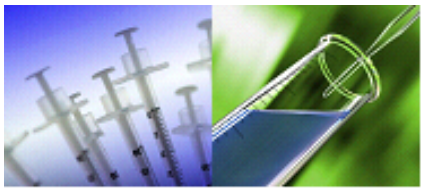


nPOD
Network for Pancreatic Organ Donors with Diabetes

## 1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for processing and storing pancreas and other serum and whole blood by the nPOD Organ Processing and Pathology Core (OPPC).

## 2 SCOPE

This SOP will be applied to all samples recovered through the nPOD program.

## 3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.

3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

## 4 EQUIPMENT and MATERIALS

The materials, equipment and forms listed in the following list are recommendations only and alternative products as suitable may be substituted for the site specific task or procedure.

Sterilized dissecting instruments (e.g., forceps, scissors, scalpels, single-sided blades)
Scale and weighing boat for pancreas
Dissection boards
Gauze sponges/paper towels
Centrifuge tubes (15, 50 ml)
Dulbecco's salt solution with penicillin/streptomycin (D-PBS, store at 4$^{o}$C)
RPMI 1640 L Glutamine and HEPES-supplemented media (Sigma, Cat. No. R7388)
Inactivated fetal bovine serum (FBS) (Sigma, Cat. No. F2442), aliquot 50 ml and store at -20$^{o}$C
100x Antibiotic/antimycotic (penicillin/streptomycin) solution (Sigma, Cat. No. A9909), aliquot 10 ml and store at -20$^{o}$C
Phosphate buffered saline (PBS)
Cassettes
10% neutral buffered formalin (NBF) in specimen container
OCT media and cryomolds, pen, tin foil
O-ring cryovials
RNAlater (Ambion) (room temperature)
Dry ice/ice bucket
Isopentane in plastic beaker/long forceps
Liquid nitrogen in Dewar flask
70% ethanol, 10-50% Clorox bleach
Tissue waste container with formalin
Sharps containers

## 5 SAFETY

5.1 Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).

5.2 Follow chemical safety procedures and dispose of waste tissues according to UF EHS guidelines.

5.3 Handle sharps (e.g., scalpels, blood tubes) carefully and dispose of properly.

5.4 Follow aseptic procedures throughout processing.

## 6 PROCEDURE

6.1 The tissues received will be identified as follows:

**Table 1. Sample Type Nomenclature and Abbreviations**

| Sample Type | Sample Type Abbreviation |
|---|---|
| Pancreas- Uncinate | PanUncinate |
| Pancreas- Head | PanHead |
| Pancreas- Body | PanBody |
| Pancreas- Tail | PanTail |
| Pancreas-Other | PanOther |
| Pancreatic Lymph Node | PLN |
| Spleen | Spleen |
| Non-pancreatic Lymph Node | NonPLN |
| Duodenum | Duo |
| Skin | Skin |
| Thymus | Thy |
| Vertebral Bodies | BM |
| Eye | Eye |
| Kidney | Kid |
| Heart | Heart |
| Sural nerve | S Nerve |
| Other organs | [truncated name] |

6.2 Aliquots from samples will be identified as follows:

**Table 2. Aliquot Type**

| Type | |
|---|---|
| DNA | OCT |
| EM-Lowicryl | Paraffin |
| EM-TAAB | Serum |
| Fresh Spleen, PLN, etc. | Vials with and without RNAlater |

6.3 Case Number Assignment

6.3.1 nPOD organ donors will be assigned sequential case numbers starting at 6000 for the University of Florida processing facility.

6.3.2 Alternate donor numbering will be used at other sites.

6.4 Aliquot Labeling

6.4.1 Cassettes for paraffin embedding

6.4.1.1 Line #1: Case ID + Block # + Sub-division where applicable (e.g., 6101-01A)

6.4.1.2 Line #2: Sample type abbreviation (Table 1)

6.4.2 OCT cryomolds

6.4.2.1 Print legibly using a ink Sharpie pen

6.4.2.2 Identify by Case ID + Block# + Sub-division and Sample Type

6.4.3 Cryovials

6.4.3.1 Line #1 and #2: As for cassettes

6.4.3.2 Line #3: Aliquot type (Table 2)

6.4.3.3 Line #4: Barcode and unique aliquot number

6.5 Data Collection

6.5.1 Collection data will be recorded in the nPOD database. Access will be limited to UF nPOD staff and will be granted by the Administration or OPPC Director.

6.5.2 Required fields during case processing include case identification, processing date and time (start and end), staff, samples and blood tubes (tube top color, number, volume of serum or whole blood), and aliquot types and numbers.

6.5.3 Optional: The Case Worksheet form will be used for manual data entry and scanned for archiving. Data will be transferred to the database by OPPC staff.

6.6 Tissue Dissection

6.6.1 Identify and record all shipment contents.

6.6.1.1 Shipment paperwork will be kept in a secure place until transferred to nPOD Administration Core.

6.6.2 Whole Blood

6.6.2.1 Note relative temperature of tubes upon receipt (room temperature is preferred).

6.6.2.2 Refer to the Isolation of PBMC SOP for further processing of whole blood.

6.6.3 Serum tubes

6.6.3.1 Centrifuge tubes at 1400 rpm for 10 minutes at room temperature.

6.6.3.2 If hemolysis observed, record the degree (i.e., light or gross) and re-centrifuge at the same settings for an additional 10 minutes.

6.6.3.3 Immediately aliquot the serum in labeled O-ring cryovials using volumes of 200 ul or more per vial. Store at -80$^{o}$ C.

6.6.3.4 Make one aliquot containing at minimum200 ul vial for autoantibody analysis (See SOP Autoantibody RIA).

6.6.3.5 Make one aliquot containing 50-100 ul for C-peptide analysis (See SOP C-Peptide Determination).

6.6.3.6 Make one aliquot containing a minimum of 200ul for autoantibody confirmation testing (SOP 22: Autoantibody Screening Process).

6.6.4 Duodenum

6.6.4.1 Dissect the duodenum from the pancreas and hold in cold buffer until processed.

6.6.4.2 To procure duodenal mucosa, use separate dissecting board and instruments. Open the duodenum and gently scrape off mucus or ingesta. Cut off several segments of mucosa.

6.6.4.3 Place duodenal mucosal segments in cassettes for paraffin processing, cryomolds for OCT blocks, and mince for use in cryovials (see 6.6.5.10). Samples for paraffin and OCT molds will be oriented so that the mucosa is perpendicular to the muscle layers after sectioning.

6.6.4.4 For OCT blocks, place mucosa on slightly frozen OCT to maintain vertical orientation then fill mold with OCT. Immerse molds in a freezing bath (dry ice or liquid nitrogen-cooled isopentane) until frozen. Hold on dry ice or immediately transfer to -80° freezer. Wrap OCT blocks in tin fold for long term storage.

6.6.5 Pancreas

6.6.5.1 Optional: Spread one stripe of blue ink on the anterior surface of the pancreas.

6.6.5.2 Tare scale with a container to hold the pancreas.

6.6.5.3 Weigh the pancreas and record.

6.6.5.4 Divide the pancreas into 3 regions (See Appendix 1).

Head: Portion adjacent to the duodenum and includes the region proximal to the notch.

Body and Tail: Equal division of remaining portion after head removed.

6.6.5.5 Weigh each region and record.

6.6.5.6 Remove a section from the Head-Body junction and a section from the Body-Tail junction to be minced for cryovials.

6.6.5.7 Mince tissues for cryovials with or without RNAlater to ~1.0 gram pieces and evenly divide pieces among cryovials to ensure uniform distribution.

6.6.5.7.1 Each vial without RNAlater will contain ~2-5 grams of tissue. Immediately snap freeze the vials in liquid nitrogen and then transfer to -80$^{o}$C for storage.

6.6.5.7.2 Each vial with RNAlater will contain about 1 gram of tissue. Mix contents with RNAlater and then equilibrate at room temperature for 30 minutes. Remix and snap freeze the vials and transfer to -80$^{o}$C for storage.

6.6.5.8 Section each pancreas region in a transverse “bread loaf” manner with alternating sections for paraffin and frozen OCT blocks.

6.6.5.9 In all cases, maintain medial to lateral/anterior to posterior orientations in cassettes as feasible depending on sample size.

6.6.5.10 Number blocks sequentially beginning with most medial section.

6.6.5.11 Sections for paraffin cassettes should be ~ 1.5 x 1.5 x 0.5 cm. Place in labeled cassettes with the label oriented to the left. The orientation of the tissues in the cassettes should be as if viewed from the donor’s midline through the long axis of the pancreas.

6.6.5.11.1 If the sections are too large to fit into one mold, cut each section in half and label cassettes A&B according to Figure 2.

6.6.5.11.2 If the sections are still too large after being cut in half, cut each section perpendicular to the previous cut and label the cassettes A-D in a clockwise manner. If necessary, the sections can be trimmed further to fit in the cassettes.

6.6.5.11.3 Place cassettes in NBF and record the fixation start time when the last cassette is placed in fixative.

6.6.5.12 Fix samples using an automatic processor (16 hours) or stop the process manually (24 ± 8 hours). Fixation is ended by transfer to 70% ethanol. Record end time when preformed manually Trim tissues intended for OCT blocks to ~0.5 x 0.5 cm and place in cryomolds with a small amount of OCT media. Freeze as above.

6.6.5.12.1 If the sections are too large, sub-divide each section as for paraffin.

6.6.6 <u>Pancreas Electron Microscopy</u>

6.6.6.1 Collect pancreas samples from the junction of the head and body and process as described in the Electron Microscopy SOP. Select regions that are comprised of mostly exocrine tissue.

6.6.7 <u>Spleen</u>

6.6.7.1 Prepare sterile RPMI* complete solution. An asterisk denotes that supplements have been added to the solution.

6.6.7.1.1 RPMI* complete solution can be used up to one month.

6.6.7.1.2 Remove and discard 50 ml from 500 ml RPMI stock solution.

6.6.7.1.3 Add 50 ml FBS to 6.6.7.1.2 above to make a final concentration of 10% FBS.

6.6.7.1.4 Add 5 ml of 100x antibiotic/antimycotic stock to 6.6.7.1.2 above to make a final dilution of 1:100.

6.6.7.1.5 Label the RPMI* complete container with preparation date, additives, and preparer's initials and store at 4$^{o}$C.

6.6.7.2 The number of cryovials prepared will depend on the spleen size and requests for fresh spleen.

6.6.7.3 Procure a 2 x 3 cm sample for cell isolation.

6.6.7.3.1 If temporary holding is needed, place sample in a culture dish with sterile D-PBS.

6.6.7.3.2 Mince the spleen sample into small (5 x 5 mm) pieces and transfer ~ 25 pieces to 50 ml tubes with RPMI media. Store minced spleen in refrigerator until shipment or use. For distribution to investigators, completely fill tubes with RPMI to avoid excessive shaking of spleen during shipment.

6.6.7.4 Place a ~0.5 gram piece of spleen in cryovial and store at -20$^{o}$C for DNA extraction (See DNA Extraction SOP).

6.6.7.5 Use remaining spleen for fixed paraffin blocks, OCT frozen blocks, and cryovials (with and without RNAlater).

6.6.8 Pancreatic Lymph Nodes (PLN)

6.6.8.1 Dissect PLN in peripancreatic fat and hold in D-PBS in a cell culture dish until collections finished.

6.6.8.2 Remove fat or connective tissues from each PLN and incise capsule if needed.

6.6.8.3 Use the following decision tree to determine which aliquots to prepare:

6.6.8.3.1 **Minimum tissue size for RPMI cell culture specimens is approximately 1 x 2 cm.**

6.6.8.3.2 If total lymph node volume is ≤ 1 x 2 cm, prepare only OCT blocks.

6.6.8.3.3 If total lymph node volume is up to 1 x 4 cm, prepare one RPMI tube for cell isolation with the remainder for OCT blocks.

Transfer minced nodes to RPMI-filled 15 ml tubes and hold in refrigerator until use.

6.6.8.3.4 If total lymph node volume is up to 1 x 5 cm, submit as RPMI and OCT blocks and collect the remainder for RNAlater vials.

For RNAlater vials, group enough nodes for 4-5 RNAlater tubes and mince together so each vial will receive an equal representation of harvested nodes.

6.6.8.3.5 If total lymph node volume is up to 1 x 6 cm, prepare additional RPMI aliquots or RNAlater vials.

6.6.9 Non-Pancreatic Lymph Nodes

6.6.9.1 As for PLN.

6.6.10 Sample Archive

6.6.10.1 All materials obtained by this program will be inventoried in the nPOD database and archived in the OPPC.

6.6.10.2 Samples will be transferred upon request by the sponsor.

## 7 REFERENCES


7.1 Clinical Association of Pathology Anatomic Pathology Manual

7.2 Campbell-Thompson, et. all. Processing of human pancreas. JoVE, in press, 2012.

7.3 SOP 26 Autoantibody RIA

7.4 SOP 59 Isolation of PBMC

7.5 SOP 71_1 Electron Microscopy

7.6 SOP 79 DNA Extraction

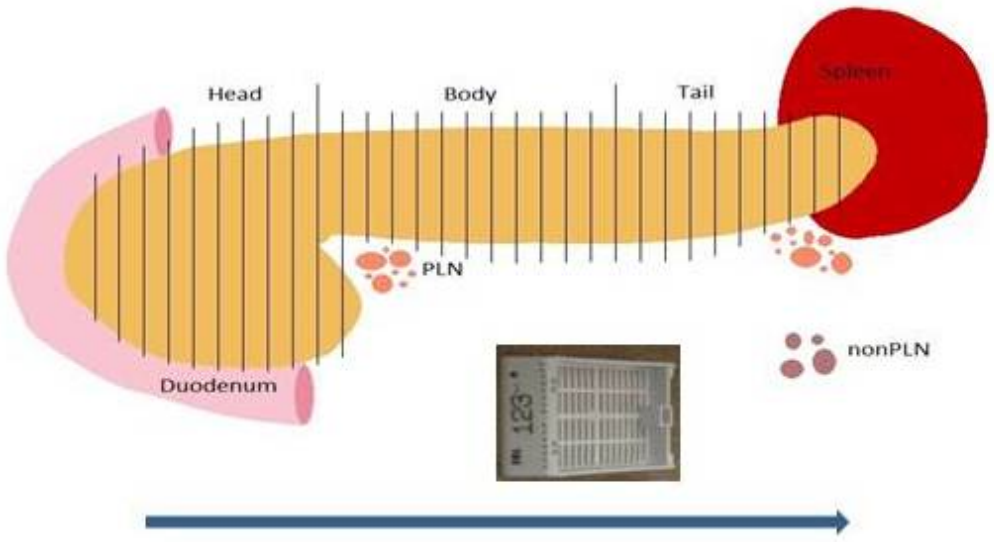
7.7 SOP 85 C-Peptide Determination


## 8 REVISION HISTORY

| Version | Date | Revision |
|---|---|---|
| 0 | 9/21/11 | Added Appendix 2 and changed text on trimming larger pancreata. AW 9/21/11 |
| 1 | 3/29/12 | Edited the pancreas processing and updated the figures in the appendices. EM 3/29/12 |
| 2 | 8/28/12 | Updated media composition for PLN processing IK 8/28/12 |

| | | | |
|---|---|---|---|
| Prepared by | Emily Montgomery | | |
| Approved by | Martha Campbell-Thompson | | |
| | Name | Signature | Date |

**Appendix 1**




Lay sections to the right and place in cassette in the same orientation.

**Pancreas:** Head, body, and tail – bread loaf so paraffin and OCT are harvested in alternating sections, reserve the sections at the head-body and body-tail junctions to be minced for cryovials.

OCT blocks: 5-10 all regions or as indicated by size with A/B and A-D subsections as necessary.

Paraffin blocks: 5-10 with A/B and A-D subsections as necessary (OCT takes precedence over paraffin if the sample size is too small).

Cryovials – 4 w/o RNALater and 6 w/RNALater of the PanHead-PanBody and PanBody-PanTail junctions.

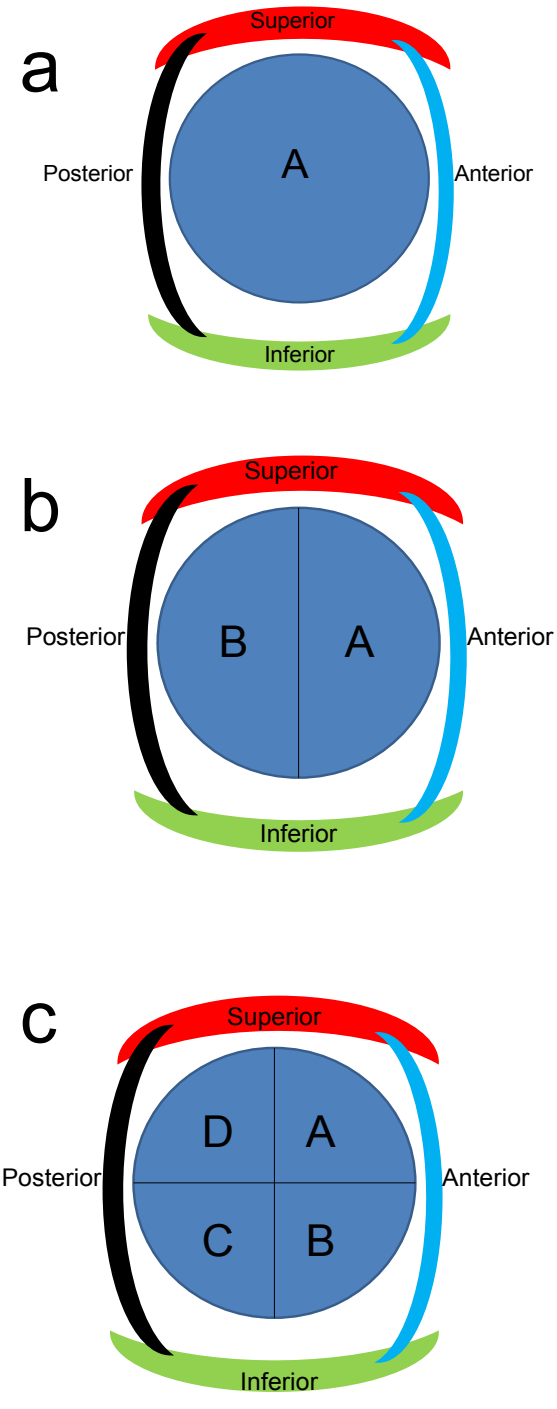
**PLN:** OCT blocks, Cryovials w/RNALater, Fresh for cells

**Spleen**: OCT blocks, Paraffin blocks, Cryovials with and without RNALater, Fresh for cells

**NonPLN:** OCT blocks, Cryovials w/RNALater, Fresh for cells

**Duodenum:** OCT blocks, Paraffin blocks, Cryovials with and without RNALater

**Appendix 2**


a
Superior
Posterior
A
Anterior
Inferior
b
Superior
Posterior
B
A
Anterior
Inferior
c
Superior
D
A
Posterior
Anterior
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B
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