Biospecimen Manual of Procedures (MOP)

Version 7

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1. Introduction

This Manual of Operating Procedures is provided for KPMP Recruitment Sites (RS) for biospecimen collection, processing, and local storage prior to shipment to the Central Biorepository. It will outline the procedures for obtaining blood, urine, and stool from KPMP participants, as well as processing each sample type and local storage for all samples.

The KPMP Research Coordinator is responsible for collecting samples from consented participants, returning samples to the local lab for processing and storage, processing select samples (where appropriate training has been received), and shipping samples to the Central Biorepository (CBR) for further redistribution, processing, and storage. In the absence of the Research Coordinator, the Site PI or designated alternate would provide this service.

1.1 Safety Precautions for Handling All Biospecimens

- Obtain and utilize necessary protective clothing/equipment when working with all specimens. Such items include but are not limited to lab coats, gloves, protective eyewear, and absorbent pads. All specimens are handled as potentially infectious agents.

- Wear disposable gloves when collecting and processing specimens. Wash hands thoroughly with disinfectant soap prior to leaving the work area. Skin cuts or abrasions should be covered.

- It is recommended that aliquoting and processing specimens be done within a biological safety cabinet (or at least behind a plexiglass splash shield, if a biological safety cabinet is unavailable).

- Use 0.1% sodium hypochlorite (i.e. diluted household bleach) to clean up spills of blood, plasma, or serum. Use this solution to clean up all lab work surfaces at the start and completion of work activities.

- Dispose of all needles and tubing in puncture-resistant sharps containers for safe disposal.

- Do not perform any pipetting by mouth for any reason.

- Avoid formation of potentially infectious aerosols by careful pipetting and centrifugation.

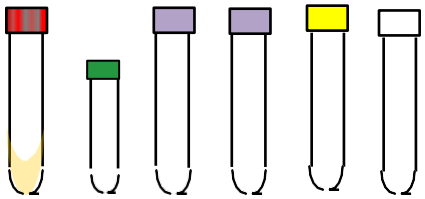
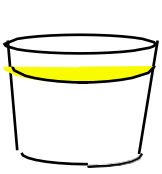
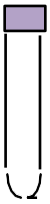
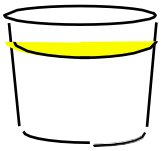
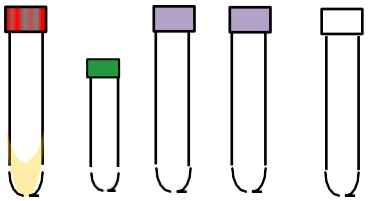
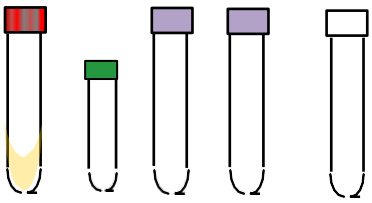
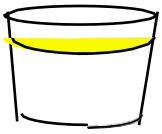
- Dispose of all used vacutainer tubes, needles and blood products in a spill proof liquid biohazard sharps container for disposal.

2. Biospecimen Sample Collection

2.1 Overview

Specimens will be collected at baseline (pre-biopsy for CKD participants; preferably pre-biopsy for AKI. See **Tables 1 & 2**), during the hospitalization for AKI participants, and at all follow-up study visits. **Figure 1** details the samples that will be collected by visit. For detailed descriptions, please refer to specific sections in the MOP for instructions pertaining to (1) collection and handling, (2) processing, and (3) aliquot and storage of each sample type.

Figure 1. Samples to be collected by visit

Visit	Blood (in draw order left to right)	Urine	Stool
Baseline (AKI and CKD)	 SST LiHep EDTA EDTA ACD PAXgene *With Buffy coat	 Spot Urine (120 mL)  Timed Urine	 Stool
Post-Biopsy (AKI only)	 EDTA *daily until discharge (up to 7 days)	 Spot Urine (20 mL) *daily until discharge (up to 7 days)	
3-month Follow-up (AKI only)	 SST LiHep EDTA EDTA PAXgene	 Spot Urine (20 mL)	
Annual Follow-Up (CKD and AKI)	 SST LiHep EDTA EDTA PAXgene *With Buffy coat for 1st annual only	 Spot Urine (20 mL)	

2.2 Allowable Sample Collection Time Windows for the Baseline Visit (AKI and CKD) and Post-biopsy Sample Collection (AKI only)

2.2.1. General principles:

The preference is for all samples to be collected as close to and before the biopsy as possible (see 'Ideal Sample Collection Time Point' column in **Tables 1 and 2**). In acknowledgment that clinical circumstances may not always make the ideal sample collection time point reasonable, **Tables 1 and 2** also outline the allowable windows, pre- and post-biopsy, in which samples may be collected.

NOTE: As a general rule, all efforts should be made to complete all baseline collections pre-biopsy, and as close to the biopsy as possible. ***If samples cannot be obtained within the allowable time window, do not discard the sample.***

2.2.2 CKD Participants:

Baseline sample collection window for CKD participants is listed in **Table 1**.

Table 1 Baseline CKD Sample Collection Window

Sample Type	Ideal Sample Collection Time Point	Allowable Pre-Biopsy Collection Window	Allowable Post-Biopsy Collection Time Point
Blood	As close to biopsy as possible	Up to 6 weeks prior to biopsy	Not allowable
Spot Urine	As close to biopsy as possible	Up to 6 weeks prior to biopsy	Not allowable
Timed 24-Hour Urine	As close to biopsy as possible	Up to 6 weeks prior to biopsy	Not allowable
Stool	As close to biopsy as possible	Up to 6 weeks prior to biopsy	Allowable up to 7 days post-biopsy, but discouraged

2.2.3 AKI Participants:

Baseline sample collection window for AKI participants is listed in **Table 2**.
AKI participants may have a central line in place for blood draw for clinical indications, which may be used for blood collection. In the absence of a central line, a venipuncture will need to be done to obtain the blood specimen; when possible, this should be timed to coincide with a clinical lab draw to reduce the number of needle sticks.

NOTE ON PRIORITIZATION: Particularly in AKI participants, the local hospital may impose daily blood draw limits for research purposes that are less than the outlined volumes in this MOP. In these scenarios, it is allowable to spread the blood collection over a few days. See **Table 5** for the revised blood collection schedule. Strive to collect all tubes pre-biopsy, but do not delay the biopsy in order to accomplish this goal. Collect as much as possible pre-biopsy. Similarly, some participants may not be able to provide both a spot and a timed urine collection in the time before the biopsy. In these scenarios, the spot urine collection should be prioritized, followed by however much of a timed urine collection (if any) is possible.

Table 2 Baseline AKI Sample Collection Window

Sample Type	Ideal Sample Collection Time Point	Allowable Pre-Biopsy Collection Time Window	Allowable Post-Biopsy Collection Time Window
Blood	As close to biopsy as possible (strongly preferred pre-biopsy!)	48 hours prior to biopsy	Allowable up to 24 hours post-biopsy but discouraged
Spot Urine	As close to biopsy as possible (strongly preferred pre-biopsy!)	48 hours prior to biopsy	Allowable up to 12 hours post-biopsy but discouraged
Timed 8-24 Hour Urine	As close to biopsy as possible **collection may not be possible*	24 hours prior to biopsy	N/A
Stool	As close to biopsy as possible **collection may not be possible*	24 hours prior to biopsy	Allowable up to 72 hours post-biopsy but preferably at the same time as spot urine is collected

Following the biopsy for AKI participants, an additional 10 mL EDTA blood sample will be collected every 24 hours post-biopsy (+/- 12 hours, as is clinically feasible) and repeat spot urine collection of 20 mL should be done every 24 hours(all +/- 12 hours, as is clinically feasible). These daily EDTA and spot urine collection should continue until discharge, or until day 7 post-biopsy. **Table 3** outlines the allowable windows for post-biopsy sample collections for AKI participants.

Table 3 Post-Biopsy AKI Sample Collection Window

Sample Type	24 Hours Post-Biopsy (+/- 12 hours)	48 Hours Post-Biopsy (+/- 12 hours)	72 Hours Post-Biopsy (+/- 12 hours)	Continue collecting q24 hours until discharge (up to day 7)
Blood	10 mL EDTA	10 mL EDTA	10 mL EDTA	10 mL EDTA
Spot Urine	20 mL Spot Urine	20 mL Spot Urine	20 mL Spot Urine	20 mL Spot Urine

3. Blood

3.1. Blood Collection & Handling Instructions

The blood draw will be done by the research coordinator or other qualified staff or phlebotomist at all sites. Blood will be drawn into several tubes.

3.1.1 Equipment

The Central Biorepository (CBR), which is located at the University of Michigan, will provide all blood collection tubes and a sample-ID specific *Blood Processing Worksheet* in the participant's KPMP kit. **All collection tubes are to be stored at room temperature prior to collection.** The Standard Blood Kit and Post-Biopsy Blood Kit that CBR provides are shown in the images below.

The recruitment site should provide the rest of the supplies needed for blood collection, listed here:

- Test tube rack to hold the blood collection tubes
- Plastic vacutainer needle holder
- Vacutainer Luer slip adaptors to connect the butterfly
- Sterile alcohol swabs
- Gauze sponges
- Tourniquet
- Bandages (Band Aids)
- Stopwatch
- Waste disposal container for sharps

3.1.2 Blood collection

Blood collection preparation should be done in the following manner:

1. Check to make sure all materials are present, in good condition and not expired, and properly labeled with appropriate kit ID. If materials are expired, set them aside and do not use. Contact the CBR via the REDCap Inventory Management tool to order replacement supplies.
2. Arrange blood collection tubes from the CBR in test tube rack, in draw order (see **Table 4**).
3. Note the Kit ID, blood draw date/time, and whether the participant was fasting on the *Blood Collection form in REDCap* (see **Section 9**).
4. Ensure the centrifuge and freezer are working properly.

The phlebotomy room should meet these criteria:

1. Ideally, blood draw should take place in an isolated room or with participants separated by room dividers.
2. The room must be equipped with all of the necessary blood specimen supplies (see **3.1.1**).
3. The centrifuge and freezer should be nearby (to minimize processing delay).

The venipuncture should follow this procedure:

1. The participant is asked whether he/she has a bleeding disorder before the blood is drawn. If such a disorder is present, ask the participant whether he/she has had blood drawn previously and if so, whether he/she had any problems with excessive bleeding or bruising at the venipuncture site. If the participant has a history of venipuncture problems, the participant should be sampled only if approved by the physician.
2. Ask the participant if he/she or his/her doctor have a preference as to which vein to use to determine whether or not he/she has been told to protect a particular vein or particular upper extremity.
3. If not, it is recommended that the medial-most antecubital vein in the dominant arm is used, assuming that the non-dominant arm will be the access arm of choice if the subject goes on to need dialysis, and that the medial most antecubital vein is not likely to be the draining cephalic vein.
4. The venipuncture is performed with a 21 gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small, walled needle which minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, he/she may be reassured to know such care is taken.
5. 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 will be the contact with the technologist who draws the blood and his/her general attitude and competence.
6. Handling participants who are extremely apprehensive about having blood drawn:
    a. Do not under any circumstances force the participant to have blood drawn.
    b. If the participant is nervous or excited, the technologist should briefly describe the procedure:
        i. “I am going to be drawing about four (4) tablespoons of blood. This blood will be used in tests for kidney function and other research analyses.”
    c. 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.
    d. 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.
    e. If the participant has “good veins” the phlebotomist can reassuringly say, “Oh, you have good veins; there should be no problem.”
7. Preparation:
    a. Remove all extra clothing and have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow).
    b. A tourniquet is used to increase venous filling. It makes the veins more prominent and easier to enter.
8. Precautions When Using a Tourniquet:
    a. The tourniquet should be on the arm for the shortest time possible.
    b. Never leave the tourniquet on for longer than two (2) minutes. To do so may result in hemoconcentration or a variation of blood test values.
    c. If a tourniquet must be applied for the preliminary vein selection, it should be released and reapplied after a wait of two minutes.

d. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.
e. Wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.
f. Tuck the end of the tourniquet under the last round. If a Velcro tourniquet is used, adhere the ends to each other.

9. Identify the vein:
    a. Palpate and trace the path of veins several times with the index finger. Thrombosed veins lack resilience, feel cord-like, and roll easily.
    b. If superficial veins are not readily apparent, have the participant close his or her fist.
    c. Lowering the extremity over the arm of the chair will allow the veins to fill to capacity.
    d. Identify the best available vein.
10. Cleanse the venipuncture site.
    a. Remove alcohol pad from its sterile package.
    b. Cleanse the vein site with the alcohol pad using a circular motion from the center to the periphery.
    c. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the participant when the venipuncture is performed.
11. Assemble the butterfly-vacutainer set.
    a. Attach the Luer adaptor to the vacutainer holder, when applicable.
    b. Attach the Luer end of the butterfly needle set to the Luer adaptor.
12. Perform venipuncture:
    a. 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 (2.3 or 5.0 cm) below the venipuncture site.
    b. With the needle bevel upward, enter the vein in a smooth continuous motion.
    c. 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.
    d. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
    e. Start a timer to measure the flow rate of blood into the first blood collection tube (see Table 4 for draw prioritization). If the flow rate in the tube is so slow that blood does not fill the first collection tube within 50 seconds, stop the blood collection and repeat on the other arm. If blood is flowing freely, the butterfly tubing can be anchored to the participant's arm using medical tape for the duration of the draw.
    f. Remove the tourniquet after blood is flowing into the second tube.
    g. 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 nor reintroduce pressure after completion of the draw.
    h. Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach another one without removing needle from vein.
    i. When the blood flow ceases, remove the tube from the holder. The shutoff valve recovers the point, stopping blood flow until the next tube is inserted.
    j. All tubes EXCEPT SERUM SEPARATOR (tiger top) should be gently mixed by inverting immediately after each tube is filled and removed from the butterfly setup.
    k. If it is not possible to collect all of the desired tubes, follow the requested order (**Table 4**) and fill each tube as completely as possible.
13. Prevent blood mixing during venipuncture.

a. Immediately invert tubes containing anticoagulant (i.e. all *except* serum separator (tiger top)). See **Figure 2.**

b. DO NOT SHAKE TUBES! Vigorous mixing or shaking can break the cells.

c. Invert each tube slowly and gently 10 times. See **Figure 2.**

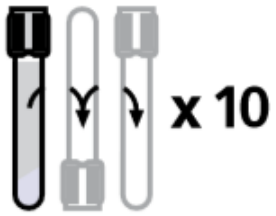


Figure 2. Tube inversion

14. If a blood sample is not forthcoming, the following manipulations may be helpful:
    a. If there is a sucking sound, the tube has lost its vacuum. Replace with a new tube.
    b. If no blood appears, move the needle slightly in hope of entering vein. Do Not Probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.
    c. The same technician should not attempt a venipuncture more than twice.
15. Finishing
    a. To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad.
    b. Discard needle with its vacutainer needle holder into a sharps container.
16. Bandage the arm.
    a. Under normal conditions:
        i. Slip the gauze pad down over the site, continuing mild pressure.
        ii. Apply an adhesive or gauze bandage over the venipuncture site after making sure blood flow has stopped.
    b. If the participant continues to bleed:
        i. Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
        ii. Wrap the gauze bandage tightly around the arm over the pad.
        iii. Tell the participant to leave the bandage on for at least 15 minutes.
17. **Precautions** –If a participant feels faint or looks faint following the blood draw:
    a. Have the person remain in the chair; if necessary, have him/her sit with head between knees.
    b. Take an ampule of smelling salts, crush it, and wave it under person's nose for a few seconds.
    c. Provide the person with a basin if he/she feels nauseous.
    d. Have the person stay reclined until their color returns and he/she feels better.
    e. Place a cold wet cloth on the back of the person's neck.
    f. If the person faints, use smelling salts to revive.
    g. If the person continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member, who will advise you on further action.

The date and time of draw, as well as whether the participant was fasting, will be documented on the *Blood Collection CRF in REDCap* (see **Section 9**). Use the form in Section 9 as a paper worksheet as needed, then transfer to REDCap.

Additionally, the method of blood collection must be documented on the *Blood Collection CRF* (see **Section 9**).

The methods of blood collection are as follows:

- venipuncture/needle stick
- arterial line
- central line

Do NOT draw from a peripheral line, unless it's freshly inserted, not previously flushed, sufficiently large caliber. Otherwise, there is risk that the sample could be impacted by contamination, dilution, and/or hemolysis.

3.1.3 Re-Draw Scenarios

The target blood draw volume is approximately 50 mL and the draw order is outlined in **Table 4**. All tubes should be **completely filled**. If a tube is under-filled, do not re-draw.

A re-draw may be warranted in certain situations:

- If damage or loss occurs to a tube for which there are no spares (e.g the PAXgene or ACD tubes), re-draw may be attempted for CKD participants. If the damage or loss impacts a tube from an AKI participant, the tube should not be re-drawn as AKI is quite dynamic and the timing of the draw in relation to the biopsy may no longer be valuable. The site should use discretion to identify a good time to approach the CKD participant about a re-draw; it could be a separate visit or during the next scheduled in-person visit. The site will need to work with the Central Biorepository to request a new tube.
- If baseline biosamples are collected and then the biopsy is delayed such that the allowable pre-biopsy collection window is surpassed (see Tables 1 and 2), re-draw may be attempted. A new kit should be selected and a new iteration of the biosample form should be generated in REDCap (use the + icon). The original samples should not be disposed of; they should be shipped to CBR and may be utilized for future quality control checks.

If delivery of the ACD tube to the CBR is delayed:

- If ACD tube is received <24 hours after draw, CBR processes as normal.
- If ACD tube is received >24 hours but <48 hours after draw, CBR should process as normal but will also notify CKD sites that they should attempt a re-draw of the tube (before or after biopsy). AKI sites do not re-draw.
    a. If the CKD site is able to re-draw the tube and the replacement arrives <24 hours after draw, CBR will process the new tube and dispose of the derivatives from the original tube (CBR will confirm disposal)
- If ACD tube is received >48 hours after draw, CBR does not process the tube. We will attempt a re-draw for CKD sites but not for AKI sites.

Table 4 Preferred Blood Collection Instructions by Type, in tube draw order

Draw Order	Vacutainer Type	Final Sample Type	Collection Instructions	Pre-Processing Instructions	Visits to Collect
Pre-Biopsy					
1	10 mL SST	Serum 	**<u>Do not</u>** invert	Store upright at room temperature for 30 minutes before processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
2	6 mL Lithium Heparin	Plasma 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
3	10 mL EDTA	Plasma & DNA 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 72 hours, 3 month, annual ***DNA for baseline and 1st annual visit ONLY***
4	10 mL EDTA	Plasma & DNA 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual ***DNA for baseline and 1st annual visit ONLY***
5	8.5 mL ACD	Peripheral blood mononuclear cells (PBMCs) 	Invert gently 8-10 times	Store at room temperature before mailing in ambient temperature mailer and sleeve. MUST GET TO CBR WITHIN 24 HOURS!	**CKD:** Baseline only **AKI:** Baseline only
6	PAXgene® RNA 2.5 mL volume	Whole blood 	Invert gently 8 – 10 times	Store at room temperature for at least 2 hours (up to 72), then transfer to -80°C	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
Post-Biopsy (AKI ONLY), Daily until Discharge					
	10 mL EDTA	Plasma 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**AKI:** Daily until discharge (up to 7 days post-biopsy)

NOTE: The PAXgene® Blood RNA tube should always be the **<u>last</u>** tube drawn in the phlebotomy procedure.

NOTE: The ACD tube should not be drawn if it is not possible to ship it to the CBR for receipt within 24 hours of the draw. The tube should not be drawn on a Friday, and should not be drawn on Thursday unless shipment to CBR is guaranteed that afternoon. If the main draw occurs late on a Thursday or anytime on a Friday, exclude the ACD tube. The ACD tube may be drawn at a later date (i.e. on the day of the biopsy for CKD participants getting study blood draws at a study visit prior to biopsy; or on the day after biopsy for AKI participants).

Table 5 Blood Collection Instructions for In-Patients with Daily Research Blood Draw Limits *ONLY* (otherwise use Table 4).

Draw Order	Vacutainer Type	Final Sample Type	Collection Instructions	Pre-Processing Instructions	Visits to Collect
Day 1					
1	10 mL EDTA	Plasma & DNA 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 72 hours, 3 month, annual ***DNA for baseline and 1st annual visit ONLY***
2	8.5 mL ACD	Peripheral blood mononuclear cells (PBMCs) 	Invert gently 8-10 times	Store at room temperature before mailing in ambient temperature mailer and sleeve. MUST GET TO CBR WITHIN 24 HOURS!	**CKD:** Baseline only **AKI:** Baseline only
Day 2					
1	10 mL SST	Serum 	**<u>Do not</u>** invert	Store upright at room temperature for 30 minutes before processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
2	6 mL Lithium Heparin	Plasma 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
3	PAXgene® RNA 2.5 mL volume	Whole blood 	Invert gently 8 – 10 times	Store at room temperature for at least 2 hours (up to 72), then transfer to -80°C	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual
Day 3					
1	10 mL EDTA	Plasma & DNA 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**CKD:** Baseline, annual **AKI:** Baseline, 3 month, annual ***DNA for baseline and 1st annual visit ONLY***
Post-Biopsy (AKI ONLY), Daily until Discharge					
	10 mL EDTA	Plasma 	Invert gently 8 – 10 times	Store on wet ice or refrigerate (4°C) until processing	**AKI:** Daily until discharge (up to 7 days post-biopsy)

Invert all tubes, except serum separator tube (tiger top), as per the collection instructions (section **3.1.2**). Tubes are inverted slowly and gently; DO NOT SHAKE TUBES. See **Figure 2** and the 'Collection Instructions' column in **Table 4.**

The 'Pre-Processing Instructions' column in **Tables 4 and 5** describe the conditions in which tubes should be handled immediately following collection:

- Blood collected in Lithium Heparin (green) and EDTA (purple) tubes should be stored at 4°C until ready to begin processing, as storage at room temperature will degrade some of the biomarkers.
- SST (tiger top) tube should remain upright at room temperature for 30 minutes prior to processing. It should then be immediately processed, but if this is not feasible, store at 4°C until able to begin processing.
- ACD (yellow) tubes should be stored and shipped at room temperature.
- PAXgene (clear) tubes must be stored at room temperature (18-25°C) for 2 hours and up to 72 hours before transfer to a -80°C freezer.

3.2. Blood Processing Instructions

The goal is to process the samples **within one hour of collection**. The maximum allowable time between specimen collection and processing is two hours. All times (venipuncture, start of centrifugation, freezer) should be recorded on the processing worksheet provided in the Kit, for later transfer to SpecTrack. Do not discard samples if the maximum allowable time to processing is exceeded; instead, process as normal, but be sure to note the times on the processing worksheet/SpecTrack.

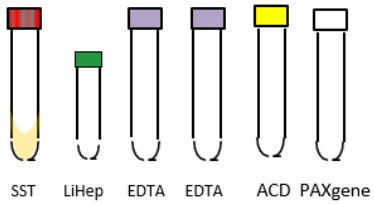
- Serum and plasma samples should complete centrifugation within 1 hour of collection and be aliquoted and frozen within 2 hours of collection.

All samples can be centrifuged concurrently with the exception of the PAXgene® Blood RNA tube (clear), ACD tube (yellow), and the stool samples **which are NOT centrifuged or processed**.

NOTE: ***Only process one participant's samples at a time: never more.*** This will help limit the chances of accidental mix-up of participant samples.

3.2.1 Equipment

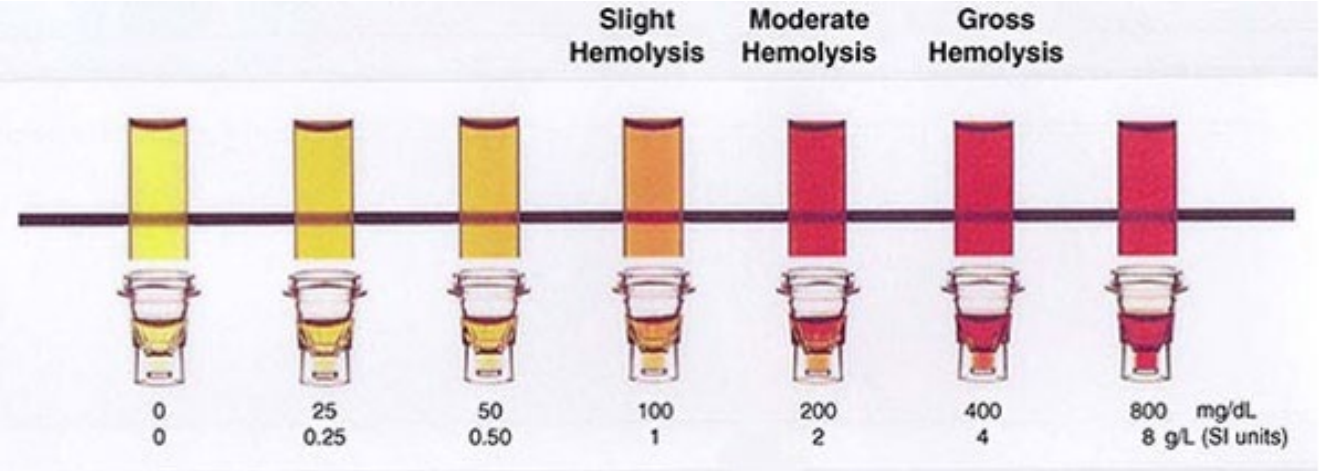
- Centrifuge capable of achieving 2000 x G and 4°C (NOTE: G is not the same as RPM; please consult your centrifuge conversion table if necessary).
- Aerosol spray covers for centrifuge buckets (or other form of cover for safety purposes, per institutional policy)
- Pipettor and pipette tips, sterile transfer pipette
- Participant's kit provided by the CBR with pre-labeled cryovials and worksheet of kit contents
- Aliquot rack
- Waste disposal containers and sharps container
- Cardboard freezer boxes and metal racks
- Freezer at -80 ˚C



- SAFETY Gear: obtain and utilize necessary protective clothing/equipment for preparing an aliquot of specimens. Such items include but are not limited to lab coats, gloves, protective eyewear, and absorbent pads.

3.2.2 Centrifuge Instructions

1. Balance the centrifuge to ensure proper performance of the instrument. If needed, use a "balance tube" filled with water to the proper level to achieve balance.
2. Once the centrifuge is loaded with samples, set the speed to 2000 x G, the time to 12 minutes and the temperature to 4°C for processing. It is recommended that aerosol spray covers for centrifuge buckets be used. ***All tubes are spun with the exception of the PAXgene RNA tube, ACD tube, and stool samples, which are not processed further locally.***
3. If the centrifuge is equipped with a brake, it may be used to accelerate the stopping time (without the brake a centrifuge typically takes about 10 minutes to stop).
4. Once the centrifuge has stopped, open the centrifuge and remove the specimens.
5. Note whether the sample has hemolyzed on the *Blood Collection CRF* (see **Section 9**). Indicate 'Yes' if there is any degree of hemolysis (i.e. if the plasma or serum is orange or red). See image below.



3.3. Blood Aliquot & Storage Instructions

3.3.1 Overview and Best Practices

1. For the centrifuged specimens, verify that the cells have been clearly separated from the serum or plasma by visibly noting the serum and plasma are distinctly above the packed red blood cells and white blood cell layers.
2. Process all specimens concurrently by type (i.e. all blood together, all urine together) to the extent possible.
3. Carefully open the blood tube using the universal precautions previously described.
4. Use a pipette to transfer the sample from the primary tube to the appropriately labeled secondary tubes/cryovials. Please pay careful attention to the accuracy of volumes pipetted. Always use a clean pipette tip for each sample type.

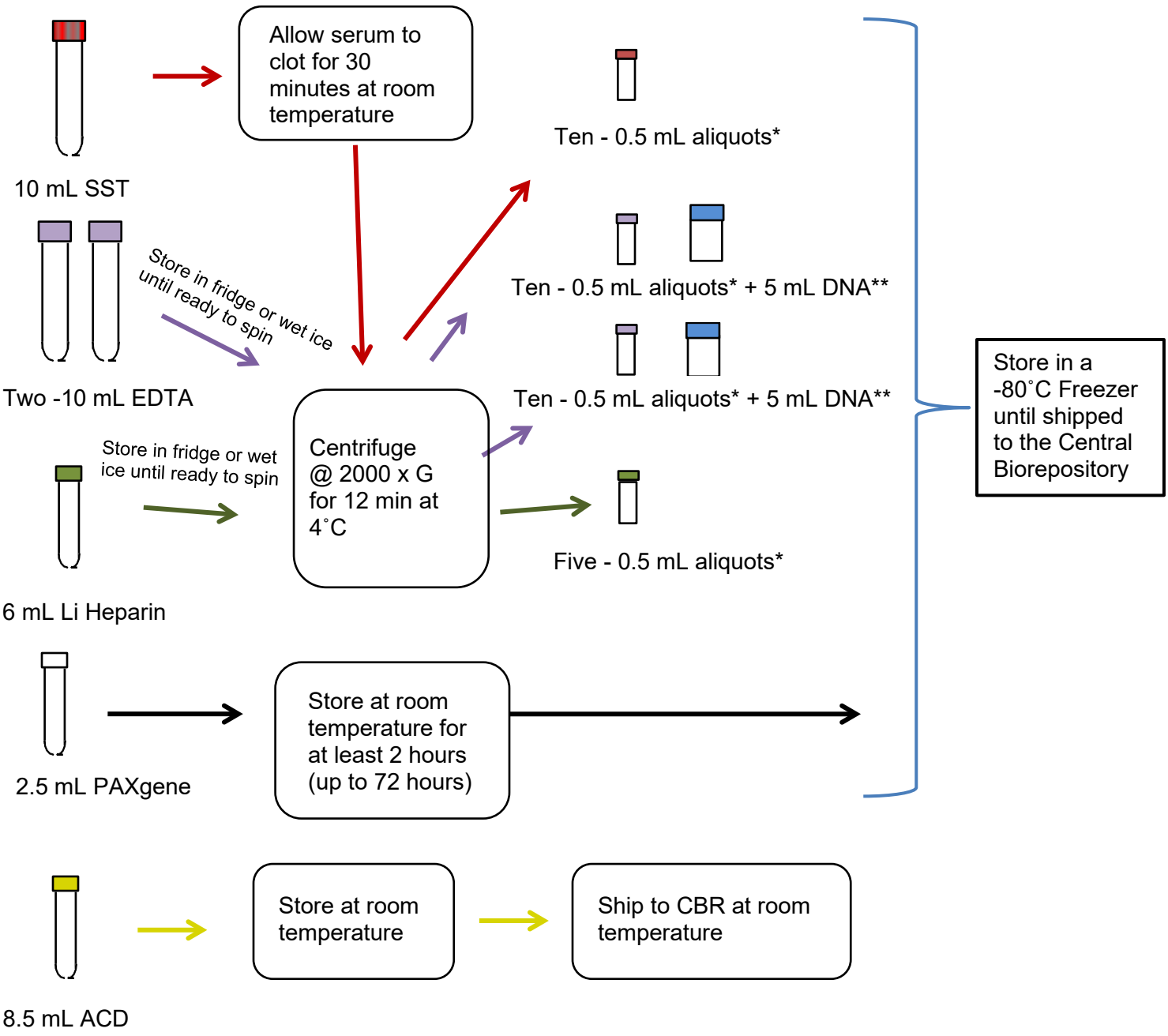
   Recommended skill: Reverse pipetting (see video at https://youtu.be/HKO4dAvb7rg)

   Reverse pipetting helps ensure proper volumes. To reverse-pipet the proper volume into the aliquot tube, set your pipet to the proper volume. Push the plunger down to the first stop (to become familiar with how far down to push for the correct volume), then push the plunger down to the second stop. Insert the pipet tip into the sample and slowly draw up until the plunger is fully extended. Move the pipet tip to the empty vial and dispense the sample by pushing down on the plunger to the first stop with the pipet tip resting against the side of the aliquot tube. For subsequent samples, keep the pipet tip at the first stop and draw up the next sample and dispense to the first stop, repeating as many times as needed. This approach keeps a small amount of sample in the pipet tip at all times and ensures that the correct amount is pipetted up and down for each sample.
5. When pipetting the plasma be very careful not to disturb the buffy coat (white cell layer) and when pipetting the serum be very careful not to disturb the serum separator layer.
6. Store all aliquots in a plastic rack or cardboard box in an upright position at -80˚C (with the exception of the ACD (yellow) tube, which is stored at room temperature) immediately following completion of processing. Label the racks or cardboard boxes with permanent marker or an adhesive label that can be easily identified. See **Section 6** for additional details about Local Storage.

Table 6: Blood processing aliquots

	Vacutainer	Volume per Aliquot	Number of Aliquots
1	10 mL SST (Tiger top)	0.5 mL for 1st nine (solid red cap); up to 1.5 mL for 10th aliquot (cap marked with red +)	10
2	6 mL Li Hep (Green)	0.5 mL for 1st four (solid green cap); up to 1.5 mL for 5th aliquot (cap marked with green +)	5
3	10 mL EDTA (Purple)	0.5 mL for 1st nine (solid purple cap); up to 1.5 mL for 10th aliquot (cap marked with purple +)	10 plasma (+ DNA at baseline and 1st annual follow-up) Do not collect DNA for the post-biopsy AKI collections.
4	10 mL EDTA (Purple)	0.5 mL for 1st nine (solid purple cap); up to 1.5 mL for 10th aliquot (cap marked with purple +)	10 plasma (+ DNA at baseline and 1st annual follow-up) Do not collect DNA for the post-biopsy AKI collections.
5	8.5 mL ACD (Yellow)	n/a	n/a
6	2.5 mL PAXgene® (White)	n/a	n/a

Figure 3: Blood Processing



**The final cryovial for SST, EDTA, and Lithium Heparin has a maximum capacity of 1.5 mL (the 10th, 10th, and 5th aliquots, respectively). Any remaining sample should be deposited in these tubes. These tubes are distinguished with a + sign on the cap.*

***DNA pellet is collected only at the baseline and 1st annual follow-up visits. Note that the pellet should not be collected for the 10 mL post-biopsy draws for AKI participants at baseline: only for the pre-biopsy baseline draws.*

3.3.2 Aliquot and/or Storage Instructions by Sample Type

3.3.2.1 Serum Separator Tube (SST) Instructions (tiger top)

1. Allow tube to clot upright for 30 minutes prior to processing.
2. Place tube in the centrifuge and spin per instructions in section **3.2.2**.
3. Pipet 0.5 mL of serum into each corresponding cryovial provided by the CBR in the participant's kit.
    a. Fill as many of the ten (10) available cryovials with serum from tiger top tubes as possible. Cryovials 1 through 9 should be filled with 0.5 mL serum. The final cryovial has 1.5 mL capacity (the cap is marked with a red + sign; the rest of the caps are solid red). Fill the final cryovial with up to 1.5 mL serum.
4. Note volumes of filled cryovials and the IDs of unused cryovials on the *Blood Processing Worksheet* in the Kit for reference later when cryovial details are noted in SpecTrack.
5. Put the cryovials into the labeled box and place them into the -80 ˚C until shipment to the CBR.

3.3.2.2 EDTA Tube Instructions (purple tops)

1. Process the tube in the centrifuge per section **3.2.2.**
2. Pipet 0.5 mL of plasma into each corresponding cryovial provided by the CBR in the participant's kit. Aspirate slowly starting at the top of the plasma. (**NOTE:** when pipetting for the baseline (pre-biopsy) and first annual follow-up visits, be sure not to allow the pipet tip to get any closer than one-half inch from the buffy coat; this will be used for the for cell pellet preparation for DNA isolation. Set aside for Step 5.) DO NOT DISCARD.
    a. Fill as many of the ten (10) available cryovials with purple caps as possible. Cryovials 1 through 9 should be filled with 0.5 mL plasma. The final cryovial has 1.5 mL capacity (the cap is marked with a purple + sign; the rest of the caps are solid purple). Fill the final cryovial with up to 1.5 mL plasma.
    b. Repeat step 2a with the contents of the second EDTA tube.
3. Note volumes of filled cryovials and the IDs of unused cryovials on the *Blood Processing Worksheet* in the Kit for reference later when cryovial details are noted in SpecTrack.
4. Put the cryovials into a labeled box and place them in the -80 ˚C freezer until shipment to the CBR.
5. **BASELINE (PRE-BIOPSY) AND FIRST ANNUAL VISIT ONLY: Packed Cell Preparation for DNA Isolation (see Figure 4):**

NOTE: the packed cell pellet for DNA extraction is collected only at the baseline (pre-biopsy) and first annual follow-up visits. For all other EDTA collections, simply aliquot the plasma into the ten cryovials, as described in step 2 above.

a. After aliquoting the top 2/3 of the plasma supernatant from the two EDTA tubes as described above (see **Figure 4B**), use a sterile soft bulb plastic transfer pipette to slow aspirate the remaining ½" layer of plasma, the buffy coat and some of the red cells from the tube (see **Figure 4C**). Take care not to aspirate the buffy coat into the bulb of the pipette.

   "Ring" the tube with the pipette by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer.

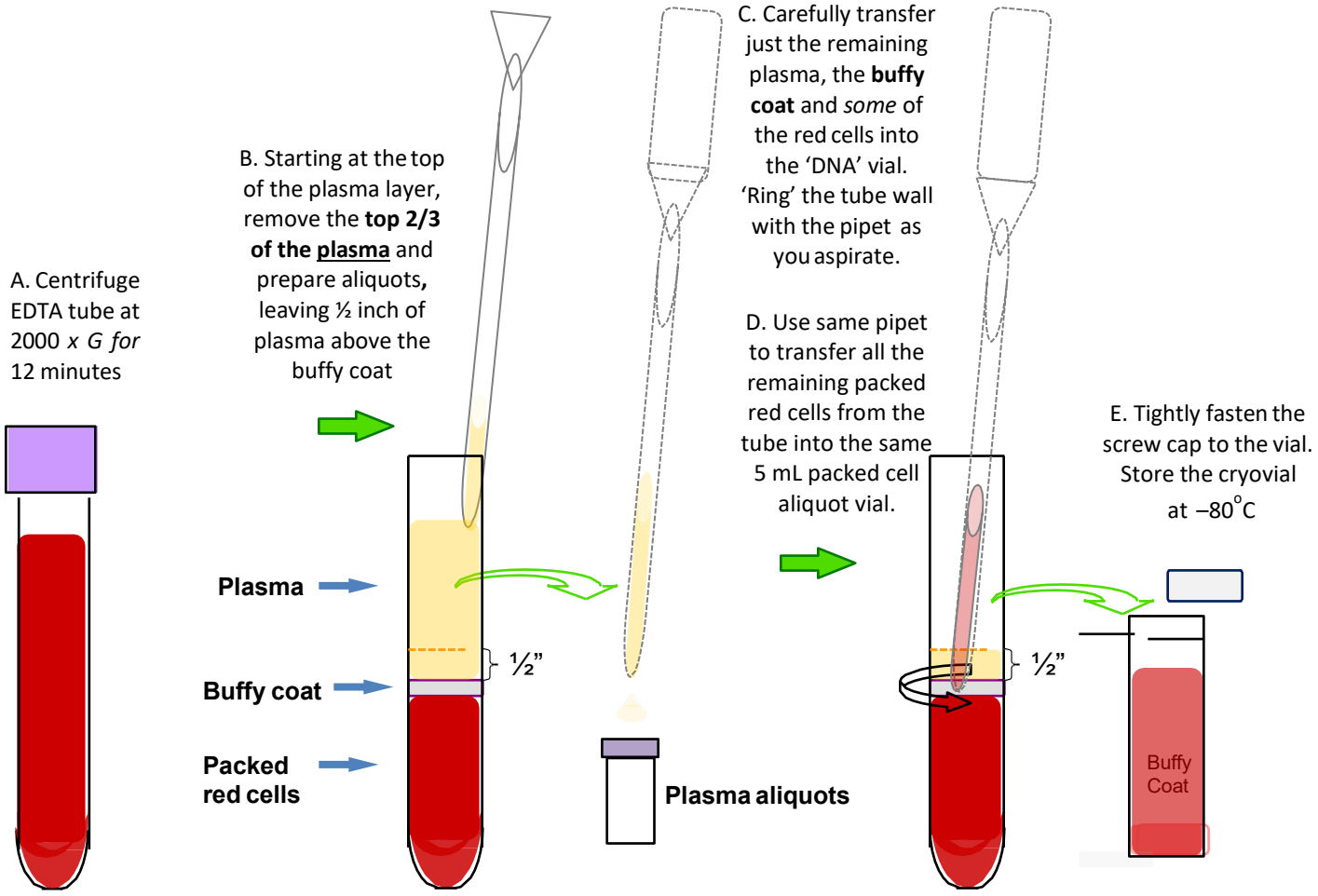
b. Dispense into the 5 mL packed cell aliquot vial (see **Figure 34C**).

c. Still using the same pipet, go back and transfer all the remaining packed red cells from the tube in to the same 5 mL packed cell aliquot vial (see **Figure 4D**). This step will ensure that all the buffy coat is adequately rinsed from the pipet.

d. Tightly fasten the screw cap on this vial and place it in the -80˚C freezer until shipment to the CBR (see **Figure 4E**).

Figure 4. Packed Cell Pellet for DNA Extraction

(Baseline (pre-biopsy) and First Annual Follow-up Visits only)



Processing the EDTA tube collected for DNA isolation:

Centrifuge the EDTA tube per instructions in section **3.2.2**. Being careful not to disturb the buffy coat and packed red cell layers, pipet the top 2/3 of the clear plasma supernatant filing the corresponding cryovials as described in step **3.3.2.2**. Leave a ½-inch layer of plasma above the buffy coat-red blood cell layers as described above. Using a sterile plastic transfer pipet, slowly aspirate the remaining ½" layer of plasma, the buffy coat and *some* of the red cells from the tube. Take care not to aspirate the buffy coat into the bulb of the pipet! 'Ring' the tube with the pipet by carefully aspirating along the wall at the buffy coat layer to ensure maximum transfer. Dispense into the 5-mL packed cell aliquot vial. Still using the same pipet, go back and transfer all of the remaining packed red cells from the tube into the same 5-mL DNA vial. This step will ensure that all of the buffy coat is adequately rinsed from the pipet. Tightly fasten the screw cap on this vial and place it in the -80°C freezer until shipment to the CBR.

This process is demonstrated in the KPMP "DNA and Urine Pellets Overview" video on YouTube:

https://www.youtube.com/watch?v=ZJraNpQjFvI

3.3.2.3 Lithium Heparin tube Instructions (green top)

1. Centrifuge samples per section **3.2.2.**

2. Pipet 0.5 mL of serum into each corresponding cryovial provided by the CBR in the participant's kit.

    a. Fill as many of the five (5) available cryovials with plasma from the green top tubes as possible. Cryovials 1 through 4 should be filled with 0.5 mL plasma. The final cryovial has 1.5 mL capacity (the cap is marked with a green + sign; the rest of the caps are solid green). Fill the final cryovial with up to 1.5 mL plasma.

3. Note volumes of filled cryovials and the IDs of unused cryovials on the *Blood Processing Worksheet* in the Kit for reference later when cryovial details are noted in SpecTrack.

4. Put the cryovials into the labeled box and place them into the -80 ˚C freezer until shipment to the CBR.

3.3.2.4 ACD tube Instructions (yellow top)*

1. Store tube at room temperature until ready for shipment to the CBR.

2. Note details on the *Blood Processing Worksheet* in the Kit for reference later when details are noted in SpecTrack.

3. Package tube in sleeve and then ambient temperature mailer for shipment to the CBR (see **Section 8** for details).

4. PBMC isolation will occur at the CBR.

Note that ACD will be shipped separately from the other tubes at room temperature and not on dry ice. See section 8 for shipping instructions.

3.3.2.5 PAXgene® RNA tube Instructions (clear top)*

1. Store the PAXgene® Blood RNA tube upright at room temperature for at least two hours.

2. Note details on the *Blood Processing Worksheet* in the Kit for reference later when details are noted in SpecTrack

3. Place the PAXgene® Blood RNA tube upright in a metal rack in a -80 ˚C freezer until shipment to the CBR.

    Do not place tubes in tight test tube containers such as Styrofoam or Plastic as this may lead the tube to break. Tubes should be loosely hanging in place in the metal rack.

4. Urine

4.1 Spot Urine Collection & Handling Instructions

A urine sample ("spot urine") will be collected at all visits.

Baseline volumes (CKD and AKI): At least 60 and ideally 120 mL of urine should be collected from the participant. If less than 60 mL is collected for the baseline spot, a replacement collection may be considered if it can be obtained pre-biopsy and does not present undue burden on the participant. The initial collection (<60 mL) should be processed normally in case a second collection is not obtained or does not surpass the initial volume. The collection of greatest volume should be kept and sent to CBR; the smaller volume may be disposed.

All other collections (post-biopsy for AKI, 3-month for AKI, and all annual follow-up for AKI and CKD): 20 mL of urine should be collected from the participant.

If there is not enough urine, do not pool urine from separate voids (i.e., from different times of the day), even if the desired volume is not achieved. Participants should do a clean catch void, if at all possible. Please refer to clean catch void instructions in section **4.1.2**. Instructions for collection when the participant has an indwelling urinary catheter are also included in section **4.1.3**.

4.1.1 Equipment

120 mL Spot urine collection kit (for baseline only):

CBR will supply the following:

- Two cups for specimen collection
- Benzalkonium chloride antiseptic towelettes
- Eight (8)-15 mL labeled conical tubes
- Thirty (30) 2 mL labeled cryovials
- Twelve (12) 5 mL labeled cryovials
- Two (2) 2 ml labeled cryovial with 0.5 ml internal volume (for pellet)
- *Spot Urine Processing Worksheet* in the Kit

The spot urine kit should be stored at room temperature.

Bulk items provided by CBR:

- RNAlater (store at room temperature)
- Pro Advantage 10 Test Urine Reagent Strips
    - Store closed container at room temperature or in the refrigerator. Keep out of direct sunlight.

Local site will supply the following:

- Soft-bulb pipettes

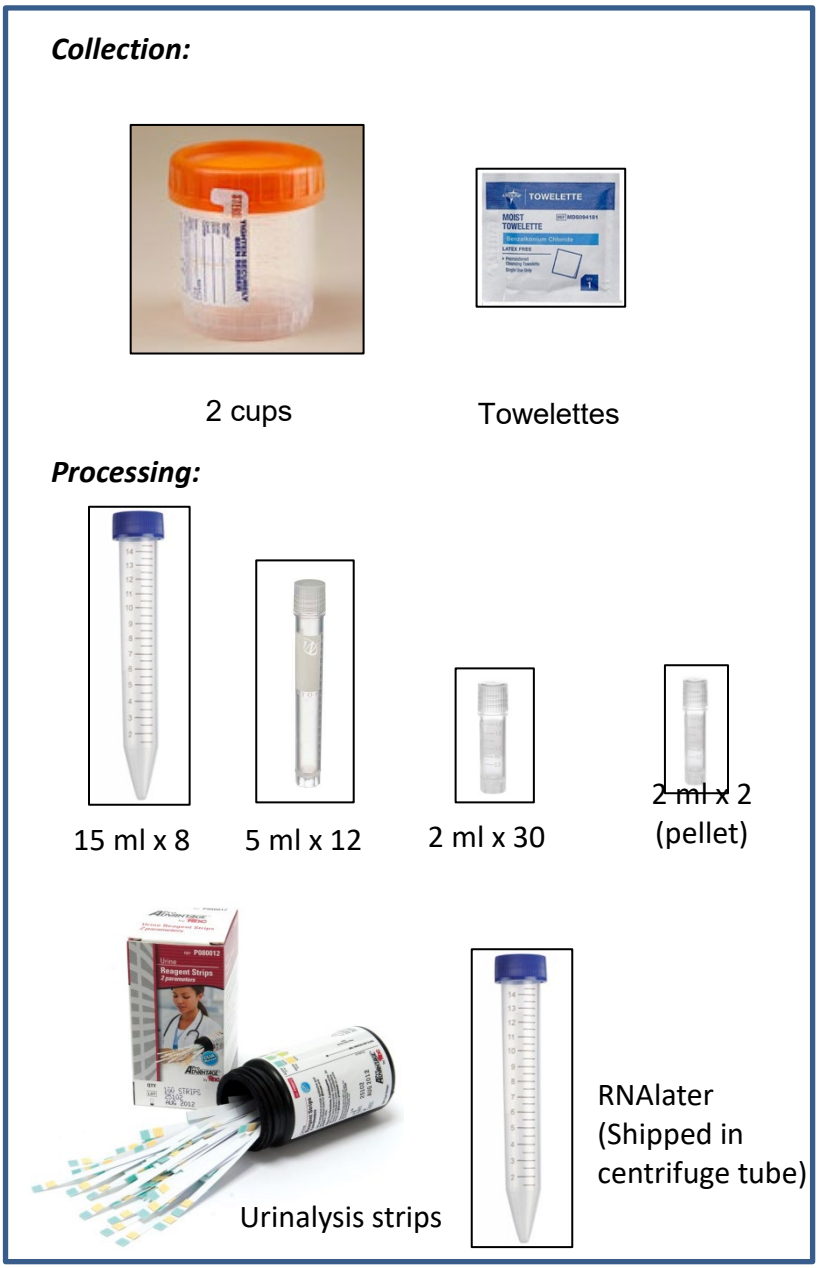


Figure 5: *120 mL Spot urine collection kit.* Cryovials will be labeled with a unique Sample ID to link to KPMP Participant ID in REDCap and SpecTrack.

Image of 120 mL spot urine collection kit above.

<u>20 mL Spot urine collection kit (for all collections other than the baseline:</u> <u>post-biopsy for AKI, 3-month for AKI, and all annual follow-up for AKI and CKD)</u>

CBR will supply the following:

- 1 cup for specimen collection
- Benzalkonium chloride antiseptic towelettes
- Two (2)-15 mL labeled conical tubes
- Ten (10) 2 mL labeled cryovials
- *Spot Urine Processing Worksheet* in the Kit

The spot urine kit should be stored at room temperature.

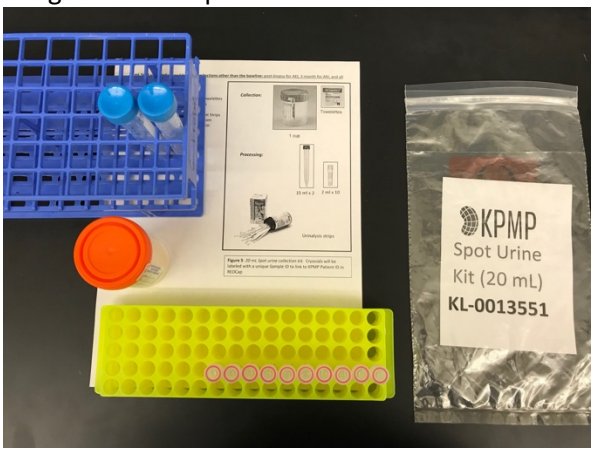
Bulk items provided by CBR:

- Pro Advantage 10 Test Urine Reagent Strips
    - Store closed container at room temperature or in the refrigerator. Keep out of direct sunlight.

Local site will supply the following:

- Soft-bulb pipettes

Image of 20 mL spot urine collection kit below:



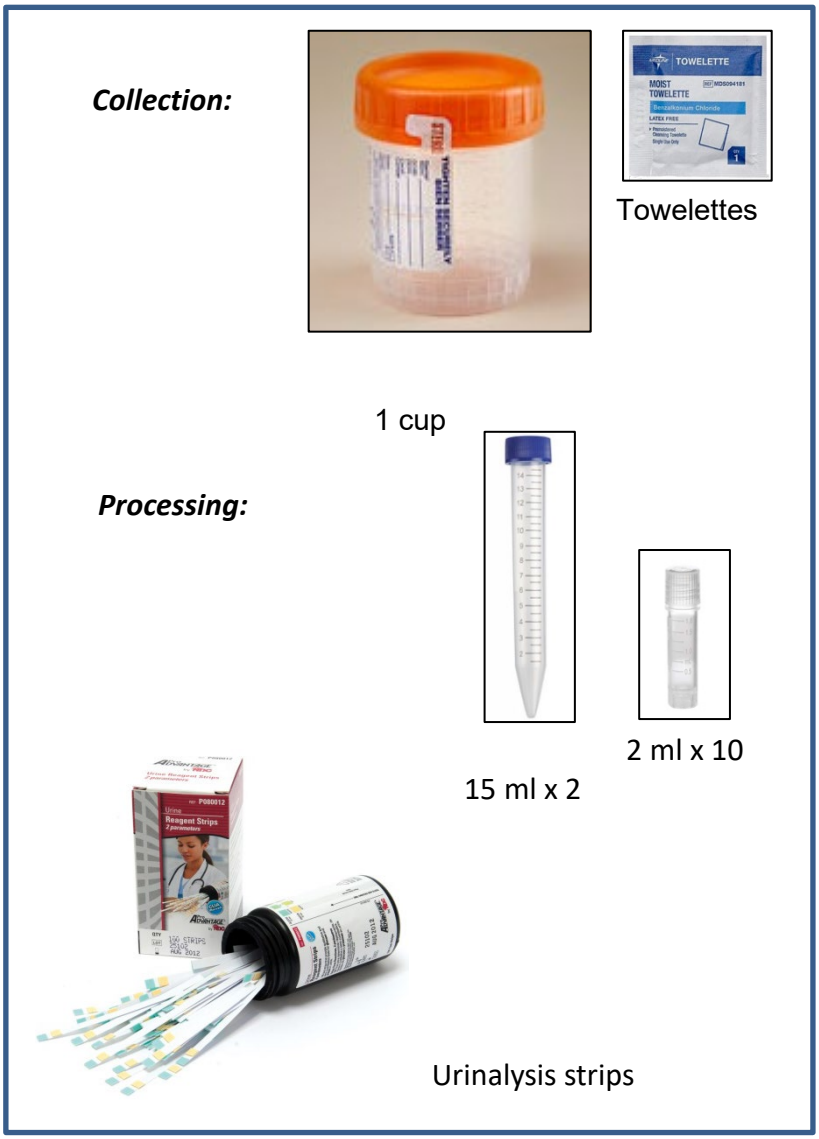


Figure 6: *20 mL Spot urine collection kit.* Cryovials will be labeled with a unique Sample ID to link to KPMP Participant ID in REDCap and SpecTrack.

4.1.2 Clean Catch Procedures

Participants should do a clean catch void, if at all possible. A clean catch specimen is a way of collecting urine that does not contain a lot of bacteria from the skin. Do not take urine from participants who have urostomy bags. Procedures for collecting a clean catch void are outlined below. See **Appendix 10.1** for Clean-catch instructions for participants giving a spot urine sample.

Instructions for Research Coordinators:

1. Give the participant the provided urine collection cup.
    a. It might be helpful to give the participant the cup in a bag which includes the towelettes.
2. Provide instructions to the participant about the collection procedures (see below and Appendix 10.1).
3. Record (on *Spot Urine CRF,* **Section 9**) the:
    a. exact time that the participant provides the urine sample
    b. the method of collection
    c. whether the participant is fasting and hours NPO
    d. total volume collected in mL
    e. if the participant menstruating - female participants only
4. Store sample on wet ice or refrigerate (4˚C) until ready to process

Instructions for Participants:

Please follow these instructions to collect a clean-catch midstream urine specimen:

1. Wash your hands with soap and water.
2. Remove the lid of the urine container, taking care not to touch the inside of the container.
3. Follow the appropriate instructions below:

FEMALE	MALE
Separate your genital folds (also called lips or labia) and gently wipe the inside of the folds with an antiseptic towelette, wiping from front to back.	Before you start urinating, gently wipe the tip of the penis around the opening with antiseptic towelette. If you have not been circumcised and still have your foreskin, gently pull the foreskin back before you wash the tip of the penis. Keep holding it back until you are finished getting the urine sample.

4. Start to urinate into the toilet, then stop. Do not collect the first amount of urine.
5. Begin urinating into the urine container; stop when it is approximately ¾ full.
6. Finish urinating into the toilet.
7. Place the lid on the urine container.
8. Bring the container to the research coordinator.

4.1.3 Participants Unable to perform a Clean Catch Void

If the participant is able to spontaneously void but unable to perform a clean catch void due to physical constraints, collect urine as outlined below.

1. Do not have the participant use antiseptic towelettes, as the cleaning agents in the towelettes may interfere with some sensitive bioassays.

2. Have the participant void into a urinal (men) or a clean hat on positioned on the toilet or on a commode (men or women).

3. Do not collect urine from a hat that is contaminated with stool.

4. Research coordinator should pour a small amount of urine into a labeled specimen cup.

5. Cap the container, avoid touching inside of container.

6. Record that the urine was not a clean catch on the *Spot Urine Collection CRF* (see **Section 9**).

4.1.5 Participants with a Foley Catheter

If participant has an indwelling urinary catheter, urine should be collected from the urine meter or the port on the catheter tubing, not from the large drainage bag. **Do not collect urine that has been sitting for more than two hours**. We recommend clamping the Foley tubing and then using a needleless system to get urine from the tube side port. A nurse caring for the participant or a trained study coordinator may collect this specimen.

1. Place a clamp (e.g. a blue plastic clamp) on the tubing to block the flow of urine into the bag.
2. Wipe the needleless port on the Foley catheter bag with an alcohol wipe and allow it to dry.
3. Access the needleless port with a Luer-lock tip syringe and aspirate back urine.
4. Transfer contents to urine container for transport back to the processing lab.
5. Record that the urine was collected from a Foley catheter.

4.1.6 Steps in Processing Spot Urines

Spot urine samples are collected at all visits for CKD and AKI participants. The target volume for baseline visits is 120 mL; for all other collections, the target volume is 20 mL.

4.1.6a Urine Dipstick Test

The urine dipstick test should be conducted on **all** spot urine samples. Readings should be recorded on the *Spot Urine CRF* (see **Section 9**).

NOTE: The urine dipstick test must be performed on whole, unspun urine! Complete this step before centrifuging, and as close to collection as possible.

Procedure:

1. Remove one strip from container and replace cap. Do not use the urine dipsticks after the expiration date. Take care not to touch the reagent areas of the strip
2. Gently swirl the urine container to mix urine well prior to testing
3. Note the color and clarity of the unspun urine on the *Spot Urine CRF* (see **Section 9**).

4. Using a soft bulb pipette, aspirate 1-2 mL of urine and quickly pipette one drop onto each pad of the strip, ensuring that all pads are soaked.
5. Remove excess urine by drawing edge of strip along the specimen container rim then blot edge of strip on a paper towel.
6. Hold strip in a level in a horizontal position. This avoids possible mixing of dyes and chemicals from adjacent pads.
7. Compare reagent pads to corresponding color chart on the bottle label. Hold the strip close to the color blocks and match carefully, reading pads from bottom (glucose) to the top (leukocytes). Record the readings on the *Spot Urine CRF* (see **Section 9**).
8. Discard the used strip after use according to local regulations.

Color changes that occur 2 minutes after dipping have no diagnostic value. Do not record. Refer to **Table 7** for proper reading time post-dip for analytes.

Note: if site has an automated analyzer for urinalysis, this may be used to obtain results of the dipstick urinalysis. In this case, the paper readout should be saved in the participant's record as source documentation.

Table 7: Post-dip analyte read time

ANALYTE	READ TIME AFTER DIP
Glucose	30 seconds
Bilirubin	30 seconds
Ketone	40 seconds
Specific Gravity	45 seconds
Blood	60 seconds
pH	60 seconds
Protein	60 seconds
Urobilinogen	Not recorded
Nitrite	60 seconds
Leukocytes	120 seconds

4.1.6b Spot urine processing for a 120 mL collection

The goal is to process the samples **within two hours of collection**. The maximum allowable time between specimen collection and processing is four hours. All times should be recorded on the processing worksheet provided in the Kit, for later transfer to SpecTrack. Do not discard samples if the maximum allowable time to processing is exceeded; instead, process as normal, but be sure to note the times on the processing worksheet/SpecTrack. When collected concurrently with blood, blood should be processed before urine.

Take note of details on the *Spot Urine Processing Worksheet* from the Kit. For a 120 mL collection (See **Figure 7**):

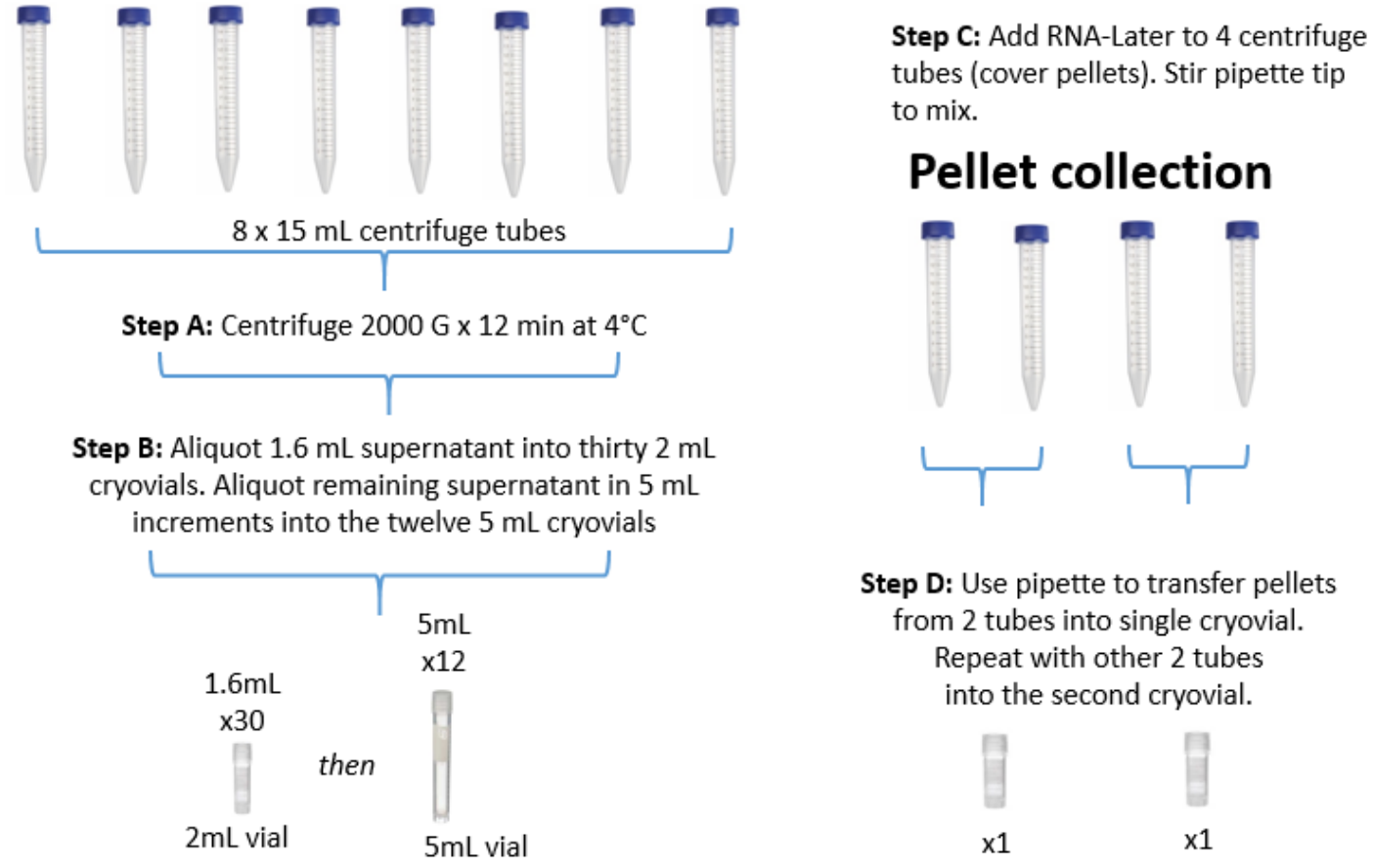
1. After completing and recording the urine dipstick test, pour 15 mL into each of the eight 15 mL centrifuge tubes. If less than 120 ml of urine is obtained, fill as many of the eight tubes as possible.
2. Centrifuge per section **3.2.2:**
    a. Balance the centrifuge to ensure proper performance of the instrument. If needed, use a "balance tube" filled with water to the proper level to achieve balance.
    b. Once the centrifuge is loaded with samples, set the speed to 2000 x G, the time to 12 minutes and the temperature to 4°C for processing. It is recommended that aerosol spray covers for centrifuge buckets be used.
3. Pipette supernatant directly from centrifuge tubes into 30 x 2 mL cryovials and 12 x 5 mL cryovials, filling the 2 mL cryovials first, and aliquoting the remaining supernatant into the larger tubes last. **Fill the 2 mL cryovials with 1.6 mL of supernatant and the 5 mL cryovials with 5 mL of supernatant.** See **Figure 7, step B.** Fill as many as possible, leaving the pellet at the bottom of the centrifuge tubes for step 5.
4. Mark on the *Spot Urine Processing Worksheet* how many vials were filled and what the pellet looks like (red, white, or not visible) for later transfer to SpecTrack.
5. Pellet collection: The pellets from four of the eight 15 mL tubes will be stored in two separate cryovials (see **Figure 7**). NOTE: The pellet collection process should be done *even if the pellet is not visible*.

a. Select four of the eight now supernatant-free 15 mL centrifuge tubes and add RNA-Later (enough to cover the pellets: approximately 25-50 uL). See **Figure 7, step C.**

b. Stir the pipette tip to mix. **Do not pipette up and down as this will cause cell breakage**.

c. With the pipette, transfer the pellets from two tubes into a single cryovial. Repeat for the other two tubes (into the second cryovial). See **Figure 7, step D.**

Figure 7. Spot Urine Processing for a 120 mL collection (Baseline only)



4.1.6c Spot urine processing for a 20 mL collection

The goal is to process the samples **within two hours of collection**. The maximum allowable time between specimen collection and processing is four hours. All times should be recorded on the processing worksheet provided in the Kit, for later transfer to SpecTrack. Do not discard samples if the maximum allowable time to processing is exceeded; instead, process as normal, but be sure to note the times on the processing worksheet/SpecTrack. When collected concurrently with blood, blood should be processed before urine.

For the 20 mL collections (i.e. post-biopsy for AKI, 3-month for AKI, and all annual follow-up for AKI and CKD):

1. After completing and recording the urine dipstick test, pour approximately 10 mL into each of the two 15 mL conical centrifuge tubes.
2. Centrifuge per section **3.2.2.:**
    a. Balance the centrifuge to ensure proper performance of the instrument. If needed, use a "balance tube" filled with water to the proper level to achieve balance.
    b. Once the centrifuge is loaded with samples, set the speed to 2000 x G, the time to 12 minutes and the temperature to 4°C for processing. It is recommended that aerosol spray covers for centrifuge buckets be used.
3. Pipette supernatant directly from centrifuge tubes into ten 2 mL cryovials.

    NOTE: aliquot *only 1.6 mL* of supernatant into the 2 mL cryovials.
4. **No pellet is collected from these samples. Pellet is <u>only</u> collected for the 120 mL baseline collection.**

4.2. Timed (8-24-Hour) Urine Collection & Handling Instructions

We will ask CKD participants to give a 24-hour timed urine sample at their baseline visit and AKI participants to give an 8-24-hour timed urine sample at their baseline visit. CKD participants can be sent home with the collection kit and instructed to return with it on the day of their biopsy. AKI participants may be collected in hospital with a nurse's assistance, as is clinically feasible.

4.2.1 Equipment

24 Hour Urine collection kit (Figure 8):

CBR will supply the following in the kit:

- 24 hour urine container/jug
- One 50 mL conical tube
- Ten (10) 5 mL cryovials
- *Timed Urine Processing Worksheet*

CBR will also provide the following in bulk:

- Urinal (male)
- Collection hat (female)

Timed urine kit contents can be stored at room temperature.

The coordinator will need to acquire a urinal or collection hat (as appropriate) from bulk storage to include in the set of materials to give participants for the timed urine collection.

The site will be responsible for the scale to weigh the container (if possible).

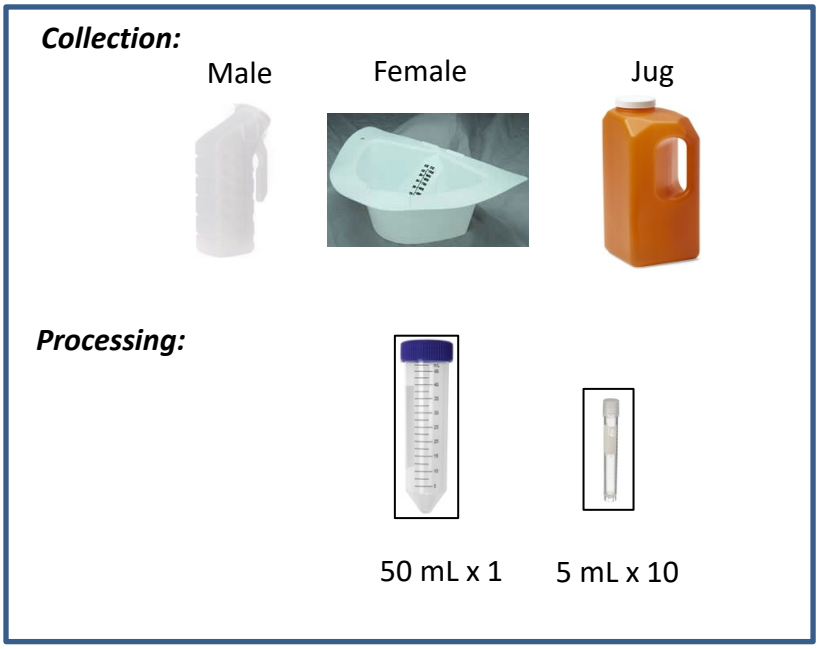


Figure 8: Timed Urine collection kit. Cryovials will be barcoded with a unique ID and include a coded link to the KPMP Participant ID

4.2.2 Collection Procedures

We will give participants a urine collection jug, along with written instructions (see **Appendix 10.2**) for collecting a timed urine sample. Women will be provided a collection 'hat', which covers the toilet seat to make collection easier, and males will be provided a urinal. We ask participants to start the timed urine collection in the morning when they wake. They flush the first void, but record the date/time, then collect all subsequent voids until the following morning where they collect the first morning void and write down the end date/time. AKI participants may provide timed samples from a Foley catheter.

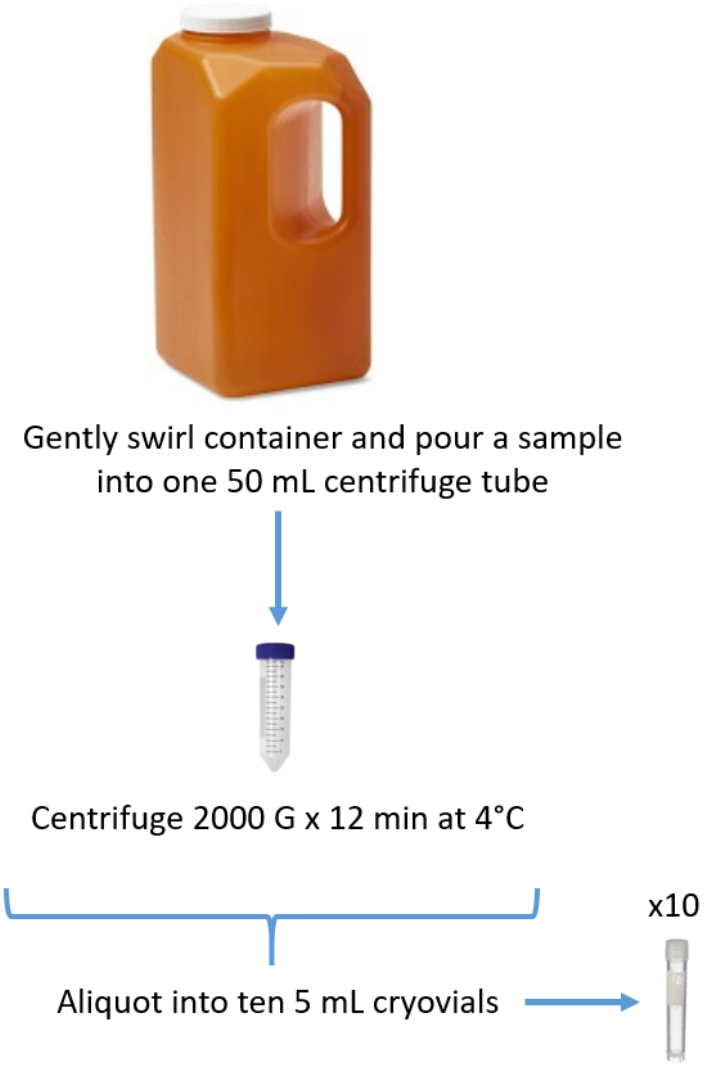
Participants will be asked to keep the jug in the refrigerator or on ice, since it must be kept cold. If kept on ice, then the ice should be at the level of the urine. If they can't collect for the full 24 hours, then ask them to do it for as long as they are able and ask them to record the start/end times on paper and on the container. The jug must be returned to the research coordinators at the conclusion of the timed collection. This can be returned at their study visit.

4.2.3 Processing

Measure the total volume of urine collected and record this on the *Timed Urine Collection CRF* (see **Section 9**). You should also measure and record the weight of the full jug, if you have a scale available. Gently swirl the container, and then pour 50 mL of urine into the 50 mL conical tube for processing. If the sample will not be processed immediately, the sample should be refrigerated or stored on ice. Centrifuge per section **3.2.2** and aliquot into ten 5-mL aliquots (**Figure 9**). Record aliquot details on the *Time Urine Processing Worksheet* for transfer to SpecTrack

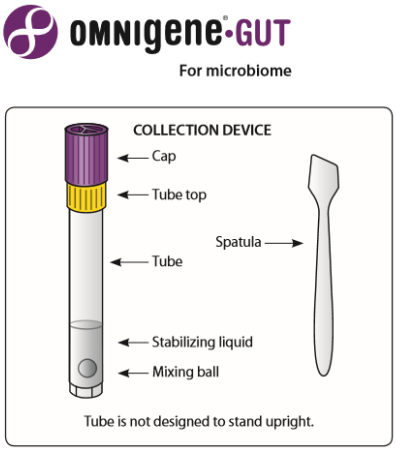
Image of Timed Urine Collection Kit (male and female options shown)

Figure 9. Timed Urine Processing

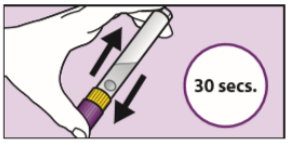


5. Stool

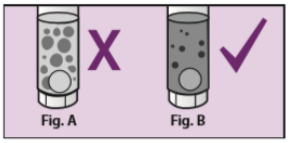
Stool specimens will be collected by study participants. Participants will be instructed on how to handle and store the samples prior to bringing them to the research team/visit. See **Appendix 10.3** for detailed Participant Stool Collection and Storage Instructions.



KPMP will use the OMNIgene-GUT system for collecting and stabilizing microbial DNA from fecal samples. Participants will collect a sample and use the provided spatula to transfer a small amount of stool into the OMNIgene-GUT tube. The tube contains a stabilizing liquid which, when well mixed with the stool, will preserve the sample for up to sixty (60) days at room temperature. During this time, the participant should return the sample to the recruitment site and the recruitment site should ship the sample to the CBR. Upon receipt, the CBR will aliquot and prepare the sample for preservation. Ideally, the specimen should be collected no more than 24 hours before the participant's study visit and/or returning samples to the research team.



7 Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.



8 The fecal sample will be mixed with the stabilizing liquid in the tube; not all particles will dissolve.

IMPORTANT: Continue shaking if **large** particles remain as shown in Figure A.

User instructions are provided in **Appendix 10.3** and should be given to the participants at the enrollment visit. Spanish versions of these user instructions are available. Research coordinators should review the instructions with participants and answer any questions. Coordinators should emphasize to participants that **the tube must be shaken vigorously for at least 30 seconds** once the specimen is added and cap replaced, in order to ensure the sample is properly stabilized. Once shaking is completed, participants will place the tube into the Ziploc bag included in the kit, seal the Ziploc bag, and return the bag and Recording Form to the coordinator at the next visit.

The remaining stool will be deposited into the toilet and the toilet paper collection accessory will be flushed. Participants should remove their gloves and wash their hands, then write the date and time of collection, as well as any notes on the Recording Form provided.

5.1. OmniGene Stool Kit Contents

The OmniGene Stool Kit will be supplied by the CBR. The OmniGene Stool Kit should be stored at room temperature at the recruitment site prior to use by the participant and also after return of the specimen.

The Kit contains the following materials, divided into one bag of materials to go to the participant, and one bag to remain at the RS until return of the sample:

Participant kit materials (see Figure 10A):

- Recording Form (Purpose: for the participant to record time and date of collection)
- User instructions from manufacturer (English provided in kit; Spanish can be printed online if needed)
- Transport bag with handles for returning samples back to the research team
- Ziploc bag for collection vial
- 1 pair of non-latex gloves
- 1 OMNIgene-GUT collection tube and spatula (Note: CBR will pre-label tube)
- 2 toilet paper accessories

Recruitment Site kit materials (see Figure 10B):
Note: do NOT give this set of supplies to the participant; they should remain at the RS until ready for use in shipping the sample to the CBR.

- Mailer for returning sample to CBR, including liquid biohazard bag with absorbent material, bubble wrap, and labeled cardboard mailer
- Kit lot insert and tracking manifest

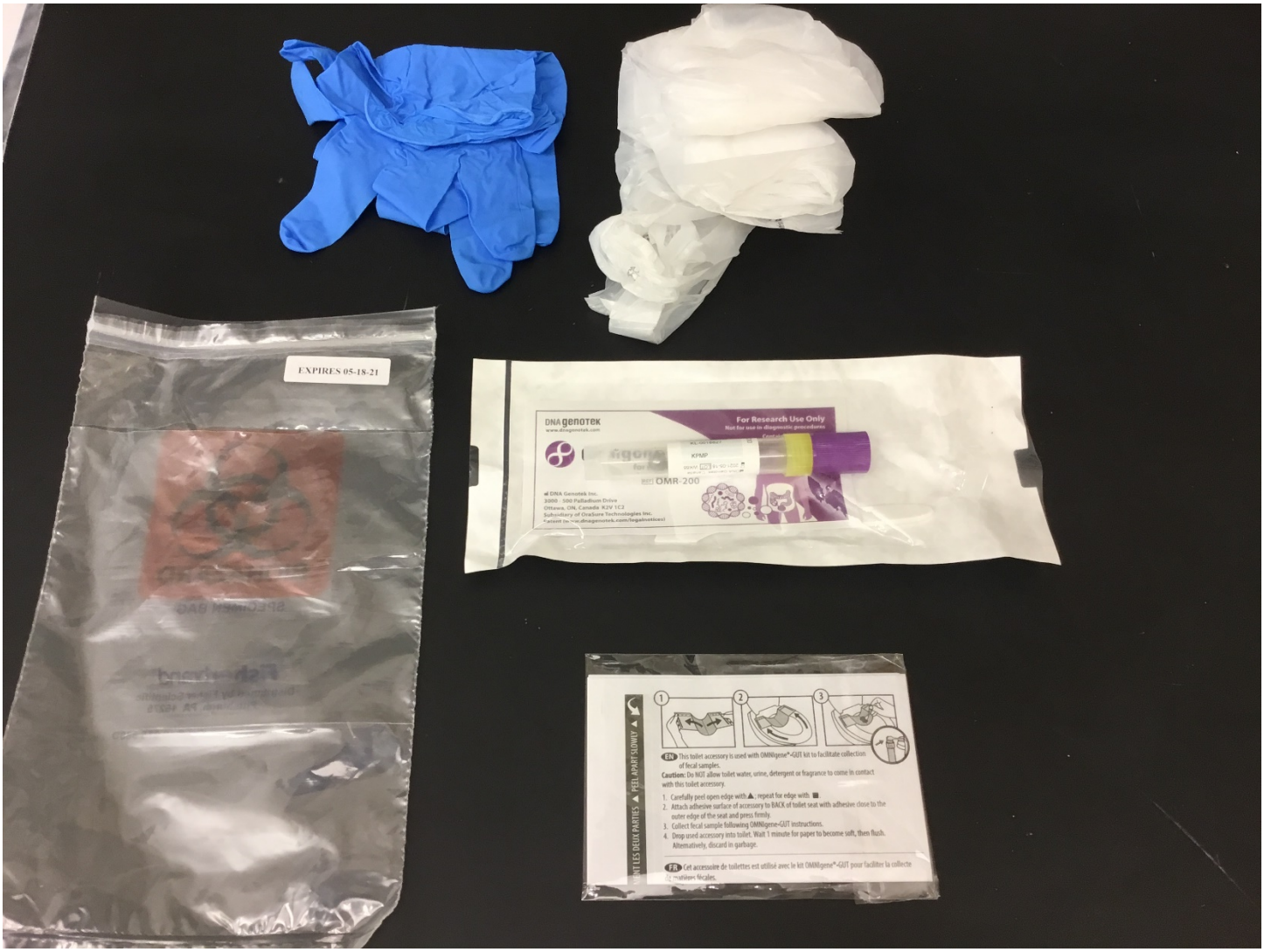


Figure 10A. Participant OmniGene Stool kit components. OMNIgene-GUT tubes will be pre-labeled with a unique Sample ID and linked to the KPMP Participant ID in REDCap. Not pictured: recording form and user instructions.

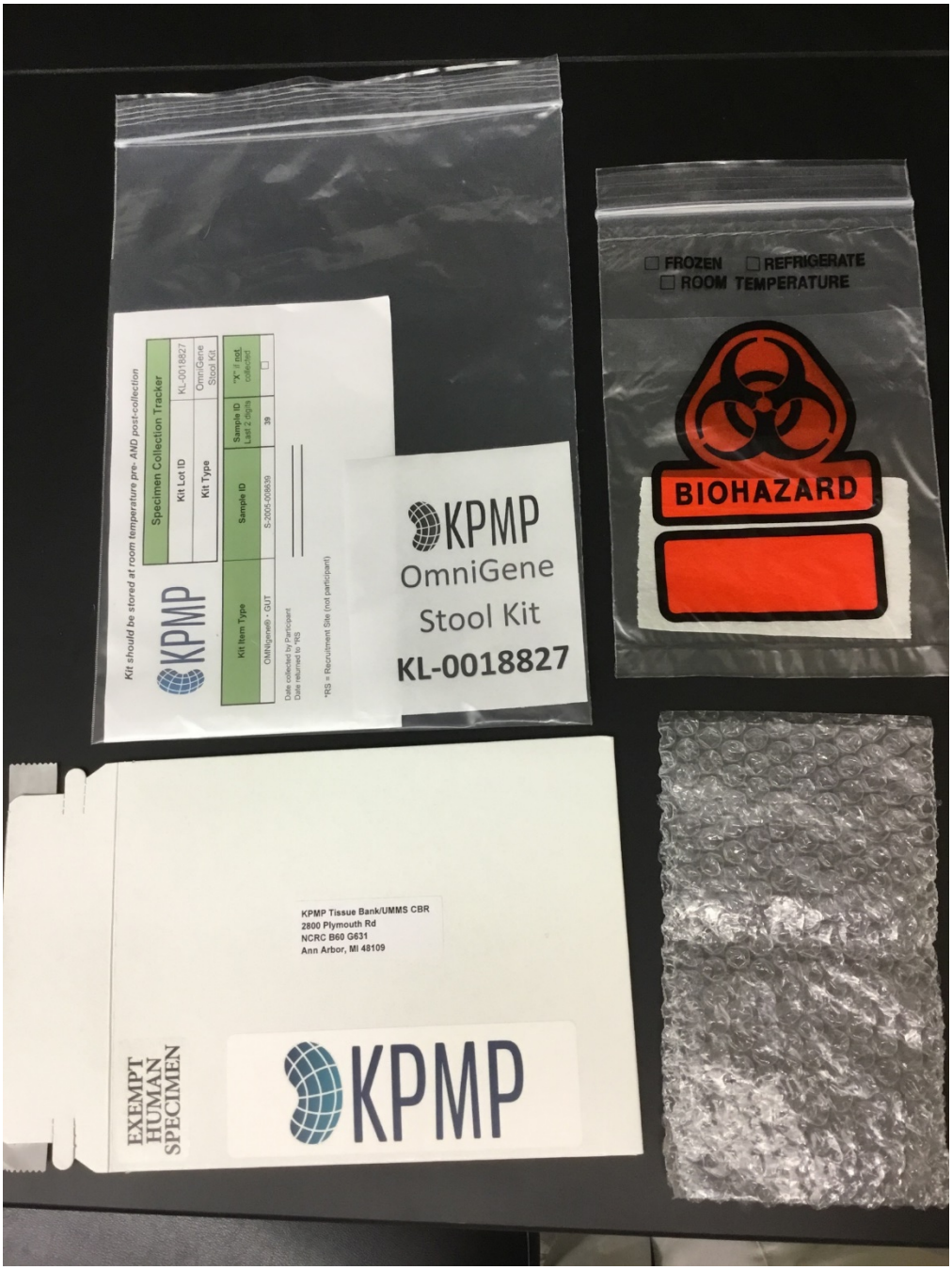


Figure 10B. Recruitment Site OmniGene Stool kit materials

Coordinator Instructions

At Enrollment Visit:

1. Obtain OmniGene Stool Kit from CBR and check expiration date on the kit bag or collection tube.
2. Remove the Recruitment Site materials from the kit and set aside for later use.
3. Verify that the User Instructions and Recording Form are included in the participant kit. If Spanish is needed, download from Basecamp. Write the participant ID on the Recording Form where indicated and return the form to the participant kit.
4. Provide the kit to the participant and provide an overview of the instructions. **Emphasize that they need to adequately shake the sample.** Show them where to note the date/time of collection on the Recording Form.

Upon Return of Sample:

1. Note sample as recorded in REDCap and input collection data from the Recording Form.

2. Store sample at room temperature until ready to ship to CBR. Use the cardboard Mailer and additional supplies set aside previously to ship the sample. **Ensure the sample arrives at the CBR within 60 days of collection**. See detailed shipping instructions in *Section 8*.

5.2. Sample Packaging by Participants

The OmniGene tube should be stored in the sealed Ziploc bag after collection. The participant should place the Ziploc bag and the completed Recording Form in the transport (grocery) bag and return the samples to the research team.

5.3 Sample Collection and Processing by Research Coordinators

Hospitalized participants (AKI participants in particular) may not be able to contribute a stool sample. If nurse assistance is available, or if the participant is ambulatory and can use a collection hat, coordinators should arrange for a nurse to page them when a participant has given a sample. The coordinator should bring the OmniGene stool collection kit and complete the spooning/shaking process in the participant's restroom, conditions permitting. The sample collection details should be recorded on the *Stool CRF* (**Section 9**) in REDCap. The sample should be kept at room temperature until shipment to the CBR.

5.3. Delivery of Sample to Research Team

Research coordinator will ideally receive the sample within 24 hours of collection. Ensure that the date and time of the specimen collection is recorded on the recording form.

5.4. Storage Instructions

Store specimens at room temperature until future shipment to the CBR.

6. Local Storage

6.1 ACD tube storage

The **ACD** tube (baseline only) should NOT be frozen locally prior to shipment to CBR.

- Store at room temperature until ready for shipment. Shipment must occur promptly. The CBR must receive the tube for processing within 24 hours of the draw.
- Record in SpecTrack (see **Section 7**)
- Package tube in "sleeve" (Styrofoam-like envelope) and then ambient temperature mailer and sleeve for shipment to the CBR. Please follow instructions in **Section 8** for shipping.

6.2 PAXgene RNA tube storage

The **PAXgene RNA** tube should NOT be frozen immediately

- Store the PAXgene RNA tube upright at room temperature (18˚C - 25˚C) for a minimum of 2 hours and a maximum of 72 hours, documenting this time on the *Blood Collection CRF in REDCap* (See **Section** 9) before transferring to freezer (-80˚C).
- Store upright loosely in a metal rack in the -80 freezer until ready for shipment. ***Do not place PAXgene tubes in tight test tube containers such as Styrofoam or Plastic as this may lead the tube to break.***
- Record in SpecTrack (see **Section 7**)

6.3 Stool sample storage

The stool (**OMNIgene-GUT** tube) should be stored at room temperature locally until ready for ambient temperature shipment to the CBR. The CBR must receive the tube for processing within 60 days of collection.

6.4 All other sample storage

Immediately freeze all other samples (blood, urine) at -80˚C after initial processing and aliquoting.

- Store upright in a labeled box until ready for shipment
- Record in SpecTrack (see **Section 7**)

7. Recording Aliquot and Shipping Details in SpecTrack

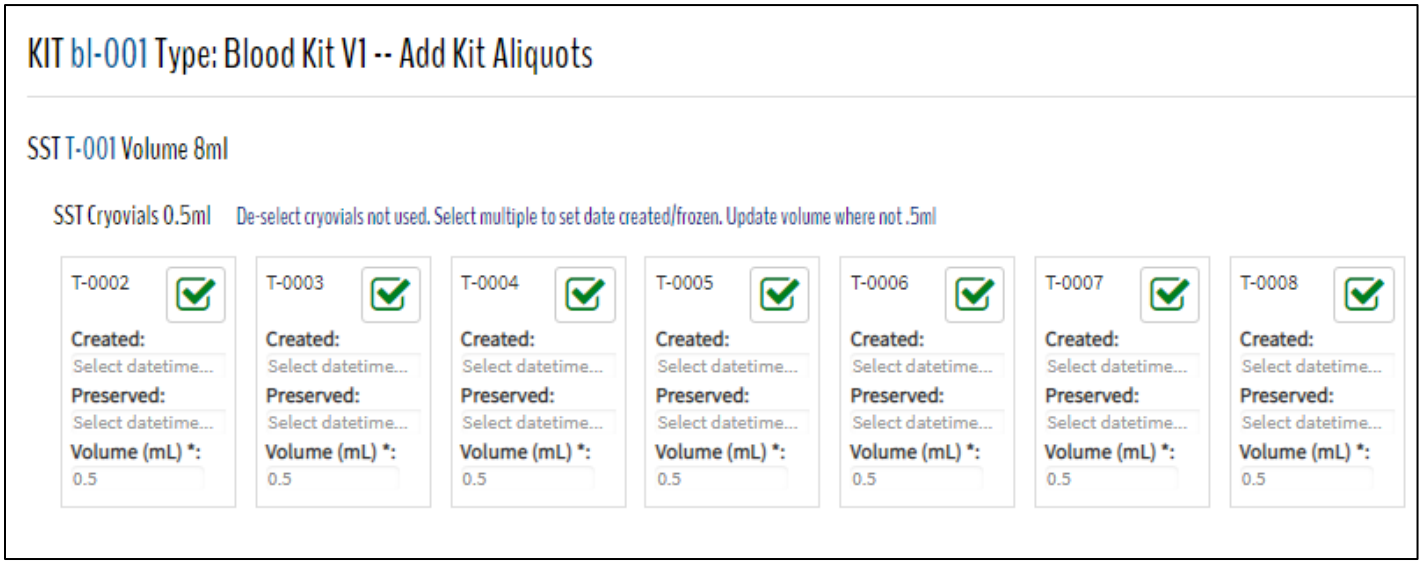
Before recording aliquot details, you must record initial specimen collection details within REDCap on the Blood, Timed Urine, Spot Urine, or Stool Collection forms (see **Section 9**). Saving these forms in REDCap will automatically transfer all data related to kit assignment and the initial specimens collected into the KPMP SpecTrack system.

7.1 Recording aliquots

To record which aliquots were filled, log into the SpecTrack system (https://specimen.kpmp.org). On the SpecTrack landing page use the Kit ID search field to look up the kit that was used for the participant.

On the kit detail page choose the 'Record Kit Aliquots' action.

This action will display a form where you can quickly record which aliquots included in the kit were used. If you did not indicate that a container was collected during the blood draw we will assume no aliquots were created and not display them.

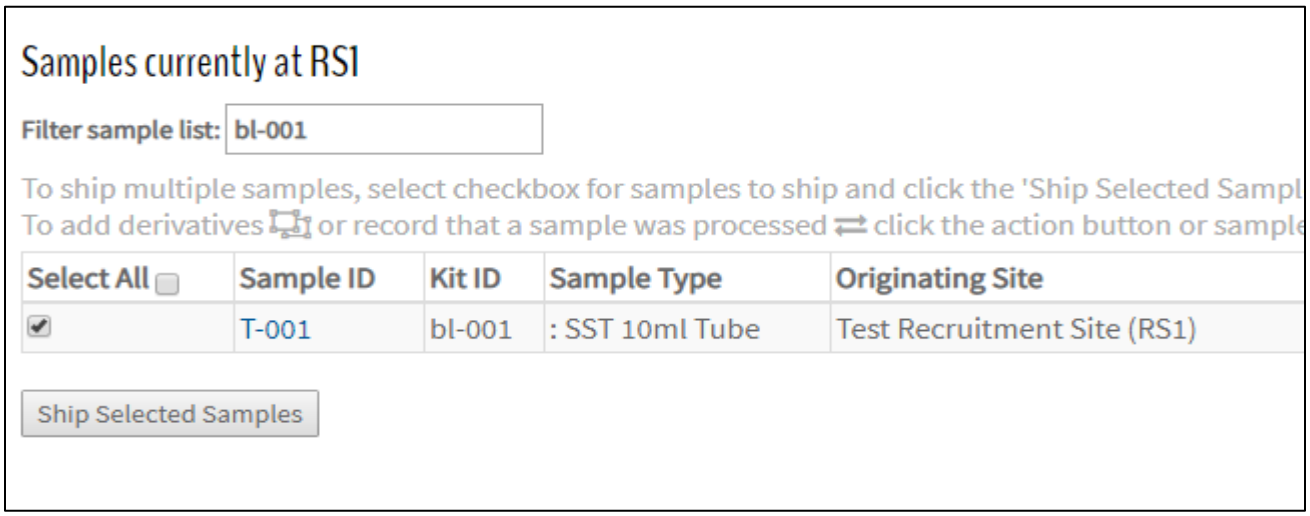


On this kit aliquot recording form you will select or de-select which aliquots were used. Volumes will be set to the default for the container; record any difference. Also record the time the aliquot was frozen for each set of aliquots. The ability to set this metadata across multiple samples at a time is supported so you don't have to enter the data for each sample individually.

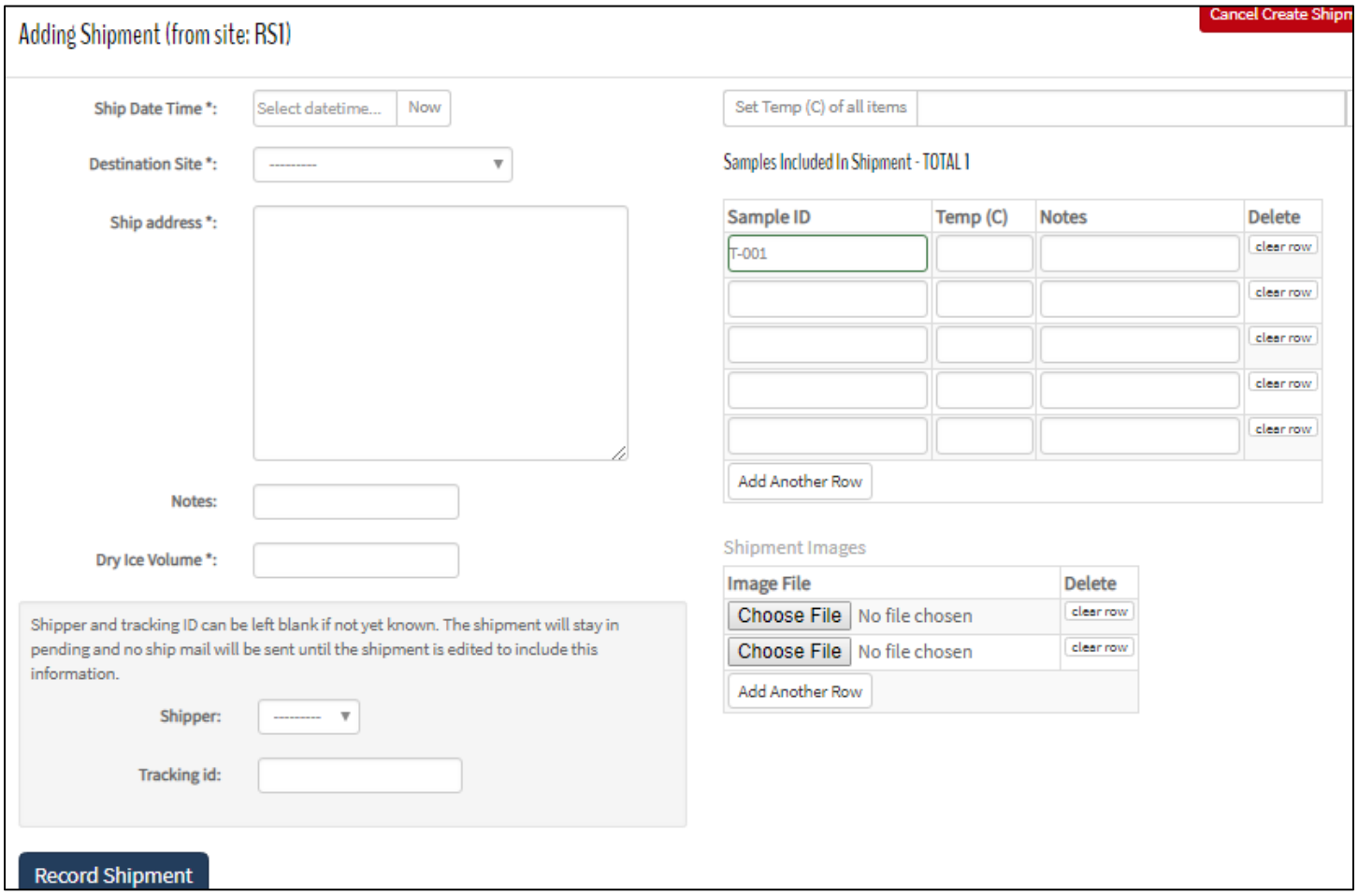
7.2 Recording sample shipments using SpecTrack

Use the 'Samples' tab within SpecTrack to view a list of all samples that have been collected at the site and have not yet been shipped.

Filter and select the samples you are planning to ship using tray ID or kit ID. Click the 'Ship Selected Samples' action.



The selected samples will be inserted into the Sample Shipment form.



Record sample temperatures, total weight of the packed box with dry ice, the site you're shipping to (address will auto-populate) and any notes about the shipment or individual samples. Upload images of the shipment contents. See **Section 8** for photo details. If you know the shipment courier and tracking ID, record it at this time. You can also return to the system to record this data after saving.

After saving the shipment data you will be able to generate a printable shipment manifest. This printed manifest should be reviewed for accuracy and included in the shipment. Be sure not to tape the box shut until you've enclosed the shipment manifest.

When the shipment data is recorded, an email notification with information about the shipment will be dispatched automatically by the SpecTrack system to both the receiving and shipping sites.

8. Central Biorepository Shipping (University of Michigan)

All biospecimen samples are shipped to the Central Biorepository (CBR) at the University of Michigan.

8.1 Contact Information

UMMS Central Biorepository
2800 Plymouth Rd.
NCRC B60 G631
Ann Arbor, MI 48109
Phone: 734-647-6285
Email: KPMP-BioRep@umich.edu

8.2 Shipping Materials

8.2.1 Provided by CBR

- KPMP Dry Ice Shipper
  - Cardboard shipping box with orientation arrows
  - Insulated shipping container (to be placed inside cardboard shipping box)
- UN1845 label
- UN3373 label
- 95 kPa bags
- Address labels (for UN1845)
- Vacutainer sleeve (for RNA PAXgene tube)
- Ambient temperature mailer and sleeve (for ACD tubes)
- Ambient temperature mailer (for OMNIgene-GUT stool tubes)
- For dry ice shipments: Cryo-Temp data logger inside Ziploc bag

8.2.2 Provided by Site

- Dry Ice (at least 14 lbs per dry ice shipment)
- Scale
- Packaging tape
- KPMP shipping manifest
- Waybill created by site on UPS or FedEx website (FedEx preferred)

Note: Blood and urine should be shipped via FedEx **Priority Overnight Delivery.** This ensures next day delivery. The CBR FedEx account number is **644377814.**

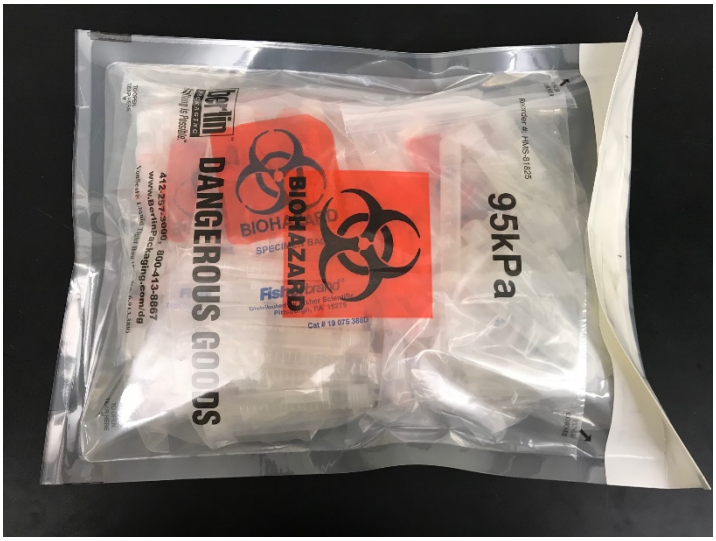
8.3 Shipping Procedure for Materials shipped on Dry Ice

1. Gather shipping materials listed above (all items except ambient temperature mailer and sleeve, which are for the ACD tube.)
2. Place UN1845 and UN3373 labels on the same side of KPMP Dry Ice Shipper (do not cover the orientation arrows.)
3. Fill out the KPMP shipping manifest in SpecTrack, indicating which samples will be included. Be sure to note the CryoTemp Data Logger ID number in SpecTrack. Print the manifest.
4. Remove biospecimen boxes from freezer.
5. Remove biospecimens from storage boxes and place them in two biohazard bags:
   a) All 0.5 mL cryovials should be placed in one bag

b) All other tubes (RNA PAXgene, 5 mL cryovials) should be placed in the 2nd bag.

i) RNA PAXgene tube should first be placed in the vacutainer sleeve

6. Place filled biohazard bags in in 95 kPa bags. Up to four biohazard bags (two participant-visits) can be placed in a single bag, and up to eight biohazard bags can be sent in a single KPMP Dry Ice Shipper. Prior to sealing the 95 kPa bag which will be the top-most bag in the box, use a magnet to activate the CryoTemp Data Logger. Once activated, ensure the logger is in a sealed Ziploc bag and then place the logger/bag inside the 95 kPa bag and seal.



7. Place filled 95 kPa bags on bottom of the empty KPMP Dry Ice Shipper. Take a photo of the biohazard bag(s) filled with sample vials resting at the bottom of the empty KPMP Dry Ice Shipper (see example image below). The image should fill the available photo area (i.e. don't take the photo too far away). The photo should demonstrate that the uppermost 95 kPa bag contains the CryoTemp Data Logger. The photo should be uploaded to SpecTrack.

8. Top off the KPMP Dry Ice Shipper with dry ice. Once filled, shake the Shipper from side-to-side to ensure dry ice has settled, and fill any space created with additional dry ice. (You may want to put the top on before shaking the Shipper).
    a) At least 15 lbs of dry ice must be included.
    b) Take another photo of the now-filled Shipper for upload to SpecTrack.
9. Place Styrofoam top on KPMP Dry Ice Shipper cooler, ensuring it "seats" properly, then place completed KPMP SpecTrack manifest on top (outside the Styrofoam inner container, but inside the outer cardboard box.) DO NOT TAPE THE STYROFOAM BOX!
    a) NOTE: if the contents of the shipment changed while you prepped it, make sure to revise and re-print the SpecTrack manifest.
10. Seal the outer cardboard box of the KPMP Dry Ice Shipper with a single piece of packaging tape. Tape should fully cover the flaps and extend at least 2 inches down the sides of the box.
11. Weigh the sealed shipment. Record the total weight and the dry ice weight for the courier (estimate the dry ice weight as the total weight minus 2 pounds (approximately the weight of the biospecimens and the KPMP shipper).
12. Add addresses (or address stickers) and dry ice weight to UN1845 labels.
    a) Be sure to write the dry ice weight on the UN1845 label, not the TOTAL weight. This must match the dry ice weight you report to UPS/FedEx when creating the shipment.
13. Create shipment on UPS/FedEx website and attach label to package. Remember to schedule shipping for Priority Overnight delivery.
14. Bring package to your UPS/FedEx pickup location.
15. Add the total weight of the package and the tracking number to SpecTrack.
16. Check inbox to ensure SpecTrack generated a shipping notification with copy to KPMP-BioRep@umich.edu

8.4 Shipping Procedure for ACD Tubes (ambient temperature)

NOTE: The ACD tube should not be drawn if it cannot make Thursday afternoon shipment to CBR. It should not be drawn on Fridays or Saturdays. The CBR must receive it for processing within 24 hours of the draw and is not staffed for processing over the weekend.

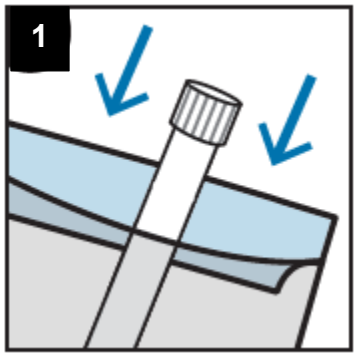
1. Gather ambient temperature mailer and sleeve.
2. Fill out the KPMP shipping manifest in SpecTrack, indicating which samples will be included.

3. Insert ACD tubes into the sleeve and take a photo for upload to SpecTrack. Then place the sleeve inside the ambient temperature mailer, along with the manifest. Take another photo for upload to SpecTrack.
4. Tape the ambient temperature mailer closed.
5. Weigh and record the weight of the filled sleeve.
6. Create shipment on UPS/FedEx website and attach label to package. Remember to schedule shipping for Priority Overnight delivery
7. Bring package to your UPS/FedEx pickup location.
8. Add the total weight of the package and the tracking number to SpecTrack.
9. Check inbox to ensure SpecTrack generated a shipping notification with copy to KPMP-BioRep@umich.edu.

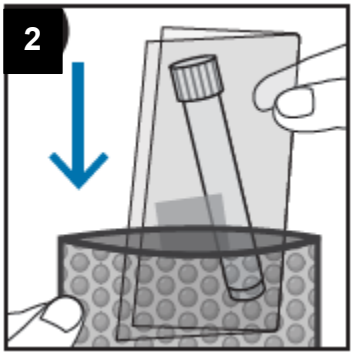
8.5 Shipping Procedure for OmniGene Stool Tubes (ambient temperature)

NOTE: The stool tubes must arrive at the CBR within 60 days post collection.

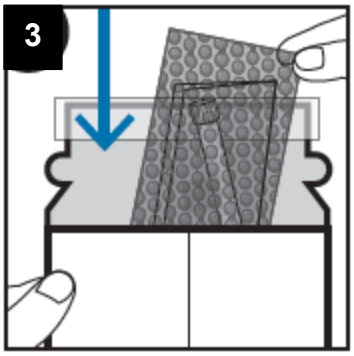
1. Gather ambient temperature mailer (included in each OmniGene Stool Kit).
2. Fill out the KPMP shipping manifest in SpecTrack, indicating which samples will be included. Print the manifest.
3. Insert the tightly capped stool tube inside provided biohazard bag with absorbent material and seal the bag:

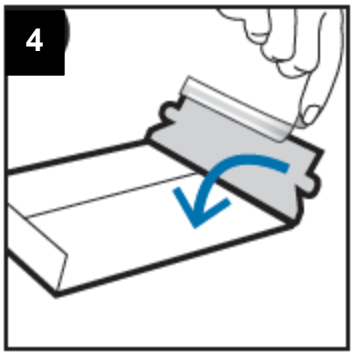


4. Fold the sealed biohazard bag with tube and place into the bubble bag. Take a photo for upload to SpecTrack.



5. Place the bubble bag, along with the SpecTrack shipping manifest into the cardboard envelope. Seal the cardboard envelope closed.





6. Weigh and record the weight of the filled mailer.
7. Create shipment on UPS/FedEx website and attach label to package. Use Ground shipping.
8. Bring package to your UPS/FedEx pickup location.
9. Add the total weight of the package and the tracking number to SpecTrack.
10. Check inbox to ensure SpecTrack generated a shipping notification with copy to KPMP-BioRep@umich.edu.

9. Biospecimen Form

Data need to be captured on the collection and processing details of biospecimens collected for KPMP. All data need to be uploaded electronically but paper forms are available to facilitate ease of capture for coordinators.

Section 9 of this MOP includes the paper version of the forms that capture data pertaining to the collection of biosamples. Coordinators wishing to use these forms to track data should print from this MOP and keep a supply on hand locally. Data collected on these Section 9 forms then need to be transferred to the corresponding CRFs in REDCap when the coordinator is able.

Following centrifugation, data need to be captured on an aliquot-level basis. The CBR is providing sample ID-specific worksheets in each individual kit (i.e. blood kits, stool kits, spot urine kit, timed urine kit) where details pertaining to aliquot volumes, times frozen, etc. can be recorded on paper. Data collected on these worksheets then need to be transferred to the corresponding aliquot space in SpecTrack when the coordinator is able.

9.1 Blood Collection

Participant Initials: _____

Visit type: __ Baseline __ 3-month (AKI ONLY) __ Follow-up (Year:__)

Participant ID: _____ Visit Date: _____

Kit ID: _____ Visit Location: __ Home health service __ In clinic or hospital __ Other

BLOOD COLLECTION

Blood Procured	☐ **Yes** ☐ **No**	**Reason if Not Collected:**	☐ Administrative Difficulties ☐ Participant Op-Out/Refused ☐ Technical Difficulties ☐ Difficult draw ☐ Vacutainer failure ☐ Concurrent large clinical draw ☐ Other, specify	**Method of blood collection:**	☐ Venipuncture (peripheral) ☐ Central venous access ☐ Arterial line ☐ Peripheral IV
Blood draw date:		**Blood draw time:**			
Draw date 2 (if applicable):		**Draw time 2 (if applicable):**			
Draw date 3 (if applicable):		**Draw time 3 (if applicable):**			
Participant fasting?	**Y / N / DK**	**Hours *NPO:** *since last meal/non-water beverage			

Tube Top	Tube Volume	Type	Tube Procured?	Volume Procured	Date Procured:	Collection Notes
Tiger (red)	**10 ml**	**SST**	**Y / N**			Do NOT invert. Store upright at RT for 30 minutes
Green	**6 mL**	**Li Heparin**	**Y / N**			Invert 8-10 times. Store on wet ice or refrigerate (4°C) until processing
Purple	**10 mL**	**EDTA**	**Y / N**			Invert 8-10 times. Store on wet ice or refrigerate (4°C) until processing
Purple	**10 mL**	**EDTA**	**Y / N**			Invert 8-10 times. Store on wet ice or refrigerate (4°C) until processing
Yellow	**8.5 mL**	**ACD**	**DO NOT FREEZE** **Y / N**		**BASELINE ONLY**	Invert 8-10 times. Store at room temperature before mailing in ambient temperature mailer and sleeve
White	**2.5 mL**	**PAXgene**	**Y / N**		***Time at room temp*** **_____ minutes***	Invert 8-10 times. Store at room temperature for at least 2 hours (up to 72), then transfer to -80°C

***Leave PAXgene at room temperature for at least 120 minutes.**

9.2 Spot Urine Collection

Participant Initials: _____

Visit type: **120 mL**: __ Baseline (AKI and CKD)
20 mL: __ hr post-bx (AKI) (collect q24 hours post-bx until discharge or day 7)
__ 3-month (AKI)
__ Follow-up (Year: __) (AKI and CKD)

Participant ID: _____ Visit Date: _____

Kit ID: _____ Visit Location: __ Home health service __ In clinic or hospital __ Other

SPOT URINE

Spot Urine Collection			
Urine Procured:	**Y / N**		
Reason if not collected?	☐ Administrative Difficulties ☐ Participant Op-Out/Refused ☐ Technical Difficulties ☐ Incontinence ☐ Participant not making urine ☐ Sample leaked ☐ Other, specify:	**Total Volume Collected**	mL
		Was this a clean catch specimen?	[ ] Yes [ ] No
		Collected via catheter?	[ ] Yes [ ] No
Date Collected:	(mm/dd/yyyy)	**Time Collected:**	(am/pm)
Participant menstruating? [Female only]	**Y / N / Don't Know**		
Participant Fasting?	**Y / N / Don't Know**	**Hours NPO**	(hours)
Color of urine	[ ] Colorless [ ] Pink [ ] Straw [ ] Red [ ] Yellow [ ] Other, specify [ ] Amber	**Urine Clarity**	[ ] Clear [ ] Hazy [ ] Cloudy

Was a Urine Dipstick Performed? [ ] Yes [ ] No ***Must be done prior to centrifugation**

Glucose	Bilirubin	Ketone	Specific Gravity	Blood	pH	Protein
[ ] Negative - [ ] 100 +- [ ] 250 + [ ] 500 ++ [ ] 1000 +++ [ ] ≥2000 ++++	[ ] Negative - [ ] 1 + [ ] 2 ++ [ ] 4 +++	[ ]Negative - [ ] 5 +- [ ] 15 + [ ] 40 ++ [ ] 80 +++ [ ] 160 ++++	[ ] 1.000 [ ] 1.005 [ ] 1.010 [ ] 1.015 [ ] 1.020 [ ] 1.025 [ ] 1.030	[ ]Negative - [ ]+- [ ]+ [ ]++ [ ]+++ [ ] 5-10 [ ] 50	[ ] 5.0 [ ] 5.5 (CCF only) [ ] 6.0 [ ] 6.5 [ ] 7.0 [ ] 7.5 [ ] 8.0 [ ] 8.5 (CCF only) [ ] 9.0	[ ] Negative - [ ] +-/15 [ ] +/30 [ ] ++/100 [ ] +++/300 [ ] ++++/2000
Urobilinogen	**Nitrite**	**Leukocytes**				
NOT RECORDED	[ ]Negative [ ]Positive (*any pink color is considered positive*)	[ ] Negative - [ ] 15 +- [ ] +/70 [ ] ++/125 [ ] +++/500				

9.3 Timed Urine Collection

Participant Initials: _____ Visit: Baseline

Participant ID: _____ Visit Date: _____

Kit ID: _____

TIMED URINE (8-24 HOURS)

Collection				
Urine Procured?	**Y / N**			
Reason if not collected?	☐ Administrative Difficulties ☐ Participant Op-Out/Refused ☐ Technical Difficulties ☐ Incontinence ☐ Participant not making urine ☐ Sample leaked ☐ Other, specify:	**Total Volume Procured**		mL
		Total Weight Procured (if scale accessible)		grams
Date collection started:	(mm/dd/yyyy)	**Time collection started:**		
Date collection completed:	(mm/dd/yyyy)	**Time collection completed:**		
Was this a complete collect?	☐ Yes, all urine voids included ☐ No, one or more voids missed	**Collected via catheter**	☐ Yes ☐ No	
Was urine stored on ice during collection?	☐ Yes ☐ No			
Participant menstruating? [Female only]	☐ **Y / N / Don't Know**			

9.4 Stool Collection

Participant Initials: _____ Visit: Baseline

Participant ID: _____ Visit Date: _____

Kit ID: _____

STOOL

Procurement	
Stool Procured?	**Y / N**
Reason if not collected?	☐ Administrative Difficulties ☐ Participant Op-Out/Refused ☐ Technical Difficulties ☐ Sample not stored properly ☐ Participant unable to provide ☐ Other, specify
Date of collection:	(mm/dd/yyyy)
Time of collection:	
Was participant on antibiotics within 7 days of collection?	☐ Yes ☐ No
Which antibiotic?	

9.5 Post-biopsy Blood Collection for AKI

Participant Initials: _____ Visit: Baseline

Participant ID: _____ Visit Date: _____

Kit ID: _____ Visit Location: __ Home health service __ In clinic or hospital __ Other

Post-Biopsy Blood Collection [AKI ONLY]

BLOOD COLLECTION

Blood Procured	☐ **Yes** ☐ **No**	**Reason if Not Collected:**	☐ Administrative Difficulties ☐ Participant Op-Out/Refused ☐ Technical Difficulties ☐ Difficult draw ☐ Vacutainer failure ☐ Concurrent large clinical draw ☐ Other, specify	**Method of blood collection:**	☐ Venipuncture (peripheral) ☐ Central venous access ☐ Arterial line ☐ Peripheral IV
Blood draw date:		**Blood draw time:**	.		
Participant fasting?	**Y / N / DK**	**Hours *NPO:** ***since last meal/non-water beverage**			

Tube Top	Tube Volume	Type	Tube Procured?	Volume Procured	Collection Notes:
Purple	**10 mL**	**EDTA**	**Y / N**		Invert 8-10 times. Store on wet ice or refrigerate (4°C) until processing

10. Appendices

10.1 Clean-Catch Instructions for Participants Giving a Spot Urine Sample

Please follow these instructions to collect a clean-catch midstream urine specimen:

1. Wash your hands with soap and water.
2. Remove the lid of the urine container, taking care not to touch the inside of the container.
3. Follow the appropriate instructions below:

FEMALE	MALE
Separate your genital folds (also called lips or labia) and gently wipe the inside of the folds with an antiseptic towelette, wiping from front to back.	Before you start urinating, gently wipe the tip of the penis around the opening with antiseptic towelette. If you have not been circumcised and still have your foreskin, gently pull the foreskin back before you wash the tip of the penis. Keep holding it back until you are finished getting the urine sample.

4. Start to urinate into the toilet, then stop. Do not collect the first amount of urine.
5. Begin urinating into the urine container; stop when it is approximately ¾ full.
6. Finish urinating into the toilet.
7. Place the lid on the urine container.
8. Bring the container to the research coordinator.

10.2 Timed (24-hour) Urine Collection Instructions for Participants

Instructions for your timed urine collection

1. **You will be given a 24-hour urine collection bottle. It will need to be kept in the refrigerator or on ice.**
2. **Always start your 24-hour urine collection in the morning, when you wake up.**
3. **Do not save the first urine of the day, but mark this as your START TIME at the bottom of this sheet.**
4. **Save all urine after this for the next 24 hours in the collection bottle. Women may use the provided collection 'hat' to cover the toilet seat and make collection easier. Men may use the provided urinal.**
5. **In order to get correct results, it is important to save every urine sample. If you forget to save any urine, please make sure to mark it at the bottom of this sheet.**
6. **The last urine sample should be as close to the starting time as possible and must be saved.**
7. **Please remember to keep urine in the collection bottle in the refrigerator or on ice. If on ice, make sure the ice is at the level of the urine. When transporting the bottle to clinic, please pack ice packs around it if it will take you longer than one hour to travel to clinic. You can make your own ice pack if needed by putting ice cubes in a Ziploc bag.**

Bring bottle to:

_____ **on** **_____**

(Clinic Location) (Next Visit Date)

If you have questions, contact:

_____ **_____**

(Coordinator Name) (Coordinator Phone Number)

PLEASE COMPLETE THE FOLLOWING INFORMATION AND RETURN THIS SHEET WITH YOUR BOTTLE.

Participant Name: _____

Collection Start Date: _____ **Start Time: _____ am / pm (circle)**

Collection Stop Date: _____ **Stop Time: _____ am / pm (circle)**

Did you miss any urine samples during this 24-hour period? _____ Y / N

If yes, how many samples did you miss? _____

Did you keep your collection bottle refrigerated or on ice? _____ Y / N

Thank you for your participation in KPMP!

10.3 Participant Instructions for Stool Specimen Collection and Storage

Collect your stool sample no more than 24 hours before your visit or return to your research coordinator.

Follow the instructions closely. If helpful, the manufacturer has provided video instructions on YouTube (search "OMNIgene.GUT collection instructions (OM-200 and OMR-200)")

- English: https://www.youtube.com/watch?time_continue=24&v=nxdAc5HJbXQ&feature=emb_logo
- Spanish: https://www.youtube.com/watch?v=JGPzvQZV1IA&feature=youtu.be&rel=0

Key points:

- If you need to urinate, do so before you begin your collection. Do not mix toilet paper, water, or soap with the sample.
- Apply the provided toilet paper accessory to your toilet seat to collect your sample.
- After your bowel movement, put on the gloves included in the kit.
- Follow the instructions provided to use the spatula to deposit a sample into the tube and cap the tube.
- Be sure to shake the closed tube as hard and fast as possible for at least 30 seconds to ensure adequate mixing.
- When you are done shaking, place the tube in the provided Ziploc bag and seal it.
- Follow the instructions to flush the toilet paper accessory and remaining stool (allow the accessory to soak up water for 1 minute before flushing).
- Discard the spatula and gloves and wash your hands.
- Fill out the recording form with the date and time you collected the stool sample, and whether you were on antibiotics within the last 7 days as applicable and/or comments section.
- Put the sealed Ziploc bag containing the tube and the completed form in the provided plastic bag. Keep the bag at room temperature and bring the bag with you to your next study visit.

KPMP Participant ID:_____

Date Collected:
_____/_____/_____

Time Collected:

_____

Were you on antibiotics within 7 days of the collection?

[ ] Yes [ ] No

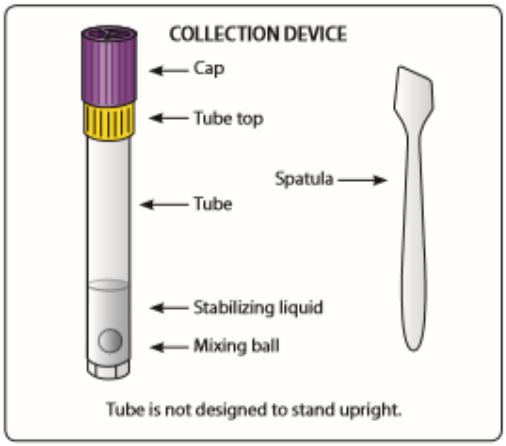
If Yes, which antibiotic:

_____

Comments:_____
_____
_____
_____
_____

OMNIgene•GUT

For microbiome



Summary and explanation of the kit:

OMNIgene•GUT provides the materials and instructions for collecting and stabilizing microbial DNA from a fecal sample.

Warnings and precautions:

- FOR EXTERNAL USE ONLY.
- Do NOT remove the yellow tube top from the tube.
- Do NOT spill the stabilizing liquid in the tube.
- Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
- If collecting a liquid fecal sample, see separately provided user instructions.
- Small items may pose a choking hazard.

Storage: 15°C to 25°C

Ship in accordance to applicable regulations covering transport of biological specimens.
See MSDS at www.dnagenotek.com

Label legend:

	Collect sample by (Use by)
REF	Catalog number
	Manufacturer
15°C 25°C	Storage instructions
	Caution, consult instructions for use
LOT	Lot number

USER INSTRUCTIONS

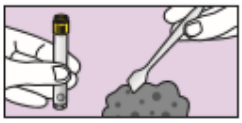
Read all instructions prior to collection

Procedure:

1 **IMPORTANT PREPARATIONS:**
- Empty your bladder before beginning the collection.
- Collect fecal sample free of urine or toilet water.
- Toilet paper or tissues may be required.

2 While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set aside for later use.

IMPORTANT:
Do NOT remove the yellow tube top.
Do NOT spill the stabilizing liquid in the tube.



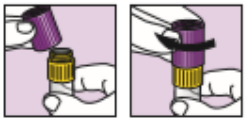
3 Use the spatula to collect a small amount of fecal sample.

4 Transfer the fecal sample into the yellow tube top. Repeat until the sample fills the yellow tube top.

IMPORTANT: Do NOT push sample into the tube.

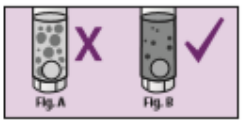
5 Scrape horizontally across the tube top to level the sample and remove any excess.

Wipe exterior of tube and top with toilet paper or tissue as needed.



6 Pick up the purple cap with the solid end facing down and screw onto the yellow tube top until tightly closed.

7 Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.



8 The fecal sample will be mixed with the stabilizing liquid in the tube; not all particles will dissolve.

IMPORTANT: Continue shaking if **large** particles remain as shown in Figure A.

9 Place spatula in original packaging or wrap in toilet paper and discard in garbage.

IMPORTANT: Send the sample for processing following the delivery instructions supplied separately by the kit provider.

OM-AC1 Toilet accessory

Used with OMNIgene®·GUT

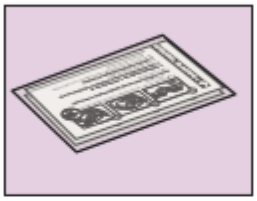
USER INSTRUCTIONS

This toilet accessory is used with OMNIgene®·GUT kit to facilitate collection of fecal samples.

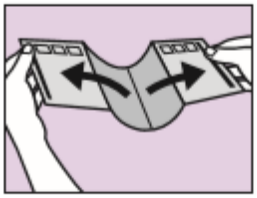
Caution: Do NOT allow toilet water, urine, detergent or fragrance to come in contact with this toilet accessory.

Storage: 15°C to 25°C

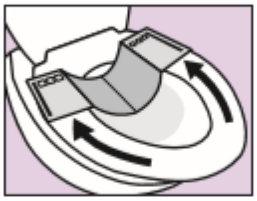
Procedure:



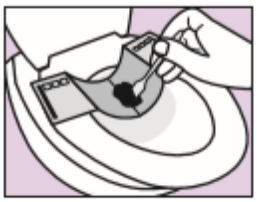
1 Two toilet accessories are provided in the event that you are unable to collect a sample on the first attempt.



2 Carefully peel open edge with ▲; repeat for edge with ■.



3 Attach adhesive surface of accessory to **BACK of toilet seat** with adhesive close to the outer edge of the seat and press firmly.



4 Collect fecal sample following OMNIgene·GUT user instructions.

5 Drop used accessory into toilet. Wait 1 minute for paper to become soft, then flush. Alternatively, discard in garbage.