3.3.6. Forms

3.3.6.1. Purpose

The purpose of the blood collection forms is to facilitate the collection of plasma and serum samples from participants in CHS. This collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the process must have, built in, features which allow monitoring of phlebotomy and other quality assurance parameters as well. Note all forms are to be completed in ink.

3.3.6.2. Description

There are actually two forms associated with blood drawing. The first is the "CHS Participant Phlebotomy Questionnaire". This form contains questions which are important for participant safety; these questions should be asked immediately prior to phlebotomy, and deal with any propensity to bleed, faint, or have an improper reaction to the glucose tolerance test.

The second form is the "CHS Phlebotomy/Processing Form". This form is broken down into five subsections which deal with quality assurance and record keeping for : a) venipuncture; b) glucose challenge; c) blood drawing; d) centrifugation; and e) aliquotting.

These forms have the following purposes:

- assure the most efficient and safest possible venipuncture, blood drawing and glucose tolerance test for CHS participants;
- data gathered will allow us to monitor the quality of the above procedures;
- the "CHS Phlebotomy/Processing Form" will accompany the samples to Vermont, allowing us to more efficiently process the samples further;
- ultimately, these forms are critical to the interpretation of the assay results in the CHS cohort and in Case/Control studies.

3.4. Preparation for Specimen Collection

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

1. Check to make sure that blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.
2. Check that each vacutainer tube is properly labeled with the appropriate participant number and number 1-7 in order of draw. A sheet of numbered labels will be provided for each

participant.
3. Check that the sample processing station is properly equipped. Every item on the checklist must be ready and in its proper position.
4. Check that each sample aliquot tube is labeled with its appropriate participant identification number and in its proper rack.
5. Check that the Participant Questionnaire and Phlebotomy/Processing Forms are labeled and included with the blood collection tray.
6. Perform quality control (Q.C.) check on Refrigerator temperature (Refrigerator Temperature Log).
7. Perform Q.C. check on freezer temperature (Freezer Temperature Log).
8. Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, wash cloths, and that blood mixer is functional.

Approximately 10 minutes before scheduled participant arrival:

1. Fill styrofoam ice bath 3/4 full with crushed ice.

At Participant arrival:

Check that the ID number on the tubes matches the participant ID.

3.5. Venipuncture

3.5.1.Precautions for Handling Blood Specimens

<u>All specimens must be handled as potentially infectious for laboratory workers.</u> Transmissions of the infectious agents including those associated with hepatitis and the acquired immunodeficiency syndrome (AIDS) via "needlestick" skin punctures have been documented.

Disposable plastic gloves and lab coats are worn when collecting and processing specimens. Hands are washed thoroughly with disinfectant soap prior to leaving the work area. Skin cuts or abrasions should be covered.

If the phlebotomist accidentally sustains a needle stick during or after phlebotomy, the wound is immediately cleansed with soap and water. The CHS physician is notified to order the analysis of the participant's serum for possible hepatitis. Needles are also stored in a locked cabinet when the clinic is closed.

A solution of 0.1% sodium hypochlorite (household bleach) is used to clean up any spills of blood, plasma, or serum and all laboratory work surfaces at the completion of work activities.

All needles and tubing are disposed of in puncture-resistant containers for safe disposal.

No pipetting by mouth is allowed; especially of any blood, plasma, or serum.

Careful pipetting and centrifugation will avoid the formation of potentially infectious aerosols.

All used vacutainer tubes and blood products are to be placed in biohazard containers for proper disposal.

3.5.2. Phlebotomy Room

The blood drawing takes place in an isolated room or participants are separated by room dividers. The room is equipped with all of the necessary blood drawing supplies. A separate counter or work table is equipped with all of the materials and vials that are used in the blood handling and processing. The centrifuge, refrigerator, and freezer should be nearby.

3.5.3. Participant Preparation

Informed consent must be obtained by the receptionist (see CHS Manual) and confirmed by the phlebotomy technologist before drawing blood. This procedure is followed to ensure that the subjects understand the purpose of blood drawing and the possible complications of venipuncture. A standard informed consent has been prepared for this study. With regard to laboratory procedures, the consent statement informs study subjects that there is a small risk of bruising at the spot on the arm where the blood is taken, and that about four tablespoons of blood are drawn. The consent statement also informs study subjects that they will be contacted if clinically important test results are abnormal.

3.5.4. CHS Participant Phlebotomy Questionnaire

Following the questionnaire format, each subject is asked whether he/she has a bleeding disorder before the blood is drawn (Ques. 1). If he/she has had any problems with excessive bleeding or bruising at a
* venipuncture site, the participant should be sampled only if approved by
* a clinic supervisor, clinic physician, or P.I.

There is no action to take if the participant has been told they have a coagulation disorder (Ques.2).

If the participant has experienced fainting spells during phlebotomy
* (Ques. 3), ask the participant the frequency of fainting spells. If the

* participant has fainted only once before, proceed with phlebotomy. If
* the participant frequently faints, consult the clinic supervisor, clinic
* physician, or P.I. before attempting the venipuncture.
Provide smelling salts, basin, cold cloth if needed.

If the participant does NOT have diabetes (Ques. 4), proceed to Question 5. If yes or don't know to Question 4, this participant does NOT receive GTT.

* If the participant is NOT fasting (Ques. 5), they do NOT receive GTT.
* Minimum fasting time required for testing is 8 hours. This person will
* be encouraged to reschedule when they are fasting. Upon rescheduling,
* the phlebotomist will redraw <u>all</u> CHS blood samples.

3.5.5. General

Blood drawing is standardized for the sitting position.

The venipuncture is performed with a 21 gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small, thin walled needle which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, he/she may be reassured to know such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with the technician who draws the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure, e.g., "I am going to be drawing about 4 tablespoons of blood. This blood will be used in tests for lipids and cholesterol and blood clotting factors. We hope to be able to use the results of these tests to predict who might have a greater risk of heart attacks".

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPREHENSIVE ABOUT HAVING BLOOD DRAWN. <u>Do not under any circumstances force the participant to have blood drawn</u>. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood. If the participant has "good veins" the phlebotomist can reassuringly say, "Oh, you have good veins; there should be no problem." Elderly patients are often aware of the difficulty they pose to phlebotomists and should receive extra consideration and detailed explanations as required.

*

3.5.6. Venipuncture Procedure

1. Arrange draw tubes in order of draw on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.
2. Apply tourniquet.
3. Examine participant's arms for the best site for venipuncture. Release tourniquet.
4. Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion form center to periphery. Allow area to dry.
5. Reapply tourniquet and start timer.
6. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.
7. With the needle bevel upward, enter the vein in a smooth continuous motion.
8. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.
9. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
10. Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly.
11. Remove the tourniquet at 2 minutes. Note the time on the P/P Form. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle.
12. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube (especially tubes #1 & 6). This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.
13. Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach another without removing the needle from vein. As each tube is filled, mix by gently inverting before placing tube on the mixer. Note: do NOT mix tubes #5 & 7. (See section Blood Mixing during Venipuncture).
14. When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted (if necessary).

15. Average venipuncture time is 3-6 minutes, but any difficulties may increase this time to 10 or 15 minutes.

3.5.7. Procedures for Difficult Draw

If a blood sample is not forthcoming, the following manipulations may be helpful.

1. If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
4. The phlebotomist should not attempt a venipuncture more than twice.
5. Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
6. If venipuncture is unsuccessful, CHS participant must be rescheduled at a later date with a different Field Center phlebotomist.

* 3.5.8. Syringe Method for Venipuncture
*
* This section outlines a syringe method of blood collection which should
* be used only under the following circumstances:
* a. Vein has collapsed during the routinge nethod of collection.
* b. Use of the syringe method would not cause undue discomfort/alarm
* to the CHS participant.
* The butterfly apparatus can be adapted to accommodate a syringe for
* collection of the remaining blood specimen in the following manner:
* 1. Pinch the butterfly tubing with your fingers and remove the Luer
* adaptor and vacutainer holder from the butterfly apparatus without
* pulling on the needle in the participant's arm.
* 2. Carefully insert and secure a 10 or 20 cc plastic syringe into the
* butterfly apparatus. The syringe plunger may be pulled prior to
* insertion onto the butterfly to loosen plunger.
* 3. Release butterfly tubing and pull on syringe plunger to allow blood
* flow into the syringe.
* 4. It may be necessary to draw more than one syringe to complete the
* collection of blood. To change syringes, pinch butterly tubing,
* remove filled syringe, and insert new syringe.
* 5. When blood collection is completed, remove butterfly needle from
* the participant's arm. Immediately transfer the contents of the

* syringe(s) to blood collection tubes placed upright in a test tube
* rack. For transfer, place a needle onto the syringe and carefully
* insert the needle through the cap into the blood collection tube.
* Gentle tapping of the syringe will draw air bubbles to the top and
* avoid a short fill. Be careful not to contaminate the needle with
* liquid/powder anticoagulant in the collection tube. Contamination
* with anticoagulant is possible if tubes were not upright during
* collection. In order to avoid contamination, it may be necessary
* to change needles prior to filling each tube type. After filling,
* the tubes are placed in their proper locations on ice or room
* temperature.
*
* It would be advisable to have another person working with the technician
* to fill the collection tubes as the phlebotomist draws the syringes.
*

3.5.9. Removing the Needle

To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into needle box.
Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.
Remove tubes from the blood mixer and place in ice (#1,3,4) and at room temperature (#2,6). Tube #5 should already be in the rack at room temperature.

3.5.10. Blood Mixing During Venipuncture

Each tube should be treated as follows:

#1 EDTA	on mixer for ~30 seconds	place in ice bath
#2 Citrate	on mixer for ~30 seconds	place in rack at R. T.
#3 Special	on mixer for ~30 seconds	place in ice bath
#4 Citrate	on mixer for ~30 seconds	place in ice bath
#5 Serum	do NOT mix	place in rack at Room Temperature
#6 EDTA	on mixer for ~30 seconds	place in rack at R. T.
#7 Serum	do NOT mix	place in rack at Room Temperature

3.5.11. Bandaging the arm.

1. Under normal conditions:
   a. Slip the gauze pad down over the site, continuing mild pressure.
   b. Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
   c. Tell the patient to leave the bandage on for at least 15 minutes.
2. If the patient continues to bleed:
   a. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
   b. Wrap a gauze bandage tightly around the arm over the pad.
   c. Tell the patient to leave the bandage on for at least 15 minutes.

3.5.12. Precautions

PRECAUTIONS WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE BLOOD DRAWING.

1. Have the person remain in the chair, if necessary have him/her sit with head between knees.
2. Provide the person with a basin if he/she feels nauseous.
3. Have the person stay reclined until the color returns and he/she feels better.
4. Place a cold wet cloth on the back of the person's neck.
5. If the person faints, use smelling salts to revive by crushing the ampule and waving it under the person's nose for a few seconds.
6. If the person continues to feel sick, contact a medical staff member who will advise you on further action.

3.5.13. Glucose Challenge

If the participant is eligible (see criteria for inelgibility below), he/she will be given the glucose to drink at this time. Administration of the GTT will be monitored by someone other than the phlebotomist.

Criteria for GTT ineligibility:

Insulin dependent diabetics will not receive GTT but will be scheduled early on for a snack.

Non-fasting participants will not receive GTT although they will
* have initial bloods drawn. They will be encouraged to return for a
* redraw of all bloods when they are fasting.
* If less than a half dose of the glucose is ingested, no GTT blood draw will be done.

The expected time of the two-hour draw is noted on the
* Phlebotomy/Processing Form and on the computer. Note that this time is
* two hours from the time the participant starts the drink. The
* participant is allowed 10 minutes to finish their drink before moving to
* their next station. Make a note of consumption time (start & finish) on
* the Phlebotomy/Processing Form. The two-hour blood sample must be drawn
* within plus or minus 10 minutes of the expected two-hour time.

3.5.14. Completing the Blood Drawing Procedure

Dispose of needle and tubing in biohazard containers. (It may be necessary to use hemostats to remove tubing from vacutainer holders).
Complete the first page of the Phlebotomy/Processing Form
Clean up the venipuncture area (if necessary)
Bring blood collection tray to the processing area with the filled vacutainer tubes.

3.6. Blood Collection Tray Checklist

Per Tray:

10	21G Butterfly needles with Luer Adaptors
10	Alcohol Swabs
15	Bandaids
15	Gauze pads
5	Vacutainer holders
2	Tourniquets
1	smelling salts
1	Timer or stopwatch
2	pencils/pens
	Latex gloves
1	Hemostats
~10 min before draw:	
1	styrofoam ice bath filled with ice

* Optional:
* 10 CC plastic syringes
* 20 CC plastic syringes
* Needles for syringes
*

Per participant:

1	Blood tube rack with 7 draw tubes (labeled and numbered)
1	CHS Participant Phlebotomy Questionnaire
1	CHS Phlebotomy/Processing Form

Have available at the Phlebotomy Station:

Basin
Cold cloth
Tube mixer
Biohazard containers
Needle/Sharps container
Paper towels

8. Form Definitions

8.1. CHS Phlebotomy/Processing Form

1. Clots in tubes:
   Solid clumps of red cells seen in anticoagulated tubes after spinning.

2. Excessive duration of draw:
   Blood collection time exceeds five minutes

3. Hematoma:
   Bruising of skin in area of venipuncture

4. Hemolyzed:
   Serum or plasma sample has a pink or reddish appearance instead of a light yellow color after centrifugation.

5. Icteric:
   Serum or plasma sample has a dark yellow or brownish appearance instead of a light yellow color after centrifugation.

6. Leakage at venipuncture site:
   Blood escaping from needle site during phlebotomy.

7. Lipemic:
   Sample appears milky white or cloudy after centrifugation.

8. Platelet stabilizer:
   Solution provided by CBAL for clear Eppendorf aliquot

9. Supine:
   Participant is lying on a flat surface.

10. Vein collapse:
    Sudden cessation of blood flow during venipuncture. Note, however, that at times a needle will adhere to the vessel wall giving the same effect. A slight movement will start flow again if this is the case.

8.2. CHS Participant Phlebotomy Questionnaire

1. Bleeding disorder:
   An abnormality of the clotting mechanism. Participant may experience excessive bleeding at the site of venipuncture.

2. Fainting spells:
   Participant may experience light headedness or loss of consciousness during or following a venipuncture.

3. Fasting:
   Participant has not has anything to eat or drink (except water) for 12 hours prior to phlebotomy.

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4. Diabetes:
   Disorder of glucose (blood sugar) metabolism resulting in elevated blood glucose levels.