

SECTION 11

BLOOD AND URINE COLLECTION, PROCESSING AND SHIPMENT

INTRODUCTION

A fasting blood sample is collected from all participants in the Women's Health Initiative at the first screening visit. All participants in the Clinical Trial (CT) also provide blood samples at their first annual visit and a subsample of participants provide blood samples at three years and every three years thereafter. All Observational Study (OS) participants provide blood samples at their three-year visit.

First-morning urine specimens are collected from all participants recruited at the three designated Clinical Centers (CCs) participating in the bone density substudy (University of Alabama, University of Arizona, and University of Pittsburgh). Participants provide a urine sample during screening, at the first annual visit, the three year visit, and every three years thereafter. (See *Vol. 1, Table 1-A1.1 - Frequency of Data Collection* for schedules of blood and urine collections.)

The CCs send specified serum samples to their local laboratory for specified analyses. The remaining serum, plasma, RBCs, buffy coat, and urine samples are shipped to the central repository, McKesson BioServices in Rockville, MD. McKesson BioServices ships selected samples to the central lab, and other selected labs, for analyses. This section describes procedures for collecting, processing, storing, and shipping blood and urine samples to McKesson BioServices.

11.1 General Guidelines

11.1.1 Safety Procedures - Precautions for Handling Blood

The WHI has adopted the Universal Precautions for blood collection, processing and shipping. These precautions are intended as optimal "guidelines." These do not apply to urine unless there is visible blood in the urine. The term *Universal Precautions* refers to an approach to infectious disease control which assumes that every direct contact with body fluids is infectious. Also refer to the Occupational Safety and Health Administration (OSHA) guidelines which should be available from your institution for handling blood specimens.

Follow all local requirements for handling and disposing of blood and materials exposed to blood. Check with your institution's Health and Safety office for any additional requirements as there may be some local variation at each CC, which may be stricter or slightly less strict than those listed below.

The following are guidelines for all blood collection and processing procedures:

- Handle all blood specimens as potentially infectious material. Transmission of the infectious agents associated with hepatitis and acquired immunodeficiency syndrome (AIDS) via "needle stick" skin punctures have been documented.
- Wear disposable plastic latex gloves when collecting and processing specimens. When drawing blood, change gloves between participants.
- Wear protective eye goggles, a face mask, a full face shield, or work behind a barrier shield at all times when processing blood. Regular glasses without splatter shields are not sufficient.
- Encourage CC staff having direct contact with blood specimens to get a Hepatitis B vaccination. Your local institution may provide Hepatitis B vaccinations for personnel handling blood specimens. The Hepatitis B vaccine provides immunity against Hepatitis B, an infectious, blood-borne disease causing inflammation of the liver with possibly serious or fatal consequences. The vaccination series involves three separate doses over six months. This protection is believed to last five to seven years at which time a booster dose may be needed.
- Wash your hands with soap and water:
 - Immediately after contact with blood or other infectious materials (even if you wore gloves).
 - Before and after using restroom facilities.
 - After you take off your gloves or other protective clothing.
 - Before and after each participant contact.
 - When you leave the work area where blood or other infectious materials are present.
- Do not use gloves if they are peeling, cracked or discolored, or if they have punctures, tears or other evidence of deterioration. Cover skin cuts or abrasions with a Band-Aid underneath the glove.
- Anyone performing a process where there is a potential for splashing of blood (e.g., the processing of blood) should wear a long-sleeved, buttoned-up, fluid-resistant lab coat.
- Use disposable lab coats, if available. Place disposable protective equipment in a labeled infectious waste container for disposal.
- Remove the disposable/non-disposable lab coat when leaving the blood draw area. The lab coat worn in the blood draw area should not be worn in other areas of the clinic.
- Tie back any long hair.
- If you accidentally sustain a contaminated (or used) needle stick, thoroughly cleanse the wound with soap and water. Notify the CC physician to order an analysis of the participant's serum for possible hepatitis

or HIV antibodies. Complete a local accident report, as needed, and follow all appropriate OSHA guidelines as dictated by your institution.

- Do not manipulate needles, scalpel blades, or other contaminated sharp objects by hand. Do not bend, break or remove needles from disposable syringes by hand. Store unused needles in a secure cabinet when the CC is closed.
- Do not recap or re-sheath needles or sharp instruments unless absolutely necessary. If you need to recap a needle, cover it immediately after use. To recap the needle, use a cap holder designed for this purpose (placed in close proximity); or use a hemostat. Do not use your hand to recap the needle since this is the most common cause of sustaining a contaminated needle stick.
- Use 0.1% sodium hypochlorite (10% household bleach) to clean up any spills of blood, serum or urine. Use this solution on all work surfaces at the end of each day. Dilute one part household bleach with nine parts water to get a 0.1% sodium hypochlorite solution. Apply with a paper towel. Do not use an aerosol spray to apply the solution.
- Deposit all used needles, blood collection tubes, transfer pipettes and pipette tips in puncture-resistant containers for safe disposal.
- Place all other used blood processing supplies and blood products in biohazard bags for disposal. Also dispose of other materials exposed to blood in biohazard bags.
- Never perform any pipetting by mouth, especially if any blood or serum is involved.
- Avoid formation of potentially infectious aerosols by carefully removing stoppers from the vacutainer tubes and by careful pipetting and centrifugation.
- Never allow food or drink in the blood drawing or processing rooms. Do not store food or drink in the same refrigerator or freezer as blood samples.
- Do not eat, drink, apply cosmetics or lip balm, or handle contact lenses in work areas where blood or potentially infectious materials are present.
- Assume all laundry to be contaminated. Wear protective gloves when handling laundry. Bag laundry at the location where it was used. Double bag if soaking through is likely.
- Label all containers of infectious waste (i.e., biohazardous and medical waste), refrigerators and freezers containing blood or other potentially infectious materials with the biohazard symbol. The biohazard symbol must be black on an orange background.
- Transport all specimens or containers of blood and other potentially infectious materials in a secondary container (e.g., plastic bag or other container having a liquid-tight seal).

11.1.2 Training and Certification (Required)

A CC staff person may draw, process and ship the blood and urine specimens after completing the WHI training and certification process. Such a staff person must become familiar with *Section 11 - Blood and Urine Collection, Processing and Shipment* and should also be familiar with local or state certification requirements. Keep a copy of any local and state requirements relating to the collection and processing of blood and urine products on file at the CC.

Because blood collection may involve a small amount of pain for the participant, it is important that phlebotomists review the techniques involved in the collection process. Phlebotomists should be highly experienced with vacutainer and butterfly blood collections, prepared to handle common problems such as fainting, and familiar with precautions to avoid exposing themselves to blood. Ideally they will be CPR certified. They should: 1) read "Collection and Handling of Laboratory Specimens: A Practical Guide" or similar phlebotomy manual; 2) wear clean white lab coats (with no blood stains) and appear generally neat and tidy; 3) wear name tags and introduce themselves to the participants before the blood draw; and 4) not chew gum or have any food items in their mouths during blood draws.

Because the study depends on the voluntary return of participants over an extended period, the phlebotomist should make every effort to make the entire procedure as easy and painless as possible. The phlebotomist should remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is to be thoroughly knowledgeable about all aspects of the procedures. This includes knowledge about the participant contact, the handling of each tube and the purpose of each sample.

11.1.3 Facilities

Phlebotomy Room

Perform blood draws in a private area such as an isolated room (blood drawing room) or in an area separated by room dividers. Equip the room with all the necessary blood drawing supplies. Use a counter or work table for all of the blood handling equipment and supplies. The blood drawing room should be clean and tidy with no obvious evidence of a previous blood draw such as used needles, blood stains, etc. A phlebotomy chair should be available for 15 to 20 minute periods to allow participants to be seated for a few minutes before the blood draw. Ideally, only the participant and phlebotomist should be in the room during the procedure.

Blood Processing Room

Equip the blood processing room with a refrigerated centrifuge, a small refrigerator and sufficient counter space for the processing of blood specimens. A -70°C freezer should be in or near the room. The room must have a sink and running water available. Use one counter area for processing the blood samples and another counter area for completing paperwork. Safety regulations state food and drink should not be kept in refrigerators or freezers in which you put the blood samples. Food should not be kept in the blood processing room even if it is not in the same refrigerator as the blood samples. Food should not be eaten in the blood processing room.

11.1.4 Equipment and Supplies (Required)

See *Section 2.3 - Equipment and Supplies* for lists of required and recommended blood collection, processing and shipment supplies. Orders for cryovials, labels and shipping supplies are filled by McKesson BioServices. Clinical Centers also have the option of ordering the three blood collection tubes from McKesson BioServices. Clinical Centers can order blood collection tubes using the Notification of WHI Shipment form. If your local or state laws require different or additional shipment supplies, contact McKesson BioServices to see if they can provide the supplies for you. When vendors change or update their catalogs, check the part numbers to be sure you are ordering the correct tubes. Some vendors have changed part numbers in the past. When receiving the royal blue-stoppered tubes, check to be sure that the "7 ml" is printed in **red** on the tube label, indicating a serum tube with no anticoagulant. A tube with green "7 ml" printed on the label indicates the tube contains heparin. Try to maintain at least a 2-month inventory of blood drawing and processing supplies. When purchasing blood collection tubes from a vendor and not ordering from McKesson, specify the expiration date so the vendor does not send you its older stock.

Calculate the quantities of disposable supplies needed based on your CC's projected workload. Allow sufficient time for the delivery of the required supplies from the vendors.

Local preferences, procedures and regulations may require a CC to have access to items that are not on the list.

11.2 Blood Collection

11.2.1 Blood Sample Labels (Required)

McKesson BioServices provides blood sample label sets with a unique 6-digit preprinted number and corresponding barcodes to use during the collection and processing of blood samples. The labels are specifically made for use in low temperature freezers. Clinical Centers use the labels from one label set to label the blood collection tubes, blood collection form, and all cryovials for one blood draw. The label on *Form 100 - Blood Collection and Processing* links the blood sample number to the participant ID and information about the blood draw such as date and time drawn.

Use one set of blood sample labels for each blood sample collected and recorded on *Form 100 - Blood Collection and Processing*. For example, one set of multiple blood sample tubes collected at one time from a participant have the same blood sample number. Blood samples collected from a participant at different times or different visits have unique blood sample numbers. Blood collected on different days or different times (> 1 hour apart) are considered different draws and require two separate Form 100s with different blood sample numbers.

Each set of blood sample labels contains the following labels, each printed with the unique 6-digit blood sample number:

- Two labels for *Form 100 - Blood Collection and Processing*, one for the front of the form and one for the back of the form. Each label also contains a barcode label corresponding to the 6-digit blood sample number and the words "Blood Form." The blood processor uses the back of the form for recording blood processing data; and the label on the back of the form assists the blood processor in properly identifying the blood sample during processing.
- Seven labels for the blood collection tubes, printed "Tube" on each of the seven labels.
- Thirteen labels for the 13 cryovials. (*Note:* Two cryovials [-01 and -15] were previously discontinued and one [-20] was added beginning 12-1-95.) Each of the thirteen labels contains the 6-digit blood sample number, a 1-letter check digit or a 2-digit cryovial number, and a corresponding barcode. The 6-digit blood sample number uniquely identifies each blood sample and the 2-digit cryovial number uniquely identifies each cryovial. Placing the blood sample number on a participant's *Form 100* identifies the blood sample as belonging to that participant. Each label also gives the color of the blood collection tube stopper and the type of the aliquot specimen. (You may use local labels for the triglyceride and CBC samples you send to your local lab.)

Beginning October 1, 1995, the buffy coat for cryovial - 20 is collected from the two light blue vacutainer tubes. The wording on the label was changed from "lavender" to "light blue".

<u>Vacutainer Stopper Color</u>	<u>Cryovial Number</u>	<u>Specimen Volume and Type</u>
Royal Blue	02	1.8 ml serum
Royal Blue	03	1.8 ml serum
Royal Blue	04	1.8 ml serum
Royal Blue	05	1.8 ml serum
Light Blue	06	1.8 ml citrate plasma
Light Blue	07	1.8 ml citrate plasma
Light Blue	08	1.8 ml citrate plasma
Royal Blue	09	Triglyceride, serum
Lavender (10 ml)	10	1.8 ml EDTA plasma
Lavender (10 ml)	11	1.8 ml EDTA plasma
Lavender (10 ml)	12	1.8 ml EDTA plasma
Lavender (10 ml)	13	Buffy Coat
Lavender (10 ml)	14	1.8 ml RBC
Lavender (2 ml)	16	CBC
Light Blue	20	Buffy Coat

Figure 11.1
Blood Sample Labels

[BARCODE] 123456 A Blood Form	[BARCODE] 123456 A Blood Form	123456 A Tube
123456 A Tube	123456 A Tube	123456 A Tube
123456 A Tube	123456 A Tube	123456 A Tube
[BARCODE] 123456-02 Royal blue tube, serum orange cap	[BARCODE] 123456-03 Royal blue tube, serum orange cap	[BARCODE] 123456-04 Royal blue tube, serum orange cap
[BARCODE] 123456-05 Royal blue tube, serum orange cap	[BARCODE] 123456-06 Light blue tube, plasma blue cap	[BARCODE] 123456-07 Light blue tube, plasma blue cap
[BARCODE] 123456-08 Light blue tube, plasma blue cap	123456-09 Royal blue tube, triglyceride, serum	[BARCODE] 123456-10 Lavender tube, plasma yellow cap
[BARCODE] 123456-11 Lavender tube, plasma yellow cap	[BARCODE] 123456-12 Lavender tube, plasma yellow cap	[BARCODE] 123456-13 Lavender tube, Buffy coat white cap
[BARCODE] 123456-14 Lavender tube, RBC red cap	123456-16 2 ml Lavender, CBC	[BARCODE] 123456-20 Light blue tube, Buffy coat white cap

11.2.2 Identification of Participants Requiring Blood Collection

11.2.2.1 Routine Visits

First Screening Visit: Collect a fasting blood sample from all participants at SV1 if they have been fasting for at least 12 hours. This includes participants interested in the CT components and participants interested in the OS.

Clinical Centers have the option to collect blood samples on participants at the Screening Visit 2 (SV2) if this fits better with their clinic flow. They may also schedule a time separate from the visit. For example, a CC may schedule SV1s and SV2s in the afternoon and not require participants to be fasting; then ask the participants to come for blood draws in the morning on a different day. If a woman has not been fasting for at least 12 hours at SV1, postpone the blood draw until SV2 and ask the woman to fast at least 12 hours before the next visit.

Perform any off-site blood draws using the same procedures as those performed at a primary clinic, including adherence to time limits and equipment for processing and freezing. See *Figure 11.3 - Guidelines for Blood Processing* for a summary of time requirements.

Follow-up Visits: The schedule of blood collection for the CT and OS is different. In addition, the women in the CT blood draw subsample are determined at the time of randomization. See *Vol. 1, Table 1-A1.1 - Frequency of Data Collection* for the schedule of blood collection and

Figure 11.2 – Blood Collection and Aliquot Schedule for tubes to collect at the indicated contacts.

Figure 11.2
Blood Collection and Aliquot Schedule

Blood Collection Tube		Three 7 ml Royal Blue		Two 4.5 ml Light Blue		One 10 ml Lavender		One 2 ml Lavender	
Cryovials		Four 1.8 ml Serum	Trig. 0.5 ml Serum	Coag Panel Three 1.8 ml Plasma	One 1.8 Buffy coat	Lipid Panel Three 1.8 ml Plasma	One 1.8 RBC	One 1.8 Buffy Coat	WBC, Hct platelet count
Study	Visit								
CT/OS	SV1	X	Hrt if Lipemic	X	X	X	X	X	X
CT	1st Annual	X	Hrt if Lipemic	X	X	X	X	X	
CT	Subsample at 3rd, 6th, and 9th Annual	X	Hrt if Lipemic	X		X			
OS	3 Year	X		X	X	X	X	X	X

11.2.2.2 Quality Assurance (QA) Samples

There are no QA blood samples to collect.

11.2.3 Volume of Blood to Collect (Required)

The volume of blood to collect depends on the type of visit.

The volume of blood to collect depends on the type of visit. *Figure 11.2 – Blood Collection and Aliquot Schedule* shows the number and type of vacutainer tubes to use at the SV1 and subsequent annual visits.

11.2.3.1 Blood Sample for the Local Lab (Required)

The analyses you request from your local lab depends on the type of visit and test needed to determine the woman's eligibility. See *Figure 11.2 – Blood Collection and Aliquot Schedule* for a summary.

At SV1:

- Collect blood for analyses of hematocrit and platelet count to be used to determine the participant's eligibility and for analysis of WBC. (Be sure to ask the lab to give a platelet count and not a platelet estimate.) As of March, 1996, hemoglobin is no longer a criterion for eligibility and is no longer required.
- If the woman is interested in HRT and her serum is lipemic, send an aliquot of serum from the royal blue tube for a triglyceride level. See *Section 11.3.3.1 - Preparation of Blood Cryovials (Required)* for details of determining lipemia.

At Year 3 for OS participants:

- Collect blood for analysis of WBC, hematocrit, and platelet count.

11.2.3.2 Routine Blood Sample for the Serum Repository (Required)

Send aliquoted cryovials to McKesson BioServices according to the schedule shown in

Figure 11.2 – Blood Collection and Aliquot Schedule. Collect approximately 42 ml of blood using royal blue, light blue, and lavender-stoppered tubes.

11.2.4 Preparation for Blood Collection

11.2.4.1 General Instructions for Participants Before Blood Draws (Required)

Before visits at which you will be drawing blood, tell each woman to prepare for the blood collection. Ask her to:

1. Fast (that is, take nothing by mouth except water) for at least 12 hours before all blood collections. To reduce the likelihood of fainting, encourage her not to initiate the fast much more than 16 hours before the blood draw. Also ask her to drink water liberally during the fasting period to prevent dehydration and reduce the likelihood of fainting.
2. Take all regular medications except for insulin or oral medication used to control diabetes. (Note that participants with diabetes may have more specific guidelines from their primary physician about blood draws.)
3. Take no aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) for 48 hours before the visit. NSAIDs that are taken regularly may be continued and taken within the 48 hours before the blood draw, consistent with the participant's usual schedule.
4. Do not smoke for at least one hour before the blood draw.
5. Perform no vigorous physical activity (such as jogging or bicycling) for at least 8 hours before the blood draw.
6. Wear clothing which allows the sleeve to be easily raised above the elbow without constricting the blood flow to forearm and hands.

11.2.4.2 Directions for Staff When Participants Do Not Follow Instructions (Required)

1. In general, do not draw the blood sample if the participant is not fasting. To be fasting the participant should not have consumed even a lifesaver, stick of gum or other sugar-containing food items during the fasting period, or has “accidentally” ingested a sip of juice, black coffee, tea, single bite of other food items, or any amount of alcohol.

For safety reasons, a participant with diabetes should be scheduled as the first draw of the day. If a participant with diabetes cannot delay taking her diabetic medications until after the blood draw, you may draw her blood even if she has recently taken the medications.

- If a participant has taken her diabetic medication or advises you that she is not fasting, reschedule the blood draw when fasting will be convenient for the participant, if she is willing to return to the CC. Do not coerce the participant to return to the CC for a blood draw.
- If she is fasting but took her diabetic medication, note that she took the medication in the comments section of *Form 100 - Blood Collection and Processing*.
- If she is not fasting and is not willing to return to the CC, you may draw the blood, being sure to record on the *Form 100 - Blood Collection* when she last ate. (See Step 1 in *Section 11.2.4.2 - Directions for Staff When participants Do Not Follow Instructions*.)

If the participant failed to initiate the fast slightly less than 12 hours before the blood draw, try to do other visit procedures first or delay the blood draw until 12 hours have passed; otherwise, reschedule the blood draw. If 12 hours will not pass before the end of the visit, ask her to come back at another time when she can fast.

Reschedule the blood draw for another date. (See Step 3 in *Section 11.2.4.5 – Participant Preparation*.) Do not coerce the participant to return to the CC for a blood draw – her adherence and retention in the study overall is more important than obtaining a fasting blood sample. In the rare circumstances where the participant is not fasting and is unable or unwilling to return to the CC, you may draw the blood, being sure to record on *Form 100 – Blood Collection and Processing* when she last ate.

2. If a woman has smoked during the hour before the blood draw, try to do other visit procedures first until an hour has passed since her last cigarette. If postponing the draw is not possible, proceed with the draw.
3. If a woman has done vigorous activity within eight hours of the blood draw, try to do other visit procedures until eight hours have passed since the exercise session. If the activity was quite recent, schedule the blood draw for another time. “Non-vigorous” physical activities include walking/jogging at less than four m.p.h. or stair climbing. Aerobics should be avoided. “Vigorous” activities include vigorous jogging or running. If the participant has indicated she has engaged in vigorous activity within 8 hours prior to her blood draws, indicate this on *Form 100 – Blood Collection and Processing*, and proceed with the blood draw.
4. If the woman's sleeve constricts the arm when rolled or pushed such that it functions as a tourniquet, ask her to remove the blouse or top and offer her a gown.
5. If the participant has indicated she has taken non-routine, non-regular aspirin or non-steroidal anti-inflammatory agents within the 48 hours prior to her blood draw, indicate this on *Form 100 - Blood Collection and Processing* and proceed with the blood draw.

11.2.4.3 Preparation Before the Visit

After the determination has been made that a participant will have a blood draw at the visit:

1. Ensure the blood drawing and blood processing areas are equipped with the proper supplies.
2. Obtain *Form 100 - Blood Collection and Processing* and a blood sample label set.
3. Apply a label with the participant's name and ID number on the form.

4. Indicate the type of visit on *Form 100 - Blood Collection and Processing*.
5. Insert the labeled *Form 100 - Blood Collection and Processing* and label set into the participant's file.

11.2.4.4 Preparation at the Visit (Required)

1. Review the Blood Request part of *Form 100 - Blood Collection and Processing* to see the type and number of tubes of blood to collect.
2. Arrange the set of tubes in a test tube rack, one rack per participant. For the first blood draw occasion, draw the tubes in the order listed below. Attempt to draw the full set of blood collection tubes as described in *Section 11.2.4.4 - Preparation at the Visit* with the tube for CBC last. If you do not draw enough sample on the first occasion, attempt a blood draw on a second occasion. At this second occasion, draw the blood samples you need for eligibility, drawing a small (2 ml) lavender tube for the CBC tube and, for HRT participants only, a royal blue tube for evaluation of triglycerides if the blood is lipemic.

Three	7 ml	Royal Blue
One	2 ml	Lavender (for CBC)
Two	4.5 ml	Light Blue
One	10 ml	Lavender

The order for filling the tubes (Royal Blue - CBC - Light Blue - Lavender) is based on the following:

Royal blue tube first: This tube is trace-element free. You draw this first to avoid the possibility of the anticoagulant in the other tubes (sodium citrate in the light blue tube and EDTA in the lavender tube) contaminating the blood in this tube via the blood or needle.

Lavender (CBC) tube: This tube is for the local CBC. You can draw this tube next to assure you obtain the CBC results needed for eligibility.

Light blue tube: This tube collects plasma for coagulation tests. You do not draw this first to avoid contamination with tissue thromboplastin response to the needle stick, which can affect the coagulation test results. If you do not need to draw the royal blue-stoppered tube, draw a small amount of blood into another royal blue tube to flush the tissue thromboplastin from the needle (you may use a less expensive red-stoppered tube if available at your CC), discard, and then draw the light blue tube for processing.

3. Prepare aluminum sleeves for the royal blue-stoppered tubes. Serum from this tube will be analyzed for carotenoids, which break down in white light. The aluminum foil cover will help protect the blood from light and prevent deterioration of the carotenoids.
4. Check the identifying information on the form and the labels to make sure the form is correctly labeled.

11.2.4.5 Participant Preparation (Required)

1. Blood drawing is standardized to the sitting position. Whenever possible, have the participant in the sitting position for five minutes immediately before venipuncture. Pulse and blood pressure measurements should be performed before the blood draw or an appropriate time thereafter (i.e., wait 30 minutes) in the **opposite** arm.
2. Each participant must sign the Initial consent authorizing the CC to draw blood at the visit before you can draw her blood. Ensure the participant understands the specifics of the blood collection as detailed in the informed consent:
 - Approximately 3 tablespoons of blood will be drawn for CBC and other chemistry tests at baseline.
 - Blood will be drawn at selected follow-up visits to the CC.

- A small risk is associated with the drawing of blood, no greater than if it were performed in a doctor's office.
 - A little discomfort may be experienced as the blood-drawing needle penetrates the skin.
 - A bruise may develop at the blood-drawing site, however, keeping pressure on the site for 1-2 minutes after the needle is removed may prevent a bruise.
 - Very rarely, an infection may develop in the arm as a result of blood drawing
3. Review the blood collection checklist on *Form 100 – Blood Collection and Processing* with the participant. See *Section 11.2.4. – General Instructions for Participants Before Blood Draws (Required)* and *Section 11.2.4.2 – Directions for Staff When Participants Do Not Follow Instructions (Required)*.
- Ask the participant if she is fasting. The minimum fasting time for the participant is 12 hours. Only water is acceptable to have during the fasting period.
 - Ask the participant if she has engaged in vigorous physical activity in the last 8 hours.
 - Ask the participant if she has taken aspirin or anti-inflammatory agents in the last 48 hours.
4. Review safety issues with the participant.
- Ask the participant whether she bleeds or bruises easily. If she has had any problems with excessive bleeding or bruising at a venipuncture site, draw her blood only if approved by a Clinic Manager, CC physician or Principal Investigator (PI).
 - Ask the participant if she has ever been told she has a disorder related to blood clotting or coagulation or is taking any anticoagulants or aspirin. Continued bleeding may be a complication if the participant has any problems related to blood clotting or coagulation. To prevent bleeding and preserve the vein, you may need to apply pressure to the site for an extended period of time after you draw the blood. Stay with the participant to ensure the bleeding has stopped. Use Coban self-adherent wrap, or the equivalent, to keep in place for 10-15 minutes over the blood draw site.
 - Ask the participant if she has ever experienced fainting spells while having blood drawn. If she has experienced fainting spells during venipuncture, ask her the frequency of fainting spells. Proceed with the venipuncture if she has fainted only once before. If she advises that she frequently faints, consult the Clinic Manager, CC physician or PI before attempting the venipuncture. Provide smelling salts, amyl nitrate, basin, or cold cloth, if needed. Have orange juice or fruit juice available to offer to the participant. It is advisable to have the participant lie down initially. Note the condition in the participant's file, and perform future blood draws while she is lying down. It is also advisable to loosen any tight clothing before drawing the blood, especially clothing around the neck. (See also *Section 11.2.6.4 - Fainting.*)

11.2.4.6 Handling Participants Who Are Extremely Apprehensive About Having Blood Drawn

Even though blood drawing is standardized for the sitting position, you may ask an extremely apprehensive participant to lie down, if there is a bed available in the CC.

Do not force the participant to have blood drawn under any circumstances. It may help to explain to the participant that the blood drawing is designed to be as painless as possible. Sometimes it helps 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 that you can check the veins in her arms, without actually drawing blood. If the participant has "good veins" you can reassuringly say, "Oh, you have good veins; there should be no problem." You may also consider using a butterfly needle to perform the draw if the participant is apprehensive. The butterfly needle is a thinner, smaller needle and may be less painful to the participant.

Have the participant sit upright with jacket or sweater removed and with the sleeves rolled up to expose the antecubital fossa (elbow).

Give the participant 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 is the blood collection process, the contact with the staff person who draws the blood, and the staff person's general attitude and competence.

This study requires the voluntary cooperation of the participants over a period of many years. Thus, the whole experience must be made as pleasant as possible. Reassure participants who are concerned about the volume of blood that the total amount of blood drawn is about three tablespoons. Also assure the participants that they donate up to 10 times as much blood (450 ml) when they donate a pint of blood.

11.2.5 Venipuncture

11.2.5.1 Wash Hands and Put on Gloves

Wash your hands with soap and water before every blood draw and put on a pair of disposable plastic latex gloves.

11.2.5.2 Assemble the Vacutainer Holder

1. Attach the needle to the vacutainer holder.
2. Place the royal blue-stoppered tube in the vacutainer holder being careful not to break the vacuum

11.2.5.3 Identify Venipuncture Site

1. Position the participant's arm on the drawing table. Extend the arm toward you, palm up. Use a padded cushion under her elbow for comfort, if appropriate.
2. Wrap the tourniquet around the arm three to four inches (7.5 to 10.0 cm) above the venipuncture site.

A tourniquet is used to increase venous filling. It makes the veins more prominent and easier to enter. **PRECAUTIONS WHEN USING A TOURNIQUET:** The tourniquet should be on the arm for the shortest time possible. **Never** leave the tourniquet on for longer than one (1) minute at a time. To do so may result in hemoconcentration or a variation in blood test values. (It was documented [Clinical Chemistry, 20:1513-1519, 1974] that changes in venous occlusion from 1 to 3 minutes led to a change in various blood components including increase in total lipids (4.7%) and cholesterol (5.1%) and decrease in other components.) If you must apply a tourniquet for the preliminary vein selection, release and reapply it after a wait of one minute. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

3. Ask the participant to make a fist.
4. Identify a vein. Use the antecubital site of either arm as first choice. The median cubital vein is the one used most frequently. If the venipuncture of this vein is unsuccessful, use the cephalic and basilic veins as the next appropriate choices, followed by veins on the back of the hand. Palpate and trace the path of veins several times with the index or middle finger. Unlike veins, arteries pulsate, are more elastic and have a thick wall. Thrombosed veins lack resilience, feel cord-like and roll easily. If you cannot readily see superficial veins, ask the participant to open and close her fist. Lowering the lower arm over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.
5. Palpate the vein. If you do not feel a vein, try another arm or site (see *Section 11.2.6.3 - Difficult Venipuncture*).
6. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.
7. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the participant when you perform the venipuncture.

8. If venipuncture becomes difficult, you may need to touch the vein again with your hand. If this happens, cleanse the site again with alcohol.

11.2.5.4 Perform Venipuncture

1. Explain the procedure to participant; for example, "I will be drawing a blood sample from your arm. You will probably feel a small prick when I insert the needle."
2. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be one or two inches (2.5 or 5.0 cm) below the venipuncture site.
3. Enter the vein in a smooth continuous motion with the needle bevel upward and parallel to the vein. Use a straight stab; do not poke around.
4. Make sure the participant's arm is in a flat and stable position.
5. Grasp the flange of the vacutainer holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
6. Remove the tourniquet after blood is flowing into the tube. If no blood enters the tube, the needle may not be positioned in the vein. See *Section 11.2.6 - Blood Collection Problems* for possible action.
7. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve from stopping blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.
8. Draw each vacutainer tube in order (three royal blue, the 2 ml lavender for CBC, two light blue, and the 10 ml lavender). Fill each tube as completely as possible, that is, until the vacuum is exhausted and the blood flow stops. If a vacutainer tube fills only partially, remove the vacutainer tube and attach another without removing the needle from the vein.
9. When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until you insert the next tube (if necessary).
10. Draw the required blood tubes and place the royal blue-stoppered tubes into sleeves of aluminum foil to protect from the light.
11. Gently invert the lavender and light blue-stopped vacutainer tubes several times to ensure proper mixing of blood with the anticoagulants before placing the tubes in the rack. Do not invert the royal blue-stoppered tube.
12. Label the form and each tube with a blood sample label immediately after you complete the blood draw. (You can best do this while the participant is holding the gauze pad over the venipuncture site; see the steps below.)

For routine samples, put the first label (printed with "Form") from a blood sample label set on the front of *Form 100 - Blood Collection and Processing* in the space indicated for Blood Sample Number and a second label (also printed with "Form") on the back of the form.

11.2.5.5 Bandage the Arm

1. Under normal conditions:
 - 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 a needle box. If using a syringe with needle, dispose entire set-up into disposable container.
 - Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma. Ask the participant to keep her arm extended. Bending her arm at the elbow increases the risk of developing a hematoma.
 - Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.

2. If the participant continues to bleed:
 - Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
 - Wrap a gauze bandage tightly around the arm over the pad.
 - Tell the participant to leave the bandage on for at least 15 minutes.
3. Wash your hands.
4. Complete the remainder of the "Blood Collection" section of *Form 100 - Blood Collection and Processing*.
5. Deliver the blood sample tubes and *Form 100 - Blood Collection and Processing* to the blood processing area.

11.2.6 Blood Collection Problems

11.2.6.1 Special Consideration for Drawing Blood in the Elderly

The elderly pose some special blood drawing problems due to fragile and small veins, especially if the samples are drawn during a fasting period. The system of drawing blood by using vacuum tubes can increase this problem, causing veins to collapse due to high pressure exerted by the vacuum tubes. Steps outlined in *Section 11.2.6.3 - Difficult Venipuncture* below may help to lessen some of the problems.

An important point in drawing blood is that the tourniquet must not occlude arterial flow; otherwise the problem of veins collapsing during the venipuncture may be accentuated. By using a syringe rather than a vacuum tube, you can control the pressure and reduce this problem. When using a smaller needle (for example, 22 gauge 1"), there is less chance of blowing (hematoma formation) a fragile vein.

11.2.6.2 Difficult-to-Identify-Venipuncture Sites

1. Determine if the vein is difficult to identify, which may occur, if:
 - The palpitated vein feels small or rolls.
 - The participant has been stuck once already.
2. If the vein is difficult to find, check the back of the hand and forearm for venipuncture sites with larger veins. You can also try one or more of the following vein-dilation methods:
 - Be sure the room is not too cool.
 - Hot pack the venipuncture site with warm, wet towels for 3-5 minutes.
 - Have the participant wash her hands in warm water for 3-5 minutes.
 - Have the participant dangle her arm at her side with the tourniquet in place for one minute.
 - Use the blood pressure cuff as a tourniquet by pumping the pressure to 60-80 mm Hg.
3. If the vein is small, try a disposable syringe and 22-gauge needle or a butterfly initially.
4. Finish the venipuncture following the procedures outlined above.

11.2.6.3 Difficult Venipuncture

If you performed the venipuncture and a blood sample is not forthcoming, you may find the following manipulations helpful:

- If there is a sucking sound, turn the needle slightly or lift the holder to move the bevel away from the wall of the vein.

- If no blood appears, move the needle slightly in hopes of entering the vein. Do not probe. If unsuccessful, release the tourniquet and remove the needle. A second attempt can be made on the other arm.
- If the vein rolls, withdraw the needle slightly without coming back through the skin and try a second thrust.
- If the vein collapses, remove the vacutainer tube, call another staff person to reapply the tourniquet, ask the participant to open and close fist, and then reinsert tube. If still no blood appears in the tube, stop the procedure and use techniques in *Section 11.2.6.2 - Difficult-to-Identify-Venipuncture Sites* above.

If this is a problem on a particular participant, try using a butterfly needle rather than a vacutainer needle to draw the blood.

The participants come to the CC visit fasting for 12 hours (nothing but water) and may be dehydrated. Encourage the participants to drink water liberally during the fast. If this is a particular problem at your CC, consider offering the participants water as they arrive at the visit.

- Loosen the tourniquet. You may have applied it 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 one minute at a time.
- Remove the tube and insert a second vacutainer tube. On rare occasions, the vacutainer tube may have lost its vacuum and thus may not draw blood into the tube even when the needle is positioned properly in the vein.
- If another technician is available, allow the other technician to attempt a venipuncture.
- If venipuncture fails with the second technician, request that the participant return at another time for a successful venipuncture. (See *Section 11.2.6.5 - Deficient Serum and Plasma Sample*.)
- Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on her part.
- No single staff member should attempt more than two venipunctures on the same participant at any single visit. See *Section 11.2.4.4 - Preparation at the Visit* for instructions on specific tubes to draw at the second blood draw occasion.

11.2.6.4 Fainting

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

- Finish drawing blood if possible but do not proceed if participant is clearly in trouble.
- Have participant lay her head on a table.
- Continue talking to participant to assess level of consciousness.
- Have participant lie down for 5-10 minutes after removing the needle; apply pressure on vein to prevent injuries from possible fall or seizure.
- Apply cool compress to her forehead.
- Have participant put a pillow or cushion under her knees.

If the participant faints:

- Withdraw the needle immediately.
- Apply pressure at the venipuncture site.
- Call for help.

- Apply a cool compress to her forehead.
- Use an ammonia capsule, if needed, by crushing the ampule and waving it under her nose for a few seconds.
- Once recovered, have the participant lie down on an exam table until she feels better.
- Take blood pressure readings to assess her recovery, if necessary.
- Offer the participant water, juice and food.

Note: If you do not collect a blood sample, reschedule the blood draw for another day.

Realize that the participant might be disoriented, embarrassed, or irritable and needs reassurance and attention. Recognize also that this incident may have an impact on future blood drawing and possibly on study adherence and must be handled well. Make a note of the difficulty in the participant's file for future reference.

11.2.6.5 Deficient Serum and Plasma Samples

Common reasons for failure to obtain enough specimen include:

- Original vacutainers not properly filled.
- Adequate time not allowed for clot formation (royal blue-stoppered tubes only).
- Failure of centrifuge to attain required relative centrifugal force. Check or ask service representative to check the relative centrifugal force (RCF) every six months or when you begin to experience more frequent insufficient sample problems.
- High hematocrit of participant. This can be more common in people with asthma or emphysema. Note this in the participant's file and draw more tubes of blood at subsequent blood draws so these participants do not have deficient samples each time.
- Over-filling the sample cryovials.

If you do not get a full royal blue-stoppered vacutainer of blood (for serum), you should try to collect an additional half-tube immediately. After centrifuging the tubes, you can combine the serum from the half-tube with the serum from the first incompletely filled royal blue-stoppered tubes. Similarly, if you do not get full light-blue or lavender tubes, you should try to collect additional tubes. However, you must fill these tubes as full as possible to get the proper proportion of blood to anticoagulant.

If you do not obtain sufficient blood in the first attempt, perform a second venipuncture (see *Section 11.2.6.3 – Difficult Venipuncture*).

Deficient Samples

There may be an occasion where the blood draw may yield less than you need to fill all the tubes. In general, blood collected from different blood draws within a short time (e.g., less than one hour) can be considered the same blood draw. Blood collected on different days or different times (> 1 hour apart) are considered different draws and require two separate *Form 100s* with different blood sample numbers. To do a second blood draw, complete a new *Form 100 - Blood Collection and Processing*, assign a new blood sample number, and answer the questions on the form as you would for any blood draw.

- **At SV1:**

For CT participants, if you do not collect enough blood samples for the triglycerides [if serum is lipemic for HRT participants] or CBC at SV1, collect additional blood for the triglycerides or CBC at SV2. At SV2 it is essential to collect sufficient samples for the local lab analysis of hematocrit and platelet count and for triglycerides (if an HRT participant's serum is lipemic) because you cannot randomize a

participant at Screening Visit 3 (SV3) without these results. It is not necessary to obtain the rest of a deficient screening sample after the participant is randomized. It is better to try to get the rest of the sample before randomization if the additional attempt would not adversely effect the participant's willingness to join the study.

For OS participants, you must obtain at least 2 ml of serum for a participant to be eligible for enrollment into OS. This means that a minimum draw of 4 ml of blood in a royal blue tube is necessary (to make 2 ml of serum). Thus, at least 2 aliquots should be sent to McKesson BioServices (1 ml each in cryovial -02 and -03; fill the cryovial to the 1.8 ml mark only if you have enough blood for 1 ml in all required aliquots). For OS, collection of the local lab has a lower priority, and attempts should be made to collect the blood for McKesson BioServices before collecting the CBC blood.

- **At Annual Visits:**

You do not need to make additional contacts or visits to collect missing blood samples.

11.2.7 Summary of Blood Collection Procedure

1. Label *Form 100 - Blood Collection and Processing* with the participant's name and ID number.
2. Obtain a set of blood sample labels and attach to *Form 100 - Blood Collection and Processing*.
3. Review the blood collection aliquot schedule portion of *Form 100 - Blood Collection and Processing* indicating the type of blood you need to collect.

At the Visit:

1. Check that *Form 100 - Blood Collection and Processing* is correctly labeled with the participant's name and ID number.
2. Review the section of the form indicating the number of tubes to draw.
3. Instruct the participant.
4. Collect the blood.
5. Apply a blood sample label to *Form 100 - Blood Collection and Processing* in the spaces indicated.
6. Label the blood collection tubes with the blood sample labels.
7. Cover the royal blue-stoppered tubes with foil.
8. Gently invert light blue and lavender tubes several times after each tube is drawn.
9. Complete the remainder of the Blood Collection section of *Form 100 - Blood Collection and Processing*. Ensure first page is completed.
10. Deliver the blood sample tubes and *Form 100 - Blood Collection and Processing* to the blood processing area.

11.3 Blood Processing

11.3.1 Timelimits and Sample Handling

Procedures and time limits for blood processing are listed below. See also *Figure 11.3 - Guidelines for Blood Processing*.

1. Stand time:
 - Allow the royal blue-stoppered tubes to stand for at least 30 minutes at room temperature. The 30-minute waiting time is necessary to allow an adequate clot to form. If you frequently find fibrin clots in the centrifuged serum samples, try letting the samples sit for 45 minutes before centrifugation.
 - Always protect blood samples for carotenoids (in royal blue-stoppered tubes) from natural and fluorescent white light. Store samples in covered containers or tubes.
2. Refrigerate time:
 - Refrigerate the royal blue (after clotting), light blue and 10 ml lavender tubes if it is not possible to centrifuge the samples within one hour after collection.
 - Set the samples in wet ice if a refrigerator is not available.
3. Centrifuge time:
 - Make every attempt to centrifuge the tubes between 30-45 minutes after collection. Set a timer, if necessary, as a reminder when the collected blood is ready for centrifugation.
 - Centrifuge the royal blue and light blue tubes within two hours of collection.
 - Centrifuge the 10 ml lavender tube within four hours of collection.
4. Aliquot time:
 - Aliquot the serum and plasma into the cryovials within 15 minutes after centrifugation.
5. Freeze time:
 - Freeze all aliquoted specimens within two hours of collection.

11.3.1.1 CBC

Do not refrigerate the tube for the CBC unless it is requested by your local lab. Send the 2 ml lavender stoppered tube to your local lab for a CBC analysis and a platelet count. Do not centrifuge the tube for the CBC. Follow your local CC procedures for completing the paperwork.

Figure 11.3
Guidelines for Blood Processing

Blood Collection Tube	Minimum Stand Time at Room Temp.	Maximum Stand Time at Room Temp. (before refrigeration)	Min. Time Post-blood Collection to Begin Centrifugation	Max. Time to Begin Centrifugation	Centrifuge Time	Post-Centrifugation Aliquot Time	Freeze Time Post Collection
Royal blue (7 ml)	30 min.	1 hr	≥ 30 min	2 hours post collection	10 minutes	15 minutes post-centrifugation	Within 2 hours post collection
Light blue (4.5 ml)	0 min	1 hr	Immediately post-collection	2 hours post collection	10 minutes	15 minutes post-centrifugation	Within 2 hours post-collection
Lavender (10 ml)	0 min	1 hr	Immediately post-collection	4 hours post-collection	10 minutes	15 minutes post centrifugation	Within 2 hours post-collection
Lavender (2 ml)	0 min	n/a	n/a	n/a	n/a	n/a	n/a

11.3.2 Operating the Refrigerated Centrifuge

1. Set the centrifuge temperature at 4°C. (A setting in the 2° to 8°C range is acceptable.)
2. Load the rotor, being careful to place balanced tubes in buckets directly opposite to each other. If necessary, fill an empty tube with water, insert stopper and use as a balance for a blood sample tube. Be sure there is enough clearance for the tube and stopper when the buckets are in horizontal position.
3. Close the centrifuge cover and lock.
4. Centrifuge for ten minutes with brake off at a speed setting that will yield a relative centrifugal force (RCF) of 1,300 xg.

You can obtain the desired RCF of 1,300 xg on various centrifuges by adjusting the revolutions per minute (RPM). You need to do this because the RPM setting of a centrifuge does not equal the RCF. The following equation shows the calculation of RCF:

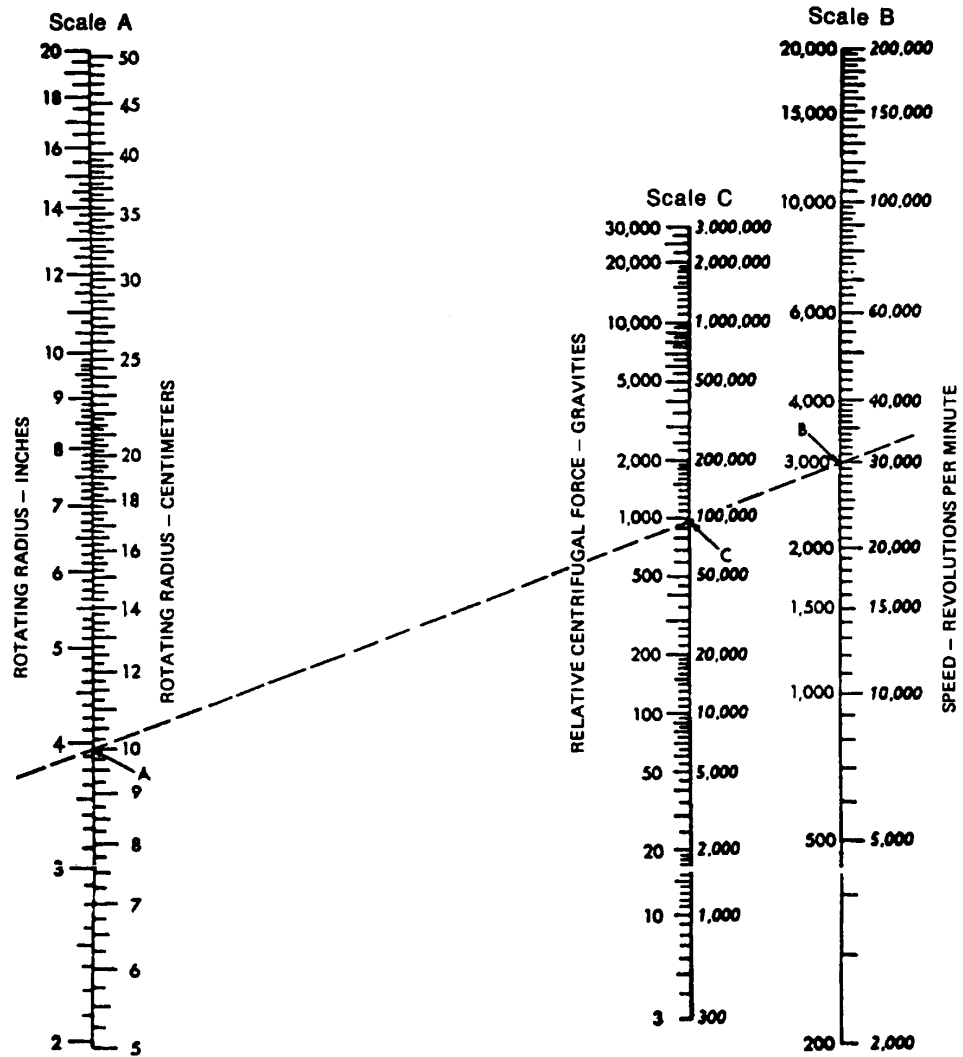
$$RCF = (1.118)(10^{-5})(r)(n^2)$$

where r = rotating radius in centimeters (the distance from the center of the centrifuge rotor to the middle of the sample tube when the tubes in the swinging buckets are at the horizontal position) and n = revolutions per minute.

Alternately, use *Figure 11.4 - Nomogram for Calculating RCF* by lining up the radius in cm and RCF = 1,300 xg with a straight edge and reading the point at which the straight edge intersects the scale for RPM. For example, to achieve RCF of 1,300 xg with a centrifuge that has a radius of 10 cm, a straight line from these points on *Figure 11.4 - Nomogram for Calculating RCF* intersects the RPM scale at 3,400 RPM.

5. Record the time you started the centrifuge on *Form 100 - Blood Collection and Processing*. Do not use the time when you removed the samples from the centrifuge.
6. The centrifuge will stop automatically at the prescribed time. Allow the centrifuge to come to a complete stop before opening the cover. Do not use the brake to slow down the centrifuge. Using the brake may cause the RBCs to become resuspended in the plasma.
7. Remove the blood sample tubes one at a time and put in the test tube rack. Be careful not to tip the tubes and disturb the red cell layer. Re-centrifuge any tube containing red blood cells in the serum or plasma.
8. Consult the Centrifuge Service manual for other guidelines and for troubleshooting.

Figure 11.4
Nomogram for Calculating RCF



*Modified from the IEC (a division of Damon Corporation, Needham Heights, Mass.) Relative Centrifugal Force Nomograph.

11.3.3 Processing Samples

You can process several blood samples at a time as long as you follow the timing guidelines listed in *Section 11.3.1 - Timelimits and Handling* above and in *Figure 11.3 - Guidelines for Blood Processing*. To keep the blood samples you are processing separate, keep the blood collection and cryovials from each participant in a separate rack. This will help you aliquot the samples into the correct corresponding cryovials.

The following is an example of how you can set up your work area and process several blood samples at one time. You may change the procedure as needed to make the processing more efficient at your CC.

- Set up a separate rack for each participant's set of blood samples. Because the cryovials fit better in a rack with smaller holes, you can use two racks for each blood sample: the one with smaller holes for the cryovials in front, and one with larger holes for the blood collection tubes directly behind the smaller rack. See *Figure 11.5 - Sample Blood Processing Rack Set-Up* below for an example set-up of tubes.
- When you first get the blood sample tubes, put the protected royal blue tubes in the appropriate racks to stand for 30 minutes and allow the blood to clot. Keep the tubes protected from light during this waiting time.
- Centrifuge the light blue and 10 ml lavender tubes for ten minutes.
- While the samples are centrifuging, label the cryovials. Place the labeled cryovials in the smaller rack. Remove the caps and lay them in front of the corresponding cryovials in the rack.
- When you have time, process the 2 ml lavender tube by completing the local lab paperwork for the CBC and platelet count as needed.
- When the centrifuge stops, place the tubes from the centrifuge in the appropriate larger racks, placing the tubes directly behind the row of cryovials into which you will be placing the plasma.
- Process the plasma from the light blue and lavender tubes as described in *Section 11.3.3.1 - Preparation of Blood Cryovials* below. Process only one set of colored tubes from one blood sample at a time (e.g., process plasma from the light blue tubes before processing plasma from the lavender tubes). This will help reduce the chance of mixing serum and different types of plasma from one participant and from mixing samples between different participants.
- After processing the plasma from the 10 ml lavender tube, process the buffy coat and RBCs as described in *Section 11.3.3.1 - Preparation of Blood Cryovials*.
- When 30 minutes have passed, centrifuge the royal blue tubes. While the tubes are centrifuging, you can complete processing of the light blue and lavender tubes and the 2 ml lavender tube for the CBC, if you have not already done so.
- Follow the procedure in *Section 11.3.3.1 - Preparation of Blood Cryovials* for processing the serum from the royal blue tube.
- Place the samples in the freezer box. Complete the Blood Processing portion of *Form 100 - Blood Collection and Processing* as you place the aliquots into the freezer box, marking the corresponding aliquot on the form, as you place the aliquot into the freezer box.

Figure 11.5
Sample Blood Processing Rack Set-Up
Rack for Blood Collection Tubes

									Back
Royal Blue									
Royal Blue		Light Blue							
Royal Blue		Light Blue		10 ml Lavender					Front

Rack for Blood Cryovials

-09		-20		-14					Back
-05				-13					
-04		-08		-12					
-03		-07		-11					
-02		-06		-10					Front

11.3.3.1 Preparation of Blood Cryovials (Required)

1. Place the 2 ml lavender-stoppered tube and the centrifuged tubes from each participant in separate racks.
2. Inspect each centrifuged tube for hemolysis. Recentrifuge any specimen that contains red cells suspended in the serum or plasma. If a specimen is badly hemolyzed, do not use the specimen for preparing aliquots for storage.
3. For HRT or HRT+DM participants, determine if you need to process an aliquot sample for triglycerides.
 - Inspect the royal blue-stoppered tube for lipemia (serum will appear opaque or milky). Use the laminated “Test Print” card and the photograph of tubes of varying triglyceride levels supplied by MRL.
 - In the photograph, the tube on the left, labeled “Normal Trig.,” contains clear serum, indicating that the triglyceride is not elevated above 300 mg/dl. The print behind the tube is clearly seen through the serum.

- The middle tube, labeled “Trig. 300,” contains serum that appears slightly turbid. Though the print behind the tube can still be seen, the print is distorted and not easily read. This tube represents a triglyceride of 300 mg/dl.
 - The tube on the right, labeled “Trig. 500,” contains serum with triglyceride of 500 ml/dl. This serum is turbid and you cannot read the print through the tube.
 - Hold the participant’s royal blue-stoppered tube up to the laminated “Test Print” card and compare the visibility of the print with the tubes in the photograph.
 - If the participant’s serum appears as turbid as the tube labeled “Trig. 500”, the serum is considered lipemic. Prepare a 0.5 ml serum aliquot as directed under step 6 below and send to your CC’s local lab for a triglyceride level. Use local CC procedures to complete the paperwork.
4. Review the Blood Request information on *Form 100 - Blood Collection and Processing* to determine the number of cryovials to prepare.
 5. Using the 13 blood sample labels for the cryovials, label each cryovial you will need. For the triglyceride level you can use vials supplied by McKesson BioServices, purchased by your CC, or supplied by the local lab. (*Note:* Each cryovial is marked with a line indicating the 1.8 ml mark. Because you should not fill the cryovials over this line, try not to cover the 1.8 ml mark with the label. Wrap the label around the cryovial, placing one side on the bottom edge of the cryovial as shown below.) Refer to *Figure 11.6 - Processing Blood Samples* for a guide for labeling cryovials.

6. **When you remove the stoppers from the blood collection tubes that do not have hemoguards, remove them behind a work shield or wear a face shield to avoid potential exposure to aerosol.**

Transfer the serum and plasma to the cryovials as described below. For each blood sample, use a different clean pipette tip for each specimen type (i.e., use one for the serum in the royal blue tube, a different one for the plasma in the light blue tubes, and a different one for plasma in the lavender tubes.)

At the time of the transfer, check to make sure that you place the correct specimen in the correct cryovial. When you fill the cryovials, do not fill the cryovial over the 1.8 ml mark on the vial. You must leave a small pocket of air at the top of the vial to prevent breakage during freezing.

- Remove the stopper from the light blue-stoppered tube.

Transfer 1.8 ml of plasma to each of three cryovials (numbered -06 to -08). If you are not certain that you have sufficient plasma to fill each of the three cryovials with 1.8 ml plasma, first fill each vial with 1.0 ml plasma then add an additional 0.8 ml plasma to each vial. Screw the lids onto the three cryovials.

Remove the buffy coat layer from both light blue tubes and place in cryovial labeled "Buffy Coat" numbered -20. To process the buffy coat, remove the buffy coat along with approximately 1.5 to 1.8 ml of the RBCs. (Including the RBCs with the buffy coat will help ensure better recovery of the WBCs used in the DNA extractions.) Screw the lid onto the cryovial.

Insert the light blue stoppers back in the blood collection tubes and place the tubes and pipette tip in a biohazard container.

- Remove the stopper from the 10 ml lavender-stoppered tube.

Transfer the plasma to the three cryovials (numbered -10 to -12). Put 1.8 ml plasma in the vials numbered -10, -11, and -12. If you are not certain that you have sufficient plasma to fill each of the three cryovials with 1.8 ml plasma, first fill each vial with 1.0 ml plasma then add an additional 0.8 ml plasma to each vial. Screw the lids onto the three cryovials, the buffy coat and RBC aliquots as follows:

1. Remove the buffy coat layer and place in the cryovial (labeled “Buffy Coat” and numbered -13.) To process the buffy coat, remove the buffy coat along with approximately 1.5 to 1.8 ml of the RBCs. (Including the RBCs with the buffy coat will help ensure better recovery of the WBCs used in the DNA extractions.) Leave sufficient RBCs in the tube to process for RBC only aliquot (-14). Screw the lid onto the cryovial.

2. Aliquot 1.8 ml of the packed RBCs into one cryovial (labeled "RBC" and numbered -14). Screw the lid on the vial.

- Remove the stopper from the royal blue-stoppered tube.

If you need a triglyceride level (as described above for HRT and HRT+DM participants), transfer 0.5 ml of serum to the local lab's cryovial (labeled "Triglyceride" and numbered -09).

Transfer 1.8 ml of serum to each of four vials (numbered -02 to -05). If you are not certain that you have sufficient serum to fill each of the four cryovials with 1.8 ml serum, first fill each of the vials with 1.0 ml serum. Then add an additional 0.8 ml serum to each vial. This will help assure that you get sample in each of the four cryovials. Screw the lids onto the four cryovials. Place the four vials in a box and cover with a lid to protect the serum from white light. Insert the royal blue stoppers back into the blood collection tubes. Place the blood collection tube and the pipette tip in a biohazard container.

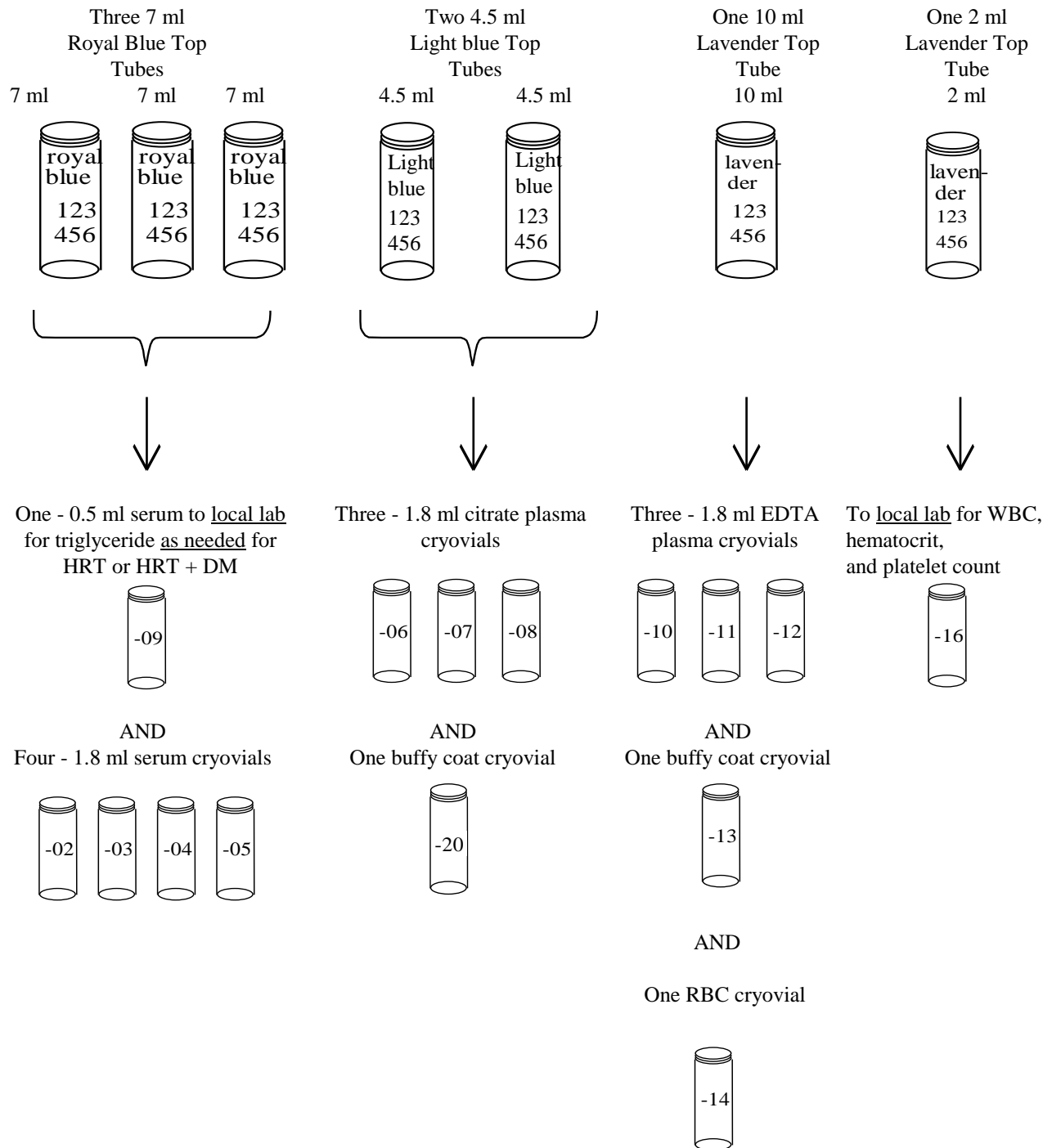
7. Check all cryovials to be sure you have correctly labeled them.
8. Label the 2 ml lavender blood collection tube with the cryovial labeled "CBC" and numbered -16. Follow CC procedures for sending the specimen to the local lab.
9. Complete the Blood Processing portion of *Form 100 - Blood Collection and Processing*.

Record the centrifuge time and time placed in the freezer for the lavender tube and corresponding cryovials.

Mark the box corresponding to each cryovial you processed to indicate you prepared the cryovial and the CBC collection tube. If you did not process a particular cryovial or process a tube for the CBC, do not mark the corresponding box. Double check each cryovial marked on *Form 100 - Blood Collection and Processing*, as you place each cryovial in the freezer box.

Figure 11.6
Processing Blood Samples

(Example for blood sample #123456)



11.3.4 Tracking Specimens for Local Lab

Follow your CC procedures for tracking CBC and triglyceride specimens, and receiving the corresponding results. Examples of activities to include in the procedures include:

- Which specimens you sent to the local lab, the date sent, and the tests ordered.
- Handling results as they return, including how to pair the results with the corresponding *Form 100 - Blood Collection and Processing*.
- Review of the results, including review for eligibility and abnormalities and handling of abnormalities that require follow-up.
- Processing the paperwork, including key-entry of the *Form 100 - Blood Collection and Processing* and the results, and filing both in the participant's file.

11.3.5 Freezing and Storing Blood Cryovials (Required)

Store the processed cryovials in labeled freezer storage boxes as described below.

1. Assign a sequential frozen shipment number to each frozen shipment you send to McKesson BioServices, starting with number "1". Do not use duplicate numbers for two batches since McKesson BioServices cannot track duplicate batch numbers. Do not skip numbers, since the skipped numbers indicate a missing or lost batch. **[Optional]**
2. Label any side of both the freezer storage box and lid with your CC number, frozen shipment number, and a box sequence number. The side you label becomes the front of the box. Start the box sequence number with "1" for each month's shipment. For example, use "43-011-2" for the CC with an ID number of 43, frozen shipment number 11, and box number 2. (Labeling the sides of the freezer boxes sent to McKesson BioServices is optional and is no longer required.) **[Optional]**
3. Insert the storage box divider into the box.
4. Place the frozen cryovials in the freezer storage box. You can put the cryovials in any order in the box. For this description, the positions in the storage box are numbered from 1 to 81, starting with the front left, and running from left to right in rows from front to back. See *Figure 11.7 - Specimen Storage Box*. Starting with position number 1 and continuing to fill the box in sequence until the box is full can help you locate a sample if necessary. If all the cryovials will not fit in one box, fill the current box and then begin filling the next storage box. You can put cryovials from different participants in the same box and samples from one participant in two separate boxes.
5. Place the box in the -70°C freezer. Samples must be frozen at least two hours before packing them for shipment to McKesson BioServices. If you do not have a -70°C freezer available, put the cryovials in a -20°C freezer immediately after aliquoting. Then transfer to a -70°C freezer as soon as possible but no longer than two days (over the weekend). Placing the samples on wet ice or dry ice does not sufficiently preserve the sample, so you must put the samples in at least a -20°C freezer. Do not thaw the samples after freezing. If frozen samples are thawed (i.e., the result of a freezer failure) contact the CCC for instructions.

If after freezing, you find a cryovial is broken, do not send it to McKesson BioServices. Place it in the biohazard container. Record the information of the breakage on a log sheet and edit the corresponding *Form 100 - Blood Collection and Processing* to indicate that you are not sending the broken sample to McKesson. A broken cryovial may indicate more than 1.8 ml of serum was added to the cryovial. Review the blood processing procedures to ensure blood processing staff do not overfill the vials.

11.3.5.1 Equipment Quality Assurance (Required)

- **Centrifuge**

Monitor the temperature of the refrigerated centrifuge daily using the temperature gauge on the centrifuge. Measure the temperature before centrifugation. Recording each temperature check on a log sheet is optional.

Once each month, check the temperature of the centrifuge using a certified temperature thermometer in the 0°C to 10°C range. Do this even if you have an internal digital thermometer, as this will then also check the accuracy of the digital thermometer. You may do this by laying the thermometer on the bottom of the centrifuge.

Once each year, check the speed of the centrifuge using a tachometer. Perform routine maintenance as suggested by the centrifuge manufacturer. A centrifuge maintenance log can be used to record these routine equipment checks and maintenance at the CC's discretion and is optional.

- **Freezer**

Monitor the temperature of the freezer daily using a certified low temperature thermometer in the -70°C range or a digit temperature display that may be on the freezer. If you use the digit display for the daily temperature checks, check the temperature of the freezer using a certified thermometer in the -80°C to -60°C range at least once each month. Recording each temperature check on a log sheet is optional.

Each month check the CO₂ tank to be sure it has not been emptied. Also test the alarm system to be sure it will sound should the freezer temperature rise above -50°C. As of January 1997, CCs are required to have an alarm system attached to each -70°C freezer. An appropriate system must have the capacity to monitor the freezer and to call multiple phone numbers should malfunction occur. See *Section 2.3.2.5 - Blood and Urine Collection, Processing, and Shipment* for recommended alarm system.

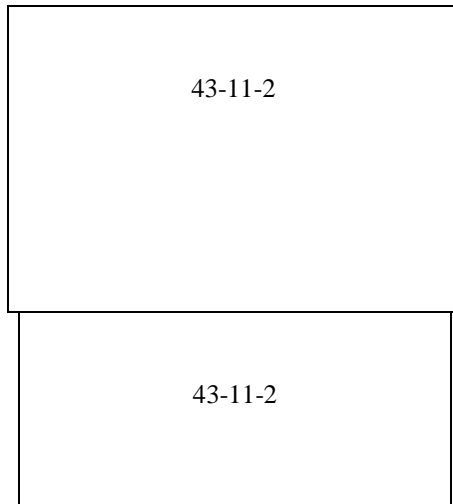
Perform routine maintenance as suggested by the freezer manufacturer such as routine freezer defrosting and record in freezer maintenance log. Make sure there are no samples in the freezer when you defrost it. You can coordinate the timing of the defrosting with the shipment of samples to McKesson BioServices; or use temporary storage in another freezer during the defrosting.

11.3.6 Summary of Blood Processing Procedures

1. Send blood sample aliquots to the local lab for WBC, hematocrit and platelet count and for triglyceride as needed (for HRT and HRT+DM participants with lipemic serum).
2. Allow royal blue tubes to sit at least 30 minutes before centrifuging. Refrigerate them and any other tubes if you cannot centrifuge within one hour. Centrifuge the royal blue tube and light blue tubes within two hours of collection. Centrifuge the 10 ml lavender tube within four hours of collection. Freeze all samples within two hours of collection.
3. Set up and label cryovials corresponding to the aliquots to be prepared.
4. Transfer serum and plasma into appropriate cryovials within 15 minutes post centrifugation.
5. Cap the vials securely.
6. Prepare the RBC and buffy coat aliquots.
7. Place cryovials in the freezer boxes and place in freezer, checking each cryovial number with the cryovials marked on *Form 100 - Blood Collection and Processing*.

**Figure 11.7
Specimen Storage Box**

Front view of box and lid



← Lid

Example:

43 - Clinical Center ID#
11 - Frozen shipment number
2 - Box 2 of this shipment

← Bottom

Top View of Box

73								81
64								72
55								63
46								54
37								45
28								36
19								27
10								18
1	2	3	4	5	6	7	8	9

Front

Numbers indicate position numbers inside of storage box

11.4 Blood Storage and Shipping

11.4.1 Shipping Schedule (Required)

Ship frozen serum samples to McKesson BioServices at least once every three months. Make shipments by mid- February, May, August, and November to ensure McKesson has sufficient time to log in the blood samples before the database consolidation at the end of the month. A quarterly shipping of blood specimens by the CC is required. CCs may make more frequent shipments at the CC's discretion. All CCs must ensure that frozen samples are shipped on a quarterly basis to McKesson BioServices regardless of the number of samples in the freezer.

Ship on a Monday, Tuesday or Wednesday. Do not ship on a day before a McKesson BioServices holiday. McKesson BioServices holidays are: New Year's Day, President's Day, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving, and Christmas.

Samples must be frozen at least two hours at -70°C before packing them for shipment. If the blood samples were drawn on the same day you plan to ship them to McKesson, to help with freezing, leave the lid off the freezer box until it is ready to be packed for shipment. If the insulated shipping container is not filled, stuff newspapers to stabilize the freezer boxes.

11.4.1.1 Mailing Instructions (Required)

Ship the fiberboard shipping box with frozen samples to McKesson BioServices by day or overnight courier to ensure receipt at McKesson BioServices within 24 hours.

There is no minimum number of cryovials to include in a shipment; ship all frozen cryovials regardless of the number of specimens that have been frozen and stored within the last collection period. However, CCs must follow current International Air Transport Association (IATA) regulations. As of January 1997, IATA Dangerous Goods Regulations have changed. The maximum allowable volume of diagnostic specimens allowed in a shipping container has increased from 500 ml to 4 liters (4000 ml). You can send up to 16 freezer boxes in the large shipping container. Include all the blood aliquots you have drawn and frozen.

Form 104 - Frozen Specimen Shipment has been replaced with the McKesson BioServices form Notification of WHI Shipment. CCs use this form to record shipping information and to order blood collection and shipping supplies. The CC is responsible for faxing the Notification of WHI Shipment form (Figure 11.7A) to McKesson when the CC sends a blood shipment. Upon receiving the blood shipment, McKesson will complete the "Confirmation of Receipt" section of the Notification of WHI Shipment form and send it to the CC. If the required WHI Notification of Shipment form is not sent to McKesson by the CC, then McKesson cannot return a Confirmation of Receipt.

11.4.1.2 Mailing Instructions for Visiting Participants (Required)

Two options may be used for ensuring blood samples are sent to McKesson when a participant has her blood drawn while she is visiting another CC:

- One option is to perform the blood draw and processing at the visiting CC and return the completed *Form 100 – Blood Collection and Processing* and frozen blood samples to the permanent CC.
- The second option is to perform the blood draw and processing at the visiting CC, return the completed *Form 100 – Blood Collection and Processing* to the permanent CC and ship the frozen blood samples directly to McKesson.

The option that you select to use should be a mutually agreeable process by both the "permanent CC" and the "visiting CC." Note that the work scope for the permanent CC is the same for both options. The work scope for the visiting CC is dependent on the option choice.

**Figure 11.7A
Notification of WHI Shipment**

NOTIFICATION OF WHI SHIPMENT
FAX TO (301) 838-9753

<p>CENTER INFORMATION:</p> <p>_____</p> <p align="center"><i>Center</i></p> <p>_____</p> <p align="center"><i>Name</i></p> <p>_____</p> <p align="center"><i>Phone Number</i></p> <p>_____</p> <p align="center"><i>Fax Number</i></p> <p>SHIPPING INFORMATION:</p> <p>Number of Shipping Boxes: _____</p> <p>Number of Freezer Boxes: _____</p> <p>Shipment date: _____</p> <p>Airbill Numbers: _____</p> <p>_____</p> <p>_____</p> <p>_____</p> <p align="center">CONFIRMATION OF RECEIPT</p> <p>Date of Receipt: _____</p> <p>Number of Boxes Received: _____</p> <p>Samples were received:</p> <p>_____ Frozen</p> <p>_____ Thawed</p> <p>COMMENTS: _____</p> <p>_____</p>	<p>SEND THE FOLLOWING SUPPLIES:</p> <p align="center">BLOOD COLLECTION SUPPLIES</p> <p>Serum Tubes (Royal Blue) 7ml No additives – for trace elements</p> <p>100 tubes/pk _____ pk</p> <p>10 tubes/pk _____ pk</p> <p>Plasma Tubes (Lavender) 10ml Power additive – EDTA (Na2)</p> <p>100 tubes/pk _____ pk</p> <p>10 tubes/pk _____ pk</p> <p>Plasma Tubes (Light Blue) 4.5ml 3.8% Buffered Citrate Solution</p> <p>100 tubes/pk _____ pk</p> <p>10 tubes/pk _____ pk</p> <p align="center">STORAGE SUPPLIES</p> <p>Cryovials: 100 vials/bag _____ bag</p> <p>Freezer Storage boxes _____ boxes includes 81 cell divider</p> <p align="center">LABLES</p> <p>Barcode Labels (Blood) _____ sets Shipped in groups of 100</p> <p>Barcodes Labels (Urine) _____ sets Shipping in sets of 100</p> <p align="center">SHIPPING SUPPLIES</p> <p>Ziplock Bags _____ groups Shipped in groups of 5</p> <p>Vial Shipping Boxes (Lg) _____ boxes Holds 8-12 freezer storage boxes</p> <p>Vial Shipping boxes (Sm) _____ boxes Holds 1-5 freezer storage boxes</p>
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Rev. 12/02

Option One

The permanent CC has responsibility for the following:

- Complete as much of the visit as possible by phone and/or mail.
- Create a new employee ID in WHILMA and give it the employee name “other CC.”
- Apply a participant ID label, complete the employee ID (“other CC”), visit type and visit year. Clip a set of blood labels to the *Form 100 – Blood Collection and Processing*.
- Send the *Form 100 – Blood Collection and Processing* to the visiting CC that will perform the blood draw along with a self-addressed envelope to facilitate the form’s return.

The visiting CC has responsibility for the following:

- Perform the blood draw. Record the name of the staff person drawing the blood alongside the “drawn by” box.
- Process and freeze the blood samples. Record the name of the staff person processing the blood alongside the “processed by” box.
- Return the completed *Form 100 – Blood Collection and Processing*, including CBC results, applicable for the OS AV3, to the permanent CC in the self-addressed envelope (or enclose it in an airtight plastic bag in the shipping container along with the blood samples).
- Ship the frozen blood samples to the permanent CC to ship to McKesson.
- Phone, FAX or email the permanent CC to advise of the shipment.

Option Two

The permanent CC has responsibility for the following:

- Complete as much of the visit as possible by phone and/or mail.
- Create a new employee ID in WHILMA and give it the employee name “other CC.”
- Apply a participant ID label, complete the employee ID (“other CC”), visit type and visit year. Clip a set of blood labels to the *Form 100 – Blood Collection and Processing*.
- Send the *Form 100 – Blood Collection and Processing* to the visiting CC that will perform the blood draw along with a self-addressed envelope to facilitate the form’s return.

The visiting CC has responsibility for the following:

- Perform the blood draw. Record the name of the staff person drawing the blood alongside the “drawn by” box.
- Process and freeze the blood samples. Record the name of the staff person processing the blood alongside the “processed by” box.
- Return the completed *Form 100 – Blood Collection and Processing*, including CBC results, applicable for the OS AV3, to the permanent CC in the self-addressed envelope.
- Email McKesson to notify them that additional blood samples from another CC will be included in the routine monthly shipment of blood. Include the permanent CC’s two digit ID number in the email.
- Place a note on the top of the styrofoam cover, on the insulating portion of the shipping box, indicating that the shipment contains blood samples from another CC.
- Place a note on the top of the freezer box indicating that blood samples from another CC are included in the freezer box.
- Ship the blood samples to McKesson with the routine monthly shipment.

11.4.2 Packaging Instructions (Required)

Pack the freezer storage boxes of frozen blood and urine samples in the shipping boxes as follows:

(The shipping box is composed of an inner styrofoam insulating portion and an outer fiberboard shell).

1. On the day of shipment, complete the Notification of WHI Shipment form to provide shipment information and/or to request supplies from McKesson. FAX the form to McKesson at 301-838-9753 and retain a copy for your records.
2. Wrap the freezer storage boxes containing the WHI samples in absorbent material (paper towels, wadding, etc.).
3. Place the freezer storage boxes in a plastic bag, either self-sealed zip-lock bag or secured with a waterproof seal. Remove as much of the air from the bag as possible and seal.
4. If you are using the smaller shipping box, place a minimum of 5-8 lbs of dry ice nuggets on the bottom. Place a maximum of 5 freezer storage boxes on top of the dry ice layer and then scatter the remaining 5-8 lbs. of dry ice nuggets around the freezer storage boxes. A minimum of 10 lbs. of dry ice and a maximum of 16 lbs. of dry ice can be used for the small shipping box. This will allow the package to remain frozen for 48 hours in case the shipment is delayed.
5. If you are using the larger shipping box, place a 8-10 lbs. of dry ice nuggets on the bottom. Place a maximum of 16 boxes on top of the dry ice layer and then scatter the remaining 8-10 lbs. of dry ice nuggets around the freezer storage boxes. A minimum of 16 lbs. of dry ice and a maximum of 20 lbs. of dry ice can be used for the large shipping box. This will allow the package to remain frozen for 48 hours in case shipment is delayed.
6. Stuff any empty space in the shipping box with newspaper or absorbent material.
7. Place the styrofoam cover on the insulating portion of the shipping box with the Notification of Shipment Form taped to the top. DO NOT TAPE the styrofoam cover to allow the dry ice gas to escape. Close and tape the outer fiberboard shell of the shipping box by sealing the top and corners of the box with waterproof tape to keep as much cool air as possible from escaping. Do not use scotch or masking tape.
8. Attach the airbill holder to the front of the box. Place the following labels on the top of the shipping box, so that none of the labels touch each other.

<u>Label</u>	<u>Location on Fiberboard Box</u>
Clinical Center's return address label	Top, upper left corner
McKesson BioServices address label	Top, lower right corner
Black-and-white class "9" label	Top, under Return address
Priority overnight label (optional)	Top, upper right corner
Diagnostic specimen label	Anywhere on top
Keep Frozen label (optional)	Anywhere on top

See Figure 11.8 - Frozen Specimen Shipping Labels to see sample labels and placement of the labels on top of the fiberboard shipping box. You must follow the current federal regulations of labeling or face a possible fine of up to \$10,000 if proper labels are not in compliance.

9. Write in the amount of dry ice placed in the box on the black-and-white class "9" label. Record the weight in pounds or kilograms. Federal Express occasionally changes the weight units on the label. Record the weight in the units requested on the label. You can use the table below to convert from one unit to the other. Federal Express will return shipments which do not show the weight of dry ice.

<u>Pounds</u>	<u>Kilograms</u>	<u>Pounds</u>	<u>Kilograms</u>
10	4.4	16	7.3
11	4.8	17	7.7
12	5.3	18	8.2
13	5.7	19	8.6
14	6.2	20	9.1
15	6.6		

10. Complete the Federal Express airbill supplied by McKesson BioServices. It will already be partially completed. Enter:

- The date of the shipment.
- Weight of dry ice (in pounds or kilograms), as requested.
- Number of packages and total weight of shipment.

As with the dry ice label, record the weight requested on the label. Federal Express supplies the airbills to McKesson BioServices, and sometimes requests the weight in pounds and other times in kilograms.

Insert the airbill in the airbill holder. See Figure 11.9 - Federal Express Airbill for Frozen Specimens.

11. To delay thawing, place the box in the -70°C freezer to wait for pick up. You don't need to do this if you pack the box within 2-3 hours of pickup and you hold the box in a controlled temperature area (for example, at room temperature and not over 80°F).
12. Notify McKesson BioServices via email (or phone if email is not possible) the day you ship the specimens. In the message, include:
 - The CC name.
 - The date of the shipment.
 - The date of expected arrival (same day or next day).
 - The airbill number from the Federal Express label.

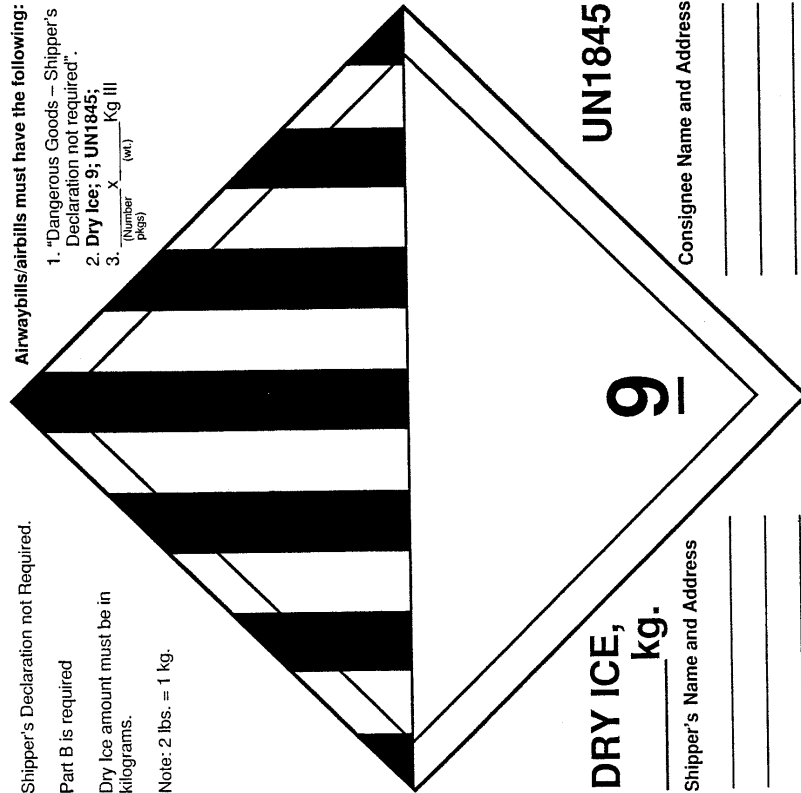
Figure 11.8
Frozen Specimen Shipping Labels



"DIAGNOSTIC SPECIMENS
PACKED IN COMPLIANCE WITH
IATA PACKING INSTRUCTION 650"

Airwaybills/airbills must have the following:
1. "Dangerous Goods – Shipper's Declaration not required".
2. **Dry Ice; 9; UN1845;**
3. (Number) X (wt) Kg III
 (plugs)

Shipper's Declaration not Required.
Part B is required
Dry ice amount must be in kilograms.
Note: 2 lbs. = 1 kg.



Donald Nolde
McKesson BioServices
625 Lofstrand Lane, Rockville, MD 20850
PH: 301-340-1620 FAX: 301-838-9753

HML-DI Printed by Labelmaster, An American Labelmark Co., Chicago, IL 60646 (800) 621-5808

11.4.3 Receipt of Frozen Specimen Shipment at McKesson BioServices

McKesson BioServices personnel will complete the Confirmation of Receipt section of the Notification of WHI Shipment form upon arrival of the shipment at the repository.

The McKesson BioServices staff will record:

- Date shipment arrived.
- Number of boxes received.
- Condition of the total shipment, for example, "thawed."

If McKesson BioServices staff do not receive the shipment on the expected day, they will trace the shipment using the airbill number.

McKesson BioServices will include a packing slip when sending blood collection and other supplies to the CC.

11.4.4 Summary of Blood Sample Shipping Procedures

1. Order dry ice.
2. Complete *Notification of WHI Shipment* form.
3. Notify McKesson BioServices of shipment by faxing them the Notification of WHI Shipment Form. Include on this form any orders for cryovials, labels, blood collection tubes and shipping supplies.
4. Pack freezer boxes in insulated shipping container with dry ice.
5. Place the insulated shipping container in the fiberboard shipping box.
6. Place Notification of WHI Shipment form in plastic bag and place in shipping box on top of the lid of the insulated shipping container.
7. Seal the fiberboard shipping box.
8. Label the box and complete Federal Express airbill.
9. Arrange for shipment.
10. McKesson will send the completed Confirmation of Receipt of WHI Shipment to the CC upon receiving the blood shipment.

11.5 Urine Collection

Participants at the three bone density CCs will be asked to provide a clean, midstream, first-morning urine samples at selected visits during the course of the study. Collect a urine sample even if the participant has a urinary track infection.

Although the midstream clean-catch technique has been a standard practice for collecting urine specimens from participants for bacterial culture purposes, results of a recent study (New England Journal of Medicine, Vol. 328, p. 289-90, 1993) demonstrated that there was no difference in the rate of bacterial contamination between midstream urine specimens obtained and those that were obtained without prior cleansing of the perineum and urethral meatus.

11.5.1 Schedule of Urine Collection

See *Vol. 1, Table 1-A1.1 - Frequency of Data Collection* for a schedule of urine collection in CT and OS participants.

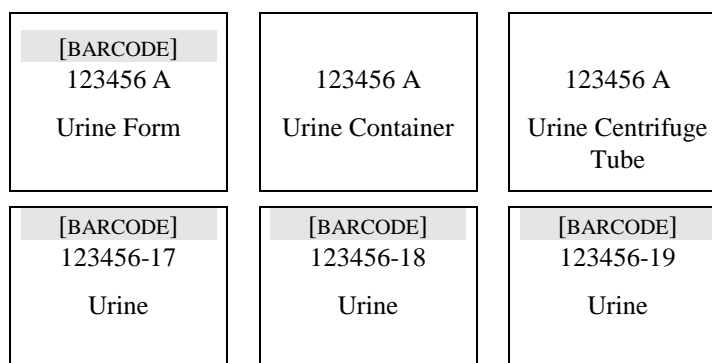
11.5.2 Urine Sample Labels

McKesson BioServices provides urine sample label sets similar to the blood sample label sets described in *Section 11.2.1 - Blood Sample Labels*. The only difference between the blood sample labels and the urine sample labels is the number of labels and the specified 2-digit cryovial numbers.

A urine sample label set consists of the following labels:

- One label for *Form 101- Urine Collection and Processing*.
- One label for the urine collection container.
- One label for the centrifuge tube.
- Three labels for the urine cryovials. Each label contains the 6-digit urine number and a 2-digit cryovial number (-17, -18, and -19) with corresponding barcodes, and "Urine" printed on the labels. See *Figure 11.10 - Urine Sample Labels*.

Figure 11.10
Urine Sample Labels



11.5.3 Preparation Before the Visit

- Determine if the participant is to provide a urine sample.
- Label a 30 ml urine container with the participant's ID barcode label.

- Give or mail to the participant the container and instructions for collecting the urine. Instruct her on how to obtain the urine specimen by giving her the urine instruction sheet. (See

Figure 11.11 - WHI Urine Home Collection for the instruction sheet.)

- • During screening, mail the labeled urine container and a ziploc bag with instructions to the participant before SV1 or give to her at an SV0 visit (if your CC conducts SV0 visits). Ask her to bring in the urine sample at SV1.
- • At designated follow-up visits, mail the labeled urine container, ziploc bag, and instructions to the participant with the self-administered forms you send her before the visit.
- Attach a urine sample label set to *Form 101 - Urine Collection and Processing* and insert the form in the participant's file with the other visit packet forms.

Figure 11.11
WHI Urine Home Collection

Instructions for Home Urine Collection
<ul style="list-style-type: none"> • Please collect a urine sample on the morning of your visit. Collect the sample in the enclosed container and place in the ziploc bag. • Collect the sample midstream when you <u>first</u> urinate in the morning sometime <u>after</u> 5 AM. • Please urinate directly into the container. Fill the container about 2/3 full. If you cannot use the container, use a clean glass jar and transfer the urine into the container. <p style="text-align: center; margin-top: 10px;">Write the time you collected the urine on the label on the container. Put the lid on the container and put the container in the ziploc bag. Then put the container in the refrigerator or on ice until you leave for the visit.</p>

11.5.4 At the Visit

If the participant brings a urine sample to the visit, label and complete the *Form 101 - Urine Collection and Processing* as follows:

- Attach the participant ID barcode label to the *Form 101 - Urine Collection and Processing*.
- Attach the label "Form" from the urine label set to the form and the label "Container" to the urine container the participant brought in.
- Ask the participant the time she collected the urine sample, and record the time in the appropriate place on *Form 101*.
- Take the labeled urine container and *Form 101 - Urine Collection and Processing* to the specimen processing area.

If the participant does not bring a urine sample to the visit, collect a sample at the visit. Note the number of prior voids (after 5 AM) on the *Form 101* next to the time processed. Label and complete *Form 101* as described above.

11.6 Urine Processing

1. Process the urine sample within 30 minutes of receipt. Refrigerate the specimens if they are not processed immediately. If urine is allowed to sit at room temperature, bacteria that may be present in the urine may multiply or chemicals and cells in the urine may deteriorate, causing changes in the composition of the urine.
2. Label a 15 ml conical plastic centrifuge tube with the urine sample label "centrifuge tube."
3. Transfer 10 ml of specimen from the urine collection container into the tube, screwing the cap on tightly.
4. Centrifuge for five minutes at 1,300 xg. Refer to *Section 11.3.2 - Operating the Refrigerated Centrifuge* for instructions on how to obtain 1,300 xg.
5. Label three cryovials with the cryovial labels (numbered -17, -18 and -19).
6. Transfer three 1.8 ml aliquots of urine supernatant into each of the three cryovials. Be careful not to fill past the 1.8 ml mark on the tube, as doing so may cause the tube to crack when frozen. Screw the lids onto the cryovials.
7. Complete the Urine Processing portion of *Form 101 - Urine Collection and Processing*. Mark the box corresponding to each cryovial you processed to indicate you prepared the cryovial.
8. Replace the urine container lid and discard the urine container in the biohazard container.

11.7 Freezing, Storing, and Shipping Urine Samples

1. Freeze the urine cryovials following identical procedures for freezing blood sample cryovials. See *Section 11.3.5 - Freezing and Storing Blood Cryovials* (Required). You can place the urine cryovials in the same box as the blood cryovials.
2. Store and ship the samples following procedures for blood storage. See *Section 11.4 - Blood Storage and Shipping*. You may place the urine cryovials in the same shipment box with the frozen blood sample cryovials.

Section 11
Blood and Urine Collection, Processing and Shipment

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