

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

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

SAMPLE COLLECTION:

Please collect the following tissues:

- **Kidney**
- **Liver**

We would appreciate collection of additional tissues as available:

- **Lung**
- **Brain**
- **Heart**
- **Placenta (from mother, if samples are collected from newborns)**

~ 75% of total available tissue for each organ (with exception of pancreas) is to be frozen and ~ 25% is to be fixed in 10% neutral buffered formalin (NBF), with one specimen placed in RNA/ater. When fixing specimens, the volume of fixative should be 10x the volume of the sample to assure proper fixation.

see detailed instructions provided below for freezing and fixation of 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
- Absorbant 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:

- Liquid nitrogen (LN $_2$) for snap-freezing of samples. Alternatively, samples may be frozen over dry-ice if LN $_2$ is not available.
- LN $_2$ vapor-phase or -80°C 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 Elena Gibson, 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:
 - Elena Gibson, RN (German certified)
 - CCRP Clinical Research Coordinator.
 - Phone: 202-476-2197 (9AM-5PM Monday-Friday)
 - E-mail: egibson@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.

- Cut the fresh kidney lengthwise (into two halves). This preserves the cortical area.
- Cut each half transversely into smaller pieces.
- Cut kidney into approximately 1cm x 1cm x 3mm specimens (10 to 15 samples) of a size that will fit into a standard histology cassette. This facilitates uniform freezing and utilizes a standard storage container.
- Place 1 of the specimens into the cup of RNA later .
- Place 25% (~4) of the dissected specimens of kidney into pre-labeled (for kidney) specimen container of 10% neutral buffered formalin.
- Place the remaining 75% (~10) of the 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 LN $_2$; alternatively, they can be frozen over dry ice or on the shelf of a -80° or -20° freezer.

Liver:

NOTE: The instructions above assume the liver is obtained at autopsy. If tissue is obtained at hepatectomy, obtain as much remnant [not needed for diagnosis] tissue as possible, section into sizes as indicated, and process as requested below.

- Cut fresh tissue into 10 to 20 (1cm x 1cm x 3mm) specimens.
- Place 1 specimen into a cup of RNA later .
- Place 25% (~4) of the dissected specimens of liver into pre-labeled specimen container of 10% neutral buffered formalin.
- Place the remaining 75% (~10) of the specimens in individual pre-labeled (see below) histology cassettes.
- Wrap each cassette in aluminum foil.
- With a Sharpie pen, write the specimen type on the foil.
- Snap-freeze by placing cassettes in a vapor phase dewar of LN $_2$; alternatively, they can be frozen over dry ice or on the shelf of a -80° or -20° freezer.

SAMPLE PROCESSING:

RNAlater Specimen Handling and Storage:

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

Fixed Specimen Handling and Storage:

- 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 -80° (preferred alternative) or non-self-defrost -20° 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 -20°C 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.

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 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/ater 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/ater.
- Wrap the lid/cap of each of the specimen containers holding fixed or RNA/ater 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/ater 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:

- Fixed and RNA/ater 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.

- **Use Federal Express # 2530-0934-9 for shipping charges.**
- **Ship to:**
 - ATTN: Kathy Sexton
 - UAB Tissue Procurement
 - 703 19th Street South, ZRB 449
 - Birmingham, AL 35294-0007
 - Phone #: 205-934-6071
- Provide e-mail notification ahead of shipment delivery to the following e-mail addresses:
 - sexton@uab.edu
 - amitchell@uab.edu
 - egibson@childrensnational.org

QUESTIONS:

General questions:

- **PI Contact: Lisa M. Guay-Woodford, MD**
 - E-mail: lguaywoo@childrensnational.org
 - Phone: 202-476-6439
- **Clinical Research Coordinator Contact: Elena Gibson, RN (German certified)**
 - E-mail: egibson@childrensnational.org
 - Phone: 202-476-2197

Technical Questions:

- Contact Kathy Sexton and reference "Core A: Hepato-Renal Fibrocystic Diseases Translational Resource"
 - E-mail: sexton@uab.edu
 - Phone: 205-934-6071