



Organ Processing and Histopathology

1.0 Purpose

The purpose of the Organ Procurement and Pathology Core (OPCC) is to process pancreas and other organs or tissues (including serum and whole blood) for storage and distribution to investigators approved by the JDRF nPOD program.

2.0 Application/Scope

- 2.1 nPOD is designed to study organs and tissues from individuals with type 1 diabetes of any duration (i.e., recent onset to long-standing disease, including Joslin “Medalist” study participants), as well as in non-diabetic persons at increased risk for the development of the disease (i.e., type 1 diabetes autoantibody positive individuals). Organ donors without diabetes will also be recovered to serve as controls for the above patients.
- 2.2 The study of cadaveric donor pancreas from individuals throughout these various stages of diabetes will help researchers learn more about the pathophysiology of type 1 diabetes.
- 2.3 Pre-diabetic donors will be identified using rapid autoantibody assays as described in the Autoantibody SOP.
- 2.4 This SOP applies to all organs provided through the nPOD program. This document provides an outline of the sample processing, storage, and distribution methods and histology and immunolocalization procedures.

3.0 References

- 3.1 Guidelines on Good Clinical Laboratory Practice ([pdf](#))
- 3.2 University of Florida Health Information Privacy office ([UF Privacy](#))
- 3.3 University of Florida, Department of Pathology, Anatomical Pathology division
- 3.4 Armed Forces Institute of Pathology Standards of Procedure ([website](#))

4.0 Associated SOPs

- 4.1 Shipping and Handling
- 4.2 New Case Protocol
- 4.3 Autoantibody
- 4.4 Molecular Pathology Core Good Lab Practice

5.0 Location/Contact Information

5.1 The location of the nPOD OPPC is:

POB 100275, Room D11-50
Department of Pathology, Immunology, and Laboratory Medicine
University of Florida
1600 SW Archer Road
Gainesville, FL, USA 32610

Lab Phone: 352-273-7737

5.2 Contact information

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5.3 Additional contact information is noted on the nPOD website: www.jdrfnpod.org.

6.0 Materials and Equipment

- 6.1 Computer, printer, scanner
- 6.2 Histobath or other snap freezing method
- 6.3 BSL 2 Biosafety hood
- 6.4 -20 and -80 Freezers
- 6.5 Refrigerator
- 6.6. Cryotank
- 6.7 Centrifuge and pipettes

7.0 Procedure

7.1 The major emphasis of the nPOD program is to isolate auto reactive T cells involved in the pathogenesis of T1D. These cells may be derived from 4 main sites: pancreatic lymph node (PLN), pancreas, spleen, and/or peripheral blood mononuclear cells. Non-pancreatic LN will be studied as well for comparison to these sites.

7.2 The test organs for the purpose of this study will be the pancreas, PLN, spleen, and non-pancreatic LN (Appendix). Additional organs may be recovered. The tissues received will be identified as follows:

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Sample	Sample Abbreviation
Pancreas-Head	PanHead
Pancreas- Uncinate	PanUncin
Pancreas-Body	PanBody
Pancreas-Tail	PanTail
Pancreatic Lymph Node	PLN
Pancreatic Lymph Node-A-D (when harvested by region)	PLN-A; etc
Spleen	Spleen
Non-pancreatic Lymph Node	NonPLN
Duodenum, kidney, other	[Truncated name]

7.3 Representative tissue samples provided through the nPOD program will be further analyzed by histology and immunohistochemistry following fixation in NBF or as fresh frozen OCT blocks.

7.4 *Case number assignment:*
Organ donors will be assigned case numbers starting at 6000.

7.5 *Specimen accessioning and labeling:* Each specimen label will contain the following:
Case ID-Block/Vial Number (ex. 6025-01)
SampleID (ex. PanHead)
Procedure, derivative, or stain as applicable (ex. Serum; Cells (with concentration and viability); H&E; DNA (concentration (ug/ul))
Optional: Sample label with a 1-D bar code will be generated from the nPOD online database for cryovials and tubes.

7.6 Data collection

7.6.1 A case work-up form (Appendix) will be used in the processing lab (D11-50) for direct data entry to record shipment information and tissues and blood samples provided. The UNOS# will be entered along with processing start and end times. Procurement information will be entered (dissection/fixation start times, organs, cells, block and/or aliquot totals, serum, blood). The completed form will be scanned to the departmental server and the original maintained according to the College of Medicine security processes for private health information.

7.6.2 Data will be recorded in the relational database “nPOD” (Access 2007 (Microsoft)). This database will be stored in a restricted folder on the Departmental server (G:/Projects/nPOD/Pathology Core/Operations). Access will be limited to UF nPOD staff and will be granted by the departmental IT group upon request from the PI. The Case table includes data from the case work up plus demographic information (CaseID, UNOS#, recovery date, X clamp time, demographics (gender, age (years), race)). Donor types will include control, autoantibody

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positive, Type 1 Diabetes (T1D), T1D Medalist, and Other. The data will be updated from the case work-up form and medical charts provided by the OPO by Administrative Core staff.

7.6.3. Each case will have a record in the Aperio Spectrum online pathology database (see section 10) for associated stained slides.

7.7 Tissue Dissection

7.7.1 Supplies and equipment

- Forceps, scissors (Metzenbalm, Mayo, other), scalpels, blades (10, 22 and single sided), and other dissecting instruments- sterilized by autoclaving or other methods
- Scale- tared with weighing container to hold pancreas
- Dissection boards
- Gauze sponges 4x4, paper towels
- 70% ethanol, disinfectant wipes
- Dulbecco's salt solution with Penicillin/Streptomycin- stored in refrigerator between uses, handled with sterile techniques
- RPMI with Pen/Strept/Glutamine/10% Serum- prepare 50 and 15 ml sterile centrifuge tubes, store prepared tubes and stock bottles in refrigerator between use, handle with sterile techniques
- Histobath or isopentane (stored in flammables cabinet)/liquid nitrogen for freezing bath, Dewar flask, plastic beaker for isopentane, long forceps, OCT, molds, cryopens for labeling
- 10% Neutral buffered formalin (NBF) in 1 quart container (~half-full)
- Cassettes and cryomolds- pre-labeled with CaseID + block number and tissue type for standard collections, additional unlabeled in case of additional collections
- Cryovials without and with RNAlater (1 ml, use sterile technique to dispense), store in a box at RT
- Tissue waste container with waste formalin for tissue disposal
- Sharps containers for blades and blood tubes

7.7.2 Follow UF EHS training procedures for universal precautions when handling human samples and use personal protective equipment (face mask, gloves, lab coat or apron). Follow all chemical safety procedures for formalin, isopentane, and liquid nitrogen. Use sterile techniques as much as feasible throughout process for obtaining samples suitable for cell cultures and DNA/RNA samples of the highest quality. The lab will be maintained according to clinical laboratory standards.

7.7.3 The organ donor shipment container will be unpacked and all materials identified and recorded. When present, the UNOS organ tag will be stapled to the case work-up form for QC purposes. All shipment paperwork

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will be kept in a secure place until given to nPOD administration core. The case work-up form will be updated with processing date and start time, procurement staff present, and organs and blood tubes (tube top color, size, number) received. Blood tubes will be stored at room temperature in D11-50 until processed by nPOD lab staff.

7.7.4 Duodenum

- 7.7.4.1 When provided with pancreas, separate the duodenum from the pancreas by blunt dissection. The duodenum will be given to the assistant or held until the primary organs have been processed. For procuring duodenal mucosa, open the duodenum, remove any mucus and ingesta by gentle scraping, and then snip off several segments of mucosa. These will be placed into cassettes for paraffin processing, molds for OCT, and cryovials for snap freezing/-80 storage.
- 7.7.4.2 The primary prosector (Pathology assistant or other nPOD staff) will then transfer the pancreas and spleen to the biosafety hood for further dissection.

7.7.5 Spleen

- 7.7.5.1 Remove spleen from pancreas and immediately procure a 2x3 cm sample for cell culture. If temporary holding is needed, place sample in a culture dish with D-PBS fluid. Mince the spleen sample using sterile technique into small pea sized pieces and transfer to the pre-filled 15 or 50 ml tubes with sterile RPMI. For tubes to be distributed to investigators, completely fill tubes with media to avoid excessive shaking of contents during shipment. The number of tubes prepared will depend on spleen sample size and numbers of requests for fresh spleen. Minced spleen will be stored at RT until shipment or use. Larger pieces may be requested for other investigators and similar processing will be followed with larger RPMI volumes.
- 7.7.5.2 The remaining spleen is given to the assistant for procuring fixed, frozen OCT, and cryovials (with and/or without RNA later) samples. Obtain one cryovial with ~1 g tissue for future genomic DNA isolation and store in holding box in -20 freezer.

7.7.6 Pancreatic Lymph Nodes (PLN; combined)

- 7.7.6.1 Proceed to dissection of all peripancreatic fat from the pancreas. After removing peri-pancreatic fat, the pancreas will be given to the assistant for weighing, transection into regions and reweighing

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followed by processing for fixed, frozen, and cryovials (with and/or without RNAlater).

7.7.6.2 Dissect all PLN from all areas of peripancreatic adipose tissue and place into Dulbecco's solution in a cell culture dish for holding and washing. Use the following decision tree for determining which specimens to prepare.

7.7.6.3 **Minimum tissue for RPMI cell culture specimens is approximately 1 X 2 cm**

If total lymph node volume is 1 X 2 cm or less, submit all nodes for OCT frozen blocks.

If total lymph node volume is up to 1 X 4 cm, submit RPMI specimen and remainder as OCT frozen blocks.

If total lymph node volume is up to 1 X 5 cm, submit RPMI and OCT specimens per # 2 and remainder in RNAlater vials. Group enough nodes for 3-4 RNAlater tubes and mince them together so each vial will receive an equal representation of harvested nodes. Volume of sample: RNAlater is ~1:10.

If total lymph node volume is up to 1 X 6 cm, submit as per # 3 with extra OCT blocks.

If total lymph node volume is greater than 1 X 6 cm, consider extra RPMI specimens.

7.7.6.4 Transfer nodes to RPMI tubes after thorough cleaning to facilitate cell isolation and hold at room temperature in hood for other staff to distribute to nPOD researchers or process for cells.

7.7.7 Pancreatic Lymph Nodes (Regional, not to be performed unless specifically requested):

7.7.7.1 The peripancreatic fat is removed in 4 sections corresponding to the four PLN regions A-D (Appendix, trimming plan). Other landmarks include the vascular groove (retroperitoneal side) without the vessels, the splenic groove on the retroperitoneal and superior edge, and the uncinate process. Dissected fat regions may be separated by placing in plastic bags with a small amount of media. The D region is of most interest to researchers, however, it is also the most often missing portion due to harvesting of vessels in this region for liver transplantation.

7.7.7.2 For each PLN region, prepare a 100mm culture dish with sterile DPBS. Dissect PLN according to 7.7.6 while maintaining regions. Follow the same decision tree for combined PLN in 7.7.6.3. to determine collections for OCT blocks and cryovials. PLN samples for cells will be placed in a 15 ml tube containing RPMI for storage

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at room temperature in the hood. Cell isolations will be performed following distribution to researchers and nPOD staff as indicated by numbers of PLN available.

7.7.8 Non-pancreatic lymph nodes (nonPLN)

7.7.8.1 NonPLN nodes will be processed exactly as for PLN and will be shipped in a separate tube from the OPO.

7.7.8.1.1 If total lymph node volume is 1 X 2 cm or less, submit all nodes for OCT frozen blocks.

7.7.8.1.2 If total lymph node volume is up to 1 X 4 cm, submit RPMI specimen and remainder as OCT frozen blocks.

7.7.9. Pancreas

7.7.9.1 Weigh the pancreas and record weight on case work-up form. The pancreas will be divided into 4 regions. The head region is defined as that portion adjacent to duodenum and includes the region proximal to the notch (Appendix). The region within the head demarcated by the notch and posterior fascial plane will be designated as the uncinata region. Remove the head and uncinata regions and return pancreas to cold media until finished with those regions. The remaining portion will be equally divided into proximal body and distal tail regions.

7.7.9.2 Section the pancreas by "bread-loaf" so that alternating sections are fixed and frozen. Tissues intended for paraffin blocks are trimmed to no more than 1.5cm x 1.5 cm pieces then placed in labeled cassettes. Cassettes will be transferred to a container of NBF for 16 hours at room temperature on a Sakura VIP processor. Record fixation start time as that when the last cassette is placed into fixative. Fixation is stopped by transfer to PBS and samples will be processed at the next available processing schedule using the program for human tissues.

7.7.9.3 Tissues intended for OCT blocks are trimmed to ~0.5 cm then placed in labeled molds with a small amount of OCT. Tissues are covered with OCT then immersed in the Histobath or liquid-N₂ cooled isopentane for rapid freezing. Blocks are transferred to a -80 freezer for storage.

7.7.9.4 Tissues intended for cryovials will be minced to ~0.1gm pieces and evenly divided between cryovials to ensure uniform distribution.

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These vials will be immediately snap frozen in liquid nitrogen or the isopentane bath then transferred to -80 freezer for storage.

7.7.9.5 Tissues intended for RNAlater cryovials will be minced as above and evenly divided into cryovials. These vials will be snap frozen after ~30 minutes equilibration in RNAlater then transferred to -80 freezer for storage.

7.7.9.6 Record total number of blocks, cryovials, and tubes on the case workup form. This information will be verified when the samples are placed into storage boxes and locations logged into the respective tables in the database. Add any additional comments as needed for quality control purposes.

7.8 The nPOD OPPC laboratory will be cleaned and restocked following each case according to EHS guidelines for handling human samples. Discarded samples and tissues will be properly disposed and hazardous waste autoclaved.

8.0 Histopathology

8.1 One outcome of these studies will be baseline histopathologic examination of the pancreas for insulinitis, pancreatitis, or other abnormalities. Fixed tissues will be submitted to the UF Molecular Pathology Core. Tissues will be processed, embedded in paraffin. Two blocks from pancreas head, body, and tail regions and one block from spleen will be sectioned as serial sections. One section will be stained with Hematoxylin and Eosin (H&E) following Good Lab Practice compliant standard operating procedures.

8.2 All stained slides will be labeled as in 7.5.

9.0 Immunohistochemistry

9.1 There will be two primary data sets in these studies, namely islet cell characterization and immunophenotyping. Islet cell characterization will include endocrine cell types (insulin, glucagon, somatostatin, pancreatic polypeptide) and beta cell replication rates will be estimated by co-expression of Ki67 (Table 2). Immunophenotyping of lymphoid organs will be determined for B (CD20) and T (CD3) cell populations, subsets of T cells (CD4, CD8), and macrophages (CD68) (Table 2).

9.2 Serial sections will be analyzed by IHC using paraffin or OCT frozen blocks as detailed in Table 2.

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Table 2

Tissue	Antigen
Pancreas	Ki67+ Insulin CD3+ Glucagon CD4, 8, 20, 68, others as requested
PLN	CD3, 4, 8, 20, 68, others as requested
Spleen	As for PLN
NonPLN	As for PLN

9.3 IHC run conditions will be optimized to provide specific staining using common detection reagents for blocking solutions (peroxidase, alkaline phosphatase), antigen retrieval, primary and secondary antibodies, chromagens, and counterstain (Appendix).

9.4 Paraffin sections will be stained in batch using the Dako Autostainer. Each run will include a reference positive control sample for quality control purposes. A section from the case will be included as a negative control using substitution of the primary antibody with primary host species IgG or serum.

9.5 Stained slides will be labeled as listed in 7.5 with the addition of the primary antibodies and assay completion date.

10.0 Slide scanning

10.1 Stained slides will be scanned using an Aperio CS Scanscope and dedicated PC computer by OPPC lab staff. The Aperio Spectrum information management system will be accessed through the http address: path-aperio.ad.ufl.edu. OPPC staff will create a case record for each donor and will input nPOD Case ID, donor type, demographics, and other pertinent information as available. Each case will have specimens consisting of each pancreas regions and other organs from which stained slides are created.

10.2 Cases will be assigned to the Data group "JDRF nPOD". Access to this datagroup is assigned through administrative rights determined by user roles. Approved researchers will be allowed read-only access to the scanned images. Access is requested by on-line form and approved by nPOD Administrative Core staff. Each user will be assigned a specific username and temporary password that will be changed at first log in by the user.

10.2 Image files will be stored on a server maintained by the Department of Pathology IT group according to UF security practices. The Department IT group will also provide back-up support for administrative functions.

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11.0 Image analysis

11.1 Image analysis will be performed using Aperio software to determine fractional stained area per pancreatic section. Data will be averaged by pancreatic region and compared within donor types. Beta cell mass will be calculated as the fractional area multiplied by weight. One-way ANOVA will be used to determine significance differences ($P < 0.05$) between donor groups.

12.0 Reports

12.1 Progress reports will be provided annually and will include organ and cell recovery data and histopathology data including tables of IHC image analysis. Additional reporting will be provided upon requested.

13.0 Archiving of Materials

13.1 All materials obtained by this program will be inventoried in the nPOD databases and will be archived in the Molecular Pathology Core lab. Materials will be transferred upon request by the sponsor.

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Appendix

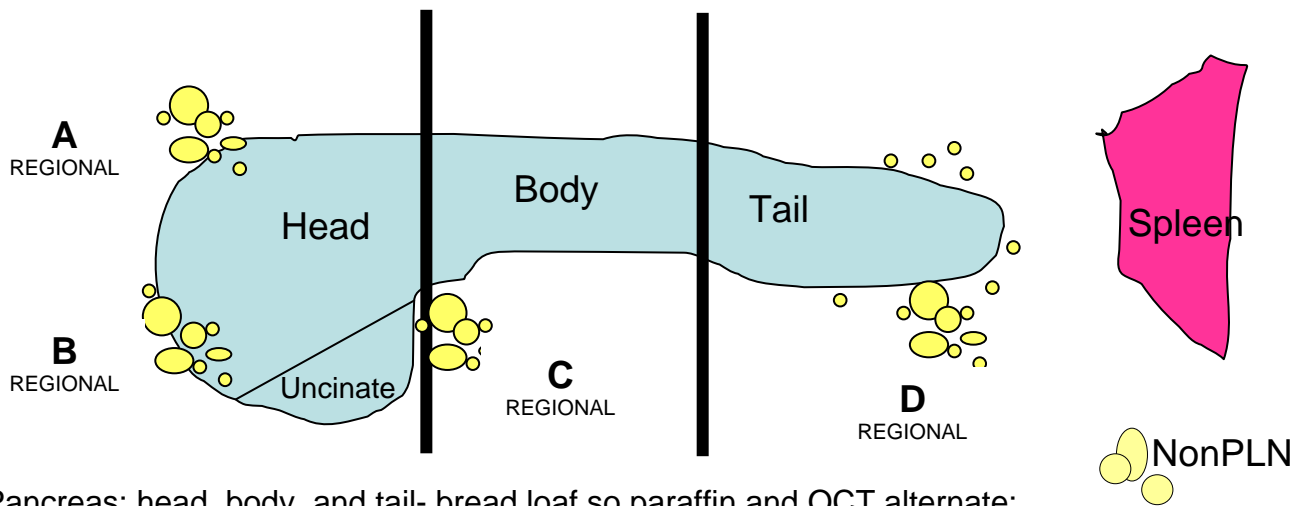
Trimming and processing workplan

Case workup form

IHC reagents

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nPOD Organ Trimming and Processing Workplan



Pancreas: head, body, and tail- bread loaf so paraffin and OCT alternate:

- OCT blocks- 5-10 all regions or as indicated by size
- Paraffin blocks- 5-10 (OCT takes precedence over fixed when organ size is small)
- Cryovials- 5 cryovials snap frozen, can also make 4 cryovials with 1ml RNAlater
- Minced fresh samples for cells collected to RPMI may be eventually requested
- Uncinate region- fixed only

PLN: Combined collections (**do not separate into the 4 regions unless specifically requested**).

Resect all nodes from all areas and place into Dulbecco's for holding and washing. Use the following decision tree for determining which specimens to prepare.

Minimum tissue for RPMI cell culture specimens is approximately 1 X 2 cm

1. If total lymph node volume is 1 X 2 cm or less, submit all nodes for OCT frozen blocks.
2. If total lymph node volume is up to 1 X 4 cm, submit RPMI specimen and remainder as OCT frozen blocks.
3. If total lymph node volume is up to 1 X 5 cm, submit RPMI and OCT specimens per # 2 and remainder in RNALater vials.
4. If total lymph node volume is up to 1 X 6 cm, submit as per # 3 with extra OCT blocks.
5. If total lymph node volume is greater than 1 X 6 cm, consider extra RPMI specimens and paraffin blocks.

Spleen: Per PLN. Do not need paraffin unless excess sample available.

NonPLN: Per PLN

Other organs: Paraffin and OCT samples and 2-3 snap-frozen cryovials without RNAlater

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Case Work-up

UNOS#: _____

nPOD#: _____

Date Received: _____

Processing Start Time: _____ am pm

Prosectors: _____

Processing End Time: _____ am pm

Tissue	Rec'd	Weight (g)	#Parafin Blocks *	#OCT Blocks	# Cryovials		Fresh Tissues #/tube size	Other
					Plain	RNAlater		
PanHead	<input type="checkbox"/>							
PanBody	<input type="checkbox"/>							
PanTail	<input type="checkbox"/>							
PanUncinate	<input type="checkbox"/>							
PanOther	<input type="checkbox"/>							
PLN	<input type="checkbox"/>							
Spleen	<input type="checkbox"/>							
NonPLN	<input type="checkbox"/>							
Duodenum	<input type="checkbox"/>							
Other	<input type="checkbox"/>							
Serum	<input type="checkbox"/>	Color: <input type="checkbox"/> Red Other: _____ Volume (ml): 1 2.5 5 10						
Whole Blood	<input type="checkbox"/>	Color(s): <input type="checkbox"/> Green <input type="checkbox"/> Purple <input type="checkbox"/> Yellow Volume (ml): 1 2.5 5 10						

*Fix Start Time: _____ am pm

Fix End Date: _____ Time: _____ am pm

Temp: RT 4'C VIP

Transferred to: PBS 70% VIP Initial/Date: _____

Comments:

OPPC IHC Reagent Listing

Reagent	Vendor
AR	
Trilogy	Cell Marque
Borg Decloaker 1X	Biocare
Target Retrieval (S1699) 10X	Dako
Blocking	
Dual Endogenous Peroxidase	Dako
Sniper	Biocare
Normal Goat Serum	Vector
Dako Protein Block	Dako
Primary Antibodies	
Ki67	Dako
Insulin	Dako
CD20,45	Dako
CD3,4,8	Dako
CD68	Dako
Glucagon	Dako
Somatostatin	Dako
Pancreatic polypeptide	Dako
Secondary Antibodies	
Biot. Gt anti GP	Vector
Biot. Hs anti Ms	Vector
Biot. Gt anti Rb	Vector
Mach 2 Gt anti Ms HRP polymer	Biocare
Mach 2 Gt anti Rb HRP polymer	Biocare
Tertiary Antibodies	
Standard and Elite HRP and AP kits	Vector
LSAB kits	Dako
Ancillary	
DAB (HRP)	Dako
cDAB, Vulcan Fast Red	Biocare
DAB, Hematoxylin	Vector
Liquid Permanent Red (AP)	Dako
Hematoxylin	Dako