VANDERBILT UNIVERSITY®

HANDEL-P Protocols for Human Pancreas Processing

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Pancreas Dissection

Pancreas weight and size

- Upon arrival, transfer the pancreas and transport solution (UW or HTK) into a dissection tray placed on ice. Transfer enough of transport solution to keep the pancreas submerged. *Note: All the reagents for pancreas processing should be prepared and ready prior to this point.*
- Dissect the pancreas by carefully removing duodenum, mesentery, fact, and spleen.
- Once the pancreas is cleaned (**Figure 1**), blot it with a sterile sponge (Covidien, 8044) and quickly take its wet weight by placing it into a clean plastic boat (**Figure 2**).
- Immediately, transfer the pancreas on an ice-cooled dissecting platform and take a photograph with a ruler as shown in **Figure 1**.

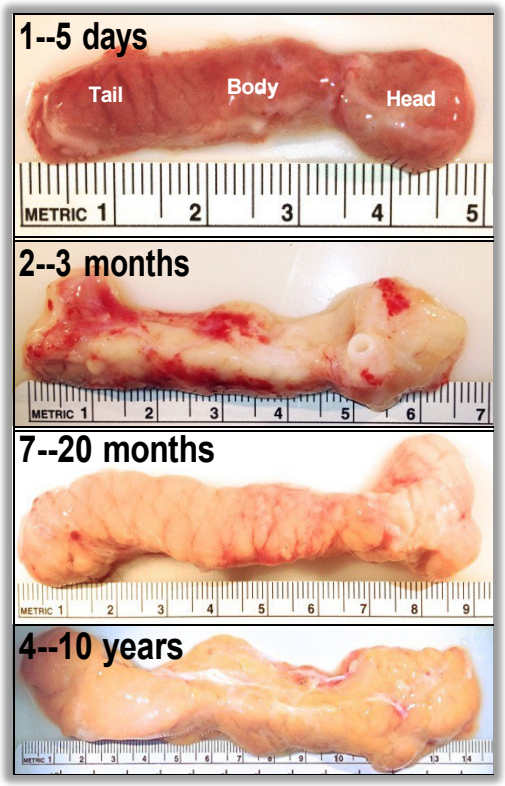

Figure 1 Example of pancreatic tissues from young donor of different age categories.

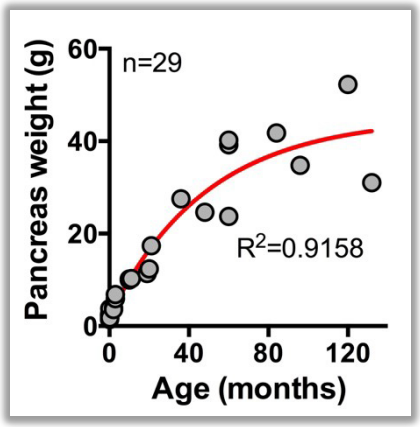

Figure 2 Range of pancreatic weights collected from neonatal and juvenile donors in the first 10 years of life.

Pancreas sectioning layout

- Once the pancreas was photographed, start collecting approximately 2 mm thick tissue cross-sections as outlined in **Figure 3**. *Note: We have been collecting sections in the head-to-tail order.*
- The number of sections and their processing will be determined on the case by case basis depending on the tissue availability in a certain age category and requirements for downstream analyses.
- Label pancreatic cross-sections following the naming nomenclature and controlled vocabularies developed for Vanderbilt LIMS, which keeps up with the position of each slice in a given anatomical location and processing procedure (see Appendix).

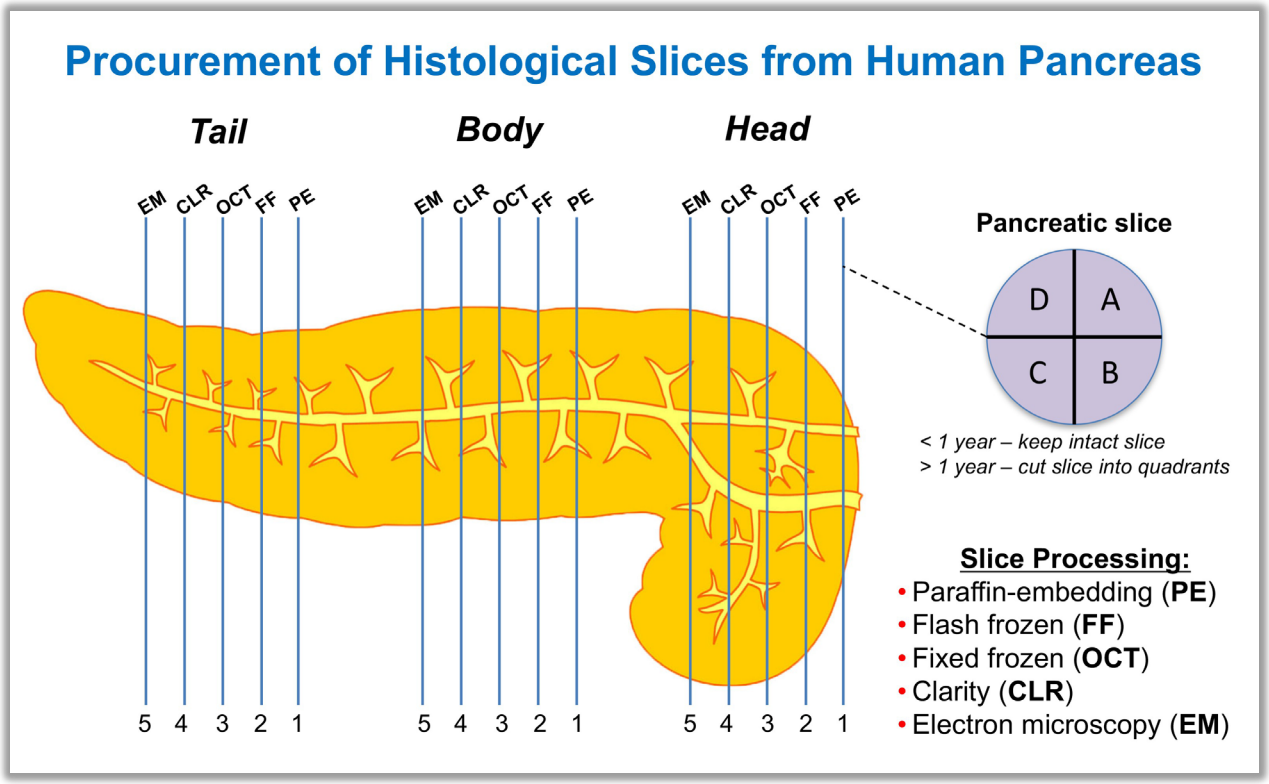

Figure 3 Map of pancreatic tissue cross-sections with reference to their anatomical location and processing procedure.

SPECIMEN LABELING EXAMPLES:

Labeling of tissue cross-sections collected from the pancreatic head would look like this:

- DonorID---ND---10y---M---PANC---PE---H1
- DonorID---ND---10y---M---PANC---FF---H2
- DonorID---ND---10y---M---PANC---OCT---H3
- DonorID---ND---10y---M---PANC---CLR---H4
- DonorID---ND---10y---M---PANC---EM---H5

If the next set of tissue cross-sections was collected from the head region, the specimen labeling would continue as follows:

- DonorID---ND---10y---M---PANC---PE---H6
- DonorID---ND---10y---M---PANC---FF---H7
- DonorID---ND---10y---M---PANC---OCT---H8
- DonorID---ND---10y---M---PANC---CLR---H9
- DonorID---ND---10y---M---PANC---EM---H10

If donor's age was > 1 year, each tissue cross-sections would be divided into quadrants and the labeling would look like this:

- DonorID---ND---10y---M---PANC---PE---H1A
- DonorID---ND---10y---M---PANC---PE---H1B
- DonorID---ND---10y---M---PANC---PE---H1C
- DonorID---10y---M---PANC---PE---H1D

Human Pancreas Processing for Paraffin-embedding (PE)

Reagents

- **100 mM PBS, 1L:** 12.07 g Na_2HPO_4 (dibasic), 2.04 g KH_2PO_4 (monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. Prepare fresh and keep at 4°C. Based on Electron Microscopy Sciences catalog, 100 mM PBS filtered through a 0.22 μm filter has a shelf life of 1 month at 4°C.
- **16% Paraformaldehyde (PFA):** Electron Microscopy Sciences, 15710. Right before fixation, prepare 4% paraformaldehyde solution. Open the vial containing 10 mL 16% paraformaldehyde stock, transfer contents of vial into a 50---mL Falcon tube, add 30 mL 100 mM PBS, mix and place the tube on ice. *Note: Prepare as many tubes as many tissue sections will be procured considering approximately 1:50 tissue to fixative ratio.*
- **70% Ethanol, 1L:** Mix 700 mL 190 Proof ethanol with 300 mL dH_2O water. *Note: Consider approximately 1:50 tissue to sucrose ratio.*

Procedure

1. Collect about 2---mm thick cross---sectional tissue pieces from the Head, Body, and Tail (see the pancreas mapping).
2. Cut each slice into four quadrants (A, B, C, D) and place all 4 tissue pieces into the same 50 mL tube. *Note: For donors < 1 year of age keep the entire slice intact.*
3. Transfer the tissue into a 50---mL Falcon tube containing 40 mL of freshly prepared fixative (4.0 % paraformaldehyde/100 mM PBS) and fix overnight (at least 18 hours) at 4°C under mild agitation using an adjustable tilt rocker (LabNet).
4. Wash the tissue four times in 40 mL of 100 mM PBS at 4°C for the period of 2---3 hours under mild agitation using an adjustable tilt rocker. Blot the tube with paper towel before adding the fresh washing solution.
5. After the last wash, add 40 mL of 70% ethanol and keep at 4°C.
6. Ship tissue in sealed tubes to Vanderbilt on wet ice.

Human Pancreas Processing for Flash Frozen Sections (FF)

Reagents

- **Liquid nitrogen:** Depending on the amount of tissue, fill a 50 mL Falcon tube with 2-5 mL of liquid nitrogen. *Note: Prepare as many tubes as many tissue sections will be procured.*

Procedure

1. Collect about 2 mm thick cross-sectional tissue sections from the Head, Body, and Tail (see the pancreas mapping).
2. Cut each slice into four quadrants (A, B, C, D) and drop each piece of tissue, one at a time, into the same 50 mL tube. *Note: For donors < 1 year of age keep the entire slice intact.*
3. Leave the 50 mL Falcon tube containing tissue on dry ice until liquid nitrogen evaporates.
4. Using forceps, transfer the frozen tissue on a pre-labeled and pre-cooled aluminum foil, wrap it up, and place in a zip-lock bag. Seal and store in a storage box at 80°C. *Note: Aluminum foil and zip-lock bag prevent the block from draying.*
5. Ship tissue blocks to Vanderbilt on dry ice.

Human Pancreas Processing for Fixed Cryosections (OCT)

Reagents

- **1X PBS/10 mM PBS, 1L:** 1.44 g Na_2HPO_4 (dibasic), 0.2 g KH_2PO_4 (monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. *Prepare from scratch or use 1X PBS without Ca/Mg, Invitrogen, 14190-144.*
- **100 mM PBS, 1L:** 12.07 g Na_2HPO_4 (dibasic), 2.04 g KH_2PO_4 (monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. Prepare fresh and keep at 4°C. Based on Electron Microscopy Sciences catalog, 100 mM PBS filtered through a 0.22 μm filter has a shelf life of 1 month at 4°C.
- **16% Paraformaldehyde (PFA):** Electron Microscopy Sciences, 15710. Right before fixation, prepare 4% paraformaldehyde solution. Open the vial containing 10 mL of 16% paraformaldehyde stock, transfer contents of vial into a 50 mL Falcon tube, add 30 mL 100 mM PBS, mix and place the tube on ice. *Note: Prepare as many tubes as many tissue sections will be procured considering approximately 1:50 tissue to fixative ratio.*
- **Sucrose:** Fisher Scientific, BP220-1. Prepare 30% sucrose solution: 15 g sucrose + 35 mL 10 mM PBS in a 50---mL Falcon tube. Place the tube on a rocker to dissolve sucrose and then keep at 4°C. Alternatively, 30% sucrose can be prepared in a larger quantity, filtered through a 0.22 μm and stored at 4°C for 1 month. *Note: Consider approximately 1:50 tissue to sucrose ratio.*

Procedure

1. Collect about 2-mm thick cross-sectional sections from the Head, Body, and Tail (see the pancreas mapping).
2. Cut each slice into four quadrants (A, B, C, D) and place all 4 tissue pieces into the same 50 mL tube. *Note: For donors < 1 year of age keep the entire slice intact.*
3. Transfer the tissue into a 50 mL Falcon tube containing 40 mL of freshly prepared fixative (4.0 % paraformaldehyde/100 mM PBS) and fix for 3 hours on ice under mild agitation using an adjustable tilt rocker (LabNet).
4. Wash the tissue four times in 40 mL of 100 mM PBS on ice over the period of 2-3 hours under mild agitation using an adjustable tilt rocker. Blot the tube with paper towel before adding the fresh washing solution.
5. Equilibrate the tissue in 40 mL of 30% sucrose/10 mM PBS at 4°C overnight. Tissue will settle to bottom of the tube. *Note: If the pancreas is not cleaned sufficiently from fat, it will never drop to bottom.*
6. Prepare a pre-labeled cryomold (VWR, 25608-916) and fill it half way with an OCT compound (VWR, 25608-930). Pour the pancreas with some sucrose into a 10 cm Petri dish. Pick the pancreas with a pair of fine forceps and blot it with Kimwips to remove an excess of sucrose. Place the tissue into the OCT-containing cryomold. Using forceps, push the tissue lightly to bottom of the cryomold. Add more OCT to fill completely the cryomold.

7. Freeze the tissue on a dry ice block. As OCT starts freezing, push again the tissue lightly to bottom of the cryomold.
8. When OCT compound is frozen, wrap the cryomold containing the tissue in a pre-labeled aluminum foil, place it a zip-lock bag, seal and store in a storage box at 80°C. *Note: Aluminum foil and zip-lock bag prevent the block from draying.*
9. Ship tissue blocks to Vanderbilt on dry ice.

Human Pancreas Processing for Clarity (CLR)

Reagents

- **1X PBS/10 mM PBS** without Ca/Mg, Invitrogen, 14190-144
- **16% Paraformaldehyde** (10x10 mL ampoule, Electron Microscopy Sciences, 15710). Right before fixation, prepare 4% paraformaldehyde solution. Open the vial containing 10 mL 16% paraformaldehyde stock, transfer contents of ampoule into a 50 mL Falcon tube, add 30 mL 1X PBS, mix and place the tube on ice. *Note: Prepare as many tubes as many tissue sections will be procured considering approximately 1:50 tissue to fixative ratio.*

Procedure

1. Collect 1-2 mm thick cross-sectional sections from the Head, Body, and Tail (see the pancreas mapping).
2. Cut each section into four quadrants (A, B, C, D) and place all 4 tissue pieces into the same 50 mL tube. *Note: For donors < 1 year of age keep the entire sections intact.*
3. Transfer the tissue into a 50 mL Falcon tube containing 40 mL of freshly prepared fixative (4.0 % paraformaldehyde/1X PBS) and fix overnight (at least 18 hours) at 4°C under mild agitation using an adjustable tilt rocker (LabNet).
4. Wash the tissue four times in 40 mL of 1X PBS at 4°C for the period of 2-3 hours under mild agitation using an adjustable tilt rocker. Blot the tube with paper towel before adding the fresh washing solution.
5. After the last wash, add 40 mL of fresh 1X PBS and keep at 4°C.
6. Ship tissue in sealed tubes to Vanderbilt on wet ice.

Human Pancreas Processing for Transmission Electron Microscopy (EM)

Reagents

- 16% Paraformaldehyde (10x10 mL ampoule, Electron Microscopy Sciences, 15710)
- 8% Glutaraldehyde (10x10 mL ampoule, Electron Microscopy Sciences, 16020)
- 200 mM Sodium Cacodylate Buffer (500 mL, Electron Microscopy Sciences, 11652)
- Right before fixation, prepare the TEM fixative containing 2% paraformaldehyde/2% glutaraldehyde in 0.1M cacodylate buffer by mixing the following components in a 50---mL Falcon tube:
    - 5 mL 16% paraformaldehyde stock
    - 10 mL 8% glutaraldehyde stock
    - 20 mL 200 mM sodium cacodylate buffer
    - 5 mL dH_2O

  Note: Prepare as many tubes as many tissue sections will be procured considering approximately 1:50 tissue to fixative ratio.

Procedure

1. Collect about 2-mm thick cross-sectional sections from the Head, Body, and Tail (see the pancreas mapping).
2. Place each slice in a separate 10 cm Petri dish containing about 10-15 mL EM fixative warmed up to room temperature. *Note: Cold depolymerizes cytoskeletal elements, so whenever possible fixative and sample should be at room temperature or 37℃ to initiate fixation.*
3. Mincing the sample using fine scissors directly in a Petri dish containing the worm fixative works best. Alternatively, the tissue can be minced by placing it into the drop of fixative on dental wax or parafilm and using fresh razor blades. *Note: Aldehyde fixatives penetrate tissue slowly, so samples must be small (less than 0.5 mm on two sides).*
4. Transfer the minced tissue into a 50 mL Falcon tube and fix in 40 mL fixative and fix for 24 hours at 4°C under mild agitation using an adjustable tilt rocker (LabNet).
5. Keep the tissue in the fixative and ship to Vanderbilt on wet ice for further processing within three days after procurement.
6. The Vanderbilt group submits the tissue in fixative to the VU EM Core for EM processing. Tissue is embedded into a Spurr resin within 7 days of fixation.

Appendix

Prepared by Jill Lindner

LIMS RECORDS – NAMING NOMENCLATURE & CONTROLLED VOCABULARIES

Powers Laboratory / Core Informatics LIMS (core_informatics_specimen_nomenclature_r20 last saved on 2/8/2017 11:01 PM)

RECORD NAMING NOMENCLATURE

In order to maintain consistency in the identification of records in the LIMS, we have adopted a nomenclature. This nomenclature begins at the entity donor level and is passed down to LIMS samples and LIMS lots. These entity names are assembled as an ordered and separated list of allowed (controlled) tokens. The LIMS is configured to automatically generate these names, as long as the primary values exist.

Please note that the entity hierarchy within the LIMS: **Donor** > **Sample** > **Lot** > **Container**

General rule is to separate tokens with hyphens: **{TOKEN}|{TOKEN}**

DONOR LEVEL NAME

Structure	{donorID})){disease state)age)disease duration)gender}
Example	

SAMPLE (SPECIMEN) LEVEL NAME

Structure	{donorID})){disease state)age)disease duration)gender}){specimen type}
Example	

LOT LEVEL NAME

Structure	{donorID})){disease state)age)disease duration)gender}){specimen type}){processing}){specimen anatomical location}
Example	

Token Definitions

{Token}	Definition	Include for	Examples	Example names
donorID	Given by facility	donor name, donor specimen name, donor lot name	A0002	A0002)ND)19y)M
disease state	diabetic status (non-diabetic)	donor name, donor specimen name, donor lot name	ND	A0002)ND)10y/F
age	donor age (e.g. 49 years)		10y	
gender	donor gender (e.g. M for male or F for female)		F	
specimen type	type of specimen (e.g. pancreas, blood, islet)	donor specimen name	PANC BLD ISL	A0002)ND)10y)F)PANC A0002)ND)10y)M)BLD
processing	type of processing (e.g. paraffin embedded, fixed frozen)	donor lot name	PE OCT	A0002)ND)10y)F)PANC)PE
specimen anatomical location	section of the specimen, (e.g. head, body, tail) This only applies to pancreas.)	donor lot name	H B T	A0002)ND)10y)F)PANC)PE)T

Notes

1. Entity names must be unique.
2. Spaces are not allowed.
3. Hyphens are used to separate tokens.
4. Each entity record name not controlled by this nomenclature will generated using a preNconfigured 2N3 letter prefix, e.g. DON20, VAE31, etc.
5. NonNdiseased donors (i.e. no disease or *ND*) will not have a disease duration in the donor name, e.g. NDN25yNF is a 25Nyear old nonNdiabetic female.
6. Donors whose age is less than one year require the label “d” or “w” or “m” for days/weeks/months of age, based on the selected “age unit” of the respective donor.
7. Any donor whose age is in weeks will have a “G” automatically inserted in front of the age, e.g. G39w would be gestational age 39 weeks.
8. In order to keep things consistent, a dash is used between each token, e.g. ABHQ115NT2DN49yN3yNF.

CONTROLLED VOCABULARIES

KEY		
Token	**Term**	**Acronym**
diabetic status	non/diabetic	ND
age	*See AGE table below*	
gender	female	F
	male	M
specimen type	blood	BLD
	islets	ISL
	lymph nodes	LN
	pancreas	PANC
	spleen	SPL
	FACS sorted cells	FACS
processing	plasma	PLA
	serum	SER
	clarity	CLR
	electron microscopy embedding	EM
	flash frozen	FF
	frozen fixed	OCT
	paraffin embedding	PE
specimen anatomical location	head	H
	body	B
	tail	T
	unknown	U

AGE		
Unit	**Abbreviation**	**Example**
gestational (weeks)	G*(number)*w	G39.9w
days	d	1d
months	m	10m
years	y	33y/16y