

BIOSPECIMEN COLLECTION

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1. Background and rationale

At the 144-Month Clinic Visit (2016-2017) we will collect baseline 12 hour fasting blood and urine samples from new cohort study participants. Samples will be archived in a state-of-the-art biorepository for future analyses. Blood will be collected in three types of tubes for specialized processing of different blood components. After processing, biospecimens will be aliquoted into cryovials and shipped to the storage repository.

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 clinical center personnel. A standard informed consent has been prepared for this study. With regard to laboratory procedures, the consent statement informs study participants how much blood will be collected and that there is a small risk of bruising at the spot on the arm where the blood is taken, of the amount of blood drawn at each clinic visit and blood storage for future DNA/RNA genetic testing.

The MOST clinic visit involves the collection of approximately 46.5mL of blood in six draw tubes and a urine specimen from participants.

1.1 Overview of blood and urine collection

Collection tubes #1 and #3: two 7.0mL EDTA lavender top tubes (yield 6.0mL plasma)
Collection tubes #2, #4 and #5: three 10.0mL red top tubes (yield 12.0mL serum)
PAXgene tube #6: one 9.5mL tube with 6.9mL of chemical additive (2.5mL blood)
Urine: urine collection cup (4.5mL urine specimen)

2. Equipment and supplies

2.1 Specimen ID labels

Specimen ID labels for each participant have a unique 5-digit specimen ID number (starting 144-month specimen ID number is 80001 for U-Iowa and 30001 for UAB). Labels with barcodes have a three-digit extension e.g., B01 after the 5-digit specimen ID number that serves as a unique identifier for each storage cryovial or PAXgene tube. Barcodes will be used to inventory, track and manage samples at the Fisher BioServices repository.

Pre-printed labels are on large rolls with two labels (side by side) per row. There are 54 labels (27 rows) for each participant (see Appendix 1). Use of the labels for the blood collection rack is described in section 2.2.2 Blood collection rack: labeling and set-up.

Nonbarcode labels (8 labels)

- Row 1: Tube #1 (Lavender Top) and Tube #2 (Red Top)
- Row 2: Tube #3 (Lavender Top) and Tube #4 (Red Top)

- Row 3: Tube #5 (Red Top) and Extra Tube (back-up for phlebotomists)
- Row 4: Urine Cup and Lab Form (laboratory processing form)

Barcode labels (46 labels)

- Row 5: one for PAX RNA (Red Top Tube) and one for PAX RNA grid form
- Rows 6 thru 11: six for plasma vials (Blue 01-06) and six for plasma grid form
- Row 12: one for buffy coat vial (Yellow 07) and one for buffy coat grid form
- Rows 13 thru 24: twelve for serum vials (Red 08-19) and twelve for serum grid form
- Row 25 thru 27: three for urine vials (Clear 20-22) and three for urine 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 (to protect from light contamination)
- Rack of 22 cryovials for the plasma, buffy coat, 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
One back-up lavender and red top vacutainer tubes	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 six 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 22 cryovials should be pre-labeled with the “vial” 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 54 specimen-specific labels per participant. After 29 labels have been used for setting up the blood collection tubes (six), aliquot rack (22) and urine cup (1), there will be 25 labels left:

- One label for the “Backup Vacutainer”
- One for the Laboratory Processing Form
- 23 labels for the Lab Storage Box Grid forms

These can be separated into two mini-sheets:

1. One label for the “Backup Vacutainer,” should be clipped to the blood collection tray.
2. Twenty-three labels for the Lab Storage Box Grid forms and one label for the Laboratory Processing Form should be clipped to the aliquot rack.

2.2.3 Description of blood collection

Each drawtube is color-coded to aid in handling.

Tubes #1 and #3 are 7.0mL lavender stoppered tubes containing 15% EDTA as the anticoagulant.

Tubes #2, #4, and #5 are 10.0mL red stoppered tubes. These tubes contain no anticoagulant so that the blood clots to form serum.

Tube #6 is a PAXgene tube is a 9.5mL PAXgene RNA collection tube with a red stopper. It is a 9.5mL tube, but only 2.5mL of blood is collected in this tube because the tube already contains approximately 6.9mL of a chemical additive that stabilizes the RNA in the collected blood.

Blood handling during venipuncture is discussed in section 2.2.5.

2.2.4 Priority of tubes

A total of approximately 46.5mL (3 tablespoons) of blood will be drawn from each participant in the six tubes. Tubes are numbered 1, 2, 3, 4, 5, 6 and arranged in the rack to be drawn in the following order of priority:

1. EDTA 7.0mL lavender top stopper
2. Serum 10.0mL red top stopper
3. EDTA 7.0mL lavender top stopper
4. Serum 10.0mL red top stopper
5. Serum 10.0mL red top stopper
6. PAXgene 2.5mL of blood in 9.5mL red stopper tube

2.2.5 Blood handling during venipuncture

Each tube should be treated as follows:

#1 and #3 EDTA Plasma: gently invert 10 to 15 times minimum, sheath to protect from light exposure, and then placed in “wet ice” bath. Process within 15 minutes (see Laboratory Processing chapter).

#2, #4, and #5 Serum: sheath to protect from light exposure, and then placed in the blood collection rack and incubate at room temperature for 30 minutes. Refrigerate after 30 minutes if not processed. Process within 60 minutes after blood draw (see Laboratory Processing chapter).

#6 PAXgene: Confirm that participant provided informed consent for RNA before filling the PAXgene tube (information should already be marked on the participant’s Laboratory Processing Form by the staff person getting informed consent). After drawing, the PAXgene tube should be gently inverted 10 times and placed on rack at room temperature for at least 2 hours. The PAXgene tube should undergo a room-temperature incubation period of at least 2 hours before the tube is placed in the -20°C freezer. If this is not possible or the PAXgene tube is inadvertently left overnight, the maximum amount of time that the tube should incubate is 36 hours. Avoid leaving the PAXgene tube to incubate over the entire weekend.

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

There are 2 electronic REDCap data collection forms for biospecimen collection:

- Urine Collection
- Blood Collection

Both forms are accessed via the MOSTv4 study website (see Data Management Operations Manual, section 6.3.2 For REDCap electronic forms and Website User's Guide Operation Manual, section 4.3 Data entry using REDCap. Mock data collection paper forms will be available on the MOST study website in case the phlebotomist prefers to collect data on a mock paper form and then enter the data into REDCap after blood and/or urine collection is completed (see Appendix 6).

Urine Collection form

Questions #1 and #2 ask the examiner to confirm that a urine specimen was collected, and to enter the 5-digit biospecimen label number assigned to the participant. If blood and urine are both collected, the 5-digit numbers on the biospecimen labels entered on the various forms for that participant MUST be the same. This is very important so we can link the 7-digit Participant ID to the correct 5-digit specimen ID number.

Question #3 asks the examiner to document 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 has insufficient volume, it is permissible to combine two specimens, i.e., second and third void, as long as neither is the first morning void. The time of urine specimen collection in military time hours and minutes is documented in Question #4.

For Questions #5a and #5b, 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 military time. Only include the total number of complete hours fasted in Question #5c 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 to fast for 12 hours and not to eat or drink anything EXCEPT WATER the morning of the clinic visit and that they would provide a second morning urine 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 #6. 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 #7. 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 #8 is an optional place to document comments about urine collection.

Blood Collection form

Phlebotomy will not be done on an arm that has had an arm graft shunt or port placed for kidney dialysis (Question #1). Participants will be asked if they have ever had a radical mastectomy or other surgery where lymph nodes were removed from the armpit (Question #2), as blood will not be drawn from the arm on the same side that had a radical mastectomy or other surgery where lymph nodes were removed from the armpit. Which arm can safely be used for phlebotomy is documented in Question #3. A urine specimen will be collected and stored for participants who cannot safely have blood drawn from either arm.

Question #4 is to document whether the participant has been ill or had a medical procedure 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.

For Questions #8a, #8b and #8c, the phlebotomist again 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 military time. Only include the total number of complete hours fasted in Question #8c ignoring any additional minutes of fasting. Record how many hours the participant has fasted. The participant was told at the telephone interview and sent written instructions that they were to fast for 12 hours and not to eat or drink anything EXCEPT WATER the morning of the clinic visit. 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, indicating the date and time that they last ate or drank anything on the Blood Collection form (Question #8c).

If blood is drawn, Question #10 will be marked “yes” and the 5-digit specimen label number assigned to the participant entered for Question #10a. In Question #10, mark “yes” or “no” to the question about whether or not each of the 6 tubes were filled to capacity.

At the end of the form the phlebotomist has an optional place to document comments about blood collection.

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. Give the list to the study coordinator so it can be documented in the participant's file.

4. Participant and exam room preparation

4.1 Phlebotomy room

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 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 (4.5mL).

Female and male participants may urinate directly into the prelabeled specimen collection cup. 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. Use the prelabeled urine collection cup.
2. Remove the cap from the urine collection cup.
3. Void directly into the collection cup until nearly full.
4. Carefully seal the cap of the collection cup 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/urine 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. Six tubes of blood are collected, for a total volume of 46.5mL of blood. Any participants who are concerned about the volume of blood should be reassured that the total amount of blood drawn is about 3 tablespoons, although it may look like more. The phlebotomist may also assure participants that they donate 10 times as much blood (450mL) when they donate a unit of blood to a blood bank.

5. Detailed procedures

5.1 Forms

Affix the Lab Form label to the Laboratory Processing Form (Teleform), write the 5-digit specimen number in the boxes under the label, and deliver the form to the lab with the samples for processing.

Data collection forms facilitate the monitoring of phlebotomy, urine collection and other quality assurance parameters. Biospecimen collection forms will be completed electronically in REDCap. Mock data collection paper forms will be available on the MOST study website in case the phlebotomist prefers to collect data on a mock paper form and then enter the data into the REDCap form after blood and/or urine collection is completed (see Appendix 6).

The Urine Collection and Blood 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.

Complete the date (“Today”), Interview Start time (“Now”) and MOST Staff ID# on the top of the REDCAP form and whether it is the first sample collection or repeat sample collection (generally only first sample collection will be done in this study).

The specimen ID has already been assigned and you will confirm that 5-digit specimen ID# on the pre-labeled tubes and the urine collection cup match. It is vital that this same specimen-specific ID be matched up on the Urine Collection form (REDCap), Blood Collection form (REDCap), and the Laboratory Processing form (upper right side of paper form) for the same study participant.

5.2 Phlebotomy

5.2.1 General Blood Collection Information

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 Blood Collection form under “Comments.”* 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 3 tablespoons of blood. We hope to be able to use the results of these tests to better understand bone and joint health."*

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 the specimen-specific bar-coded "Extra Tube" labels on that tube after completing phlebotomy or remove the label from the tube that failed and place it on the backup tube.
- 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.
- Keep tubes #1 and #3 (Lavender top-for plasma) below the level of the venipuncture site to minimize the unlikely chance EDTA contamination.
- As tubes #1 and #3 (Lavender top-for plasma) are removed, mix by gently inverting at least 10 to 15 times, sheath to protect from light, and place in wet ice bath.
- As tubes #2, #4, and #5 (Red top-for serum) are removed, sheath the tube to protect from light and place in rack on the blood collection tray at room temperature.
- Tube #6: **Confirm that the participant consented to RNA testing before drawing blood in the PAXgene Tube**. Complete Blood Collection form Question #9, "Did participant

consent to RNA testing?”. After the blood is drawn, the PAXgene tube should be inverted gently 10 times and placed on rack at room temperature for at least 2 hours.

- 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 Blood Collection form. This includes marking if any blood was drawn (Question #10), entering the Biospecimen label number (Question #10a), confirming that collection tubes were filled (Question #10b), and writing comments about any difficulties with blood collection under “Comments”. It is extremely important that the 5-digit Specimen ID assigned to the participant be entered accurately in Question #10a (please double-check that the numbers were entered correctly before submitting the form).
- Clean up the venipuncture area (if necessary).
- Bring blood collection tray to the processing area with the filled vacutainer tubes, labels, 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 sample collection on the Blood Collection form.

5.2.8 Other possible problems

1) Not all tubes are collected (blood flow ceases, difficult venipuncture, etc.): make notations of difficulties on the Blood Collection 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 #10b of the Blood Collection 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

Urine Collection form:

- Specimen 5-digit ID# entered (Question #10a)
- Correctly calculated total fasting time (Question #8c)

Phlebotomy preparation:

- Blood collection trays properly prepared
- Blood draw tubes properly labeled
- Questions on Blood Collection form asked
- Hepatitis B vaccination given or offered to all personnel handling blood
- Confirmed that participant consented to RNA testing to determine that PAXgene tube will be filled (information marked on Laboratory Processing Form)

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 tubes 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:

- Tubes #1 and #3 gently inverted at least 10-15 times minimum, put in sheath and then placed in ice bath
- Tubes #2, #4 and #5 put in sheath and then placed in rack at room temperature
- Confirmation that participant consented to RNA testing before tube #6 blood collection** (information marked on Laboratory Processing Form)
- Tube #6 gently inverted 10 times and then placed in rack at room temperature















































Phlebotomy form properly filled out

- Arm safe to use for phlebotomy correctly filled out (Question #3)
- Documentation of tube status (Question #10b)

9. Data collection forms

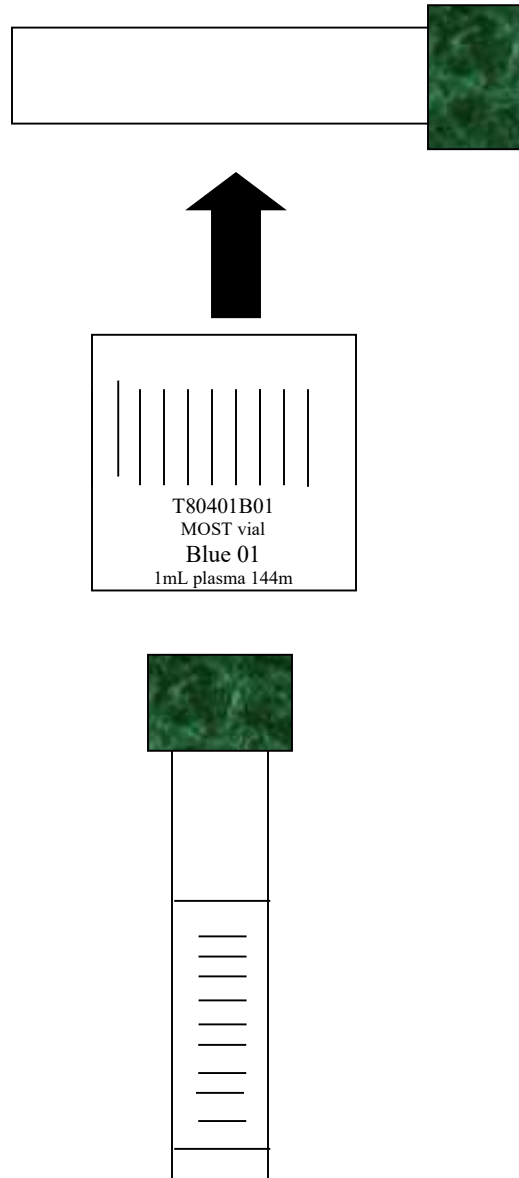
Please see the Overview of the 144-month Follow-up Visit Operations Manual chapter for an overview of the data collection forms, information on whether each form is in REDCap or TELEForm, and where the forms can be accessed on the study website.

Appendix 1 Labels

Row 9	 T80401B04 MOST vial Blue 04 1mL plasma 144m	 T80401B04 MOST grid Blue 04 1mL plasma 144m	Row 18	 T80401R13 MOST vial Red 13 1mL serum 144m	 T80401R13 MOST grid Red 13 1mL serum 144m	Row 27	 T80401C22 MOST vial Clear 22 1.5mL urine 144m	 T80401C22 MOST grid Clear 22 1.5mL urine 144m
Row 8	 T80401B03 MOST vial Blue 03 1mL plasma 144m	 T80401B03 MOST grid Blue 03 1mL plasma 144m	Row 17	 T80401R12 MOST vial Red 12 1mL serum 144m	 T80401R12 MOST grid Red 12 1mL serum 144m	Row 26	 T80401C21 MOST vial Clear 21 1.5mL urine 144m	 T80401C21 MOST grid Clear 21 1.5mL urine 144m
Row 7	 T80401B02 MOST vial Blue 02 1mL plasma 144m	 T80401B02 MOST grid Blue 02 1mL plasma 144m	Row 16	 T80401R11 MOST vial Red 11 1mL serum 144m	 T80401R11 MOST grid Red 11 1mL serum 144m	Row 25	 T80401C20 MOST vial Clear 20 1.5mL urine 144m	 T80401C20 MOST grid Clear 20 1.5mL urine 144m
Row 6	 T80401B01 MOST vial Blue 01 1mL plasma 144m	 T80401B01 MOST grid Blue 01 1mL plasma 144m	Row 15	 T80401R10 MOST vial Red 10 1mL serum 144m	 T80401R10 MOST grid Red 10 1mL serum 144m	Row 24	 T80401R19 MOST vial Red 19 1mL serum 144m	 T80401R19 MOST grid Red 19 1mL serum 144m
Row 5	 T80401PAX MOST vial Red Top Tube PAX RNA 144m	 T80401PAX MOST grid Red Top Tube PAX RNA 144m	Row 14	 T80401R09 MOST vial Red 09 1mL serum 144m	 T80401R09 MOST grid Red 09 1mL serum 144m	Row 23	 T80401R18 MOST vial Red 18 1mL serum 144m	 T80401R18 MOST grid Red 18 1mL serum 144m
Row 4	T80401 Urine Cup	T80401 Lab Form	Row 13	 T80401R08 MOST vial Red 08 1mL serum 144m	 T80401R08 MOST grid Red 08 1mL serum 144m	Row 22	 T80401R17 MOST vial Red 17 1mL serum 144m	 T80401R17 MOST grid Red 17 1mL serum 144m
Row 3	T80401 Red Top Tube #5	T80401 Extra Tube	Row 12	 T80401Y07 MOST vial Yellow 07 buffy coat 144m	 T80401Y07 MOST grid Yellow 07 buffy coat 144m	Row 21	 T80401R16 MOST vial Red 16 1mL serum 144m	 T80401R16 MOST grid Red 16 1mL serum 144m
Row 2	T80401 Lavender Top Tube #3	T80401 Red Top Tube #4	Row 11	 T80401B06 MOST vial Blue 06 1mL plasma 144m	 T80401B06 MOST grid Blue 06 1mL plasma 144m	Row 20	 T80401R15 MOST vial Red 15 1mL serum 144m	 T80401R15 MOST grid Red 15 1mL serum 144m
Row 1	T80401 Lavender Top Tube #1	T80401 Red Top Tube #2	Row 10	 T80401B05 MOST vial Blue 05 1mL plasma 144m	 T80401B05 MOST grid Blue 05 1mL plasma 144m	Row 19	 T80401R14 MOST vial Red 14 1mL serum 144m	 T80401R14 MOST grid Red 14 1mL serum 144m

Appendix 2 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
- 6 Vacutainer holders
- Complete set of extra, unlabeled collection tubes
- 2 Tourniquets
- 1 Smelling salts
- 2 Pencils/pens
- Latex gloves
- 1 Hemostat
- 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 6 draw tubes labeled
- 5 Blood draw tube sheaths
- 22 Cryovials (6 blue cap, 1 buffy coat, 12 red cap, and 3 clear cap) with labels affixed
- 1 MOST Urine Collection Form (REDCap)
- 1 MOST Blood Collection Form (REDCap)
- 1 MOST Laboratory Processing Form (TELEForm)
- 1 Urine cup labeled
- Label (Extra Tube) for back-up vacutainer (1)
- Label for Laboratory Processing form (1)
- Labels for grid forms (22)

At the Phlebotomy Station:

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

Appendix 4 Precautions When a Participant Feels FaintPRECAUTIONS 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

Specimen collection supplies	# per participant	sample type	vendor: catalog #
7.0mL EDTA vacutainer	2	plasma	VWR: Catalog #: VT6450 (Supplier #: 366450) or equivalent from preferred vendor
10.0mL serum vacutainer	3	serum	Fisher: 23-021-018 or equivalent from preferred vendor
PAXgene vacutainer blood collection tubes 9.5mL tube (2.5mL blood with 6.9mL PAXgene blood RNA preservative solution)	1	RNA	Fisher: 23-021-01 (BD brand) 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: 22-289-953 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 (22 cryovials per participant)	22 cryovials		Determined by clinical center

Note: Educational discounts should apply. Please carefully review the specifications prior to placing an order. Contact the UCSF Coordinating Center if you have concerns about equipment on this list. Labels, cryovials, cryovial caps and cryovial storage boxes (not PAXgene 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

VWR
Radnor Corporate Center
Building One
Suite 200
P.O. Box 6660
100 Matsonford Road
Radnor, PA 19087-8660
1-800-932-5000
www.us.vwr.com

Appendix 6 Mock Data Collection Paper Forms

NOTE: Do not fax this form into the data system. This data must be entered into the Urine Collection REDCap form for submission to UCSF.

Blood Collection

Visit	MOST ID #	Acrostic	Date Exam Completed	Staff ID#
<input type="radio"/> BL/144m	<input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/>	<input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/>	<input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> / <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> / <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <small>Month Day Year</small>	<input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/> <input style="width: 30px; height: 20px; border: 1px solid black;" type="text"/>
<input type="radio"/> First exam <input type="radio"/> Repeatexam 1 <input type="radio"/> Repeatexam 2 <input type="radio"/> Repeatexam 3				

Time stamp start: : (military time)

1. Have you ever had an arm graft shunt or port for dialysis?

Yes No Don'tknow/Refused

Go to Question #3 and mark "Neither."

1a. Which side?

Right Left Both

Do NOT draw blood on right side.

Do NOT draw blood on left side.

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'tknow/Refused

Go to Question #3 and mark "Neither."

2a. Which side?

Right Left Both

Do NOT draw blood on right side.

Do NOT draw blood on left side.

Do NOT draw blood on either side. Go to Question #3 and mark "Neither."

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.

3. Have you had an illness in the past week requiring antibiotics, hospitalization, or steroids?

Yes No Don'tknow/Refused

4. Do you bleed or bruise easily?

Yes No Don'tknow/Refused

5. Have you ever been told you have a disorder related to blood clotting or coagulation?

Yes No Don'tknow/Refused

6. Have you ever experienced fainting spells while having blood drawn?

Yes No Don'tknow/Refused

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

(Examiner Note: Ask question even if already asked for urine collection.)

a. Date: / /

Month Day Year


b. Time: : (military time)

c. How many hours has participant fasted?

(Examiner Note: Proceed with the blood draw even if participant has not fasted.)

Hours

Visit	MOST ID #	Acrostic
<input type="radio"/> BL/144m	<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"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>



Blood Collection (continued)

8. Did participant consent to RNA testing?

Yes

No

Collect blood in Tube #6
(PAXgene RNA, 9.5mL)

Do not collect blood in
Tube #6 (PAXgene RNA)

9. Was any blood drawn?

Yes

No

Please describe why not in Question #10.

9a. Biospecimen label number assigned to participant:

(Examiner note: If blood and urine both collected, label number must be the same.)

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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9b. Were tubes filled to specified capacity?

(Examiner Note: wrap all tubes in foil or place in sheath to protect from light.)

1. Tube 1 (Lavender Top EDTA Plasma, 7mL)

Yes No

2. Tube 2 (Red Top Serum, 10mL)

Yes No

3. Tube 3 (Lavender Top EDTA Plasma, 7mL)

Yes No

4. Tube 4 (Red Top Serum, 10mL)

Yes No

5. Tube 5 (Red Top Serum, 10mL)

Yes No

6. Tube 6 (Red Top PAXgene RNA, 9.5mL)

(Examiner note: If participant consented to RNA testing (Question #8), collect Tube #6.)

Yes No

10. Comments:

Time stamp stop: : (military time)

NOTE: Do not fax this form into the data system. This data must be entered into the Urine Collection REDCap form for submission to UCSF.

Urine Collection

Visit	MOST ID #	Acrostic	Date of Exam Completed	Staff ID#
<input type="radio"/> BL/144m	<input type="text"/>	<input type="text"/>	<input type="text"/> / <input type="text"/> / <input type="text"/> Month Day Year	<input type="text"/>



First exam Repeat exam 1 Repeat exam 2 Repeat exam 3

Time stamp start: : (military time)

- Was a urine specimen obtained?
 Yes No (Go to Questions #8.)
- Biospecimen label number assigned to participant:
(Examiner note: If blood and urine both collected, label number must be the same.)
- 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:
 Yes
 No

Second:
 Yes
 No

Third:
 Yes
 No

Fourth or
 Yes
 No

- What time was the urine specimen collected?
(Examiner note: If two specimens are combined, please write the later of the two times.)
 : (military time)

- What is the date and time you last ate or drank anything except water?
(Examiner note: Ask question even if already asked for blood collection.)
a. Date: / /
Month Day Year
b. Time: : (military time)
c. How many hours has participant fasted?
 Hours
- Place of urine collection:
 Home
 Clinic
- What time did you get up for the day today?
 : (military time)
- Comments:

Time stamp stop: : (military time)