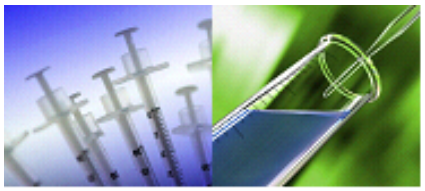


nPOD
Network for Pancreatic Organ Donors with Diabetes

CASE PROCESSING

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for processing and storing pancreas and other tissues including 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.

Sterile dissecting instruments (forceps, scissors, scalpels)
Scale and weighing boats
Dissection boards
Sterile gauze sponges/paper towels
Centrifuge tubes (15 ml, 50 ml)
Dulbecco's Phosphate Buffered Saline (D-PBS), Mg2+ Ca2+ free (Invitrogen, Cat. No. 10010-023), store at 4°C
Complete culture media (DMEM/F12 50/50 with L-Glutamine (Corning Cat. No. 10-090-CV + 10% Fetal Bovine Serum (Corning, Cat. No. MT35016CV + 1x Anti/Anti)
Inactivated fetal bovine serum (FBS), aliquot 50 ml and store at -20°C
100x Antibiotic-Antimycotic solution, 10,000 I.U./ml Penicillin 10,000 µg/ml Streptomycin 25 µg/ml Amphotericin B (Corning, Cat. No. 30-004-CI), aliquot 5 ml and store at -20°C
Uni-cassettes (Tissue-Tek®)
Label printers (cab EOS1, Brady BSP31 Label Attachment System)
10% neutral buffered formalin (NBF) in specimen container
O.C.T. ™ compound (Tissue-Tek®) and cryomolds, aluminum foil
Cryovials with O-rings (FisherSci, Cat. No. 12-565-163N)
RNAlater® (Ambion™), store at 25°C
Pipettes and sterile filter tips (200 µl, 1000 µl)
Dry ice and ice bucket
2-Methylbutane with dry ice in ice bucket and long forceps
70% ethanol
10-50% Clorox bleach (6% Sodium hypochlorite)
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- Head	PanHead/PH
Pancreas- Body	PanBody/PB
Pancreas- Tail	PanTail/PT
Pancreas-Other	PanOther
Pancreatic Lymph Node	PLN
Spleen	Spleen/Sp
Non-pancreatic Lymph Node	NonPLN/nPLN
Duodenum	Duo
Skin	Skin
Thymus	Thy
Vertebral Bodies	BM
Eye	Eye
Kidney	Kid
Heart	Heart
Sural nerve	S Nerve
Whole Blood	Whole Blood
Other organs	[truncated name]

6.2 Aliquots from samples will be identified as follows:

Table 2. Aliquot Type

Type	
Cells	
DNA	OCT
	Paraffin
	PBMC
EM-4%PF	RNA
EM-2%PF+1%GA	
	Vials
Fresh Spleen, PLN, etc.	Vial 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)

6.4.1.3 Line #3: Barcode

6.4.2 O.C.T. cryomolds

6.4.2.1 Print legibly using permanent ink

6.4.2.2 Line #1 and #2: As for cassettes

6.4.3 Cryovials

6.4.3.1 Line #1 Case ID + aliquot number

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

6.4.3.3 Line #3: Aliquot type (Table 2)

6.4.3.4 Line #4: Barcode

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, date received, processing date and time (start and end), staff, samples, pancreas section weights, blood tubes (tube top color, number, volume of serum or whole blood), aliquot types and numbers, and tissue quality and recovery feedback comments.

6.5.3 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 Blood Processing and Tissue Dissection

6.6.1 <u>Identify and record all shipment contents.</u>

6.6.1.1 <u>Photograph exterior of shipment container, including UNOS label and contents checklist sticker</u>

6.6.1.2 <u>Photograph interior of container</u>

6.6.1.2.1 <u>Include all tissue and documentation received</u>

6.6.1.2.2 <u>Include any packing abnormality (ie. Melted ice, missing items)</u>

6.6.1.3 <u>In the event of any shipment error, contact on-call administration staff who will notify the OPO</u>

6.6.1.4 <u>Complete the Recovery Feedback section on the Case Worksheet</u>

6.6.2 <u>Whole Blood</u>

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

6.6.3 <u>Serum tubes</u>

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 300ul or more per vial. Store at -80^{o} C.

6.6.3.4 Make one aliquot containing at minimum 200ul vial for autoantibody analysis (See SOP Autoantibody RIA).

6.6.3.5 Make one aliquot containing 50-100ul 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 <u>Tissue Separation</u>

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

6.6.4.2 Dissect the spleen from the pancreas and hold in cold buffer until processed.

6.6.4.3 Clean the pancreas of peripancreatic fat and collect in cold buffer for pancreatic lymph node dissection.

6.6.5 Spleen

6.6.5.1 Prepare sterile DMEM/F12* complete culture media. An asterisk denotes that supplements have been added to the solution.

6.6.5.1.1 DMEM/F12 complete solution can be used up to one month.

6.6.5.1.2 Remove and discard 50 ml from 500 ml DMEM/F12 stock media.

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

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

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

6.6.5.2 Procure spleen tissue for cell isolation.

The number of tubes with tissue for cell isolation will depend on the spleen size and requests for fresh spleen sample.

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

6.6.5.2.2 Mince the spleen sample into small (5 x 5 mm) pieces and transfer to 50 ml tubes with complete DMEM/F12 media up to the 25ml line. Store minced spleen in refrigerator until shipment or use. For distribution to investigators, completely fill tubes with complete DMEM/F12 media to avoid excessive shaking of spleen during shipment.

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

6.6.6 Pancreas

6.6.6.1 Spread one stripe of blue ink on the anterior surface of the pancreas.

6.6.6.2 Tare scale with a container to hold the pancreas tissues.

6.6.6.3 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.

6.6.6.4 Weigh each region and record.

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

6.6.6.6 Mince tissues for cryovials with or without RNAlater to small (3x3mm) pieces and evenly divide pieces among cryovials to ensure uniform distribution.

6.6.6.6.1 Immediately snap freeze the vials without RNAlater in dryice/isopentane bath or liquid nitrogen and then transfer to -80^{o}C for storage.

6.6.6.6.2 For vial with RNALater, mix tissue contents with RNAlater buffer and then equilibrate at room temperature for 15 minutes. Snap freeze the vials and transfer to -80^{o}C for storage.

6.6.6.7 Section each pancreas region in a transverse "bread loaf" manner with alternating sections for paraffin and frozen OCT blocks (See Appendix 1).

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

6.6.6.9 Number blocks sequentially beginning with most medial section.

6.6.6.10 Place sections in labeled cassettes. 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 (See Appendix 1).

6.6.6.10.1 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.

6.6.6.10.2 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. (See Appendix 2)

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

6.6.6.11 Fix samples using an automatic processor or manually for 24 hours. For pancreas with high fat content, fixation time should be increased (40±8 hours). Fixation is ended by transfer to 70% ethanol. Record end time when preformed manually.

6.6.6.12 Sections intended for OCT blocks are placed in cryomolds with a small amount of OCT media. Freeze as above.

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

6.6.7 Optional: Pancreas Electron Microscopy

6.6.7.1 Collect pancreas samples from the head and body junction and body and tail junction and process as described in the Electron Microscopy SOP.

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 Make two paraffin blocks using two medium PLNs or several small PLN.

6.6.8.4 Make two OCT block using two medium PLNs or several small PLN.

6.6.8.5 Remaining PLNs should be divided between cell isolation, fresh shipments, snap-frozen vials and RNALater snap-frozen vials.

6.6.9 Non-Pancreatic Lymph Nodes

6.6.9.1 As for PLN.

6.6.10 Duodenum

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

6.6.10.2 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.

6.6.10.3 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/isopentane or liquid nitrogen-cooled isopentane) until frozen. Hold on dry ice or immediately transfer to -80° freezer. Wrap OCT blocks in tin foil fold for long term storage.

6.6.11 Sample Archive

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

6.6.11.2 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

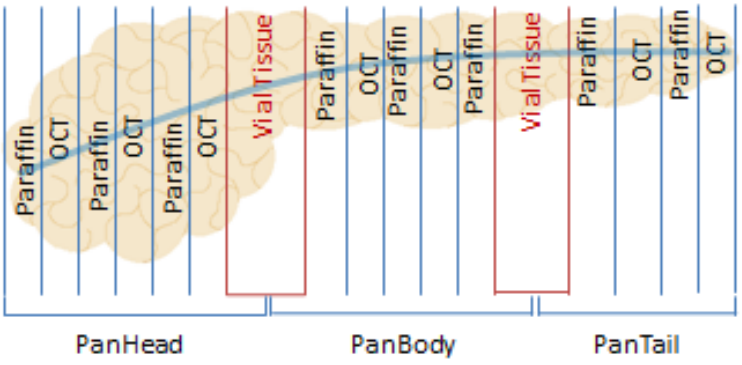
7.7 SOP 85 C-Peptide Determination

8 REVISION HISTORY

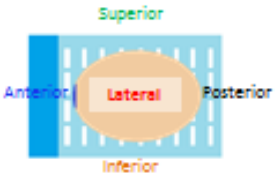
Version	Date	Revision
1	9/21/11	Added Appendix 2 and changed text on trimming larger pancreata. AW 9/21/11
2	3/29/12	Edited the pancreas processing and updated the figures in the appendices. EM 3/29/12
3	8/28/12	Updated media composition for PLN processing IK 8/28/12
4	6/11/15	Updated reagents, added tissue separation, removed processing of duodenum mucosa, removed weighing entire pancreas, removed reference to tissue weights in vials, updated manual fixation procedure, changed order of SOP to reflex order of organs processed in real time. Updated Appendix 1 and 2.
5	9/30/15	Updated reagents, Table 1 and 2, aliquot labeling, data collection, and blood processing and tissue dissection to reflect current procedures. Added additional package documentation steps (section 6.6.1).

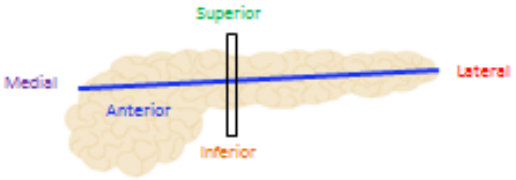
Prepared by	Tiffany Philips		
Edited by	Maria Peterson		9/30/15
Approved by			
	Name	Signature	Date

Appendix 1



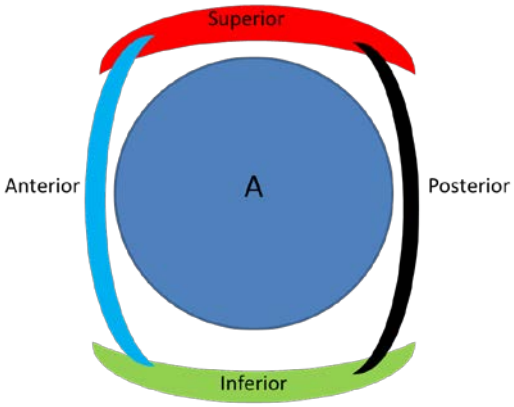
Pancreas Head, Body, and Tail are sectioned so paraffin and OCT blocks are collected in alternating sections. Sections at the Head/Body junction and Body/Tail junction are to be minced for vials.





Appendix 2

a



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