**Core A: Hepato-Renal Fibrocystic Diseases Translational Resource**

**Collection and preparation of Human Tissue Samples from patients with ARPKD or other Hepato-Renal Fibrocystic Diseases**

**SAMPLE COLLECTION:**

**Tissue may be collected at autopsy, or from a nephrectomy or hepatectomy.**

**KIDNEY**

* ~ 25% of total available tissue should be collected fresh, under sterile conditions.
* ~ 50% of total available tissue is to be frozen.
* ~ 25% is to be either fixed in 10% neutral buffered formalin (NBF) or placed in RNA*later*.

*See detailed instructions provided below for collection and processing of the specimens.*

Biological specimens that contain chemical preservatives such as ethanol, formaldehyde or formalin are regulated as hazardous materials by both the U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA). Hazardous materials are generally defined as any substance that could adversely affect the safety of the public, handlers or carriers during transportation. Shipments of hazardous materials must be packaged, marked and accompanied by a dangerous goods declaration.

**Neutral Buffered Formalin** - If the solution is <10%, there is no limit to the amount that can be used, and the solution does NOT fall under the dangerous goods category. There is also no limit of volume for the outer package.

Personnel shipping samples must be trained in IATA regulations.

**SUPPLIES:**

**Sites are provided with a “kit” from this Study that includes the following:**

* Standard histology cassettes
* Aluminum foil
* Black Sharpie Marker
* Specimen containers of 10% neutral buffered formalin (NBF)
* Specimen containers of RNA*later* solution
* Absorbent pads
* Biohazard ziplock baggies
* Parafilm
* Shipping containers (x2) (with insulated inner container and outer cardboard box)
* Packing material (bubble wrap)
* Packaging tape
* Appropriate labels and inserts:
  + Biohazard labels
  + UN 1845 Dry ice labels
  + UN 3373 Biohazard Substance, Category B labels
  + Exempt Human Specimen Label

**Supplies the collection sites must provide:**

* Wet ice.
* Sterile specimen containers (for fresh tissue).
* Liquid nitrogen (LN2) for snap-freezing of samples. Alternatively, samples may be frozen over dry-ice if LN2 is not available.
* LN2 vapor-phase or -80oC mechanical freezer for storage of samples until shipped. Alternatively, samples can be stored over dry ice.

**PATIENT UNIQUE IDENTIFIER AND SAMPLE LABELING:**

**Patient Study Identification Number:**

* Sites must contact Jasmine Jaber, the Study Coordinator, to obtain a Patient Study ID # for labeling of the sample containers.
* This will allow the samples to be provided to the study as de-identified specimens without patient identifiers.
* Contact Information:
  + Jasmine Jaber
  + CCRP Clinical Research Coordinator.
  + Phone: 202-476-2838 (9AM-5PM Monday-Friday)
  + E-mail: [jjaber@childrensnational.org](mailto:jjaber@childrensnational.org)

**Labeling of specimen containers:**

* Each cassette should be labeled as follows:
  + Lip – Patient Study ID #
  + One Side - Date of specimen collection
  + Other side: Tissue Type & Disease (if applicable
* Each specimen container of 10% NBF should be labeled with:
  + Patient Study ID #
  + Date of specimen collection
  + Tissue Type & Disease (if applicable)
* Each specimen container of RNA*later* should be labeled with:
  + Patient Study ID #
  + Date of specimen collection
  + Tissue Type & Disease (if applicable)

**SAMPLE PREPARATION:**

**Kidney:**

*NOTE: The instructions below assume the kidney is obtained at autopsy. If tissue is obtained at nephrectomy, obtain as much remnant [not needed for diagnosis] tissue as possible, preserving the cortical area. Section into sizes as indicated and process as requested below.*

* Please use sterile technique for tissue handling.
  + Cut the fresh kidney lengthwise (into two halves) and then cut each half transversely (4 quarters)
  + Place one quarter of the kidney in a sterile bag and tie securely to prevent leakage.
* Place the bag with kidney tissue into a biohazard bag filled with **wet ice** and tie securely to prevent leakage.
  + Cut the remaining kidneyinto approximately 1cm x 1cm x 3mm specimens (~20 samples or more if possible) of a size that will fit into a standard histology cassette. This facilitates uniform freezing and utilizes a standard storage container.
  + Place ~5 specimens into the cup of RNA*later*.
  + Place ~5 dissected specimens of kidney into pre-labeled (for kidney) specimen container of 10% neutral buffered formalin.
  + Place the remaining specimens in individual pre-labeled (see above) histology cassettes.
  + Wrap each cassette in aluminum foil.
  + With a Sharpie pen, write the specimen type (kidney) on the foil.
  + Snap-freeze by placing cassettes in a vapor phase dewar of LN2; alternatively, they can be frozen over dry ice or on the shelf of a -80o or -20o freezer.

**SAMPLE PROCESSING:**

**Fresh Tissue:**

* Samples should be held on **wet ice** until packaged for shipment.

**RNA*later* Specimen Handling and Storage:**

* Specimens in RNAlater should remain at room temperature until packaged for shipment.

**Fixed Specimen Handling and Storage:**

* For fixing specimens, the volume of fixative (10% NBF) should be 10X the volume of the sample to assure proper fixation.
* Specimens in 10% NBF should remain at room temperature until packaged for shipment.

**Frozen Specimen Handling and Storage:**

* Samples should be snap-frozen in LN2 vapor phase. Alternatively, samples may be frozen over dry-ice, if LN2 is not available.
* If neither LN2 nor dry ice is readily available, specimens may be placed on the shelf of a -80o (preferred alternative) or non-self-defrost -20o freezer until dry ice is available.
* For storage, place all frozen specimens from one organ into a separate biohazard ziplock baggie labeled with the organ name (e.g., kidney) and the patient ID #.
* Do the same for frozen specimens for each organ, such that all of the frozen specimens for each organ are grouped together in individual baggies.
* These bags of frozen samples should be stored in a LN2 freezer, -80°C freezer, or on dry ice until packaged for shipment. If a LN2, -80°C freezer or if dry ice is not immediately available, specimens may be stored at -20oC in **a non-self-defrost** freezer until they can be placed over dry ice.

**Patient Demographic Sheet and Shipment Checklists:**

* Complete the form, providing information about the patient from whom specimens were collected.
* Make two copies of the form, one of which is to be kept for your records.
* Of the remaining two, place one in the shipment with the frozen specimens and the other in the shipment with the fixed specimens.

**PACKAGING:**

**Fresh Specimen Packaging**

* Utilize the styrofoam mailing box provided.
* Place absorbent pads at the bottom of the box to prevent from possible leaking.
* Place the bag with the kidney samples and **wet ice** along with a second sealed bag of **wet ice** into a polystyrene bag and tie securely to prevent leakage. This outer bag will minimize leakage, a serious concern for Federal Express.
* Place the bag containing kidney samples and wet ice into the styrofoam mailing box and seal.
* Place the styrofoam mailing box into the outer cardboard box and seal securely with packaging tape.
* To the outside of the cardboard shipping container, add the label, “Exempt Human Specimen.”
* Apply appropriate courier label for shipment.
* **Ship overnight for next day delivery.**

**Frozen Specimen Packaging:**

* Utilize an insulated shipping container that includes an outer cardboard box.
* Cover bottom of insulated container with a layer of dry ice.
* Place biohazard bags (containing specimens) on dry ice, and cover with another layer of dry ice so that bags are sandwiched between dry ice (a layer at bottom and on top).
* At least 7 to 10 lbs of dry ice should be used to package the shipment.
* Fill any empty space in shipping container with waded brown paper (from the dry ice delivery bag) or brown paper towels. (*This slows evaporation of dry ice and prevents specimens from being damaged in transit by jostling*.)
* Place lid on insulated shipping container, but DO NOT seal. (*NOTE: It is very important to allow enough ventilation in the packaging to allow the carbon dioxide gas to be released during transit. Improper ventilation may cause the gas from the dissipating dry ice to build up inside the package and could cause the package to explode. For this reason,* ***Styrofoam packaging containing dry ice should never be sealed***.)
* Place insulated shipping container into the outer cardboard box.
* Apply Biohazard Label to top of insulated container, so that it will be visible to anyone opening the box
* Before the outer box is taped shut, place one of the copies of the “Patient Demographic Sheet” with the “Frozen Specimen Shipment Checklist” on top of the insulated container.
* Tape cardboard box securely with at least 2 strips of packaging tape.
* Make sure shipping labels are applied to shipping container as follows:
  + - UN 1845 Dry ice label, (*Apply to outside shipping container*)
    - UN 3373 Biohazard Substance, Category B (*Apply to outside shipping container)*
    - Exempt Human Specimen Label (*Apply to outside shipping container*)

**Fixed and RNA*later* Specimen Packaging:**

* Each set of fixed specimens per organ should be in a separate specimen container of 10% NBF, so you will have one cup of kidney specimens, one cup containing liver, etc.
* Similarly, you should have one container per organ in RNA*later*.
* Wrap the lid/cap of each of the specimen containers holding fixed or RNA*later* tissue with parafilm to prevent leakage.
* Place absorbent pad into an empty biohazard ziplock bag.
* Place the parafilm wrapped containers holding the 10% NFB fixed specimens into one of the biohazard bags (to which absorbent pad was added) and seal bag securely.
* Place biohazard bag into a second biohazard bag so that the containers are double-bagged.
* Place the parafilm wrapped containers holding the RNA*later* specimens into a separate biohazard bag (to which absorbent pad was added) and seal bag securely.
* Place biohazard bag into a second biohazard bag so that the containers are double-bagged.
* Place absorbent pad in bottom of insulated inner shipping container.
* Place biohazard bags (containing specimens) into insulated container.
* Fill any empty space remaining in the container with packing material (e.g., bubble wrap, wadded paper towel or brown paper) to keep specimens from jostling during shipment.
* Place lid on insulated shipping container. Seal lid securely with packaging tape.
* To biohazard label, for “Hazard Identity,” write “human tissue.” Add biohazard label to top of inner shipping container.
* Place insulated shipping container into outer cardboard box.
* Place copy of “Patient Demographic Sheet” and “Fixed Specimen Shipment Checklist” on top of insulated container.
* Close outer cardboard box, taping securely with packaging tape.
* To the outside of the cardboard shipping container, add the label, “Exempt Human Specimen.”
* Apply appropriate courier label for shipment.

**SAMPLE SHIPPING:**

* Fresh specimens should be shipped the same day as collection. If tissues are collected a on a Friday or the day prior to a holiday, see specific shipping instructions from LM Guay-Woodford.
* Fixed and RNA*later* specimens should be shipped the same day as collection, unless that day is a Friday or the day prior to a holiday. In that case, hold at ambient temperature until the next business day.
* Please ship all frozen shipments and ambient fixed specimen shipments Federal Express overnight Monday-Thursday (except Holidays).
* Do NOT ship to delivery on a Saturday or on a holiday.
* Provide e-mail notification ahead of shipment delivery to the following e-mail addresses:
  + [jjaber@childrensnational.org](mailto:jjaber@childrensnational.org)

**QUESTIONS:**

**General questions:**

* **PI Contact: Lisa M. Guay-Woodford, MD**
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  + Phone: 202-476-6439
* **Clinical Research Coordinator Contact**: **Jasmine Jaber** 
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