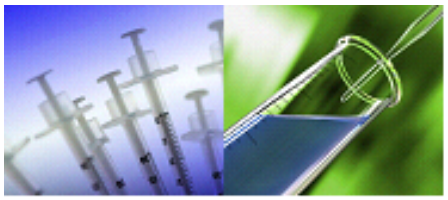

nPOD
Network for Pancreatic Organ Donors with Diabetes

STANDARD OPERATING PROCEDURE

Case Processing

OPPC-SOP-57

Prepared by:	Tiffany Philips	**Original Effective Date:**	07/07/2007
Revised by:	Maria Beery	**Version Effective Date:**	04/16/2021
Reviewed by:	Irina Kusmartseva	**Reviewed Date:**	01/30/2018
Approved by:	Irina Kusmartseva	**Approved Date:**	04/16/2021

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Network for Pancreatic Organ Donation with Diabetes (nPOD)
BMSB Room J586
P.O. BOX 100275
Gainesville, FL 32610

CASE PROCESSING

POLICY: Use universal safety precautions when handling human samples and use appropriate personal protective equipment. Follow biohazardous and chemical safety procedures and dispose of waste tissues according to UF EH&S guidelines. Handle sharps carefully and dispose in sharps containers. Follow aseptic procedures throughout processing.

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).

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

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

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

EQUIPMENT & MATERIALS: The materials, equipment and forms in the following list are recommendations only and alternative products may be substituted for the site specific task or procedure.

- Kimberly-Clark Benchtop Protector (Fisher, Cat. No. 15-235-101)
- Leica L'Absorbs towel (Leica, Cat. No. 3803240)
- Double Edge Pathology Blades for Pathco Handle (Fisher, Cat. No. 23-720-200)
- Dissection Board (Fisher, Cat. No. 36114)
- Dissecting Forceps (Fisher, Cat. No. 13-812-40)
- Dissecting Scissors (Fisher, Cat.No. 08-940)
- Margin Marking Dye, Blue (American Master Tech, Cat.No. STD2227BL)
- Wooden Applicators with Cotton tips (Fisher, Cat. No. 23-400-115)
- Microtubes with Silicone O-ring (VWR, Cat. No. 89004-302)
- Tissue-Tek O.C.T. Compound Media (VWR, Cat.No. 25608-930)
- Invitrogen RNALater™ Stabilizing Solution (Invitrogen, Cat. No. AM7021), store at room temperature
- D-PBS 1X without Ca^{2+} or Mg^{2+} (Fisher, Cat. No. MT21040CM), store at 4°C
- DMEM/F12 50/50 Media, with L-Glutamine and 15mM HEPES, (Fisher, Cat. No. MT10092CM)
- Antibiotic-Antimycotic solution, 100x, 10,000 I.U./ml Penicillin 10,000 µg/ml Streptomycin 25 µg/ml Amphotericin B (Fisher,

- 32 oz. Screw Top Polypropylene Histology Container (Fisher, Cat. No. 22-026-315)
- 52 oz. Plastic Container with Lid (Fisher, Cat. No. 02-544-127)
- 10% Neutral Buffered Formalin (NBF) (Fisher, Cat. No. 23-245-685)
- Cassettes (Lab Storage Systems, Cat. No. EC-0112)
- Disposable Base Molds, 15x15mm, 24x24mm, 30x24mm, 37x24mm (Fisher, Cat.No. 22-050-159 to 162)
- Parafilm M™ Wrapping Film (Fisher, Cat.No. S37440)
- Kimberly-Clark Fluidshield Fog-Free Protective Mask (Fisher, Cat. No. 19-003-495)
- Disposable Lab Coats
- Nitrile gloves
- Permanent marker
- Biosafety cabinet
- Cat. No. MT30004CI), aliquot 5 ml and store at -20°C
- HyClone Fetal Bovine Serum (Fisher, Cat. No. MT35016CV), aliquot 50 ml and store at -20°C
- High-Performance centrifuge tubes, 15 and 50 ml, Sterile (VWR, Cat. No. 89039-666 and 89039-658)
- Sterile Nunc Cryotubes (Thermo Sci, Cat No. 375418)
- Weigh boats
- Pipettes and sterile filter tips (20 µl, 200 µl, 1000 µl)
- Dry ice
- 2-Methylbutane
- Magic Touch 2™ Ice Pans, 9L with lid (Bel-Art, Cat.No. M16807-9104)
- 70% ethanol
- Concentrated bleach
- Biohazard Sharps containers
- Label printers (cab EOS1, Brady BSP31 Label Attachment System)
- Centrifuge
- Microtube racks
- Balance, 200g

PROCEDURE:

1.0 Identification of tissue and aliquots from samples

1.1 Sample Type Nomenclature and Abbreviations:

Sample Type	Sample Type Abbreviation
Pancreas- Head	PanHead/PH
Pancreas- Body	PanBody/PB
Pancreas- Tail	PanTail/PT
*Pancreas- Uncinate *inactive*	*PanUnc/PU *inactive*
Pancreas- Other	PanOther/PO
Pancreas- Transplant	Pan-Tx
Pancreatic Lymph Node	PLN
Spleen	Spleen/Sp
Non-pancreatic Lymph Node	NonPLN/nPLN
Duodenum	Duo
Adipose- Pancreatic	Adipose
Thymus	Thy

Vertebral Bodies/Bone Marrow	VB/BM
Kidney	Kid
Heart	Heart
Plasma	Plasma
Serum	Serum
Other tissues	[truncated name]

1.2 Aliquots from samples will be identified as follows:

Aliquot type	
Cells	OCT-4%PF
DNA	Paraffin
EM-4%PF	PBMC
EM-2%PF-1%G	PF
Fresh-15ml media/-50ml media	RNA
Islet	Vial
OCT	Vial RNALater

2.0 Case number assignment

2.1 nPOD organ donors will be assigned sequential case numbers starting at 6000 for the University of Florida processing facility.
2.2 nPOD-T transplant donors will be assigned sequential case numbers starting at 3000.
2.3 HANDEL-P and HANDEL-I donors will be assigned sequential case numbers starting at HDL001.
2.4 Gastrointestinal project donors will be assigned sequential case numbers starting at G1000.
2.5 SARS-CoV-2 donors will be assigned sequential case numbers starting at COV-01
2.6 Alternate donor numbering will be used at other sites.

3.0 Aliquot labeling

3.1 Refer to SOP Tissue Sample Archiving

3.2 Cassettes for paraffin embedding
- 3.2.1 Line #1: Case ID + Block # + Sub-division where applicable (e.g., 6101-01A)
- 3.2.2 Line #2: Sample type abbreviation (Table 1)
- 3.2.3 Line #3: Barcode

3.3 O.C.T. cryomolds
- 3.3.1 Line #1 and #2: As for cassettes

3.4 Cryovials and Fresh tissue
- 3.4.1 Line #1 Case ID + aliquot number
- 3.4.2 Line #2: Sample type abbreviation (Section 1.1)
- 3.4.3 Line #3: Aliquot type (Section 1.2)
- 3.4.4 Line #4: Barcode

4.0 Data collection

4.1 Data will be recorded in the nPOD databases. Access will be limited to UF nPOD staff and will be granted by the Administration or OPPC Director.
4.2 Use a single line to strikethrough corrections, then initial and date.
4.3 Identify and record all shipment contents.

4.3.1 Photograph interior of container if there is any packing abnormality (ie. melted ice, missing items).

4.3.2 In the event of any shipment error, contact on-call administration staff who will notify the OPO.

4.3.3 Complete the Recovery Feedback section on the Case Worksheet.

4.4 Completely fill out the case worksheet form during processing.

4.4.1 After the case has been processed, archive the case worksheet and enter all data following SOP 52 Case Data Management and SOP 55 Sample Data Management.

5.0 Sterile media preparation

5.1 Complete media

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

5.1.2 Add 50ml FBS to the 450ml DMEM/F12 for a final concentration of 10% FBS.

5.1.3 Add 5ml 100X antibiotic/antimycotic stock.

5.1.4 Label the sterile media container with preparation date, additives, and preparer's initials. Store at 4°C for up to one month.

5.2 Phosphate-Buffered saline

5.2.1 Add 5ml 100x antibiotic/antimycotic stock to 500ml 1x D-PBS (without Ca^{2+} or Mg^{2+}).

5.2.2 Label the sterile D-PBS container with the preparation date, additive, and preparer's initials. Store at 4°C for up to one month.

6.0 Fixation and freezing preparation

6.1 Add 5-10 lbs dry ice pellets to an ice pan and cover.

6.2 Add 5 lbs dry ice pellets and vial racks to a second ice pan, fill pan ~5 cm with 2-Methylbutane and cover. Ensure enough 2-Methylbutane is added such that vials are 75% submerged.

6.3 Pour 10% NBF into two 52 oz. plastic containers until half full, then secure lids.

6.3.1 Label one container with case ID number, date, and "10% Neutral Buffered Formalin" for cassettes.

6.3.2 Label second container as "Waste Tissue, 10% Neutral Buffered Formalin".

6.4 Prepare RNALater tubes by filling 50mL conical tubes with 20-30mL RNALater solution. Label each tube with the Case ID and sample type for PanHead, PanBody, Spleen, and Duodenum.

7.0 Blood processing and tissue dissection

7.1 Whole Blood and Cell Isolation

7.1.1 Refer to SOP 59 Isolation of PBMC for further processing of whole blood, SOP 60 Isolation of Cells from Spleen, Thymus, and Lymph Nodes, and SOP 62 Bone Marrow Cell Isolation.

7.1.1.1 If a third staff member is present for case processing, cell isolation may be started at this time with PBMC processed first, followed by spleen, PLN, thymus, and bone marrow.

7.1.1.2 If one or two staff members are present, perform cell isolations after all other tissue samples have been processed.

7.2 Serum tubes

7.2.1 Centrifuge tubes at 1300 x g for 10 minutes at room temperature.

7.2.2 If hemolysis observed, record the degree (i.e., light or gross) and re-centrifuge at the same settings for an additional 5 minutes.

7.2.3 Aliquot 300 µl of serum into the first three labeled O-ring microtubes, then 700 µl into subsequent aliquots. Snap freeze in 2-methylbutane/dry ice, then place on dry ice.

7.2.4 Aliquots 1 through 3 will be used for autoantibody, C-peptide, and QC analysis. Refer to SOP 85 C-Peptide Determination and SOP 22 Autoantibody Screening Process.

7.3 Tissue Separation

7.3.1 Lay out the en-bloc pancreas with duodenum and spleen attached on the dissection board in the biosafety cabinet. If thymus or bone marrow received, keep in original container until processing.

7.3.2 Remove duodenum, spleen, and peri-pancreatic fat using blunt dissection technique and surgical dissection tools as needed.

7.3.3 Place duodenum, spleen, and peri-pancreatic fat in cold UW storage solution.

7.4 Spleen

7.4.1 Cut one 10cmx20cm piece of spleen for vials, paraffin, and O.C.T. blocks and set aside.

7.4.2 Divide the remainder of spleen in 15-20g aliquots, minced into 5x5mm pieces, and transfer to 50ml conical tubes with 35ml of complete media. Secure the tops and wrap with paraffin.

7.4.2.1 The number of aliquots is determined by investigator requests, with each investigator receiving a minimum of one 50ml tube. Reserve 2 aliquots for OPPC cell isolation.

7.4.3 Process the 10cm x 20 cm piece of spleen into snap frozen vials, paraffin blocks, and O.C.T. blocks.

7.4.3.1 Snap frozen vials

7.4.3.1.1 Mince 0.5 grams of tissue and place in vial.

7.4.3.1.2 Secure the top of the vial and place in the dry ice/2-methylbutane bath until all tissue has been processed.

7.4.3.1.3 For vials with RNALater, add minced tissue to the prepared RNALater conical tube. Fill with additional RNALater solution up to 40mL, close the tube, and invert to ensure proper contact with tissue. Use Parafilm to secure the top of the tube, and store horizontally in processing room fridge at 4°C overnight.

7.4.3.2 Paraffin and O.C.T. blocks

7.4.3.2.1 Place 0.5cm thick sections for paraffin in labeled cassettes, then place cassettes in container with 10% NBF labeled with the case ID.

7.4.3.2.2 Dispense enough O.C.T. media in the cryomold for tissue to remain in place, then place tissue on top of media in the center of the mold. Add O.C.T. until the tissue is covered ensuring minimal bubble formation.

7.4.3.2.3 Place the cryomold in the dry ice/2-methylbutane bath, 30-60 seconds, or until O.C.T. molds stop bubbling, then transfer the frozen block to the dry ice container.

7.5 Pancreas

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

7.5.2 Tare scale with a weigh boat to hold the pancreas regions.

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

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

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

7.5.3.3 Weigh each region and record. **Do not weigh pancreas if whole pancreas is not intact**

7.5.4 Remove a 1cm section from the Head-Body junction and a 1cm section from the Body-Tail junction to be minced for microtubes with and without RNALater.

7.5.4.1 Microtubes for Head-Body junction will be labeled as PanHead, while tubes for Body-Tail junction will be labeled as PanBody.

7.5.4.2 Mince tissue to small (3x3mm) pieces and evenly divide pieces among tubes to ensure uniform distribution. Proceed as in step 7.4.3.1.

7.5.5 Remove a 0.5cm section from the Body-Tail junction for the nPOD slices project, and transfer to cell culture dish with sterile ECS buffer. Process for fresh pancreas slice generation, distribution, and functional assays according to SOP 58 Vibratome Slicing and SOP 56 Perifusion of Tissue Slices.

7.5.6 Section each pancreas region in a transverse "bread loaf" manner with alternating 0.5cm sections for paraffin and frozen OCT blocks (See Appendix 1). Section PanTail first, followed by PanBody and PanHead.

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

7.5.7 Number blocks sequentially beginning with most medial section.

7.5.8 Place sections in labeled cassettes and cryomolds. 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).

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

7.5.8.2 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).

7.5.9 Place sections in cryomolds as in steps 7.4.3.2.2, maintaining orientation.

7.5.9.1 If the sections are too large, sub-divide each section as in step 7.5.9.

7.6 Optional: Pancreas Electron Microscopy

7.6.1 Collect pancreas samples from the head/body junction and body/tail junction and process as described in the SOP 71 Electron Microscopy.

7.7 Pancreatic Lymph Nodes (PLN)

7.7.1 Dissect PLN from peri-pancreatic fat and hold in D-PBS in a cell culture dish.

7.7.2 Remove fat or connective tissues from each PLN.

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

7.7.4 Make two OCT blocks using two medium PLN or several small PLN.

7.7.5 Remaining PLN should be divided between cell isolation, fresh shipments, snap-frozen vials and RNALater snap-frozen vials depending on investigator requests and sample inventory needs.

7.8 Non-Pancreatic Lymph Nodes

7.8.1 See 7.7, as for PLN.

7.9 Duodenum

7.9.1 Open the duodenum from inferior to superior and flush the mucosa.

7.9.2 Cut 15-20 5mm wide transverse segments of duodenum

7.9.3 Snap frozen vials

7.9.3.1 Mince 1 strip of duodenum per vial.
7.9.3.2 Proceed as in step 7.4.3.1.
7.9.4 Paraffin and O.C.T. blocks, proceed as in 7.4.3.2.
7.9.4.1 Further divide duodenum segments to fit in the cassettes. Soak histology paper in 10% NBF. Place each duodenum segment on a pre-soaked histology paper mucosa-side up. Fold the histology paper to cover the segment, and close the cassette.

7.10 Thymus
7.10.1 Dissect into 2 equal sections.
7.10.1.1 Cut one section into several pieces and transfer to 50ml conical tube with 35ml sterile complete media. Refer to SOP 60 for cell isolation.
7.10.1.2 Divide the second section into fourths to make 2 paraffin and 2 O.C.T. blocks, following step 7.4.3.2.

7.11 Bone Marrow
7.11.1 Refer to SOP 62 Bone Marrow Cell Isolation.

7.12 Other tissue
7.12.1 Make 2 paraffin and 2 O.C.T. samples as in 7.4.3.2.
7.12.2 Make 2 vials with and 2 without RNALater as in 7.4.3.1 depending on tissue type and amount available.

7.13 Sample Archiving and Processing Completion
7.13.1 Fix cassettes using an automatic processor or manually for 24 hours at room temperature in 1-2 L of 10% NBF with magnetic ~~stirring~~ stir bar. Record fixation start time.
7.13.1.1 Formalin volume must be at least 15-20 times greater than tissue volume. Ensure cassettes are completely covered.
7.13.1.2 For pancreas with high fat content, fixation time must be increased (40±8 hours).
7.13.2 Record fixation end time and transfer cassettes to tissue processor. Follow tissue processing protocol for pancreas or fatty pancreas. If tissue processor is unavailable, transfer cassettes to a 52oz container labeled with the Case ID number, “nPOD” and the date, then fill with 70% ethanol. Place container in processing room fridge until tissue processor is available.
7.13.3 Process cassettes to paraffin according to tissue processing protocol for pancreas or fatty pancreas.
7.13.4 Transfer all snap frozen materials and O.C.T. blocks to a clear bag labeled with the Case ID number. Store at -80°C until tissue archiving.
7.13.5 After overnight equilibration at 4°C, tissue in RNALater tubes should be removed from the solution. Transfer tissue to labeled O-ring microtubes and add fresh RNALater solution before snap-freezing in dry ice/2-methybutane bath. Once vials are completely frozen, they may be stored with the rest of the case frozen materials as in 7.14.4.
7.13.6 Materials obtained by this program will be inventoried in the nPOD database and archived in the OPPC according to SOP 55 Case Data Management, SOP 52 Sample Data Management, and SOP Tissue Sample Archiving.
7.13.7 Dispose of any remaining tissue according to SOP 66 Specimen Disposal.
7.13.8 Sterilize all work surfaces using bleach, then wipe surfaces with 70% ethanol to remove residue. Wash all surgical tools and dissection boards, then autoclave tools to sterilize. Place dissection boards, pens, pipettes, camera, and other

non-autoclavable items in biosafety cabinet and expose to UV radiation for at least 30-45 minutes to decontaminate.

7.13.9 Dispose of all biohazardous waste according to UF EH&S guidelines.

REFERENCES:

1.0 Related Documents and Procedures

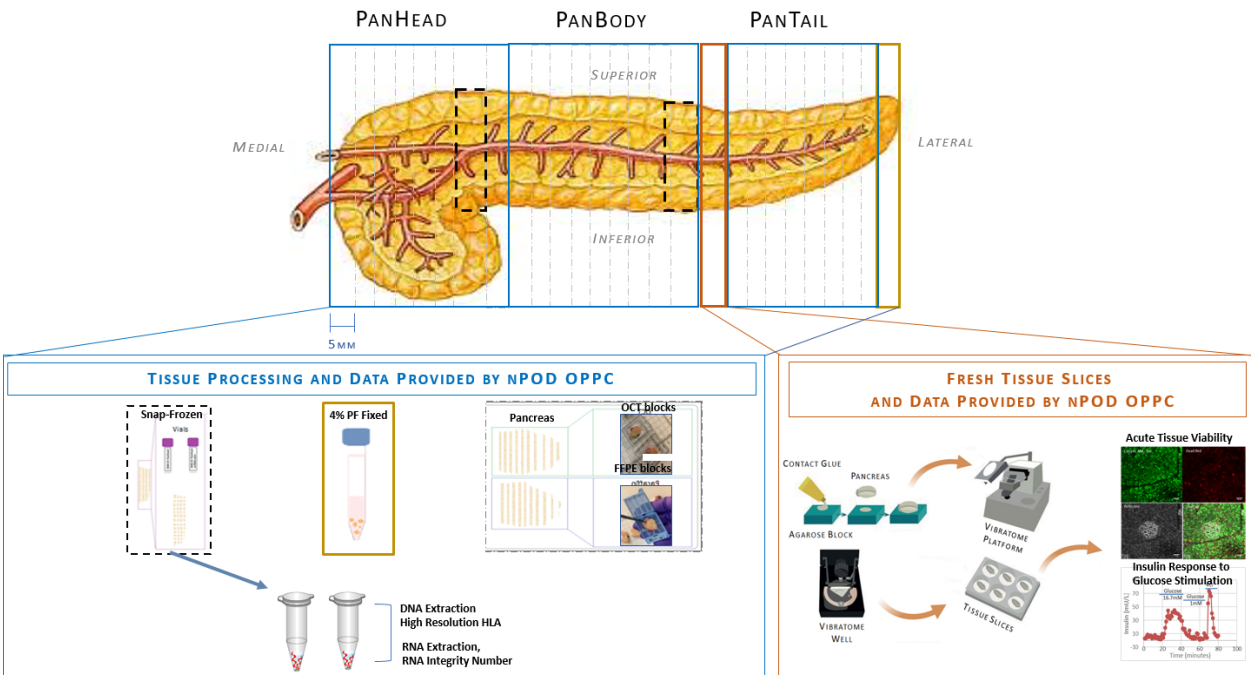
1.1 Clinical Association of Pathology Anatomic Pathology Manual
1.2 UF Biological Waste Disposal Policy
1.3 Campbell-Thompson, et. al. Processing of human pancreas. JoVE, 2012.
1.4 SOP 22 Autoantibody Screening
1.5 SOP 52 Sample Data Management
1.6 SOP 55 Case Data Management
1.7 SOP 58 Vibratome Slicing
1.8 SOP 59 Isolation of PBMC
1.9 SOP 60 Isolation of Cells from Spleen, Thymus, and Lymph Nodes
1.10 SOP 62 Bone Marrow Cell Isolation
1.11 SOP 66 Specimen Disposal
1.12 SOP 71 Electron Microscopy
1.13 SOP 85 C-Peptide Determination
1.14 SOP 56 Perifusion of Tissue Slices
1.15 GDL Tissue Sample Archiving

REVISION HISTORY

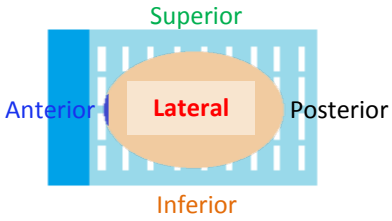
Version	Date	Revision
1	9/21/11	Added Appendix 2 and changed text on trimming larger pancreata. AW 9/21/11
2	3/29/12	Edited the pancreas processing and updated the figures in the appendices. EM 3/29/12
3	8/28/12	Updated media composition for PLN processing IK 8/28/12
4	6/11/15	Updated reagents, added 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).
6	7/24/16	Updated reagents, Table 1 and 2, data collection, tissue processing to clarify. Added Appendix 1 workflow chart.
7	4/7/21	Removed Appendix 1. Revised Appendix 2 (renamed as Appendix 1). Updated reagents, supplies, tissue processing, added tissue slices to pancreas processing.

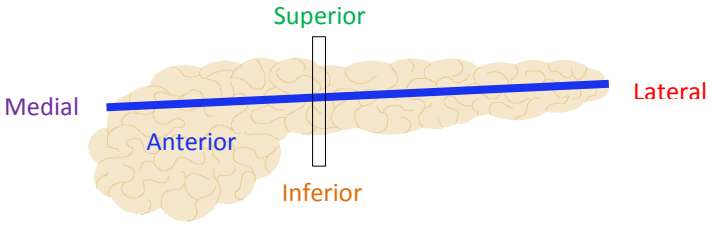
	Name	Signature	Date
Prepared by:	Tiffany Philips		6/11/15
Approved by:	Irina Kusmartseva		4/16/21
Edited by:	Maria Beery		4/16/21

Appendix 1



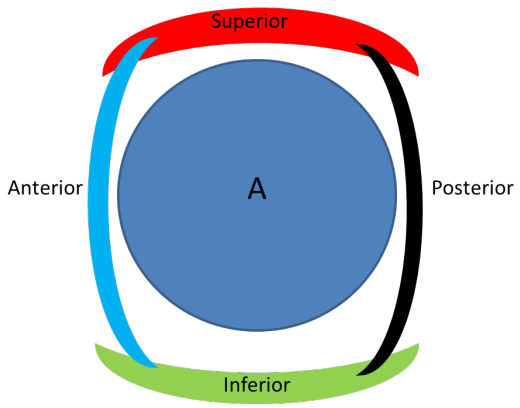
Pancreas Head, Body, and Tail are sectioned so paraffin and OCT blocks are collected in alternating sections. Sections at the Head/Body junction and Body/Tail junction are to be minced for vials.





Appendix 2

a



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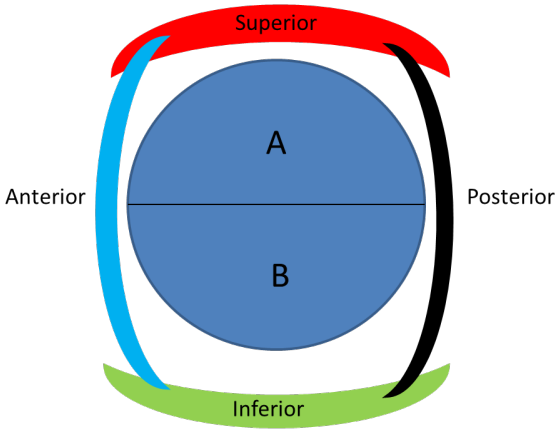

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