure for calibrating the glucometer and steps to follow in obtaining a glucose reading. (Consult with the operations manual, which can be obtained from Lifescan, Inc. 1-800-227-8862) A video and training will be provided at the initial training session. Thereafter, training will be provided on-site. See Appendix A of this volume (p. IV-A-1 below) for additional instructions.

Labeling Collection Tubes and Samples:

Prior to venipuncture, a label showing the date and time of collection and participant ID number should be written by the phlebotomist.

Pre-numbered and bar-coded labels will be provided to the study sites. Take care to select the correct number depending on whether the samples are being collected from the participant as a QA sample or for a Courtesy visit.

To properly label vacutainers and shipping vials, the white section of the label must be applied (first) to the tube laterally with the clear end wrapped over the white section of the label after the label is wrapped around the tube.

**Module III: Venipuncture Procedure**

This module will include the following:

- Correct Venipuncture procedures

Posture During Blood Draws:

A participant should be seated during blood draws. However, if the participant is clearly uncomfortable with the blood drawing situation, because of a previous fainting episode or a fear of fainting, have the participant lie down provided the
blood draw can proceed within 10 minutes. This is to ensure that blood is collected before body fluid shifts occur, which could alter plasma concentrations of outcome variables. Therefore, it is desirable that less than 10 minutes elapse between the participant’s lying down and completion of the blood draw.

Difficult Venipunctures:

There will be several common situations in which vascular access may be difficult. These will include but are not limited to the following:

- Palpated vein feels small or rolls.
- Excess subcutaneous tissue and fat lies over veins.
- Participant complains of being stuck more than once on a previous visit (no single staff person will attempt more than three venipunctures on a single participant at a single clinic visit) or has had a bad experience elsewhere.
- Participant has been stuck once already and none of the usual veins are palpable.

All reasonable efforts should be made to collect a blood sample, including use of a 23 gauge needle if that is the only means available to obtain a sample, e.g., in the case of a child or elderly person. If the participant experiences any of the above problems, and is agreeable to a repeat attempt, you may try the following procedure:

- Check back of hand and forearm for venipuncture sites with larger veins.
- Attempt one or more vein dilation methods:
  1. Hot pack venipuncture site with a warm, wet towel or apply heating pad for 3-5 minutes.
  2. Have participant hold hand in warm water for 3-5 minutes.
  3. Have participant dangle arm at side with tourniquet in place for one minute.
  4. Use blood pressure cuff as a tourniquet by pumping pressure to 60-80 mm Hg.
  5. Be sure room is not too cool.

1) Position the participant in comfortable chair in an environment free from distraction.

2) Query the participant about their fasting state. Example: "When was the last time you ate or drank anything except water?" The participant should be fasting for about 12 hours. Record the time since the last food or beverage was consumed. If subject is not fasting, record time and note in comment section what foods or beverages were consumed that morning. Be sure to include any
additives like cream, sugar, or artificial sweeteners if a beverage was consumed. Regardless of fasting state, proceed with drawing procedure.

3) Inform the participant about the procedure. Explain the procedure to the participant, e.g., "I will be drawing a blood sample from your arm today. You will probably feel a small prick when I insert the needle."

4) Assemble all materials; have extra tubes within reach.

5) Blood samples will be collected from Strong Heart Study participants using conventional vascular access with a multi-draw Vacutainer (butterfly) needle and collection of the blood sample into Vacutainer tubes.

6) The antecubital site of the left arm will be used as the first choice for venipuncture. The median cubital vein is the one most frequently used. If the venipuncture of this vein is unsuccessful, the cephalic and basilic may be the next appropriate choice, followed by veins on the back of the hand. For known mastectomy participants, avoid use of an arm where there was axillary lymph node dissection.

7) Be sure all necessary supplies and equipment are available and set up in advance. Note visit type and type of Vacutainer tubes required. Label tubes with participant ID# and date and time of collection. Complete all lab forms before specimen collection. Ensure that all necessary equipment is functioning properly.

8) Be sure a full length lab coat is worn and buttoned. Wash hands and put on protective gloves.

9) Fit luer adapter needle at end of collection set into Vacutainer sleeve and place the first collection tube into sleeve/hub.

10) Position participant's arm on the drawing table. Extend the arm toward you, palm up.

11) Apply tourniquet 3 inches above a venipuncture site. If it is necessary to apply a tourniquet for preliminary vein selection, release it for two minutes and reapply immediately before entering the vein. 

12) Pull skin taut 2 inches below site to keep vein from rolling.

13) Palpate vein. (A vein feels like an elastic tube and returns when pressure is applied). If the presence of a vein is questionable, remove or loosen the tourniquet. If no radial pulse can be felt, the tourniquet is too tight.

\[\text{Tourniquet must not be in place more than two minutes.}\]
tourniquet. If the structure remains, it probably was not a vein; if it
disappears assume it was a vein. Another technique to assist in locating a
vein is to moisten the skin with alcohol as it will decrease the friction and
may aid in the palpation of a vein. If no vein is felt, try other arm or another
site (See section on "Difficult Venipunctures").

14) Cleanse skin over vein thoroughly using a circular motion from center to
periphery. Wipe alcohol with new 2x2 gauze to dry the area.

DO NOT TOUCH SKIN AFTER CLEANSING.

15) With the bevel of the needle in upright position, enter vein. Hold needle in
the same direction as vein and at a 15-degree angle to vein. Insert the
multidraw needle bevel up, parallel to vein. Use a straight smooth
movement through the skin; do not poke around. The needle is sterile; do
not touch it while performing venipuncture. If vein rolls, withdraw needle
slightly without coming completely out of the arm and try a second
attempt. If the vein collapses, remove the needle and tourniquet. Apply
slight pressure to the puncture site. Try another site and/or call another
staff person to assist. After a new location has been determined, usually
the other arm, begin the procedure again. Reapply the tourniquet,
possibly have participant open and close the fist, swab areas with alcohol
and dry, then reinsert the tube. If there is still no blood, stop the
procedure and use techniques in section on "Difficult Venipunctures."

16) If the phlebotomy is successful, draw required blood tubes. After blood begins to
flow, secure butterfly with a piece of tape and loosen the tourniquet. Place tubes
in conditions as specified in the instructions.

If blood does not begin to flow, try the following:

a) Move the needle slightly in or out.

b) Rotate needle slightly or lift needle to move bevel away from the wall of
the vein.

c) Try another tube.

d) Loosen tourniquet; blood flow may be impeded if tourniquet is too tight.

e) Be sure to watch for signs of hematoma or swelling from the vein. If there
is any indication of hematoma or swelling, immediately remove tourniquet
and needle. Place 2x2 gauze over the site, and apply pressure and/or ice
pack on site for 5 minutes. If the first attempt to obtain blood is
unsuccessful (with the subject's permission) try again on the opposite arm.

The same technician should not attempt a venipuncture more than
three times.

17) When first tube is filled, remove tube and replace with the next tube. Invert all
filled tubes except SST tubes 8-10 times and place on ice. DO NOT place SST
tube on ice.
18) Proceed with collection of tubes in this order. Label all tubes:

- **Fasting:**
  1. (3) Red top (SST) tubes
  2. (1) or (2) Light Blue top (Citrate) tubes
  3. (1) Gray top (Sodium fluoride) tubes
  4. (4) Lavender top (EDTA) tubes

19) After drawing the last tube, remove the tourniquet. Use clean gauze to apply slight pressure to arm and withdraw needle, then immediately apply pressure to site. Apply gentle pressure to the site.

20) Request participant apply pressure at site for 3-5 minutes while leaving the arm straight at the elbow. This is more important than elevating the arm or bending the elbow, which some participants might do automatically.

21) Confirm that bleeding has stopped, and apply a pressure bandage at venipuncture site. If bleeding has not stopped, elevate arm and continue to apply pressure until it stops.

22) Check preprinted labels and tubes, making sure the ID# and tube designation are correct.

23) Dispose of entire needle set-up into a proper biohazard disposal container. *Never try to re-cap a needle since this puts you at risk for a needle puncture.*

24) Check site. If blood oozes from the site, have the participant apply pressure to the site 1-2 minutes longer or as long as is necessary, elevating arm above head. Apply Band-Aid.

25) Give the participant labeled urine specimen cup and instruct him to void into the container. Inform him/ her where to leave the container.

26) Remove gloves, wash hands, and proceed to next participant.
Realize that the participant might be disoriented, embarrassed, or irritable and may need additional attention. Recognize also that this incident will have an impact on future blood drawing, and possible adherence through the study, and must be handled with reassurance. Make a note in the participant's file so that clinic staff will be aware of the situation in the future.

Finish venipuncture following procedures outlined above, if possible. If multiple attempts at venipuncture are unsuccessful, do not reschedule the participant unless both the technician and the participant agree that this is an unusual situation and that there is a high probability of obtaining a sample on the first try at another visit.

If Fainting Episodes Are Experienced:

If participant shows signs of becoming faint (loss of color in the face, unusual sweating on the forehead) or reports feeling dizzy:

- Finish drawing blood if possible but do not proceed if participant is clearly in trouble.
- Remain calm and call for help.
- Have participant lay head on table or move participant into a fully reclined position, if possible.
- Have participant prop feet up on pillow or cushion and elevate participant's legs above her head.
- Continue talking to participant to assess level of consciousness.
- Prevent injuries from possible fall or seizure.
- Have participant lie down for 5-10 minutes after removing the needle; apply pressure on vein.
- Apply cool compress to forehead.
- Use ammonia capsule if needed.
- Keep participant in a reclined position until the subject feels better.
- Taking blood pressure readings to assess recovery may be worthwhile.
- Offer participant water, juice and food after they have recovered.

Urine Sample Collection:

1. Containers for routine collection should be clean and hold about 50 ml in volume and
must have a tight-fitting lid.

2 The participant’s privacy should be assured and a clean bathroom available.

3 Instruct the participant to perform the following steps:

- Remove cap from the labeled container before beginning urination
- Void directly into toilet and after stream is steady, pause.
- Begin stream again and fill approximately half of the cup.
- Finish urinating, firmly place cap on container and return sample to the study person.

Flow charts summarizing processing procedures are in Appendix B-1A through B-1C.
General Sample Collection:

### Table I: General Instructions for Sample Processing of Blood & Urine Samples

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<th>Collection Tube</th>
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| **Serum Storage** | 1. Let stand at room temperature for 20 minutes so blood can clot. If samples cannot be processed within the hour, refrigerate sample or place on ice.  
2. Centrifuge at 3000 rpm (1000xG) for 10 minutes.  
3. Place approximately 1 ml of serum sample in each of the appropriate 2ml-cryovials and label. |
| **Serum Storage** | 1. *This vacutainer must be allowed to fill completely with blood at the time of collection.*  
2. After collection gently invert 8-10 times. Place on ice or refrigerate immediately.  
3. Centrifuge at 3000 rpm (1000xG) for 10 minutes at 4°C.  
4. Place approximately 1 ml of plasma sample in each of the appropriate 2ml-cryovials and label. |
| **Plasma Storage** | 1. After collection gently invert 8-10 times. Place on ice or refrigerate immediately.  
2. Centrifuge at 3000 rpm (1000xG) for 10 minutes at 4°C.  
3. Place approximately 1 ml of plasma sample in each of the appropriate 2ml-cryovials and label. |
| **Plasma Storage** | 1. After collection, gently invert 8-10 times, place on ice or refrigerate immediately.  
2. Tube #1: Prior to centrifuging, mix well and pipette approximately 1 ml of whole blood and place in each appropriate 2-ml cryovial and label. Re-cap tube #1.  
3. All three tubes: Centrifuge at 3000 rpm (1000xG) for 10 minutes at 4°C. First, place approximately 1 ml of plasma sample in each of the appropriate 2-ml cryovials and label. Then, remove the buffy coat using the *Purple top tube buffy coat isolation protocol* as follows:  
   | **Buffy Coat:**  
   1. After plasma has been removed, there should be about 1/8th inch of plasma remaining on top of the buffy coat.  
   2. With either a glass or plastic pipette, place the tip of the pipette at the bottom of the small plasma layer just slightly above the buffy coat. Also, rest the pipette against the glass inside edge of the vacutainer tube.  
   3. Slowly draw up the buffy coat by moving the pipette in a circular motion around the inside of the vacutainer.  
   4. Remove all of the buffy coat from one tube and place in a 2.0 ml cryovial (orange cap).  
   5. Cap cryovial firmly, apply label.  
   6. With each tube repeat steps 1-4 using a different pipette for each tube. Use a new clean pipet for each tube. Do not mix the buffy coats between cryovials, ie only one buffy coat from one tube per cryovial. |
| **Urine Storage** | 1. Do not centrifuge.  
2. After collection, place on ice or refrigerate immediately.  
3. Place 1 ml of urine sample in each of the appropriate 2-ml cryovials and label. |
### Table II: Participant Collection Instructions

<table>
<thead>
<tr>
<th>Collection Tube Type</th>
<th>Tests</th>
<th>Sample Type</th>
<th>Storage/Shipping Requirement</th>
<th>Cryovial Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10 ml SST (red/gray tiger top)</td>
<td>Serum</td>
<td>Frozen</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chem Profile, Lipids, Insulin, CRP, FFA</td>
<td>Serum</td>
<td></td>
<td>2 ml-red cap vial</td>
</tr>
<tr>
<td></td>
<td>Storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.5 ml Lt blue or 2.7ml Lt blue</td>
<td>Na Citrate Plasma</td>
<td>Frozen</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>Na Citrate Plasma</td>
<td>Frozen</td>
<td>2 ml-blue cap vial</td>
</tr>
<tr>
<td></td>
<td>Storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.0 ml Gray</td>
<td>NaFl Plasma</td>
<td>Frozen</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0 hour (fasting) glucose</td>
<td></td>
<td></td>
<td>2 ml-black cap vial</td>
</tr>
<tr>
<td>3</td>
<td>10 ml Purple</td>
<td>Whole Blood Buffy coat</td>
<td>Frozen</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>HemoglobinA1c</td>
<td>EDTA Plasma EDTA Plasma</td>
<td>Frozen</td>
<td>2 ml-neutral cap vial</td>
</tr>
<tr>
<td></td>
<td>DNA Isolation</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td></td>
<td></td>
<td>2 ml-orange cap vial</td>
</tr>
<tr>
<td></td>
<td>EDTA Storage</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 ml-purple cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 ml-purple cap vial</td>
</tr>
<tr>
<td>1 cup Random Urine</td>
<td>Albumin/Creatinine</td>
<td>Urine</td>
<td>Frozen</td>
<td>2</td>
</tr>
<tr>
<td>Storage</td>
<td></td>
<td></td>
<td></td>
<td>2 ml-yellow cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 ml-yellow cap vial</td>
</tr>
</tbody>
</table>
### Table III: QA Collection Instructions:

<table>
<thead>
<tr>
<th>Collection Tube Type</th>
<th>Tests</th>
<th>Sample Type</th>
<th>Storage/Shipping Requirement</th>
<th>Cryovial Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 10 ml SST</td>
<td>Chem Profile, Lipids, Insulin, CRP FFA</td>
<td>Serum</td>
<td>Frozen</td>
<td>4 2 ml-red cap vial</td>
</tr>
<tr>
<td>1 4.5 ml Lt blue or</td>
<td>Fibrinogen</td>
<td>Na Citrate Plasma</td>
<td>Frozen</td>
<td>2 2 ml-blue vial</td>
</tr>
<tr>
<td>2 2.7 ml Lt blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 4 ml Gray</td>
<td>0 hour (fasting) glucose</td>
<td>NaFl Plasma</td>
<td>Frozen</td>
<td>2 2 ml-black cap vial</td>
</tr>
<tr>
<td>1 10 ml Purple</td>
<td>HemoglobinA1c, Leptin</td>
<td>Whole Blood EDTA Plasma</td>
<td>Frozen</td>
<td>2 2 ml-neutral cap vial 2 2 ml-purple cap vial</td>
</tr>
<tr>
<td>1 cup Random Urine</td>
<td>Albumin/Creatinine</td>
<td>Urine</td>
<td>Frozen</td>
<td>2 2 ml-yellow cap vial</td>
</tr>
</tbody>
</table>

Strong Heart Study V  07/01/06  IV-19  Laboratory Manual
Table IV: Courtesy Collection Instructions

<table>
<thead>
<tr>
<th>Collection Tube Type</th>
<th>Tests</th>
<th>Sample Type</th>
<th>Storage/Shipping Requirement</th>
<th>Cryovial Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 10 ml SST</td>
<td>Chem Profile, Lipids, Insulin, CRP, FFA</td>
<td>Serum</td>
<td>Frozen</td>
<td>4 2 ml-red cap vial</td>
</tr>
<tr>
<td></td>
<td>Storage</td>
<td>Serum</td>
<td>Frozen</td>
<td>8 2 ml-red cap vial</td>
</tr>
<tr>
<td>1 4.5 ml Lt blue or</td>
<td>Fibrinogen</td>
<td>Na Citrate Plasma</td>
<td>Frozen</td>
<td>1 2 ml-blue cap vial</td>
</tr>
<tr>
<td>2 2.7ml Lt blue</td>
<td>Storage</td>
<td>Na Citrate Plasma</td>
<td>Frozen</td>
<td>1 2 ml-blue cap vial</td>
</tr>
<tr>
<td>1 4 ml Gray</td>
<td>0 hour (fasting) glucose</td>
<td>NaFl Plasma</td>
<td>Frozen</td>
<td>2 2 ml-black cap vial</td>
</tr>
<tr>
<td>3 10 ml Purple</td>
<td>HemoglobinA1c</td>
<td>Whole Blood</td>
<td>Frozen</td>
<td>3 2 ml-neutral cap vial</td>
</tr>
<tr>
<td></td>
<td>DNA Isolation</td>
<td>Buffy Coat</td>
<td>Frozen</td>
<td>3 2 ml-orange cap vial</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>EDTA Plasma</td>
<td>Frozen</td>
<td>2 2 ml-purple cap vial</td>
</tr>
<tr>
<td></td>
<td>EDTA Storage</td>
<td>EDTA Plasma</td>
<td>Frozen</td>
<td>8 2 ml-purple cap vial</td>
</tr>
<tr>
<td>1 cup Random Urine</td>
<td>Albumin/Creatinine</td>
<td>Urine</td>
<td>Frozen</td>
<td>2 2 ml-yellow cap vial</td>
</tr>
<tr>
<td></td>
<td>Storage</td>
<td>Urine</td>
<td>Frozen</td>
<td>4 2 ml-yellow cap vial</td>
</tr>
</tbody>
</table>

Module IV: Quality Assurance Sample Collection:

As part of the Quality Assurance process of this study, there is a need to assure that all the steps from the time that blood is collected to the time that results are reported are correct. To accomplish this, replication of unknown samples will be necessary by performing blind duplicate testing of samples. Blind duplicate samples, otherwise known as quality assurance (QA) samples, will be obtained from participants as follows:

1. Collect blind duplicate samples at a frequency of every 20th participant.

2. Collect blind duplicate samples only for the tests listed in Table III above.

3. In order to label the blind duplicate samples, the numbering system for these QA samples is similar to the Study ID and consists of 6 digits with the first digit corresponding to the center (1-SD, 2-OK, 3-AZ), the second digit will be a "3" to
indicate that the sample is a QA and the 4-digit participant ID number. The Coordinating Center should receive at monthly intervals the matching participant ID and corresponding QA for analysis. This list should not be made available to the Core Laboratory.

Processing and Shipping QA samples

These samples should be treated the same as the regular participant samples and be included in regular shipments with the participant and courtesy samples. DO NOT note the corresponding (regular) participant number anywhere on the form to go to the lab.

3.0 Sample Storage and Shipment

Module I: Equipment Maintenance

This module will include the following:

° Proper maintenance of equipment

The proper care of equipment promotes the life of any piece of equipment and will reduce the possibility of downtime while waiting for repair. Included in the proper maintenance of equipment is the requirement of taking temperatures of refrigerators and freezers.

• Refrigerators and Freezers

Storage requirements for samples include keeping samples at the proper temperature until samples are shipped. Never store samples in a self-defrost freezer. At each site, there should be a temperature log to record the temperatures of the room, all refrigerators and all freezers that hold samples. By recording and evaluating temperatures each day, you will see temperature fluctuation that is a signal that some part is not working properly and downtime is inevitable. It is also advisable to locate a maintenance/repair company that services your unit in the area before a problem is experienced. If temperatures begin to fluctuate, the repair service should be called in to evaluate the problem. It may be a simple repair like a door seal or it may require ordering a part. In any case, detecting the problem early will give you time to have the repair done while still maintaining samples at proper temperatures. In addition to recording temperatures, all refrigerators and freezers require routine maintenance. Follow manufacturer guidelines.

• Centrifuges

Like refrigerators and freezers, there are many makes and models of centrifuges. Follow manufacturer guidelines for the care of your centrifuge. In addition, locate a service company that can do maintenance and repairs. Find
this company before a problem occurs. In addition, once a month the inside bowl of the centrifuge should be cleaned with a disinfectant. Always wear gloves, safety glasses and a lab coat when performing this task.

Module II: Storage Requirements

This module will include the following:

- Proper storage
- Shipping instructions
- Proper packaging of samples
- Proper completion of FedEx airbill
- Notification of shipment to the lab

One important precaution which should always be kept in mind when handling samples is that all blood, except for the SST tube, should be cooled (either in the refrigerator or on ice) as soon as the samples are collected. They should be kept cold until processing is complete and samples are properly stored. After the SST tube is completely clotted (20-30 minutes) it should also be kept cool if it cannot be processed within the hour. Plasma should be separated from the cells within the hour. Plasma samples should not be allowed to freeze and thaw during any of the handling steps.
**Module III:  Shipping Instructions**

**Table V:  Shipping Instructions for All Visit Types (Participant, QA & Courtesy)**

PML = Penn Medical Laboratory  
SFBR = Southwest Foundation for Biomedical Research

<table>
<thead>
<tr>
<th>Collection Tube Type</th>
<th>Tests</th>
<th>Sample Type</th>
<th>Storage/Shipping Requirement</th>
<th>Lab to Ship to:</th>
<th>Cryovial Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 10 ml SST</td>
<td>Chem Profile, Lipids, Insulin, CRP, FFA Storage</td>
<td>Serum</td>
<td>Frozen</td>
<td>PML</td>
<td>4 2 ml-red cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>Frozen</td>
<td>PML</td>
<td>8 2 ml-red cap vial</td>
</tr>
<tr>
<td>1 4.5 ml Lt blue or 2 2.7 ml Lt blue</td>
<td>Fibrinogen Storage</td>
<td>Na Citrate Plasma Na Citrate Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>1 2 ml-blue cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>1 2 ml-blue cap vial</td>
</tr>
<tr>
<td>1 4 ml gray</td>
<td>0 hour (fasting) glucose</td>
<td>NaFl Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>2 2 ml-black cap vial</td>
</tr>
<tr>
<td>3 10 ml Purple</td>
<td>HemoglobinA1c DNA Isolation Leptin EDTA Storage</td>
<td>Whole Blood Buffy Coat EDTA Plasma EDTA Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>3 2 ml-neutral cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buffy Coat EDTA Plasma EDTA Plasma</td>
<td>Frozen</td>
<td>SFBR</td>
<td>3 2 ml-orange cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA Plasma EDTA Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>2 2 ml-purple cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA Plasma EDTA Plasma</td>
<td>Frozen</td>
<td>PML</td>
<td>8 2 ml-purple cap vial</td>
</tr>
<tr>
<td>1 cup Random Urine</td>
<td>Albumin/Creatinine Storage</td>
<td>Urine</td>
<td>Frozen</td>
<td>PML</td>
<td>2 2 ml-yellow cap vial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Frozen</td>
<td>PML</td>
<td>4 2 ml-yellow cap vial</td>
</tr>
</tbody>
</table>
• **Supplies Required for Shipping**

• **Frozen Samples:**

  - Shipping Log Form
  - Polyfoam shipping containers with cardboard cartons
  - FedEx Shipping Labels
  - Biohazard bags
  - Dry Ice
  - Paper Towels for wrapping Storage Boxes
  - Newspaper or Styrofoam chips - for filling empty container space to prevent rattling
  - 3/4" Scotch Brand Filament Tape

**Note:** 20 lbs of dry ice gives some insurance against thawing if the package is delayed a few hours.

• **Preparation of Samples for Shipment to Penn Medical Lab:**

  ° Study laboratory requisitions stapled to extra unused labels for each set of samples must accompany each shipment.

  ° Each is printed on two-part carbonless form.

  ° Keep the last copy for your records and send the original with the samples. When your shipment is received, lab technicians at each laboratory will perform an inventory to be certain that all samples in the box correspond to those indicated on the shipping log. If the lab finds any discrepancies, they will call you to ask for your assistance in identifying extra samples or find lost samples.

• **Packing Shipping Containers**

  ° All samples are to be packed according to DOT regulations and in compliance with shipper’s requirements. This includes the following:

  ° Label the exterior of all shipping boxes according to the shipper's requirements. Boxes must have dry ice labels with the amount of dry ice marked on the label and orange-red labels with “Perishable” printed.

  ° Sort specimens to be sent to Penn Medical Lab or Southwest Foundation for Biomedical Research (See Table V above).

  ° Place approximately 20 pounds of dry ice at the bottom of the shipping box.

  ° Place packing material (i.e., chux, Styrofoam “peanuts” or newspaper) on top of dry ice.
• Place samples in biohazard bags with forms in pocket of bag on top of packing.

• Check all of the specimens in the box against the Shipping Log Form to be sure there are no transcription errors or missing specimens.

• Add more packing material if there is additional space so samples cannot bounce around the box while in shipment.

• Place "Class 9" (dry ice) labels on the outside of the cardboard shipping carton and record the amount enclosed.

• Place polyfoam lid on box.

• Close cardboard lids.

• With ¾” tape secure the cardboard lid closed.

• Prepare FedEx air bill.

• Samples will be shipped by priority air so that they arrive at the laboratory WITHIN 24 HOURS. ONLY SHIP SAMPLES MONDAY through WEDNESDAY.

• Retain a copy of the air bill as a receipt for tracking and auditing purposes.

• The day of shipment, fax (202-877-7342), call 202-877-5040 or email the laboratory to inform them that a package is being sent.

• Please give the following information:
  - Date samples will be shipped
  - The name of the person responsible for shipping the package and a phone number where the call can be returned if needed
  - Number of shipping boxes sent
  - FedEx tracking number
This information will allow the lab to track the package quickly if it does not arrive as planned.

- If you have any question regarding samples or shipment to Penn Medical Lab:
  
  Sophia Rushton-Reid: Phone: 202-877-8379  
  Fax: 202-877-7342  
  Email: Sophia rushton-reid@medstar.net  
  
  Shipping/Receiving Dept: Phone: 202-877-5040 or 202-877-5055  
  Technical Area: Phone: 202-877-5630

- If you have any question regarding samples or shipment to Southwest Foundation for Biomedical Research Lab:
  
  Shelly Cole: Phone: 210-258-9688  
  Fax: 210-670-3334  
  Email: scole@darwin.sfbr.org

- **Holiday Schedule:**

**Penn Medical Laboratory is closed on the following holidays:**

<table>
<thead>
<tr>
<th>Holiday</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>President’s Day</td>
<td>February 20, 2006</td>
<td>February 19, 2007</td>
<td>February 18, 2008</td>
<td>February 16, 2009</td>
<td>February 15, 2010</td>
</tr>
</tbody>
</table>
SFBR Laboratories are closed on the following holidays:

<table>
<thead>
<tr>
<th>Holiday</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiesta Friday</td>
<td>April 28, 2006</td>
<td>April 27, 2007</td>
<td>April 25, 2008</td>
<td>April 24, 2009</td>
<td>April 23, 2010</td>
</tr>
</tbody>
</table>

The following recommendations are made regarding maintenance and operation of the One-Touch Sure Step Flexx meter in order to ensure accurate readings of blood glucose.

There are 2 types of One Touch equipment (for Home use and for Hospital use). Hospital equipment requires daily QC checks, and this is also required by SHS. Please record the QC in your daily log. Be sure to use only Hospital products including the hospital test strips.

Excerpts taken from Sure Step Flexx meter operator’s guide:

General Care

To keep the meter in good operating condition, you must keep it clean and handle it with care. Follow these simple rules:

- Keep the test strip holder and lens area clean.
- Keep the meter dry and avoid exposing it to extremes in temperature and humidity.
- Do not take apart the meter.
- If you drop the meter, inspect it for obvious damage.
- Perform a quality control test prior to
- Running a patient blood glucose test.

Cleaning

When to Clean the Meter

- If dirt, blood, or lint is present.
- When an error message appears and the troubleshooting solution indicates cleaning the meter.
- As defined by your institution’s infection control policies.

Cleaning the Outside of the Meter

Clean the outside of the meter with a cloth dampened with a 10% bleach solution. Follow with a cloth moistened with water to remove residual bleach. Dry the meter thoroughly. Refer to “Cleaning Agents” on page 100 for other solutions that can be used to clean the outside of the meter.

▲ CAUTION: Do not get water inside the meter. Never immerse the meter or hold it under running water because it will damage the meter.

Cleaning the Test Strip Holder and Lens

To clean the test strip holder (cover and base), lens area, and contact points, use a 10% bleach solution followed by water. Dry thoroughly.

▲ CAUTION: Do not use alcohol, glass cleaners, or any cleansers containing abrasives, phenol, or ammonia to clean the test strip holder or lens area because it will damage the meter parts.
1 Press down on the left side of the test strip holder. This releases the holder, allowing you to slide it from the meter.

2 Wipe the test strip holder cover and base with a cotton swab or soft cloth dampened with a 10% bleach solution. Be sure to thoroughly wipe the gray area on the inside cover. Clean both sides of the base. Follow with a cotton swab dampened with water to remove residual bleach.

▲ CAUTION: Bleach residue left on the test strip holder may lead to an error message or an inaccurate, high result.

3 Dry the test strip holder with a soft cloth or lint-free tissue. Close the holder and set it aside.

4 Using a cotton swab or soft cloth dampened with a 10% bleach solution, wipe the lens area and contact points. Wipe this area even if it doesn’t appear to be dirty. Use a cloth moistened with water to remove residual bleach.

Be careful not to scratch the lens area or get water inside the meter, contact points, lens area

5 Dry the lens area gently with a clean, soft, lint-free cloth or tissue. Remove any lint.

6 Slide the closed test strip holder into the meter. Push the holder until it clicks into place.

7 Turn on the meter. If an error appears, check to make sure you installed the test strip holder correctly.

8 If necessary, wipe the scanner lens with a soft cloth dampened with water.

9 Perform a quality control test to verify that the result is within the expected range.

Changing the Batteries
The meter is powered by three size AA alkaline batteries. The LOW BATTERY message appears when the battery power is too low to perform a test. Change the batteries when the message appears. You may want to change the batteries if the battery bar on the status screen is low. Although the meter will continue to accurately perform glucose tests until the LOW BATTERY message appears, barcode scanning and data transfer may be affected before the message appears.

▲ CAUTION: Do not use rechargeable batteries.

■ NOTE: Neither the LOW BATTERY message nor the removal of batteries affects the test results stored in meter memory.

1 Turn off the meter.

2 Turn the meter over. Press down on the battery door latch and lift open the door.

3 Remove the batteries from the battery compartment and dispose of them according to your institution’s guidelines.

4 Insert three new size AA batteries, matching the positive + end of each battery with the + signs inside the battery compartment.

■ NOTE: The battery life depends on how quickly the meter is powered off after each test. Under optimal conditions—using the barcode scanner to enter information and turning off the meter immediately after test results are displayed and recorded—you can expect approximately 1000 tests.

5 Replace the battery compartment door.

6 Turn on the meter to verify power.

Adjusting the Screen Contrast
You can increase or decrease the meter’s LCD screen contrast.

1 Select Setup from the Main Menu.

2 Select LCD Contrast from the Setup Menu.

The options available at the Setup Menu are different for stand-alone meters and meters configured by a DataLink workstation.

3 Press the arrows to increase or decrease the LCD contrast.
A change occurs each time you press the arrow. The gauge within the bar gives you an approximate indication of the current setting.

4 When you are satisfied with the setting, press OK to save the setting and return to the Setup Menu, OR, press the back or menu key to ignore any changes and return to the Setup Menu.

Setting the Date and Time
You can set the date and time for meters operating in stand-alone mode.

1 Select Setup from the Main Menu.
2 Select Date/Time Entry from the Setup Menu.
   The Date/Time Entry option is available for stand-alone meters only.
3 Enter the time using the 24-hour format (hours:minutes). Press OK to save the setting and advance to the date screen.
   To exit the screen without changing the time, press back or menu key
4 Enter the date using the mm/dd/yy (month/day/year) format. Press Ok to save the setting and return to the Setup Menu.
   To exit the screen without changing the date, press back or menu.

Performing a Patient Test
Setting Up the Meter
1 Press the power button to turn on the meter.
2 The status screen appears. Press Cont to continue.
3 Select Patient Test from the Main Menu.
4 Enter your operator ID and press OK.
   If the meter is equipped with the optional barcode scanner, you may scan the barcode on your ID badge.
5 Enter the patient’s ID and press OK.
   If the meter is equipped with the optional barcode scanner, you may scan the patient’s barcode.
   ▲ NOTE: Carefully enter the ID. An accurate patient ID is imperative for transferring the correct results to the medical record.
   ❑ Operator ID required for all tests
   ❑ Maintain ID for ___ min. after power off
   If your ID appears at the operator ID screen, press OK to confirm it is correct. If the ID is not correct or does not appear, enter your ID.
   ❑ Patient ID required
6 Select the test strip lot number (and code) from the list displayed.
   The last test strip lot number selected appears at the top of the list. Use the arrow(s) to scroll through the list, if necessary. If the meter is equipped with the optional barcode scanner, you may scan the barcode on the test strip bottle label.
   ▲ CAUTION: To obtain accurate results, you must enter the correct test strip lot number (and code) for each new test.

The Patient Test screen appears with messages prompting you to apply blood to the strip and insert the strip.
   ❑ New reagent entry at workstation only
   The meter may be set to allow you to enter new reagent information. If the test strip lot number does not appear in the list, press the Entr Lot# button and enter the test strip lot number, control code, and control ranges.

Applying Blood Sample to the Test Strip
The puncture site must be cleaned and thoroughly dried before obtaining the sample. Thus, the participant will be asked to wash his/her hands with soap and water and dry them. Then the staff will apply alcohol to the puncture site and wait for the alcohol to dry. A drop of capillary blood will be obtained by puncturing the finger using a lancing device. Follow your institution’s policy and procedure guidelines for blood collection.

7 Apply the blood to the center of the pink test square as follows:

**Finger Stick**

Carefully touch the center of the pink test square to the drop of blood on the patient’s finger. The test area will quickly absorb the blood.

8 Check the white pad on the front of the test strip and the confirmation dot on the back of the test strip.

- If the white pad becomes completely saturated, you have applied too much blood for an accurate result. Repeat the application with a new test strip.
- The confirmation dot should be completely blue. If white patches or streaks are visible, you have not applied enough blood for an accurate result. Repeat the application with a new test strip.

▲ CAUTION: Do not apply additional blood to the test strip or you may get an inaccurate result.

If white patches or streaks continue to appear after you have repeated the test and used a larger volume of blood, call the LifeScan Healthcare Professional Line at 1 800 524-7226.

**Inserting the Test Strip**

9 Firmly insert the test strip all the way into the test strip holder until it stops (the confirmation dot should be facing down).

▲ CAUTION: If you fail to completely insert the test strip, the test may start; however, you may receive an inaccurate, low result.

▲ CAUTION: You have up to **2 minutes** to insert the test strip after the blood is applied. If you insert the test strip after 2 minutes, you may get an inaccurate result or an error message. Discard the test strip and repeat the test with a new test strip.

**Patient Results**

The patient test result appears in approximately 30 seconds.

◆ IMPORTANT: Do not remove the test strip until the countdown is complete.

If an error message appears, refer to Chapter 7, Troubleshooting, for information.

A glucose range may be defined by your system administrator. Results that fall above or below the limits of this range appear as follows:

- **CRITICAL HIGH** indicates a result above the range.
- **CRITICAL LOW** indicates a result below the range.
- **HIGH** indicates a result greater than 500 mg/dL.

◆ IMPORTANT: When the result is greater than 500 mg/dL and proper procedures are followed, the meter will display HIGH in virtually all cases. Additionally, the confirmation dot will be darker than the 350-mg/dL sample color dot on the test strip bottle Color Chart. This indicates hyperglycemia. Follow your institution’s policies for treatment.

10 Remove the test strip and dispose of it according to your institution’s policies and procedures.

11 You may press **Entr Note** and choose 1 to 3 comments that correspond to the patient’s current situation. Press OK, Or, press menu and continue testing.
■ NOTE: Results tagged with the comment “Procedure Err” are not included in any patient reports.

If you have problems using the meter to test patient samples, or if the meter malfunctions, call the LifeScan Healthcare Professional Line at 1 800 524-7226.

☐ Require message entry for patient tests outside the critical limits. You may be required to select a comment when a patient test result falls outside the limits of the range.

Record the glucose reading (from the Sure Step Flexx screen) on form S7, Sample Collection Checklist, item #1 (Fasting One-Touch Sure Step Flexx glucose result.)

Complete the rest of the sample collection checklist (form S7)
APPENDIX B-1A
Flow Charts Summarizing Processing Procedures

PARTICIPANT VISIT

Draw Tubes in this order: 

- Chem Profile, Lipids, Insulin, CRP, Fibrinogen
- Fasting Glucose
- A1c, Leptin, DNA

Spin at 3000 rpm (1000 x G) for 10 minutes, place ~1 ml into each 2 ml Cryovial.

Place on ice

Check to see that the caps and labels are secure. Store all samples at -70°C.

See shipping instructions in Lab Manual.

March 26th 2006
Penn Medical Lab
SHS V
**APPENDIX B-1B**

**Flow Charts Summarizing Processing Procedures**

**QA VISIT**

---

**Strong Heart Study V**

**SHS Processing Procedures**

---

**Draw Tubes in this order > > >**

- **Chem Profile, Lipids, Insulin, CRP**, 10 ml SST
- **OR**, 10 ml SST

---

**Spin at 3000 rpm (1000 x G) for 10 minutes.**

- **Place on ice**
- **Spin**

---

**Check to see that the caps and labels are secure. Store all samples at -70 C.**

**See shipping instructions in Lab Manual.**

---

March 26th 2006

Pen Med Lab

SHS V
APPENDIX B-1C
Flow Chart Summarizing Processing Procedures

COURTESY VISIT

**Draw Tubes in this order:**

- Chem Profile, Lipids, Insulin, CRP,
- Fibrinogen
- Fasting Glucose
- A1c, Leptin, DNA

**Place on ice**

Spin at 3000 rpm (1000 x G) for 10 minutes. Place ~1 ml into each 2 ml Cryovial.

**Urine Tests**
Mix gently before transferring to Cryovials. Do NOT spin!!

Never overfill Cryovials. Leave small dead space filled with air.

Check to see that the caps and labels are secure. Store all samples at -70°C. See shipping instructions in Lab Manual.
APPENDIX B-2

Strong Heart Study DNA and Sample Storage Policy and Procedures
Presented by the SHS Ethics Committee
and adopted by the
SHS Steering Committee
New York City
February 14, 2002

1. Objectives

Penn Medical Laboratory (Washington, DC) is the custodian for plasma, serum, and urine samples of participants in all phases of the Strong Heart Study. Southwest Foundation for Biomedical Research (San Antonio, Texas) is the custodian for DNA samples. Henceforth the term “PML/SWF” will refer to the respective laboratories with regard to either blood or urine derived samples (Penn Medical Laboratory) or DNA samples (Southwest Foundation for Biomedical Research). PML/SWF are charged with inventory and safe storage of these samples under optimal conditions to insure stability of analytes. PML/SWF cannot release these samples unless directed by the Strong Heart Study Steering Committee and under current guidelines of the Indian Health Service, National Heart Lung and Blood Institute and all relevant Institutional Review Boards (Human Use). Samples can be released to foster specific meritorious and ethical research of cardiovascular disease and pulmonary disease and their risk factors as outlined in the Strong Heart Study consent forms. The specific use is subject to scientific review of the Strong Heart Study Steering Committee and the NHLBI. Released samples can only be used for the approved measurements by the designated investigator, and unused samples are to be returned in good condition to the PML/SWF with documented history of the uses of each sample including a log of freeze thaw cycles. Consistent with SHS consent forms, the samples will not be used for profit, patenting and or commercial purposes, and cells will not be kept growing and will not be cloned.

Policies and procedures described in this document are designed to:

- Release authorized samples only after appropriate review as laid out in Section 4 of this document.
- Release samples after receipt of the signed *Strong Heart Study Sample Use Agreement* (Appendix B3 below).
- Insure sample integrity by keeping the samples in appropriate storage conditions and documenting the history of those storage conditions.
- Insure that the samples are secure and safe from unauthorized use.
- Insure confidentiality of the sample donor in accordance with study guidelines.
- Maintain records of samples stored, removal, freeze thaw cycles, and their placement to insure efficient retrieval.
- Follow procedures to insure that samples are released appropriately and transferred under conditions, which insure sample integrity.
Maintain records indicating where, when and why samples were released.
Insure that disposal or destruction of samples is done in accordance both with good laboratory practice and the guidelines of the Strong Heart Study participants.

2. Sample Storage Conditions:

A. Buffy Coats, plasma, urine, serum, and DNA

Samples are stored in airtight, gasketed vials at -70 to -80°C (-20°C for DNA). Vials are filled leaving at least 0.5 cc airspace at the top of each vial. DNA is stored under conditions known to preserve integrity and quality of DNA (i.e. a non-frostless freezer). Vials are marked in indelible ink on freezer-safe labels with Strong Heart Study participant number, date of collection and PML/SWF Sequence number. The freezers are locked and the key is the responsibility of the laboratory supervisor.

B. Database, sample inventory

The laboratory maintains a computerized database containing the following data on each stored sample: date of receipt, condition on receipt, number of vials, approximate volumes of each sample, freezer location, sample type (DNA, buffy coat, serum, plasma, urine, etc.), release date, release destination, release purpose, return date, return volume, freeze thaw cycles logged, misc. notations. PML/SWF will maintain records of freezer temperatures. Temperatures are manually logged on all workdays by the technical staff and reviewed for drift. Periodic maintenance as recommended by the freezer manufacturer will be kept available for inspection. Records of freezer malfunction and maintenance will also be made available.

C. Damaged storage samples

Communication to the Strong Heart Study Steering Committee: At the request of the Steering Committee, PML/SWF will notify the Steering Committee of sample damage evidenced by thawing or breakage of samples. Computerized and paper logs of samples will include such events.

3. Disposal of Samples

Samples will be disposed at the direction of the Steering Committee by routine laboratory methods. Prior to this, a request will be made to appropriate tribes regarding culturally correct methods of disposal of damaged or non-usable samples and the laboratory will make a reasonable attempt to cooperate with those requests. Any procedures used for disposal of samples must be consistent with Good Laboratory Practice, and minimize biohazard contamination.

4. Release of samples
A. Procedures for acting on requests. Administrative pathway for release of samples:

Requests are presented in writing to the Strong Heart Study Steering Committee. Requests are judged by their scientific merit, potential benefit to the Indian Communities, and consistency with human use guidelines (as outlined in the signed consent) specific to the Strong Heart Study. Requests for Strong Heart Study samples must be specific. Strong Heart Study samples must not be used for additional measurements unless additional written approval is received from the Strong Heart Study Steering Committee. All uses must be consistent with the participant consent of the Strong Heart Study.

Request for Strong Heart Study samples must be made in writing to the Strong Heart Study Steering Committee and should justify the volume of sample requested and whether previously unused (never thawed) samples are necessary. Requests should be brief and generally follow guidelines used in scientific proposals:

- rationale,
- hypotheses,
- specific aims,
- background,
- methods and
- planned analyses.

Study participants and participating tribes will be notified by the Strong Heart Study Newsletter when new tests are done using stored specimens. The investigators will write articles in the newsletter describing what tests are being done and how they will increase understanding of CVD or pulmonary disease in American Indians. Scientific articles resulting from the laboratory studies of the stored specimens will be reviewed and approved by the SHS publications committee, all participating tribes, by NHBLI and by the Phoenix, Aberdeen and Oklahoma Area IRBs prior to publication.

This policy will not preclude obtaining explicit tribal and/or IRB approvals in the event that ancillary studies are proposed which would require re-contact of participants or other issues that would suggest consultation with appropriate IRBs or tribal governments.

B. Release instructions to PML/SWF:

Written requests to release samples (Request to Release Samples – Appendix B4 below) will be made by the Strong Heart Study Steering Committee after review of scientific merit and ethical considerations. The written request must confirm that all

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2 Scientific merit will include the originality of the research, value to the tribal communities and participants, and quality of the measurements proposed.
appropriate reviews have been made. Samples to be released must be identified by date or phase of collection, volume or number of vials to be released, shipping destination and contact person, and Strong Heart Study IDs.

PML/SWF will maintain records of requests for a period of 15 years. These records will be made available to the Strong Heart Study sponsor and tribal governments upon request.

C. Technical procedure for releasing samples

Samples are removed from storage only by PML/SWF employees who are trained in safe sample handling. Written logs of the samples requested are used to locate and remove samples. Each sample found is logged onto the table and these data are promptly transferred into the computer database. The removed samples and the list are reviewed by the PML/SWF technical supervisor. Discrepancies are logged and resolved. Samples requested which are not found are logged and investigated to insure consistency between the data base and sample inventory. See PML/SWF Sample Request Log – Appendix B5 below.

The sample shipment is coordinated with the receiving laboratory to insure safe receipt of the requested samples. The requesting laboratory must acknowledge the receipt and condition of the samples upon arrival. Any discrepancies between the number and amount of samples approved for use by the requesting laboratory and those received must be reported by the requesting laboratory within one month of receipt of the samples.
The release of Strong Heart Study samples is subject to the following policies and procedures. No samples will be released until the investigator agrees to the following policies and procedures approved by the Strong Heart Study Steering Committee:

1. Samples can be released to foster specific meritorious and ethical research as outlined in the Strong Heart Study consent forms. The specific use is subject to prior approved scientific review of the Strong Heart Study Steering Committee and the NHLBI. The laboratory releases samples only after written instructions are received from the Steering Committee.

2. Released samples can only be used for the approved measurements in the specified laboratory and unused samples are to be returned in good condition to PML/SWF with documented history of the uses of each sample including a log of freeze thaw cycles. The investigator must supply PML/SWF with the name, phone number, E-mail address and shipping address of the person responsible for receiving the samples.

3. The samples will be released for a period of ___ days ending on ____________(dd/mm/yyyy). At the termination of this period, the investigator must either return the samples to PML/SWF or request and receive permission from the Strong Heart Study Steering Committee for a specified extension to complete the analyses.

4. Samples must be returned to the PML/SWF with any remaining material at the completion of the approved use period as described above. Samples should be returned in their original containers with the original label. Samples are to be shipped under conditions specified by the Medical or Technical Director of the PML/SWF. Unused samples must not be discarded.

5. Data derived from the use of these samples is the joint property of the Strong Heart Study Steering Committee and the investigator. Publication of the results of these investigations is subject to the policies and prior approval of the Strong Heart Study Publications Committee, the NIH and the appropriate tribal councils.

6. The investigator acknowledges and abides by the informed consent document limiting use of these specimens for the study of cardiovascular and lung diseases and their risk factors and specimens will only be used for those purposes. The samples will not be used for profit, patenting and or commercial purposes, and cells will not be kept growing and will not be cloned.

I have read the Strong Heart Study Sample Storage policies and understand that the samples must be used only for uses approved in writing by the Strong Heart Study Steering Committee. I agree to abide by the limitations set forth in these policies.

_____________________________  _________________________
printed name                          date

_____________________________
signature

_____________________________
address

_____________________________
address

_____________________________
phone number

gity, state, zip

_____________________________
e-mail address
Date: ______________________

A request to the Penn Medical Laboratory is made to release the following samples (attach list or table if necessary):

Minimum volume needed for each sample: ____________ µL.

Type of sample:  □ plasma  □ serum  □ buffy coat  □ urine  □ other: ____________

OK to use previously thawed samples?  □ Yes  □ No

To:
name of investigator: ____________________________________________________________
shipping address: ______________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
phone contact: ________________________________________________________________
E-mail address: ________________________________________________________________

Purpose of the Request:

Steering Committee Chair__________________________________________________________
__________________________ signature ______________ date

When should the samples be returned to the Penn Medical Laboratory? ________________
__________________________ date

for PML lab use (attach log of sample request v. those actually sent):
samples pulled and shipped on: _________________ (mm/dd/yyyy)
technician ___________________________________ signature

supervisor ___________________________________ signature
**PML/SWF Sample Request Log**

Sample Request Tracking number: ________________________
Technician: ________________________ (pulling samples)
Technician: ________________________ (replacing samples)

<table>
<thead>
<tr>
<th>sample requested SHS ID, phase</th>
<th>found?</th>
<th>Sent (date)</th>
<th>Notes</th>
<th>returned date/volume</th>
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## APPENDIX C
### Strong Heart Family Study
#### SHS V

<table>
<thead>
<tr>
<th>Penn Medical Laboratory</th>
<th>Southwest Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>108 Irving Street, NW</td>
<td>Department of Genetics</td>
</tr>
<tr>
<td>Annex 2</td>
<td>7620 NW Loop 410</td>
</tr>
<tr>
<td>Washington, DC 20010</td>
<td>San Antonio, TX 28227-5301</td>
</tr>
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**PARTICIPANT SAMPLE FORM**

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<thead>
<tr>
<th>Exam No:</th>
<th>Collection Date: (mm/dd/yy)</th>
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<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Redraw:</th>
<th>Yes □ No □</th>
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<tbody>
<tr>
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<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Condition</th>
<th>Sample Type</th>
<th>Lab to Receive Samples</th>
<th>Transfer Vial Type (# &amp; color cap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chem Profile, Lipids, Insulin, CRP, FFA</td>
<td>Frozen</td>
<td>Serum</td>
<td>PML</td>
<td>12 2ml-red cap vial</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Frozen</td>
<td>NaCitr Plasma</td>
<td>PML</td>
<td>2 2ml-blue cap vial</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>Frozen</td>
<td>NaFl Plasma</td>
<td>PML</td>
<td>2 2ml-black cap vial</td>
</tr>
<tr>
<td>HemoglobinA1c</td>
<td>Frozen</td>
<td>Whole Blood</td>
<td>PML</td>
<td>3 2ml-neutral cap vial</td>
</tr>
<tr>
<td>Leptin</td>
<td>Frozen</td>
<td>EDTA Plasma</td>
<td>PML</td>
<td>10 2ml-purple cap vial</td>
</tr>
<tr>
<td>Urine Creatinine &amp; Microalbumin</td>
<td>Frozen</td>
<td>Urine</td>
<td>PML</td>
<td>6 2ml-yellow cap vial</td>
</tr>
<tr>
<td>DNA isolation</td>
<td>Frozen</td>
<td>Buffy Coat</td>
<td><strong>SFBR</strong></td>
<td>3 2ml-orange cap vial</td>
</tr>
</tbody>
</table>

**Site Comments:**

**Lab Comments:**

**Date and Time Samples received:**

**Processing Technician Initials:**

---

Strong Heart Study V 07/01/06  IV-C-1  PML/SWF Participant Sample Form