STANDARD OPERATING PROCEDURE

nPOD Kidney Pilot Case Processing

OPPC-SOP-01

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KIDNEY CASE PROCESSING

POLICY: Use universal safety precautions when handling human samples and use appropriate personal protective equipment. Follow biohazardous and chemical safety procedures and dispose of waste tissues according to UF EH&S guidelines. Handle sharps carefully and dispose in sharps containers. Follow aseptic procedures throughout processing.

PURPOSE: The purpose of this Standard Operating Procedure (SOP) is to outline procedures for processing and storing kidney and other human samples including serum and whole blood by the UF Organ Processing and Pathology Core (OPPC).

SCOPE: This SOP will be applied to all samples recovered through the CARE- program.

RESPOSIBILITIES: Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
Laboratory personnel - are responsible for reading and understanding this SOP and related documents and performing these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

EQUIPMENT & MATERIALS: The materials, equipment and forms in the following list are recommendations only and alternative products may be substituted for the site specific task or procedure.

- Kimberly-Clark Benchtop Protector (Fisher, Cat. No. 15-235-101)
- Leica L'Absorbs towel (Leica, Cat. No. 3803240)
- Double Edge Pathology Blades for Pathco Handle (Fisher, Cat. No. 23-720-200)
- Pathco Handle (Fisher, Cat.No. NC9552049)
- Dissection Board (Fisher, Cat. No. 36114)
- Dissecting Forceps (Fisher, Cat. No. 13-812-40)
- Dissecting Scissors (Fisher, Cat.No. 08-940)
- Microtubes with Silicone O-ring (VWR, Cat. No. 89004-302)
- Tissue-Tek O.C.T. Compound Media (VWR, Cat.No. 25608-930)
- Invitrogen RNALater™ Stabilizing Solution (Invitrogen, Cat. No. AM7021), store at room temperature
- D-PBS 1X without Ca^{2+} or Mg^{2+} (Fisher, Cat. No. MT21040CM), store at 4°C
- DMEM/F12 50/50 Media, with L-Glutamine and 15mM HEPES, (Fisher, Cat. No. MT10092CM)
- Antibiotic-Antimycotic solution, 100x, 10,000 I.U./ml Penicillin

- TruSlice Specimen Cut Up System (CellPath, CBA-0100-00A)
- Black Diamond™ Disposable Pathology Knives (CellPath, CAA080101A)
- 32 oz. Screw Top Polypropylene Histology Container (Fisher, Cat. No. 22-026-315)
- 52 oz. Plastic Container with Lid (Fisher, Cat. No. 02-544-127)
- 10% Neutral Buffered Formalin (NBF) (Fisher, Cat. No. 23-245-685)
- 16% Paraformaldehyde (Electron Microscopy Sciences, 15710)
- 8% Gluteraldehyde (Electron Microscopy Sciences, 16020)
- 200 mM Sodium Cacodylate Buffer (Electron Microscopy Sciences, 11652)
- Embedding LWS Uni Cassettes (Electron Microscopy, Cat.No. 62500-GR)
- Tissue-Tek Mega Cassette (Electron Microscopy, Cat.No. 62512-30)
- Disposable Base Molds, 15x15mm, 24x24mm, 30x24mm, 37x24mm (Fisher, Cat.No. 22-050-159 to 162)
- Disposable Deep Base Mold for Macro-Cassette (Electron Microscopy, Cat.No. 62353)
- Tissue-Tek O.C.T. Compound Media (VWR, Cat.No. 25608-930)
- Parafilm M™ Wrapping Film (Fisher, Cat.No. S37440)
- Kimberly-Clark Fluidshield Fog-Free Protective Mask (Fisher, Cat. No. 19-003-495)
- Disposable Lab Coats
- 10,000 µg/ml Streptomycin 25 µg/ml Amphotericin B (Fisher, Cat. No. MT30004CI), aliquot 5 ml and store at -20°C
- HyClone Fetal Bovine Serum (Fisher, Cat. No. MT35016CV), aliquot 50 ml and store at -20^{o}C
- High-Performance centrifuge tubes, 15 and 50 ml, Sterile (VWR, Cat. No. 89039-666 and 89039-658)
- Sterile Nunc Cryotubes (Thermo Sci, Cat No. 375418)
- 100 mm x 20 mm Petri dishes (Fisher, 08-772E)
- Weigh boats
- Pipettes and sterile filter tips (20 µl, 200 µl, 1000 µl)
- Dry ice
- 2-Methylbutane
- Magic Touch 2™ Ice Pans, 9L with lid (Bel-Art, Cat.No. M16807-9104)
- Integra Miltex Surgical Instrument Cleaner (Fisher, Cat.No. 12-460-424)
- PDI™ Super Sani-Cloth™ Germicidal Disposable Wipes (Fisher, Cat. No. 23-100-124)
- Diversey™ Virex® Tb Disinfectant (Office Depot, Cat.No. 898168)
- 70% ethanol
- Concentrated bleach (6% sodium hypochlorite)
- Biohazard sharps containers
- Label printers (cab EOS1, Brady BSP31 Label Attachment System)
- Centrifuge
- Adjustable tilt rocker
- Microtube racks
- Balance, 200g
- Nitrile gloves
- Permanent marker
- Biosafety cabinet

PROCEDURE:

1.0 Identification of tissue and aliquots from samples

1.1 Sample Type Nomenclature and Abbreviations:

Table 1. Sample Type Nomenclature and Abbreviations

Sample Type	Sample Type Abbreviation
Right Kidney Superior Pole	RKSP
Right Kidney Inferior Pole	RKIP
Right Kidney Lateral Surface	RKLS
Right Kidney Cortex	RKC
Right Kidney Medulla	RKM
Left Kidney Superior Pole	LKSP
Left Kidney Inferior Pole	LKIP
Left Kidney Lateral Surface	LKLS
Left Kidney Cortex	LKC
Left Kidney Medulla	LKM
Renal Artery	Renal Artery
Whole Blood	PBMC
Whole Blood	Plasma
Serum	Serum

1.2 Aliquots from samples will be identified as follows:

Table 2. Aliquot Type

OCT
Paraffin
Vial
EM

2.0 Case number assignment

2.1 Kidney organ donors will be assigned sequential case numbers for the University of Florida processing facility.

3.0 Aliquot labeling

3.1 Refer to SOP Tissue Sample Archiving

3.2 Cassettes for paraffin embedding
- 3.2.1 Line #1: Case ID + Block # + Sub-division where applicable (e.g., 6101-01A)
- 3.2.2 Line #2: Sample type abbreviation (Section 1.1)
- 3.2.3 Line #3: 2D Barcode

3.3 O.C.T. cryomolds
- 3.3.1 Line #1 and #2: As for cassettes

3.4 Cryovials and Fresh tissue
- 3.4.1 Line #1 Case ID + aliquot number
- 3.4.2 Line #2: Sample type abbreviation (Section 1.1)
- 3.4.3 Line #3: Aliquot type (Section 1.2)
- 3.4.4 Line #4: 2D Barcode

4.0 Data collection

4.1 Sample processing and donor data will be recorded in the nPOD databases. Access will be limited to UF Organ Processing and Pathology Core staff and will be granted by the Administration or OPPC Director. All collected data pertaining to research activities will be made available through the online nPOD database.

4.2 Use a single line to strikethrough corrections, then initial and date.

4.3 Identify and record all shipment contents.
- 4.3.1 Photograph interior of container if there is any packing abnormality (ie. melted ice, missing items).
- 4.3.2 In the event of any shipment error, contact on-call administration staff who will notify the OPO.
- 4.3.3 Complete the Recovery Feedback section on the Case Worksheet.

4.4 Completely fill out the case worksheet form during processing.
- 4.4.1 After the case has been processed, archive the case worksheet and enter all data following SOP 52 Case Data Management and SOP 55 Sample Data Management.

5.0 Sterile media and fixative preparation

5.1 Complete media
- 5.1.1 Remove and discard 50ml from 500ml DMEM/F12 media.
- 5.1.2 Add 50ml FBS to the 450ml DMEM/F12 for a final concentration of 10% FBS.
- 5.1.3 Add 5ml 100X antibiotic/antimycotic stock.
- 5.1.4 Label the sterile media container with preparation date, additives, and preparer's initials. Store at 4°C for up to one month.

5.2 Phosphate-Buffered saline
- 5.2.1 Add 5ml 100x antibiotic/antimycotic stock to 500ml 1x D-PBS (without Ca^{2+} or Mg^{2+}).
- 5.2.2 Label the sterile D-PBS container with the preparation date, additive, and preparer's initials. Store at 4°C for up to one month.

5.3 Transmission Electron Microscopy fixative
- 5.3.1 Prepare 2% paraformaldehyde/2% glutaraldehyde in 0.1M cacodylate buffer in 50mL conical tubes with one tube per tissue sample collected. At least one additional tube is needed for mincing sample in fixative prior to transfer.
  - 5.3.1.1 Mix the following components in each 50 mL conical tube:
    - 5.3.1.1.1 5 mL 16% paraformaldehyde stock
    - 5.3.1.1.2 10 mL 8% glutaraldehyde stock
    - 5.3.1.1.3 20 mL 200 mM sodium cacodylate buffer

5.3.1.1.4 5 mL dH_2O

6.0 Fixation and freezing preparation

6.1 Add 5-10 lbs dry ice pellets to an ice pan and cover.

6.2 Add 5 lbs dry ice pellets and vial racks to a second ice pan, fill pan ~5 cm with 2-Methylbutane and cover. Ensure enough 2-Methylbutane is added such that vials are 75% submerged.

6.3 Pour 10% NBF into two 52 oz. plastic containers until half full, then secure lids.

6.3.1 Label one container with case ID number, date, and "10% Neutral Buffered Formalin" for cassettes.

6.3.2 Label second container as "Waste Tissue, 10% Neutral Buffered Formalin".

7.0 Tissue dissection and blood processing

7.1 Whole Blood

7.1.1 Mix one tube well by inversion, then aliquot 100 µl for HbA1c testing using the DCA Vantage.

7.1.2 Refer to UF OPPC SOP 59 Isolation of PBMC for further processing of whole blood into PBMC. Refer to SOP 59 Isolation of PBMC for further processing of whole blood into PBMC.

7.1.2.1 To prepare plasma aliquots: Centrifuge desired quantity of tubes at 1300 x g for 10 minutes at room temperature.

7.1.2.2 If hemolysis observed, record the degree and re-centrifuge at the same settings for an additional 5 minutes.

7.1.2.3 Aliquot 700 µl of plasma into labeled O-ring microtubes. Snap freeze in 2-methylbutane/dry ice then place on dry ice.

7.2 Serum

7.2.1 Centrifuge tubes at 1300 x g for 10 minutes at room temperature.

7.2.2 If hemolysis observed, record the degree (i.e., light or gross) and re-centrifuge at the same settings for an additional 5 minutes.

7.2.3 Aliquot 300 µl of serum into the first three labeled O-ring microtubes, then 700 µl into subsequent aliquots. Snap freeze in 2-methylbutane/dry ice, then place on dry ice.

7.2.4 Aliquots 1 through 3 will be used for autoantibody, C-peptide, and QC analysis. Refer to SOP 85 C-Peptide Determination and SOP 22 Autoantibody Screening Process.

7.3 Kidney

7.3.1 Remove and discard surrounding adipose and capsule using blunt dissection technique and surgical dissection tools as needed.

7.3.2 Take a photo of the kidney and vasculature. Record kidney weight.

7.3.3 Section kidney on a transverse plane into three regions: superior pole, lateral surface, and inferior pole (Appendix 1).

7.3.4 Process kidney into flash frozen vials, FFPE blocks, OCT blocks, and transmission electron microscopy samples. Sample equally from kidney poles and lateral regions for each sample type (Appendix 1).

7.3.5 Flash frozen vials.

7.3.5.1 Mince cortex and place in 8 microtubes, 0.5g of tissue per vial.

7.3.5.2 Place the vial in the dry ice/2-methylbutane bath for 120 seconds, then transfer to the dry ice container.

7.3.6 FFPE and OCT blocks

7.3.6.1 Alternate sections for FFPE and OCT blocks, with the sample spanning from capsule to renal pelvis.

7.3.6.2 Place 3-5 mm thick sections of medulla and cortex for paraffin processing in labeled cassettes, then place cassettes in container with 10% NBF labeled with the case ID.

7.3.6.3 Record the processing start time when the first cassette is placed in fixative and end time when the last cassette is placed in fixative. If kidney is cystic, make 1-2 paraffin blocks of cysts.

7.3.6.4 Place enough OCT media to cover the bottom surface of the cryomold, then place 3-5 mm thick section of medulla and cortex on top of media in the center of the mold. Add OCT until the tissue is covered.

7.3.6.5 Place the cryomold in the dry ice/2-methylbutane bath for 120 seconds, then transfer the frozen block to the dry ice container.

7.3.7 Transmission electron microscopy (TEM)

7.3.7.1 Collect 2 mm x 2mm x 2mm thick section of cortex and place each section in a separate 10cm Petri dish containing 10-15mL TEM fixative at room temperature.

7.3.7.2 Mince each tissue sample into cubes using microdissection scissors or sterile scalpel directly in the petri dish with fixative. Tissue pieces must be less than or equal to 0.5mm on two sides.

7.3.7.3 Transfer minced tissue to conical tube with 40 mL of room temperature fixative. Fix for 24 hours at 4°C on an adjustable tilt rocker.

7.3.7.4 Tissue should be embedded in Spurr resin within 7 days of fixation.

7.4 Vasculature

7.4.1 Identify the renal artery and section on a transverse plane at 3 mm intervals.

7.4.2 Make 2 FFPE and 2 OCT samples as in 7.3.6.

7.4.3 Make 2 flash frozen vials as in 7.3.5 depending on amount of tissue available.

7.5 Sample Archiving and Processing Completion

7.5.1 Fix cassettes using an automatic processor or manually for 48 hours at room temperature in 1-2 L of 10% NBF with magnetic stirring. Record fixation start time.

7.5.1.1 Formalin volume must be at least 20 times greater than tissue volume. Ensure cassettes are completely covered.

7.5.2 Record fixation end time and transfer cassettes to tissue processor. Follow tissue processing protocol for kidney. If tissue processor is unavailable, transfer cassettes to a 52oz container labeled with the Case ID number, and the date, then fill with 70% ethanol. Place container in processing room fridge until tissue processor is available.

7.5.2.1 Set tissue processor for “kidney”.

7.5.2.2 Embed tissue into paraffin after processing

7.5.3 Make serial sections from two FFPE blocks per region and stain using H&E, Periodic Acid Schiff, Sirius Red and Jones silver stain.

7.5.4 Transfer all snap frozen materials and OCT. blocks to a clear bag labeled with the Case ID number. Store at -80°C until tissue archiving.

7.5.5 Send sample to Dr. Fogo’s lab at the Vanderbilt medical center for further processing and analysis by electronic microscopy.

7.5.6 Materials obtained by this program will be inventoried in the nPOD database and archived in the OPPC according to SOP 55 Case Data Management, SOP 52 Sample Data Management, and SOP Tissue Sample Archiving.

7.5.7 Dispose of any remaining tissue according to SOP 66 Specimen Disposal.

7.5.8 Sterilize all work surfaces using bleach or VirexTb then wipe surfaces with 70% ethanol to remove residue. Wash all surgical tools and dissection boards using surgical instrument cleaner, then autoclave tools to sterilize. Place dissection boards, pens, pipettes, camera, and other non-autoclavable items in biosafety cabinet and expose to UV radiation for at least 30-45 minutes to decontaminate.

7.5.9 Dispose of all biohazardous waste according to UF EH&S guidelines.

REFERENCES:

1.0 Related Documents and Procedures

1.1 Clinical Association of Pathology Anatomic Pathology Manual

1.2 UF Biological Waste Disposal Policy

1.3 Campbell-Thompson, et. al. Processing of human pancreas. JoVE, 2012.

1.4 SOP 22 Autoantibody Screening

1.5 SOP 26 Autoantibody Radioimmunoassay

1.6 SOP 52 Sample Data Management

1.7 SOP 55 Case Data Management

1.8 SOP 59 Isolation of PBMC

1.9 SOP 66 Specimen Disposal

1.10 SOP 85 C-Peptide Determination

1.11 GDL Tissue Sample Archiving

REVISION HISTORY

Version	Date	Revision
0	1/24/17	Created working SOP
1	7/31/17	Updated working SOP
2	5/8/18	Updated working SOP

Prepared by	Maria Beery		
Edited by	Irina Kusmartseva		
Approved by	Dr. Kusmartseva		09/17/2021
	Name	Signature	Date

Appendix 1

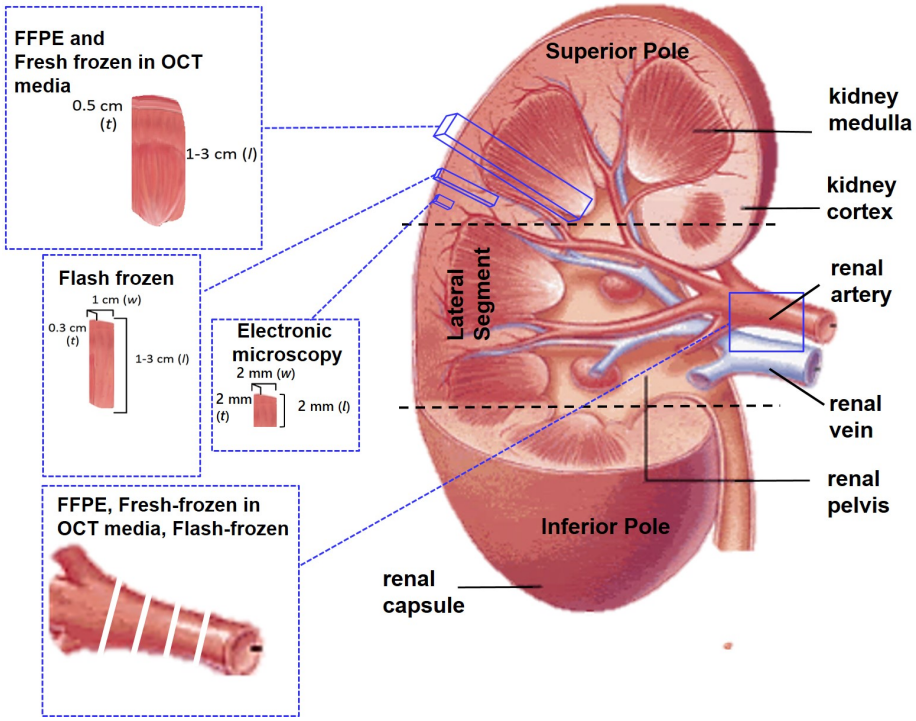

Figure 1. *Proposed scheme of the kidney sample preparation.* Both fixed and cryopreserved kidney blocks (top) should be 0.5 cm thick x 1 cm wide x 1-3 cm long and span from capsule to renal pelvis, including cortex and medulla. Flash frozen and transmission electron microscopy sections (bottom) should be sectioned from cortex only.