

**SPECIMEN COLLECTION**

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## 1. Background and rationale

During the MOST baseline clinic visit we will collect fasting blood for plasma, serum, and buffy coat samples for analysis of Vitamin C, Vitamin D, Vitamin E, PTH (parathyroid hormone), future genetic analyses, and archiving. We will also collect a urine specimen for future analyses.

During the MOST 30-month follow-up clinic visit we will collect a fasting blood (for plasma and serum) and a second morning urine specimen for future analyses. We will collect these specimens from a sample of study participants only. The Data from Prior Visits reports will let you know who is selected for specimen collection at the 30-month visit. Participants were told at the time of the Telephone Interview that they were selected for specimen collection and sent Previsit Instructions telling them to not eat or drink anything other than water (except for regular medications and vitamins) prior to their clinic visit. For all clinic visits, participants selected for specimen collection are to be scheduled for their clinic visit prior to 9am.

The MOST baseline clinic visit involves the collection of approximately 44mL of blood in four draw tubes and a urine specimen from participants. The MOST follow-up clinic visit involves the collection of approximately 15mL of blood in two drawtubes and a urine specimen from participants. Since the study depends on the voluntary return of participants over an extended period of time, every effort must be made to make the entire procedure as easy and painless as possible both for the participants and for the Field Center personnel.

Participants will complete a Human Subject Protection informed consent. The consent statement should inform the study participants that there is a small risk of bruising at the spot on the arm where the blood is taken and that about two tablespoons of blood are drawn at the baseline visits and one tablespoon of blood is drawn at follow-up visits.

### 1.1 Overview of blood and urine collection

#### Baseline visit

<b>Collection tube #1 and #3:</b> 7mL EDTA lavender tops for plasma
-Vitamin C
-Vitamin D
-plasma for future analyses
-buffy coat for future DNA analyses
<b>Collection tubes #2 and #4:</b> 15mL red tops for serum
-Vitamin E
-PTH
-serum for future analyses
<b>Urine:</b> urine collection cup (4.5 mL urine specimen)
-urine for future analyses

**Follow-up visit**

<b>Collection tube #1:</b> 3 to 5mL EDTA lavender top (yield 1.5 mL plasma)
<b>Collection tube #2:</b> 7 to 10mL red top or tiger top (yield 3.5 mL serum)
<b>Urine:</b> urine collection cup (2.0 mL urine specimen)
-urine for future analyses

**2. Equipment and supplies****2.1 Sample ID labels**

You will be supplied with barcode specimen ID labels to use for labeling forms, drawtubes, urine specimen cup, PCA mixing tube, cryovials, and the Cryovial Storage Box Grid form. A sample of the labels can be found in Appendix 1. All labels have a barcode and the same 5-digit specimen-specific ID number. *The process of matching the participant-specific 7 digit MOST ID# to the specimen-specific 5 digit barcode ID is crucial to being able to use the data collected from laboratory tests and will be reviewed in detail in section 5.*

**Labels for baseline visit**

- Four for pre-labeling the 4 draw tubes
- Two extras for back-up vacutainers
- One for the urine specimen cup
- One for the tube that will be used to mix the plasma with PCA.
- One for the “Specimen Collection form”
- One for the “Laboratory Processing form”
- 25 for cryovials
- 25 for the Cryovial Storage Box Grid form

**Labels for follow-up visit**

- Two for pre-labeling the 2 draw tubes
- Two extras for back-up draw tubes
- One for the urine specimen cup
- One for the collection form
- One for the laboratory processing form
- 14 for cryovials
- 14 for the Cryovial Storage Box Grid form

## 2.2 Blood collection trays and tubes

- Blood drawing trays are prepared one day in advance
- Stocked with a full supply of blood drawing equipment
- Labeled tubes/cryovials and organization of specimen labels for 4 to 6 participants
- “Wet ice” bath container (“wet ice” = 1:1 ratio of crushed ice + water)
- Rack of blood collection tubes for each participant
- Foil for wrapping blood collection tubes after filled (baseline visit only)
- Sheath for filled blood collection tubes (follow-up visit only)
- Rack of 25 cryovials for the serum, plasma, buffy coat, and urine aliquots (baseline visit only)
- Rack of 14 cryovials for the serum, plasma, and urine aliquots (follow-up visit only)

### 2.2.1 Blood collection tray

The collection tray itself is made of hard plastic, which is unbreakable and can be easily cleaned. The tray has compartments, which are filled with the following supplies:

Alcohol swabs	Band-Aids
Tourniquets (2)	Smelling salts
21G Butterfly needles with Luer adapter	Scissors
23G Butterfly needles with Luer adapter	Gauze
Back-up vacutainers (lavender & red top)	Adhesive tape
Vacutainer holders	Pens
Needle/sharps container	Latex gloves
Container for “wet ice” bath filled ~10 min before draw	
Sheaths for blood collection tubes (30-month visit only)	

### 2.2.2 Blood collection rack: labeling and setup

The day before the scheduled study visit, all the necessary blood draw and laboratory processing supplies for each participant will be prepared. The blood collection tubes should be pre-labeled with the specimen-specific ID labels and placed in the tube rack. This rack will fit into the blood collection tray. The cryovials should be pre-labeled with the barcode specimen-specific ID labels and placed in the aliquot rack. Label orientation on the cryovial is shown in Appendix 2.

#### Baseline visit

There are a total of 60 specimen-specific labels per participant. After 29 labels have been used for setting up the blood collection rack (4) and the aliquot rack (25), there will be 31 labels left:

- 2 labels for the “Backup Vacutainers”
- 1 label for the “Specimen Collection Form” label
- 1 label for the “Laboratory Processing Form”
- 1 label for the urine cup
- 1 label for plasma/PCA mixing tube

- 25 labels for the cryovial storage box grid sheet

These can be separated into 2 mini-sheets:

1. The “Backup Vacutainers,” urine cup, “Specimen Collection Form,” and “Laboratory Processing Form” labels should be clipped to the blood collection tray.
2. The plasma/PCA mixing tube and Cryovial Storage Box Grid form labels should be clipped to the aliquot rack.

### Follow-up visit

There are a total of 35 specimen-specific labels per participant. After 16 labels have been used for setting up the blood collection rack (2) and the aliquot rack (14), there will be 19 labels left:

- 2 labels for the “Backup Vacutainers”
- 1 label for the “Specimen Collection Form” label
- 1 label for the “Laboratory Processing Form”
- 1 label for the urine cup
- 14 labels for the cryovial storage box grid sheet

These can be separated into 2 mini-sheets:

1. The “Backup Vacutainers,” urine cup, “Specimen Collection Form,” and “Laboratory Processing Form” labels should be clipped to the blood collection tray.
2. The Cryovial Storage Box Grid form labels should be clipped to the aliquot rack.

### 2.2.3 Description of blood collection

Each drawtube is color-coded to aid in handling.

#### Baseline visit

Tubes #1 and 3 are 7mL lavender stoppered tubes containing 15% EDTA as the anticoagulant. After drawing, the EDTA tube is inverted 10 to 15 times minimum, wrapped in foil and immediately placed on "wet ice."

Tubes #2 and 4 are 15mL red stoppered tubes. These tubes contain no anticoagulant so that the blood clots to form serum. After drawing the blood, the tubes are immediately wrapped in foil and then placed in the rack at room temperature.

#### Follow-up visit

Tube #1 is a 3mL to 5mL lavender stoppered tube containing 15% EDTA as the anticoagulant. After drawing, the EDTA tube is inverted 10 to 15 times minimum, placed in a sheath to protect it from light exposure and immediately placed on "wet ice."

Tube #2 is a 7mL to 10mL red stoppered tube. This tube contains no anticoagulant so that the blood clots to form serum. After drawing the blood, the tube is immediately placed in a sheath to protect it from light exposure and then placed in the rack at room temperature.

## 2.2.4 Priority of tubes

### Baseline visit

A total of approximately 44 mL of blood will be drawn from each participant in 4 tubes. Tubes are numbered 1 to 4 and arranged in the rack to be drawn in the following order of priority:

1. EDTA 7mL           lavender top
2. Serum 15mL       red top
3. EDTA 7mL           lavender top
4. Serum 15mL       red top

### Follow-up visit

A total of approximately 15 mL (1 Tablespoon) of blood will be drawn from each participant in 2 tubes. Tubes are numbered 1 and 2 and arranged in the rack to be drawn in the following order of priority:

1. EDTA 3-5mL           lavender top
2. Serum 7-10mL       red top

## 2.2.5 Blood mixing during venipuncture

Each tube should be treated as follows:

**EDTA:** invert 10 to 15 times minimum, foil wrapped (baseline) or sheathed (follow-up) to protect from light exposure, and then placed in "wet ice" bath. Process within 15 minutes (see Laboratory Processing chapter).

**Serum:** foil wrapped (baseline) or sheathed (follow-up) to protect from light exposure, and then placed in blood collection rack at room temperature for 30 minutes.

Refrigerate after 30 minutes if not processed. Process within 60 minutes after blood draw (see Laboratory Processing chapter).

## 3. Safety issues and exclusions

### 3.1 Precautions for handling blood specimens

In accordance with the OSHA regulations on blood-borne pathogens (see OSHA regulations manual), the following laboratory safety protocols are recommended for the field centers:

- Non-permeable lab coats, latex gloves, and face shields should be used when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.
- 'Universal Precautions' should be followed when handling any blood products.

- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak proof container. Never recap or break needles.
- Hepatitis B vaccine should be offered to all unvaccinated technicians handling blood and documentation of vaccination or technicians declining to be vaccinated should be kept.

## 3.2 Participant precautions and exclusions

**3.2.1 Participant biospecimen collection questionnaires** See section 9 for copies of forms for all visits. At the baseline visit, all participants are eligible for fasting blood and urine collection. At the 30-month follow-up visit only selected study participants will have fasting blood collection and fasting second morning void urine collection. The Eligibility for Biospecimen Collection form is completed to determine eligibility for biospecimen collection. Question 1 asks the examiner to determine if the participant is in the specimen collection cohort for this visit. This information is provided on the 30-Month Clinic Visit Data from Prior Visits Report. If the participant was not selected, urine is collected only if a female participant requires a urine pregnancy test, and blood is not collected. If the participant was selected for biospecimen collection, the examiner continues to the Urine Collection Form.

Urine Collection form: For all visits, the bar code label is placed in the appropriate area of the form and the 5-digit ID from the bar code label is entered in the text box. Questions are asked about fasting, whether a urine specimen was collected and the time of urine collection.

For all visits except baseline, the examiner is asked to confirm that a urine specimen was collected, and whether the urine was a first, second, third or fourth (or later) urine void. The goal is to collect a second morning urine void. Do not aliquot a first morning void unless a later void is not obtained. If one void is an insufficient volume, it is permissible to combine two specimens, i.e. second and third void, as long as neither is the first morning void.

Document the time of the urine collection in nonmilitary time hours and minutes, and whether it was am or pm.

The examiner asks the participant and documents the date and time that the participant last ate or drank anything other than water. Please document the hour in nonmilitary time and mark am or pm. If two specimens are collected, document the time that the later specimen was collected. The participant was told at the telephone interview and written instructions were sent to the participant before the clinic visit telling them not to eat or drink anything EXCEPT WATER the morning of the clinic visit and to provide a second morning void at the clinic. All biospecimen collection cohort participants were to be scheduled for clinic visit at or before 9:00 am. Even if a participant has not fasted, you should go ahead and collect the blood and urine, indicating the date and time that they last ate or drank anything on the Urine Collection form.

Ask the participant what time they got up for the day and document the response. Some participants wake up at an early time and then return to bed. If the hour is early in the morning,

please confirm that the time is when they actually stayed up for the day. There is an optional space to document comments about urine collection.

At the 30-month visit only, mark whether or not a pregnancy test is required to determine if it is safe to obtain x-rays and MRI examinations. When a urine pregnancy test is required, mark whether the test was positive, negative or if the participant refused the test. A female participant that has a positive pregnancy test or refuses a test is not eligible for radiographs (x-rays) or MRI examinations.

Phlebotomy form: Phlebotomy will not be done on an arm that has had a shunt or port placed for kidney dialysis. Female participants will be asked if they have ever had a radical mastectomy as blood will not be drawn from the arm on the same side that a radical mastectomy was done. A urine specimen will be collected and stored for participants who cannot safely have blood drawn from either arm. Which arm can safely be used for phlebotomy is documented.

The follow-up visit questionnaire documents whether the participant has been ill in the past week requiring antibiotic treatment, hospitalization, or treatment with steroids. All visit questionnaires ask participants if they bleed or bruise easily. They are asked if they have ever been told that they have a disorder related to blood clotting or coagulation. If participants report that they have had problems with excessive bleeding or bruising at a venipuncture site, use your own judgment to decide whether or not a clinic physician or nurse supervisor should be consulted.

If the participant has experienced fainting spells during phlebotomy, ask the participant the frequency of fainting spells. If the participant frequently faints, again, use your own judgment to determine whether or not a consultation with the clinic physician or nurse supervisor is necessary. Provide smelling salts, basin, and a cold cloth if needed. See Appendix 4 for precautions when a participant feels faint.

### **3.3 Participant refusal to provide urine sample or refusal of phlebotomy**

Rarely, a participant will refuse phlebotomy or to provide a urine sample. Please keep a list of the MOST ID #s of any of these participants and identify which test they refused.

## **4. Participant and exam room preparation**

### **4.1 Phlebotomy room**

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



## 4.2 Preparation for phlebotomy

Preparation for phlebotomy is done in the following manner. Early morning, before any participants arrive:

- Check to make sure that the blood collection tray is properly equipped. Every item on the checklist (Appendix 3) must be ready before proceeding.
- Check that the vacutainer tubes are properly labeled with the specimen-specific ID labels.
- Check that the specimen processing station is properly equipped (see Laboratory Processing chapter).
- Make sure the phlebotomy room is tidy and stocked with extra smelling salts, basin, and disposable washcloths.

Approximately 10 minutes before scheduled participant arrival:

- Fill “wet ice” bath container 1/2 full with a 1:1 ratio of crushed ice and water.

## 4.3 Preparation of participants for urine collection

Urine will be collected on all participants selected for biospecimen collection whenever possible. Collection will be the second void of the day whenever possible. *Urine should be collected before venipuncture*. Samples should not be collected after exertion or an acute fluid load; however, participants should be encouraged to stay hydrated even while fasting for the visit. Participants having difficulty with producing a urine specimen may be offered a glass of water, and another urine specimen may be collected later in the visit to bring the volume up to the required amount.

Female and male participants may urinate directly into the specimen collection container. Containers must have a tight-fitting lid to prevent leakage during transportation.

### 4.3.1 Instructions for participants

The participant’s privacy should be assured. They should perform the following steps:

1. Have equipment ready with label on specimen cup.
2. Remove the cap from the collection container.
3. Void directly into the collection container until nearly full.
4. Carefully seal the cap of the container so that it is tight and leak proof.

#### 4.4 Preparation of participants for phlebotomy

It should be stressed that this study requires the voluntary cooperation of the participants. People are donating both time and blood on a purely voluntary basis, with no reward other than the knowledge that they are contributing to progress in medicine. Thus, the whole experience must be made as pleasant as possible. Four tubes of blood (44mL) are collected at baseline and two tubes of blood (15mL) are collected at follow-up visits. Any participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is about 2 tablespoons (baseline) or 1 tablespoon (follow-up visits), although it may look like more. The phlebotomist may also assure participants that they donate 450 mL when they donate a unit of blood to a blood bank which is 10 times as much blood (baseline) and 30 times as much blood (follow-up visits).

### 5. Detailed procedures

#### 5.1 Forms

The Eligibility for Biospecimen Collection (30-month follow-up visit only) and specimen collection forms are in section 9. **These forms provide a vital link between the specimen-specific ID# and the participant's study ID#, and facilitate the collection and archiving of plasma, serum and urine biological samples from participants.** The Eligibility for Biospecimen Collection form is completed to confirm that the participant was selected for specimen collection. The participant is no longer eligible if a total knee replacement was done, so that question is asked prior to proceeding with the measurement. The collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the forms facilitate the monitoring of phlebotomy and other quality assurance parameters. All forms must be completed in black ink.

The specimen collection forms have the following purposes:

1. Assure the most efficient and safest possible venipuncture for participants.
2. Allow the monitoring of the quality of the above procedures.
3. Allow efficient processing of the samples.
4. Provide information critical to the interpretation of the assay results.

The participant will arrive at the phlebotomy station with their MOST ID# and acrostic preprinted on the top of their specimen collection and Laboratory Processing forms. Complete the date and MOST Staff ID# on the top of both of the forms.

The specimen ID has already been assigned and you will note that 5 digit ID# on the prelabeled tubes and the urine collection cup is correct. It is vital that this same specimen-specific ID be matched up with the participant's MOST ID# on the specimen collection form and the Laboratory Processing form. There will be a small sheet of labels clipped to the rack of vacutainers with a "Collect form" label and a "Lab Form" label. Affix the bar-coded "Collect

Form” label to the specimen collection form and write that number in the space provided. This should be done before drawing any blood, to insure that this critical task is not forgotten.

## 5.2 Phlebotomy

### 5.2.1 General

The venipuncture is performed with a 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. A 23- gauge needle may be used, if necessary, for a difficult draw, *but this must be noted on the specimen collection form under “Comments on blood collection/phlebotomy.”* The butterfly has a small, thin-walled needle, which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, they may be reassured to know such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with the technician who draws the blood and their general attitude and competence.

If the participant is nervous or excited, the technician briefly describes the procedure. Sample script (baseline visit): *"I am going to be drawing about 2 tablespoons of blood. This blood will be used in tests for Vitamins C, E, and D. We hope to be able to use the results of these tests to better understand how nutrition relates to knee problems."* Sample script (follow-up visits): *"I am going to be drawing about 1 tablespoon of blood. We hope to be able to use the results of these tests to better understand knee osteoarthritis."*

### 5.2.2 Handling participants who are extremely apprehensive about having blood drawn

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

### 5.2.3 Venipuncture procedure

- Wear Latex gloves and a lab coat.
- Arrange draw tubes in order of draw (see below) on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.
- Apply tourniquet.

- Examine participant's arms for the best site for venipuncture. Generally the antecubital vein is preferred, if feasible. Release tourniquet.
- Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.
- Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be 1 or 2 inches below the venipuncture site.
- With the needle bevel upward, enter the vein in a smooth continuous motion.
- Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.
- Grasp the flange of the vacutainer holder and push the tube forward until the blunt end of the needle punctures the stopper, exposing the full lumen of the needle.
- Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly.
- Remove the tourniquet at 2 minutes. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. If blood flow ceases after the tourniquet is removed, it may be reapplied for another 2 minutes.
- 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 and stopping of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.
- Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach one of your extra, backup tubes of the same type without removing the needle from the vein. Be sure to place one of the specimen-specific bar-coded "X-tra Lav top" or "X-tra Red top" labels on that tube after completing phlebotomy.
- When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until the next tube is inserted.
- As lavender top tube (plasma) is removed, mix by gently inverting at least 10 to 15 times, wrap in foil or sheath to protect from light, and place in wet ice bath.
- As red top tube (serum) is removed, wrap in foil or sheath to protect from light and place in rack on the blood collection tray.
- Average venipuncture time is 3 to 6 minutes, but any difficulties may increase this time to 10 or 15 minutes.

#### 5.2.4 Removing the needle

- To remove the needle (after the tourniquet is removed), lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply gentle pressure to the site with a gauze pad. Discard needle into a puncture-proof sharps container.
- Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma.

### 5.2.5 Bandaging the arm

Under normal conditions:

- Slip the gauze pad down over the site, applying mild pressure.
- Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
- Tell the participant to leave the bandage on for at least 15 minutes.

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.

### 5.2.6 Completing the blood drawing procedure

- Dispose of needle and tubing in the appropriate biohazard needle sharps containers.
- Complete the specimen collection/phlebotomy form. This includes checking which collection tubes were filled, time of blood draw, and writing comments about any difficulties with the phlebotomy under “Comments on blood collection/phlebotomy.”
- Clean up the venipuncture area (if necessary).
- Bring blood collection tray to the processing area with the filled vacutainer tubes and Laboratory Processing form.

### 5.2.7 Procedures for difficult draw

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

- If there is a sucking sound, turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.
- If no blood appears, move needle slightly in hope of entering the vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
- Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.
- DO NOT attempt a venipuncture more than twice unless a participant encourages you to do so.
- Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on their part.
- If venipuncture is unsuccessful, participant should be rescheduled at a later date, preferably with a different phlebotomist.
- Document any problems with venipuncture and sample collection on the specimen collection/phlebotomy form. In particular, note whether a vein other than one of the antecubital veins was used.

### 5.2.8 Other possible problems

1) Not all tubes are collected (blood flow ceases, difficult venipuncture, etc.) *Always fill the collection tubes in the order specified.* Make notations of difficulties on the specimen collection/phlebotomy form. If the participant is willing, another attempt should be made to complete the draw.

2) Collection tube does not fill: First, try another tube of the same type. A partially filled plasma tube is **not** acceptable if less than 2/3 full. *Do not send partially filled plasma tubes for processing.* Partially filled tubes for serum are okay, but will result in a reduced number of aliquots. If a tube is not completely filled, check “No” on the specimen collection/phlebotomy form.

## 6. Procedures for performing the measurements at home

If the clinical centers choose to schedule participants for an afternoon appointment, participants may perform the urine specimen collection at home (second morning void), refrigerate, place the specimen in a paper bag, and then bring the sample to the clinic within 24 hours.

## 7. Alert Values/Follow-up

All specimens will be stored for later analyses. No reports will be available to the participants.

## 8. Quality assurance

### 8.1 Training requirements

Clinical experience with phlebotomy is mandatory. Additional training should include:

- Read and study manual
- Observe procedure by experienced examiner
- Discuss problems and questions with local expert or QC officer

### 8.2 Certification requirements

- Complete training requirements
- Explain what to do for difficult venipuncture
- Recite measures to take for fainting participant
- Conduct phlebotomy on volunteer or participant while being observed by QC officer using QC checklist

### 8.3 Quality assurance checklist

Eligibility for Biospecimen Collection form: (30-month visit only)

- Eligibility is determined correctly

Specimen/Urine Collection form:

- Specimen ID barcode label affixed to upper right corner and 5-digit ID# entered
- Fasting time correctly calculated

Phlebotomy preparation:

- Blood collection trays properly prepared
- Blood draw tubes properly labeled
- Questions on Specimen Collection/Phlebotomy form asked
- Hepatitis B vaccination given or offered to all personnel handling blood

Venipuncture properly carried out:

- Script properly delivered
- Non-permeable lab coats, gloves, and face shields used
- Preparation of venipuncture site correctly done
- Venipuncture smoothly done
- Tubes filled in proper priority order
- Plasma tube at least 2/3 full
- Tourniquet removed at 2 minutes
- Needle removed and arm bandaged correctly
- Needle and tubing appropriately disposed

Tubes mixed and handled correctly after filling:

- Lavender top tube inverted at least 10-15 times minimum, wrapped in foil/put in sheath and then placed in ice bath
- Red top tube wrapped in foil/put in sheath and then placed in rack at room temperature


Specimen/Phlebotomy form properly filled out

- Arm safe to use for phlebotomy questions correctly filled out
- Documentation of tube status and time of draw correctly filled out

9. Data Collection Forms

9.1 Baseline visit forms

### Specimen Collection

 30953	MOST ID # [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ] <i>Office Use Only</i>	Acrostic [ ][ ][ ][ ][ ][ ][ ][ ][ ]	Date Form Completed [ ][ ] / [ ][ ] / 200[ ][ ] Month / Day / Year	Staff ID# [ ][ ][ ][ ][ ][ ][ ][ ][ ]
--------------------------------------------------------------------------------------------	-----------------------------------------------------------------------	-----------------------------------------	--------------------------------------------------------------------------	------------------------------------------

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**1** What is the date and time you last ate or drank anything except water?

a. Date: [ ][ ] / [ ][ ]  
          Month      Day

b. Time: [ ][ ] : [ ][ ]     am  
          Hours    Minutes     pm

c. How many hours has participant fasted? [ ][ ] Hours  
*(Note: Proceed with the blood draw even if participant has not fasted.)*

**7** **Bar Code Label**

Enter ID from Bar Code label:  
[ ][ ][ ][ ][ ][ ][ ][ ][ ]

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**2** Do you bleed or bruise easily?  
 Yes    No    Don't know/Refused

**3** Have you ever been told you have a disorder related to blood clotting or coagulation?  
 Yes    No    Don't know/Refused

**4** Have you ever had a shunt or port for kidney dialysis?  
 Yes    No    Don't know/Refused

Which side?  
 Right     Left     Both  

Draw blood on left.

Draw blood on right.

Do NOT draw blood.

**8** Was any blood drawn?  
 Yes     No

Please describe why not: \_\_\_\_\_

Were tubes filled to specified capacity?  
*(Note: wrap all tubes in foil.)*

Tube	Volume	Filled to Capacity
1. EDTA	7mL	<input type="radio"/> Yes <input type="radio"/> No
2. Serum	15mL	<input type="radio"/> Yes <input type="radio"/> No
3. EDTA	7mL	<input type="radio"/> Yes <input type="radio"/> No
4. Serum	15mL	<input type="radio"/> Yes <input type="radio"/> No

Time of blood draw: [ ][ ] : [ ][ ]     am  
                                 Hours    Minutes     pm

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**5** Have you ever had a radical mastectomy?  
*(Female participants only.)*  
 Yes     No     Don't know/Refused

Which side?  
 Right     Left     Both  

Draw blood on left.

Draw blood on right.

Do NOT draw blood.

**9** Was any urine collected?  
 Yes     No

Please describe why not: \_\_\_\_\_

Time of urine collection: [ ][ ] : [ ][ ]     am  
                                 Hours    Minutes     pm

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**6** Have you ever experienced fainting spells while having blood drawn?  
 Yes    No    Don't know/Refused

**10** Comments on blood or urine collection:  
\_\_\_\_\_  
\_\_\_\_\_















MOST ID #	Acoustic	Staff ID#
<input type="text"/>	<input type="text"/>	<input type="text"/>



**Laboratory Processing**

First sample collection    Repeat sample collection

Time at start of EDTA plasma processing:  :   am  
Hours Minutes  pm

Collection Tubes	Cryo #	Vol.	Cap	Condition of cryovial (mark only one)				
<b>#1 EDTA plasma tube</b>								
-plasma	01	0.5	V	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-plasma	02	0.5	V	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-plasma	03	0.5	V	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled

Ending time of EDTA plasma aliquoting:  :   am  
Hours Minutes  pm

Bar Code Label

Enter ID from Bar Code label:

Time at start of serum processing:  :   am  
Hours Minutes  pm

Collection Tubes	Cryo #	Vol.	Cap	Condition of cryovial (mark only one)				
<b>#2 Serum tube</b>								
-serum	04	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	05	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	06	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	07	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	08	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	09	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled
-serum	10	0.5	R	<input type="checkbox"/> OK	<input type="checkbox"/> H	<input type="checkbox"/> P	<input type="checkbox"/> B	<input type="checkbox"/> not filled

Ending time of serum aliquoting:  :   am  
Hours Minutes  pm

<b>Urine</b>								
-urine	11	0.5	C	<input type="checkbox"/> OK	<input type="checkbox"/> P	<input type="checkbox"/> not filled		
-urine	12	0.5	C	<input type="checkbox"/> OK	<input type="checkbox"/> P	<input type="checkbox"/> not filled		
-urine	13	0.5	C	<input type="checkbox"/> OK	<input type="checkbox"/> P	<input type="checkbox"/> not filled		
-urine	14	0.5	C	<input type="checkbox"/> OK	<input type="checkbox"/> P	<input type="checkbox"/> not filled		

H=Hemolyzed P=Partial B=Both V=Violet R=Red C=Clear



Appendix 1 Specimen Labels

Baseline Visit (page 1 of 2)

 B00001 00001 MOST Tube 1 Lav top-15 mL	 B00001 00001 MOST Tube2 Red top - 15 mL	 B00001 00001 MOST vial Yellow 01 0.5 mL PCA	 B00001 00001 MOST grid Yellow 01 0.5 mL PCA	 B00001 00001 MOST vial Violet 06 1.0 mL plasma	 B00001 00001 MOST grid Violet 06 1.0 mL plasma
 B00001 00001 MOST Tube 3 Lav top - 7 mL	 B00001 00001 MOST Tube2 Red top - 15 mL	 B00001 00001 MOST vial Yellow 02 0.5 mL PCA	 B00001 00001 MOST grid Yellow 02 0.5 mL PCA	 B00001 00001 MOST vial Violet 07 1.0 mL plasma	 B00001 00001 MOST grid Violet 07 1.0 mL plasma
 B00001 00001 MOST X-tra Lav top - 7 mL	 B00001 00001 MOST X-tra 15mL Red top	 B00001 00001 MOST vial Violet 03 1.0 mL plasma	 B00001 00001 MOST grid Violet 03 1.0 mL plasma	 B00001 00001 MOST vial White 08 buffy coat	 B00001 00001 MOST grid White 08 buffy coat
 B00001 00001 MOST Urine Cup	 B00001 00001 MOST Mixing Tube	 B00001 00001 MOST vial Violet 04 1.0 mL plasma	 B00001 00001 MOST grid Violet 04 1.0 mL plasma	 B00001 00001 MOST vial Red 09 1.0 mL serum	 B00001 00001 MOST grid Red 09 1.0 mL serum
 B00001 00001 Collect form	 B00001 00001 Lab form	 B00001 00001 MOST vial Violet 05 1.0 mL plasma	 B00001 00001 MOST grid Violet 05 1.0 mL plasma	 B00001 00001 MOST vial Red 10 1.0 mL serum	 B00001 00001 MOST grid Red 10 1.0 mL serum

 B00001 00001 MOST Tube 1 Lav top-15 mL	 B00001 00001 MOST Tube2 Red top - 15 mL	 B00001 00001 MOST vial Yellow 01 0.5 mL PCA	 B00001 00001 MOST grid Yellow 01 0.5 mL PCA	 B00001 00001 MOST vial Violet 06 1.0 mL plasma	 B00001 00001 MOST grid Violet 06 1.0 mL plasma
 B00001 00001 MOST Tube 3 Lav top - 7 mL	 B00001 00001 MOST Tube2 Red top - 15 mL	 B00001 00001 MOST vial Yellow 02 0.5 mL PCA	 B00001 00001 MOST grid Yellow 02 0.5 mL PCA	 B00001 00001 MOST vial Violet 07 1.0 mL plasma	 B00001 00001 MOST grid Violet 07 1.0 mL plasma
 B00001 00001 MOST X-tra Lav top - 7 mL	 B00001 00001 MOST X-tra 15mL Red top	 B00001 00001 MOST vial Violet 03 1.0 mL plasma	 B00001 00001 MOST grid Violet 03 1.0 mL plasma	 B00001 00001 MOST vial White 08 buffy coat	 B00001 00001 MOST grid White 08 buffy coat
 B00001 00001 MOST Urine Cup	 B00001 00001 MOST Mixing Tube	 B00001 00001 MOST vial Violet 04 1.0 mL plasma	 B00001 00001 MOST grid Violet 04 1.0 mL plasma	 B00001 00001 MOST vial Red 09 1.0 mL serum	 B00001 00001 MOST grid Red 09 1.0 mL serum
 B00001 00001 Collect form	 B00001 00001 Lab form	 B00001 00001 MOST vial Violet 05 1.0 mL plasma	 B00001 00001 MOST grid Violet 05 1.0 mL plasma	 B00001 00001 MOST vial Red 10 1.0 mL serum	 B00001 00001 MOST grid Red 10 1.0 mL serum

Specimen Labels: 30-Month Follow-up Visit (page 1 of 2)

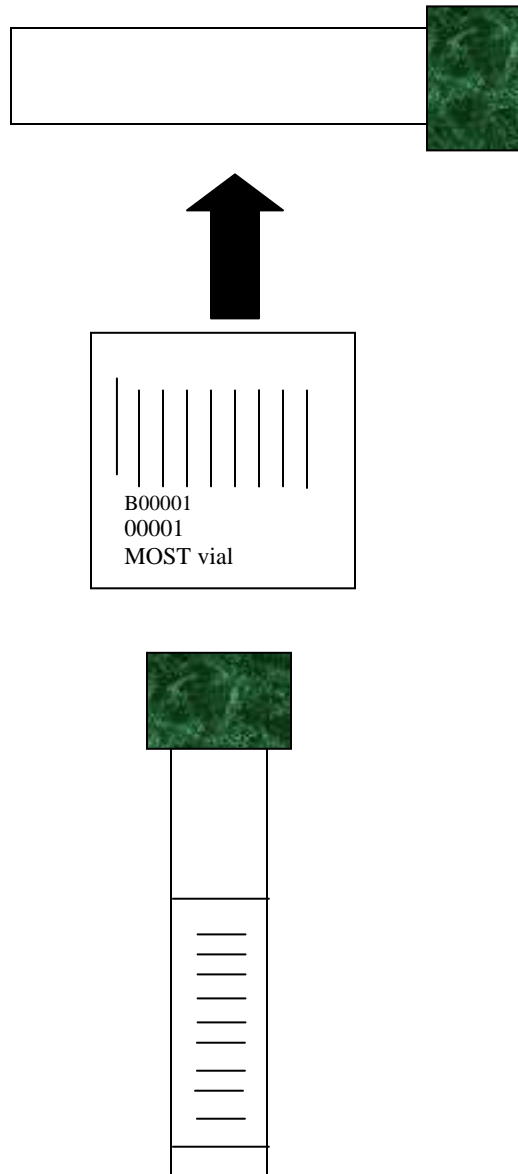
 00001 ##### MOST vial Violet 01 0.5 plasma-30M	 00001 ##### MOST grid Violet 01 0.5 plasma-30M	 00001 ##### MOST vial Red 06 0.5 serum-30M	 00001 ##### MOST grid Red 06 0.5 serum-30M	 00001 ##### MOST vial Clear 11 0.5 urine-30M	 00001 ##### MOST grid Clear 11 0.5 urine-30M
 00001 ##### MOST vial Violet 02 0.5 plasma-30M	 00001 ##### MOST grid Violet 02 0.5 plasma-30M	 00001 ##### MOST vial Red 07 0.5 serum-30M	 00001 ##### MOST grid Red 07 0.5 serum-30M	 00001 ##### MOST vial Clear 12 0.5 urine-30M	 00001 ##### MOST grid Clear 12 0.5 urine-30M
 00001 ##### MOST vial Violet 03 0.5 plasma-30M	 00001 ##### MOST grid Violet 03 0.5 plasma-30M	 00001 ##### MOST vial Red 08 0.5 serum-30M	 00001 ##### MOST grid Red 08 0.5 serum-30M	 00001 ##### MOST vial Clear 13 0.5 urine-30M	 00001 ##### MOST grid Clear 13 0.5 urine-30M
 00001 ##### MOST vial Red 04 0.5 serum-30M	 00001 ##### MOST grid Red 04 0.5 serum-30M	 00001 ##### MOST vial Red 09 0.5 serum-30M	 00001 ##### MOST grid Red 09 0.5 serum-30M	 00001 ##### MOST vial Clear 14 0.5 urine-30M	 00001 ##### MOST grid Clear 14 0.5 urine-30M
 00001 ##### MOST vial Red 05 0.5 serum-30M	 00001 ##### MOST grid Red 05 0.5 serum-30M	 00001 ##### MOST vial Red 10 0.5 serum-30M	 00001 ##### MOST grid Red 10 0.5 serum-30M	 00001 ##### MOST-30M Collect Form	 00001 ##### MOST-30M Lab Form
 00001 ##### MOST-30M X-tra Tube Lav top 3-5 mL	 00001 ##### MOST-30M X-tra Tube Red top 7-10 mL				

 00001 ##### MOST-30M Tube 1 Lav top 3-5 mL	 00001 ##### MOST-30M Tube 2 Red top 7-10 mL
 00001 ##### MOST-30M Urine Cup	 END OF SET



Appendix 2 Specimen Label (Placement on Cryovial)

**MOST**  
**Label Orientation on Cryovial**



**Appendix 3 Phlebotomy Checklists****Baseline Visit****Blood Collection Tray Checklist**

## Per Tray:

- 10 21G Butterfly needles with Luer Adapters
- 5 23G Butterfly needles with Luer Adapters
- 10 Alcohol Swabs
- 15 Band-Aids
- 15 Gauze pads
- 5 Vacutainer holders
- complete set of extra, unlabeled collection tubes
- 2 Tourniquets
- 1 Smelling salts
- 2 Pencils/pens
- Latex gloves
- 1 Hemostats
- 1 Adhesive tape
- 1 Scissors

## ~10 min before draw:

- 1 container with “wet ice” bath = ½ ice and ½ water

## Per participant:

- 1 Blood tube rack with 4 draw tubes labeled and arranged according to draw priority (1 through 4)
- 4 Pieces of aluminum foil
- 25 Cryovials (2 yellow cap, 5 violet cap, 1 white cap, 14 red cap, and 3 clear cap) with labels affixed
- 1 MOST Specimen Collection Form
- 1 MOST Laboratory Processing Form
- 1 Urine cup labeled
- Labels for back-up vacutainers (2), forms (2) and mixing tube (1)

## At the Phlebotomy Station:

- Emesis Basin  Needle/Sharps container
- Cold cloth  Paper towels
- Biohazard containers

**Phlebotomy Checklist: Follow-up Visit**

## Blood Collection Tray Checklist

## Per Tray:

- 10 21G Butterfly needles with Luer Adapters
- 5 23G Butterfly needles with Luer Adapters
- 10 Alcohol Swabs
- 15 Band-Aids
- 15 Gauze pads
- 5 Vacutainer holders
- complete set of extra, unlabeled collection tubes
- 2 Tourniquets
- 1 Smelling salts
- 2 Pencils/pens
- Latex gloves
- 1 Hemostats
- 1 Adhesive tape
- 1 Scissors

## ~10 min before draw:

- 1 container with “wet ice” bath = ½ ice and ½ water

## Per participant:

- 1 Blood tube rack with 2 draw tubes labeled
- 2 Blood draw tube sheaths
- 14 Cryovials (3 violet cap, 7 red cap, and 4 clear cap) with labels affixed
- 1 MOST Urine Collection Form
- 1 MOST Phlebotomy Form
- 1 MOST Laboratory Processing Form
- 1 Urine cup labeled
- Labels for back-up vacutainers (2) and forms (2)

## At the Phlebotomy Station:

- Emesis Basin
- Cold cloth
- Biohazard containers
- Needle/Sharps container
- Paper towels

**Appendix 4 Precautions when a participant feels faint****PRECAUTIONS WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING  
THE BLOOD DRAWING**

- Have the participant remain in the chair; if necessary have them sit with their head between their knees.
- Provide the participant with a basin if they feel nauseated.
- Have the participant stay sitting until the color returns and they feel better.
- Place a cold wet cloth on the back of the participant's neck.
- If the participant faints, use smelling salts to revive by crushing the ampoule and waving it under the participant's nose for a few seconds.
- If the participant continues to feel sick, contact a medical (nursing) staff member who will advise you on further action.

**Appendix 5 Specimen Collection Supply Lists**  
**Specimen Collection Supply List: Baseline Visit**

Specimen collection supplies	# per participant	sample type	vendor: catalog #	\$ price/unit
7 mL EDTA vacutainer	2	plasma	Fisher: 02-683-71 Allegiance: TBD	\$19.03/ 100 \$171.50/case
15 mL serum vacutainer	2	serum	Fisher: 02-685C Allegiance: TBD	\$31.65/ 100 \$258.91/case
vacutainer blood collection set 21G 3/4"	1	all	Fisher: 02-664-1 Allegiance: TBD	\$78.24/50 \$236.54/200
vacutainer blood collection set 23G 3/4"	back-up	all	Fisher: 02-664 Allegiance: TBD	\$78.24/50 \$236.54/case
vacutainer blood collection tube holders	1	N/A	Fisher: 02-665-110 Allegiance: TBD	\$115.14/72 pk
urine specimen containers	1	N/A	Fisher: 13-771-64 Allegiance: TBD	\$104.85/500
vacutainer tube rack (4 vacutainers per participant, tube sizes 13mm and 16mm)	4	all	Fisher: 14-793-11 Allegiance: TBD	\$40.80 each
Blood collection tray (10 compartments)	1		Determined by field center	
Aliquot racks (25 cryovials per participant)	4		Determined by field center	

Note: Prices are from the catalogs. Educational discounts should apply.

\*Cryovials and cryovial cap inserts provided by Coordinating Center

Fisher Scientific  
 Fisher HealthCare  
 9999 Veterans Memorial Drive  
 Houston, Texas 77038  
 1-800-640-0640  
[www.fishersci.com](http://www.fishersci.com)

Allegiance (Scientific Products)  
 1430 Waukegan Road  
 McGaw Park, Illinois 60085  
 1-800-964-5227  
[www.allegiance.net](http://www.allegiance.net)

## Specimen Collection Supply List: 30-Month Follow-up Visit

Specimen collection supplies	# per participant	sample type	vendor: catalog #	\$ price/unit
3mL or 5mL EDTA vacutainer	1	plasma	Fisher: 02-685-113; 02-683-73 or equivalent from preferred vendor	\$32.12/ 100 \$281.46/case  \$30.43/ 100 \$249.45/case
7mL or 10 mL serum vacutainer	1	serum	Fisher: 02-684-18; 02-683-93 or equivalent from preferred vendor	\$27.06/ 100 \$221.46/case  \$31.34/ 100 \$256.98/case
vacutainer blood collection set 21G 3/4"	1	all	Fisher: 02-664-1 or equivalent from preferred vendor	\$81.03/50 \$244.97/200
vacutainer blood collection set 23G 3/4"	back-up	all	Fisher: 02-664 or equivalent from preferred vendor	\$81.03/50 \$242.57/200
vacutainer blood collection tube holders	1	N/A	Fisher: 02-665-110 or equivalent from preferred vendor	\$118.79/72 pk
urine specimen containers	1	N/A	Fisher: 22-610-130 or equivalent from preferred vendor	\$200.73/500
vacutainer tube rack (2 vacutainers per participant, tube sizes 13mm and 16mm)	4	all	Fisher: 14-793-11 or equivalent from preferred vendor	\$52.75 each
Blood collection tray (10 compartments)	1		Determined by clinical center	
Aliquot racks (14 cryovials per participant)	4		Determined by clinical center	

Note: Prices are from the Fisher Scientific catalog. Educational discounts should apply.

\*Cryovials, cryovial caps and cryovial storage boxes provided by Coordinating Center.

Fisher Scientific  
Fisher HealthCare  
9999 Veterans Memorial Drive  
Houston, Texas 77038  
1-800-640-0640  
[www.fishersci.com](http://www.fishersci.com)