65. 1 nPOD ISLET ISOLATION CASE PROCESSING

1. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for processing pancreas and associated organs in conjunction with islet isolation for the nPOD Islet Isolation Pilot Program.

2. SCOPE

This SOP will be applied to all samples recovered through the nPOD Islet Isolation Pilot Program and designed to be used in conjunction with SOP IS-001(nPOD) “Human Pancreatic Islet Isolation For nPOD Islet Pilot Project.”

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.

Kimberly-Clark Benchtop Protector, Fisher Scientific #15-235-101
Leica Biosystem 3P L-absorb towel, Leica #3803240
Double Edge Pathology Blades for Pathco Handle, Fisher Scientific #23-720-200
Disposable Scalpel Handle with Blade 20/Pk, Fisher Scientific #5-0949-246
Margin Marking Dye, Blue, American Master Tech #STD02204E
Wooden Applicators with Cotton tips, Fisher Scientific #14-959-96B
32 oz. screw top polypropylene histology container, Fisher Scientific #22-026-315
52 oz. Plastic Container with Lid, Fisher Scientific #02-544-127
Formalin, 1:10 dilution (buffered), Fisher Scientific #23-245-685
Cassettes for embedding, blue, Lab Storage Systems #EC-0112
Disposable Base Mold, Fisher Scientific #22-050-161
Microtubes with Silicon O-ring, VWR #89004-300
Tissue-Tek OCT Compound Media, Fisher Scientific #14-373-65
Ambion RNALater Solution, Ambion #AM7021
HyClone D-PBS 1X without Ca or Mg, Fisher Scientific #SH3002801
DMEM/F12 1:1 Modified, Fisher Scientific #SH30261.01
Anti-Anti, Antibiotic-Antimycotic 100X, Invitrogen #15240-062, aliquot 5ml and store at -20°C

Ammonium Chloride Solution, StemCell #7800
HyClone Fetal Bovine Serum, Fisher Scientific #SH30088031H, aliquot 50ml and store at -20°C
High-Performance centrifuge tubes (15, 50 ml), sterile, VWR, # 89039-666; #89039-65
Permanent marker (labeling OCT, etc), Fisher Scientific # 13383C
Kimberly-Clark Fluidshield Fog-Free Protective Mask, Fisher Scientific #18-806
Polyethylene Apron, Fisher Scientific # 19-181-529
Gloves
Ultra Clorox Germicidal Bleach
Ethanol
Biological Safety Hood
Centrifuge, room temperature
Organ/Tissue Scale
Dissecting Board, Fisher Scientific #36-114
Pathco Blade Handle, Fisher Scientific #NC9552049
Specimen Forceps, VWR #82027-438
Walter Stern Dissecting Scissors 310-045, LabSource 25876009
Drummond Pipet-Aid Filler/Dispenser, Fisher Scientific #13-681-15E
Pipettes, ThermoScientific Finnpipette F2, 20-200 µl, Fisher Scientific #14-386-319
Pipettes, ThermoScientific Finnpipette F2, 100-1000 µl Fisher Scientific # 13-386-320
ART Barrier Pipette tips 200ul, sterile, Thermo Scientific # 2279
ART Barrier Pipette tips 1000ul, sterile, Thermo Scientific # 2069
Biohazard Sharps Container
Styrofoam container
Liquid nitrogen

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 properly.
   5.4. Follow aseptic procedures throughout processing

6. PROCEDURE
   6.1. Preparation for sample processing
      6.1.1.Case number assignment
         6.1.1.1. Donors accepted as part of the nPOD Islet Isolation Pilot Project will be assigned case numbers in sequence with those at the University of Florida nPOD processing facility.
      6.1.2. Aliquot labeling
         6.1.2.1. Cassettes for paraffin embedding
            6.1.2.1.1. Line #1: Case ID + Block #

6.1.2.1.2. Line #2: Sample type abbreviation

6.1.2.2. OCT cryomolds

6.1.2.2.1. Print legibly using a Sharpie permanent marker

6.1.2.2.2. Identify by Case ID, Block #, and sample type

6.1.2.3. Cryovials and Fresh Tissue

6.1.2.3.1. Line #1: Case ID + vial #

6.1.2.3.2. Line #2: Sample type abbreviation

6.1.2.3.3. Line #3: Aliquot type

6.1.2.3.4. Line #4: Barcode and unique aliquot number

6.1.3.Sterile media preparation

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

6.1.3.2. Add 50ml FBS to the 450ml RPMI stock solution for a final concentration of 10% FBS.

6.1.3.3. Add 5ml 100X Antibiotic/antimycotic stock to the solution in 6.1.3.2.

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

6.1.4.Liquid Nitrogen freezing bath

6.1.4.1. Pour liquid nitrogen into Styrofoam container and cover.

6.1.4.2. Place vial rack in the liquid nitrogen filled Styrofoam container and cover.

6.1.5.10% NBF container

6.1.5.1. Pour 10% NBF into a 52 oz. plastic container and secure the lid.

6.1.6.Data Collection

6.1.6.1. Unpack shipment and record contents.

6.1.6.1.1. If any of the expected items were not received, notify the administrator on-call immediately.

6.1.6.2. Manually fill out the Case Worksheet form during the case processing.

6.1.6.2.1. After case has been processed scan, upload, and archive the completed worksheet. Manually enter the case information into the nPOD database.

6.2. Tissue Dissection

6.2.1.1. Receive one 1.5 cm wide piece of pancreas from the PanHead/Body junction, and one 1.5 cm wide piece of pancreas from the distal portion of the PanTail from islet isolation staff and move to hood.Make 4 blocks per 1.5cm region, 2 paraffin and 2 OCT.

6.2.1.1.1. Alternate paraffin and OCT as seen in Appendix 1.

6.2.1.1.2. Maintain the same tissue orientation for all blocks.

6.2.1.1.3. Paraffin blocks

6.2.1.1.3.1. Place the tissue in the cassette.

6.2.1.1.3.2. Attach and secure the top of the cassette.

6.2.1.1.3.3. Place the cassette containing the tissue in the container of 10% NBF.

6.2.1.1.4. OCT blocks

6.2.1.1.4.1. Place enough OCT media to cover the bottom surface of the cryomold.

6.2.1.1.4.2. Place tissue on top of the media in the center of the mold and add OCT media until the tissue is covered.

6.2.1.1.4.3. Place the filled cryomold on the top of the rack in the container with liquid nitrogen until frozen.

6.2.1.1.4.4. Once frozen, move the OCT block to the polyurethane container with dry ice only.

6.2.2. Receive whole spleen and duodenum from islet isolation staff and move to hood.

6.2.3.Dissect the spleen into 2 regions.

6.2.3.1. 10cm x 20cm piece to be processed for cryovials, paraffin blocks, and OCT blocks.

6.2.3.2. The second piece is split into 5g aliquots, minced and transferred to 50ml conical tubes that contain 35ml of fresh complete sterile media. Secure the top and wrap with parafilm.

6.2.3.2.1. The number of aliquots is determined by the number of investigators that will be receiving fresh spleen.

6.2.4. Process 10cm x 20 cm piece of spleen into snap frozen cryovials, paraffin blocks, and OCT blocks.

6.2.4.1. Snap frozen cryovials

6.2.4.1.1. Without RNALater

6.2.4.1.1.1. Mince 0.5 grams of tissue and place in vial.

6.2.4.1.1.2. Secure the top of the vial and place in the vial rack in the container with liquid nitrogen.

6.2.4.1.2. With RNALater

6.2.4.1.2.1. Add 1ml RNALater to each vial

6.2.4.1.2.2. Mince 0.5 grams of tissue and place in vial.

6.2.4.1.2.3. Secure the top of the vial and allow the tissue and RNALater to equilibrate to room temperature for 30 minutes.

6.2.4.1.2.4. Place the vial in the vial rack in the container with liquid nitrogen.

6.2.4.2. Paraffin blocks

6.2.4.2.1. Place the tissue in the tissue cassette.

6.2.4.2.2. Attach and secure the top of the cassette.

6.2.4.2.3. Place the cassette containing the tissue in the container filled with 10% NBF.

6.2.4.3. OCT Blocks

6.2.4.3.1. Place enough OCT media to cover the bottom surface of the cryomold.

6.2.4.3.2. Place tissue on top of the media in the center of the mold and add OCT media until the tissue is covered.

6.2.4.3.3. Place the filled cryomold on top of the rack in the container with liquid nitrogen until frozen.

6.2.5.In the hood, open the duodenum and gently scrape off mucus and ingesta.

6.2.6.Cut several 1” segments of mucosa.

6.2.6.1. Fresh tissue for distribution

6.2.6.1.1. Fill a conical tube to the 15ml mark with 1” segments of mucosa. Add fresh sterile media until the volume reaches 50ml.

6.2.6.2. Snap frozen cryovials

6.2.6.2.1. Without RNALater

6.2.6.2.1.1. Mince a 1” strip of duodenum and place in vial.

6.2.6.2.1.2. Secure the top of the vial and place the vial in the vial rack in the container with liquid nitrogen.

6.2.6.2.2. With RNALater

6.2.6.2.2.1. Add 1ml RNALater to vial

6.2.6.2.2.2. Mince a 1” strip of duodenum and place in vial.

6.2.6.2.2.3. Secure the top of the vial and allow the tissue and RNALater to equilibrate to room temperature for 30 minutes.

6.2.6.2.2.4. Place the vial in the vial rack in the container with liquid nitrogen.

6.2.6.3. Paraffin blocks

6.2.6.3.1. Place a single 1” segment per cassette.

6.2.6.3.2. Attach and secure the top of the cassette.

6.2.6.3.3. Place the cassette containing the tissue in the container filled with 10% NBF.

6.2.6.4. OCT blocks

6.2.6.4.1. Place enough OCT media to cover the bottom surface of the cryomold.

6.2.6.4.2. Place a 1” segment of duodenum on top of the media in the center of the mold and add OCT media until the tissue is covered.

6.2.6.4.3. Place the filled cryomold on top of the rack in the container with liquid nitrogen.

6.2.7. In the hood, place the peri-pancreatic fat in the 32oz screw top polypropylene histology container. Fill the remaining volume of the container with fresh sterile media. Secure the top and wrap with parafilm.

6.2.8. If thymus is received, dissect it into 2 equal sections.

6.2.8.1. The first section is to be cut into several pieces and transferred to 50ml conical tubes that contain 35ml of fresh complete sterile media. Secure the top and wrap with parafilm.

6.2.8.2. The second section is to be cut in half and used to make 2 paraffin blocks and 2 OCT blocks.

6.2.8.3. Paraffin blocks

6.2.8.3.1. Place the tissue in the cassette.

6.2.8.3.2. Attach and secure the top of the cassette.

6.2.8.3.3. Place the cassette containing the tissue in the container filled with 10% NBF.

6.2.8.4. OCT Blocks

6.2.8.4.1. Place enough OCT media to cover the bottom surface of the cryomold.

6.2.8.4.2. Place tissue on top of the media in the center of the mold and add OCT media until the tissue is covered.

6.2.8.4.3. Place the filled cryomold on top of the rack in the container with liquid nitrogen.

6.2.9. After all tissue in the hood has been processed, centrifuge serum tubes at 1400 rpm for 10 minutes at room temperature.

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

6.2.10. Aliquot the serum into labeled O-ring cryovials using volumes of 300ul or more per vial. Store at -80°C.

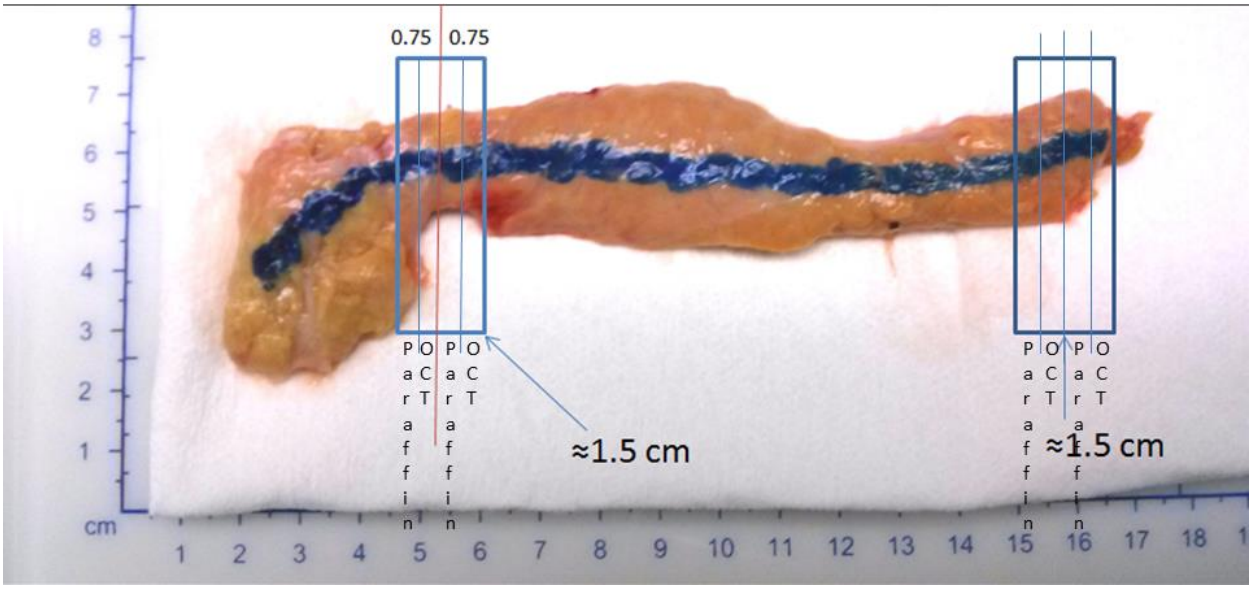
6.2.10.1. Set aside 3 aliquots for autoantibody and C-peptide analysis. Refer to SOP 85 C-Peptide Determination, and SOP 22 Autoantibody Screening Process.

6.2.11. All sample types that were frozen in the liquid nitrogen bath should be stored in a -80°C freezer until they are shipped to the OPPC Lab on dry ice.

6.2.12. The cassettes for paraffin processing are to be kept in 10% NBF for 24 hours and then processed according to the SOP 57.3 Case Processing.

6.2.13. Dispose of any remaining tissue. Refer to SOP 66 Specimen Disposal.

Appendix 1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