STANDARD OPERATING PROCEDURE

Title: Collection and Preservation of Kidney or Liver Transplant Biopsy for ITN Study	
SOP Number: KC-SOP-001	Version: 2.0
Effective Date: 12/1/2011	Page 1 of 12
All Kidney and Liver Transplant Trials	SOP Owner (Department): ITN (TADA)

Approval

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Immune Tolerance Network

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1. PURPOSE

The purpose of this document is to ensure the proper collection, processing and transport of kidney and liver biopsy specimens for Mechanistic Assays performed in ITN Clinical Trials.

2. RESPONSIBILITY

The Assay Development Group, in TADA, with assistance from the staff at the Thomas Starzl Research Pathology Laboratory, University of Pittsburgh (ITN Tissue Pathology Core) provided guidance in writing the standard operating procedures (SOP) for the proper processing and handling of kidney and liver biopsy samples at clinical sites. Modifications to this SOP will be reviewed by TADA and staff at the UPMC laboratory.

The ITN Trial Implementation Manager (TIM) will distribute this SOP number KC-SOP-001, to the appropriate Site Laboratory Personnel, ensure that laboratory personnel are properly trained, and confirm that all reagents and supplies needed are available.

The appropriate site laboratory personnel* will prepare the biopsy samples once they are collected by a Transplant Surgeon or other technical personnel. These designated lab personnel will be responsible for reading the SOP, and assuring that the specimens are properly handled and shipped to the correct location. Any deviations from the SOP must be documented and forwarded to the ITN TIM.

***NOTE: Designated site laboratory personnel may include the ITN site coordinator, the lab coordinator, a nurse, or any personnel who have been appropriately trained to perform this procedure.**

3. DEFINITIONS AND ABBREVIATIONS

ITN	Immune Tolerance Network

ITN Tissue Pathology Core	Thomas Starzl Research Pathology Lab, University of Pittsburgh
TIM	Trial Implementation Manager
TADA	Tolerance Assay and Data Analysis
FCB	For Cause Biopsy
SOP	Standard Operating Procedure
LN2	Liquid Nitrogen
OCT	Optimum Cutting Temperature - embedding medium
NBF	Neutral Buffer Formalin

4. REAGENTS, EQUIPMENT AND MATERIALS

The following reagents and supplies are provided by ITN as specified in the lab manual.

Title	Vendor	Catalog #
RNALater (1.5 ml vials)	Ambion/Invitrogen Provided by Fisher	AM7022
Tissue Tek OCT Compound	Tissue Tek	4583
Vials, cryogenic, 1.8 ml , sterile	CIC Provided by Fisher	T311-2P
24x24x5mm Base Molds (Cryomolds)	Fisher	22-038217
Requisition forms and labels for tubes and slides	Fisher/ITN	N/A
Shipping Containers	Fisher/ITN	N/A
Preprinted Fed Ex Air bills	Fisher/ITN	N/A

The following reagents and supplies are not provided by ITN

Title	Vendor	Catalog #
Formaldehyde	Fisher	F79-1

100% Ethyl Alcohol	Fisher	BP2818-100
Primary collection tray	Available in any pathology laboratory	N/A
15 ml conical vials	Fisher	05-539-1
Liquid nitrogen storage tank	Thermo Forma	8030
Liquid nitrogen canister	Lab Line	2123
TELFA Non-adherent Dressing or Pad	Kendall/Covidien	1050
Sterile Forceps	Multiple suppliers	N/A
Scalpel	Multiple suppliers	N/A
Aluminum Foil	Multiple suppliers	N/A
Small Airtight bags (for cryomolds)	Multiple suppliers	N/A
Styrofoam Float	Multiple suppliers	N/A
Ruler (suggested to measure size of biopsy piece)	Multiple suppliers	N/A

5. PROCEDURE

5.1 Collection of biopsy samples

This SOP focuses primarily on the steps following biopsy collection, as samples are forwarded to the designated laboratory personnel. In preparation for the collection, a designated person (laboratory technician or study coordinator) must be available with the appropriate containers to transport samples collected during the procedure. Biopsies are collected from the transplanted

organ after the transplant has occurred and in some cases from the donor organ prior to transplant**.** A biopsy sample collected prior to transplant, from the donor organ is considered to be a donor biopsy. The donor biopsy is usually collected one time before transplant when the organ is initially harvested. Protocol biopsies are performed on transplant participants post-transplant at intervals listed in the schedule of events (SOE). Biopsy samples may be collected at time points not listed in the schedule of events, and are referred to as "for cause biopsies" (FCB). This protocol describes the processing of all biopsies from both donor and participant.

5.1.1 Donor biopsy samples

Donor biopsy samples may be taken as a wedge or a core and are usually obtained on the day of transplant after the organ is removed from the donor. Please see the trial specific protocol for the type (wedge or core) and size of the donor biopsy preferred for that protocol. The biopsy tissue collected for mechanistic purposes will be transferred into a container, separate from the biopsy piece required for clinical assessment. The tissue will then be transported to the laboratory by the designated lab personnel for further processing. The sample will be separated into pieces allotted for the assays specific to a particular trial, including pathology, immunohistochemistry, isolation of RNA, and storage.

5.1.2 Patient biopsy samples

Patient biopsy samples (protocol or FCB) are collected as a core or several cores. It is recommended that these core biopsies be collected percutaneously under ultrasound guidance with a 16 gauge needle. Please see individual protocol for specific size required. Sample will be separated into portions and allocated for trial specific assays, RNA isolation, storage requirements or other needs.

5.1.3 Specimen handling for transport from the OR to the laboratory

Biopsy samples handed to the designated lab personnel should be placed on a TELFA pad or other non-stick pad (DO NOT USE GAUZE) moistened with distilled water and held on wet ice until sectioning and processing of the sample can begin.

Note: Samples should be processed and stored at the appropriate temperature as quickly as possible, ideally less than 15 minutes. If transport from the OR to the laboratory and processing will exceed this time limit, processing should be performed at the bedside. Holding sample temporarily on wet ice should not pose a freezing danger to the sample.

5.2 Processing of biopsy samples in the laboratory

Biopsy samples MUST be processed immediately. The designated lab personnel will prepare reagents and supplies to process samples when notified of a biopsy. Ideally, each biopsy will be

sectioned into at least three pieces: (1) formalin preserved for histology and pathology, (2) OCT preserved for immunohistochemistry, and (3) RNALater preserved for gene expression studies. Section 5.2 will describe general processing and shipping instructions for each of these three pieces. Depending on the protocol or type of biopsy there may be additional pieces required for local analysis. Please see the specific protocol and lab manual for direction on the sizes and priority order of each piece.

5.2.1 Biopsy processing in formalin for routine diagnostic assessment and histochemical analysis

Formalin fixing is performed to preserve tissues prior to further processing. One portion of the core biopsy from the patient, or wedge from donor is preserved in formalin for routine diagnostic purposes. Please see the trial specific lab manual to determine the size of the piece required for this assay.

10% neutral buffered formalin (NBF) is a standard fixative used in diagnostic settings. Immediately after dissection, the tissue is placed in a pre-labeled vial and submerged in 10% NBF solution.

In most cases, the pathology sample for a protocol biopsy will be preserved in formalin and shipped directly to the ITN Tissue Pathology Core. However, there are a few cases in which the site will not send a formalin piece, but process the biopsy locally and send a subset of stained and unstained slides to the ITN Tissue Pathology Core. These include any for cause biopsy, or in the instance that the site's local pathology laboratory requires slides from a protocol biopsy. Also, if there is not adequate sample collected to distribute for all mechanistic purposes, any tissue collected will be saved for RNALater and OCT block (see trial specific guidelines for minimum required pieces). Instead of a formalin piece, slides prepared from the piece collected for the clinical assessment will be shipped for mechanistic purposes.

In all cases that a formalin piece is not obtained and shipped, a subset of stained and unstained slides will be shipped to the ITN Tissue Pathology Core after local analysis; the exact items shipped will depend on the specific protocol. Please see instructions in the specific laboratory manual on biopsy processing. All previously stained slides will be returned to the site within 4 weeks.

5.2.1.1 Preparation of tissue in Formalin

a. Fill a 15 ml conical tube with 10% NBF.
b. Add the specimen to the tube and **make sure that the tissue is completely submerged**
c. Store ambient until shipped.

5.2.1.2 Preparation of 10% NBF

Sodium phosphate monobasic	40 gm
Sodium Phosphate dibasic	65gm
Formaldehyde (37-40% solution)	1 Liter
Distilled water	9 Liters

Mix well, label appropriately with date and concentration. Solution should be prepared fresh and should not be stored for longer than 2 weeks.

Safety: Formaldehyde is corrosive and carcinogenic. Work in well ventilated areas, use lab coat, gloves and goggles.

5.2.1.3 Preparation of Slides (See 5.2.1 and specific protocol for cases in which slides will be sent instead of formalin piece)

The ITN Tissue Pathology Core lab suggests that any slides sent for mechanistic studies: be cut to the following thickness:

Liver biopsy: 4-5 microns.
Kidney biopsy: 3-4 microns

Please see specific protocol or lab manual for any further instructions on the thickness of slides.

5.2.1.4 Storage and shipping of formalin sample or slides for histochemical/pathological analysis.

The type of tissue and biopsy and the preparation of the sample will affect how it is shipped to the ITN Tissue Pathology Core. Refer to the trial specific lab manual for further shipping instructions for each piece.

Formalin preserved: Wrap the tube in bubble wrap and pack it in an ITN ambient shipper according to packing instructions enclosed in the box itself or the ITN lab manual. Ship to the ITN Tissue Pathology Core the same day M-Th. If collected Fri – Sun, hold at room temperature for Monday shipment.

Slides: Place the slides in the slide container provided by ITN. Pack it in an ITN ambient shipper according to enclosed packing instructions enclosed in the box itself or the ITN lab manual. Ship to the ITN Tissue Pathology Core the same day M-Th. If collected Fri – Sun, hold at room temperature for Monday shipment.

5.2.2 Biopsy processing in OCT for immunohistochemical analysis

WARNING! SAFETY CAUTION:
There are TWO major risks associated with the use and handling of LN2 which present potential hazards. Always follow your institution guideline in the safety and handling of LN2. Liquid Nitrogen (LN2) is extremely cold (< -196 degrees C).At normal atmospheric pressure LN2 vaporizes at -196 ◦C (-320 ◦F). LN2 itself, and every object in direct contact with it, will rapidly produce frost – burns and frostbite. During this vaporization process LN2 expands to produce a large volume of gaseous N2. Asphyxiation may result from this large volume of gaseous N2 displacing the oxygen present in the atmosphere of any small unventilated room.

One section of the biopsy will be frozen for immunohistochemical analysis. Fresh tissue must be used for freezing. **Do not use tissue that has been formalin treated**. Please see the trial specific lab manual to determine the size of the piece required for this assay.

5.2.2.1 Preparation of tissue in cryomold with OCT.
There are three possible ways to prepare tissue in OCT depending on the facilities at each institution. Please choose the best way available.

5.2.2.1.1 – BEST OPTION – Immediate access to Liquid Nitrogen (LN2) Post Biopsy

a. Fill a Styrofoam cooler half-way with LN_2.
b. Place a Styrofoam tube holder, with holes punched through, into the LN2.
c. Lay cryomolds atop of Styrofoam float allowing vapors to reach mold but not touching LN2.
d. DO NOT submerge in LN2 or use freeze spray.
e. Apply thin coat of OCT media in cryomold.
f. Orient the core in thin layer and allow to freeze slightly for about 1 minute. “Slight freeze” is complete when media is slightly opaque in color.
g. Add OCT media to fill cryomold well.
h. Allow cryomolds to sit over LN2 vapors until frozen.
i. Once frozen, wrap entire labeled cryomolds containing OCT block in aluminum foil. Transfer to airtight bag.
j. Store in -70°C freezer (at least overnight) until ready to be shipped.

5.2.2.1.2 – GOOD OPTION – Immediate access to Dry Ice Post Biopsy

a. Fill a Styrofoam cooler or an ITN frozen shipper with dry ice.
b. Wedge cryomolds in dry ice and/or adjust for media to freeze evenly in the bottom of the mold. DO NOT SUBMERGE BIOPSY PIECE IN DRY ICE AT THIS POINT.
c. Apply thin coat of OCT media in cryomold.
d. Orient the core in thin layer and allow to freeze slightly for about 1 minute. “Slight freeze” is complete when media is slightly opaque in color.
e. Add OCT media to fill cryomold well.
f. Completely submerge cryomold with dry ice and allow to sit for 20 minutes.
g. Once frozen, wrap entire labeled cryomolds containing OCT block in aluminum foil. Transfer to airtight bag.
h. Store in -70°C freezer (at least overnight) until ready to be shipped.

5.2.2.1.3 – ADEQUATE OPTION – Immediate access to -70°C Freezer Post Biopsy

a. Apply thin coat of OCT media in cryomold.
b. Orient the core in thin layer and transfer to -70°C freezer allowing it to slightly freeze for about 1 minute. “Slight freeze” is complete when media is slightly opaque in color.
c. Remove from freezer and add OCT media to fill cryomold well.
d. Return cryomolds to -70°C freezer and allow to sit for 20 minutes.
e. Once frozen, wrap entire labeled cryomolds containing OCT block in aluminum foil. Transfer to airtight bag.
f. Store in -70°C freezer (at least overnight) until ready to be shipped.

5.2.2.2 Storage and shipping of the OCT piece for immunohistochemical analysis

Depending on the trial, this sample may be shipped to the ITN Repository or directly to the ITN Tissue Pathology Core. Please see the trial specific lab manual for the destination of this sample. This sample should be stored in a -70◦ C (no defrosting) freezer until ready to ship.

Wedge the cryomold (covered with foil and inside the airtight bag) inside a small cardboard cryovial box by bending the edges of the grid towards the side of the box. If there is more than one cryomold, the grid may be completely removed from the box. Secure the lid to the box with a rubber band and pack in an ITN frozen shipper according to packing instructions enclosed in the box itself or the ITN lab manual. Ship to the appropriate core on dry ice.

If shipping to the ITN Repository, ship Mon – Wed.
If shipping to the ITN Tissue Pathology Core, ship Mon – Thurs.

Otherwise hold at -70°C for Monday shipment.

5.2.3 Biopsy processing in RNALater for Gene expression analysis

One piece of the biopsy is preserved in RNALater media for gene expression analysis. Do not use samples that have been formalin treated for this purpose, only fresh samples can be used. Please see the trial specific lab manual to determine the size of the piece required for this assay.

5.2.3.1 Specimen processing in RNALater

d. Fill a cryovial with 800 µl of RNALater
e. Add the specimen to the vial and **make sure that the tissue is completely submerged**
f. Let the tissue soak overnight at 4◦C.

If not shipped the day after collection, transfer to -70°C freezer until shipped.

5.2.3.2 Storage and shipping of the RNALater sample for gene expression analysis

The sample should sit at 4°C in RNALater for no longer than 24 hours.
If shipping the day after collection, sample can be transferred from 4°C to dry ice for shipment. If not shipping the next day, transfer the sample from 4°C to -70°C after 24 hours and ship to ITN Repository on dry ice within 2 weeks of collection.

When ready to ship, place the frozen vial in a small cryovial box and pack it in an ITN frozen shipper according to packing instructions enclosed in the box itself or the ITN lab manual. Ship to ITN Repository on dry ice Mon – Wed ONLY. Otherwise hold at -70°C until Monday for shipment.

6. REVISION HISTORY

Version	Effective Date	Section	Description of Revisions/Justifications
1.0	05.29.2009	*	• SOP Format Change
1.0	05.29.2009	*	• Addition of Table of Contents
1.0	05.29.2009	2	• Addition of Responsibility
2.0	12.01.2011	ALL	Update to cover both kidney and liver biopsies and bring procedures up to date.