KPMP
Pathology MOP

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Abbreviation list

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

A. Gross examination and triaging of the renal biopsy

A.1 Purpose

All the procedures described here will be performed in the procedure suite by trained tissue processing personnel from the Participant 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 (MOP). 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).

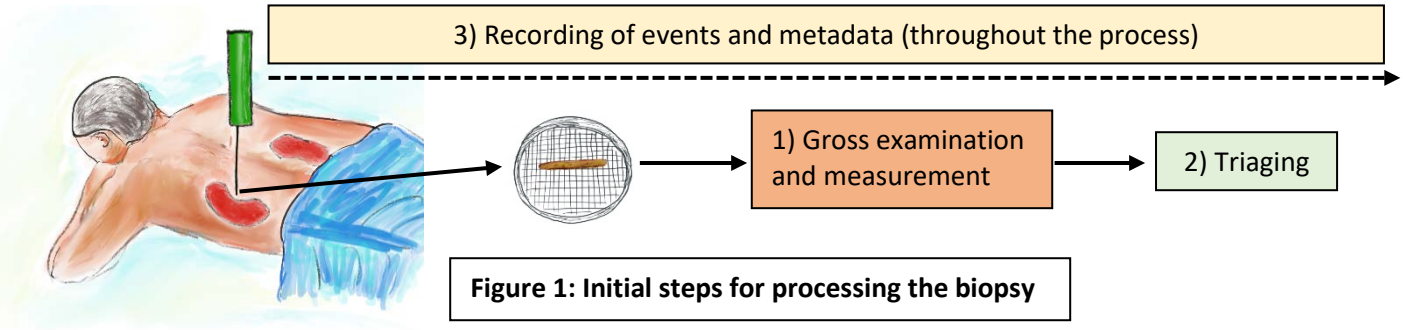

Figure 1: Initial steps for processing the biopsy

A.1.a 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.

A.1.b. 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 adequate enough to enter the analytical pipeline.

A.1.c. Purpose of recording of event 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. 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.

A.2. Renal Biopsy supplies for triaging, handling and shipping of the 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 DCC, and the KPMP tablet with camera (to take pictures and collect data in the Kidney Biopsy Procedure Details Case

Report Form (CRF 1). 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), 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 diagnostic biopsy kit and the tissue interrogation biopsy kit are described below.

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

A.2.a. Checklist

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

A.2.a.1. Equipment for handling the renal biopsy:

- The KPMP renal biopsy cart (see A.2.a.2 and figure 2)
- The KPMP tablet (with camera)
- The KPMP renal biopsy kit (A or B) (see A.2.a.3 and A.2.a.4 and figures 3 and 4)
- A paper copy of the KPMP Biopsy Procedure CRF as backup, printed on the same day as the biopsy procedure (Always use the electronic CRF unless technical issues prevent it)

A.2.a.2. KPMP renal biopsy cart supplies (Figure 2)

- Bucket with 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.
  - 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 idea is that the OCT cassettes in which the tissue will be frozen can be placed on the dry ice for uniform freezing. Ensure 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 solutions and corresponding vial, petri dish and sterile PBS cold)
- Mr. Frosty freezing container (Nalgene, PN#5100-0001), containing isopropanol (will need to be procured at the recruitment site)
- 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)
- RNaseZap solution (to clean all surfaces and instruments prior to handling the biopsy) (Thermo Fisher Scientific AM9780)

- Sterile 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
- Clean pack of paper towels to wipe the surfaces with RNaseZap
- RNase free water
- 70% ethanol in spray bottle
- Bench paper pads to cover the workspace on the top of the cart before laying out the supplies
- Sterile Petri dishes

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 prepare the cart before going to the biopsy suite is 30 minutes.

A.2.a.3. KPMP renal biopsy Kit A components: (see Figure 3)

Kit A - Diagnostic core (Core #1):

- Container 1: 10% neutral buffered formalin (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 (EMS cat. no. 16537-16) for EM
- Razor Blades

Kit A – Tissue Interrogation Research core(s):

- 2 Cryomold cassettes: Tissue-Tek Cryomold (Standard, #4557) [for Core #2] (frozen core 2 will be placed in small Ziploc bag (Container 4))
  - One cryomold is for 'bathing' only; other is marked with a blue label
  - Ziploc bag for container 4
- Container 5, Tube #1: 1 Orange cap pre-labeled Cryovial (Cryostor) (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416) (Container 5) [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)

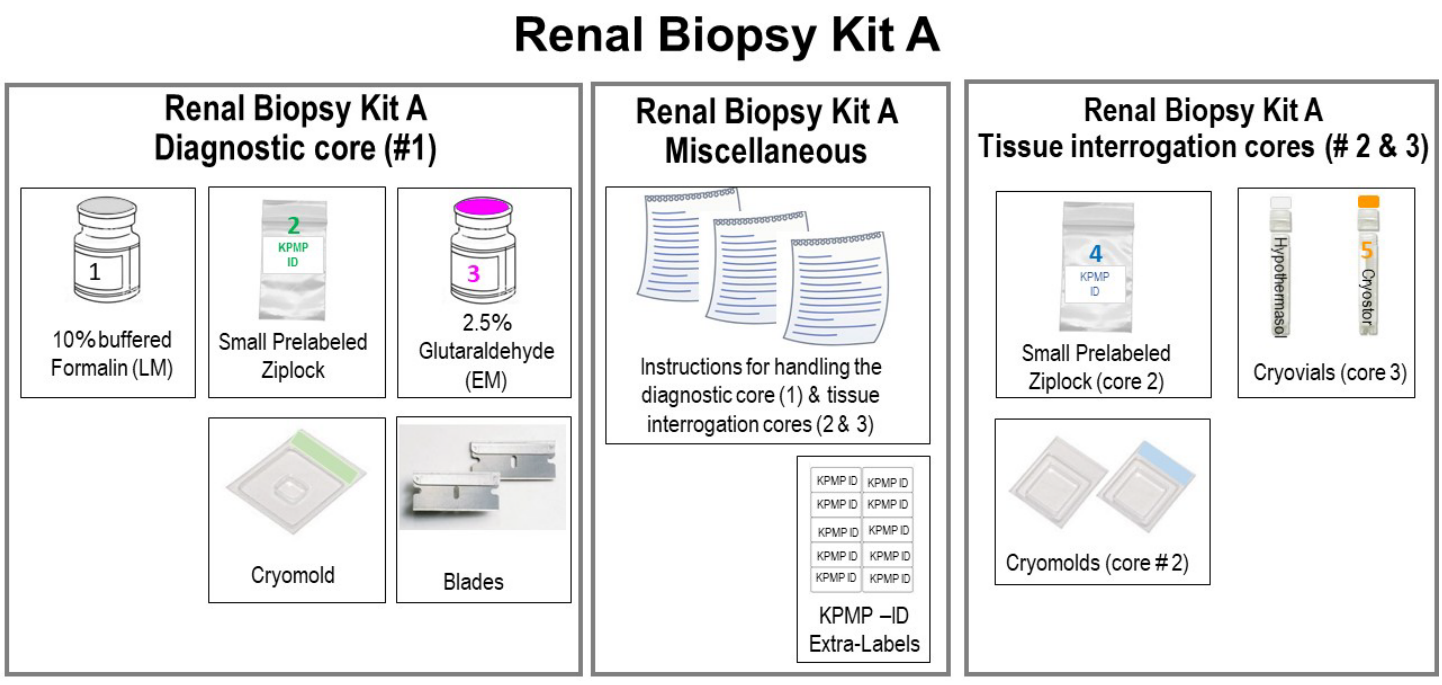

Figure 3: KPMP renal biopsy Kit A. The itemized list of the supplies in the renal biopsy kit is illustrated.

A.2.a.4. KPMP renal biopsy Kit B components (see Figure 4)

Kit B - Diagnostic core section (same as in kit A):

- Container 1: 10% neutral buffered formalin (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 (EMS cat. no. 16537-16) for EM
- Razor Blades

Kit B – Tissue Interrogation core section:

- 2 Cryomold cassette: Tissue-Tek Cryomold (Standard, #4557) [for Core #2] (frozen core 2 will be placed in small Ziploc bag (Container 4))
  - One cryomold is for 'bathing' only; other is marked with a blue label
  - Ziploc bag for container 4
- 1 red cap pre-labeled Cryovial (container 6) (~2 mL, Corning Incorporated, Cryogenic Vial: Cat. No. 431416) [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)

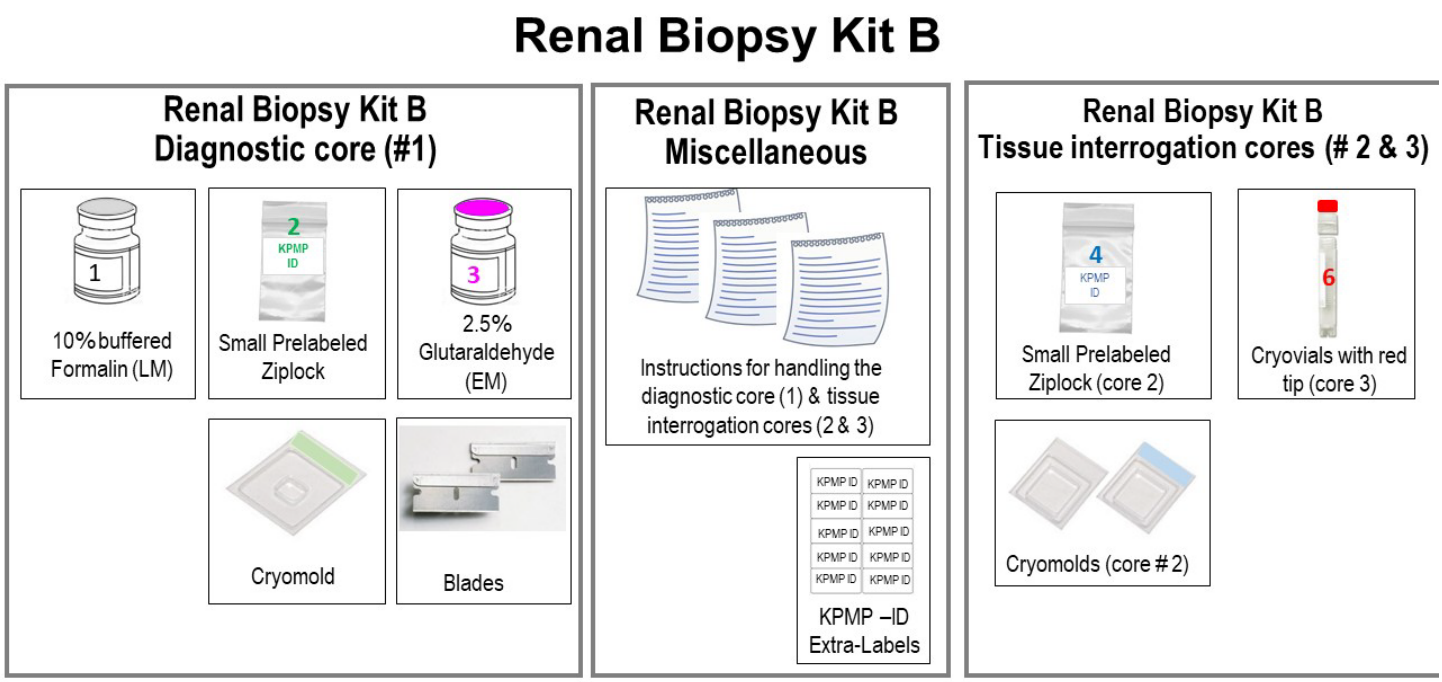

Figure 4: KPMP renal biopsy Kit B. The itemized list of the supplies in the renal biopsy kit is illustrated.

A.2.a.5. Metadata forms (CRF 1)

- ✓ KPMP Kidney Biopsy Procedure Details CRF in REDCap (with paper back-up)

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.

A.2.b. 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 A.2.a.1) 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, surface and external surface of solutions containers before starting the procedure, see below A.2.b.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.

A.2.b.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.2.b.1.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.

A.2.b.1.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.

- o 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.
- o 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 does not need to be wiped and may be placed down after wiping the rest of the bucket with the paper towel.
- o 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.
- o Wipe with clean paper towel
- o Set the timer for 2:00 minutes and wait a full 2 minutes for the area to dry completely.
- o Spray with RNase free water and dry with a clean paper towel.

A.2.b.1.c. *Covering:*
Place clean bench pads (2-3 blue pads) on the cart or on the surface where tissue processing will occur and set up the wet ice bucket.

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

A.2.c. General Protocol for initial handling of the tissue *(see also section B.2)*

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 core will be designated as the diagnostic core (Core 1), unless it does not meet the criteria discussed in section A.3.c-e. 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.

Prepare the working space before starting:

- Set up all the material necessary for each step
- Clean all supplies and surface (see A.2.b.1)

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.

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.

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 triaging and decision tree below). 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.

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 and CRF 1 (Kidney Biopsy Procedure Details) see Appendix A.

A.3.a 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 or similar may be provided to maximize the ability of the personnel to see cortex. Recruitment site pathologists are welcome to provide their own method to aid in seeing cortex, such as a dissecting microscope. However any light source used to see the core MUST NOT produce heat (e.g. LED). Heating the tissue WILL alter the molecular signatures. 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 CRF 1 (Kidney Biopsy Procedure Details, see appendix A). The gross photos will be labeled with the biopsy core specific KPMP participant code identifier and uploaded electronically via REDCap.

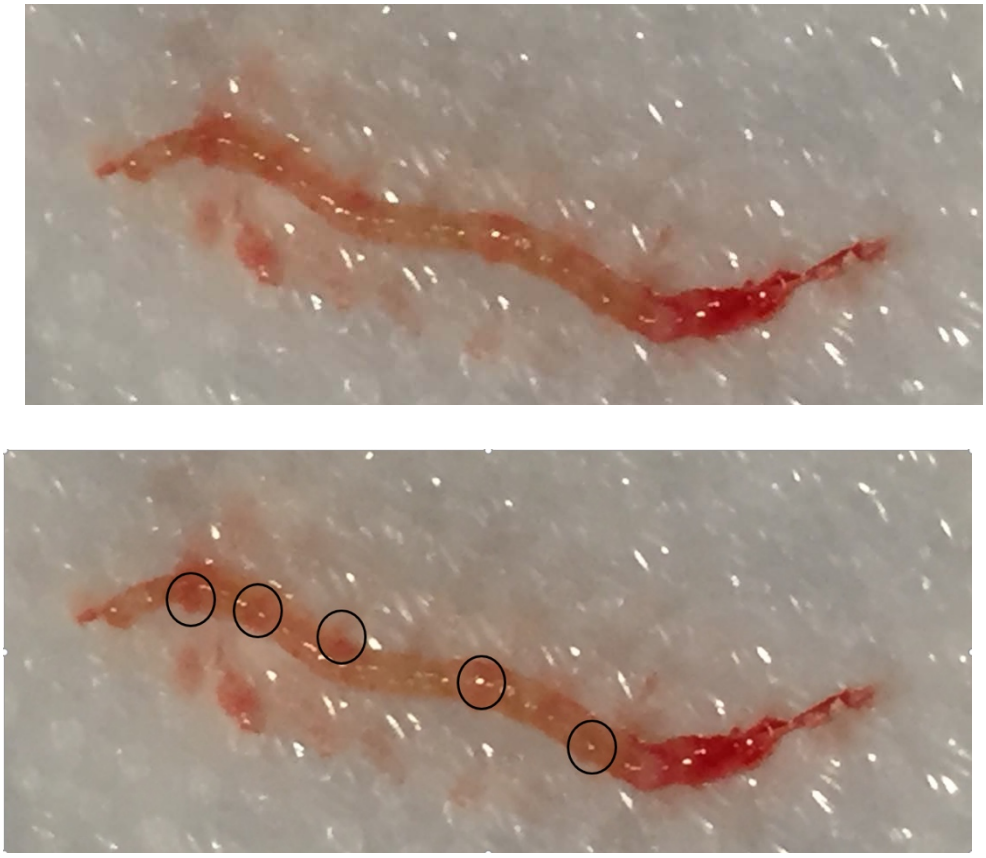

Figure 6: Imaging and recording of the fresh tissue for gross examination. The upper panel shows a digital image of a fresh biopsy taken by a portable digital camera. Gross examination of the biopsy reveals the presence of multiple discrete red areas (black circles in lower panel), which represent glomeruli 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.

A.3.b Triaging

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 TIS MOP.

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 (Hypothermasol 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 Hypothermasol or liquid nitrogen, (2nd) place core #1 formalin and gluataraldehyde, 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).

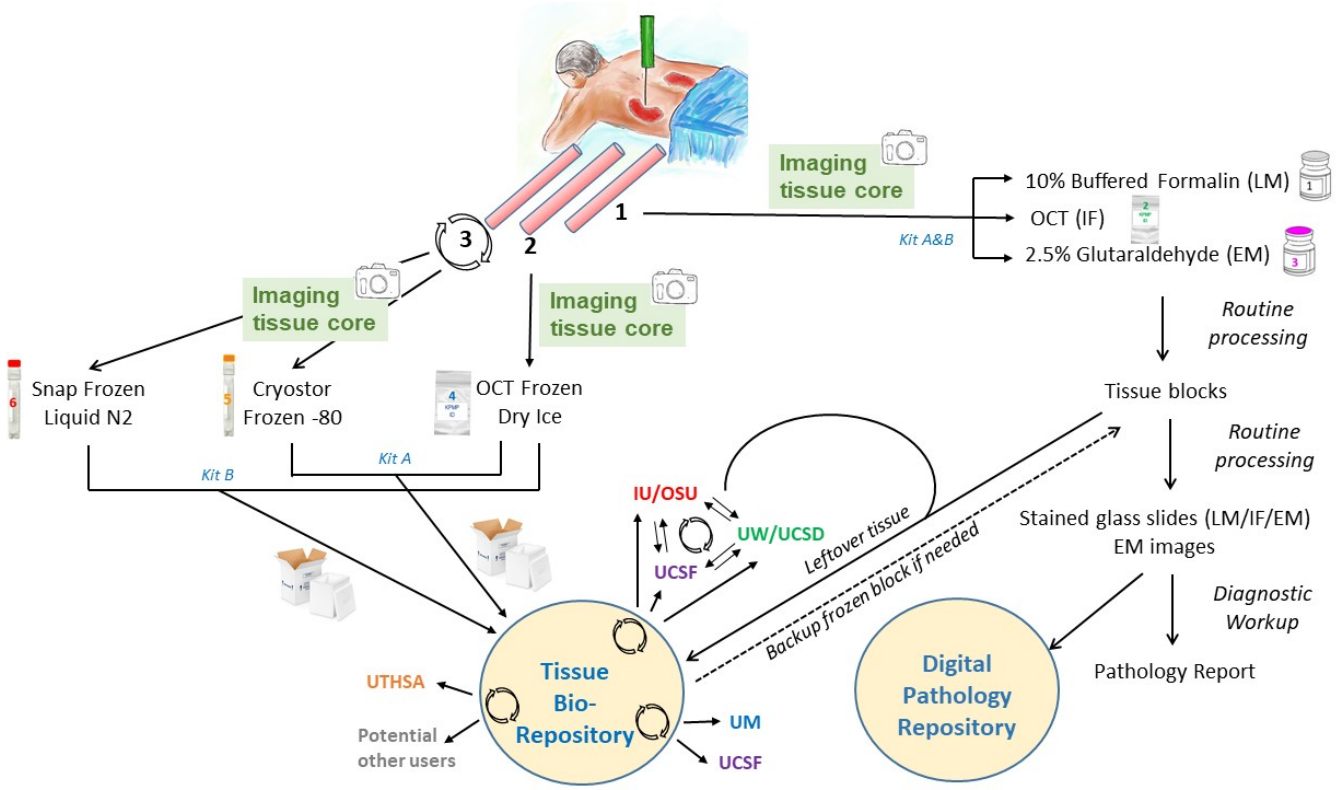

Figure 7: Overall kidney biopsy schema for triaging of the cores for downstream analysis and interrogation. The goal for KPMP ideally is to obtain 3 cores from each participant. Core 1 will be used for diagnostic purposes, and core remnants may be used for research. Cores 2 and 3 are solely used for 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 TIS MOP.

A.3.c. Decision tree for triaging

The guiding principles detailed in sections A.3.d-f 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).

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 A.3.d), then this core will be immediately allocated to the diagnostic workflow (section B) 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). Note that you do not need to wait for the 'best' core for the diagnostic core.

- *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 A.3.d.

- 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 A.3.e.

- In case of only one core, then it will be handled according to A.3.f.

The time of each biopsy pass, whether tissue was obtained, and how it was triaged is recorded (see appendix A; CRF 1: Kidney Biopsy Procedure Details). Note that biopsies may come quite quickly if the operator with the biopsy gun is experienced, as they are in KPMP. It is acceptable to keep biopsy cores on chilled PBS soaked Telfa pad until all cores are out before beginning triage. If the recruitment site team is comfortable processing biopsy cores as they come, that is also acceptable.

A.3.d. Three cores biopsy specimen.

A.3.d.1. 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 B) 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 A.3.f.2). 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, according to the rotation protocol (see Figure 7 and section C for details). The goal will be to triage the tissue cores within 5 minutes post harvesting.

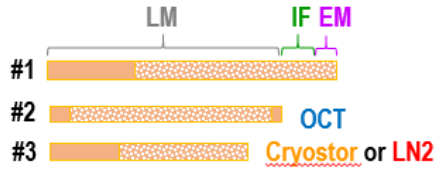

Figure 8: triaging of the renal biopsy when 3 cores are obtained, with at least one ≥1.4cm in length and renal cortex is visualized.

A.3.d.2. Renal cortex not visualized (in all three specimen): 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 - 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 C for details).

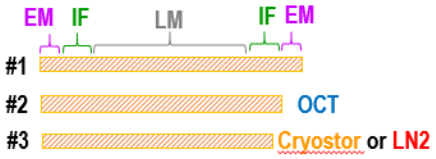

Figure 9: triaging of the renal biopsy when 3 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is not visualized.

A.3.d.3. 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.

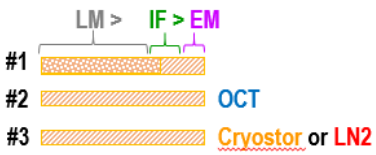

Figure 10: triaging of the renal biopsy when 3 cores are obtained, all measuring <1.4cm.

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 C for details).

A.3.e. Two cores biopsy specimen.

A.3.e.1. 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 according to the rotation scheme (see Figure 7 and section C for details). The goal will be to triage the tissue within 5 minutes from harvesting and record this time.

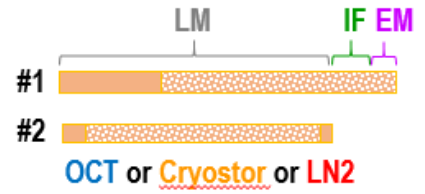

Figure 11: triaging of the renal biopsy when 2 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is visualized.

A.3.e.2. Renal cortex not 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 tissue bio-repository (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 C for details). The goal will be to triage the tissue within 5 minutes from harvesting and record this time.

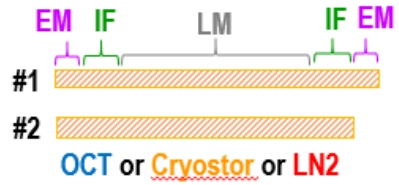

Figure 12: triaging of the renal biopsy when 2 cores are obtained, with at least one ≥1.4cm in length, and renal cortex is not visualized.

A.3.e.3. 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 central tissue biorepository and distributed to the tissue interrogation sites according to a rotation schedule (see Figure 7 and section C for details).

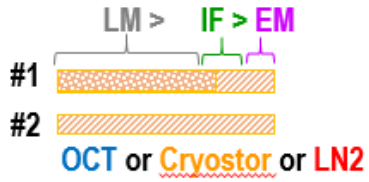

Figure 13: triaging of the renal biopsy when 3 cores are obtained, all measuring <1.4cm.

A.3.f. One core biopsy specimen.

A.3.f.1. 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.

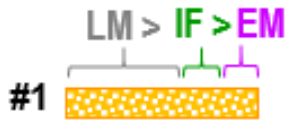

Figure 14: triaging of the renal biopsy when 1 core, at least one ≥1.4cm in length, is obtained, and renal cortex is visualized.

A.3.f.2. Core measures < 0.5 cm: The entire tissue will be fixed in 10% neutral buffered formalin (container 1) to be processed for Light Microscopy.

A.3.f.3. Renal cortex not visualized: The entire tissue will be fixed in 10% neutral buffered formalin (container 1) to be processed for Light Microscopy.

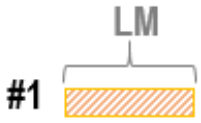

Figure 15: triaging of the renal biopsy with a single core obtained, and no cortex is identified or the tissue is <5cm in length.

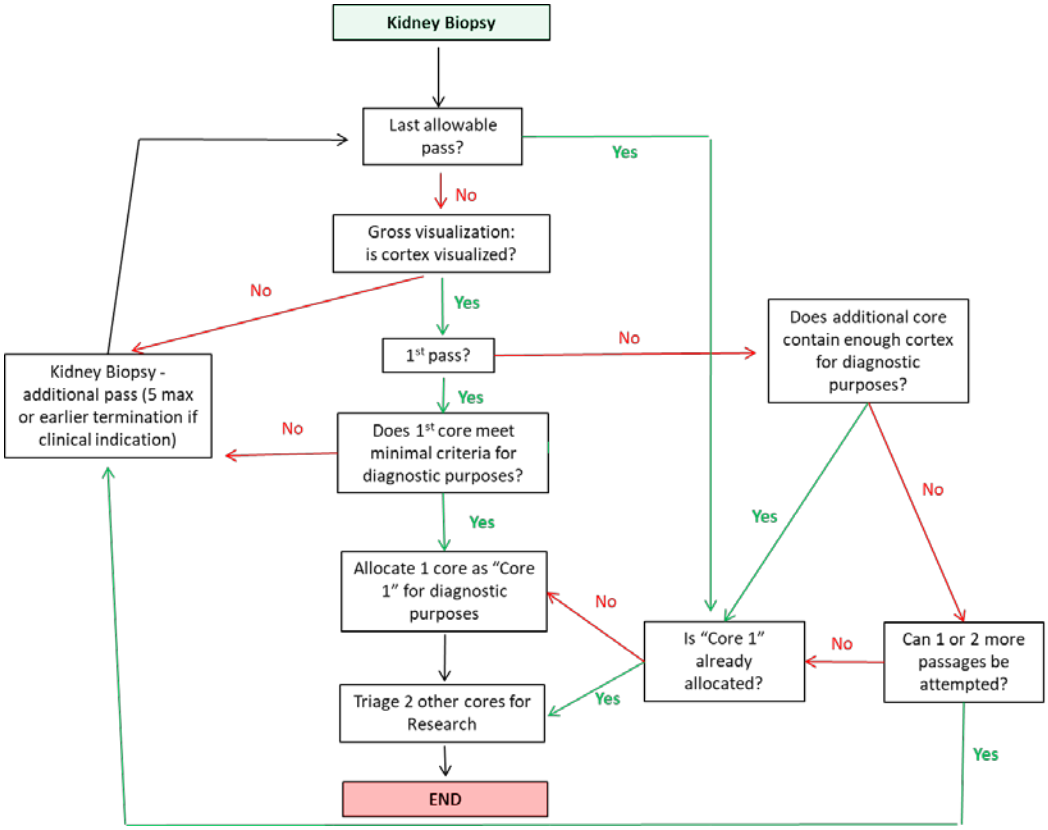

Figure 16: Decision tree to guide the triaging process based on the biopsy progress and the anticipated tissue workflow (see text for details). Research cores will only be committed to processing after the diagnostic core is secured.

A.4. Recording of events from harvesting to processing and quality control

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 A.2.a.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 and Specimen Tracking Systems. The Kidney Biopsy Procedure Details CRF (CRF 1) will be completed upon conclusion of the procurement phases (gross evaluation, triaging, processing, storage) of the tissue for diagnostic and tissue interrogation purposes (see Appendix A for details on the metadata list and CRF 1).

B. Handling and processing of the diagnostic core

B.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.

B.1.a. Handling of the diagnostic core:

This step occurs at the time of the renal biopsy procedure (see triage, section A.3) 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.

B.1.b. 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.

B.1.c. 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.

B.2. Handling of the diagnostic core at the time of the renal biopsy procedure

B.2.a. 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 A).

B.2.b. Materials (see Figures 2, 3, and 4 and section A.2.a.1-3):

From the renal biopsy kit A or B (A.2.a.2 and 3)

- 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 #4565) with OCT embedded frozen tissue for IF (to be placed in small Ziploc bag).
- Ziploc bag
- Container 3: 2.5% Glutaraldehyde with pink label (EMS cat. no. 16537-16) for EM
- Razor Blades to cut IF and EM pieces
- Instructions

From the renal biopsy cart (A.2.a.1)

- Optimum Cutting Temperature medium O.C.T. compound: Tissue-Tek (#4583)
- Sterile 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

B.2.c. Protocol

B.2.c.1.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.

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 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.Measure the full length of the core (i.e. do not exclude fat or other components suspected to be non-renal tissue when taking the measurement).

Tissue is assessed for diagnostic adequacy per above criteria (section A.3). Record whether cortex is visualized or not in the Kidney Biopsy Procedure Details CRF. Include comments about core composition (i.e. fat) in the CRF, if applicable. These comments are very helpful for the tissue interrogation sites.

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 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 for later use as a research core and await additional tissue.

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 A.3.

One blade technique: 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.2.c.2. 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.

B.2.c.3. 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 9A). Briefly bathe the tissue in the cassette (few seconds) (Figure 9 A') 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 9B) 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 9B'). 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 10. 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. Ensure good contact with the bottom of the cryomold on the crushed dry ice so the tissue freezes evenly.**

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. Discard the sterile wooden applicator on the bench pad.

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.

B.2.c.4. 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.

B.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 B.2. All key events occurring in the specimen procurement stages will be time-stamped (see Appendix A, CRF 1: Kidney Biopsy Procedure Details) by the RS dedicated staff as noted in **bold** font in section B.

After the procurement of all the cores and their processing described in section B.2 and C (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 E.

B.4. Local processing workflow (default option)

Kidney tissue obtained at the KPMP Participant Recruitment Sites (RS) is triaged as described in section A.3. 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.

B.4.a. 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 stained as follows, for a total of 8 slides per KPMP diagnostic biopsy core, 2 Hematoxylin & Eosin (H&E), 2 periodic acid Schiff (PAS), 2 Masson's Trichrome (TRI), and 2 Jones Methenamine Silver (SIL). Please process the 12 sections thus:

1. H&E
2. PAS
3. TRI
4. SIL
5. unstained (blank)
6. unstained (blank)
7. unstained (blank)
8. unstained (blank)

9. H&E
10. PAS
11. TRI
12. 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 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.

B.4.b. 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 section should be obtained from the OCT block. Do not collect any unstained slides.

B.4.c. 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.

B.4.d Diagnostic core: follow the tissue overview

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 E), relabeled with the KPMP subject ID, and uploaded into KPMP REDCap System
- All glass slides are de-identified (see section E), relabeled with a unique KPMP sample ID (provided with the kit; ensure there is no overhang when applying the label), 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 F). **Do not send immunofluorescence glass slides to the CBR.**

- The jpeg images of immunofluorescence are de-identified (see section E), updated to include the KPMP sample ID that was previously applied to the slide and uploaded into REDCap (see section F).
- The electron microscopy digital images (jpeg) are de-identified (see section E), updated to include the KPMP sample ID that was applied to the slide and uploaded in REDCap (see section F).
- 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 E & G, and Appendix F).
- The OCT frozen tissue remnant block will have been placed in a pre-labeled cryomold with a unique KPMP sample ID during the biopsy procedure. Please return the remnant block to this container and verify that it still retains this label and is de-identified. Do not place the OCT remnant block in a cryovial. If the labeled cryomold is inadvertently lost, place the remnant block in a small Ziploc with the KPMP sample ID label on the outside, and then place this Ziploc inside a second Ziploc (double bagging protects the label from loss during shipment). 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 G and Appendix F).
- 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 G and Appendix F).

B.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 B.4, and undergoes high-resolution quality control assessment centrally, as indicated in section I. 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 (see section I), the decision to recut and stain the paraffin block (see B.4.a) 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.

B.5.a. 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 (according to Pilot 2 criteria) for computational imaging, the de-identified FFPE blocks are retrieved from the KPMP CBR and sent to the CPL in case any further processing (cutting and staining) is required.

B.5.b. 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 E) and reviewed by the central core pathologist for assignment of diagnostic category and high-resolution adequacy assessment (see section I).

C. Handling of the research core(s) at the time of the renal biopsy procedure for downstream analysis by TIS

C.1 Purpose. This section describes methods for processing of the clinical biopsy tissue procured by the RS for use by the TIS in their OMICS, imaging and other interrogation technologies. 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 TIS. 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 A.3 and Figure 8)

C.2. Fresh frozen OCT embedded block preparation (for Core 2, container 4)

C.2.a Applications:

Histology, multiplex ISH and IF, proteomics, single nucleus DropSeq, 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.

C.2.b Materials

From the renal biopsy Kit A or B

- 2 Cryomold cassettes: Tissue-Tek Cryomold (Standard, #4557) [for Core #2] (frozen core 2 will be placed in small Ziploc bag (Container 4))
  - One cryomold is for ‘bathing’ only; other is marked with a blue label
- Small Ziploc bag (container 4) to hold the cryomold cassettes containing OCT embedded frozen tissue
- 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

From 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 A.2.a 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 phosphate buffered saline (PBS), pre-chilled on ice
- Sterile dropper to transfer reagents to keep biopsy moist
- Sterile Petri dish 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

NOTE: Perform procedures with RNase reducing precautions (clean and sterile where it can be. Follow the RNase elimination method above using RNaseZap (section A.2.b).

C.2.c. Protocol: Tissue embedding in OCT and OCT frozen block preparation

Make sure items in biopsy kit A are on the cart workspace.

C.2.c.1. Before the biopsy starts, take the one of the cryomolds (the 'bathing' cryomold) and completely fill it with OCT (Figure 9 A). Replace the cap on the OCT bottle when not in use. Keep it at room temperature on the cart workspace.

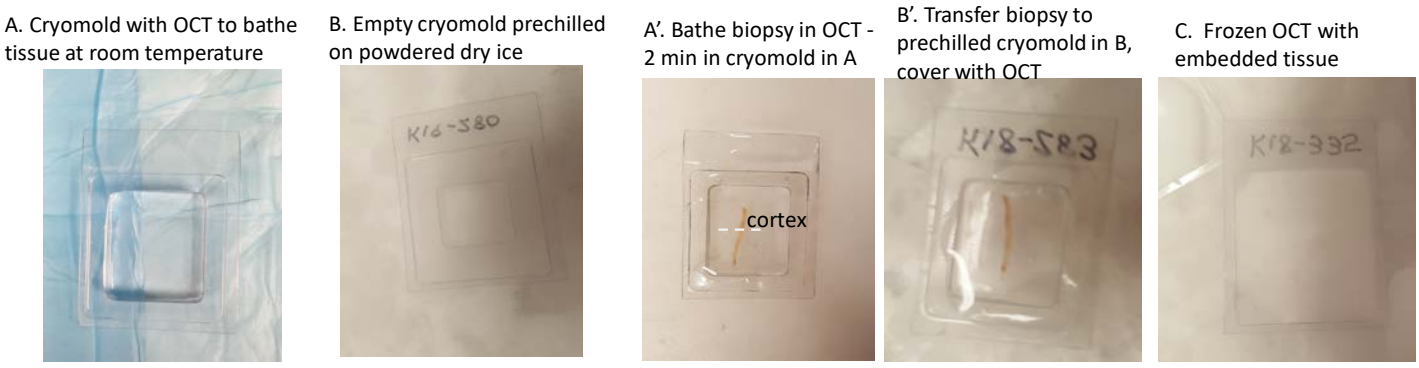

Figure 9. Preparation of cryomolds for fresh frozen OCT embedding of research core.

C.2.c.2. 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 9B).

C.2.c.3. **Record the time** when the biopsy gun containing the tissue is removed from the participant (time stamp in the Biopsy Procedure CRF).

C.2.c.4. 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.

C.2.c.5. 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 A.3.a for the diagnostic core biopsy. The photo is uploaded in the Biopsy Procedure Details CRF. The length of the core is recorded in the Kidney Biopsy Procedure Details CRF. Measure the full length of the core (i.e. do not exclude fat or other components suspected to be non-renal tissue when taking the measurement). Record whether cortex is visualized or not in the Kidney Biopsy Procedure Details CRF. Include comments about core composition (i.e. fat) in the CRF, if applicable.

C.2.c.6. Transfer the biopsy tissue gently with a sterile wooden applicator into the OCT bathing cassette kept at room temperature (Figure 9A). Briefly bathe the tissue in the cassette (few seconds) (Figure 9 A') by gently swishing around the tissue with a sterile wooden applicator (without squishing the tissue).

C.2.c.7. 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 9B) 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 9B'). Pouring the OCT over the biopsy core directly can reduce unwanted movement of the tissue. 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 10. 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. Ensure good contact with the bottom of the cryomold on the crushed dry ice so the tissue freezes evenly.** Replace the cap on the OCT bottle when not in use.

NOTE: avoid bubbles; can move the bubbles out of the way with forceps or sterile wooden applicators.

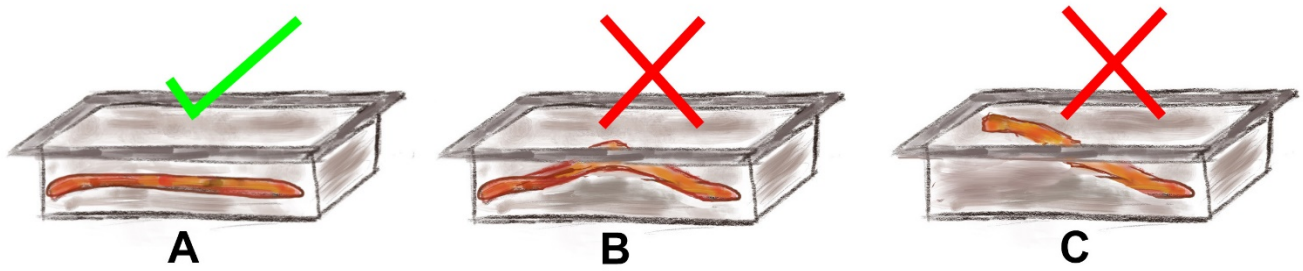

Figure 10: Proper orientation of the tissue during OCT freezing. A shows the proper orientation whereby the tissue is laid flat and parallel to the bottom surface. B and C are examples of incorrect freezing, whereby the tissue is not flat or not parallel to the surface.

C.2.c.8. 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.

C.2.c.9. 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. Keep the block chilled and store it in a freezer box at -80 °C 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 section G). Take photograph(s) using KPMP tablet for documentation as a part of quality assessment and control.

C.3. Fresh frozen biopsy tissue in Cryostor (Core 3, container 5)

C.3.a. *Applications:*

Single cell RNA sequencing

C.3.b. Materials

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)
- 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

From 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

C.3.c. Protocol: Preservation in Cryostor CS10 and freezing

Make sure items in biopsy kit A are on the cart workspace.

C.3.c.1. Place the orange cap ‘cryostor’ cryovial and the ‘HypoThermosol’ cryovial with 1.5 mL HypoThermosol (tube #1 of container 5) and Cryostor CS10 (tube #2) into wet ice vertically.

C.3.c.2. **Record the time** when the biopsy gun containing the tissue is removed from the participant (in Biopsy Procedure CRF).

C.3.c.3. 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.

C.3.c.4. 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 A.3.a. for the diagnostic core biopsy. The photo is uploaded in the Biopsy Procedure Details CRF.The length of the core is recorded in the Kidney Biopsy Procedure Details CRF. Measure the full length of the core (i.e. do not exclude fat or other components suspected to be non-renal tissue when taking the measurement). Record whether cortex is visualized or not in the Kidney Biopsy Procedure Details CRF. Include comments about core composition (i.e. fat) in the CRF, if applicable.

C.3.c.5. 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). **Record time of when the tissue is placed in the cryovial with HypoThermosol.** 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

C.3.c.6. 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. **Record time.**

C.3.c.7. Keep the vials upright on wet ice for 15 – 20 minutes to allow penetration of the solution to the tissue.

C.3.c.8. 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.

C.3.c.9. Transfer cryovial containing CryoStor CS10 (tube #2, container 5) preserved tissue to Mr. Frosty Freezing container containing isopropanol (**Record time in the Tissue Tracking CRF**) and place container at -80°C (**Record time in the Tissue Tracking CRF**) for about 24 hours (minimum of 12 hours). The isopropanol in Mr. Frosty Freezing container should be changed every 5 uses. Note: keep a container with dry ice handy to place the Mr Frosty in if it is not possible to reach the -80C freezer in time. 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 -80C freezer.

C.3.c.10. Next day, transfer the cryovial containing CryoStor CS10 (tube #2, container 5) cryopreserved tissue for storage in liquid nitrogen. Use long forceps to hold the cryovial during transfer. **Record the time of transfer to freezer or liquid nitrogen. If liquid nitrogen storage is not available, leave the specimens at -80°C until shipping to CBR. Aim to ship to the CBR quickly and ideally no later than one week after collection.**

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

C.4. Fresh biopsy tissue frozen in liquid nitrogen (Core 3, container 6)

C.4.a. Applications:

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

C.4.b. Materials:

From the renal biopsy Kit B

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

From 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

C.4.c. Protocol: Freezing in liquid nitrogen (LN2)

Make sure items in biopsy kit B are on the cart workspace.

C.4.c.1. Fill the liquid nitrogen storage Dewar with liquid nitrogen

C.4.c.2. Keep container 6 cryovial on wet ice.

C.4.c.3. **Record the time** when the biopsy gun containing the tissue is removed from the participant in the Biopsy Procedure CRF.

C.3.c.4. 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.

C.3.c.5. 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 A.3.a for the diagnostic core biopsy. The photo is uploaded in the Biopsy Procedure Details CRF.The length of the core is recorded in the Kidney Biopsy Procedure Details CRF. Measure the full length of the core (i.e. do not exclude fat or other components suspected to be non-renal tissue when taking the measurement). Record whether cortex is visualized or not in the Kidney Biopsy Procedure Details CRF. Include comments about core composition (i.e. fat) in the CRF, if applicable.

C.3.c.6. 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.

C.4.c.3. Immediately plunge container 6 into liquid nitrogen. **Record time in Biopsy Procedure Details.** The cryovial can be transported to the laboratory in liquid nitrogen (although make sure it is not all evaporated). The cryovial is then stored in -80 °C until shipped to CBR on dry ice.

C.5. Distribution of the research core(s) to the CBR (at the time of the renal biopsy procedure), for storage before shipment to TIS

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 C.2 to C.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 section G for details). Triaging details and metadata will be recorded in the Kidney Biopsy Procedure Details CRF (CRF 1) and Tissue Tracking 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.

C.6. Shipping research core(s) to Central Biorepository (CBR) for storage and distribution to TIS

C.6.a. Materials

C.6.a.1 Provided by CBR

- KPMP insulated dry ice shipper (For frozen cores and OCT blocks)
  - Cardboard shipping box with orientation arrows
  - Insulated shipping container (to be placed inside cardboard shipping box)
- UN1845 label
- Address labels (for UN1845)
- Exempt human specimen label
- Biohazard and Ziploc bags from the Pathology Kit

C.6.a.2 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

C.6.b. 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 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 freezer box, 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 15 pounds). (See Section G and Appendix F).

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 at the bottom of the empty KPMP Dry Ice Shipper. Fill the shipper with at least 15 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 11. All dry ice shipments must include a Cryo-Temp Ultra Low Temperature Data Logger to record temperatures during transit to the CBR.

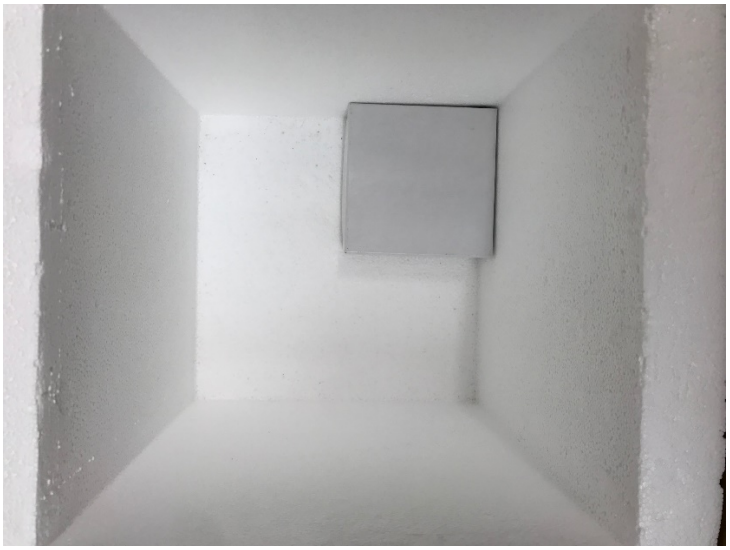

Figure 11. Example shipment photo

The details of the samples being shipped are entered in the SpecTrak 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 system to the qualified TISs according to the workflow in Figure 7 is compliant with the KPMP protocol and agreement among TIS investigators. The rotation schedule will be executed by the CBR through communication lines with DCC, and RS and TIS personnel.

C.7. Distribution of the research cores from the KPMP Central Biorepository (CBR) → Tissue Interrogation Sites

C.7.a. Materials

See section G.4.c

C.7.b Overall Procedure

See section G.4.c

D. Reporting of diagnostic work-up (by RS pathologists)

D.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.

D.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 central pathologists will assign the disease categories by accessing the KPMP REDCap System, CRF 3 (see section I and Appendix H).

For the list of disease categories see Section H.2 and Appendix E.

D.3. Local processing workflow (default option)

D.3.a. RS phase:

Upon triaging of the tissue (see section A.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 B.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. The pathology material is then retrieved, de-identified and transferred to the DVC (see section E) for scanning and uploading into the KPMP-DPR (see section F).

D.3.b. The KPMP central pathology core phase:

The KPMP central renal 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 H.2 and Appendix E), and for high-resolution evaluation of adequacy (see section I and Appendix H).

D.4. Central processing workflow (alternative option)

The central processing workflow will be implemented only in those cases where the histology preparations are not in compliance with KPMP quality control and protocol.

D.4.a. RS phase: same as D.3.a.

D.4.b. The KPMP central pathology core phase: same as D.3.b.

D.4.c. 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 will be sent to the Central Pathology Laboratory for re-cutting and staining according to the standardized KPMP protocols (See section B.4.a). Stained glass slides are then scanned and uploaded into the KPMP DPR.

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

E.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:

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

E.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.

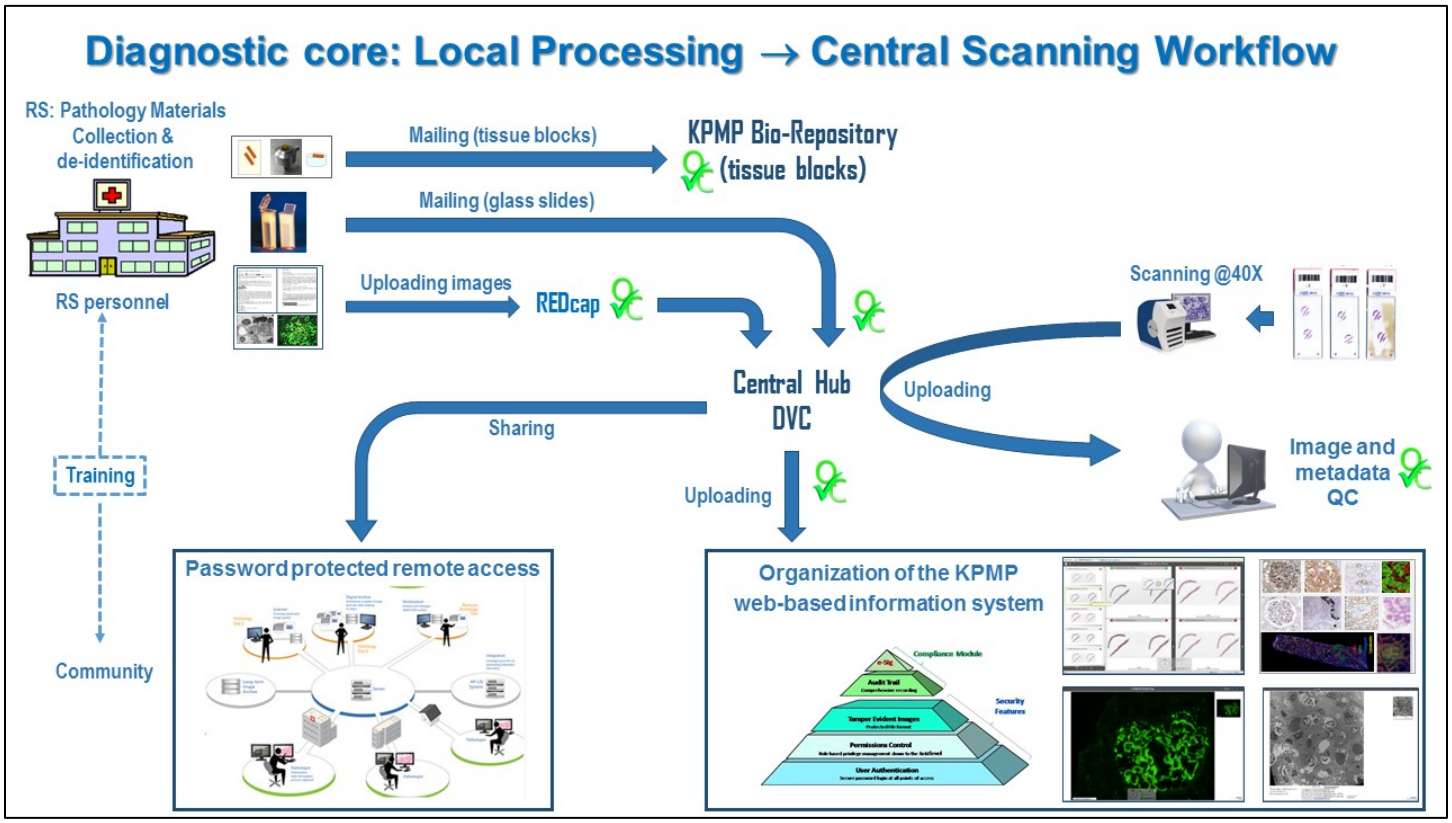

Figure 11: Local processing workflow

E.2.a. Pathology material

E.2.a.1 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)

E.2.a.2. Electron Microscopy material (EM)

- Copy of digital EM images (jpeg format preferred)

E.2.a.3. Immunofluorescence material (IF)

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

E.2.a.4. Copy of the de-identified pathology report PDF.

E.2.a.5. Paraffin blocks (Histology)

E.2.a.6. Frozen tissue blocks (IF)

E.2.a.7. Plastic blocks (EM)

E.2.b. 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 (supplied with the kit) must be placed on each slide and tissue block. The corresponding sample ID should be recorded on each image before scanning or shipping. See instructions in Appendix D.

Caution: cropping portions of digital images may be reversible, please ensure that the information is truly deleted. Please see Appendix D: De-identification Instructions for how to permanently remove identifiers on 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.

E.2.b.1. Reports:

All participant, hospital (address, logo, etc.), and physician (pathologist and nephrologist) identifiers need to be masked before uploading into KPMP REDCap or when a copy is mailed with the pathology materials. See specific instructions for de-identification in Appendix D.

In addition, 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.

E.2.b.2. Glass Slides:

All glass slides require a KPMP sample ID label (supplied with the kit) 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.

Be sure that the KPMP sample ID label completely covers any identifiers on the side AND that the labels do not hang over the edges of the slide. Place the KPMP sample ID label over your local site's participant identifier taking note of the slide "level". Please refer to Appendix D.

E.2.b.3. Electron Microscopy Images:

Save the digital images updated with the corresponding KPMP sample ID and the number of the image (1of10, 2of10 etc.). See specific instructions for de-identification in Appendix D.
Note: Do not make any modifications to the file compression, size, or layers. All images should be saved as *.jpg.

E.2.b.4. Immunofluorescence Digital Images:

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, or others), and the number of the image (1of10, 2of10 etc.).

E.2.b.5. 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	

*When applicable

E.2.c. 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 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).

E.2.c.1. Items Provided by KPMP DCC and CBR to RS study coordinators and other relevant personnel

- Training materials
- DPR certification of training
- KPMP sample ID 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
- For 4^{o}C shipment (for FFPE and plastic blocks)
    - KPMP small insulated 4^{o} shipper
    - Cold gel packs for shipper
    - Small Ziploc bags (for each FFPE Block) (put with KPMP 4 degree shipper)
- For ambient shipment (for glass slides)
    - Glass slide containers

E.2.c.2. 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

E.2.c.3. Preparing Glass Slides for Shipment: Glass slides should be placed in an appropriate slide storage box for shipping (red arrow in Figure 12 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).

Figure 12: 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.

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.

E.2.c.4. Mailing of tissue blocks to 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 F)
- 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 Section G and Appendix F).

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.2.c.5. Uploading of the pdf of the report and jpeg images of immunofluorescence and electron microscopy into REDcap:

A password-protected access to REDcap will be provided to RS study coordinators to upload the de-identified pdf of the report, the de-identified jpeg images of immunofluorescence and electron microscopy.

E.2.d. Scanning of the stained glass slides into whole slide images.

See Section F

E.2.e. Mailing of Pathology Material Back to RS:

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.

E.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 B.3). The overall protocol for the Central Processing Pathway is similar to the Local Processing Pathway protocol, with the exception of the workflow for the paraffin block(s).

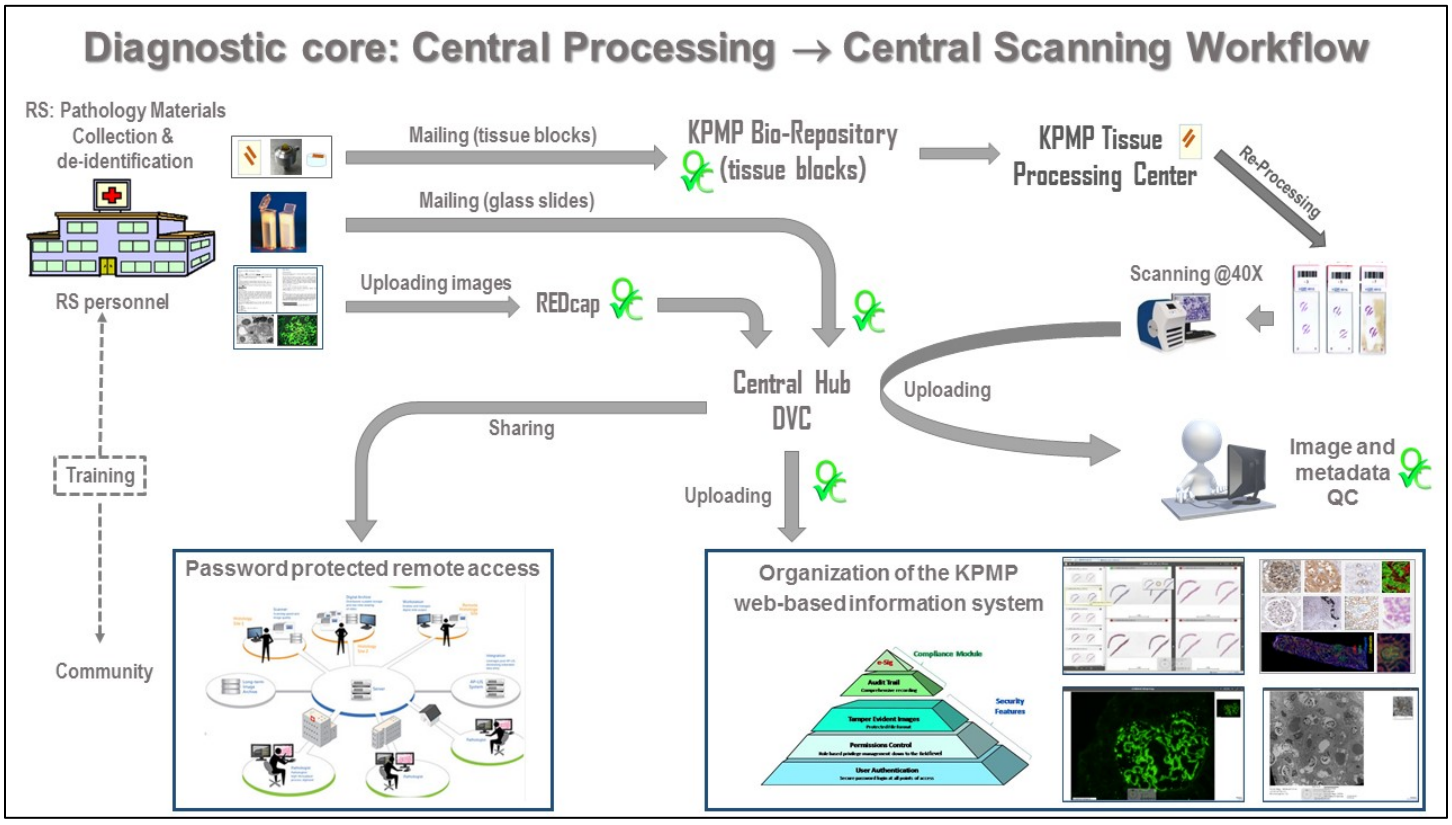

Figure 13: Central processing workflow

E.3.a. Pathology Materials generated at the RS

See section E.2.a

E.3.b. Pathology Material: Handling and De-Identification

See section E.2.b.

E.3.b.1 Reports: See section E.3.b.1.

E.3.b.2. Glass Slides: See section E.3.b.2.

E.3.b.3. Electron Microscopy Images: See section E.2.b.3.

.

E.3.b.4. Immunofluorescence Digital Images: See section E.2.b.4.

E.3.b.5. Tissue blocks: See section E.2.b.5.

E.3.c. Transfer of Pathology Materials to the KPMP CBR and DVC (Central Hub)

See section E.2.c

E.3.c.1. Items Provided by KPMP DCC to RS study coordinators and other relevant personnel.
See section E.2.c.1.

E.3.c.2. Items Provided by KPMP Site.
See section E.2.c.2.

E.3.c.3. Preparing Glass Slides for Shipment.
See section E.2.c.3.

E.3.c.4. Mailing of tissue blocks to KPMP Central Biorepository.
See section E.2.c.4.

E.3.c.5. Uploading of the pdf of the report and jpeg images of immunofluorescence and electron microscopy into REDcap.
See section E.2.c.5.

E.3.d. Scanning of the stained glass slides into whole slide images.

See Section F

E.3.e. *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 C.3). Upon reprocessing, the new set of stained glass slides are scanned and uploaded into the KPMP DPR (see section F), and the paraffin blocks are transferred back to the KPMP CBR.

E.3.f. *Mailing of original stained glass slides back to RS*

See section E.2.e.

F. The KPMP Digital Pathology Repository

F.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 see sections E):

- 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).

F.2. Quality Control of diagnostic pathology material received at the CBR and DVC

As described in E.2.b, 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. Two sets of quality control will be performed:

F.2.a. 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.

F.2.a.2. 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. An automated email will be generated so that the DVC personnel will be alerted and ready to check the individual image against the itemized list in REDCap.

F.2.b. 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 E.2.b. The QC processes will be documented in the CRF 2 ("Dx Core - .jpg Images QC").

F.2.c. QC in preparation for scanning

All glass slides will be inspected cracks or chips in the slide, overhanging labels, tape, or excess mounting medium that may affect the fit of the slide in the scanning mechanism and impede operation. Slides will be carefully wiped with a soft cloth (e.g. microfiber) to remove loose debris, water spots, or fingerprints from the upper and lower surface. For difficult stains, the cloth will be dampened with water or alcohol solution to clean. The QC processes will be documented in the CRF 2 ("Dx Core - .jpg Images QC").

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

F.3. Scanning of 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 AT2 high volume scanners (Leica Biosystems) set to scan at 40x magnification and generate .SVS format files.

F.4. Quality control for Whole Slide Images (WSI)

Quality control for generation of digital .SVS files of whole-slide images will be performed following these steps and recorded in the CRF 2 ("Dx Core - .jpg Images QC") (see Appendix H):

F.4.a. QC for focus

F.4.a.1. Review the whole slide images – or at least the entire scanned tissue area – at a low magnification (e.g. 4x), checking for visible out-of-focus areas or misalignments. Misalignments will typically be visible as a "stitch line" through the image, where the scanned stripes that make up the full image meet.

F.4.a.2. Zoom into the magnification at which the high-resolution image was captured, and review in a single horizontal path across the tissue section at its widest point, one field of view at a time. This will allow you to check for smaller areas of poor focus or subtler misalignments.

F.4.a.3. Perform the same field-by-field review in the vertical direction, from one edge of the tissue to the other in an unbroken line, checking again for any out-of-focus areas.

F.4.a.4. Spot check a few areas in the slide at high magnification, particularly any regions where the tissue is a different thickness or where there are any defects in the slide. These are the areas most likely to have poor focus.

*F.*4.a.5. Check the color of the whole slide image and compare to original slide to assess fidelity of the scanning.

F.5. 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.

G. The KPMP Central Biorepository (CBR)

G.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.

G.2. Renal 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 renal biopsy tissue and slides only from:

a) 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.
b) Deidentified stained slides derived from the diagnostic core (core #1), as described in Section E.
c) 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.
d) 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.

NOTE: Please refer the Laboratory Manual of Procedures for blood, urine, and stool processing and shipment.

G.3. KPMP CBR Personnel and Duties:

*G.3.a.*CBR Organization

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.

G.3.b. Key Personnel and Duties

G.3.b.1. 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.

G.3.a.2 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.

G.3.c. 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.

G.4. Shipping, Receiving, Storage, and Distribution at the KPMP Central Biorepository (CBR)

G.4.a. Transferring of tissue and slides from Recruitment Sites (RS) → KPMP Central Biorepository (CBR)

G.4.a.1. 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.

G.4.a.2. Shipping of diagnostic core tissue blocks and research core(s) from RS to KPMP CBR

As previously described, (see Section E), 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 C.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 E .2.c.4 and Section C.7 for preparing outbound shipment.

G.4.a.3. *Receiving the diagnostic core tissue blocks and research core(s) from RS*

Upon receipt, the paraffin block will be placed at 4^{o}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.

G.4.a.4. *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.

G.4.b. Specimen types and storage conditions (See Appendix F)

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.

G.4.b.1 Paraffin blocks (diagnostic core#1)

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.

G.4.b.2 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.

G.4.b.3 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.

G.4.b.4 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.

G.4.b.5 Snap frozen cores (research core #2)

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.

G.4.b.6 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.

G.4.c. Distribution of the research cores from the KPMP Central Biorepository (CBR) → Tissue Interrogation Sites (TIS)

G .4.c.1. Materials

- Styrofoam Shipping Box
- Pre-paid return Shipping label
- Dry Ice (at least 14 lbs. per dry ice shipment)
- UN1845 label
- Exempt human specimen label
- Scale
- Packaging tape
- KPMP shipping manifest
- Waybill created on UPS or FedEx website

G.4.c.2. Overall Procedure

At the appropriate time, either through an established rotation schedule, or upon request from KPMP DCC, specific samples will be retrieved from the appropriate storage location and prepared for shipment to TIS. 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.

TIS will receive the following types of preserved tissue:

- UCSD-WU:
  - Frozen in OCT
- IU-OSU:
  - Frozen in OCT
- UTHSA-PNNL-EMBL:
  - Snap frozen in liquid nitrogen
- UM-Broad-Princeton:
  - Frozen in Cryostor
  - FFPE
- UCSF:
  - Frozen in Cryostor
  - Frozen in OCT
  - FFPE

Pulling specimens from Storage location

The tissue bank coordinator will create a pick list of specimens in LabVantage to be sent to the TIS using details provided by the DCC. 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 de-identified OCT block(s) or research cores in cryovials are placed in an appropriately configured freezer box within an insulated container with dry ice and mailed to the KPMP TIS (See Appendix F)

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**.**

G.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.

G.5.a. Quality control of biospecimens received at 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. Discrepancies include:

a) Damaged shipping boxes
b) Shipping conditions inappropriate for the sample type
c) Shipment arrival at the incorrect temperature
d) Too little dry ice, gel pack, etc.
e) Sample labels missing/incorrect
f) Damaged internal boxes
g) Cracked vials
h) Cracked OCT blocks
i) Melted FFPE blocks
j) Samples thawed

G.5.b. Quality control for HIPAA compliance of the diagnostic pathology material (tissue blocks) and research cores for the KPMP Central Biorepository (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:

a) Compliance with HIPAA regulations
b) Compliance with study protocol
c) Completeness of the material received
d) 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.

H. Kidney Tissue Atlas Libraries

H.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.

H.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.

H.2.a. Classes of Conventional Diagnostic Categories

H.2.a.1. Primary diagnoses are defined as the disease categories most responsible for the majority of the histologic changes and clinical presentation.

H.2.a.2. Secondary diagnoses are defined as the disease categories either superimposed on the primary diagnosis or underlying condition that contributes to the current clinical presentation.

H.2.a.3. 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.

H.2.b. List of conventional disease categories – See appendix E.1.

H.2.c. Assignment of *Conventional Diagnostic Categories Workflow:*

The KPMP central core renal pathologists are provided password-protected access to the KPMP-DPR for pathology materials digital review and to the KPMP REDCap System. The KPMP core pathologists will assign the disease categories and class by accessing the KPMP REDCap System.

H.2.c.1. Disease category assignment: Each case will be reviewed by a KPMP core pathologist for disease category and class assignment and completion of the "Dx Core - Disease Categories CRF" (Appendix H – CRF 3). A drop-down list of primary, secondary and tertiary disease categories will be available in the CRF 3 for standardization of language. If category assignment is different from official pathologic diagnosis, a second KPMP renal pathologist will review the case to confirm the disease category assignment. If there is no agreement between the two KPMP pathologists, the case will be reviewed by the KPMP central pathology core group (see section G).

H.2.c.2. High resolution evaluation of adequacy: Each case will be reviewed by a KPMP core pathologist for high-resolution adequacy and completion of the "Central Path Quality Metrics Assessment Dx Core CRF" (see Appendix H – CRF 4).

H.3. Descriptors Library

The KPMP pathology working group generated a list of standardized morphologic features that can be detected visually (observational data). The descriptor library is inclusive of all glomerular, tubulointerstitial and vascular normal and abnormal features. The library will be used to morphologically profile participant kidney tissue using visually detected observational data. The list of descriptors was created using published data (B.E. reproducibility data).

H.3.a. The descriptor library: See appendix E.2.

H.3.b. The descriptor scoring workflow:

The KPMP descriptor scoring core pathologists are provided password protected access to the KPMP-DPR for pathology materials digital review and to the KPMP REDCap System. The KPMP descriptor scoring core pathologists will score each renal biopsy tissue by accessing the KPMP DPR and record the findings into a scoring matrix, by accessing the KPMP REDCap System (Appendix H – CRF 6).

H.4. Vectors Library

The KPMP vector library working group generated a library of vectors for cellular and extracellular feature extraction. The vector library includes algorithms for normal and abnormal structures in glomeruli, tubulointerstitium and vasculature compartments.

H.4.1. The vectors library: See appendix E.3.

H.4.2. The vectors assignment:

I. Quality Control – Whole slide images from the diagnostic and tissue interrogation cores

I.1. Purpose

To evaluate pre-analytic and analytic variables that may interfere with quality of imaging, pathology interpretation, and computational image analysis. To determine if additional processing (central processing workflow option) will be required (see sections B and C).

I.2. Adequacy of pathology material provided or generated at the KPMP CPL

The pathology committee generated guidelines and a quality control CRF ("Dx Core - .jpg Images QC" CRF 2) to evaluation adequacy of pathology material preparation. See Appendix H. The data collected will be recorded by accessing the KPMP REDCap System.

I.2.a. Adequacy of histology preparations: The KPMP core pathologists will access the password-protected KPMP DPR. Digital whole slide images will be reviewed and histology adequacy assessed. Three levels of high-resolution assessment will be provided: A) presence of pre-analytic and analytic artifact, B) type of tissue present (cortex, medulla, other tissue), C) high resolution assessment of glomeruli, tubulointerstitium, and vessels. The high resolution assessment will be performed on the formalin-fixed and paraffin-embedded sections from the diagnostic core and tissue interrogation core(s) when applicable. See Appendix H CRF 4 and CRF 5 (Central Path Quality Metrics Assessment Dx Core and Interrogation Core CRFs).

I.2.b. Adequacy of frozen sections preparations: The KPMP core pathologists will access the password-protected KPMP DPR to review frozen sections stained with H&E, and the KPMP REDCap System for report review. High resolution evaluation of adequacy of the tissue processed for immunofluorescence will include recording the number and type of antibodies used (report and JPEG images), and tissue characteristics (frozen H&E). The high resolution assessment for tissue characteristics will be performed on the OCT-embedded frozen sections from the diagnostic core and tissue interrogation core(s) when applicable. See Appendix H CRF 4 and CRF 5 (Central Path Quality Metrics Assessment Dx Core and Interrogation Core CRFs).

I.2.c. Adequacy of electron microscopy preparations: The KPMP core pathologists will access the password-protected KPMP DPR to review digital electron microscopic images and Toluidine blue stained thick section whole slide images. Three levels of high-resolution assessment will be provided: A) presence of pre-analytic and analytic artifact, B) type of tissue present (cortex, medulla, other tissue), C) high- resolution assessment of glomeruli. See Appendix H CRF 4 and CRF 5 (Central Path Quality Metrics Assessment Dx Core and Interrogation Core CRFs).

I.3. Quality Control for HIPAA compliance of the diagnostic pathology material

I.3.a. Quality control for HIPAA compliance of the diagnostic pathology material for the KPMP DPR

All KPMP personnel with direct access to participant identifying information are required to complete HIPAA training. All data and specimens will be accessible to KPMP in a de-identifiable manner except individuals involved directly in the activities related to participant recruitment and specimen processing at their respective sites. The verification of completion of appropriate CITI training modules and HIPAA training according to the role in the KPMP will be verified by the KPMP administration and the central IRB. The DVC 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 study IE. DVC personnel will ensure consistency in formatting of the labels for all RS participant materials prior to uploading into the KPMP DPR. KPMP core pathologists will access the password-protected KPMP DPR and KPMP REDCap System to confirm that participant materials are compliant with HIPAA regulations. QC for HIPAA compliance will be recorded in the "Dx Core - .jpg Images

QC" CRF (CRF 2) at the time of high-resolution assessment of adequacy. See Appendix H. Any physical records or logbooks with records of tissue tracking with identifiable information will be behind two layers of locked access (example, 2 locked doors, 1 locked door and 1 locked file cabinet or drawer). If identifiable information is found to have been shared with individuals that are not authorized, the incident will be reported to the 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. While many of these aspects are discussed elsewhere, here the DVC will specifically ensure that the vials, slides or tissue blocks received have identifiers such as name, date of birth, social security number, medical record number, surgical pathology accession number are masked. Electronic media such as emails are another source through which there can be breach of confidentiality. Scrutiny of information in the Specimen Tracking System will also be done as above and breaches notified. The individuals who are found to have accidently violate 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. 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.

I.3.b. Quality control for HIPAA compliance of the diagnostic pathology material for the KPMP tissue bock biobank

I.4. Quality Control for Whole Slide Images

Contemporary whole slide imaging workflow still represents an unresolved challenge, in that there exist a wide range of scanning appliances, all with their characteristic optical scanning properties and associated file formats. The optical point spread function of many devices is suboptimal, creating a possibility where insufficient spatial information will be captured from the study slide sets in the process of being digitized. Additionally, as the DICOM whole slide image file format has not yet been universally adopted by most whole-slide imaging vendors, the 15 or so formats in extant use represent a substantial challenge the KPMP for realizing image data interoperability. Moreover, the concomitant need for longitudinal data stewardship is also impacted if the KPMP is unable to ultimately select a single sustainable file type.

Recognizing these limitations, the DVC proposes the use of a centralized file scanning and data archival/curation service, by which the greatest degree of a) image first-time quality and b) long-term image interoperability can be realized.

Towards these goals, the Digital Pathology Unit (DPU) of the Division of Pathology Informatics at the University of Michigan already houses a high-throughput whole slide image scanning facility, along with integrated use of semi-automated computational tools for assessing image quality of batch-scanned jobs, prior to final acceptance. In addition, this unit already has the capability of natively encoding resultant image data into one of the three standard file formats (tiff, bigtiff and DICOM), effectively resolving the interoperability challenge.

The Quality Control of WSI will be recorded in the "Dx Core - .jpg Images QC" CRF (CRF 2) (see section F and Appendix H)

I.4.a. Automated Scanning Pipeline:

Upon receipt of batches of slides, arranged as study sets, DPU staff, will visually inspect, document, clean and queue slide for subsequent scanning on one of the two calibrated Leica/Aperio AT2 high-throughput scanners.

Resultant scans will be queued on the DPU's WSI automated Quality Assessment Light Microscopy Pipeline (QALM-Pipe) for evaluation of 11 spatial and colorimetric indicators of quality (see Appendix F), generating a score sheet that can be subsequently reviewed by both DPU staff and KPMP core pathologists. See Appendix H.

I.4.b. Review of Scan Pipeline Quality Metrics:

Adequacy of resultant WSI scan data: The DPU staff, working in tandem with the KPMP core pathologists at UM (and other institutions, by use of remote imaging technology will access the password-protected staging area, for spot checking of computationally assigned scan grades, making use of the QALM-Pipe data provided for each scan. Those images that are deemed to be not meeting the minimum threshold for quality will be remanded back to the DPU team for rescanning, along with suitable comments and directives, indicating the nature of the deficiency and the recommended course of action needed to result in an acceptable scan. Alternatively, evaluators may decide that the image artifacts present are beyond salvage and may recommend that that data set no be considered for promotion to the one or both of the KPMP Data Lake and/or KPMP Knowledge Environment. See Appendix H.

APPENDICES

Pathology MOP

APPENDIX A: Gross examination and triaging

A.1 Quality Assessment and Control Parameters. Pertinent metadata and pre-analytical parameters associated with specimens under interrogation are collected at the time of renal biopsy procedure. These data will be recorded manually in the renal biopsy suite, and entered electronically using the CRF 1, by accessing the REDCap and Specimen Tracking Systems. For several of the items in each category there will be additional options to select (for example, list of comorbidities, lab value fields, clinical diagnosis etc.). These are presented in more detail in the Recruitment Site MOP. Majority of the features listed are applicable to resected tissue and biopsy. The online entry system will provide options for data entry that are pertinent to the type of specimen being used (for example, only fields relevant to biopsy show when biopsy is selected). Note: By design of the KPMP MOPs, there is overlap of several of these items among the clinical, pathology and TIS MOP.

A.2 Quality Control Measures at Procurement

A.2.1. Participant Data

1. Consent
2. Subject Source
3. Subject Source ID
4. Age
5. Race
6. Sex
7. Kidney Function
8. Vitals
    a) Systolic Blood Pressure
    b) Diastolic Blood Pressure
    c) Temperature
    d) Heart Rate
    e) Respiratory Rate
9. Weight
10. Height
11. BMI
12. Pathology Diagnosis
13. Comorbidities
14. Meds
15. Lab Values
16. Clinical Diagnosis
17. Social History
    a) Smoking
    b) Drinking
    c) Recreational Drugs

A.2.2. Procedure Data

1. Type
    a) Nephrectomy (partial, total)
    b) Donor (deceased, living)
    c) Needle Biopsy (size, procedure)
    d) Wedge Biopsy
2. Site of excision
    a) Laterality (left, right)

   b) Location (superior, middle, inferior)
   c) Distance from tumor
3. Recruitment Site
4. Medication Anesthesia
5. Time stamp of procedure
   a) Retrieval time (out of the body)
   b) Transport time (OR/procedure room to lab if applicable)
   c) Processing time
6. Tissue collection reagents and supplies (*standardize supplies*)
   a) Container (bucket, cryovial, cryo mold)
   b) Media (normal saline, PBS, dry ice, preservative solution)

A.2.3. Tissue Spatial Coordinates
1. Cortex vs. Medulla
2. Gross measurements
3. Orientation
4. Image (before processing)
5. Morphological/Physical state (intact/fragmented) (support by a photograph)

A.2.4. Storage/Processing Parameters (procurement)
1. Time Variables
   a) Total time processing
   b) Time - processing to storage
   c) Retrieval time
   d) Total Storage time (Retrieval time – time at storage)
   e) Shipment date/time
2. Storage Conditions (procurement)
   a) Medium used (RNAlater, Cx media – Cryostor, 10% serum-DMSO culture media, Wisconsin solution, OCT - dry ice frozen, buffered formalin, 4% PFA, methanol)
   b) Freezing conditions Temperature (dry ice, LN2, slow freeze -80 ^{o}C RT, 4 ^{o}C, -20 ^{o}C, -80 ^{o}C)
   c) Container type (cryovial, cryomold, histology cassettes)
   d) Time when stored
   e) Time retrieved
   f) Time shipped (standardize supplies, reagents)

A.2.5. Sample Preparation/processing Parameters (for procurement)
1. Time Variables
   a) Total time processing for technology
   b) Time: processing to storage
   c) Storage time
   d) Shipment date/time
2. Storage/processing Conditions (procurement)
   a) Medium used (RNAlater, Cx media – cryostor, 10% serum-DMSO culture media, Wisconsin solution, OCT< dry ice, formalin, 4% PFA, meOH)
   b) Freezing conditions Temperature (dry ice, LN2, slow freeze -80 ^{o}C RT, 4 ^{o}C, -20 ^{o}C, -80 ^{o}C
   c) Container type (cryovial, cryomold, histology cassettes)
   d) Time when stored
   e) Time shipped (standardize supplies, reagents)

A.2.6. Tissue Composition (fixed or unfixed)
Low Resolution Assessment

a) % cortex vs. medulla
b) Medullary ray
c) Glomerular number
d) % Glomerular sclerosis (global, focal)
e) % tubular atrophy
f) % fibrosis
g) State of Vessels
h) Evidence of inflammation
i) Other morphological features (necrosis, congestion, autolysis, hemorrhage, cautery)
j) Tumor present
k) Other tissue

A.2.7. Shipping Parameters (for TIS)

1. Time Variables
   a) Storage time
   b) Shipment date/time
   c) Shipment site
2. Documentation (image)

CRF 1 – Kidney Biopsy Procedure Details

BIOPSY PROCEDURE AND TISSUE DATA (to be completed by Research Coordinator during the Biopsy)

Confidential

Kidney Biopsy Procedure Details

Study ID	_____

Biopsy Kit

Pre-biopsy safety checklist was not signed off!

Participant has not passed all safety criteria.

Type of Kit used:	○ A ○ B
Renal Biopsy Kit Number	(The Kit ID should be in the format: KL-9999999 "KL-" followed by 7 digits)
Renal Biopsy Kit Number	(The Kit ID should be in the format: KL-9999999 "KL-" followed by 7 digits)
Re-enter kit ID	_____

Kit IDs must match.

View kit info in SpecTrack here after saving the form: [bp_kit_id_2]

Enter sample IDs The sample ID should be in the format: S-9999-999999 "S-" followed by 4 digits, a dash, and 6 digits

Vial 2 Sample ID - Cryomold with OCT for IF - GREEN	_____
Vial 4 Sample ID - Cryomold with OCT - BLUE	_____
Vial 5 Sample ID - Cryostor Frozen -80 - ORANGE	_____
Vial 6 Sample ID - Snap Frozen Liquid Nitrogen - RED	_____

Confidential

Procedure Data	
Biopsy Date	_____
Operator name	_____ (Should be dropdown list)
Pathology Tech Name	_____
Procedure Type	○ Percutaneous ○ Open
Procedure guidance	○ CT ○ Ultrasound ○ Other
Specify procedure guidance	_____
Needle gauge	○ 16 gauge ○ other
Other needle gauge	_____
Will cores be measured in millimeters or centimeters?	○ mm ○ cm
Site of excision	
Laterality	○ Left ○ Right
Location	○ Upper pole ○ Middle ○ Lower pole
Length (mm)	
Length (cm)	
Passes attempted	
Pass 1	_____
Passes	
Approximate cortex length (mm)	
Approximate cortex length (cm)	

Confidential

Pass 1 attempted?	◯ Yes ◯ No
Harvest time	_____ (Use military time)
Length	_____ (If no tissue obtained, enter 0)
Renal Tissue obtained?	◯ Yes ◯ No ◯ Unknown ◯ No tissue obtained
Cortex visualized?	◯ Yes ◯ No ◯ Unknown
Approximate cortex length	_____
Cortex length > core length	
If length > 0 tissue was obtained	
1st Pass image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	◯ Yes ◯ No
1st Pass 2nd image	
Is another image needed?	◯ Yes ◯ No
1st Pass 3rd image	
Pass 2	_____
Pass 2 attempted?	◯ Yes ◯ No
Harvest time	_____ (Use military time)
Length	_____ (If no tissue obtained, enter 0)

Confidential

Renal Tissue obtained?	◯ Yes ◯ No ◯ Unknown ◯ No tissue obtained
Cortex visualized?	◯ Yes ◯ No ◯ Unknown
Approximate cortex length	_____
Cortex length > core length	
If length > 0 tissue was obtained	
2nd Pass image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	◯ Yes ◯ No
2nd Pass 2nd image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	◯ Yes ◯ No
2nd Pass 3rd image	
Pass 3	_____
Pass 3 attempted?	◯ Yes ◯ No
Harvest time	_____ (Use military time)
Length	_____ (If no tissue obtained, enter 0)
Renal Tissue obtained?	◯ Yes ◯ No ◯ Unknown ◯ No tissue obtained
Cortex visualized?	◯ Yes ◯ No ◯ Unknown

Confidential

Approximate cortex length	_____
Cortex length > core length	
If length > 0 tissue was obtained	
3rd Pass image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	◯ Yes ◯ No
3rd Pass 2nd image	
Is another image needed?	◯ Yes ◯ No
3rd Pass 3rd image	
Pass 4	_____
Pass 4 attempted?	◯ Yes ◯ No
Harvest time	_____ (Use military time)
Length	_____ (If no tissue obtained, enter 0)
Renal Tissue obtained?	◯ Yes ◯ No ◯ Unknown ◯ No tissue obtained
Cortex visualized?	◯ Yes ◯ No ◯ Unknown
Approximate cortex length	_____
Cortex length > core length	
If length > 0 tissue was obtained	

Confidential

4th Pass image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	○ Yes ○ No
4th Pass 2nd image	
Is another image needed?	○ Yes ○ No
4th Pass 3rd image	
Pass 5	_____
Pass 5 attempted?	○ Yes ○ No
Harvest time	_____ (Use military time)
Length	_____ (If no tissue obtained, enter 0)
Renal Tissue obtained?	○ Yes ○ No ○ Unknown ○ No tissue obtained
Cortex visualized?	○ Yes ○ No ○ Unknown
Approximate cortex length	_____
Cortex length > core length	
If length > 0 tissue was obtained	
5th Pass image	(To take a photo of the core - Click - Upload Document - Then - Choose File - Then - Take Photo)
Is another image needed?	○ Yes ○ No
5th Pass 2nd image	

Confidential

Is another image needed?	○ Yes ○ No
5th Pass 3rd image	

Diagnostic Core

Was a diagnostic core obtained?	○ Yes ○ No
Diagnostic core obtained in Pass number	○ 1 ○ 2 ○ 3 ○ 4 ○ 5
Diagnostic core - Vial 1 (Formalin for LM) - GRAY	
Time tissue placed in Vial 1	_____ (Record in military time (e.g. 5pm=17:00))
Diagnostic core - Vial 2 (for cryomold with OCT for IF) - GREENSample ID [bp_v2_sample_id]	
Tissue obtained for Vial 2	○ Yes ○ No
Time tissue placed on dry ice	_____ (Record in military time (e.g. 5pm=17:00))
Diagnostic core - Vial 3 (2.5% glutaraldehyde for EM) - PINK	
Tissue obtained for Vial 3	○ Yes ○ No
Time placed in Vial 3	_____ (Record in military time (e.g. 5pm=17:00))

Tissue Interrogation Core 2

Was a 2nd core obtained?	○ Yes ○ No
Core obtained in pass number	○ 1 ○ 2 ○ 3 ○ 4 ○ 5
Vial 4 (for cryomold with OCT) - BLUESample ID [bp_v4_sample_id]	

Confidential

Time tissue placed on dry ice	_____ (Record in military time (e.g. 5pm=17:00))

Tissue Interrogation Core 3

Was a 3rd core obtained?	○ Yes ○ No
Core obtained in Pass number	○ 1 ○ 2 ○ 3 ○ 4 ○ 5

Kit A

Vial 5 (Cryostor Frozen -80) - ORANGE Sample ID [bp_v5_sample_id]

Time tissue placed in hyperthermosol	_____ (Record in military time (e.g. 5pm=17:00))
Time tissue placed in Vial 5	_____ (Record in military time (e.g. 5pm=17:00))

Kit B

Vial 6 (Snap Frozen Liquid Nitrogen) - REDSample ID [bp_v6_sample_id]

Time tissue frozen	_____ (Record in military time (e.g. 5pm=17:00))

Did the biopsy have to be stopped?	○ Yes ○ No
Why was the biopsy stopped?	☐ Bleeding complication ☐ Participant request ☐ Other
Why was the biopsy stopped?	

Biopsy Procedure Details	
Anesthesia used	○ Local ○ Conscious sedation
Medications given	☐ Lidocaine ☐ Versed ☐ DDAVP ☐ Fentanyl ☐ Other ☐ None of the above
Versed dosage	_____
Versed dosage units	○ mg ○ Other
Other dosage units	_____
Fentanyl dosage	_____
Fentanyl dosage units	○ mcg ○ mg ○ Other
Other dosage units	_____
Other medications given with dosages	_____
Trocar used?	○ Yes ○ No
Gel foam applied?	○ Yes ○ No
Pressure held at biopsy site?	○ Yes ○ No
How long was pressure held at biopsy site (minutes)?	_____
Has bleeding stopped from the surface needle insertion site?	○ Yes ○ No
According to the RS MOP, pressure should be help until surface bleeding stops (~5 minutes). If surface bleed persistence seems unusual, consider reporting an AE.	
Immediate post-procedure imaging done?	○ Yes ○ No

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Type of imaging performed	○ Ultrasound ○ CT
Evidence of hematoma?	○ Yes ○ No
Hematoma size (mm)	_____
Was any part of the procedure outside of expected protocol?	○ Yes ○ No
How was the procedure outside of expected protocol?	
Enter any notes or comments (e.g. initial impressions of core composition, whether fat is included, etc.)	
There should be an image uploaded for each pass where there was tissue obtained.	
Why was there no image for pass 1?	_____
Why was there no image for pass 2?	_____
Why was there no image for pass 3?	_____
Why was there no image for pass 4?	_____
Why was there no image for pass 5?	_____
Is there and image for pass 1 -pass attempted, tissue obtained, and no image = '0' -otherwise = '1'	_____

Is there and image for pass 2 -pass attempted, tissue obtained, and no image = '0' -otherwise = '1'	_____
Is there and image for pass 3 -pass attempted, tissue obtained, and no image = '0' -otherwise = '1'	_____
Is there and image for pass 4 -pass attempted, tissue obtained, and no image = '0' -otherwise = '1'	_____
Is there and image for pass 5 -pass attempted, tissue obtained, and no image = '0' -otherwise = '1'	_____
Since there were [bp_nbr_passes_with_tissue] passes with tissue obtained, was tissue from extra passes placed in formalin?	◯ Yes ◯ No
Number of passes with tissue	_____

Remember to record the time that research core materials are placed in the -80C freezer in the Tissue Tracking CRF.

Confidential

Tissue Tracking

Study ID

_____

Mr. Frosty Timestamp

Time vial 5 placed in Mr Frosty

_____
(Vial 5 (Cryostor Frozen -80) - ORANGE)

Recruitment Site Storage

Vial 4 - Cryomold with OCT - BLUE If vial 4 indicated as collected on biopsy procedure form, record RS storage arrival below.

Date/Time Vial 4 arrived in RS storage

_____

Storage temperature (C)

_____

Vial 5 - Cryostor Frozen -80 - ORANGE If vial 5 indicated as collected on biopsy procedure form, record RS storage arrival below.

Date/Time Vial 5 arrived in RS storage

_____

Storage temperature (C)

_____

Vial 6 - Snap Frozen Liquid Nitrogen - RED If vial 6 indicated as collected on biopsy procedure form, record RS storage arrival below.

Date/Time Vial 6 arrived in RS storage

_____

Storage temperature (C)

_____

This section is to explain any potential damage to specimens.

Vial 1 (Formalin for LM) - GRAY

Vial 1 was [tt_vial_1_time] minutes from harvest to formalin. Please enter any notes about the delay getting the specimen into the formalin.

Do you think damage occurred to the specimen in vial 1?

- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 1

Vial 2 (for cryomold with OCT for IF) - GREEN

Vial 2 was [tt_vial_2_time] minutes from harvest to dry ice. Please enter any notes about the delay getting the specimen onto the dry ice.

Do you think damage occurred to the specimen in vial 2?
- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 2

Vial 3 (2.5% glutaraldehyde for EM) - PINK

Vial 3 was [tt_vial_3_time] minutes from harvest to glutaraldehyde. Please enter any notes about the delay getting the specimen into the glutaraldehyde.

Do you think damage occurred to the specimen in vial 3?
- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 3

Vial 4 - Cryomold with OCT - BLUE

Vial 4 was [tt_vial_4_time] minutes from harvest to dry ice. Please enter any notes about the delay getting the specimen onto the dry ice.

Do you think damage occurred to the specimen in vial 4?
- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 4

Confidential

Vial 5 - Cryostor Frozen -80 - ORANGE

Vial 5 was [tt_vial_5_time] minutes from harvest to hyperthermosol. Please enter any notes about the delay getting the specimen into the hyperthermosol.

Do you think damage occurred to the specimen in vial 5?
- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 5

Vial 6 - Snap Frozen Liquid Nitrogen - RED

Vial 6 was [tt_vial_6_time] minutes from harvest to liquid nitrogen. Please enter any notes about the delay getting the specimen into the liquid nitrogen.

Do you think damage occurred to the specimen in vial 6?
- ○ Yes
- ○ No

Please explain why you think damage may have occurred to the specimen in vial 6

vial 1 time	_____
vial 2 time	_____
vial 3 time	_____
vial 4 time	_____
vial 5 time	_____
vial 6 time	_____

APPENDIX B: Processing the diagnostic core

Pending: a copy of the individual processing protocols in each of the RS.

APPENDIX C: Handling of the research core(s) at the RS for shipping to CBR and downstream analysis by the TIS

APPENDIX D: De-identification of diagnostic pathology material for shipment to the CBR and DVC

D.1. De-identification Instructions

Participant identifiers should be deleted on all documents and replaced with KPMP Sample ID.

D.2. Electronic Pathology 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:

1. Participant hospital identifier-including hospital address and telephone number
2. Ordering physician's name/initials
3. Pathologist name/initials
4. Other information deemed identifiable: for example, in the comment identifiers of the treating nephrologist e.g. "the case was discussed with Dr. John Smith"

Information can be de-identified by completely deleting the text or inserting continuous XXX's in place of identifiable details.

All pages should be labeled with the KPMP Sample ID number followed by the number of the page as "XofY" where X is a page counter and Y is the total number of pages.

Figure 1: De-identified Pathology Report Example

HISTORY:

The participant is a 17 year old with a several year history of proteinuria and non-dysmorphic hematuria. [redacted] sister has similar signs and symptoms. Urinary protein: creatinine ration has ranged from 5 to 6; serum albumin has been 2.5 to 3.5 gm/dL, serum creatinine to 0.7 mg/dL. [redacted] has edema. ANA is positive, otherwise all serologies negative.

Gross:
---
1. Labeled with participant's name and hospital registration number. Received in formalin are three tan cores ranging from 0.5 cm to 1.0 cm. (3ns)
2. Labeled with participant's name and hospital registration number. Received in [redacted] fixative are for, 0.1 cm, tan-gray, soft tissue bits submitted for immunofluorescence. (4ns)
3. Labeled with participant's name and hospital registration number. Received in glutaraldehyde are four tan cores 0.1 cm each; held for processing in the EMlab. (4ns)

[redacted]

MICROSCOPIC:
LIGHT MICROSCOPY:

Sections are stained with H&E, PAS, trichrome and Jones-Silver. There are three cores of renal cortex that contain thirty glomeruli, eight of which are globally sclerotic. An additional six glomeruli display focal segmental sclerosis with obliteration of capillary lumina, excess matrix and adhesion to Bowman's capsule. The remaining glomeruli appear mildly enlarged with patent capillary lumina and glomerular basement membranes of normal thickness and contour. Tubular atrophy and interstitial fibrosis involves approximately 10% of the renal cortex. Collections of foam cells can be seen in the interstitium in a multifocal pattern throughout the tissue. Mild tubular injury can be identified in patchy locations. A few small arteries are sampled and appear normal.

IMMUNOFLUORESCENCE
MICROSCOPY:

Direct immunofluorescence examination of the renal biopsy specimen (two glomeruli) with FITC-conjugated antisera for IgG, IgA, IgM, C3, C4, C1q, albumin and fibrinogen revealed: Controls for IgG, IgA, IgM, and C3 are positive.

IgG: Negative. IgA:
Negative. IgM:
Negative.
C3: Focal glomerular positivity (nonspecific).
C1q: Negative.
C4: Negative. Albumin:
Negative.
Fibrinogen: Negative.
Kappa: Negative. Lambda:
Negative.

ELECTRON
MICROSCOPY:

All of the tissue submitted for electron microscopy was embedded and examined at the thick section level. One available glomerulus is selected for ultrastructural evaluation.

The ultrastructural preservation of the tissue is adequate. Glomerular capillary lumina are patent and mesangial areas are normal in size and cellularity. The glomerular basement membranes are diffusely thickened with an average thickness of 116 nm (range 79 to 142 nm). A few portions of the basement membrane display mild textural irregularities including internal lucent rarefactions and slight lamellation. Some portions of the basement membrane are focally thickened and wrinkled. Podocyte foot process effacement involves approximately 70% of the glomerular capillary surface. Podocytes appear hypertrophic and reactive with lipid and protein resorption droplets and focal microvillous transformation.

MICROSCOPIC
DIAGNOSIS:

Renal biopsy, light, fluorescence and electron microscopy:

1. Focal segmental and global sclerosing glomerulopathy, moderate, with diffuse thinning and segmental lamellation and thickening, consistent with hereditary nephritis.
2. Tubular atrophy and interstitial fibrosis, mild.

COMMENT:

The immunofluorescence findings provide evidence against glomerular disease of the immune complex type. Electron microscopy reveals predominant thinning and focal thickening of the basement membranes with some irregularities. This, in conjunction with the light microscopic findings of focal and global glomerulosclerosis and multifocal collections of interstitial foam cells, and a hereditary nephritis often have a predominantly thin basement membrane phenotype, as appears in this case.

[redacted]

I, [redacted] the signing staff pathologist, have personally examined and interpreted the slides from this case.

D.3. Redacting a PDF

1) From the Tools page in the PDF, choose the  *Redact* icon
2) Select text to redact
   a. Click *Mark for Redaction* from the **Redact** ribbon
   b. Click *Text & Images*

Figure 2: Example of Redact Ribbon

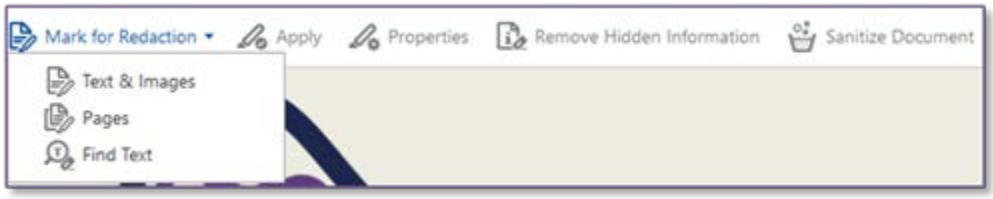


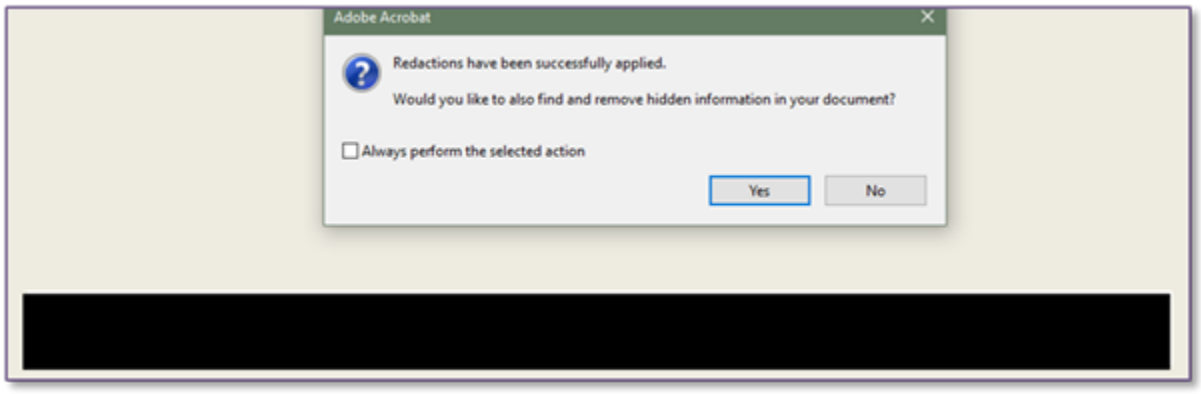
Figure 3: Example of Using Redaction Tools

c. Select text and/or images to be redacted

d. Click on *Apply* from the **Redact** ribbon
    i. See figure 7 for example of **Redact** ribbon
e. Click on *Ok* and *Yes* on the pop ups if you have selected the correct information to redact

Figure 4: Example of Message when "Apply" is Clicked

Figure 5: Example of Message When Redaction is Successful



a. Save document
    i. When you save/save as, "Redacted" will automatically appear in the file name

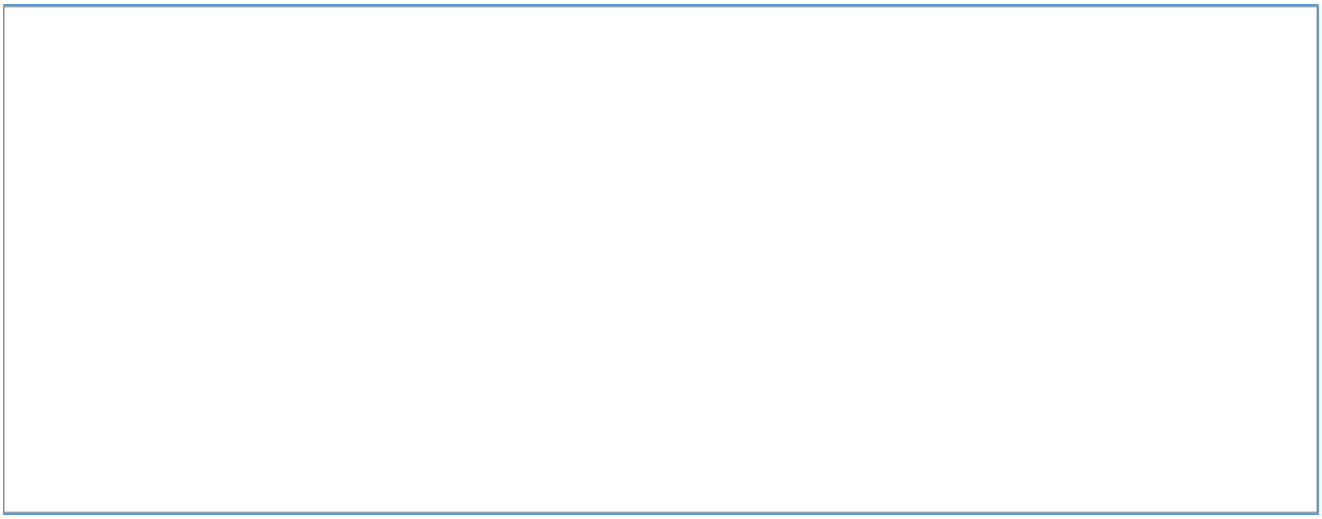
D.4. Glass Slides

All glass slides require a KPMP sample ID label to cover the original label on the glass slide. New pre-printed labels from the KPMP pathology kit include the following section that will need to be completed:

- Level indicator: to be completed by hand on site 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 Figure 6 below.
- Stain indicator: to be completed by hand on site as able to be determined. Please work with your local site pathology department to determine how this is indicated.

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 the KPMP sample ID label *over* your local site's participant identifier taking note of the slide "level". Please refer to Figure 6 below for reference.

Figure 6: Sample of slides before and after de-identification

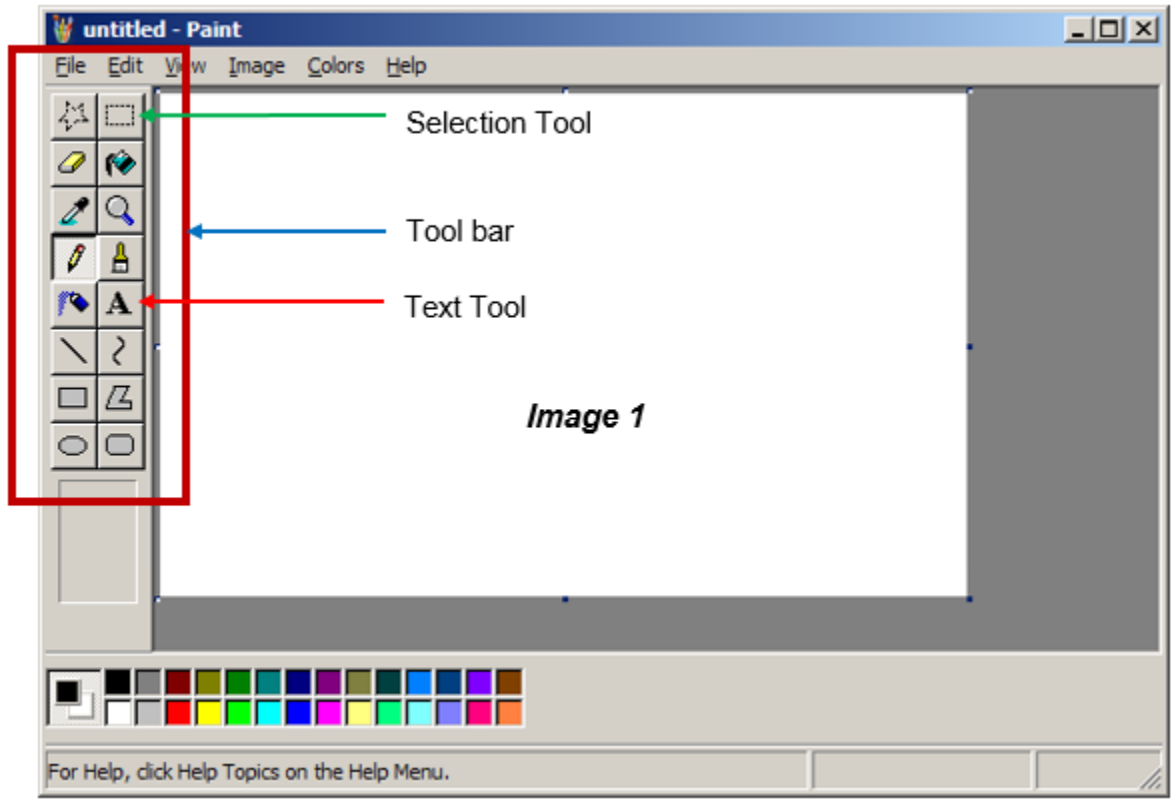


D.5. EM Digital image de-identification

Upon receiving digital images from your site's pathology department, save a copy in a local folder on your work station:

a. Open "PAINT" or a similar free image-adjusting software.

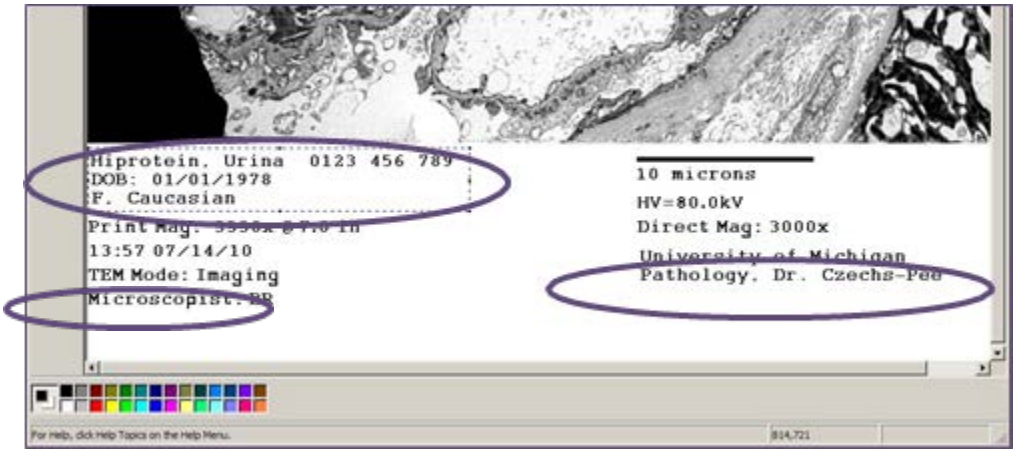
Figure 7



b. Open the EM document and review the information, noting any details which are “identifying”.

c. Review the image:

Figure 8



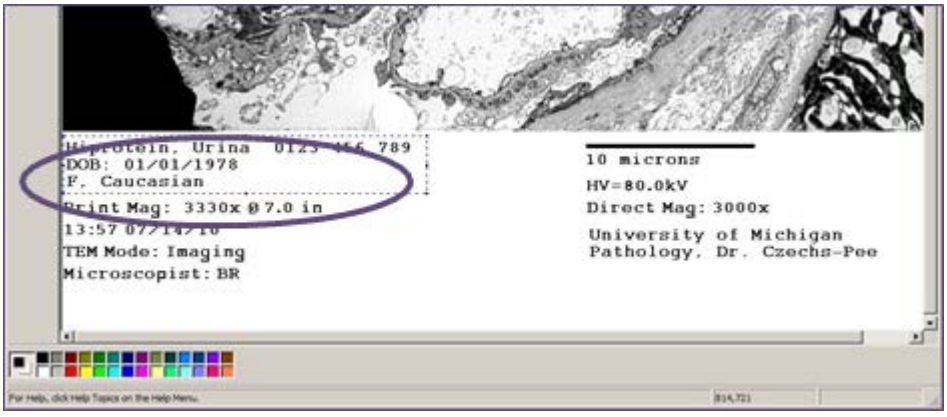
As shown in the image above, the participant's name (Urina Hiprotein), Medical Record (0123 456 789,) date of birth (01/01/1978), the site identification (University of xxxxxx), the pathologist (Dr. Czechs-Smith), and the microscopist initials (BR) are embedded at the bottom of the image. Any information that is not pertinent to the image magnification or modality and time of scoping must be removed, and includes:

- Participant's name or initials
- Participant's medical record
- Participant's Date of birth
- Gender Identification (F/M)
- Racial Identification
- Site identification by name or address
- Pathologist's name or initials
- Microscopist' s name or initials

d. Selecting/deleting identifiable information:
   - From the tool bar on the left of ***Image 1*** (this may vary at your site, depending on default settings), click on the "Select" tool. When over the image, the arrow becomes a cross-hair.

   - Move your mouse over the beginning of the text to be removed and hold the click key. Hold the click key on the mouse while dragging to select all text you would like to delete (see dashed box below).

Figure 9



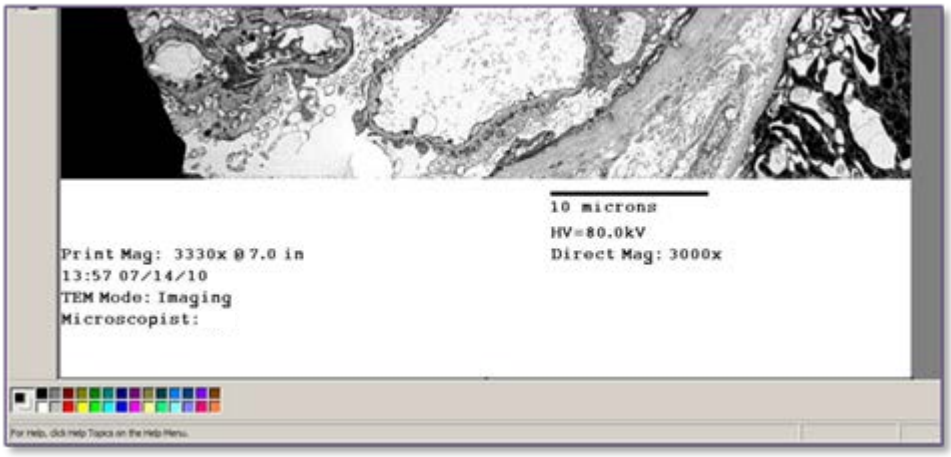
e. Release the mouse and the selected area will remain.

f. The selection can be deleted by:

- Right clicking on the mouse and selecting "Cut"; or
- From the top menu bar, selecting "Edit" and choosing "Cut"; or
- Pressing "Ctrl-X" simultaneously

g. Repeat step 4 above as necessary to remove all identifying information until the image resembles:

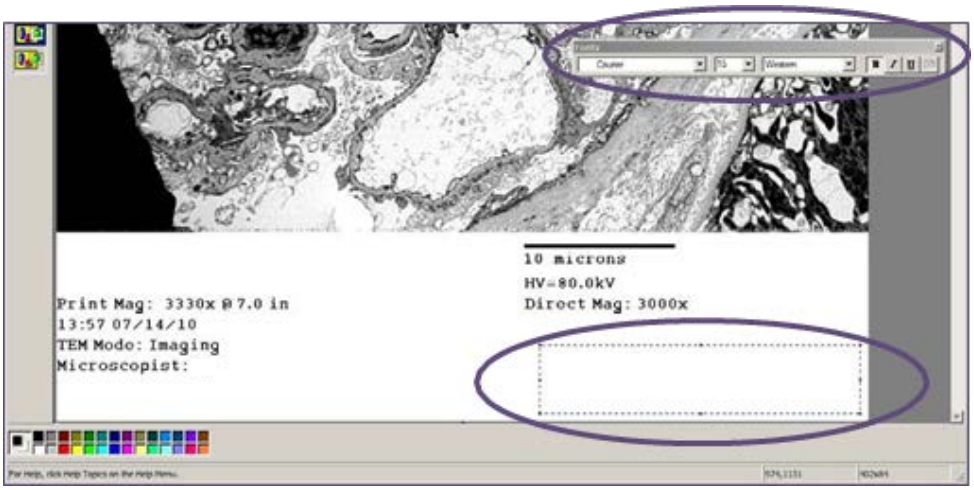
Figure 10



h. Relabeling EM images with KPMP Sample ID:

- From ***Image 1*** above, locate the "Text Tool". Click on the tool and then move the mouse to the LOWER RIGHT CORNER of the image. Drag a rectangle by clicking on the mouse and enlarging the box (see below):

Figure 11



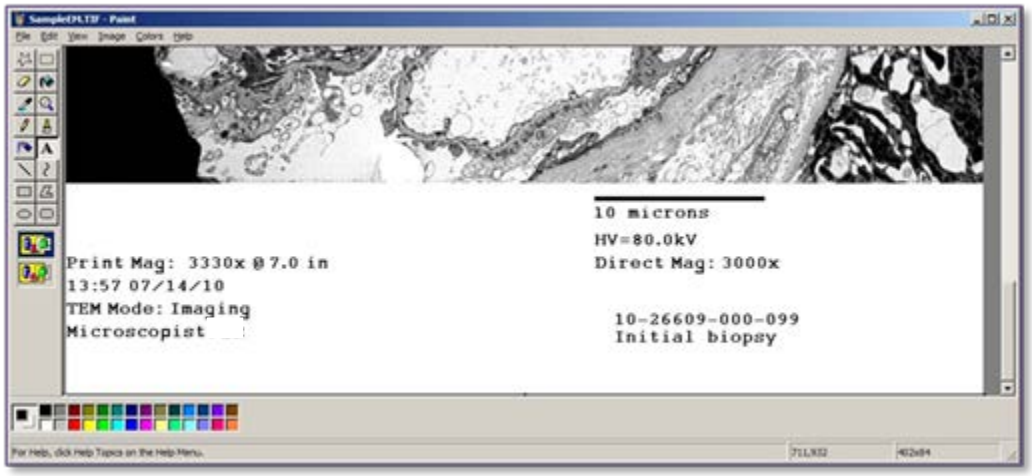
- Once the box is drawn, release the mouse key and type the following details:

  - Participant Sample ID. If you are unable to see the Text toolbar above, you may need to click the "View" dropdown menu and select "Text Toolbar". Please use the following:
  - Courier

- Western
- 15 point
- Bold

o The final study image should resemble as follows:

Figure 12



h. Taking care to NOT overwrite the original image (do not just click “save”), please save the modified image by selecting:
File >Save as>

o The file name should be the Participant Study ID (xxx-xxxx) appended with the image type and number of the total quantity of images as follows:
- *KPMP Study ID*_EM_1of4
- *KPMP Study ID*_EM_2of4
- *KPMP Study ID*_EM_3of4
- *KPMP Study ID*_EM_4of4

Note: please save de-identified images a *.jpg file type

D.6. Immunofluorescence Digital Image De-Identification

As indicated above, IF images are open with any free image adjusting software, and saved as Jpeg format, labeled with the study ID_*antibody*_1ofx. Choice of antibodies generally labeling the IF images: IgG, IgG1, IgG2, IgG3, IgG4, IgM, IgA, C3, C1q, C4, kappa, lambda, albumin, fibrinogen, PLA2R, etc. Example of IF image label:

- *KPMP Study ID*_IgG_1of4
- *KPMP Study ID*_IgA_2of4
- *KPMP Study ID*_C3_ 3of4
- *KPMP Study ID*_kappa_4of4

Figure 13

KPMP Study ID_IgG_1of2 KPMP Study ID _ID_C3_2of2

APPENDIX E: The KPMP Digital Pathology Repository (DPR)

Pending

E.1 List of conventional disease categories

E.2 The descriptor library

E.3 The vector library

APPENDIX F: KPMP Biorepository of Tissue Blocks

Conditions for shipping renal biopsy materials to or from CBR

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

F.1 Shipping procedure for materials shipped on dry ice

Materials

Provided by CBR

- KPMP Dry Ice Shipper
  - Cardboard shipping box with orientation arrows
  - Insulated shipping container (to be placed inside cardboard shipping box)
- UN1845 label (dry ice label)
- Address labels (for UN1845)
  Exempt human specimen label
- Ziploc bags from the pathology kit

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 (all frozen tissue samples (e.g. OCT molds, Cryostor, LN) can be in the same 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)). *See example photo below.* Seal the outer Ziploc bag and place it in the pre-chilled freezer box. Close the freezer box.

6. 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).*

7. Top off the KPMP Dry Ice Shipper with dry ice. Once filled, shake the Shipper from side-to-side to ensure dry ice has settled, and ***fill any space created with additional dry ice***. (You may want to put the top on before shaking the Shipper).
    a) At least 14 lbs. of dry ice must be included.

b) 

8. Place Styrofoam top on KPMP Dry Ice Shipper cooler, ensuring it “seats” properly. DO NOT TAPE THE STYROFOAM BOX!
9. Weigh the shipment and record the total weight and the dry ice weight (the dry ice weight is the total weight minus the weight of the biospecimens and the KPMP shipper, which should weigh about 2 lbs.)
10. Place completed KPMP Manifest in the KPMP Dry Ice Shipper (outside the Styrofoam inner container, but inside the outer cardboard box).
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!
    b) Note: All dry ice shipments should be shipped via FedEx **Priority Overnight Delivery**. This ensures next day delivery. The CBR FedEx account number is 644377814.
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

F.2 Shipping 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 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.
    a. Ensure the FFPE and plastic blocks have already been tracked as derivatives in SpecTrack!
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. Note: All gel pack shipments should be shipped via FedEx **Priority Overnight Delivery**. This ensures next day delivery. The CBR FedEx account number is 644377814.
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.

F.3 Renal biopsy material storage conditions at 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

Type of sample	Preservation, support medium, or stain	Number Per Participant	Additional container	Storage Temperature
	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 G: KPMP Kidney Tissue Atlas libraries

CRF 2: Quality control for HIPAA compliance on tissue blocks received at the KPMP Central Biorepository center

CRF 3: See Appendix H – CRF 3 for high resolution evaluation of adequacy of tissue – Disease Category assignment

CRF 4: See Appendix H – CRF 4 for high resolution evaluation of adequacy of tissue - QC diagnostic core– histology (includes paraffin-embedded, frozen, and plastic sections)

CRF 5: See Appendix H – CRF 5 for high resolution evaluation of adequacy of tissue – tissue interrogation core(s) – histology

CRF 6: Scoring matrix for descriptors

APPENDIX H: Quality Control – Whole slide images from the diagnostic and tissue interrogation cores

CRF 2: Dx Core - .jpg Images QC

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Dx Core - .jpg Images QC

Study ID _____

The pathology report and images have been uploaded into the "Pathology Images Upload" form. Open that form and download the pathology report and images to examine them. Then answer the following questions.

Has a complete pathology report been received?
◯ Yes
◯ No

Enter any notes regarding the missing path report. Once the report has been received, click Yes on the question below.

Has a complete pathology report been received?
◯ Yes
◯ No

Has the **PHI** been redacted from the path report?
◯ Yes
◯ No

Enter any notes regarding remediating any **PHI** found in the path report. Once the **PHI** removal has been verified, click Yes on the question below

Has a complete and redacted path report been received?
◯ Yes
◯ No

Immunofluorescence / Immunohistochemistry jpeg images

Are the Immunofluorescence / Immunohistochemistry jpeg images complete and accurate as sent?
◯ Yes
◯ No

If the images are not complete and accurate, enter the discrepancies in the note box below and take steps to remediate. Once the remediation is done, answer the question below to verify that the images are complete and accurate now.

Are the Immunofluorescence / Immunohistochemistry jpeg images complete and accurate now?
◯ Yes
◯ No

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Electron microscopy jpeg images

Are the electron microscopy jpeg images complete and accurate?
- ◯ Yes
- ◯ No

If the images are not complete and accurate, enter the discrepancies in the note box below and take steps to remediate. Once the remediation is done, answer the question below to verify that the images are complete and accurate now.

Are the electron microscopy jpeg images complete and accurate now?
- ◯ Yes
- ◯ No

Review the EM jpg images - or at least the entire scanned tissue area - at a low magnification (e.g. 4x), checking for visible out-of-focus areas or misalignments. Misalignments will typically be visible as a "stitch line" through the image, where the scanned stripes that make up the full image meet.

Zoom into the magnification at which the high-resolution image was captured, and review in a single horizontal path across the tissue section at its widest point, one field of view at a time. This will allow you to check for smaller areas of poor focus or subtler misalignments.

Perform the same field-by-field review in the vertical direction, from one edge of the tissue to the other in an unbroken line, checking again for any out-of-focus areas.

Spot check a few areas in the slide at high magnification, particularly any regions where the tissue is a different thickness or where there are any defects in the slide. These are the areas most likely to have poor focus.

Check the color of the whole slide image and compare to original slide to assess fidelity of the scanning.

Electron microscopy jpeg images

Quality of electron microscopy jpeg images
- ◯ Good
- ◯ Satisfactory
- ◯ Poor

Notes about EM image quality

CRF 3: Dx Core – Disease Categories

Confidential

Dx Core - Disease Categories

Study ID	_____
Primary clinical pathologic disease category	_____
Secondary clinical pathologic disease category	_____
Tertiary clinical pathologic disease category	_____

CRF 4: Central Path Quality Metrics Assessment Dx Core

Confidential

Central Path Quality Metrics Assessment Dx Core

Study ID _____

Formalin-Fixed/Paraffin-Embedded (FFPE) (LM Processed) from Dx core - Container 1

How many whole slide images from FFPE tissue are there?

- ◯ None
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 11
- ◯ 12

Whole Slide Image 1 of [pqc_ff_nbr]

Sample ID _____ (Whole Slide Image ID)

Stain Used

- ◯ H&E
- ◯ PAS
- ◯ TRI
- ◯ SIL
- ◯ Other

Other stain used _____

Overall Quality

- ◯ Acceptable
- ◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

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Tissue Fragments	
Maximum number of tissue fragments in specimen	_____
% cortex	_____
% medulla	_____
% other tissue	_____
Total percent is [pq_ff_tf_total]. It should equal 100	
total percent	_____ (Total % of cortex + medulla + other)

Glomeruli	
total # glomeruli (count first and last level)	_____
average # glomeruli per level	_____
total # globally obliterated glomeruli	_____
Number of obliterated glomeruli cannot be more than total glomeruli	
average # globally obliterated glomeruli	_____

Tubulointerstitium	
Features of acute injury	◯ Yes ◯ No ◯ Unable to assess
Features of chronic injury	◯ Yes ◯ No ◯ Unable to assess

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Vessels

Interlobular	◯ Yes ◯ No (Well-defined internal elastic lamina layer)
Arcuate	◯ Yes ◯ No
Arterioles	◯ Yes ◯ No

Frozen sections from OCT block (IF) from Dx Core - Container 2

How many frozen section whole slide images are there from OCT block of Dx core with H&E?	◯ None ◯ 1 ◯ 2 ◯ 3 ◯ 4

Whole Slide Image 1 of [pqc_if_nbr]

Sample ID

_____
(Whole Slide Image ID)

Whole Slide Image 2 of [pqc_if_nbr]

Sample ID

_____
(Whole Slide Image ID)

Whole Slide Image 3 of [pqc_if_nbr]

Sample ID

_____
(Whole Slide Image ID)

Whole Slide Image 4 of [pqc_if_nbr]

Sample ID

_____
(Whole Slide Image ID)

Immunofluorescence	◯ IgG ◯ IgA ◯ IgM ◯ C3 ◯ C1q ◯ Alb ◯ Fib ◯ kappa ◯ lambda

Confidential

Tissue fragments	
Maximum number of tissue fragments in specimen	_____
% cortex	_____
% medulla	_____
% other tissue	_____
Total percent is [pq_if_tf_total]. It should equal 100	
total percent	_____ (Total % of cortex + medulla + other)

Glomeruli	
total # glomeruli	_____
average # glomeruli per level	_____
total # globally obliterated glomeruli	_____
Number of obliterated glomeruli cannot be more than total glomeruli	
average # globally obliterated glomeruli	_____

Thick sections from glutaraldehyde-fixed/plastic embedded blocks (EM) from Dx core - Container 3

How many thick section whole slide images are there from glutaraldehyde-fixed/plastic embedded blocks (EM) from Dx core?	◯ None ◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6

Confidential

Whole Slide Image 1 of [pqc_em_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality

◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 2 of [pqc_em_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality

◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 3 of [pqc_em_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality

◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 4 of [pqc_em_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality

◯ Acceptable
◯ Unacceptable

Confidential

	Good	Satisfactory	Poor
Color Intensity	○	○	○
Section Thickness	○	○	○
Section Integrity	○	○	○

Whole Slide Image 5 of [pqc_em_nbr]

Sample ID

_____
(Whole Slide Image ID)

Overall Quality

○ Acceptable
○ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	○	○	○
Section Thickness	○	○	○
Section Integrity	○	○	○

Whole Slide Image 6 of [pqc_em_nbr]

Sample ID

_____
(Whole Slide Image ID)

Overall Quality

○ Acceptable
○ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	○	○	○
Section Thickness	○	○	○
Section Integrity	○	○	○

Tissue Fragments

% cortex

_____

% medulla

_____

% other tissue

_____

Total percent is [pq_em_tf_total]. It should equal 100

total percent

_____
(Total % of cortex + medulla + other)

Confidential

Glomeruli
total # glomeruli _____
average # glomeruli per level _____
total # globally obliterated glomeruli _____
Number of obliterated glomeruli cannot be more than total glomeruli
average # globally obliterated glomeruli _____

CRF 5: Central Path Quality Metrics Assessment Interrogation Core
Confidential

Central Path Quality Metrics Assessment Interrogation Core

Study ID _____

Frozen sections from OCT block from tissue interrogation core stained with HE - Container 4

How many frozen section whole slide images are there from OCT block of tissue interrogation core?

- ◯ None
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6

Whole Slide Image 1 of [pqc_tic_nbr]

Sample ID _____ (Whole Slide Image ID)

Overall Quality

- ◯ Acceptable
- ◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 2 of [pqc_tic_nbr]

Sample ID _____ (Whole Slide Image ID)

Overall Quality

- ◯ Acceptable
- ◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Confidential

Whole Slide Image 3 of [pqc_tic_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality
◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 4 of [pqc_tic_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality
◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 5 of [pqc_tic_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality
◯ Acceptable
◯ Unacceptable

	Good	Satisfactory	Poor
Color Intensity	◯	◯	◯
Section Thickness	◯	◯	◯
Section Integrity	◯	◯	◯

Whole Slide Image 6 of [pqc_tic_nbr]

Sample ID

_____ (Whole Slide Image ID)

Overall Quality
◯ Acceptable
◯ Unacceptable

Confidential

	Good	Satisfactory	Poor
Color Intensity	○	○	○
Section Thickness	○	○	○
Section Integrity	○	○	○

Tissue Fragments

% cortex

_____

% medulla

_____

% other tissue

_____

Total percent is [pq_tic_tf_total]. It should equal 100

total percent

_____

(Total % of cortex + medulla + other)

Glomeruli

total # glomeruli

_____

average # glomeruli per level

_____

total # globally obliterated glomeruli

_____

Number of obliterated glomeruli cannot be more than total glomeruli

average # globally obliterated glomeruli

_____

Tubulointerstitium

Features of acute injury

○ Yes
○ No
○ Unable to assess

Features of chronic injury

○ Yes
○ No
○ Unable to assess

E.4. Frozen sections from OCT block from tissue interrogation core stained with HE

Container 4

Definitions

Overall quality

Assess as acceptable (1) or unacceptable (0). Expert pathologist assessment of the overall utility of the stained slide including pre-analytic quality (dehydration, crush). Is the slide of sufficient quality to be used for diagnostic purposes?

Score remaining metrics as good (2), satisfactory (1), or poor (0).

Color intensity

- Good – Stain shows good contrast between nucleus and cytoplasm. Details are readily visible (i.e. glomerular basement membranes stain clearly with silver).
- Satisfactory – Slide staining quality may show less distinct contrast and color brilliance, but is suitable for diagnosis
- Poor – tissue did not take up the stain evenly or adequately and is suboptimal for diagnostic evaluation.

Section thickness

- Good (2) – section is cut at the appropriate 2-3 micron thickness and is sufficient for diagnosis
- Satisfactory (1) – section shows variations in thickness, but is still sufficient for diagnosis.

Section integrity

- Good (2) – section demonstrates an absence of chatter, folding, or holes
- Satisfactory (1) – section shows some evidence of chatter, folding, or holes, but is still acceptable for diagnosis.
- Poor (0) – section shows significant chatter, folding, or holes and is unacceptable for diagnosis.

Features of acute injury: degenerative and regenerative changes, loss of proximal tubular brush border, luminal irregular profiles, pathologic vacuolization of tubular epithelium, inflammation and edema.
Features of chronic injury: interstitial fibrosis and tubular atrophy.
Interlobular artery: renal arteries that branch at right angles from the arcuate arteries, course between medullary rays and give rise to afferent arterioles.
Arcuate artery: renal arteries that curve along the corticomedullary junction, ascend the lateral surface of the renal pyramids and terminate mid-renal lobe. These arteries do not anastomose.

CRF 6: Scoring matrix for descriptors (see appendix G)