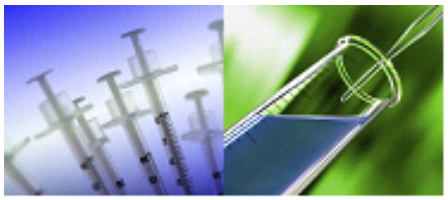

nPOD
Network for Pancreatic Organ Donors with Diabetes

STANDARD OPERATING PROCEDURE

Histology

OPPC-SOP-70

Prepared by:	Lynda Schneider	**Original Effective Date:**	07/01/2007
Revised by:	Myriam Padilla, Leyla Evans	**Version Effective Date:**	05/06/2021
Reviewed by:	Maria Beery	**Reviewed Date:**	04/15/2021
Approved by:	Irina Kusmartseva	**Approved Date:**	05/06/2021

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

HISTOLOGY

POLICY: Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron). Use chemical and physical safety precautions when working with paraformaldehyde and sharps, respectively.

PURPOSE: The purpose of this Standard Operating Procedure (SOP) is to outline procedures for histological preparation of nPOD samples.

SCOPE: This SOP will be applied to nPOD samples in paraffin or OCT blocks.

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 to perform these tasks in accordance with the SOPs.

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

- 10% neutral buffered formalin (NBF)(Fisher Scientific)
- Specimen containers
- Paraffin Wax (McCormick Paraplast X-tra)
- Xylene
- Ethanols (EtOH)- 70%, 95%, 100%
- Tissue processor (automatic), cassette basket, paraffin dispenser
- Embedding station, molds, forceps
- Superfrost Plus slides and slide boxes
- Gemini Autostainer, slide racks with reagent containers
- Hematoxylin 7211, Eosin-Y, Bluing Reagent, Clarifier 1 (Richard-Allan Scientific)
- OCT media
- Cytoseal XYL (Richard-Allan Scientific)
- Coverslips
- Slide label printer
- RNAase Zap, gauze pads (4x4)

PROCEDURE:

1.0 Tissue Processing

1.1 Tissue samples are processed and divided into paraffin and frozen blocks.

1.2 Freeze tissue samples intended for OCT sectioning in 2-methylbutane/dry ice bath. Reserve and store 30% of these blocks. Set aside available blocks for sectioning and staining, following the OCT protocol according to Appendix 3.

1.3 Fix samples intended for paraffin blocks in cassettes in 10% NBF for 24 hours either in a programmable tissue processor or manually. Increase fixation time (40±8 hours) for tissue with high fat content.

1.4 On the Case Submission Form, record fixation start time as the time when the last cassettes are placed in fixative.

1.5 After fixation, hold samples in 70% ethanol.

1.5.1 For manual processing, decant formalin into waste container and place samples into ethanol. Record the manual fixation end time on the Case Submission Form.

1.5.2 Routine processing protocol performed according to Appendix 1.

1.6 Embed samples as placed in cassettes to maintain orientation (see SOP Case Processing).

1.7 After embedding, reserve and store 30% of every sample type. Set aside available blocks for sectioning and staining.

1.8 Label slides with CaseID, block number, slide number, sample type, and date using a slide printer, cassette marker or by pencil.

1.9 Place block in microtome to face block and expose the entire tissue then hold on wet ice for at least 5 minutes.

1.10 Cut 4 um serial sections from blocks 02 and 04 from Pancreas Head, Body, and Tail regions, as well as block 01 from Spleen for IHC and H&E staining.

1.10.1 Blocks 02 Pan Head – minimum 6 slides
1.10.2 Blocks 04 Pan Head – minimum 5 slides
1.10.3 Blocks 02 & 04 Pan Body –minimum 4 slides
1.10.4 Blocks 02 & 04 Pan Tail – minimum 4 slides
1.10.5 Block 01 Spleen - minimum 3 slides

Note: Some cases require different blocks for sectioning.

1.11 Pick up serial sections in order and place one section per slide in the middle to lower half of the slide without tissue touching the slide edges. Orient the section on the slide to mirror tissue within the cassette.

1.12 Air-dry the slides overnight at room temperature.

1.13 Stain the first serial section of each block with Hematoxylin and Eosin (H&E) according to protocol shown in Appendix 2.

1.13.1 When first sectioning any uncut block, cut and stain an H&E, then cut serial sections as needed.

1.14 Stain serial sections accordingly: Ki67+Insulin, CD3+Glucagon, CD45+Somatostatin. For Pan Head blocks only, stain fifth serial section for Pan Polypeptide.
1.14.1 Follow Immunopathology SOP.

1.15 Coverslip sections with mounting media and glass coverslip.

1.16 Evaluate sectioning and stain quality on all slides and correct inadequacies by re-sectioning and staining.

1.17 Label stained slide as per cassette labeling according to SOP Case Processing.

1.18 Scan stained slides according to SOP Online Pathology.

1.19 Reseal block face with paraffin and inventory.

1.20 For OCT blocks, cut 5 um sections.
1.20.1 For H&E staining, air-dry for 30 minutes and fix in 95% Alcohol for 20 minutes.
1.20.2 Coverslip section with mounting media and glass coverslip.
1.20.3 Evaluate sectioning and stain quality on all slides and correct inadequacies by re-sectioning and staining.

1.21 Fixation, processing, and staining by consistent procedures will provide optimal comparisons between cases for immunopathology and image analyses.

2.0 For Paraffin and OCT Service Request Sectioning

2.1 Section blocks requested on service request after request has been approved by OPPC lab Director
2.2 Review and perform special requests when feasible.
2.3 Use Superfrost plus slides.
2.4 Cut serial slides when possible and re-inventory blocks.
2.5 For OCT, immediately place cut sections in slide box and store in -80C. For Paraffin let slides air-dry overnight before storing in slide box at room temperature.

3.0 Sectioning Under RNase-free Conditions

3.1 Use new slide box and clean with RNase Away, 70% ethanol, and 100% ethanol using clean gauze pad with each reagent.
3.2 Place opened slide box in hood and run UV light program.
3.3 Open a new package of gauze 4x4 squares and use only for RNase-free sectioning.
3.4 Clean the entire cutting area of the microtome (i.e., chuck, blade holder, stage, and tray) with RNase Away using clean gauze squares.
3.5 Wipe down the same area with 70% ethanol with clean gauze and then with 100% ethanol.
3.6 Cut each block with a new blade.
3.7 Change gloves between each block and wipe gloves with RNase Away followed by 70% ethanol.
3.8 Clean the blade holder and any area that might contact section for each new block sectioned under RNase-free conditions.
3.9 If cutting slabs, use sterile forceps to help guide into appropriate tube.

4.0 Cryostat Decontamination

4.1 Keep interior clean by using 70% ethanol and new 4x4 gauze pads.

4.2 Before running decontamination, turn off cryostat until defrosted.
4.3 Run the "Decontaminate" feature on the cryostat, leaving all instruments such as chucks, forceps, and paint brush inside the chamber to also be decontaminated.
4.4 Use appropriate decontamination solution.

REFERENCES:

1.0 Related Documents and Procedure

1.1 Clinical Association of Pathology Anatomic Pathology Manual
1.2 SOP 57 Case Processing
1.3 Online Pathology
1.4 SOP 91 Researcher Service Requests
1.5 SOP 72 Immunopathology

REVISION HISTORY

Version	Date	Revision
1	4/12/12	Lynda Schneider – adjusted slide counts, changed Permount for Cytoseal XYL in materials, changed 10% NBF for acetone in OCT
2	11/19/14	Marcela Gomez, Myriam Padilla – updated protocol, changed Paraffin wax to McCormick, adjusted H&E protocol Appendix 2 for Gemini Autostainer.
3	06/16/15	Myriam Padilla- added OCT block processing and adjusted slide count
4	04/16/21	Updated formatting

	Name	Signature	Date
Prepared by:	Myriam Padilla		6/25/2015
Approved by:	Irina Kusmartseva		5/6/2021

Appendix 1

nPOD Paraffin Fixation and Processing Program

Station #	Solution*	Conc.	Time	Set Temp.	P/Vacuum	Mixer
1	Formalin	10%	24:00±8:00	_____	off	slow
2	EtOH	70%	Hold**	_____	off	off
3	EtOH	70%	0:35	_____	P/V	slow
4	EtOH	80%	0:35	_____	P/V	slow
5	EtOH	95%	0:35	_____	P/V	slow
6	EtOH	95%	0:35	_____	P/V	slow
7	EtOH	100%	0:35	_____	P/V	slow
8	EtOH	100%	0:35	_____	P/V	slow
9	Xylene	100%	0:45	_____	P/V	slow
10	Xylene	100%	0:45	_____	P/V	slow
11	Paraffin	_____	0:45	60°C	P/V	slow
12	Paraffin	_____	0:45	60°C	P/V	slow
13	Paraffin	_____	0:45	60°C	P/V	slow
14	Paraffin	_____	0:45	60°C	P/V	slow

*Maintenance according to manufacturer's recommendation.

**Duration of holding time dependent on desired end time to start embedding.

Appendix 2

nPOD Paraffin Section H&E Staining Schedule (Gemini Autostainer)

	Station	**TIME**	**Maintenance**	**Purpose**
Oven		20 min		Melt paraffin wax
Xylene	**1**	3 min	Rotate daily or change as needed	Deparaffinization
Xylene	**2**	3 min	Rotate daily or change as needed	Deparaffinization
Xylene	**3**	3 min	Rotate daily or change as needed	Deparaffinization
100% EtOH	**4**	1 min	Rotate daily or change as needed	Xylene clearing
100% EtOH	**5**	1 min	Rotate daily or change as needed	Xylene clearing
95% EtOH	**6**	1 min	Rotate daily or change as needed	Rehydration
Water	Wash 1	1 min	Self-maintenance	Rehydration
Hematoxylin 7211	**21**	3 min	Change weekly or as needed	Nuclear stain
Water	Wash 2	1 min	Self-maintenance	Remove excess
Clarifier 1	**23**	45 sec	Change weekly or as needed	Differentiation
Water	Wash 3	1 min	Self-maintenance	Remove clarifier
Bluing Reagent	**22**	1 min	Change weekly or as needed	Convert Hemalum to blue color
Water	Wash 4	1 min	Self-maintenance	Return pH to neutral
95% EtOH	**24**	30 sec	Change daily or as needed	Dehydration
Eosin-Y	**25**	30 sec	Change weekly or as needed	Cytoplasmic stain
100% EtOH	**15**	1 min	Rotate daily or change as needed	Remove excess Eosin
100% EtOH	**16**	1 min	Rotate daily or change as needed	Dehydration
100% EtOH	**17**	1 min	Rotate daily or change as needed	Dehydration
Xylene	**18**	1min	Rotate daily or change as needed	Clearing
Xylene	**19**	1min	Rotate daily or change as needed	Clearing
Xylene	**20**	1min	Rotate daily or change as needed	Clearing
Xylene	**C-Exit**	Hold	Rotate daily or change as needed	Exit
Xylene	**D-Exit**	Hold	Rotate daily or change as needed	Exit

Times may vary depending on the chemicals in use and may be adjusted to achieve an optimum result.

Appendix 3

nPOD Frozen Section H&E Staining Schedule (Gemini Autostainer)

	Station	**TIME**	**Maintenance**	**Purpose**
Water	Wash 1	30 sec	Self-maintenance	Rehydration
Hematoxylin 7211	**21**	30 sec	Change weekly or as needed	Nuclear stain
Water	Wash 2	1 min	Self-maintenance	Remove excess
Clarifier 1	**23**	30 sec	Change weekly or as needed	Differentiation
Water	Wash 3	1 min	Self-maintenance	Remove clarifier
Bluing Reagent	**22**	30 sec	Change weekly or as needed	Convert Hemalum to blue color
Water	Wash 4	30 sec	Self-maintenance	Return pH to neutral
95% EtOH	**24**	30 sec	Change daily or as needed	Dehydration
Eosin-Y	**25**	6 sec	Change weekly or as needed	Cytoplasmic stain
100% EtOH	**15**	20 sec	Rotate daily or change as needed	Remove excess Eosin
100% EtOH	**16**	30 sec	Rotate daily or change as needed	Dehydration
100% EtOH	**17**	1 min	Rotate daily or change as needed	Dehydration
Xylene	**18**	1min	Rotate daily or change as needed	Clearing
Xylene	**19**	1min	Rotate daily or change as needed	Clearing
Xylene	**20**	1min	Rotate daily or change as needed	Clearing
Xylene	**C-Exit**	Hold	Rotate daily or change as needed	Exit
Xylene	**D-Exit**	Hold	Rotate daily or change as needed	Exit

Times may vary depending on the chemicals in use and may be adjusted to achieve an optimum result.