KPMP Pathology

Manual of Procedures (MOP)

Version 7

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Abbreviation List

CBR	Central Biorepository
CRF	Case Report Form
CPL	Central Processing Laboratory
DCC	Data Coordinating Center
DVC	Data Visualization Center
DPR	Digital Pathology Repository
EM	Electron Microscopy
EHS	Environmental Health & Safety
FFPE	Formalin Fixed Paraffin Embedded
IF	Immunofluorescence
IHC	Immunohistochemistry
ISH	In Situ Hybridization
IATA	International Air Transport Association
LIMS	Laboratory Information Management System
LV	LabVantage
LM	Light Microscopy
LN2	Liquid Nitrogen
MOP	Manual of Procedures
OCT	Optimal Cutting Temperature compound
O_2	Oxygen gas
RS	Participant Recruitment Site
PBS	Phosphate Buffered Saline
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
TIS	Tissue Interrogation Site
U-M	University of Michigan
UMMS	University of Michigan Medical School

1. Gross examination and triaging of the renal biopsy

This Manual of Operating Procedures outlines the procedures for collection, processing, local storage, and shipment to the Central Biorepository (CBR) of KPMP kidney biopsies. It also outlines procedures for quality and feature assessment of the biopsy tissue.

All the procedures described here will be performed in the procedure suite by trained tissue processing personnel from the KPMP Recruitment Sites (RS). For each participant enrolled in the KPMP, the target is to obtain three biopsy cores from the renal cortex, to be used for tissue interrogation, conventional diagnostic work up, and imaging. The biopsy procedures including participant consent, participant preparation, site of the biopsy and the actual procedure are described in the Recruitment Site Manual of Procedures (OPS002). Each time a biopsy pass is performed by the nephrologist or radiologist, the specimen obtained will be handed over to the trained tissue processing personnel for further processing, which is detailed in this MOP. This process requires 3 phases: 1) gross examination; 2) triaging; and 3) recording of events for quality control and metadata collection (Figure 1).

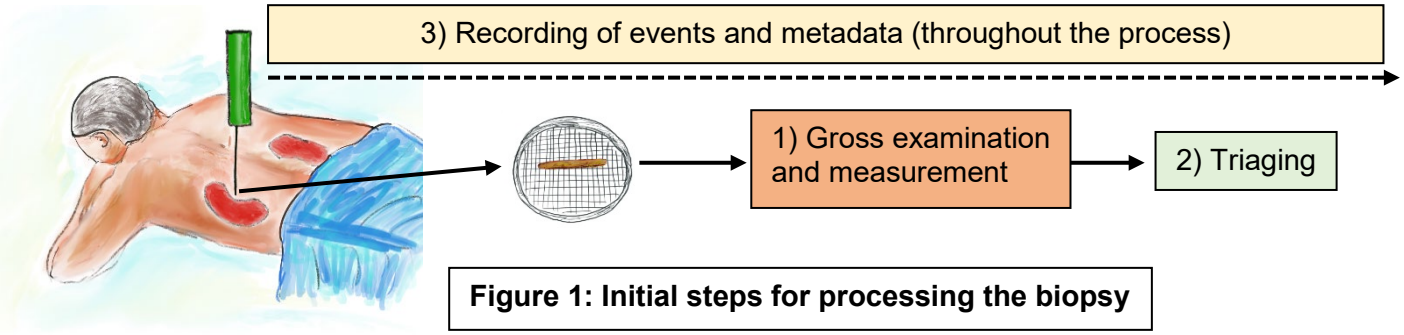


Figure 1: Initial steps for processing the biopsy

1.1 Purpose

1.1.1. Purpose of gross examination

The purpose of the gross examination, or ex-vivo imaging, is to determine if each of the biopsy cores is composed of renal parenchyma, has a sufficient length (size), and contains an acceptable amount of renal cortex before being triaged into the various transport media and fixatives as per protocol. The ex-vivo imaging will also provide information on structural changes of the renal tissue.

1.1.2. Purpose of triaging

The purpose of triaging is to prioritize the assignment and transfer of each renal biopsy core, as quickly as possible, to the appropriate medium or solution that will stabilize it and limit loss of molecular or cellular elements that are crucial to the success of downstream applications. This has to be balanced by the need to qualify the tissue by gross examination, and assure that its structure, composition and state are well characterized for subsequent analysis.

1.1.3. Purpose of recording events and metadata

Each of the steps taken during gross examination and triaging need to be recorded. This includes pertinent metadata and pre-analytical parameters associated with specimens under interrogation. These parameters will be collected at the time of the renal biopsy procedure in the renal biopsy suite and electronically entered into fillable forms by accessing the KPMP REDCap System. Paper CRFs are available in the Research Coordinators team on Basecamp. The purpose of the collection of this information is to increase robustness of the phenotype detail that will ultimately aid in interpreting the molecular data associated with the specimen and the participant.

1.2 Supplies for triaging, handling, and shipping renal biopsy cores

Trained personnel (the research coordinator, in most cases) will prepare a renal biopsy cart (Figure 2). The biopsy cart will be maintained in a designated location decided by the local PI and transported to the renal biopsy procurement suite together with biopsy Kit A or B (Figures 3 & 4), as instructed by the kit rotation schedule, and the KPMP tablet with camera (to take pictures and collect data in the Kidney Biopsy Procedure Details Case Report Form. All supplies in the renal biopsy cart (with the exception of wet and dry ice, and other

materials readily available at the RS, e.g. gloves, liquid nitrogen, isopropanol, ethanol), and the renal biopsy kits for the diagnostic core and tissue interrogation cores will be provided by the Central Hub to each of the participant Recruitment Sites. Each biopsy kit will be pre-labeled with a unique KPMP Kit ID, and the relevant vials/containers in each kit will be pre-labeled with a unique KPMP Sample ID that is tied to the Kit ID. The itemized list of the supplies that are part of the renal biopsy cart, and the supplies contained in the biopsy kits are described below.

The RS sites are expected to have temporary storing facilities including a well-monitored -80°C freezer and cardboard freezer boxes; the RS are also expected to have access to a 4°C refrigerator to store kit and cart components requiring refrigeration.

1.2.1. Checklists

Note: Estimate about 30 minutes to prepare the biopsy cart and supplies to be taken to the biopsy procurement suite and prepare a clean RNase-free environment in the procurement suite.

The following checklist needs to completed and ready immediately prior to entering the renal biopsy suite:

A. Equipment for handling the renal biopsy:

- The KPMP renal biopsy cart with all supplies (Figure 2 and section B below)
- The KPMP tablet (with camera)
- The KPMP renal biopsy kit (A or B) (Figures 3 and 4)
- A paper copy of the ***KPMP Biopsy Procedure CRF*** and ***Tissue Triage CRF*** as backup, printed on the same day as the biopsy procedure (Always use the electronic CRF in REDCap unless technical issues prevent it. Paper CRFs are available on Basecamp.)

B. KPMP renal biopsy cart supplies (Figure 2)

- Bucket(s) with lid containing powdered dry ice
  - Will be used to freeze containers 2 (diagnostic core tissue for IF) and 4 (tissue interrogation core) in OCT, and transport these to the pathology and research laboratories. Most hospitals will send the research cores to a different area than the diagnostic core, which first goes to the pathology laboratory. These hospitals need to have two separate containers for dry ice. A small lidded Styrofoam box with crushed dry ice is advised for taking the diagnostic container to the pathology lab.
  - Before going to the renal biopsy procurement suite have powdered dry ice ready in the dry ice bucket (Prepared by using a mallet to thoroughly crush dry ice pellets in a canvas bag and transferring to the dry ice bucket). The OCT cassettes in which the tissue will be frozen should be placed on the dry ice to pre-chill for uniform freezing. Ensure the dry ice is finely crushed and the surface of the dry ice is level, so the OCT cassettes will rest flat.
- Ice tray or bucket with wet ice (to keep Hypothermosol and Cryostor vials (for Kit A biopsies), the cryovial for flash frozen LN (for Kit B biopsies), petri dishes and sterile PBS cold)
- *For Kit A Biopsies only:* Mr. Frosty freezing container (Nalgene, PN#5100-0001), containing isopropanol (will need to be procured at the Recruitment Site)
- *For Kit B Biopsies only:* A transportable Dewar flask (1 L) containing liquid nitrogen for container 6 (core 3) (LN will need to be procured at the recruitment site)
- Optimum Cutting Temperature (OCT) medium, Tissue-Tek (#4583) [for container 2 & 4]
- Long forceps (to handle frozen cryoblocks and cryovials. Clean with RNaseZap prior to use)
- Sterile wood applicators
- Sterile Telfa pads (to collect the biopsy core from the biopsy gun and then transfer to the petri dish)
- Sterile 1X phosphate buffered saline (PBS), keep chilled on ice bucket
- Sterile dropper (plastic pipettor) to transfer sterile PBS on the biopsy and keeping it moist
- Cryostor solution (Sigma, PN#C2874-100mL)
- Hypothermosol solution (Sigma, PN#H4416-100mL)
- Gloves (disposable)

- Dry ice gloves (use to prepare powdered dry ice mentioned above)
- Extra fine-tip alcohol resistant lab markers (VWR, PN#52877-310)
- Sharpie permanent marker
- Timer
- RNaseZap solution (to clean all surfaces and instruments prior to handling the biopsy) (Thermo Fisher Scientific AM9780)
- Clean pack of paper towels to wipe the surfaces with RNaseZap
- RNase free water
- 70% ethanol in spray bottle (Ethanol will need to be procured at the recruitment site)
- Bench paper pads to cover the workspace on the top of the cart before laying out the supplies
- Sterile Petri dishes
- Optional: laminated triage reference document
- Approved microscope with cold LED light source for tissue visualization

Figure 2: Supplies in the renal biopsy cart. The itemized list of the supplies is indicated above, which should be used as a checklist. The cart is stored in a designated location by the PI. The local KPMP personnel will maintain the cart in appropriate conditions at all times. The solutions requiring refrigeration (kept in a designated 4C refrigerator) will be placed in wet ice bucket on the cart prior to going to the biopsy suite. Dewar to be filled with LN and Mr. Frosty with isopropanol prior to going to the biopsy suite. Estimated time to clean and prepare the cart before going to the biopsy suite is 30 minutes.

C. KPMP renal biopsy Kit A components (Figure 3)

- *Kit A - Diagnostic core (Core #1):*
  - Container 1: 10% neutral buffered formalin marked with gray cap (NBF) (Fisher Scientific cat. no 22-126-346) for LM
  - Container 2: Cryomold marked with green label (biopsy – tissue tek #4557) with OCT embedded frozen tissue for IF cut from the diagnostic Core #1 (to be placed in small Ziploc bag).

        - Ziploc bag
        - One unlabeled cryomold for ‘bathing’ only
    - Container 3: 2.5% Glutaraldehyde marked with pink cap (EMS cat. no. 16537-16) for EM
    - Razor Blades
- *Kit A – Tissue Interrogation Research core(s):*
    - Container 4: Cryomold marked with blue label (Tissue-Tek Standard, #4557) for frozen Core #2 (to be placed in small Ziploc bag)
        - Ziploc bag
        - One unlabeled cryomold for ‘bathing’ only
    - Container 5, Tube #1: 1 Cryovial marked with orange cap (Cryostor) (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416, or similar) [for Core # 3 Cryostor preservation]
    - Container 5, Tube #2: 1 HypoThermosol cryovial [for Core # 3 Cryostor preservation]
- *Kit A – Miscellaneous:*
    - Extra Labels (stickers) with KPMP sample IDs (for labeling derivative materials from the diagnostic core following the local pathology work-up)

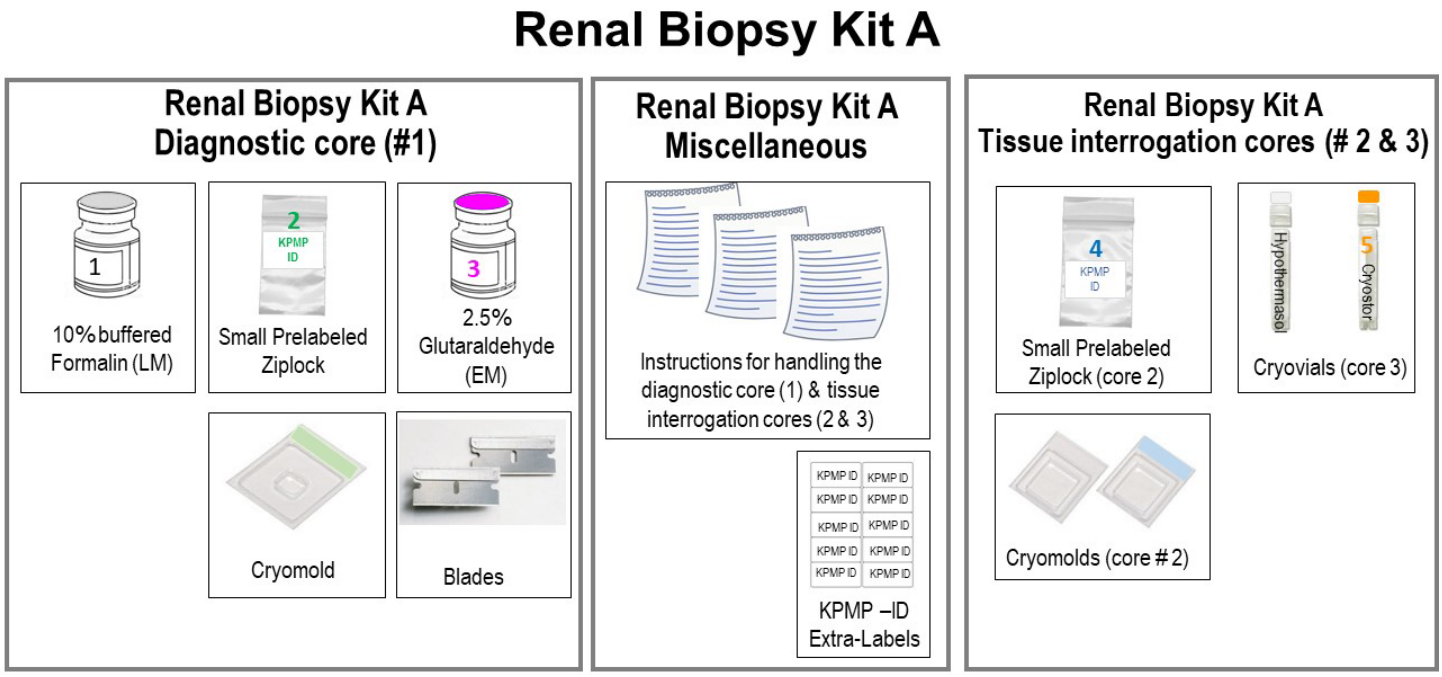


Figure 3: KPMP renal biopsy Kit A. The itemized list of the supplies in the renal biopsy kit is illustrated.

D. KPMP renal biopsy Kit B components (Figure 4)

- *Kit B - Diagnostic core (same as in Kit A):*
    - Container 1: 10% neutral buffered formalin marked with gray cap (NBF) (Fisher Scientific cat. no 22-126-346) for LM
    - Container 2: Cryomold marked with green label (biopsy – tissue tek #4565) with OCT embedded frozen tissue for IF cut from the diagnostic Core #1 (to be placed in small Ziploc bag).
        - Ziploc bag
        - One unlabeled cryomold for ‘bathing’ only
    - Container 3: 2.5% Glutaraldehyde marked with pink cap (EMS cat. no. 16537-16) for EM
    - Razor Blades
- *Kit B – Tissue Interrogation Research core:*

- Container 4: Cryomold marked with blue label (Tissue-Tek Standard, #4557) for frozen Core #2 (to be placed in small Ziploc bag)
  - Ziploc bag
  - One unlabeled cryomold for 'bathing' only
- Container 6: 1 pre-labeled Cryovial marked with red cap (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416, or similar) [for Core # 3 liquid nitrogen preservation]

○ *Kit B – Miscellaneous (same as in Kit A):*

- Extra Labels (stickers) with KPMP sample IDs (for labeling derivative materials from the diagnostic core following the local pathology work-up)

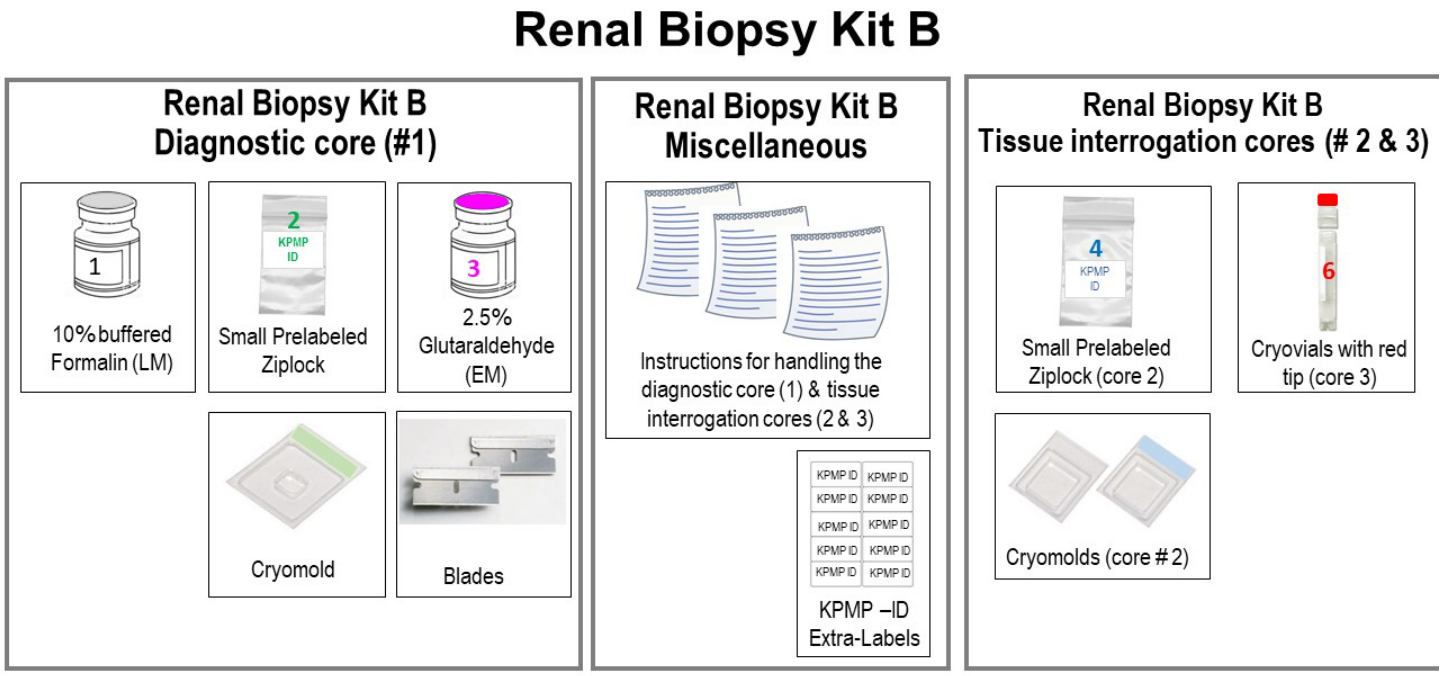


Figure 4: KPMP renal biopsy Kit B. The itemized list of the supplies in the renal biopsy kit is illustrated.

E. Metadata case report forms

○ KPMP ***Kidney Biopsy Procedure Details CRF*** and ***Tissue Triage CRF*** in REDCap (with paper back-ups; available on Basecamp)

1.3 Preparing for handling the renal biopsy cores

The renal biopsy dedicated tissue processing personnel will be trained by the Central Hub. The designated trained tissue processing personnel will review the renal biopsy supplies checklist (renal biopsy cart and kits) before going to the renal biopsy suite. The supplies (see section 1.2) are transported to the renal biopsy procurement suite at the time of the renal biopsy procedure.

The renal biopsy dedicated personnel or research coordinator(s) will clean the instruments, cart surface and external surface of solutions containers before starting the procedure, see below (section 1.3.1). To prepare designated area for tissue processing in the renal biopsy procurement suite, trained tissue processing personnel should be gowned with appropriate personal protective equipment including gloves and masks.

1.3.1. Procedure for making a RNase free working area (use for preparing the cart before placing items on it and the working area in the biopsy suite)

Note on creating RNAse free work area: All processing conditions should be done with RNase reducing precautions. All surfaces that will be used in specimen handling should be decontaminated at the beginning of every procedure with RNaseZap solution (**Caution – do not use on corrodible metal surfaces)**. This solution

is used to remove RNases from instruments, apparatus, countertops, plastic and glassware. Do not use on surfaces incompatible with ethanol or RNaseZap. There are 3 major steps to follow:

A. Inspection:

- First, inspect the biopsy suite and select the best area that will be used for the biopsy processing area. This may be the area where the cart itself will be positioned if there is not designated space in the biopsy suite.

B. Cleaning:

- Clear and set up space to be used for receiving and processing the biopsy cores obtained from the clinical staff. Put gloves on.
- Clean space (surface) initially by spraying 70% ethanol (prior to placing the items on the cart and working area in the suite), and wipe dry with a clean paper towel.
- Clean by spraying 70% ethanol on the items (supplies) that will be placed on the working space (e.g. ice buckets, forceps, vials and solution containers). The underside of the bucket needs to be wiped too.
- After space has dried completely, apply RNaseZap to the entire working surface area and supplies (i.e. items on the cart needed for tissue processing) with spray bottle.
- Wipe with clean paper towel
- Set the timer for 2:00 minutes and wait a full 2 minutes for the area to dry completely.
- Spray with RNase free water and dry with a clean paper towel.

C. Covering:

- Place clean bench pads (2-3 blue pads) on the cart or on the surface where tissue processing will occur.

NOTE: Ensure that the work area is not contaminated following these steps. Do not touch the work area without clean gloves following the cleaning process.

1.3.2. General protocol for initial handling of the tissue

These are general steps expected to occur as soon as a biopsy pass is complete, and the clinical staff has a biopsy core that is excised from the participant. The core needle is taken to the designated area for tissue processing. In most cases, it is expected that the first pass will be designated as the diagnostic core (Core 1), unless it does not meet the criteria discussed in section 1.5. In this case, it is possible that the diagnostic core will be obtained from later passes and that the initial tissue may be designated for tissue interrogation (see decision tree below); thus to preserve integrity of RNA and protein place the cores in ice-cold sterile PBS in the petri dish during triaging. The diagnostic and research core workflows are described in Section 2 and 3 of this MOP. The Biopsy Reference checklist in Basecamp serves as a reference guide to the key steps for pre-, intra-, and post-biopsy operations, with emphasis on best practices.

A. Prepare the working space before starting:

- Set up all the material necessary for each step
- Clean all supplies and surface (see section 1.3.1)

B. Maintain a clean environment at all times:

- Wear disposable gloves at all times.
- To prevent contamination of the needle, which will be re-used for the subsequent passes, as soon as the tissue is harvested and brought to the KPMP personnel, the tissue will be transferred (gently deposited) from the needle onto the sterile telfa pad.
- Unwrap the sterile cotton-tipped applicator, and use one to transfer the core biopsy tissue from the sterile telfa pad to the Petri dish as stated below
- Touch the tissue using sterile cotton-tipped applicators (removal from biopsy needle) or sterile wooden applicators (for other maneuvers). **DO NOT** reuse flat wooden applicators, especially if they touched a media such as formalin, OCT, or glutaraldehyde. Use a new sterile wooden applicator at each step touching the tissue.

C. Handle the tissue gently:

- When using any wooden instrument to move the biopsy, let the tissue gently adhere to the tip and gently transfer it into the Petri dish. Do not use forceps to handle the tissue.
- Keep the tissue cold by placing the Petri dish that has been pre-chilled on wet ice. Keep the tissue well hydrated (moist) by adding sterile saline phosphate solution (about 100 µl at a time) (dropping the solution on top of the tissue without touching it). This will help keep the tissue intact while taking photographs and during triaging.

D. General rules for triaging:

- An assessment will then be performed by the trained tissue processing personnel to decide if the tissue will go towards diagnosis (Core 1) or will be committed to research (Cores 2 and 3) (see section 1.5).
    - Note: The number of passes does not correspond to the numerical core designation. For example, the diagnostic core (Core 1) could be obtained from biopsy pass #4.
- It is not advisable to commit the core obtained from first pass to research processing before securing the diagnostic core, because there is no guarantee that a better core, or additional core, will be obtained after the first pass. Therefore, all research cores will be committed only after the diagnostic core is secured and during this wait period place the cores in ice-cold sterile PBS in the petri dish.

E. Deidentification and labeling (post-processing):

- When adding a KPMP sample ID label to any sample or slide created at the RS, ensure that the Kit ID of the extra labels is the same as that of the pre-labeled samples, i.e. do not swap extra labels between kits.

For data collected in the metadata form see the ***Kidney Biopsy Procedure Details CRF***.

1.4 Assessment for adequacy by gross examination

A KPMP tablet camera, of minimum 3 Megapixel resolution, will be used at the participant bedside to document the gross morphology of the kidney biopsy core tissues (Figure 6). KPMP trained tissue processing personnel will assess the gross appearance of the kidney biopsy tissue and determine presence or absence of renal cortex and medulla. A digital microscope will be provided to maximize the ability of the personnel to see cortex. A minimum of 1 gross digital photo will be taken of each kidney biopsy core (diagnostic core and research cores). The number of photos taken will be recorded in the ***Kidney Biopsy Procedure Details CRF***. The gross photos will be labeled with the biopsy core specific KPMP participant code identifier and uploaded electronically via REDCap.

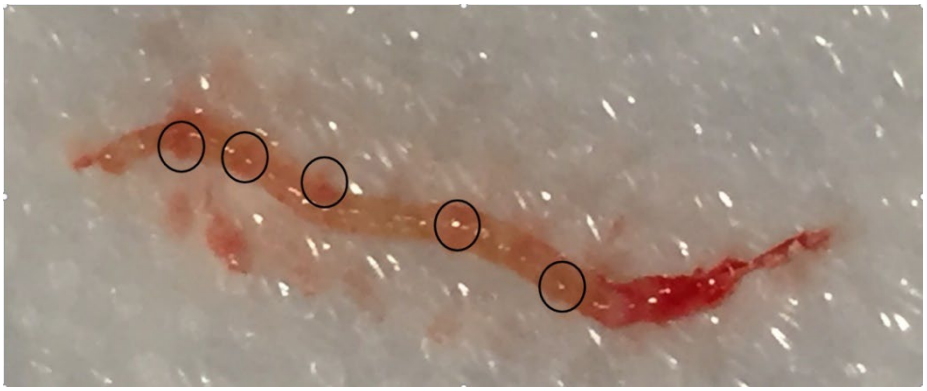


Figure 6: Imaging and recording of the fresh tissue for gross examination. The upper panel shows a digital image of a fresh biopsy taken by a portable digital camera. Gross examination of the biopsy reveals the presence of multiple discrete red areas (black circles in lower panel), which represent glomeruli within the cortex. The cortical area harboring these glomeruli will be measured to ensure the presence of a sufficient amount for diagnostic and research purposes.

1.5 Triaging of kidney biopsy cores

According to the KPMP protocol, up to 3 kidney biopsy cores (5 is the maximum number of passes allowed) will be obtained from each participant enrolled in KPMP. The tissue will be triaged after gross examination as illustrated in Figure 7 and in the section below. The workflow and prioritization illustrated below has the scope to assure that adequate amount of tissue is processed for diagnostic studies and molecular analysis, and to ensure that the tissue cores are preserved in the appropriate conditions/media for processing by the local RS pathology laboratory for diagnostic purposes and for use by the TIS for the molecular studies. The specific processing steps at each TIS are described in the site-specific protocols in the protocols.io KPMP workspace.

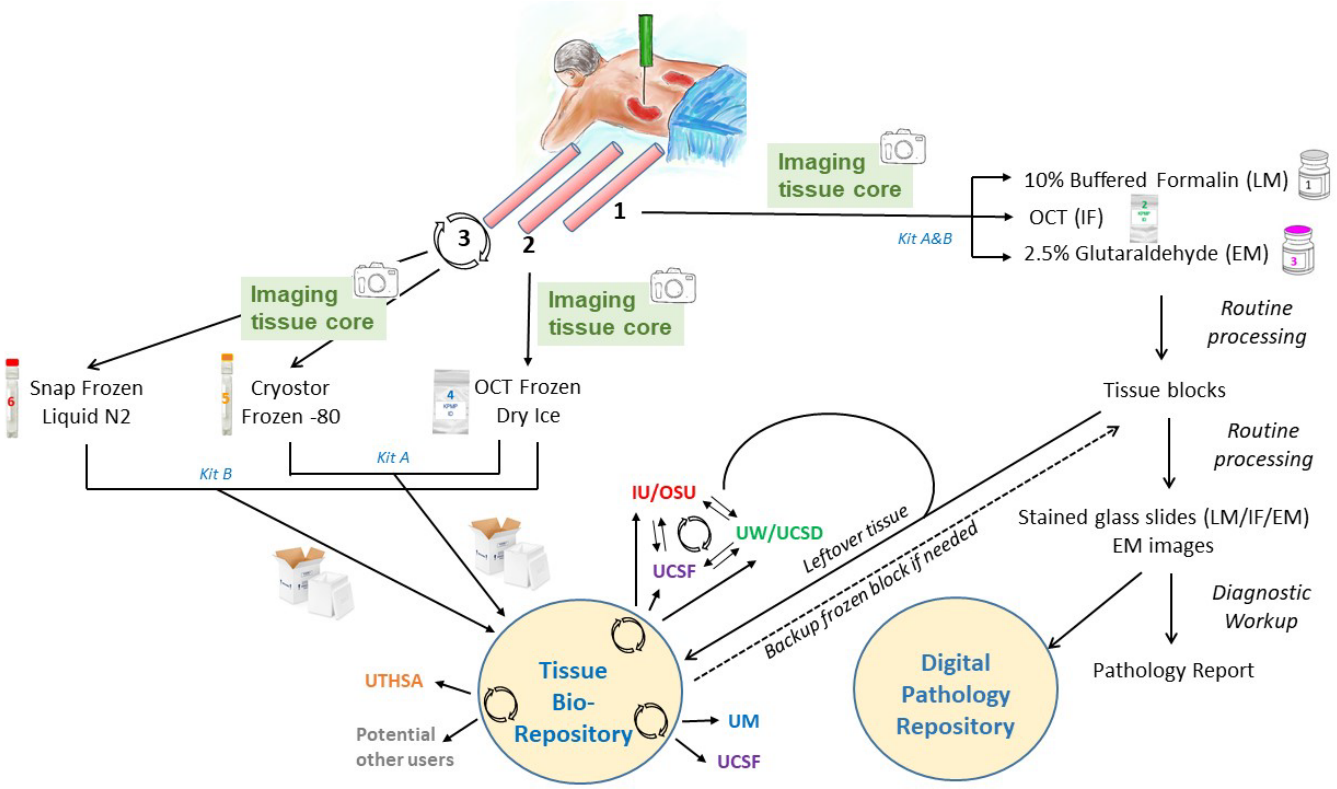


Figure 7: Overall kidney biopsy schema for triaging of the cores for downstream analysis and interrogation. The goal for KPMP ideally is to obtain 3 cores from each participant. Core 1 will be used for diagnostic purposes, and core remnants may be used for research. Cores 2 and 3 are solely used for research. At the time of procurement, it is expected that each RS will promptly process and transfer all tissue cores to the appropriate medium or condition as described above, suitable for temporary storage and shipment. This is detailed in section B. Downstream processing at each TIS is described in the site-specific protocols in the protocols.io KPMP workspace.

SUGGESTED TRIAGING APPROACH: If the biopsy cores are obtained in relatively rapid succession, it is likely best to wait until all 3 desired cores are out of the participant and on the chilled telfa pads before any triage decisions are made. Biopsy length measurements and pictures could be obtained while biopsy passes are occurring, or wait until the biopsy operator is done with the passes. When ready, each pass can be renamed as core #1, 2, or 3 based on decisions as described below. When ready to process each core, **FIRST** place research core #3 in its preservative (HypoThermosol or liquid nitrogen depending on if Kit A or B, respectively). **SECOND**, segment core #1 (diagnostic) as described into portions for LM, IF, and EM, and place tissue portions into formalin and glutaraldehyde (DO NOT FORGET to use a new sterile wooden applicator each time tissue is touched – if any formalin or glutaraldehyde touches tissue to be used for molecular analysis, it will ruin the tissue). Now only the tissue portions to freeze in OCT are remaining, so **THIRD**, freeze the research core #2 in OCT and then the last portion of the diagnostic core #1 in OCT, as described. **Thus** the suggested order of tissue core preservation is: (1st) place core #3 in HypoThermosol or liquid nitrogen, (2nd) place core #1 formalin and glutaraldehyde, and (3rd) freeze research core #2 in OCT and then the remaining

portion of core #1 in OCT. Note that this triaging approach is suggested, not required. The goal is to reduce time from harvest to placement in fixative for all cores.

Record the time each biopsy core comes out of the body. Record the time (in minutes) it takes for each biopsy core to go in its initial processing step:

- For diagnostic core (Core 1) time interval to transfer to formalin, OCT-embedded tissue on dry ice, and glutaraldehyde.
- For OCT frozen research core (Core 2), time interval to place OCT bathing cryomold on dry ice.
- For Core 3, time to transfer to HypoThermosol and CryoStor (Kit A) or when cryovial is placed in liquid nitrogen (Kit B).

1.5.1. Decision tree for triaging

The guiding principles detailed in sections 1.5.2-4 and the course of the biopsy procedure itself will likely influence the triaging process. However, considering the sequence of events in real-time, a decision on the fate of each core may need to be taken without the knowledge of the number or the quality of the other cores. The overall approach should assure first that appropriate tissue is assigned to standard diagnostics before committing the other cores to research. Therefore, to guide appropriate triage and minimize variability between RS while assuring that adequate diagnostic tissue is obtained, we propose the following decision tree to guide the triaging process (Figure 8).

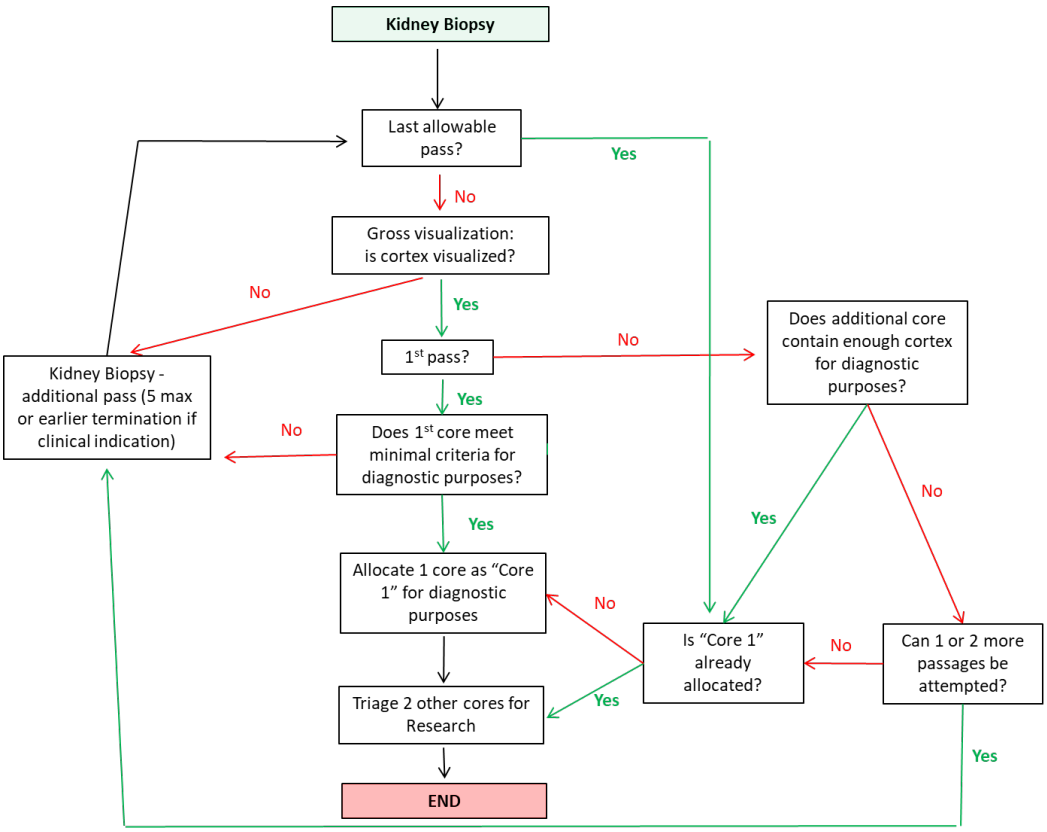


Figure 8: Decision tree to guide the triaging process based on the biopsy progress and the anticipated tissue workflow (see text for details). Research cores will only be committed to processing after the diagnostic core is secured.

Explanation of the decision tree:

After each pass, the fate of the core may depend on the number of remaining passes. If a core should fragment, it is treated as if it were one core for triaging purposes.

- *Scenario 1:* If the first pass yields a core with criteria fit to be a diagnostic core (discussed in section 1.5.2), then this core will be immediately allocated to the diagnostic workflow (section 2) and the remainder of the cores (ideally 2 cores), will be allocated immediately for tissue interrogation (Core 2 frozen in OCT, and Core 3 in Cryostor or snap frozen (Kit A or B).

- *Scenario 2:* If the first pass yields a core that is not optimal for diagnosis, then that core will need to be temporarily kept in the petri dish in ice-cold sterile PBS until the second core is obtained. If the second core obtained is fit to be the diagnostic core, then this second core will be designated as the diagnostic Core 1, and the first obtained core will be then immediately processed for research, likely frozen in OCT (Core 2). The third core will be immediately committed to research as Core 3**.** It is not advisable to commit the core obtained from first pass to research processing before securing the diagnostic core, because there is no guarantee that a better core, or additional core, will be obtained after the first pass. Therefore, all research cores will be committed only after the diagnostic core is secured. If the diagnostic core is secured first, then the biopsy cores obtained from additional passes will be immediately processed for research.
- *Scenario 3:* In the case that the first and the second cores are not optimal for diagnosis, then both cores will be temporarily kept in ice-cold sterile PBS in the petri dish until the third core is obtained. If the third core is optimal for diagnosis, it will be immediately committed as diagnostic Core 1, and the two initial cores will be then designated immediately for research. If all 3 cores do not fit the criteria for diagnostic core, then the diagnostic core will be determined based on the presence of any cortical tissue or the length of the biopsy, as outlined in section 1.5.2.
- In the case that only two cores are obtained, one will be slated for diagnostic purpose (Core 1) and the other for Core 2 processing, as outlined in 1.5.3.
- In case of only one core, then it will be handled according to 1.5.4.

The time of each biopsy pass, whether tissue was obtained, and how it was triaged is recorded in the ***Kidney Biopsy Procedure Details CRF***.

1.5.2. Triaging three biopsy cores

A. Renal cortex visualized: If the first tissue core is adequate for diagnostic workup (cortex visualized and the core is ≥1.4 cm in length) or when after two or more passes, two or three tissue cores are obtained, with at least one considered adequate for diagnostic workup, are obtained, the tissue core assigned for diagnostic workup is processed as a diagnostic core (see section 2.1.1) The diagnostic core will be divided in three parts with a minimum of 0.1-0.2 cm of cortex fixed in 2.5% glutaraldehyde for electron microscopy studies (container 3), 0.3 cm cortex snap frozen in OCT for immunofluorescence studies (container 2 – Ziploc bag), and the remaining amount of tissue fixed in 10% neutral buffered formaldehyde (formalin) (container 1) (see Figure 3, 4, and 7). If the amount of cortex is less than 0.5cm, the entire core goes toward light microscopy and placed in formalin (see also section 1.5.3). The remaining cores are immediately triaged as tissue interrogation cores 2 and 3. Cores 2 and 3 will be placed in the appropriate media or fixatives and shipped to the CBR for distribution to the TIS (see Figure 7 and section 3 for details). The goal will be to triage the tissue cores within 5 minutes post harvesting.

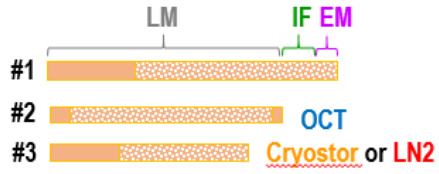


Figure 9: triaging of the renal biopsy when 3 cores are obtained, with at least one ≥1.4cm in length and renal cortex is visualized.

B. Renal cortex not visualized (in all three specimens): If renal cortex is not visualized in any of the 3 cores, and at least one core measures ≥1.4 cm, then this core will be designated as the diagnostic core, and the remaining two cores will be designated as tissue interrogation cores 2 and 3 (Figure 7). A minimum of 0.1-0.2 cm of tissue from both tips of the diagnostic core will be placed in container 3 (2.5% glutaraldehyde) to be processed for electron microscopy studies, a 0.3 cm of tissue from both tips will be frozen in OCT, to be processed for immunofluorescence studies (container 2 – Ziploc bag), and the remaining tissue will be placed in container 1 (10% neutral buffered formaldehyde -

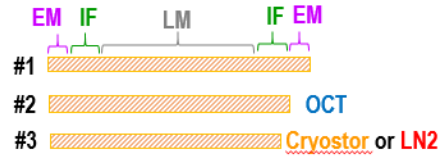


Figure 10: triaging of the renal biopsy when 3 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is not visualized.

formalin), to be processed for Light Microscopy. The remaining two cores will be designated for tissue interrogation and triaged/processed as cores #2 and #3 (see Figure 7 and section 2 for details).

C. All biopsy cores <1.4 cm: A minimum of 0.5 cm of cortex needs to be placed into container 1 (10% neutral buffered formaldehyde - formalin) for Light Microscopy processing. A minimum of 0.2 cm cortex needs to be frozen in OCT to be processed for Immunofluorescence (container 2 – Ziploc bag). A fragment of tissue measuring 0.1 cm cortex needs to be placed in container 3 (2.5% glutaraldehyde) to be processed for Electron Microscopy.
In the event of scarce tissue availability, the following prioritization protocol will be followed: Light Microscopy takes precedence over Immunofluorescence and Electron Microscopy, and Immunofluorescence takes precedence over Electron Microscopy (LM > IF > EM). Cores #2 and #3 will be triaged for tissue interrogation (see Figure 7 and section 3 for details).

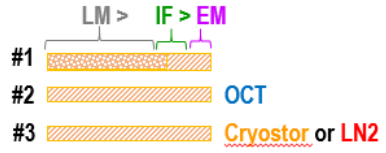


Figure 11: triaging of the renal biopsy when 3 cores are obtained, all measuring <1.4cm.

1.5.3. Triaging two biopsy cores

A. Renal cortex visualized: In the situations when a third core cannot be obtained, renal cortex can be visualized grossly or imaged, and the length of the biopsy cores is ≥1.4 cm, the core will be entirely processed for diagnostic purposes (as Core 1). The second biopsy core is embedded in OCT (container 4), frozen on dry ice and shipped to the central biorepository where it will be distributed to the TISs (see Figure 7 and section 3 for details). The goal will be to triage the tissue within 5 minutes from harvesting and record this time.

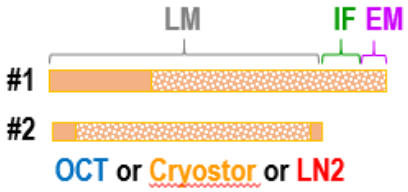


Figure 12: triaging of the renal biopsy when 2 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is visualized.

B. Renal cortex <u>not</u> visualized: If renal cortex is not visualized in any of the 2 cores obtained, and at least one core measures ≥1.4 cm, then this core will be designated as the diagnostic core (Core 1), and the remaining core will be designated as Core 2 for freezing in OCT and shipped to the Central Biorepository (Figure 7). A minimum of 0.1-0.2 cm of tissue from both tips of the diagnostic core will be placed in container 3 (2.5% glutaraldehyde) to be processed for electron microscopy studies, a 0.3 cm of tissue from both tips will be frozen in OCT, to be processed for immunofluorescence studies (container 2 – Ziploc bag), and the remaining tissue will be placed in container 1 (10% neutral buffered formaldehyde - formalin), to be processed for Light Microscopy (see Figure 7 and section 3 for details). The goal will be to triage the tissue within 5 minutes from harvesting and record this time.

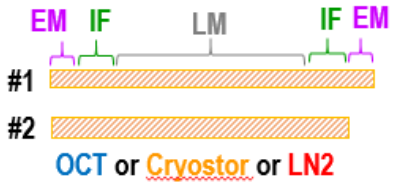


Figure 13: triaging of the renal biopsy when 2 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is not visualized.

C. All biopsy cores <1.4 cm: A minimum of 0.5 cm of cortex needs to be placed into container 1 (10% neutral buffered formaldehyde - formalin), to be processed for Light Microscopy. A minimum of 0.2 cm cortex needs to be frozen in OCT to be processed for Immunofluorescence (container 2 – Ziploc bag). A fragment of tissue measuring 0.1 cm cortex needs to be placed in container 3 (2.5% glutaraldehyde) to be processed for Electron Microscopy. In the event of scarce tissue availability, the following prioritization protocol will be followed: Light Microscopy takes precedence over Immunofluorescence and Electron Microscopy, and Immunofluorescence takes precedence over Electron Microscopy (LM > IF > EM). The second biopsy core is placed in the appropriate media or fixatives for molecular studies, shipped to the Central Biorepository, and distributed to the tissue interrogation sites (see Figure 7 and section 3 for details).

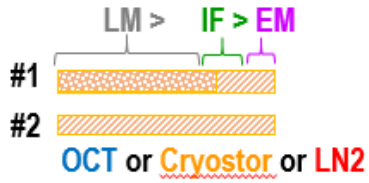


Figure 14: triaging of the renal biopsy when 2 cores are obtained, all measuring <1.4cm.

1.5.3. Triaging one biopsy core

A. Core measures >0.5 cm: A minimum of 0.5 cm of cortex needs to be fixed in 10% neutral buffered formalin (container 1) to be processed for Light Microscopy. A minimum of 0.2 cm needs to be frozen in OCT and processed for Immunofluorescence analysis (container 2 – Ziploc bag); and 0.1 cm cortex needs to be fixed in 2.5% glutaraldehyde (container 3) to be processed for Electron Microscopy. Light Microscopy takes precedence over Immunofluorescence and Electron Microscopy, and Immunofluorescence takes precedence over Electron Microscopy (LM > IF > EM). The goal will be to triage the tissue within 5 minutes from harvesting and record this time.

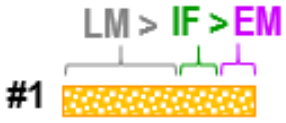


Figure 15: triaging of the renal biopsy when a single core, at least ≥0.5cm in length, is obtained, and renal cortex is visualized.

B. Core measures <0.5 cm: The entire tissue will be fixed in 10% neutral buffered formalin (container 1) to be processed for Light Microscopy.

C. Renal cortex <u>not</u> visualized: The entire tissue will be fixed in 10% neutral buffered formalin (container 1) to be processed for Light Microscopy.

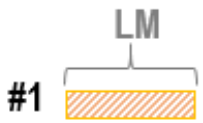


Figure 16: triaging of the renal biopsy when a single core obtained, and no cortex is identified or the tissue is <5cm in length.

<u>1.6 Recording of events from harvesting to processing and quality control</u>

Each of the steps taken during gross examination and triaging are recorded. This includes pertinent metadata and pre-analytical parameters associated with specimens under interrogation. The tissue processing committee created a list of parameters to be collected as metadata and for quality control at the time of the renal biopsy procedure. A copy of the metadata forms is included in the renal biopsy kit (see section 1.2.1), however, it is expected that in most cases these parameters will be collected at the time of the biopsy procedure in an electronic form directly, by accessing the KPMP REDCap. The ***Kidney Biopsy Procedure Details CRF*** will be completed upon conclusion of the procurement phases (gross evaluation, triaging, processing, storage) of the tissue for diagnostic and tissue interrogation purposes.

2. Handling and processing of the diagnostic core

2.1 Purpose

To prepare the diagnostic renal biopsy tissue for pathologic evaluation. This process includes three steps: handling of the tissue at the time of the renal biopsy procedure, transportation of the vials containing the tissue to the RS pathology laboratory, and processing of the tissue. The Biopsy Reference checklist in Basecamp serves as a reference guide to the key steps for pre-, intra-, and post-biopsy operations, with emphasis on best practices.

2.1.1. Handling of the diagnostic core

This step occurs at the time of the renal biopsy procedure (see triage, section 1.5) in the renal biopsy procurement suite (e.g. biopsy suite, interventional radiology, or operating room), and aims to collect adequate amounts of tissue in the appropriate media, to guarantee sufficient material for the diagnostic work-up by LM, IF, and EM.

2.1.2. Transportation of the diagnostic core to the RS pathology laboratory

This step aims to rapidly deliver the tissue obtained in the renal biopsy procurement suite in the appropriate media to the local pathology laboratory for processing for diagnostic purposes.

2.1.3. Processing the diagnostic core

This step is performed in the RS pathology laboratory, and serves to transform the tissue received in the RS pathology laboratory into material suitable for examination using LM, IF, and EM, while maximizing tissue available for downstream analysis with techniques compatible with standardized clinical pathologic tissue processing protocols. Once this step is complete, any remaining blocks and slides will be de-identified and shipped to the CBR. IF and EM images will also be obtained, de-identified, and uploaded to REDCap. The RS pathologist should complete the top portion of the paper Pathology Image Upload CRF, and both the Disease Categories Assignment and Immunofluorescence Metadata CRFs in full directly in RECap.

2.2 Handling of the diagnostic core at the time of the renal biopsy procedure

2.2.1. Applications

For histologic, immunofluorescence, and electron microscopy analysis of the diagnostic core. Upon triaging (following the decision tree illustrated in Figure 8), the diagnostic core is placed in the appropriate media as illustrated in Figure 7.

Note: Estimate about 30 minutes to prepare the biopsy cart and supplies to be taken to the biopsy procurement suite and prepare a clean RNase free environment in the procurement suite (see section 1).

2.2.2. Materials (see Figures 2, 3, and 4 and section 1.2.1)

A. From the renal biopsy Kit A or B

- Container 1: 10% neutral buffered formalin with gray label (NBF) (Fisher Scientific cat. no 22-126-346) for LM
- Container 2: Cryomold with green label (biopsy – tissue tek #4557) with OCT embedded frozen tissue for IF (to be placed in small Ziploc bag).
  - Ziploc bag
  - Bathing mold
- Container 3: 2.5% Glutaraldehyde with pink label (EMS cat. no. 16537-16) for EM
- Razor Blades to cut IF and EM piecesz

B. From the renal biopsy cart

- Optimum Cutting Temperature medium O.C.T. compound: Tissue-Tek (#4583)
- Sterile 1X phosphate buffered saline (PBS), pre-chilled on ice
- Long forceps to handle the cryomold with frozen IF tissue embedded in OCT
- Powdered Dry ice bucket
- Wet ice bucket that contains sterile PBS

- Disposable gloves to handle biopsy processing steps
- Gloves to handle dry ice
- Bucket with wet ice
- Sterile wooden applicators
- Sterile Dropper
- Sterile Telfa Pad
- Sterile Petri dish pre-chilled on wet ice bucket to triage the biopsy tissue

2.2.3. Protocol

A. Handling the diagnostic core for triaging (use protective gloves)

- Preparation:
  - Make sure containers in biopsy kit A or B are on your cart workspace:
    - Place the 10% buffered formalin container (container 1, gray) on the cart workspace or in the ice bucket.
    - Place the 2.5% glutaraldehyde container (container 3, pink) on the cart workspace, or in the ice bucket
    - Place the green labeled cryomold and Ziploc bag (container 2) in the workspace on the dry ice.
    - Place the unlabeled bathing cryomold on the cart workspace
  - In the renal biopsy procurement suite place the sterile telfa pad in the pre-chilled petri dish and place on ice.
  - Wearing protection gloves, saturate the sterile telfa pad with sterile 1X saline phosphate solution by using the dropper.
- Biopsy is obtained:
  - **Record the time** when the biopsy gun containing the tissue is removed from the participant (time stamp in the ***Kidney Biopsy Procedure Details CRF***).
- Handling the biopsy:
  - Wearing protection gloves, the individual obtaining the biopsy tissue transfers the tissue core from the needle onto the sterile PBS-soaked telfa pad in the petri dish by washing the biopsy needle with sterile saline. If necessary, use the wooden end of a sterile, cotton-tipped applicator to help remove the biopsy from the needle. Never touch the needle with anything that is not sterile.
  - The research coordinator will quickly take a picture of the core using the KPMP tablet with the ruler in view. Use sterile wooden applicators as needed to properly place the tissue. The length of the core is recorded in the ***Kidney Biopsy Procedure Details CRF***. *Include fat when measuring the core length.* If unsure, measure it all and/or rely on the pathologist.
  - Tissue is assessed for diagnostic adequacy by the TPP per above criteria (section 1.4). Record whether cortex is visualized or not in the ***Kidney Biopsy Procedure Details CRF***. Include comments about core composition (ie fat) in CRF if applicable.
    - If tissue qualifies to be used as a diagnostic core, proceed with diagnostic core processing.
    - If tissue fails diagnostic qualification, place the core in the petri dish (on wet ice) aside for later use as a research core and await additional tissue. Be sure to track which core came from which pass.
    - If the second core also fails diagnostic qualification, place the second core aside (on wet ice) for later use as a research core and await additional tissue.
  - Once the diagnostic core is identified, assign it from the pass number in REDCap.
  - For diagnostic qualified tissue, with the tissue in the petri dish, using the one or two blades technique, separate the tissue in three parts (when possible) according to the criteria discussed in section 1.5.
    - <u>One blade technique:</u> use one blade to cut through the tissue by moving the blade's sharp corner from one side to the other of the core, or by pressing down

the blade with the sharp edge parallel to the cutting surface and then sliding the blade toward the operator.

- Two blades technique: place the corner of each blade on opposite side of the biopsy core keeping both blades parallel to each other and adherent to each other. Hold the blades from the opposite not sharp corner. The sharp corner of the blade held by the left hand should be on the right side of the tissue core and the sharp corner of the blade held by the right hand on the left side of the tissue core. Cut the tissue by pulling away the blades keeping them parallel and close to each other.

B. Handling tissue for light microscopy

- Using a sterile wooden applicator, collect the fragment of tissue devoted to histology analysis, and drop it in the container with formalin (grey cap – container 1). **Record this timepoint in the *Kidney Biopsy Procedure Details CRF*.** Close the container and place it on wet ice. Discard the wooden applicator on the bench pad.

C. Handling tissue for immunofluorescence

- Before the biopsy is obtained, fill the bathing cryomold with OCT at room temperature. Replace the cap on the OCT bottle when not in use. When ready to embed, transfer the biopsy tissue gently with a sterile wooden applicator into the OCT bathing cassette kept at room temperature (Figure 17A). Briefly bathe the tissue in the cassette (few seconds) (Figure 17A') by gently swishing around the tissue with a sterile wooden applicator (without squishing the tissue).
- Briefly remove the pre-labeled green cryomold from the dry ice and place on the flat cart surface. Transfer the tissue after bathing into the pre-labeled green cryomold (Figure 17B) and with the help of a wooden applicator, orient the tissue so it is flat and parallel to the bottom surface and pour OCT to the brim, ensuring it completely covers the tissue but does not overflow (Figure 17B'). In the example shown, the cortex is oriented towards the side with the label. Representation of the proper orientation of the tissue is shown in Figure 18. This is a crucial step as improper orientation can result in loss of tissue or fragmentation during cryosectioning**. Immediately return the cryomold containing OCT embedded tissue to the dry ice and record the time in the *Kidney Biopsy Procedure Details CRF*. Take care to gently 'nestle' the cassette into the dry ice, ensuring the bottom of the cryomold is in full contact with the dry ice.** Discard the sterile wooden applicator on the bench pad.
- When completely frozen (i.e. when the clear OCT becomes frozen white solid), place the cryomold into the small pre-chilled Ziploc (container 2) using the long forceps or hands with clean gloves to hold the cassette from one edge. The goal is to refrain from touching the mold and instead handling the cassette with the edges.
- Keep the Ziploc with the cryomold containing the tissue (container 2) on dry ice until delivered to the pathology laboratory. Keep the dry ice bucket covered.

D. Handling tissue for electron microscopy

- Using a new wooden applicator, collect the fragment of tissue devoted to electron microscopy analysis, and place it into the container with glutaraldehyde (container 3 – pink cap). Close the container and place it on wet ice. **Record the time.** Discard the sterile wooden applicator on the bench pad.

The pathology personnel will collect the 3 containers with the diagnostic core fragments to transport them to pathology as per local standardized protocol.

2.3 Transportation of diagnostic core to RS pathology laboratory

It is expected that the initial processing of the cores, including the initial gross evaluation, will occur at the time of harvesting in the biopsy procurement suite. The allocation of each tissue core and tissue fragments to the appropriate media will occur in a rapid manner with each core triaged within 5 minutes. Before transportation, the diagnostic core will have already been transferred to 10% formalin (for LM), frozen in OCT (for IF, container #2) or 2.5% glutaraldehyde (container #3). The detailed protocol for handling the diagnostic core is described

in section 2.2.3. All key events occurring in the specimen procurement stages will be time-stamped (***Kidney Biopsy Procedure Details CRF***) by the RS dedicated staff as noted in **bold** font in section 2.2.3.
After the procurement of all the cores and their processing described in section 2.2 and 3 (for research cores), the staff immediately brings the materials for processing of the diagnostic core to the RS pathology laboratory and then follows the subsequent procedures for storage and shipping as detailed in section 5 and Appendix C.

2.4 Local processing workflow (default option)

Kidney tissue obtained at the KPMP Participant Recruitment Sites (RS) is triaged as described in section 1.5. The portion of the diagnostic core placed in neutral buffered formaldehyde (formalin) is processed for light microscopy; the portion of the diagnostic core snap frozen embedded in an optimal cutting temperature (OCT) compound is processed for immunofluorescence microscopy; the tissue placed in 2.5% glutaraldehyde is processed for electron microscopy. Ensure that glass slide labels and coverslips are put on straight and that there is no overhang. A pathology workup should be conducted on core 1 even if the core is suspected of containing no renal tissue.

2.4.1. Light microscopy standardized protocol

Tissue placed in 10% neutral buffered formalin is processed into formalin fixed paraffin embedded tissue blocks (FFPE) according to the local RS pathology laboratory protocols. **Record the time when tissue is removed from formalin.** The paraffin blocks are sectioned at 2-3 microns and are processed to obtain 12 slides, in this order and staining:

1. Hematoxylin & Eosin (H&E)
2. periodic acid Schiff (PAS)
3. Masson's Trichrome (TRI)
4. Jones Methenamine Silver (SIL)
5. Unstained (blank)
6. Unstained (blank)
7. Unstained (blank)
8. Unstained (blank)
9. Hematoxylin & Eosin (H&E)
10. periodic acid Schiff (PAS)
11. Masson's Trichrome (TRI)
12. Jones Methenamine Silver (SIL)

The first set of four slides are sectioned at 2-3 microns each after initial facing of the block. Two sections per slide. A step section consisting of 4 blank 2-3 micron sections is then performed and the blanks saved (one section per slide). This is followed by an additional set of four sections at 2-3 microns each, 2 sections per slides, for a total of 12 slides (8 stained and 4 unstained). All sectioning and staining steps are performed using local RS laboratory protocols. Slides are reviewed by the local RS pathologist and a standard pathology report is generated (see section 4). The local RS pathologist may order additional sections and/or stains for diagnostic purposes per his or her professional judgement. If additional testing is desired, the RS can use one of the four unstained slides that were already prepared. When necessary, additional special stains or immunohistochemistry will be performed to reach a conclusive diagnosis.

2.4.2. Immunofluorescence standardized protocol

Tissue previously frozen in the renal biopsy procurement suite using OCT on dry ice (powdered) is processed for immunofluorescence analysis per local RS pathology laboratory protocols. The OCT frozen section tissue block is cryosectioned for staining with fluorescein conjugated anti-IgG, IgA, IgM, C3, C1q, fibrin, albumin, kappa light chain, and lambda light chain antibodies using local RS pathology laboratory protocols. Immunofluorescence stained slides are examined by the local RS pathologist. Using standard clinical pathology interpretation, each stain is scored on an intensity scale of 0-3+ and the location (glomerular, tubular, vascular, interstitial) and pattern (granular, linear) of positive staining is recorded in the local RS pathologist report for routine diagnosis. When necessary, additional immunofluorescence stains will be

performed to reach a conclusive diagnosis. No more than 0-2 H&E stained sections should be obtained from the OCT block. Do not collect any unstained slides.

The site research coordinator will provide the site pathologist with a paper version of the ***Pathology Image Upload CRF*** (Paper CRF Basecamp folder). The site pathologist will fill out the 'Frozen section H&E' section and indicate which IF antibodies yielded positive staining. Trace staining will be considered a positive result. If the site pathologist wants to upload an image, they should indicate the stain as positive for the purposes of the ***Pathology Image Upload CRF***. The site research coordinator will transpose the completed paper form into REDCap and will upload the corresponding, de-identified and KPMP-labeled immunofluorescence images (see section 2.4.4).

Associated metadata of immunofluorescence slides will be captured in the ***Pathology Immunofluorescence Metadata CRF***. Recruitment site pathologists are responsible for completing this CRF and recording metadata (location, distribution, pattern, intensity) *only* for antibodies that yield positive staining directly in REDCap. The site research coordinator will send an email link to the CRF a day after the kidney biopsy occurs. The ***Pathology Immunofluorescence Metadata CRF*** should be completed soon after the site pathologists looks at the IF slides under the microscope, ideally within 2 weeks of the biopsy.

2.4.3. Electron microscopy standardized protocol

Tissue placed in 2.5% glutaraldehyde is processed for electron microscopy using local RS pathology laboratory protocols. Toluidine blue stained thick plastic sections are reviewed by the local RS pathologist as per routine clinical pathologic examination. Thin sections are cut and electron microscopy grids created per local RS pathology laboratory protocols. Electron microscopic examination is performed and digital photographs of representative glomeruli and tubulointerstitium are obtained per local RS pathology laboratory routine process. A minimum of 10 glomerular photomicrographs are taken. A minimum of 5 photomicrographs of tubular epithelium, 2 of arteries/arterioles if present, and 3 of interstitium are taken. At least 2 photomicrographs of tubular epithelium must be taken at 30,000x to visualize the mitochondria. The remaining photomicrographs are taken at lower magnification. The ultrastructural digital photomicrographs are examined by the local RS pathologist and the findings incorporated into the pathology report.

2.4.4. Diagnostic core: Site research coordinator checklist

The day after the biopsy the study coordinator will complete the following steps:

- Provide a paper copy of the first two pages of the Pathology Images Upload CRF to the site pathologist.
- Send a survey link for the case Pathology Immunofluorescence Metadata CRF and the Dx Core Disease Category Assignment CRF to the site pathologist.

Upon completion of the routine pathology interpretation and reporting, the study coordinator will complete the following steps (***could be from one to four weeks from the time the diagnosis is rendered***):

- The pathology report is de-identified (see section 5), relabeled with the KPMP subject ID, and uploaded into the ***Pathology Images Upload CRF*** in REDCap.
- Transpose the paper copy of the ***Pathology Images Upload CRF*** completed by the site pathologist into REDCap.
- All glass slides are de-identified (see section 5), relabeled with a unique KPMP sample ID (provided with the kit; place a blank label under the KPMP label and ensure there is no overhang), recorded in SpecTrack as derivatives, and shipped to the CBR for transfer to the DVC for scanning and uploading into the KPMP DPR (see section 6). Do not send immunofluorescence glass slides to the CBR.
- The jpeg images of immunofluorescence are de-identified (see section 5), updated to include the KPMP sample ID that was previously applied to the slide and uploaded into the ***Pathology Images Upload CRF*** in REDCap (see section 6).
- The electron microscopy digital images (jpeg) are de-identified (see section 5), updated to include the KPMP sample ID that was applied to the slide and uploaded into the ***Pathology Images Upload CRF*** in REDCap (see section 6).

- The FFPE blocks are de-identified, relabeled with a unique KPMP sample ID label (supplied with the kit), recorded in SpecTrack as derivatives and shipped at 4˚C to the KPMP CBR for storage (see sections 5 & 7).
- The OCT frozen tissue remnant block will have been placed in a pre-labeled container with a unique KPMP sample ID during the biopsy procedure. Please return the remnant block to this container and verify that it still retains the label and is de-identified. Record in SpecTrack (as the parent block of the glass slides derived from it) and ship on dry ice to the KPMP CBR for temporary storage before transfer to TIS for downstream analytic techniques (see section 7).
- The plastic tissue blocks are de-identified, relabeled with a unique KPMP sample ID, recorded in SpecTrack as derivatives and shipped at 4°C to the KPMP CBR for storage (see section 7).

2.4.4. Diagnostic core: Site pathologist checklist

Upon completion of the routine pathology interpretation and reporting, the site pathologist will complete the following steps for each case (***within three weeks from the time of biopsy***):

- Complete the 'Frozen Section H&E (Immunofluorescence specimen) Overview' section of the ***Pathology Images Upload CRF*** provided by the site research coordinator and indicate which IF antibodies yielded positive results in the paper version. Trace staining is considered positive. Return the completed paper CRF to the research coordinator for entry in REDCap.
- Complete the ***Pathology Immunofluorescence Metadata CRF*** directly in REDCap soon after looking at the IF slides under the microscope, ideally within two weeks of biopsy. The site research coordinator will send an email link to the CRF a day after the kidney biopsy occurs and you will receive weekly reminders until the form is complete. This CRF captures metadata (location, distribution, pattern, intensity) and should be completed *only* for antibodies that yield positive staining directly in REDCap. Each antibody requires a new instance of the form.
    - *Note:* Pathologists can navigate to the Contact Information CRF to verify the participant in question. If pathologists cannot access the Contact Information CRF they should contact kpmpdcc@uw.edu to request REDCap access.
- Complete the ***Dx Core Disease Category Assignment CRF*** directly in REDCap, ideally within two weeks of biopsy. The site research coordinator will send an email link to the CRF a day after the kidney biopsy occurs and you will receive weekly reminders until the form is complete. This CRF captures *major* patterns and we don't expect more than 2 instances for each compartment: glomerular, tubulointerstitial, vascular. Each presenting pathology requires a new instance of the form.
    - *Note:* Pathologists can navigate to the Contact Information CRF to verify the participant in question. If pathologists cannot access the Contact Information CRF they should contact kpmpdcc@uw.edu to request REDCap access.

2.5 Central processing workflow (alternative option)

Upon completion of the routine local renal biopsy interpretation and reporting, the renal biopsy material follows the pathway indicated in section 2.3. In the event the local RS pathology laboratory-derived glass slides scanned into whole slide images are not compliant with established QC metrics for imaging, the decision to recut and stain the paraffin block (see 2.4.1) at the KPMP Central Processing Pathology Laboratory (CPL) may be considered; however, since the remaining tissue is limited the decision will be made on a case-by-case basis.

2.5.1. The KPMP Central Processing Laboratory (CPL)

The KPMP CPL is located at the University of Michigan and is affiliated with the KPMP CBR. When whole slide images are considered inadequate for computational imaging, the Central Hub will consider retrieving the de-identified FFPE blocks from the CBR and for further processing (cutting and staining) by the CPL.

2.5.2. Light microscopy standardized protocol

If the decision is made to reprocess the FFPE blocks, then the FFPE blocks received from the KPMP CBR will be sectioned at 2-3 microns thickness, per the CPL standard protocol. Stains needed are determined based on

which stains from the local RS pathology laboratories were out of compliance. Sections are stained for H&E, PAS, TRI, and SIL as needed. Slides are scanned (see section 5) and can be reviewed by the central core pathologist for assignment of diagnostic category and high-resolution adequacy assessment as needed.

3. Handling of the research core(s) at the time of the renal biopsy procedure for downstream analysis by tissue interrogation technologies

3.1 Purpose

This section describes methods for processing of the clinical biopsy tissue procured by the RS for use by sites with tissue interrogation technologies (TIS and other). These processing methods are the first version, and additional modifications will be added in subsequent versions depending on the feasibility and validation data from the interrogation technologies. The various methodologies for processing the fresh tissue and shipping to the Central Biorepository for storage and distribution to the TIS are described below. Each of the methodologies will be performed at the time of the renal biopsy procedure (bedside). Personnel at each RS will be trained accordingly. The research cores (Core 2, 3) will be allocated after diagnostic core has been identified (Core 1) (see section 1.5 and Figure 7 & 8). The Biopsy Reference checklist in Basecamp serves as a reference guide to the key steps for pre-, intra-, and post-biopsy operations, with emphasis on best practices.

3.2 Fresh frozen OCT embedded block preparation (Core 2, Container 4)

3.2.1. Applications

Histology, multiplex ISH and IF, proteomics, transcriptomics, laser capture microdissection, label free imaging, 3D cytometry

Metadata: Main metadata items include time stamp of the procurement, time tracking of processing, temperature record, procedure details, gross observations and documentation including spatial coordinates of the specimen, specimen quality assessment, specimen disposition and gross imaging documentation.

3.2.2. Materials (see Figures 2, 3, and 4 and section 1.2.1)

A. From the renal biopsy Kit A or B

- Container 4: Cryomold marked with blue label (Tissue-Tek Standard, #4557) for frozen Core #2 (to be placed in small Ziploc bag)
  - Ziploc bag
  - One unlabeled cryomold for 'bathing' only
- Cryovials for biopsy tissue processed in Cryostor (orange cap) and flash frozen in liquid nitrogen (red cap)
- Sterile telfa pad for transferring biopsy tissue between the biopsy gun and the sterile petri dish for triaging

B. From the renal biopsy cart

- Optimum Cutting Temperature medium O.C.T. compound: Tissue-Tek (#4583)
- Bucket for dry powdered dry ice (use mallet to crush the dry ice pellets as indicated in section 1.2.1 above prior to going to the biopsy suite)
- Gloves to handle biopsy processing steps
- Gloves to handle dry ice
- Sterile wooden applicators
- Sterile telfa pad to transfer tissue from biopsy gun to the petri dish
- Sterile 1X phosphate buffered saline (PBS), pre-chilled on ice
- Sterile dropper to transfer reagents to keep biopsy moist
- Sterile Petri dishes pre-chilled on wet ice bucket to triage the biopsy tissue
- Long forceps to handle the frozen cryomolds and the frozen cryovials
- Extra fine-tip lab markers
- KPMP tablet with camera

3.2.3. Protocol: Tissue embedding in OCT and OCT frozen block preparation

A. Handling the research core (use protective gloves)

- Preparation:
  - Make sure containers in biopsy kit are on your cart workspace

- Before the biopsy starts, take the one of the cryomolds (the 'bathing' cryomold) and completely fill it with OCT (Figure 17A). Keep it at room temperature on the cart workspace.
- Before the biopsy procedure begins place the pre-labeled (blue) cryomold (empty) on powdered dry ice (do not touch the inside well of the cryomold with your hand and do not let anything, such as dry ice, go inside the well). This will be the container (container #4) in which tissue will be embedded (Figure 17B).

o Biopsy is obtained:
- **Record the time** when the biopsy gun containing the tissue is removed from the participant (time stamp in the ***Kidney Biopsy Procedure Details CRF***).

o Handling the biopsy:
- Wearing gloves, the individual obtaining the biopsy tissue transfers the tissue core from the needle onto the chilled PBS-soaked sterile telfa pad in the petri dish by washing the biopsy needle with sterile saline. If necessary, use the wooden end of a sterile, cotton-tipped applicator to help remove the biopsy from the needle. Never touch the needle with anything that is not sterile.
- Add a few drops of the PBS with the sterile dropper if the tissue appears dry. Take a photograph of the tissue core with the KPMP tablet for documentation as discussed in section 1.4 for the diagnostic core biopsy. The photo is uploaded in the ***Biopsy Procedure Details CRF***.
- Transfer the biopsy tissue gently with a sterile wooden applicator into the OCT bathing cassette kept at room temperature (Figure 17A'). Briefly bathe the tissue in the cassette (few seconds) by gently swishing around the tissue with a sterile wooden applicator (without squishing the tissue).
- Briefly remove the pre-labeled blue cryomold from the dry ice and place on the flat cart surface. Transfer the tissue after bathing into the pre-labeled blue cryomold (Figure 17B) and with the help of a wooden applicator, orient the tissue so it is flat and parallel to the bottom surface and pour OCT to the brim, ensuring it completely covers the tissue without overflow (Figure 17B'). In the example shown, the cortex is oriented towards the side with the label. Representation of the proper orientation of the tissue is shown in Figure 18. This is a crucial step as improper orientation can result in loss of tissue or fragmentation during cryosectioning**. Immediately return the cryomold containing OCT embedded tissue to the dry ice and record the time in the *Kidney Biopsy Procedure Details CRF*.**
  - NOTE: avoid bubbles; can move the bubbles out of the way with forceps or sterile wooden applicators.
- Keep the cassette on powdered dry ice for freezing. Keep the dry ice bucket covered to keep a cold environment. Avoid flash freezing as that could result in tissue fracturing and thus loss of integrity.
- When completely frozen (i.e. when the clear OCT becomes frozen white solid), place the cryomold into the small pre-chilled Ziploc using the long forceps or hands with clean gloves to hold the cassette from one edge. The goal is to refrain from touching the mold and instead handling the cassette with the edges. Seal the Ziploc, taking as much air out as possible. Transfer the samples into a freezer box in the -80°C freezer within 90 minutes after fixation until ready ship overnight packed in dry ice shipping container. To avoid damaging the block during transit, place it securely in a pre-chilled freezer box with dry ice pellets in direct contact with the OCT block and pack in a container with dry ice (see appendix C). Take photograph(s) using KPMP tablet for documentation as a part of quality assessment and control.

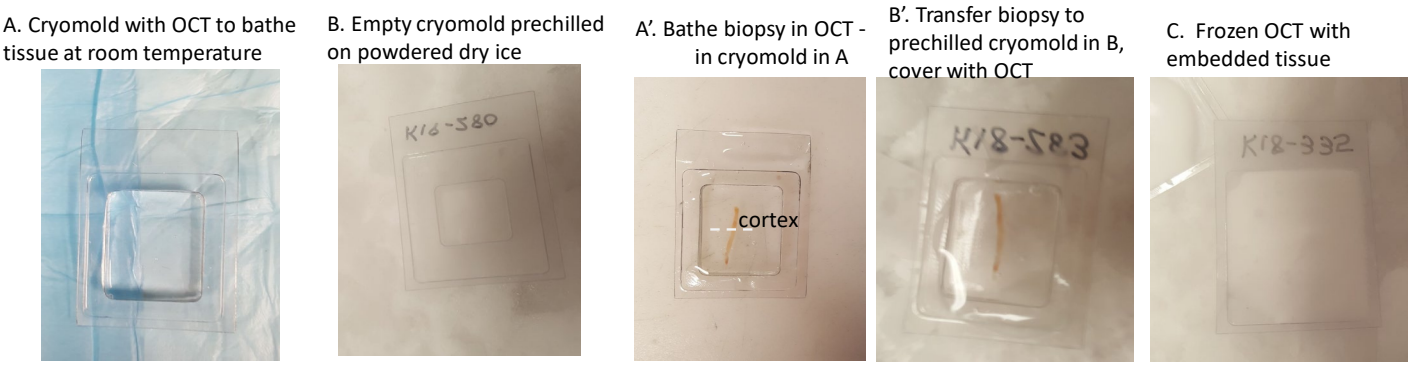


Figure 17. Preparation of cryomolds for fresh frozen OCT embedding of research core.

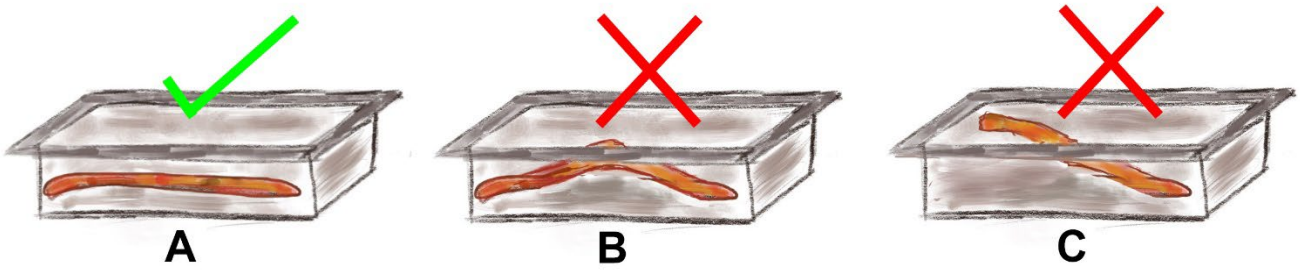


Figure 18: Proper orientation of the tissue during OCT freezing. A shows the proper orientation whereby the tissue is laid flat and parallel to the bottom surface. B and C are examples of incorrect freezing, whereby the tissue is not flat or not parallel to the surface.

3.3 Fresh frozen biopsy tissue in Cryostor (Core 3, Container 5)

3.3.1. Applications

Single cell RNA sequencing

3.3.2. Materials (see Figures 2, 3, and 4 and section 1.2.1)

A. From the renal biopsy Kit A

- Container 5, Tube #1: 1 orange cap pre-labeled cryogenic vial (Cryostor) (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416, or similar)
- Container 5, Tube #2: 1 HypoThermosol cryovial [for Core # 3 Cryostor preservation]
- Sterile telfa pad for transferring biopsy tissue between the biopsy gun and the petri dish for triaging
- Instructions

B. From the renal biopsy cart

- Bucket for wet ice
- Liquid nitrogen
- Wet ice
- HypoThermosol (Sigma, PN#H4416-100mL) solution in cryovial (tube #1)
- Cryostor CS10 solution Sigma, (PN#C2874100mL) in the cryovial Container 5 (tube #2)
- Mr. Frosty Freezing Container (Nalgene, PN#5100-0001)
- Isopropanol for freezing Core 3
- Sterile wooden applicators
- Sterile phosphate buffered saline (PBS), pre-chilled on ice
- Sterile dropper to transfer PBS and keep the biopsy moist
- Gloves to handle biopsy processing steps
- Sterile gauze
- Long forceps

- Extra fine-tip Sharpie
- Tablet with camera
- KPMP Tablet to take photos and record time
- Sterile Petri dish

3.3.3. Protocol: Preservation in Cryostor CS10 and freezing

A. Handling the research core (use protective gloves)

- Preparation:
  - Make sure containers in biopsy kit A are on your cart workspace
  - Place the orange cap 'cryostor' cryovial with 1.5 mL Cryostor CS10 (tube #2 of container 5) and the 'HypoThermosol' cryovial with 1.5 mL HypoThermosol (tube #1 of container 5) into wet ice vertically
- Biopsy is obtained:
  - **Record the time** when the biopsy gun containing the tissue is removed from the participant (time stamp in the ***Kidney Biopsy Procedure Details CRF***).
- Handling the biopsy:
  - Wearing gloves, the individual obtaining the biopsy tissue transfers the tissue core from the needle onto the sterile PBS-soaked chilled telfa pad in the petri dish by washing the biopsy needle with sterile saline. If necessary, use the wooden end of a sterile, cotton-tipped applicator to help remove the biopsy from the needle. Never touch the needle with anything that is not sterile.
  - Add a few drops of the PBS with the sterile dropper if the tissue appears dry. Take an image with the KPMP tablet for documentation as discussed in section 1.4 for the diagnostic core biopsy.
  - Using a sterile wooden applicator immediately transfer the renal biopsy specimen to the cryovial with HypoThermosol and cap the tube (tube #1 of container 5). Do not leave the wooden applicator in the tube. **Record time of when the tissue is placed in the cryovial with HypoThermosol in *Biopsy Procedure Details CRF*.** Keep the tube upright on wet ice until ready to transfer to cryopreservation with Cryostor. Set the timer for 15 minutes as a reminder to transfer to CryoStor. Aim should be to incubate tissue in HypoThermosol for *no more than 20 min*.
    - NOTE: Do not freeze the renal tissue and do not leave at room temperature
  - Using a sterile wooden applicator, transfer the renal biopsy specimen to the orange cap cryovial containing CryoStor CS10 (tube #2 of container 5). Cap the tube. Do not leave the wooden applicator in the tube. **Record time when the tissue is placed in the cryovial with CryoStor in *Biopsy Procedure Details CRF*.**
  - Keep the vials upright on ice for 15 – 20 minutes to allow penetration of the solution to the tissue.
  - Fill Mr. Frosty Freezing container with room temperature isopropanol to level indicated on the container. Note: this should be done in advance of the biopsy, during the cart preparation. The isopropanol in Mr. Frosty Freezing container should be changed every 5 uses.
  - Transfer cryovial containing CryoStor CS10 (tube #2, container 5) preserved tissue to Mr. Frosty Freezing container containing isopropanol (**Record time in *Tissue Tracking CRF***) and place container at -80°C within 90 minutes of fixation (**Record time in *Tissue Tracking CRF***) for about 24 hours (minimum of 12 hrs). Once the CryoStor tube is placed in the Mr Frosty, the Mr Frosty must immediately be placed in either the dry ice container (interim solution) or the -80°C freezer.
    - Note: Keep a container with dry ice handy to place the Mr Frosty in if it is not possible to reach the -80°C freezer in time.
  - Next day, remove the cryovial containing CryoStor CS10 (tube #2, container 5) cryopreserved tissue from the Mr Frosty container (**record time in *Tissue Tracking CRF*)** and move the Cryostor tube for storage in liquid nitrogen. Use long forceps to hold the cryovial during transfer. **If liquid nitrogen storage is not available, leave the**

specimens at -80°C until shipping to CBR. (see appendix C for shipping instructions but note that the Cryostor must be shipped within 7 days of procurement.)

- Note: Sample can be shipped on dry ice before or after transfer to liquid nitrogen

3.4 Fresh biopsy tissue frozen in liquid nitrogen (Core 3, Container 6)

3.4.1. Applications

Histology (H&E and autofluorescence), MALDI-MS imaging, bulk metabolomics, lipidomics, and proteomics.

3.4.2. Materials (see Figures 2, 3, and 4 and section 1.2.1)

A. From the renal biopsy Kit B

- 1 red cap pre-labeled cryogenic vial (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416, or similar) (container 6)

B. From the renal biopsy cart

- Powdered dry ice bucket
- Liquid nitrogen Flask (with liquid nitrogen)
- Sterile PBS pre-chilled on ice, **record temperature of ice**
- Sterile cotton-tipped applicators
- Sterile telfa pad
- Sterile wooden applicators
- Sterile dropper to transfer PBS and keep the biopsy moist
- Gloves to handle biopsy processing steps
- Gloves to handle dry ice
- Long forceps to hold the container 6 while transporting from liquid nitrogen to storage
- KPMP tablet to record time and photos
- Sterile Petri dish pre-chilled on ice
- Sterile chilled phosphate buffered saline (PBS), pre-chilled on ice to keep biopsy tissue moist
- Sterile Petri dish
- Instructions

3.4.3. Protocol: Freezing in liquid nitrogen (LN2)

A. Handling the research core (use protective gloves)

- Preparation:
  - Make sure containers in biopsy kit B are on your cart workspace
  - Fill the liquid nitrogen storage Dewar with liquid nitrogen
  - Keep container 6 cryovial on wet ice.
- Biopsy is obtained:
  - **Record the time** when the biopsy gun containing the tissue is removed from the participant (time stamp in the ***Kidney Biopsy Procedure Details CRF***).
- Handling the biopsy:
  - Wearing gloves, the individual obtaining the biopsy tissue transfers the tissue core from the needle onto the PBS-soaked chilled sterile telfa pad in the petri dish by washing the biopsy needle with sterile saline. If necessary, use the wooden end of a sterile, cotton-tipped applicator to help remove the biopsy from the needle. Never touch the needle with anything that is not sterile.
  - Add a few drops of the PBS with the sterile dropper if the tissue appears dry. Take an image with the KPMP tablet for documentation as discussed in section 1.4 for the diagnostic core biopsy.
  - Remove excess PBS moisture by briefly transferring the tissue using a sterile wooden applicator from the PBS-saturated telfa pad onto a sterile, dry telfa pad just before transferring it to the cryovial.
  - Using a sterile wooden applicator transfer the renal biopsy specimen to the red cap cryovial (container 6) pre-chilled on wet ice and cap it. Do not leave the wooden applicator in the tube.

- Immediately plunge container 6 into liquid nitrogen. **Record time in the *Biopsy Procedure Details CRF*.** The cryovial can be transported to the laboratory in liquid nitrogen (although make sure it is not all evaporated). Transfer the cryovial into the -80°C freezer within 90 minutes after fixation **(record time in the *Tissue Tracking CRF*)** until shipped to CBR on dry ice. (see appendix C for shipping instructions)

3.5 Shipping the research core(s) to the CBR for storage before shipment to interrogation sites

Upon triaging, the research cores or fragments of tissue will be placed in appropriate vials and transport media per the tissue processing protocol of each TIS specific technology (See Figure 7 for initial processing steps and section 3.2 to 3.4). Each vial or container is pre-labeled by the CBR with the KPMP sample ID. No participant identifiers will be linked to the individual biopsy tissue. The de-identified cores for the tissue interrogation will be shipped first to the CBR (see **Appendix C** for details). Triaging details and metadata will be recorded in the ***Kidney Biopsy Procedure Details CRF*** and uploaded into KPMP REDCap. Shipment details are recorded in SpecTrack and a shipment manifest will be generated. A printed copy of this manifest should be included in the shipping package, in addition, an automated email containing the manifest is generated to alert the Central Biorepository of the incoming tissue.

3.5.1. Materials

A. Provided by CBR

- Frozen Biopsy Shipment Kit
  - KPMP Dry Ice Shipper (For frozen cores and OCT blocks)
    - Insulated Styrofoam/Cardboard hybrid shipping container & KPMP label
    - Gray foam insert
  - UN1845 label w/ CBR address (for dry ice shipments)
  - Exempt human specimen label
- Biohazard and Ziploc bags from the Pathology Kit
- Cryo-Temp (Madge Tech) Data Logger inside Ziploc bag

B. Provided by Recruitment site

- Dry ice (at least 14 lbs. per dry ice shipment)
- Freezer boxes
- Scale
- Packaging tape
- KPMP shipping manifest
- Waybill created on UPS or FedEx website

3.5.2. Overall Procedure

The research cores should be shipped within one week of collection. Cores should only be shipped on Monday, Tuesday, or Wednesday, to limit arrivals at the CBR close to the weekend. Research cores should be shipped using Priority Overnight shipping for next day delivery from FedEx.

Each processing step above lists conditions under which the samples will be stored or shipped. Shipping supplies are provided by the Central Hub. Briefly:

- OCT-embedded frozen blocks for research (core 2) (in Ziploc bags)**,** Cryostor-processed biopsy tissue in cryovials, and flash frozen-liquid nitrogen samples in cryovials are secured in a pre-chilled cardboard freezer box with a pre-chilled, activated temp data logger, before being placed in a Styrofoam shipping box with dry ice. There should be enough dry ice to withstand the expected shipping duration, plus two days more (minimum of 14 pounds). (see appendix C).

The samples to be shipped are photographed at the time of shipping and also upon receipt (for upload into SpecTrack). Take a photo of the samples resting on a thin layer of dry ice at the bottom of the KPMP Dry Ice

Shipper. Fill the shipper with at least 14 pounds of dry ice, then take another photo quickly before putting the top on the Shipper. The image should fill available photo area (i.e. don't take the photo too far away). See example photo below in Figure 19.

Figure 19: Example shipment photo

The details of the samples and data logger being shipped are entered in the SpecTrack by the person preparing the shipment. This includes the **shipping condition** (e.g. on gel pack or dry ice), and a manifest of the materials. An automatic email will alert the CBR of the transfer of tissue. The recipient at the CBR will measure **and record the weight of dry ice** remaining in the frozen material box, and **record whether the FFPE samples are still cool.**

The Central Hub will provide centralized data warehousing and specimen management to ensure that tissue distribution to the qualified TISs as outlined in Figure 7 is compliant with the KPMP protocol and agreement among TIS investigators. A list of available samples at the CBR can be viewed in SpecTrack after logging in with institutional credentials.

3.6 Distribution of the research cores from the KPMP Central Biorepository (CBR) to Tissue Interrogation Sites

Information about the distribution of research cores from the CBR to sites with interrogation technologies can be found in the Tissue Interrogation MOP (TIS001). Briefly, TISs are responsible for submitting tissue requests from the CBR via SpecTrack no later than *9a PT/12p ET the Friday before the week* they wish to receive samples. The requesting TIS is responsible for talking to the other TISs that use the same tissue type to achieve group consensus before submitting the request. The CBR will send TIS samples on *Wednesdays for requests submitted on time*. Late requests will be fulfilled on Wednesday of the following week.

4. Reporting of diagnostic work-up by RS Pathologists

4.1 Purpose

To provide a clinical-pathologic conventional diagnosis following the renal biopsy. An official pathology report will be generated by the Recruitment Sites (RS) pathologists for diagnostic purposes, inclusion in the participant medical record, and disease categorization for KPMP enrollment.

4.2 Standardized language for conventional diagnostic categories

A harmonized list of conventional disease categories was established by the KPMP pathology working group and includes standardized language for clinical pathologic diagnosis. Criteria to determine whether each disease category represents a primary, secondary or tertiary process were also established by the KPMP pathology working group. The KPMP site pathologists will assign the disease categories for each of their cases using the Dx Disease Category Assignment CRF (see Section 8).

The full list of disease categories is available on Basecamp.

4.3 Local processing workflow (default option)

4.3.1. Recruitment site phase

Upon triaging of the tissue (see section 1.3), core 1 of the renal biopsy is accessioned in the RS pathology laboratory reporting system, and processed according to the RS local protocol, modified in compliance with KPMP standardization of processing (see section 2.2). The formalin-fixed and paraffin-embedded sections on glass slides (light microscopy), the OCT-embedded frozen sections (immunofluorescence microscopy), and the toluidine blue thick sections and digital images from the ultrastructural analysis (electron microscopy) will be delivered to the RS pathologist for interpretation, and labeled with RS pathology laboratory accessioning number and participant identifier, as per local protocol. Upon review of the pathology materials and interpretation, a formal pathology report is issued by the RS pathologist and included in the participant medical record as per protocol. The de-identified pathology report is retrieved by the study coordinator and uploaded into the KPMP REDCap System. See **Appendix D** for a checklist to facilitate the hand off of diagnostic core 1 derivatives from pathology to site coordinators. The pathology material is then retrieved, de-identified and transferred for scanning and uploading into the KPMP DPR (see section 6).

4.4 Central review and alternative processing workflow

4.4.1 The KPMP central pathology review

The KPMP central pathologists are provided password-protected access to the KPMP DPR for pathology materials digital review and KPMP REDCap System for review of the pathology report, assignment of the disease categories and classes (see section 6.2).

4.4.3. Central processing of paraffin blocks

In those cases where the histology preparation does not pass the KPMP quality control for imaging (independently of adequacy for diagnostic purposes), the de-identified formalin-fixed, paraffin-embedded tissue block(s) stored at the CBR can be sent to the Central Pathology Laboratory for re-cutting and staining according to the standardized KPMP protocols (See section 2.4). Stained glass slides are then scanned and uploaded into the KPMP DPR.

5. Handling of diagnostic pathology material for shipment to the CBR and DVC

5.1 Purpose

To collect, de-identify, and transfer to the DVC via the CBR (both located at U-M, adjacent to each other), the following pathology material:

- Stained formalin-fixed & paraffin-embedded sections for whole slide image (WSI) scanning
- Digital images of routine immunofluorescence and electron microscopy
- Pathology reports
- Tissue blocks

5.2 Local processing pathway (default option)

Cases are processed in the pathology laboratory at the recruitment site pathology laboratory, according to local protocols. Local protocols have been standardized as much as possible for pre-analytic, analytic and post-analytic steps across all sites. Once the diagnostic work-up is completed, the renal biopsy is interpreted and reported by the recruitment site pathologists, the pathology materials are collected by the recruitment site study coordinators, and de-identified prior transferring to the CBR and DVC as follows:

- The stained glass slides (formalin-fixed-paraffin embedded sections) are shipped to the CBR for transfer to the DVC.
- The tissue blocks are shipped to the CBR.
- The PDF of the report and jpeg images of the immunofluorescence and electron microscopy are uploaded into REDCap.

Shipment details and metadata are recorded in KPMP SpecTrack, the specimen tracking system at the DCC. Uploading of documents are recorded in an automatic email generated to alert the DCC personnel.

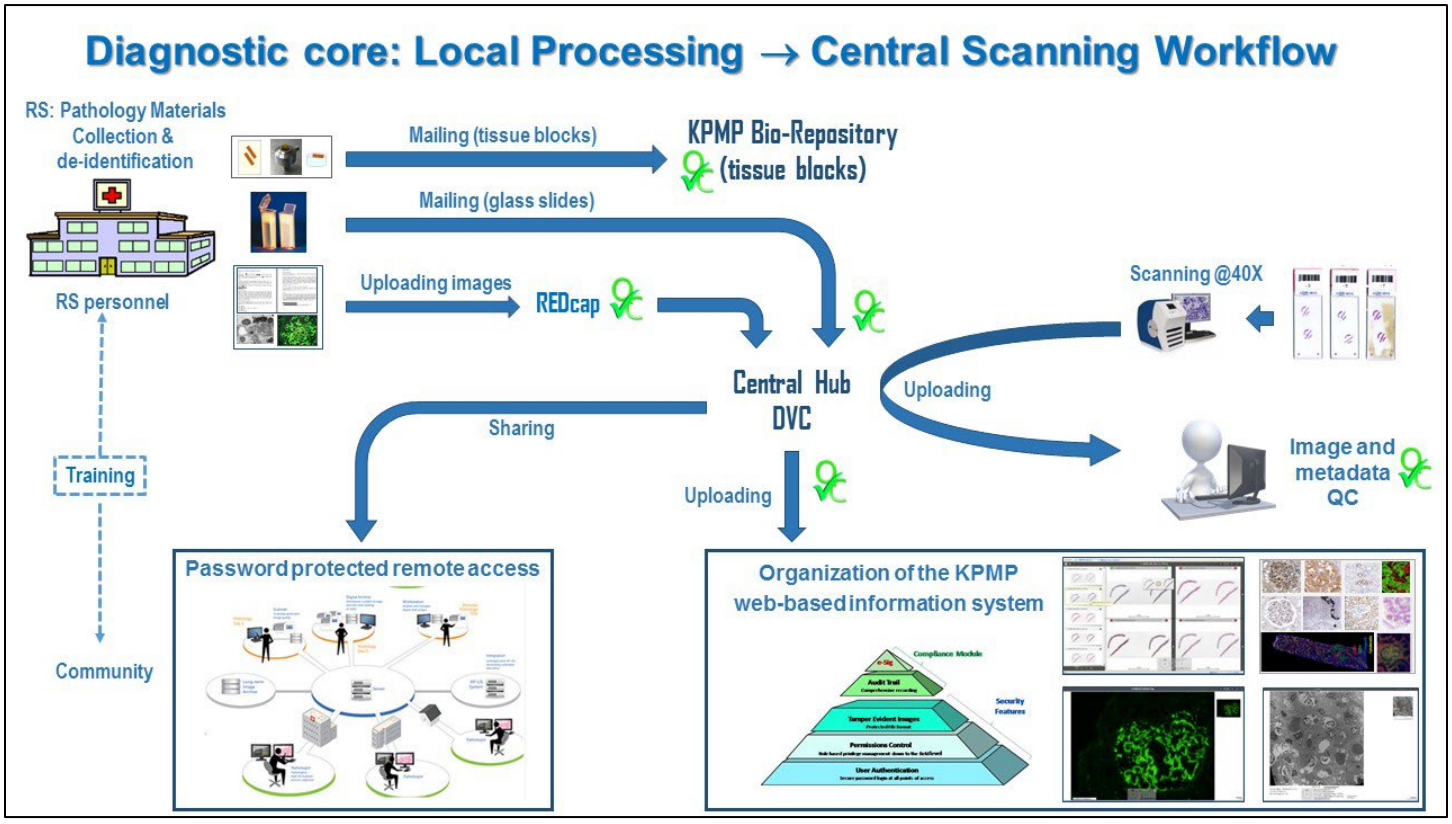


Figure 20. Local processing workflow

5.2.1 Pathology materials

A. Glass slides

- All available light microscopy glass slides processed for Light Microscopy (LM)
- Thick sections of tissue processed for electron microscopy (EM)
- H&E from frozen sections of tissue processed for immunofluorescence (IF)

B. Electron microscopy (EM) material

- Copy of digital EM images (jpeg format preferred)

C. Immunofluorescence (IF) material

- Copy of IF images if available (same CD as EM; jpeg preferred)

D. Copy of the de-identified pathology report PDF

E. Paraffin blocks (Histology)

F. Frozen tissue blocks (IF)

G. Plastic blocks (EM)

5.2.2 Pathology material handling and de-identification

All participant, hospital, and microscopist identifiers must be covered on all glass slides, block containers and deleted from each EM and IF image or EM print, at the recruitment site, prior to shipment to CBR or image upload. A unique KPMP sample ID label must be placed on top of an additional blank label (both labels are supplied with the kit) on each slide and tissue block. The corresponding sample ID should be recorded on each image before scanning or shipping. Participant identifiers should be deleted on all documents and replaced with KPMP Sample ID.

Caution: cropping portions of digital images may be reversible, please ensure that the information is truly deleted. Please see below for instructions on how to permanently remove identifiers from a digital image.

Note: Any biopsy with one or more slides, prints, reports, and images with personal identifiers will be returned to the originating site (including portions that have been de-identified) for corrections.

A. Reports

- If your site has an electronic record of the kidney biopsy report, make a copy of the report saving the new document as <Participant_Initials_PATH_REPORT.doc>.
- Remove all identifying information from the new saved report including:
  - Participant hospital identifiers including hospital address and telephone number
  - Ordering physician's name/initials
  - Pathologist name/initials
  - Other information deemed identifiable (ex. Identifiers in the comments, including mention of the treating nephrologist)
- Information can be de-identified by completely deleting the text or inserting continuous XXX's in place of identifiable details.
- If the diagnostic report is a PDF: Use the Redact tool to select text and/or images to be removed and save the redacted file.
- All pages should be labeled with the KPMP sample ID number and all pages should be labeled as "X of Y", where X is a page counter and Y is the total number of pages in the report.

B. Glass slides

- All glass slides require a KPMP sample ID label on top of a blank label (supplied with the kit) used to cover the original label on the glass slide. Pre-printed labels from the KPMP participant kit include the following data fields that may be pre-printed or will need to be completed:
  - Level indicator: to be completed onsite as it appears in the original glass slide label. This will appear as a whole number and represents the cross-sectional level of the tissue section relative to the full tissue sample (see section 2.2: Glass Slides).
  - Type of Stain: (H&E, PAS, TRI, Silver, etc.) should be added to the label when possible, and must be recorded in the KPMP Specimen Tracking System

(SpecTrack) when derivatives are recorded. If the stain is not known, use the "Unknown" or "Other" option.

- Before the KPMP sample ID label is placed over the existing label, make sure the stain and level information has been indicated on the slide label. Then place a blank label followed by the KPMP sample ID label *over* your local site's participant identifier taking note of the slide "level".
- Be sure that the KPMP sample ID label completely covers any identifiers on the side and is attached to the slide without any overhang.

C. Electron microscopy images

- Upon receiving digital images from the local pathology department, save a copy in a local folder on your work station.
- Open the image in "Paint" or a similar image-adjusting software:
  - Any information that is not pertinent to the image magnification or modality and time of scoping must be removed including: participant name/initials, MRN, DOB, sex (M/F), race, site name/address, pathologist name/initials, microscopist name/initials.
  - Use the Select tool by clicking and dragging a dashed box to select all the text to be deleted.
  - Right click or select 'Edit' from the menu and select 'Cut'. Repeat as needed to remove all identifying information.
  - Relabel the EM images with KPMP Sample ID using the 'Text' tool. Select the tool and click and drag a rectangle in the LOWER RIGHT CORNER of the image.
  - Use Courier, Western, 15 point, Bold font (Text toolbar can be found in the 'View' dropdown menu) to type the Participant Sample ID.
- Do NOT overwrite the original image.
- Save the modified image by selecting File>Save as>. The file name should be updated with the corresponding KPMP sample ID and the number of the image out of the number of total EM images (1of10, 2of10 etc.).
  - Example EM file name: *KPMP Sample ID*_1of4
  - *Note:* Do not make any modifications to the file compression, size, or layers. All images should be saved as *.jpg.

D. Immunofluorescence digital images

- Copy IF images need to be saved as *.jpg files. All identifiers should be masked (generally participant's name and surgical pathology number).
- Each IF image is saved and updated to include the corresponding KPMP sample ID, antibody used (IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, C3, C4, C1q, albumin, kappa, lambda, fibrinogen, PLA2R or others), and the number of the image out of the number of total IF images (1of10, 2of10 etc.).
  - Example IF file name: *KPMP Sample ID*_kappa_2of4

E. Tissue blocks

- Each paraffin, OCT and plastic block should be de-identified and relabeled with a unique KPMP sample ID label (supplied with the kit).

Table 1. Pathology material collected from recruitment site pathology laboratories or at the Central Processing Pathology Laboratory

Pathology material category	Pathology material collected	Transferring mechanism	Repository
Glass slides	All de-identified glass slides from paraffin-embedded tissue processed for LM	Mailing to KPMP CBR	KPMP Digital Pathology Repository

	All de-identified thick sections of tissue processed for EM*	Mailing to KPMP CBR	
	H&E from frozen sections of tissue processed for IF*	Mailing to KPMP CBR	
Digital images	Jpeg images of immunofluorescence*	Uploading into REDcap	
	EM jpeg images*	Uploading into REDcap	
Report	Copy of de-identified pathology report (pdf)	Uploading into REDcap	Central Hub – not visible to public
Tissue blocks	De-identified paraffin blocks (Histology)	Mailing to the CBR	CBR
	De-identified frozen tissue blocks (IF)*	Mailing to the CBR	
	De-identified plastic blocks (EM)*	Mailing to the CBR	

5.2.3 Transfer of pathology materials to the KPMP DVC and CBR (Central Hub)

All de-identified pathology material is shipped to the KPMP Central Hub, where:

- The pathology report uploaded in REDCap will be QC'd for completeness and HIPAA compliance at the DVC.
- The EM and IF images uploaded in REDCap will be QC'd for completeness and HIPAA compliance and then uploaded into the DPR in the appropriate case folder at the DVC.
- The glass slides and EM and IF images will be shipped to the CBR and transferred to the DVC, both at U-M. The glass slides will be QC'ed for completeness and HIPAA compliance, scanned into whole slide images, and uploaded into the digital pathology repository (DPR).
- The tissue blocks (paraffin, OCT, and plastic) will be QC'd for HIPAA compliance at the CBR, where they will be stored until needed for additional analysis (see section F and G).

A. Items provided by KPMP DCC and CBR to RS study coordinators and other relevant personnel

- Training materials
- DPR certification of training
- KPMP sample ID and blank labels
- Access to KPMP REDCap and Specimen Tracking Systems
- For Dry ice shipment (for OCT blocks)
  - KPMP insulated dry ice shipper (for OCT blocks)
  - UN1845 label (dry ice label)
  - Address labels (for UN1845)
  - Exempt human specimen label
  - Madge Tech Data Logger
- For 4°C shipment (for FFPE and plastic blocks)
  - KPMP small insulated 4°C shipper
  - Cold gel packs for shipper
  - Small Ziploc bags (for each FFPE Block) (put with KPMP 4°C shipper)

- For ambient shipment (for glass slides)
    - Glass slide containers

B. Items provided by KPMP Recruitment Site

- ALL pathology materials for enrolled participant
- Thin width sharpie or permanent marking pen for writing on slide labels
- Wide label sharpie (if de-identifying hard copies of path reports)
- Padded envelope for mailing
- Return mailing label with full return address
- Dry ice for OCT, cryostor, and LN shipments (at least 14 lbs. per dry ice shipment)
- Freezer boxes
- Scale
- Packaging tape
- KPMP shipping manifests
- Waybill created on UPS or FedEx website

C. Preparing glass slides for shipment

- Glass slides should be placed in an appropriate slide storage box for shipping (red arrow in Figure 21 indicates best option) and taped closed. Ensure that the slides are placed securely in their positions, i.e. if shaken, they should not move freely. Place a piece of paper or tissue in the box as needed to fill empty space and prevent rattling. Use a small piece of tape to secure the lid shut. Place the box of slides into a packed envelope for shipment to the KPMP CBR (Central Hub).
- All slides and other derivatives generated by the path lab are recorded in SpecTrack and then a shipping/transfer manifest is completed in SpecTrack containing the itemized list of stained glass slides to be transferred to the KPMP CBR and DVC. An automated email is generated to inform the CBR personnel of the upcoming transfer of pathology material.

Figure 21: Preparing the glass slides for Mailing: Glass slides containers come in all sort of size and shape. The best option for glass slide shipment is indicated in the red box (red arrow) in the figure above, although other options are acceptable as well. Please use padded envelope if available to ship pathology material.

D. Mailing of tissue blocks to the KPMP Central Biorepository

- The de-identified paraffin, OCT and plastic blocks are mailed to the KPMP CBR by RS study coordinators. The identifying case numbers on the block will be hidden by affixing a preprinted label, supplied by the CBR as part of the sample collection kit.
- The de-identified OCT block(s) containing the frozen tissue are placed in an appropriately configured freezer box, in contact with dry ice pellets, within an insulated container with dry ice and mailed to the KPMP biorepository. (See appendix C)
- The de-identified FFPE block(s) and de-identified plastic block(s) are placed in KPMP ID labeled Ziploc bags (following de-identification and recording of derivatives in SpecTrack), then in jewelry boxes before being placed in a Styrofoam box cooled by cold gel packs. The cold gel packs prevent melting in the event that the box experiences high external temperatures. (See appendix C)
- The **shipping condition** (e.g. on gel pack, dry ice or ambient) of samples being shipped is entered in the Specimen Tracking System by the person preparing the shipments. A shipping manifest is completed in SpecTrack and contains the itemized list of materials to be transferred to the KPMP CBR. An automated email is generated to inform the DVC and/or CBR personnel of the upcoming transfer of pathology material. Upon receipt at the CBR, the CBR staff will record **the condition of the samples**, including **the weight of dry ice** remaining, and whether the **FFPE blocks are still cool**. CBR staff will record whether any

slides were broken, and other QC parameters as described below. All samples will be examined for HIPAA compliance.

E. Uploading of the pdf of the report and jpeg images of immunofluorescence and electron microscopy into REDCap

- RS study coordinators will login to the KPMP REDCap project to upload the de-identified pdf of the report, the de-identified jpeg images of immunofluorescence and electron microscopy in the Pathology Images Upload CRF.

F. Scanning of the stained glass slides into whole slide images

- The Central Biorepository will scan the stained glass slides received from Recruitment Sites for upload into the Digital Pathology Repository. See Section 6 for more information.

G. Mailing of pathology material back to Recruitment Sites

- Upon completion of the scanning and QC processing, the stained glass slides may be mailed back to the RS pathology laboratory, if required. A return shipping manifest is completed prior to mailing in the KPMP Specimen tracking software, and an automated email is generated to inform the RS of the incoming return of pathology material.

5.3 Central Processing Pathway (Alternative Option)

In the event that the local RS pathology laboratory derived glass slides are not compliant with established QC metrics (see section I), the FFPE block is re-processed in the KPMP Central Processing Laboratory for cutting and staining. Thus, the de-identified formalin-fixed and paraffin-embedded blocks are sent to the KPMP CBR as per protocol; however, before being stored they are redirected to the KPMP Central Processing Pathology Laboratory (CPL) where additional sections are cut and stained for scanning and uploading into the KPMP DPR (see section 2.3). The overall protocol for the Central Processing Pathway is similar to the Local Processing Pathway protocol outlined in Section 5.2, with the exception of the workflow for the paraffin block(s).

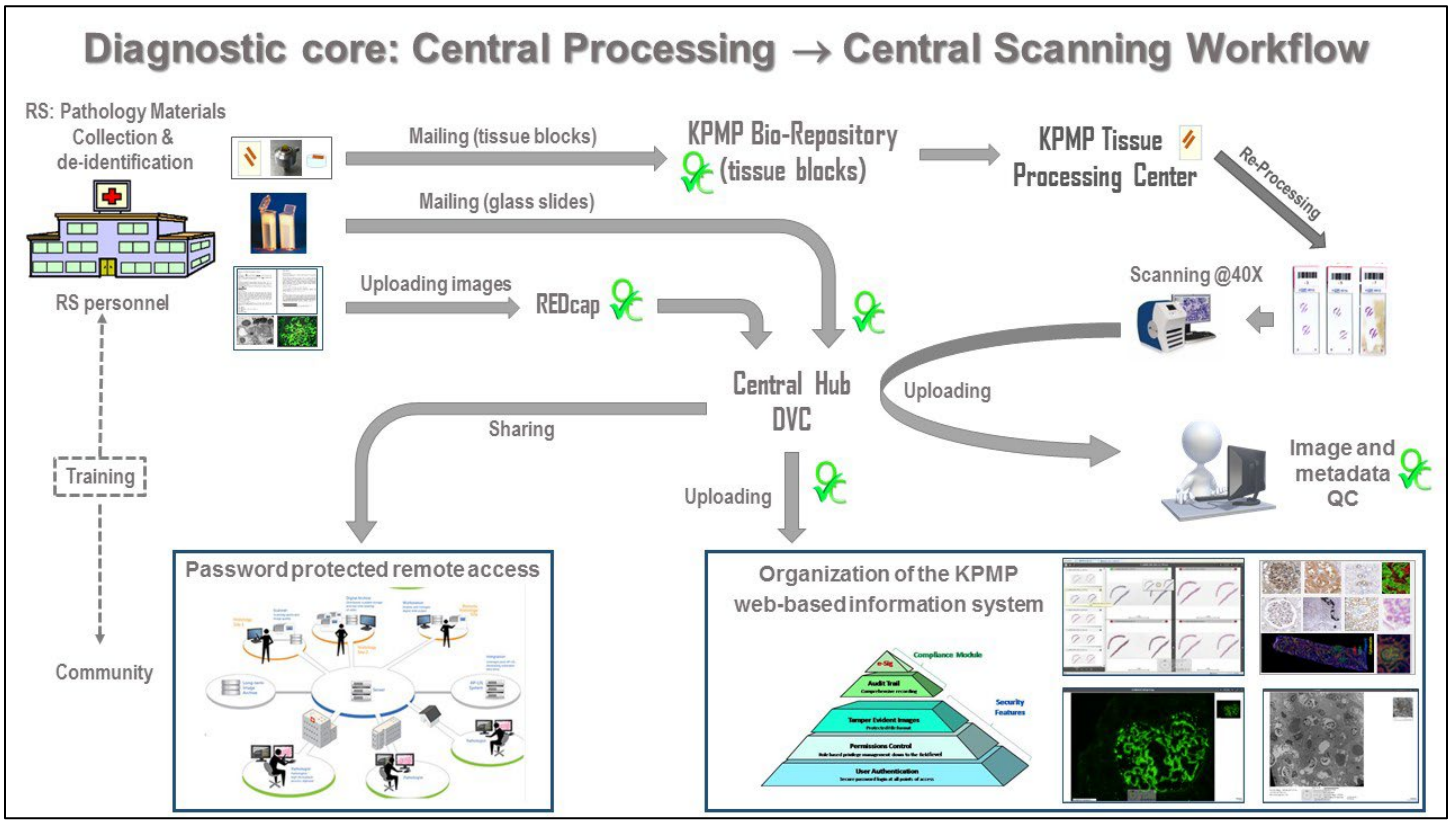


Figure 22. Central processing workflow

5.3.1 Transfer of paraffin blocks to the KPMP Central Processing Pathology Laboratory

The de-identified paraffin block is transferred from the CBR to the DVC, where it is re-processed at the KPMP Central Processing Pathology Laboratory (see section 3.3). Upon reprocessing, the new set of stained glass slides are scanned and uploaded into the KPMP DPR (see section 6), and the paraffin blocks are transferred back to the KPMP CBR.

6. The KPMP Digital Pathology Repository

6.1 Purpose

The purpose of the KPMP Digital Pathology Repository (DPR) is to store and make available to KPMP users digital images of all pathology materials used to make the clinical diagnosis for KPMP participant. The KPMP DPR is part of the DVC and located at the University of Michigan. The imagery included in the DPR is listed below (also described in section 5):

- Whole-slide digital images of glass slide-mounted sections (2-3 microns) of formalin-fixed, paraffin-embedded (FFPE) tissue stained with H&E, PAS, Jones silver, and trichrome.
- Whole-slide digital images of glass slide-mounted sections (~1 micron) of plastic embedded tissue (known as 'thick sections') stained with toluidine blue.
- Whole-slide digital images of glass slide-mounted sections (~5 microns) of OCT embedded frozen tissue stained with H&E.
- Digital images from immunofluorescence tissue (jpeg).
- Digital images from electron microscopic evaluated tissue (jpeg).

6.2 Quality control of diagnostic pathology material received at the CBR and DVC

As described in 5.2.2, all material will be de-identified and listed in the shipping manifest at the RS prior to transfer to the CBR. Upon receipt of all the diagnostic pathology material, the shipping/transfer manifest will be reviewed against the material received. The following quality control measures will be performed:

- QC for shipment/transfer:
  - At the CBR, the material received in the mail will be recorded and checked against the itemized list uploaded in the shipping manifest in SpecTrack, generated at the RS.
- Images:
  - Similarly, at the DVC, the number of individual image ID of immunofluorescence and electron microscopy images and PDF of the reports that are directly uploaded into REDCap will be captured in the REDCap CRF.
- QC for de-identification and relabeling
  - Each sample type (glass slides, digital images, pathology report) will be reviewed for proper de-identification of sensitive participant information (HIPAA compliant) and assignment of correct KPMP case identifier. Errors on slides will be corrected at the CBR, and errors on images and pathology report will be corrected at the DVC. The correct KPMP case identifier added as described in 5.2.2.

Upon completion of the QC of the material received, an email will be automatically generated in SpecTrack as feedback information to the RS.

6.3 Workflow for scanning stained glass slides into whole slide images (WSI)

Stained glass slides will be scanned in University of Michigan Pathology Core to generate whole-slide images. This is done by Aperio GT450 high volume scanners (Leica Biosystems) set to scan at 40x magnification and generate .SVS format files.

#	Step	Owner
1	Slides sent to Central Biorepository (CBR)	RS Pathologist
2	Do a QC file of manifest sample ID to actual sample ID by checking the manifest against the slide (based on barcode only) for every sample shipment we get that is more than 5 samples - (samples are still checked for consistency if 5 samples or less - no QC file is created).	CBR

	Check the stain type on the slide vs stain type on SpecTrack manifest for consistency when received into CBR. Check level type on the slide vs the manifest upon receipt	
3	Make sure that each sample type (kidney biopsy, block, glass slide) is properly de-identified of sensitive participant information (HIPAA-compliant) and is assigned a correct KPMP case identifier. • Errors on slides are corrected at the CBR* (see below for Physical issues that are corrected) . • Please note this is written on our work instructions: (errors on images and pathology report are corrected at the DVC). • See section 5.2.2 of this Pathology MOP for how to correctly add the KPMP case identifier.	CBR
4	Review slides for physical issues*: • Inspect slides for overhanging labels, tape, or excess mounting medium that may affect the fit of the slide in the scanning mechanism and impede operation (Leica Biosystems) • Check for cracks or chips in the slide, particularly at the corners. Any loose glass shards could fall off during scanning and damage the scanning mechanism (Leica Biosystems) • Review for excess mounting medium that can affect scanning CBR notifies RS of any damage or issues with slides upon receipt and records the event in the Issue Tracker. CBR Notifies the DVC before stained slides are sent and includes any issues deemed that could affect slide scanning.	CBR

5	Prepare slides for transfer to DVC for slide scanning:
	• Carefully wipe slides with a soft cloth (microfiber) to remove loose debris, water spots, or fingerprints from upper and lower surface.
	  ○ For difficult stains, dampen cloth (Kim-wipes or similar will do) with water or 70% alcohol solution to clean.
	  ○ If slide label, excess compound media or cover slip is hanging off the edge remove what is possible with a razor blade. There should not be a label on the bottom of the slide, if there is, remove it.
	• Take a picture of all slides for upload to SpecTrack shipment.
	• Ship the slides on SpecTrack to the DVC and print out the manifest.
	• Once the slides have been delivered to Pathology for scanning, complete the shipment by indicating inter-facility in the shipment lower left box in SpecTrack. Also receive the shipment created in SpecTrack under the DVC domain.
	• Create a Scan Slide Request by following the instructions on the website: https://www.pathology.med.umich.edu/digital-pathology/sample-submission-form
	• Place the slides on the white trays provided by the DVC and place a copy of the slide request on top of the slides. If there are issues with either stained or unstained slides (i.e. broken slides, unreadable, etc.) notify and take direction from DCC or UM Pathology Committee.
	• Pick up the slides where they were dropped off once receiving an email from the DPR.
	• Create a shipment from the DVC to the CBR and receive the slides.
	• Create shipment in SpecTrack (see specimen.kpmp.org/help)
	  ○ Update shipment information and Upload pictures.
	  ○ Record weight of package (if applicable)
		CBR

	○ Update date and time of when actually shipped. Use the shipping/tracking manifest created in SpecTrack to record transportation and location changes of the slides, tissue blocks, and biopsy tissue samples between CBR, DVC, and TIS. ○ Save changes ○ Place samples into temporary location in LV	
8	Slides are digitized	UMich Pathology
9	Digitized slides are placed in a pathology directory (path-rslide: Z:\PROD\Images\Hodgin_eSlide Manager\KPMP slides scan). The slides are put into sub-folders based on the date of the request from the CBR. [If you don't have access to this drive, you'll need to work with the scanning facility to get set up. DVC Data Managers need access to this.]	UMich Pathology
10	Spreadsheet that maps digitized images to the specimen ID and a variety of other metadata is generated by the scanner and placed in the associated path-rslide directory sub-folder	UMich Pathology
11	KPMP central pathology team checks regularly for new scans in pathology directory.	KPMP central pathology team
12	KPMP central pathology team opens up each .svs file in a slide image viewer (ie. Aperio ImageScope, QuPath, etc): 1. Cross-check the label against the information in SpecTrack and the tracking spreadsheet. Any discrepancies between slides, what is recorded in SpecTrack, and expectations outlined in the Pathology MOP need to be resolved. Expected samples include: ○ Fixed Slide: Stain: HE ○ Fixed Slide: Stain: PAS ○ Fixed Slide: Stain: Silver ○ Fixed Slide: Stain: Toluidine Blue ○ Fixed Slide: Stain: Trichrome ○ Fixed Slide: OCT: Stain: HE Typically, we expect 2 HE, 2 PAS, 2 Silver, 2 Trichrome, 0-2 OCT Frozen, and at least one 1 Toluidine Blue. Decide what to do if expected slide types are not present. 2. Look for issues with glass slide (dirt, smudges, bubbles under coverslip) that may impair visualization of the tissue core. Some problems, such as tissue folds, cannot be fixed without recutting the paraffin block and restaining, so they will be ignored. If problems exist that do not cover the tissue core, in the surrounding white space only, then it is probably ok to proceed. To fix, slide could be cleaned or re-coverslipped and rescanned. Most of these issues are picked up by QC when slides received at CBR, by Ninive 3. Look for scanning problems: out-of-focus areas of entire core, scanning light/dark 'lines', etc. Can request rescanning	KPMP central pathology team
13	KPMP central pathology team emails DVC Data Managers to indicate which new scans are ready for loading.	KPMP central

		pathology team

6.4 Uploading of whole slide images and digital images into the DPR

After passing quality control steps as outlined above, digital whole slide image files will be uploaded to the DPR. From there they are accessible via the KPMP Whole Slide Image Viewer. Users will need login and password to access them.

7. The KPMP Central Biorepository (CBR)

7.1 Purpose

KPMP is developing a highly complex tissue interrogation protocol to maximally extract information from research renal biopsy tissue donated by our participating participants. Ensuring tissue integrity and seamless distribution of the material from recruitment sites to the tissue interrogation network is of utmost importance for the success of the network. Both central hub and NIDDK leadership agree that a central tissue distribution unit is the most efficient way to serve this critical purpose for KPMP.

The role of the KPMP Central Biorepository (CBR) with respect to pathology specimens is to:

1) Facilitate shipment and tracking of kidney biopsy tissue samples from Recruitment Sites (RS) whenever enrolled participants undergo kidney biopsy
2) Receive, QC, and distribute the renal biopsy tissue segments as required by the Tissue Interrogation Sites
3) Properly store the kidney biopsy tissue to maintain tissue integrity
4) Facilitate shipment and tracking of kidney biopsy tissue samples to and from Tissue Interrogation Sites (TIS) for use by those sites according to their expertise

The KPMP CBR is a central tissue sample storage and distribution location for all kidney biopsy tissue within KPMP. The KPMP biorepository will be located within the University of Michigan Medical School Central Biorepository to take advantage of the significant expertise, processes and infrastructure already in place. The UMMS CBR is accredited by the College of American Pathologists Biorepository Accreditation Program. It is distinct from the KPMP Digital Pathology Repository, also located at the University of Michigan. A detailed description of all biorepository services can be found in the KPMP Biobank Plan. Biobank services and capabilities in support of pathology tissue processing services, storage and distribution to TISs are outlined here. Details may overlap between the Pathology MOP and the Biobank plan.

7.2 Kidney biospecimens sent to the KPMP Central Biorepository (CBR)

The biospecimens that are collected to be sent to the KPMP CBR for storage and/or distribution to the TIS include the kidney biopsy tissue and slides only from:

- The diagnostic core (core #1): upon processing and reporting of the diagnostic core at the RS, the paraffin (histology), frozen (immunofluorescence), and plastic (electron microscopy) blocks are transferred to the KPMP CBR for storage (see section E). In those cases where re-processing (cutting and staining of the paraffin block), the paraffin block will be temporarily transferred to the central tissue processing laboratory at U-M for reprocessing, and subsequently re-transferred to the KPMP CBR for storage (see section E). This movement will be recorded in SpecTrack.
- De-identified stained slides derived from the diagnostic core (core #1), as described in Section E.
- The research core(s) (cores # 2 & 3): as soon as the renal biopsy is performed, the research tissue core(s) is/are placed in the appropriate support media for tissue interrogation and transferred by mail to the KPMP CBR for QC, temporary storage and distribution, according to the protocol.
- Specimen collection kits containing uniquely identified collection and storage prepared by the CBR and sent to the RS in advance of the biopsy. In support of the biopsy collections, CBR will provide RS with consumable materials to stock the biopsy carts and return shipping boxes.

Only individuals trained and certified to ship biological specimens will prepare, package, and ship tissue specimens. Please refer to the Biospecimen Manual of Procedures (OPS003) for blood, urine, and stool processing and shipment.

7.3 KPMP Central Biorepository (CBR) Personnel and Duties

The CBR is comprised of eleven staff, including a Director and Associate Director, several Laboratory Technicians, a QA Manager, a Business Manager, and an Administrative Assistant. The Director is responsible for the overall performance of the CBR as the biobank and logistical hub for KPMP. In addition, one Laboratory Technician will be dedicated to the KPMP as a Tissue Bank Coordinator (see below). While the primary aspects of KPMP support will be accomplished through the Director and TBC, all CBR staff will

support the KPMP in a variety of ways, including lab management, kit production, quality assurance, billing and general administration.

7.3.1 Key personnel and duties

- Director of Central Biorepository
  - The Director is responsible for the administration and operation of the KPMP CBR. The Director's role will include organization and oversight to ensure that acquisition, storage, processing, and disbursement of tissues occurs without loss or waste, that there is immediate and central electronic and physical accessioning of the tissue, and that concept development includes consideration of pathologic/histologic issues and methodology of each TIS. The Director will work in close concert with KPMP pathologists who will provide disciplined pathology review and determine tissue adequacy as necessary.
- Tissue Bank Coordinator
  - This individual is responsible for day-to-day operations of KPMP activities at the CBR, including coordination of supplies to the RSs and overall management of the specimen receipt from RS and distribution to TIS. S/He is also responsible for electronic and physical accessioning of KPMP specimens into freezers, verification of sample integrity and QC, writing and updating appropriate SOPs. This person has extensive experience in biobanking operations and logistics coordination of activities among multiple performance sites, and LIMS operations. Finally, the TBC reports jointly to the CBR Director, and the KPMP Pathologist in the DVC and CPL at University of Michigan.

7.3.2 Information technology support

The CBR utilizes LabVantage LIMS for location and inventory management of barcoded samples stored at the CBR. CBR will use this system and additional software (see below), for management of KPMP samples. CBR will electronically create biospecimen collection kits in LabVantage, where each biospecimen storage container will be given a unique, barcoded sample identifier. Kits will be sent out to RS. After biospecimen collection and shipping back to the CBR, LV will be used to record receipt of each biospecimen, to record storage conditions, and for specimen location management. Each temperature-controlled unit, including ambient, is given a name in LabVantage reflecting the storage condition and unit number (e.g. LN2 #1). Biospecimens stored at CBR are traceable to a precise storage location by storage unit name and number, shelf number within the unit, rack number within the shelf, box number in the rack and position within the box (e.g. A1, A2, A3, etc.). LV access is privileged and role-based; the system "times-out" after 15 minutes to prevent unauthorized access and provide maximum security. All activity in LV is recorded in a detailed audit log that can be accessed only by system administrators. Full database backups are performed on a weekly basis, differential backups are performed on a nightly basis, and log backups are performed on an hourly basis. For the KPMP project, LV will only be used by CBR staff.

While the CBR uses LV for kit creation and specimen location management, the primary specimen database for the KPMP project will be SpecTrack, designed by DCC staff in Seattle. LabVantage and SpecTrack will utilize common specimen identifiers to manage between the systems. KPMP-dedicated staff in the CBR will be trained in the operation of both data systems.

For details regarding the CBR facilities, training, equipment maintenance and monitoring, safety and additional services, see the KPMP biobank plan.

7.4 Shipping, receiving, storage, and distribution at the KPMP Central Biorepository

7.4.1 Transferring of tissue and slides from Recruitment Sites (RS) to the Central Biorepository

A. Records of shipping events

- As many individual shipping manifests as needed will be generated in SpecTrack to record all shipping events, and to discern where each specimen for each participant is located at any given time.
- Glass slides pipeline:

- A shipping/tracking manifest will be created to track the glass slides from diagnostic biopsy cores from RS to the CBR/DVC for scanning into whole slide images (discussed in section E). Movement between CBR and DVC will be recorded in the shipping manifest. SpecTrack should again be used to generate the shipping manifest to ship slides back to RS after scanning at DVC is complete.

- Tissue block pipeline:
  - A shipping/tracking manifest will be created to track the tissue blocks from diagnostic biopsy cores from RS to the KPMP-CBR, from the KPMP-CBR to the KPMP central tissue processing laboratory for re-cutting and staining of the paraffin blocks, and back to the KPMP-CBR (see section E), and for distribution to the TIS when indicated.
  - When central cutting and staining is not indicated, a shipping/tracking manifest will still be created to track the tissue blocks from diagnostic biopsy cores from RS to the KPMP-CBR, within the KPMP-CBR for temporary storage, from the KPMP CBR to TIS.

B. Shipping of diagnostic core tissue blocks and research core(s) from RS to CBR

- As previously described, (see Section 5), upon completion of the tissue processing and reporting, the paraffin, frozen, and plastic blocks from the diagnostic core are de-identified and re-labeled with the KPMP sample ID. The tissue blocks are packaged and mailed to the KPMP CBR. Similarly, after the renal biopsy procedure, the research cores are placed in the appropriate support media and mailed to the KPMP CBR (see section 3.7). A shipping manifest is generated for each of the specimens mailed to the KPMP CBR and shipment tracked by SpecTrack. An automated email is generated to alert the CBR of the transfer. See Section 5.2.3 and Section 3.6 for preparing outbound shipment.

C. Receiving the diagnostic core tissue blocks and research core(s) from RS

- Upon receipt, the paraffin block will be placed at 4°C storage locations and plastic blocks will be moved to room temperature storage. All the other samples will be kept on dry ice until placed in the appropriate freezer, except during required handling. Upon receipt of the diagnostic core tissue blocks and the research core(s), specimens will be cross-checked with Spec-Track manifest, and electronically recorded as received. A QC check will be performed, including inspection for appropriate shipping conditions, maintenance of samples at the appropriate temperature, etc. The specimens will then be permanently or temporarily stored according to specimen type and protocol. Specific storage locations will be assigned by LabVantage.

D. Receiving the diagnostic slides from RS

- Slides received from the RS will be cross-checked with the SpecTrack manifest and electronically recorded as received. The CBR tissue bank coordinator will place the slides in temporary room temperature storage locations, assigned by LabVantage, and held there until transfer to the DVC for scanning. When the slides are ready to be transferred to the DVC, the slides will be checked out of LabVantage. The movement between departments will be tracked in SpecTrack.

7.4.2 Specimen types and storage conditions (See Appendix B)

The LabVantage system will be used to record the modality and location of storage for each specimen as follows. Each specimen will have been labeled with the KPMP identifier, and a unique specimen identifier, which will be used by the system to track locations. The storage unit number (which identifies the unit as -80°C freezer, LN2 tank, 4°C, RT), shelf number, rack number, box number, and position within the storage box for each sample is assigned by LV. Any movement within the CBR, and upon exit from the CBR is recorded in LV, in addition to SpecTrack.

A. Paraffin blocks (diagnostic core #1)

- Paraffin blocks will be stored individually in small Ziploc bags at 4°C in standard 2” cardboard boxes placed in a desiccated space in steel racks in the refrigerator. Upon receipt, the paraffin blocks will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. Approximately 5% of the total holdings will be visually inspected weekly for moisture accumulation, discoloration of the paraffin, or growth of any type in the bags.

Refrigerator temperature will be continuously monitored via the TempTrak system. The storage location and metadata will be recorded using LabVantage.

B. OCT embedded frozen tissue blocks (diagnostic core #1)

- OCT-embedded frozen tissue blocks will be stored individually in small Ziploc bags in standard 2" cardboard freezer boxes placed in steel racks within the -80°C freezer. Upon receipt, the frozen tissue blocks will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. The Cryofreezer temperature will be continuously monitored with the TempTrak system. The storage location and metadata will be recorded in LabVantage.

C. Plastic blocks (diagnostic core #1)

- Plastic blocks will be stored in foam rubber blocks within a multi-drawer unit at room temperature (18-24°C). Upon receipt, the plastic blocks will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. Room temperature will be continuously monitored with the TempTrak system. The storage location and metadata will be recorded in LabVantage.

D. OCT embedded frozen tissue blocks (research core #2)

- OCT-embedded frozen tissue blocks will be stored individually in small Ziploc bags in standard 2" cardboard freezer boxes placed in steel racks within the -80°C freezer. Upon receipt, the frozen tissue blocks will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. The Cryofreezer temperature will be continuously monitored with the TempTrak system. The storage location and metadata will be recorded in LabVantage.

E. Snap frozen cores (research core #3)

- Snap frozen tissue in cryovials will be stored in standard 2" cardboard boxes placed in steel racks within the -80°C freezer. Upon receipt, the snap frozen tissue will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. Cryofreezer temperature will be continuously monitored with our TempTrak system. The storage location and metadata will be recorded in LabVantage.

F. Cryostor preserved tissue (research core #3)

- CryoStor CS10 preserved tissue in cryovials will be stored in LN_2 vapor (below -150°C) in standard 2" cardboard boxes placed in steel racks within the cryofreezer. Upon receipt, the tissue in cryovials will be accessioned into the LabVantage LIMS by scanning the specimen barcode and a location assigned. Cryofreezer temperature will be continuously monitored with our TempTrak system. The storage location and metadata will be recorded in LabVantage.

7.4.3 Distribution of the research cores from the KPMP Central Biorepository to Tissue Interrogation Sites (TIS)

Information about the distribution of research cores from the CBR to sites with interrogation technologies can be found in the Tissue Interrogation MOP.

Upon receipt of a tissue request from a TIS, the CBR will retrieve the samples from the appropriate storage location and prepare them for shipment to the requesting site. Samples will be recorded as checked out and shipped in LV. In addition, the tissue bank coordinator will record the time and modality of shipment from the CBR to the TIS in SpecTrack.

The Tissue Bank Coordinator will create a pull list of the samples in Lab Vantage once a TIS site has put a request in SpecTrack. The OCT blocks will be placed in dry ice coolers after retrieval from the freezer during shipment preparation. LV will be updated to show that the specimens have been removed from their storage location for shipment to the TIS. The tissue bank coordinator will also create a shipment manifest in SpecTrack, including the sample identifiers. The de-identified research cores are mailed to the KPMP TIS by the CBR tissue bank coordinator.

The samples to be shipped are photographed in the shipping box at the time of shipping and also upon receipt. The details of the samples being shipped are entered in the Specimen Tracking System by the CBR tissue

bank coordinator. This includes the **shipping condition** (e.g. dry ice), and a manifest of the materials. An automatic email will alert the TIS of the transfer of tissue**.**

7.5 Quality Control

The KPMP-CBR and DCC will organize training sessions and provide materials to train all personnel how to process and handle specimens for shipping and receiving, and to preserve them in the best possible method as listed in each TIS Manual of Procedures. KPMP CBR will perform quality checks on all specimens received at CBR from RS to ensure adequate documentation regarding the fitness for use of the materials in their proposed downstream applications.

In addition, KPMP will protect participant privacy throughout the course of the project, and perform quality control checks to ensure that direct participant identifiers are not accidentally shared across the network.

7.5.1 Quality Control of biospecimens received at the KPMP CBR

The KPMP CBR personnel will inspect all pathology materials received from the RS, and will use SpecTrack to record any physical discrepancies observed in shipping or sample conditions upon receipt. The CBR uploads an image of received OCT blocks in SpecTrack. Discrepancies include:

- Damaged shipping boxes
- Shipping conditions inappropriate for the sample type
- Shipment arrival at the incorrect temperature
- Too little dry ice, gel pack, etc.
- Sample labels missing/incorrect
- Damaged internal boxes
- Cracked vials
- Cracked OCT blocks
- Size of core in OCT block is as expected
- Melted FFPE blocks
- Samples thawed

7.5.2 Quality control for HIPAA compliance of the diagnostic pathology material (tissue blocks) and research cores for the KPMP CBR

The KPMP CBR personnel will inspect all pathology materials received from the RS to ensure that all participant identifiers are masked and labeled with a KPMP participant specific sample ID. KPMP core pathologists at the DVC will apply a quality control protocol to assure:

- Compliance with HIPAA regulations
- Compliance with study protocol
- Completeness of the material received
- Good preservation of the tissue in the appropriate media

While many of these aspects are discussed elsewhere, here the KPMP CBR personnel will specifically ensure that the vials, slides or tissue blocks received at U-M do not have identifiers such as name, date of birth, social security number, medical record number, surgical pathology accession number readily visible, although they may be masked. Scrutiny of information in the Specimen Tracking System will also be done as above and breaches identified. If identifiable information is found to have been shared with individuals that are not authorized, the incident will be reported to the RS site PI, primary site from where the participant was recruited and to the sIRB. The notification process to individual (s) affected by breach of confidentiality will be performed per the guidelines set by the sIRB depending on the extent of breach and individuals affected. The individuals who are found to have accidently violated HIPAA will be asked to retake HIPAA training and repeated violations would be grounds for revoking access to KPMP activities for the violator until significant amends have been in place and these will be discussed in participation with the PI and the sIRB. While the CBR will have primary responsibility for verifying that there is no identifiable information on any specimen, the entire KPMP has a responsibility to report notice of any breach of privacy.

Additional Note: It is recommended that sites do not exchange participant private information through electronic media such as email and instead use role-based privileges on secure central database being established by the DVC. In cases where it is unavoidable to share information through email or cloud-based applications, this should be only done using HIPAA certified servers. The devices with access to these applications should be encrypted and registered with the institution. Any disclosure to individuals that do not have access to HIPAA information will be reported as discussed above.

8. Kidney Tissue Atlas Library

8.1 Purpose

To establish a consistent and standardized approach to profile normal and abnormal kidney structures. The individual libraries will collect a variety of information from diagnoses used in routine clinical practice, morphologic observational data, and assembly of algorithms used to identify individual cellular and extracellular structures. The data contained in the individual libraries will be used to map the omics-data generated by the tissue interrogation sites into individual kidney structures to create the kidney tissue atlas.

8.2 Conventional Disease Categories Library

Multiple classes of diseases are identified during routine renal biopsy interpretation and indicate various degrees of contribution to the clinical presentation. The KPMP pathology working group defined criteria for primary, secondary and tertiary prioritization of conventional diagnostic categories.

A list of conventional diagnostic categories was established by the KPMP pathology working group and includes standardized language for clinical pathologic diagnosis. The conventional diagnostic categories refer to broad disease classes (i.e. diabetic nephropathy, IgA nephropathy, FSGS, etc.), and do not include quantitative metrics, staging or scoring.

The library will be used to categorize individual participant kidney tissue using conventional disease classes and terminology.

8.2.1 Classes of Conventional Diagnostic Categories

- Primary diagnoses are defined as the disease categories most responsible for the majority of the histologic changes and clinical presentation.
- Secondary diagnoses are defined as the disease categories either superimposed on the primary diagnosis or underlying condition that contributes to the current clinical presentation.
- Tertiary diagnoses are defined as the disease categories either superimposed on the primary diagnosis or underlying condition that are considered incidental findings or minor contributors to the current clinical presentation.

8.2.2 Assignment of conventional diagnostic categories workflow

The local RS pathologist will complete the ***Dx Core Disease Category Assignment CRF*** directly in REDCap, ideally within two weeks of biopsy. The site research coordinator will send an email link to the CRF a day after the kidney biopsy occurs and the site pathologist will receive weekly reminders until the form is complete. This CRF captures *major* patterns and we don't expect more than 2 instances for each compartment: glomerular, tubulointerstitial, vascular. Each presenting pathology requires a new instance of the form and the pathologist has the option to select whether the observed disease pattern is primary, secondary, tertiary, or other.

8.3 Descriptors Library

The KPMP pathology working group is in the process of generating a list of standardized morphologic features that can be detected visually (observational data). Current ongoing efforts for the descriptor library are focused on tubulointerstitial features. Future efforts will include the addition of glomerular and vascular features. The library will be used to morphologically profile participant kidney tissue using visually detected observational data.

8.3.1 Tubulointerstitium descriptor scoring pilot protocol

The KPMP pathologists will access the KPMP DPR to view and score the digital whole slide images. Two pathologists will score each case.

1. Login to the Digital Pathology Repository (dpr.kpmp.org)
2. Select the KPMP ID number for the biopsy to be scored from the drop-down menu and select 'View Slides'
3. Select the menu icon in the upper left corner and navigate to the image labeled Sample ID_PAS_2of2

4. Turn the grid lines on and off by selecting the toggle in the menu
    - *Note: The grid squares are 500 X 500 microns*
5. Open the tubulointerstitium scoring spreadsheet specific to the case to be scored
    - *Note: Each case should have its own excel file/scoring sheet. The grid coordinates for each patch to be scored will be populated in the 'PATCHES' row of the spreadsheet. The default is to score the left section on the slide unless a visual inspection suggests the right section is superior in quality*
6. Specify the compartment ('C' – cortex; 'M' – medulla'; 'C/M' – cortical medullary junction) for each grid square
    - *Note: The default is 'C' – cortex*
7. For each grid square specify whether each descriptor listed is present ('1') or absent ('0')
8. Once scoring is complete for a case save the file as 'KPMP_[Sample ID]_Tub-Int Scoring_[Initials]_[Date]' and upload to Basecamp.

9. Appendices

Section 9 of this MOP includes appendices

Appendix A. Pathology Case Report Forms

- Kidney Biopsy Procedure Details CRF
- Pathology Images Upload CRF
- Pathology Immunofluorescence Metadata CRF
- Dx Core Disease Category Assignment CRF

Appendix B. KPMP Biorepository of Tissue Blocks

Table 2. Shipping conditions for renal biopsy materials to or from the CBR.
**Data loggers should be used in all dry ice shipments.*

Sample Type	Preparation detail/support medium/storage container	Internal shipping container	Shipping Condition	External Shipping Container
FFPE	Histology cassette	2-in cardboard jewelry box	Gel pack	Styrofoam
Plastic blocks	N/A	2-in cardboard jewelry box	Gel pack	Styrofoam
OCT blocks	OCT mold and Ziploc	2-in cardboard freezer box	Dry ice*	Styrofoam
CryoStor-core	2-ml cryovial	2-in cardboard freezer box	Dry ice*	Styrofoam
Flash-frozen core	2-ml cryovial	2-in cardboard freezer box	Dry ice*	Styrofoam
Glass slides	N/A	Slide shipping container	Ambient	Padded envelope

Table 3. Storage conditions for renal biopsy material at the CBR.

Type of sample	Preservation, support medium, or stain	Number Per Participant	Additional container	Storage Temperature
Diagnostic Block	FFPE	1	Ziploc	+4°C plus dessicant
Diagnostic Block	OCT	1	Ziploc	-80°C
Diagnostic Block	Plastic	1	Foam rubber	RT
Research Core #2	OCT	1	Ziploc	-80°C
Research Core #3 A	CryoStor	1	2 ml cryovial	LN2 vapor
Research Core #3 B	N/A	1	2 ml cryovial	-80°C
Stained slides	Hematoxylin & Eosin (H&E),	2	Slide catalog	RT
	Periodic Acid Schiff (PAS),	2	Slide catalog	RT
	Masson's Trichrome (TRI)	2	Slide catalog	RT
	Jones Methenamine Silver (SIL)	2	Slide catalog	RT
Other slides as needed	TBD	TBD	Slide catalog	+4°C plus dessicant

Appendix C. Shipping procedure for biopsy materials shipped on dry ice and at 4°C

C.1 Shipping biopsy materials on dry ice

Materials

Provided by *CBR:*

- KPMP Dry Ice Shipper Frozen Biopsy Shipment Kit
    - KPMP Dry Ice Shipper (For frozen cores and OCT blocks)
        - Insulated Styrofoam/Cardboard hybrid shipping container & KPMP label
        - Gray foam insert
    - UN1845 label w/ CBR address (for dry ice shipments)
    - Exempt human specimen label
- Biohazard and Ziploc bags from the Pathology Kit
- Cryo-Temp (Madge Tech) Data Logger inside Ziploc bag

Provided by *Shipping Site*

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

Procedure

1. Gather shipping materials listed
2. Place UN1845 and exempt human biospecimen 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.
4. Add a small amount of dry ice pellets to the bottom of a small freezer box (roughly a single layer of pellets)
5. Remove selected biopsy materials from the appropriate freezer and place them in a pre-chilled (at-80°C or on dry ice) Ziploc bag.
    a. Note: All frozen tissue samples (OCT molds, Cryostor, LN) can be in the same larger Ziploc. Make sure that the OCT molds are in their own, smaller Ziploc bag (should already be done, immediately following freezing in the biopsy suite) before it goes in the larger bag. *See example photo below.*

6. Seal the outer Ziploc bag and place it in the pre-chilled freezer box. Place the pre-chilled, activated temp data logger in the same pre-chilled freezer box that contains the biopsy tissue. Close the freezer box.
7. Place a thin layer of dry ice pellets in the insulated shipper. Place freezer box on top of the dry ice at the bottom of the insulated shipper. Take a photo of the specimens in the bottom of the box for upload to SpecTrack (*see example image below).*

8. Fill the KPMP Dry Ice Shipper with dry ice approximately 3 inches from the top. Leave enough space for the gray foam to be inserted while ensuring samples are fully covered with dry ice.
9. Weigh the shipment and record the total weight and the dry ice weight. On average the *total* weight of the box should be around 25-30 lbs with a dry ice weight of 15-20 lbs.
10. Place completed KPMP Manifest in the KPMP Dry Ice Shipper (on top of the gray foam insert).
11. 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.
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.
    a. Be sure to return to SpecTrack and record the shipment tracking number and package weight. It is imperative to weigh the box for dry ice shipments: this is a key quality control check!
14. Bring package to your UPS/FedEx pickup location.
15. Check inbox to ensure SpecTrack generated a shipping notification with copy to KPMP-BioRep@umich.edu

C.2 Using Data Loggers for dry ice shipments

Materials

Provided by *CBR:*

- Madge Tech Data Logger
- Two magnets

Helpful troubleshooting resources

- Product User Guide: https://www.madgetech.com/wp-content/uploads/2019/08/CryoTemp-pug.pdf
- Training Materials: https://3.basecamp.com/3770084/buckets/3935715/vaults/2752800547

Best practices for using Data Loggers:

- Data loggers should be used in ***all*** dry ice shipments.

- Data loggers are kept inside Ziploc bags and stored at room temperature until ready to pre-chill before shipment.
- Data loggers must be 'activated' prior to use in shipments using the magnets provided by CBR.
- The Ziploc baggy is meant to protect the data logger from excessive water exposure. Should the bag tear, please notify the CBR to receive a replacement bag.
- The data logger and Ziploc bag should be wiped dry prior to placing it with the samples.
- Data loggers will require yearly calibration. The CBR will contact the TISs to collect the loggers for calibration.

C.2.1 Temperature Data Logger activation and shipment with samples

1. 1-2 hours before shipments the data logger should be pre-chilled in the Ziploc bag at -80°C.
    - *Note: The data logger should not be kept in the freezer for longer than a couple hours as it will affect the battery life over time.*
2. Retrieve the pre-chilled data logger in the Ziploc bag from the -80°C.
3. Place the provided magnet in front of the 'START MARK' circle on the data logger and wait for the following light sequence:
    - OK light blinks green multiple times
    - Green, yellow, red lights blink quickly in succession
4. The data logger is now ready to record. You should see a green light that will blink intermittently signaling that the logger is recording.
    - *Note: If the green light is not blinking intermittently, the logger has not started recording. Try to activate it once more with the magnet and if that doesn't not work, contact the CBR.*
5. Place the activated data logger in the pre-chilled 5x5x2" freezer box that contains the biopsy tissue.
    - *Note: Do not ship the magnets. Store them locally.*
6. Take a picture for upload in SpecTrack.
7. Each data logger has a unique ID affixed to the front of the device. Data loggers are treated as sample inventory. When selecting shipments contents in SpecTrack be sure to select the logger ID in addition to the IDs of the samples included in the shipment.
8. Enter the data logger ID once more in the "Temperature Logger ID" field.

C.2.2 Return of inactive Data Loggers to the CBR

1. Data loggers no longer in use should be returned to the CBR via ground shipping.
2. Fill out the data logger shipment information on SpecTrack to ship to the CBR.
3. Remember to print the manifest and include it with the shipment.

C.3 Shipping biopsy materials (FFPE blocks) at 4°C

Materials

Provided by *CBR:*

- KPMP Small 4º C Insulated Shipper
    - Cardboard outer box
    - Styrofoam cooler inner box
- Two Gel Packs (**chilled to +4°C prior to shipping, i.e. in the fridge NOT the freezer!**)
- Small Ziploc bags (for each FFPE and plastic Block), labeled with the KPMP sample ID (from the Pathology Kit)
- Exempt human specimen label
- Jewelry boxes

Provided by *Shipping Site*

- Packaging tape
- Scale
- Rubber band
- Waybill created on UPS or FedEx website

Procedure

1. Fill out a KPMP Shipping Manifest in SpecTrack indicating samples to be included in shipment.
2. Gather shipping materials listed above. **Make sure the gel packs are chilled (in fridge, NOT freezer).**
3. Obtain the blocks. Ensure the FFPE and plastic blocks have already been de-identified and re-labeled with the KPMP sample ID! The blocks should already be in labeled Ziploc bags.
4. Place exempt human biospecimen labels on of KPMP 4˚C insulated shipper.
5. Place FFPE and/or plastic block bags in jewelry box.
6. Close the jewelry box and secure it with a rubber band.
7. Place a cold gel pack on the bottom of the shipper, followed by the box of FFPE, followed by another cold gel pack on top, so the FFPE box is “sandwiched” between them.
8. Place the top back on the Styrofoam cooler.
9. Place the completed KPMP Manifest on top of the Styrofoam inner box shipper (outside the Styrofoam inner box, but inside the outer cardboard box.)
10. Seal the outer cardboard box with the packing tape and weigh the package. The package weight for these shipments is for the courier only, not for KPMP purposes, so an estimate is OK (but dry ice shipments must be weighed for KPMP).
11. Create UPS/FedEx waybill and affix to the package.
12. Bring package to UPS/FedEx pickup location.
13. Be sure to return to SpecTrack and record the shipment tracking number.
14. Email KPMP-BioRep@umich.edu to confirm you have dropped off the package.

Appendix D. Pathology Checklist: Hand-off of KPMP Diagnostic Core 1 Derivatives from local site pathology to site coordinator

Biopsy Date:

Participant ID:

Biopsy Kit ID:

Section 1. Did the biopsy result in procurement of formalin fixed tissue for light microscopy?

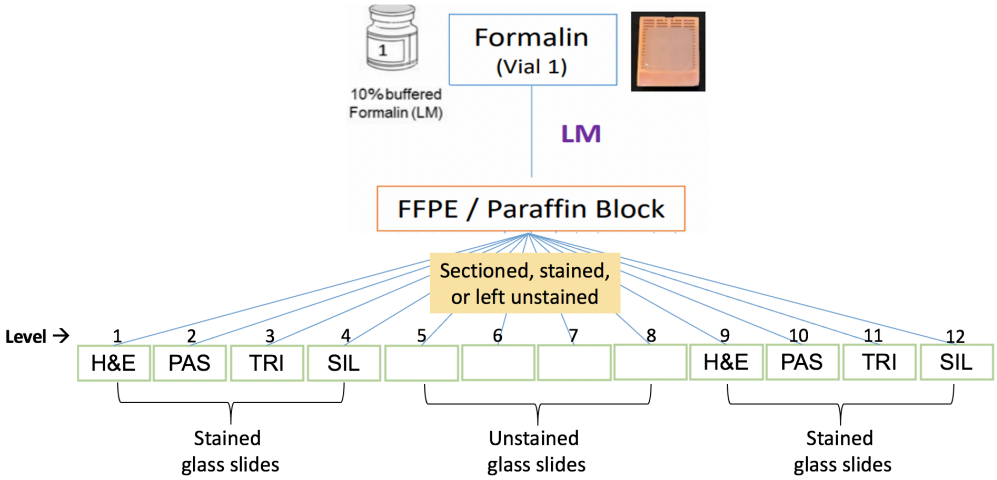
- ☐ No. Go to Section 2.
- ☐ Yes. If Yes, complete this workflow:

Reference: Pathology MOP section "Light microscopy standardized protocol".

For KPMP, the paraffin blocks are sectioned at 2-3 microns and are processed to obtain 12 slides, in this order and staining:

1. H&E (also known as Hematoxylin & Eosin)
2. PAS (also known as periodic acid Schiff)
3. TRI (also known as Masson's Trichrome)
4. SIL (also known as Jones Methenamine Silver)
5. Unstained (blank)
6. Unstained (blank)
7. Unstained (blank)
8. Unstained (blank)
9. H&E (also known as Hematoxylin & Eosin)
10. PAS (also known as Periodic acid-Schiff)
11. TRI (also known as Masson's Trichrome)
12. SIL (also known as Jones Methenamine Silver)

The first set of four slides are sectioned at 2-3 microns each after initial facing of the block. Two sections per slide. A step section consisting of 4 blank 2-3 micron sections is then performed and the blanks saved (one section per slide). This is followed by an additional set of four sections at 2-3 microns each, 2 sections per slides, for a total of 12 slides (8 stained and 4 unstained). All sectioning and staining steps are performed using local RS laboratory protocols. Slides are reviewed by the local RS pathologist and a standard pathology report is generated (see section D). The local RS pathologist may order additional sections and/or stains for diagnostic purposes per his or her professional judgement. If additional testing is desired, the RS can use one of the four unstained slides that were already prepared (please make a note that this occurred). When necessary, additional special stains or immunohistochemistry, will be performed to reach a conclusive diagnosis.



<u>Hand-off workflow:</u> Did we obtain the 8 stained glass slides and 4 unstained glass slides?

- **If Yes, hand-off slides for de-identification, KPMP re-labeling, logging in SpecTrack, and shipment to CBR.**
  - Make sure all patient identifiers are removed or hidden (MRN, DOB, participant name, hospital name, pathologist name, accession numbers, local barcodes, etc.).
  - If you are placing the KPMP label over top of the existing label, make sure to add a blank (empty) label between the two to lower the chance that text will show through the old label when scanned.
  - SpecTrack is set up to autopopulate with standard specimen hierarchy, based on the MOP. Verify that the sample type listed in SpecTrack aligns with the slide label.
    If changes or mistakes occur in the pathology lab, be sure to update SpecTrack to match.
    - Example: A common example is that the pathologist needs to make another slide for LM and uses one of the middle 4 unstained slides from the KPMP set. If this happens, update “sample type” in ST to match. SpecTrack is programmed to “expect” that Sample ID to be unstained, based on its Level. You need to update it and write in stain info
- **If No, hand-off the slides you DO have and de-identify, re-label, log, and ship as described above. Identify the slides you do NOT have and explain why these are missing.**
  - The coordinator will need to add this info to SpecTrack to explain the incomplete derivatives
  - Reasons expected derivates are missing might include: insufficient sample to create slides, sample damage, etc.

Walk through slides checklist below for guidance:

- **How many FFPE (formalin-fixed, paraffin-embedded) Blocks were created?** If more than 1, make sure to note which slides were cut from which parent block so hierarchy can be logged in SpecTrack.

1. H&E (also known as Hematoxylin & Eosin)
   - ☐ Procured.
     - Match to KPMP label KPMP_HE_L1
   - ☐ Not procured.
     - Reason:
2. PAS (also known as periodic acid Schiff)

- ☐ Procured.
  - Match to KPMP label KPMP_PAS_L2
- ☐ Not procured.
  - Reason:

3. TRI (also known as Masson's Trichrome)
   - ☐ Procured.
     - Match to KPMP label KPMP_TRI_L3
   - ☐ Not procured.
     - Reason:
4. SIL (also known as Jones Methenamine Silver)
   - ☐ Procured.
     - Match to KPMP label KPMP_SIL_L4
   - ☐ Not procured.
     - Reason:
5. Unstained (blank)
   - ☐ Procured.
     - Match to KPMP label KPMP_L5
     - *Note: if necessary for diagnostic purposes, the RS can use ONE of the four unstained slides for additional testing. Identify which level was used and write the stain used on the slide label.*
   - ☐ Not procured.
     - Reason:
6. Unstained (blank)
   - ☐ Procured.
     - Match to KPMP label KPMP_L6
     - *Note: if necessary for diagnostic purposes, the RS can use ONE of the four unstained slides for additional testing. Identify which level was used and write the stain used on the slide label.*
   - ☐ Not procured.
     - Reason:
7. Unstained (blank)
   - ☐ Procured.
     - Match to KPMP label KPMP_L7
     - *Note: if necessary for diagnostic purposes, the RS can use ONE of the four unstained slides for additional testing. Identify which level was used and write the stain used on the slide label.*
   - ☐ Not procured.
     - Reason:
8. Unstained (blank)
   - ☐ Procured.
     - Match to KPMP label KPMP_L8
     - *Note: if necessary for diagnostic purposes, the RS can use ONE of the four unstained slides for additional testing. Identify which level was used and write the stain used on the slide label.*
   - ☐ Not procured.
     - Reason:
9. H&E (also known as Hematoxylin & Eosin)
   - ☐ Procured.
     - Match to KPMP label KPMP_HE_L9
   - ☐ Not procured.
     - Reason:
10. PAS (also known as periodic acid Schiff)
    - ☐ Procured.

- Match to KPMP label KPMP_PAS_L10

☐ Not procured.
- Reason:

11. TRI (also known as Masson's Trichrome)
☐ Procured.
- Match to KPMP label KPMP_TRI_L11

☐ Not procured.
- Reason:

12. SIL (also known as Jones Methenamine Silver)
☐ Procured.
- Match to KPMP label KPMP_SIL_L12

☐ Not procured.
- Reason:

13. Were any additional slides created?
☐ No
☐ Yes. If so, use the kit labels KPMP_L13-20. Write the level and stain type used on the slide.
- 13. Stain:
- 14. Stain:
- 15. Stain:
- 16. Stain:
- 17. Stain:
- 18. Stain:
- 19. Stain:
- 20. Stain:

☐ Do we have any leftover FFPE/paraffin block?
- Yes. Hand-off block for de-identification, KPMP re-labeling, logging in SpecTrack, and shipment to CBR
  - Match to KPMP label KPMP_FFPE_Block
  - Label should go on cassette lip
  - *Note: there are three KPMP_FFPE_Block labels included per biopsy kit. You may have only one block or multiple blocks, depending on pathology lab handling.*
- No. If No, why not:

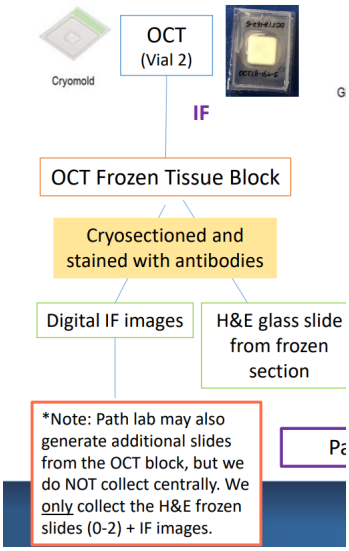
Section 2. Did the biopsy result in procurement of diagnostic OCT?

☐ No. Go to Section 3.
☐ Yes. If Yes, complete this workflow:

Reference: Pathology MOP section "Immunofluorescence standardized protocol".

For KPMP, The OCT frozen section tissue block is cryosectioned for staining with fluorescein conjugated anti-IgG, IgA, IgM, C3, C1q, fibrin, albumin, kappa light chain, and lambda light chain antibodies using local RS pathology laboratory protocols. Immunofluorescence stained slides are examined by the local RS pathologist. Using standard clinical pathology interpretation, each stain is scored on an intensity scale of 0-3+ and the location (glomerular, tubular, vascular, interstitial) and pattern (granular, linear) of positive staining is recorded in the local RS pathologist report for routine diagnosis. When necessary, additional immunofluorescence stains will be performed to reach a

conclusive diagnosis. No more than 0-2 H&E stained section should be obtained from the OCT block. Do not collect any unstained slides.



<u>Hand-off workflow:</u> Did we obtain the H&E stained glass slides from the frozen section?

- **If Yes, hand-off 0-2 slides for de-identification, KPMP re-labeling, logging in SpecTrack, and shipment to CBR. Additional H&E stained frozen OCT slides beyond 2 are not needed. If they exist, keep them.**
  - Make sure all patient identifiers are removed or hidden (MRN, DOB, participant name, hospital name, pathologist name, accession numbers, local barcodes, etc.).
  - If you are placing the KPMP label over top of the existing label, make sure to add a blank (empty) label between the two to lower the chance that text will show through the old label when scanned.
- **If No, explain why not.**
  - The coordinator will need to add this info to SpecTrack to explain the incomplete derivatives

Walk through slides checklist below for guidance:

1. H&E frozen
   - ☐ Procured.
     - Match to KPMP label KPMP_FR_HE_1
   - ☐ Not procured.
     - Reason:
2. H&E frozen
   - ☐ Procured.
     - Match to KPMP label KPMP_FR_HE_1
   - ☐ Not procured.
     - Reason:
3. If more H&E or other slides were obtained, do NOT ship to the CBR

- ☐ Do we have any leftover OCT block?
  - Yes. Return it to its original KPMP cryomold when possible. If not possible, note that it changed containers in SpecTrack. Hand-off block for de-identification, KPMP re-labeling, and shipment to CBR
    - Match to KPMP label KPMP_FR_Block (OCT)
  - No. If No, why not (*note that the options most likely to be chosen are 'Used' and 'Moved Container')*:
    - Used
    - Moved container
    - Destroyed
    - Lost
    - Derived
    - Damage/not usable
    - Exhaust on Create
    - Empty

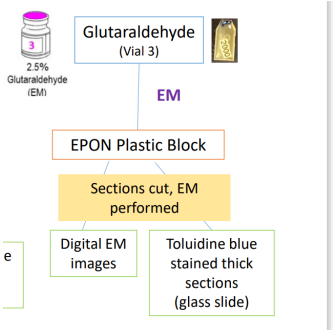
- ☐ Did we obtain the IF images?
  - Yes.
    - Pathologist to complete the Frozen Section H&E (Immunofluorescence specimen) Overview' section of the Pathology Images Upload form and return to coordinator.
    - Pathologist will also provide images with a **Positive** stain to the coordinator for de-identification, KPMP re-labeling, and upload to REDCap Pathology Images Upload CRF. Provide up to 10 images per antibody.
    - Pathologist to complete the Pathology Immunofluorescence Metadata CRF in REDCap, ideally within 2 weeks of biopsy. Coordinator will send an email link to the CRF a day after the biopsy. This CRF captures metadata (location, distribution, pattern, intensity) and should be completed only for antibodies that yield positive staining directly in REDCap. Each antibody requires a new instance of the form
  - No. If No, why not:

Section 3. Did the biopsy result in procurement of glutaraldehyde?

- ☐ No
- ☐ Yes. If Yes, complete this workflow:

Reference: Pathology MOP section "Electron Microscopy standardized protocol".

Tissue placed in 2.5% glutaraldehyde is processed for electron microscopy using local RS pathology laboratory protocols. Toluidine blue stained thick plastic sections are reviewed by the local RS pathologist as per routine clinical pathologic examination. Thin sections are cut and electron microscopy grids created per local RS pathology laboratory protocols. Electron microscopic examination is performed and digital photographs of representative glomeruli and tubulointerstitium are obtained per local RS pathology laboratory routine process. A minimum of 10 glomerular photomicrographs are taken. A minimum of 5 photomicrographs of tubular epithelium, 2 of arteries/arterioles if present, and 3 of interstitium are taken. At least 2 photomicrographs of tubular epithelium must be taken at 30,000x to visualize the mitochondria. The remaining photomicrographs are taken at lower magnification. The ultrastructural digital photomicrographs are examined by the local RS pathologist and the findings incorporated into the pathology report.



- **How many plastic/EPON Blocks were created?** If more than 1, make sure to note which slides were cut from which parent block so hierarchy can be logged in SpecTrack.

<u>Hand-off workflow:</u> Did we obtain the toluidine blue stained thick sections glass slide(s)?

- **If Yes, hand-off slides for de-identification, KPMP re-labeling, and shipment to CBR. 8 labels are included. You might not use all of them.**
  - Make sure all patient identifiers are removed or hidden (MRN, DOB, participant name, hospital name, pathologist name, accession numbers, local barcodes, etc.).
- **If No, explain why not.**
  - The coordinator will need to add this info to SpecTrack to explain the incomplete derivatives

Walk through slides checklist below for guidance:

1. Toluidine blue stained thick sections glass slide(s)
   - ☐ Procured.
     - How many were procured? (1-8): ___
     - Match slides to KPMP labels KPMP_EM_Thick. Be sure to track slide level on the label.
   - ☐ Not procured.
     - Reason:

☐ Do we have any leftover EPON/plastic block(s)?
- Yes. Hand-off block for de-identification, KPMP re-labeling, logging in SpecTrack, and shipment to CBR
  - Match to KPMP label KPMP_Plastic_BlockEM. If there are >1 block, be sure to map which slide came from which block.
  - Block should be stored in small Ziploc bag. One label per block/bag. Two bags of size 1.5” x 2” are provided in the Refrigerated Shipment Kits for this purpose.
- No. If No, why not:

☐ Did we obtain the EM images?
- Yes. Provide images to the RC for de-identification, KPMP re-labeling, and upload to REDCap Pathology Images Upload CRF. Provide up to 60 images.
- No. If No, why not:

Section 4. Other

- Pathologist to complete the Dx Core Disease Category Assignment CRF in REDCap, ideally within 2 weeks of biopsy. Coordinator will send an email link to the CRF a day after the biopsy. This CRF captures major patterns and we don't expect more than 2 instances for each compartment: glomerular, tubulointerstitial, vascular. Each presenting pathology requires a new instance of the form.