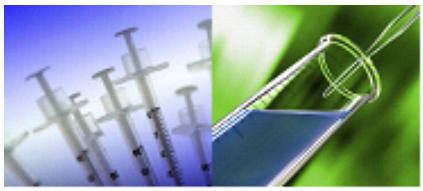


nPOD
Network for Pancreatic Organ Donors with Diabetes

HISTOLOGY

1 PURPOSE

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

2 SCOPE

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

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.

10% neutral buffered formalin (NBF)(Fisher Scientific), specimen containers
Phosphate buffered saline (PBS)
Paraffin Wax (Richard-Allan Scientific – Type 9)
Xylene
Ethanols (EtOH)- 70%, 80%, 95%, 100%
Tissue processor (automatic), cassette basket, paraffin dispenser
Embedding station, molds, forceps
Superfrost Plus slides and slide boxes
Leica Autostainer XL, 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
Compressed air, RNAase Zap, gauze pads (4x4), Clorox

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) according to the MSDS.

6 PROCEDURE

6.1 Fix samples intended for paraffin blocks in cassettes in 10% NBF for 24± 8 hours either in a programmable tissue processor or manually.
6.2 Record fixation start time as the time when the last cassettes are placed in fixative.
6.3 After fixation, hold samples in 70% ethanol.

6.3.1 For manual processing, decant formalin into waste container, wash cassettes once with PBS, and place into ethanol. Record the manual fixation end time on the case worksheet.

6.3.2 Routine processing schedules are performed according to Appendix 1.

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

6.5 Cut 4 um serial sections from blocks 02 and 04 from pancreas head, body, and tail regions, as well as block 01 from spleen.

6.5.1 Block 02 PanHead – minimum 4 slides

6.5.2 Block 04 PanHead –minimum 3 slides

6.5.3 Blocks 02 & 04 PanBody and PanTail – minimum 2 slides

6.5.4 Block 01 Spleen - minimum 2 slides

6.6 Place block in microtome such that the beveled edge with cassette label is to the left in the microtome chuck. Face block to expose the entire tissue then hold on wet ice for at least 5 minutes.

6.7 Label slides with caseID, block number, slide number, and sample type using a slide printer or by pencil.

6.8 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.

6.9 Air dry the slides overnight at room temperature.

6.10 Stain the first serial section of each block with hematoxylin and eosin (H&E) according to schedule shown in Appendix 2.

6.10.1 Section one slide and stain by H&E when first sectioning any block and when re-sectioning block for service requests to record morphology throughout the entire block. Label stained slides with date.

6.11 Coverslip sections with mounting media and glass coverslip.

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

6.13 Label stained slide as for cassette labeling according to SOP Case Processing.

6.14 Scan stained slides according to SOP Online Pathology.

6.15 Reseal block face with paraffin and inventory.

6.16 Make serial sections of OCT blocks (at 5 um) as for paraffin sections.

6.16.1 For H&E staining, air-dry overnight and post-fix in -20^{o}C acetone for 10 minutes.

6.16.2 Coverslip, QC, and scan as for paraffin.

6.17 Reserve 30% of every sample type. Store reserved paraffin blocks in non-defrosting -20°C freezer.

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

<u>For Service Request Sectioning</u>

6.19 Section blocks requested on service request after request has been approved by OPPC lab manager

6.20 Review and perform special requests when feasible.

6.21 Cut serial slides as above and replace in inventory.

<u>Sectioning Under RNase-free Conditions</u>

6.22 Spray the entire microtome and slide areas with compressed air to remove dust and other debris.

6.23 Open a new package of gauze 4x4 squares and use only for RNase-free sectioning.

6.24 Clean the entire cutting area of the microtome (i.e., chuck, blade holder, stage, and tray) with xylene using clean gauze squares.

6.25 Clean the same area with 50% aqueous bleach with clean gauze.
6.26 Wipe down the same area with 70% ethanol with clean gauze.
6.27 Cut each block with a new blade.
6.28 Change gloves between each block.
6.29 Clean the blade holder and any area that might contact section for each new block sectioned under RNase-free conditions.
6.30 If cutting at 20 um thickness, use a sterile wooden applicator stick to help guide into a tube.
6.31 Use a new slide box and wipe down exterior with RNAaseZap. Keep box closed except during placement of slides.

OCT Block sectioning

Cryostat

6.32 Keep interior clean with 50% bleach, followed by 70% ethanol, using new 4x4 gauze sponges for cleaning.
6.33 Run the "Decontaminate" feature on the cryostat, leaving all instruments such as chucks, forceps, and paint brush inside the chamber to also be decontaminated.
6.34 Fix block to chuck with OCT and section according to investigator's requests
6.35 Use Superfrost plus slides and immediately transfer to -70°C freezer for optimal morphology and for storage until shipping on dry ice.

OCT Sectioning Under RNase-free Conditions

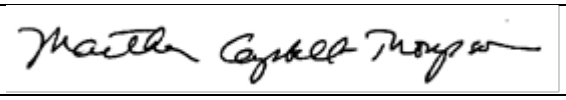
6.36 As above with change in blades and gloves between each new block.
6.37 Use a new slide box and wipe down the slide box with RNase Zap. Immediately transfer slides or thick sections to -80° freezer until shipped on dry ice.

7 REFERENCES

7.1 Clinical Association of Pathology Anatomic Pathology Manual
7.2 SOP 57 Case Processing
7.3 SOP 73 Online Pathology
7.4 SOP 91 Researcher Service Requests

8 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

Prepared by	Lynda Schneider		
Approved by	Martha Campbell-Thompson		5/10/12
	Name	Signature	Date

Appendix 1

nPOD Paraffin Fixation and Processing Program

Station #	Solution*	Conc.	Time	Set Temp.	P/Vacuum	Mixer
1	Formalin	10%	16:00	_____	off	off
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 (Leica Autostainer)

	Station	TIME	Maintenance	Purpose
1. Oven		15 min		Melt paraffin wax
2. Xylene	1	3 min	Rotate every 200 or sooner	Deparaffinization
3. Xylene	2	3 min		Deparaffinization
4. Xylene	3	3 min		Deparaffinization
5. 100% EtOH	4	1 min	Rotate every 200 or sooner	Xylene clearing
6. 100% EtOH	5	1 min		Xylene clearing
7. 95% EtOH	6	1 min	Rotate every 200 or sooner	Rehydration
8. 95% EtOH	7	1 min		Rehydration
9. Water	Wash 1	1 min		Rehydration
10. Hematoxylin 7211	8	3 min	Filter day of use Change every month	Nuclear stain
11. Water	Wash 2	3 min		Remove excess
12. Clarifier 1	9	45 sec	Change day of use	Differentiation
13. Water	Wash 3	1 min		Remove clarifier
14. Bluing Reagent	10	1 min	Change every 200 or sooner	Convert Hemalum to blue color
15. Water	Wash 4	1 min		Return pH to neutral
16. 95% EtOH	11	30 sec	Change every 200 or sooner	Dehydration
17. Eosin-Y	12	45 sec	Change every 400 or sooner	Cytoplasmic stain
18. 100% EtOH	13	1 min	Rotate every 200 or sooner	Remove excess Eosin
19. 100% EtOH	14	1 min		Dehydration
20. 100% EtOH	15	1 min		Dehydration
21. Xylene	16	1 min	Rotate every 200 or sooner	Clearing
22. Xylene	17	1 min		Clearing
23. Xylene	Exit	Hold		Clearing
End program				

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