

Feb 08, 2021

Human Pancreas Histopathology Assessment

Forked from Initial Rapid Pathology Assessment of Kidney Tissue

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1 Works for me

dx.doi.org/10.17504/protocols.io.br7tm9nn

Human Cell Atlas Method Development Community Optical Clearing of Tissue 1 more workspace



SUBMIT TO PLOS ONE

ABSTRACT

DRAFT

Purpose:

The purpose of this protocol is to outline proper procedures for fixed specimen processing using an automatic paraffin processor and sectioning and staining for histopathological review of normalcy.

Scope

This protocol applies to human pancreas specimens that will be processed to paraffin blocks for HuBMAP Tissue Mapping Centers.

Expected Outcome:

Pancreas samples are processed properly and efficiently to whole slide images for histopathology review.

Nuclei should stain blue. Cytoplasm of acinar cells should stain pink with blue zymogen granules. The large blood vessels should demonstrate clear differentiation of RBCs when present, surrounding collagen, and endothelial nuclei with scant cytoplasm.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Campbell-Thompson, ML, Montgomery EL, Foss RM, Kolheffer KM, Phipps G, Schneider L, Atkinson MA. Collection protocol for human pancreas. Journal Visual Experimentation, 2012 May 23;(63). pii: 4039. doi: 10.3791/4039. PMID:22665046

DOI

dx.doi.org/10.17504/protocols.io.br7tm9nn

PROTOCOL CITATION

Martha Campbell Thompson 2021. Human Pancreas Histopathology Assessment. **protocols.io** https://dx.doi.org/10.17504/protocols.io.br7tm9nn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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FORK NOTE

This protocol defines minimal histopathology criteria of a normal human pancreas.

FORK FROM

Forked from Initial Rapid Pathology Assessment of Kidney Tissue, Jamie Allen

KEYWORDS

HuBMAP, pancreas, H&E, whole slide image (WSI), islet, acinar cells, vasculature, pancreatic duct, duct cells

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CREATED

Feb 08, 2021

LAST MODIFIED

Feb 08, 2021

PROTOCOL INTEGER ID

47059

GUIDELINES

Wear PPE when handling fixative, ethanols, and xylene.

Pour solutions in a fume hood.

Label all containers with contents and warning labels.

Use formaldehyde spill kit for spills up to 1 liter and call EHS for larger spills.

Melted paraffin is hot (~60'C). Handle with care.

Filter, change, and/or rotate solutions as required.

MATERIALS TEXT

Equipment:

Paraffin processor (VIP or suitable substitute)

Specimen wire baskets

Paraffin wax

Ethanols (EtOH)- 70%, 80%, 95%, 100%

Xylene

Chemical waste containers

Microtome

Waterbath

Blades

Superfrost Plus slide

Leica Autostainer XL

Hematoxylin

Eosin-Y

Bluing reagent

Clarifier

EtOH and xylene

Slide rack and trays

Whole slide scanner (Aperio CS, other)

Windows computer and Microsoft Excel for reviewing and recording histopathology reviews, sample inventory

Paraffin block archive

Stained slide archive

SAFETY WARNINGS

All reagents should be handled with the proper PPE. For detailed information, contact the MSDS website for the chemical.

1	Process human pancreas for fixed paraffin samples (dx.doi.org/xxx) and submit to the University of Florida Molecular Pathology Core (https://molecular.pathology.ufl.edu/) or another GLP-compliant histology laboratory for routine paraffin embedding.
2	From 1 block of each region, create 4 μm paraffin sections and apply to Superfrost Plus slide or suitable alternative.
3	Stain sections with H&E (dx.doi.org/xxx).
4	Scan slides with whole slide digital scanner at 20x and save in default file format (Aperio .svs or Zeiss .cvs) or other lossless format (.tiff).
5	Assess and record the following qualitative information for each section: 1. Endocrine pancreas: a. Islet numbers (normal, reduced, increased), sizes (normal range from small to large but majority medium sized), and morphology (normal oval outline with clear demarcation from surrounding acinar and duct cells). i. Yes/No: amyloid, fibrosis, hydropic degeneration. 2. Exocrine pancreas: a. (0=none, 1=mild, 2=moderate, 3=severe) pancreatitis (subtype: acute, chronic), autolysis, fibrosis, fatty infiltration, neoplasia (including PanIN) 3. Additional features: a. Vascular network: arteriosclerosis, calcification vessels a. Excretory system: duct dilation, protein plugging, stones
6	Optional: analyze tissue sample using ImageScope or QuPath for total section area, % fat infiltration.
7	Based on histopathological evaluations, pancreas will be used when:
	All islet and exocrine features are scored at 0-1 No pancreatitis
8	Inventory and archive paraffin blocks and H&E slides at room temperature.
9	Quality control: Processor and staining solutions are changed at regular intervals to ensure adequate processing and staining of tissues. Temperatures and solution changes/rotations are recorded on the Paraffin Processor Use and Maintenance form