

## BIOSPECIMEN COLLECTION

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## 1. Background and rationale

During the MOST follow-up clinic visits we will collect a fasting blood (for plasma and serum) and a second morning urine specimen for future analyses from all study participants except those who have had bilateral knee replacements or those who meet exclusion criteria indicating that blood cannot be safely drawn. The Data from Prior Visits reports will indicate those participants that are not eligible due to total knee replacement. Participants were told at the time of the Follow-up Telephone Interview that the clinic visit would include fasting specimen collection and sent Pre-visit Instructions telling them to not eat or drink anything other than water (except for regular medications and vitamins) prior to their clinic visit. Participants selected for specimen collection are to be scheduled for their clinic visit prior to 9 am.

The MOST clinic visit involves the collection of approximately 15 mL 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 for the participants.

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 one tablespoon of blood is drawn.

### 1.1 Overview of blood and urine collection

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

The collection tube volume will be selected by laboratory personnel at the study site based on what is required to yield the plasma and serum required from the blood draw.

## 2. Equipment and supplies

### 2.1 Sample ID labels

Each clinic will be supplied with barcode specimen ID labels to use for labeling forms, drawtubes, urine specimen cup, 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 Coordinating Center will match the participant-specific 7-digit MOST ID# to the specimen-specific 5-digit barcode ID. This process is crucial to being able to use the data collected from laboratory tests and is reviewed in detail in section 5.*

**Labels**

- Two for pre-labeling the two 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 four to six participants
- “Wet ice” bath container (“wet ice” = 1:1 ratio of crushed ice + water)
- Rack of blood collection tubes for each participant
- Sheath for filled blood collection tubes
- Rack of 14 cryovials for the serum, plasma, and urine aliquots

**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	

**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 two 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 14 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.

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

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

These can be separated into two 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.

Tube #1 is a 3 mL to 5 mL 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 7 mL to 10 mL 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

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

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

### 2.2.5 Blood mixing during venipuncture

Each tube should be treated as follows:

- #1 **EDTA:** invert 10 to 15 times minimum, sheathed to protect from light exposure, and then placed in “wet ice” bath. Process within 15 minutes (see Laboratory Processing chapter).
- #2 **Serum:** sheath 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

The Data from Prior Visits report must be checked to see if the participant has had bilateral total knee replacements (see Question 2 on the Initial Knee Pain and Urine Collection Form). Those participants who have undergone bilateral knee replacements are ineligible for blood and urine collection; mark “Yes” for Question 2 for these participants.

Urine Collection form: Place the bar code label “MOST 60-month Collect Form” in the upper left corner of the Initial Knee Pain and Urine Collection form and enter the 5-digit ID from the bar code label in the text box to the right of the label. Questions #3 and #3a ask the examiner 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.

Question #3b asks the examiner to document the time of the urine collection in nonmilitary time hours and minutes, and whether it was am or pm.

For Questions #3c the examiner asks the participant the date and time that the participant last ate or drank anything other than water and documents the response. Document the hour in nonmilitary time and mark am or pm. Only include the total number of complete hours fasted in Question #3ciii, ignoring any additional minutes of fasting. If two specimens are collected, document the time that the later specimen was collected. Record how many hours the participant has fasted. The participant was told at the telephone interview and sent written instructions that they were 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 participants are to be scheduled for their clinic visit at or before 9:00 am. Even if a participant has not fasted, 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. Mark the location of the urine collection (clinic or home) in Question 3d. Urine collection is to take place at the clinic, but in rare circumstances, urine collection may have taken place at home prior to the clinic visit.

Ask the participant what time they got up for the day and document the response in Question #4. 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.

Question #5 is an optional place to document comments about urine collection.

Phlebotomy form: Phlebotomy will not be done on an arm that has had a shunt or port placed for kidney dialysis (Question #1). Participants will be asked if they have ever had a radical mastectomy (Question #2), 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 in Question #3.

Question #4 is to document whether the participant has been ill in the past week requiring antibiotic treatment, hospitalization, or treatment with steroids. Question #5 asks if they bleed or bruise easily. Question #6 asks 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 judgment to decide whether or not a clinic physician or nurse supervisor should be consulted.

If the participant has experienced fainting spells during phlebotomy (Question #7), 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 (see Appendix 3) must be ready before proceeding.
- Check that the two 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

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. Two tubes of blood are collected, for a total volume of 15 mL of blood. Any participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is about 1 tablespoon, although it may look like more. The phlebotomist may also assure participants that they donate 30 times as much blood (450 mL) when they donate a unit of blood to a blood bank.

### **5. Detailed procedures**

#### **5.1 Forms**

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 Urine Collection and Phlebotomy 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 acoustic preprinted on the top of their Urine Collection, Phlebotomy, and Laboratory Processing forms. Complete the date and MOST Staff ID# on the top of both of the forms and whether it is the first sample collection or repeat sample collection. The initial pain questions should be asked of all participants prior to the collection of blood.

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 on both the Urine Collection form (upper left side of form) and the Laboratory Processing form (upper right side of form) for the same study participant. There will be a small sheet of labels clipped to the rack of vacutainers with a "MOST 60M Collect form" label and a "MOST 60M Lab Form" label. Affix the bar-coded "Collect Form" label to the Urine Collection form in the upper left corner 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 Phlebotomy form under (Question 10) "Comments on phlebotomy."* The butterfly has a small, thin-walled needle, which minimizes trauma to the skin and vein. The use of 12-inch 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: *"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. • 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 before or 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 Tube Lav top 3-5 mL" or "X-tra Tube Red top 7-10 mL" 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 tube #1 (Lavender top-for plasma) is removed, mix by gently inverting at least 10 to 15 times, sheath to protect from light, and place in wet ice bath.
- As tube #2 (Red top-for serum) is removed, sheath the tube 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 1 to 2 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 Phlebotomy form. This includes checking which collection tubes were filled, time of blood draw (Question #9), and writing comments about any difficulties with the phlebotomy under “Comments on phlebotomy” (Question #10).
- 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 2 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 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.): make notations of difficulties on the 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” (not filled to capacity) on Question #9 of the 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

### Initial Knee Pain and Urine Collection form:

- Initial knee pain (Question #1) completed
- Specimen ID barcode label affixed to upper left corner and 5-digit ID# entered
- Data from Prior Visits Report checked to determine if participant is eligible for biospecimen collection (Urine collection form Question #2)
- Question #3c correctly calculated total fasting time

### Phlebotomy preparation:

- Blood collection trays properly prepared
- Blood draw tubes properly labeled
- Questions on 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 before or at 2 minutes
- Needle removed and arm bandaged correctly
- Needle and tubing appropriately disposed

### Tubes mixed and handled correctly after filling:


- Tube #1 inverted at least 10-15 times minimum, put in sheath and then placed in ice bath

- Tube #2 put in sheath and then placed in rack at room temperature

Phlebotomy form properly filled out

- Question #3 (arm safe to use for phlebotomy) correctly filled out
- Question #9 (documentation of tube status and time of draw) correctly filled out

9. Data collection forms



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### Initial Knee Pain and Urine Collection

Visit	MOST ID #	Acrostic	Date of Phlebotomy	Staff ID#
<input type="radio"/> 60-month <input type="radio"/> 84-month	<input type="text"/>	<input type="text"/>	<input type="text"/> / <input type="text"/> / 20 <input type="text"/> <input type="text"/> <small>Month Day Year</small>	<input type="text"/>

First sample collection     Repeat sample collection

**Bar Code Label**

**Enter ID from Bar Code label:**

3. Was a urine specimen obtained?

Yes     No → Go to Question #5 and explain.

3a. Which void(s) was collected?

*(Examiner note: Mark **all** that apply; if one void is insufficient volume, it is permissible to combine two specimens, as long as neither is the first morning void.)*

First     Second     Third     Fourth or later

Try to obtain a second-void specimen before noon and before the participant leaves the clinic. Do not aliquot first-void specimen unless later void not obtained.

3b. What time was the urine specimen collected?

*(Examiner note: If two specimens are combined, please write the later of the two times.)*

:      am     pm  
Hours Minutes

3c. **Ask participant:** What is the date and time you last ate or drank anything except water?

i. Date:  /  /   
Month Day Year

ii. Time:  :      am     pm  
Hours Minutes

iii. How many hours has participant fasted?

Hours

3d. Place of urine collection:  Home     Clinic

1. While you are sitting here now, are you experiencing any pain in your joints or muscles?

Yes     No     Refused or unable to answer

a. Where is the pain located? *(Mark **all** that apply.)*

<p style="text-align: center;"><b>Left side</b></p> <input type="radio"/> Buttock <input type="radio"/> Hip <input type="radio"/> Thigh <input type="radio"/> Knee <input type="radio"/> Leg <input type="radio"/> Ankle <input type="radio"/> Foot <input type="radio"/> Other <i>(Please specify: _____)</i>	<input type="radio"/> Back	<p style="text-align: center;"><b>Right side</b></p> <input type="radio"/> Buttock <input type="radio"/> Hip <input type="radio"/> Thigh <input type="radio"/> Knee <input type="radio"/> Leg <input type="radio"/> Ankle <input type="radio"/> Foot <input type="radio"/> Other <i>(Please specify: _____)</i>
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b. Did the participant report pain in either knee?

Yes     No

**Examiner Note: REQUIRED: Show Card #27 and ask participant to . . .**

i. Please rate the knee pain that you have by pointing to the number on this card. "0" means "No pain" and "10" means "Worst pain you can imagine."

0    1    2    3    4    5    6    7    8    9    10

2. Has participant had bilateral knee replacement(s)?

**Examiner Note: Check Data from Prior Visits Report.**

Yes     No

Do not obtain biospecimens.

**Ask participant:**

4. What time did you get up for the day today?

:      am     pm  
Hours Minutes

5. Comments on urine collection:

\_\_\_\_\_

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MOST Follow-up  
Clinic Visit Workbook  
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**Phlebotomy**

Visit	MOST ID #	Acrostic	Date of Phlebotomy	Staff ID#
<input type="radio"/> 60-month <input type="radio"/> 84-month	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> / <input type="text"/> / 20 <input type="text"/> <input type="text"/> Month Day Year	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

First sample collection   
  Repeat sample collection



Now I'm going to ask you two questions to see whether it is safe to draw your blood.

1. Have you ever had an arm graft shunt or port for kidney dialysis?  
 Yes     No     Don't know/Refused

Go to Question #3 and mark "Neither."

Which side?

Right     Left     Both

Right: Draw blood on left side.  
 Left: Draw blood on right side.  
 Both: Do NOT draw blood on either side. Go to Question #3 and mark "Neither."

2. Have you ever had a radical mastectomy or other surgery where lymph nodes were removed from your armpit?  
 Yes     No     Don't know/Refused

Go to Question #3 and mark "Neither."

Which side?

Right     Left     Both

Right: Draw blood on left side.  
 Left: Draw blood on right side.  
 Both: Do NOT draw blood on either side. Go to Question #3 and mark "Neither."

3. Which arm(s) can safely be used for phlebotomy?  
 (Examiner Note: Refer to Questions #1 and #2.)

Right     Left     Either     Neither

Do NOT draw blood. Go to Procedure Checklist and mark appropriate bubble.

4. Have you had an illness in the past week requiring antibiotics, hospitalization, or steroids?  
 Yes     No     Don't know/Refused

5. Do you bleed or bruise easily?  
 Yes     No     Don't know/Refused

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

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

8. What is the date and time you last ate or drank anything except water?

(Examiner Note: Do not repeat question if already asked for urine collection.)

a. Date:  /  /   
 Month Day Year

b. Time:  :   am  
 pm  
 Hours Minutes

c. How many hours has participant fasted?  
 Hours

9. Was any blood drawn?  
 (Examiner Note: Proceed with the blood draw even if participant has not fasted.)

Yes     No

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

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

Tube	Volume	Filled to Capacity
1. EDTA	3 - 5 mL	<input type="radio"/> Yes <input type="radio"/> No
2. Serum	7 - 10 mL	<input type="radio"/> Yes <input type="radio"/> No

Time of blood draw:  :   am  
 pm  
 Hours Minutes

10. Comments on phlebotomy:

\_\_\_\_\_

\_\_\_\_\_









Visit	MOST ID #	Acrostic	Staff ID#
<input type="radio"/> 60-month <input type="radio"/> 84-month	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<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 <u>one</u> )				
<b>#1 EDTA plasma tube</b>								
-plasma	01	0.5	V	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-plasma	02	0.5	V	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-plasma	03	0.5	V	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> 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 <u>one</u> )				
<b>#2 Serum tube</b>								
-serum	04	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	05	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	06	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	07	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	08	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	09	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled
-serum	10	0.5	R	<input type="radio"/> OK	<input type="radio"/> H	<input type="radio"/> P	<input type="radio"/> B	<input type="radio"/> not filled

Ending time of serum aliquoting:  :   am  
 Hours Minutes  pm

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

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



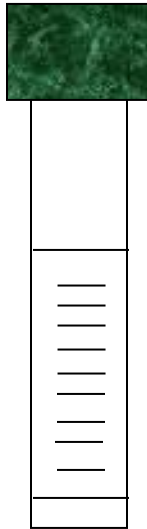
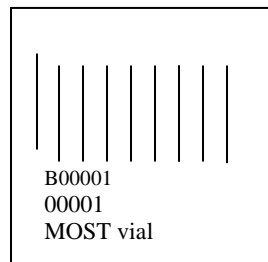
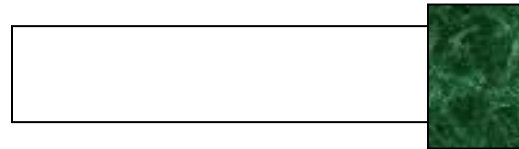
**Appendix 1 Specimen Labels (page 1 of 2)**

 00001 ##### MOST vial Violet 01 0.5 plasma-60M	 00001 ##### MOST grid Violet 01 0.5 plasma-60M	 00001 ##### MOST vial Red 06 0.5 serum-60M	 00001 ##### MOST grid Red 06 0.5 serum-60M	 00001 ##### MOST vial Clear 11 0.5 urine-60M	 00001 ##### MOST grid Clear 11 0.5 urine-60M
 00001 ##### MOST vial Violet 02 0.5 plasma-60M	 00001 ##### MOST grid Violet 02 0.5 plasma-60M	 00001 ##### MOST vial Red 07 0.5 serum-60M	 00001 ##### MOST grid Red 07 0.5 serum-60M	 00001 ##### OST vial Clear 12 0.5 urine-60M	 00001 ##### MOST grid Clear 12 0.5 urine-60M
 00001 ##### MOST vial Violet 03 0.5 plasma-60M	 00001 ##### MOST grid Violet 03 0.5 plasma-60M	 00001 ##### MOST vial Red 08 0.5 serum-60M	 00001 ##### MOST grid Red 08 0.5 serum-60M	 00001 ##### MOST vial Clear 13 0.5 urine-60M	 00001 ##### MOST grid Clear 13 0.5 urine-60M
 00001 ##### MOST vial Red 04 0.5 serum-60M	 00001 ##### MOST grid Red 04 0.5 serum-60M	 00001 ##### MOST vial Red 09 0.5 serum-60M	 00001 ##### MOST grid Red 09 0.5 serum-60M	 00001 ##### MOST vial Clear 14 0.5 urine-60M	 00001 ##### MOST grid Clear 14 0.5 urine-60M
 00001 ##### MOST vial Red 05 0.5 serum-60M	 00001 ##### MOST grid Red 05 0.5 serum-60M	 00001 ##### MOST vial Red 10 0.5 serum-60M	 00001 ##### MOST grid Red 10 0.5 serum-60M	 00001 ##### MOST-60M Collect Form	 00001 ##### MOST-60M Lab Form

 00001 ##### MOST-60M X-tra Tube Lav top 3-5 mL	 00001 ##### MOST-60M X-tra Tube Red top 7-10 mL
 00001 ##### MOST-60M Tube 1 Lav top 3-5 mL	 00001 ##### MOST-60M Tube 2 Red top 7-10 mL
 00001 ##### MOST-60M Urine Cup	END OF SET

Appendix 2 Specimen Label (Placement on Cryovial)

# MOST Label Orientation on Cryovial



**Appendix 3 Phlebotomy Checklist**

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 List**

<b>Specimen collection supplies</b>	<b># per participant</b>	<b>sample type</b>	<b>vendor: catalog #</b>
3 mL or 5 mL EDTA vacutainer	1	plasma	Fisher: 02-683-99B; 02-683-56 or equivalent from preferred vendor
7 mL or 10 mL serum vacutainer	1	serum	Fisher: 02-685-2A; 02-685-112 or equivalent from preferred vendor
vacutainer blood collection set 21G 3/4"	1	all	Fisher: 02-664-1 or equivalent from preferred vendor
vacutainer blood collection set 23G 3/4"	back-up	all	Fisher: 02-664 or equivalent from preferred vendor
vacutainer blood collection tube holders	1	N/A	Fisher: 02-665-110 or equivalent from preferred vendor
urine specimen containers	1	N/A	Fisher: 22-610-130 or equivalent from preferred vendor
Blood collection tray (10 compartments)	1		Determined by clinical center
Aliquot racks (14 cryovials per participant)	4		Determined by clinical center

Note: Educational discounts should apply. Please carefully review the specifications prior to placing an order. Contact the UCSF Coordinating Center (MOSTHelpdesk@psg.ucsf.edu) if you have concerns about equipment on this list. Cryovials, cryovial caps and cryovial storage boxes are provided by the UCSF Coordinating Center.

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