PREPARATION AND SHIPPING OF VEINS

Histology Core Facility
Dr. Charles Alpers
University of Washington
Seattle

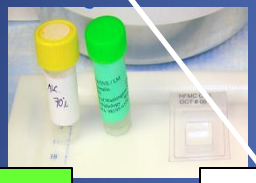
Preparation, shipping, processing

Alpers Histology Core

Data

Data Coordinating Center (DCC),

Ships materials, labels,shipping kits



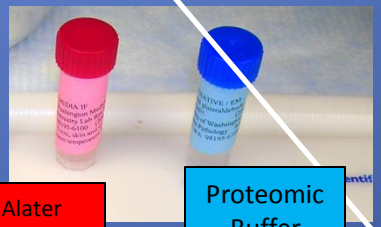
10% Buffered Formalin

OCT Frozen Tissue

Ships tissue for processing and analysis

Clinical Center Vein sample collection

Ultrasound Core (US)



RNAlater Media

Proteomic Buffer

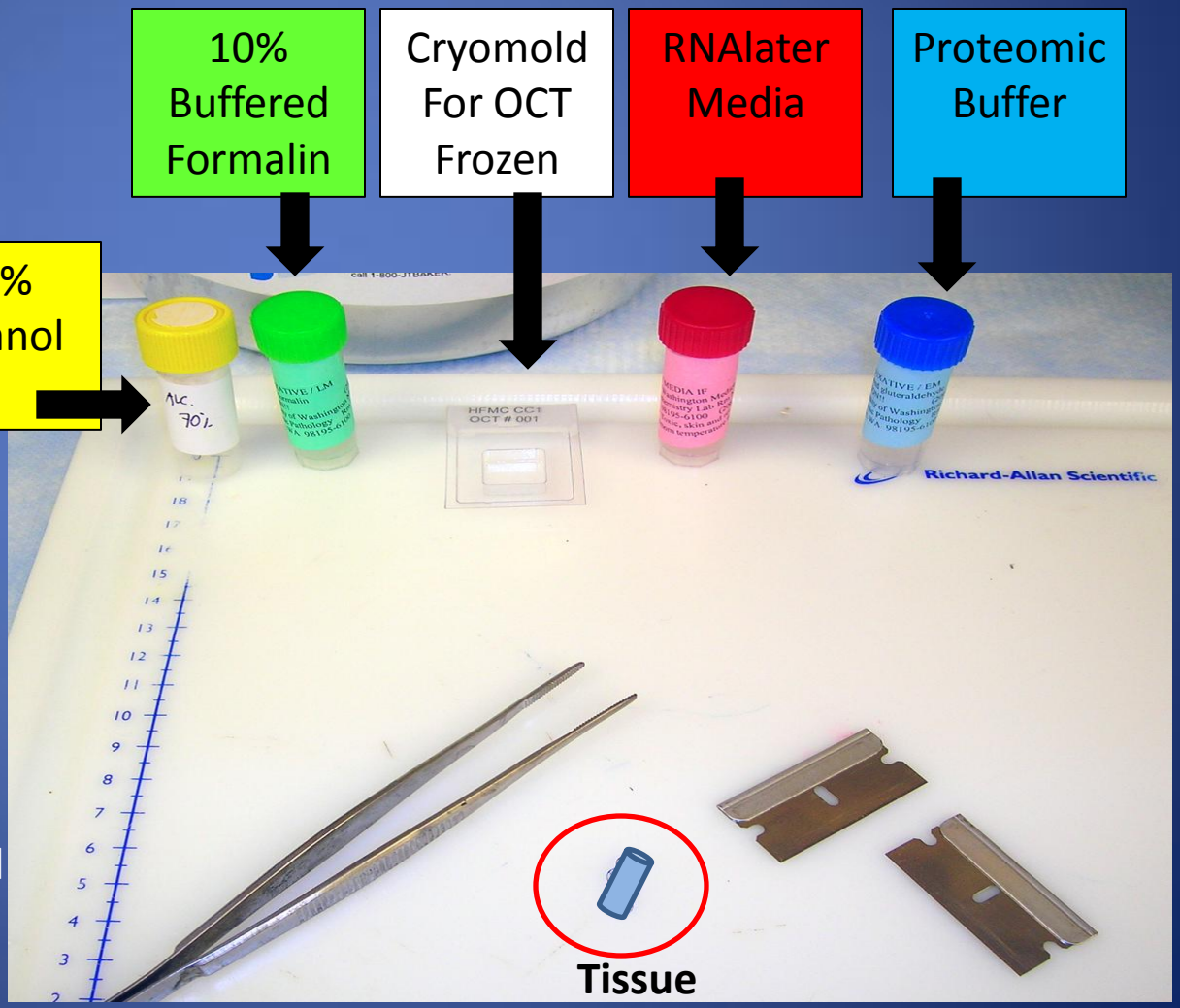
Processed tissue

NIDDK Central Biosample Repository

Required materials and preparations

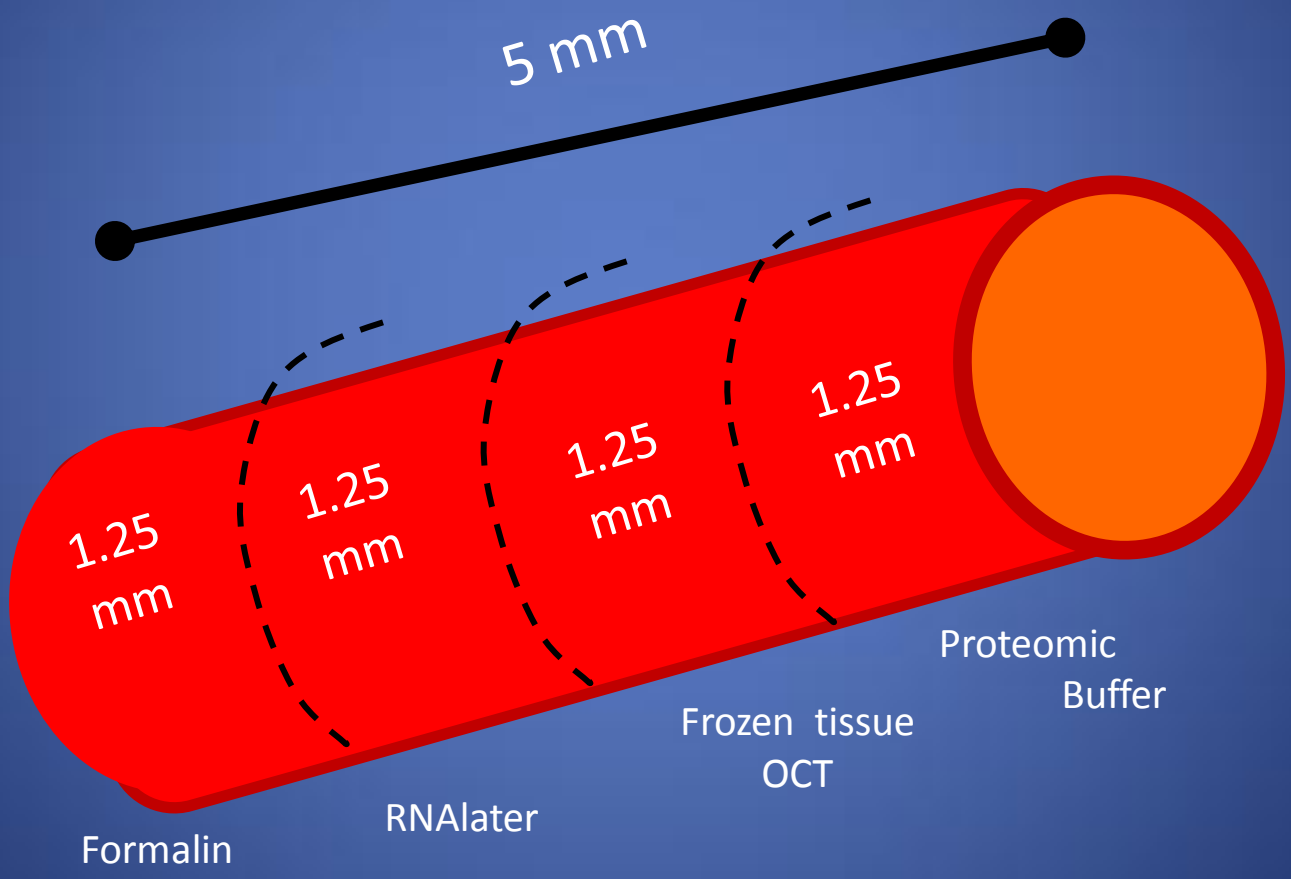
1. Color coded and labeled 2ml tubes with solution (supplied by Alpers Lab)
2. Cryomolds , dry ice, aluminium foil.

3. Disposable blades, forceps, gloves, permanent histopen (StatmarkPen), cutting board (or disposable Petri dishes), cryotubes.



Tissue preparation

1. Following excision of vein, divide into 4 even transverse sections using clean blades.



What if ?

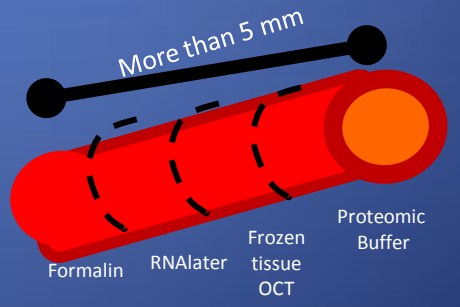
Q. There is less than 5mm of tissue.

A. Keep the thickness of the slices at 1.25 minimum.
If one slice of tissue (1.25mm thick) is available, preserve it in Formalin
If two slices of tissue are obtained – preserve in Formalin and Frozen OCT
If three slices of tissue are obtained – use for Formalin, Frozen OCT, RNAlater
if four slices of tissue are obtained – collect Formalin, Frozen OCT, RNAlater, and Proteomics

1. **Formalin**
2. **Formalin , Frozen OCT**
3. **Formalin, Frozen OCT, RNAlater**
4. **Formalin, Frozen OCT, RNAlater, Proteomics**

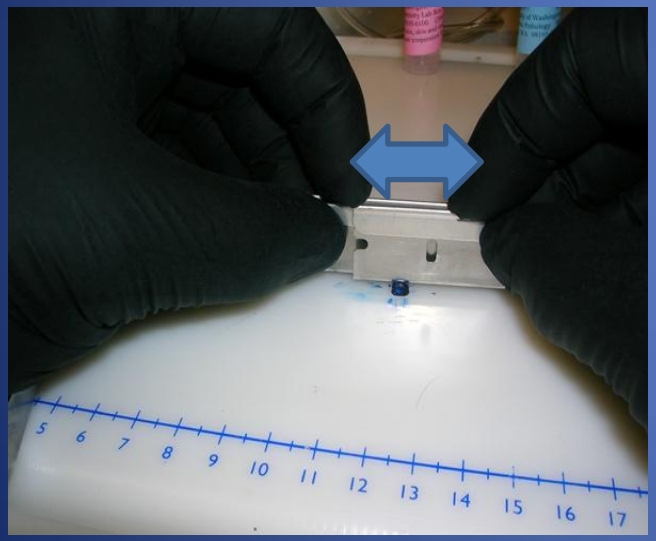
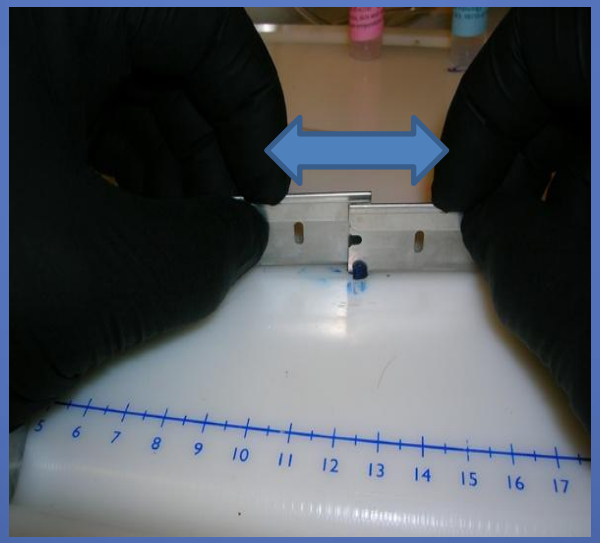
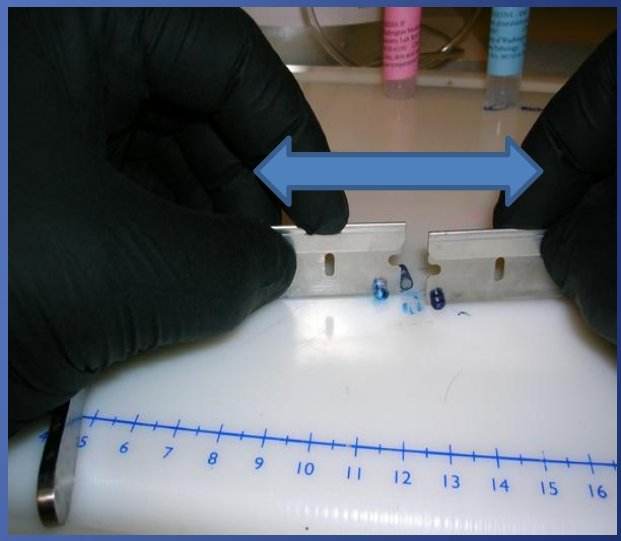
Q. I have more than 5mm of tissue.

A. Divide the available tissue into the 4 even slices.



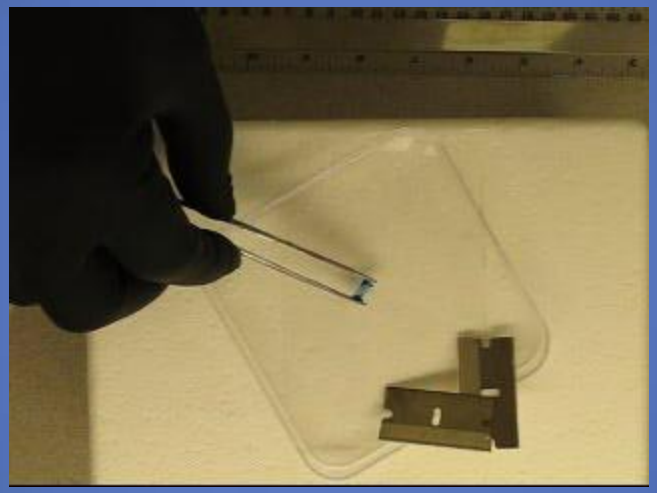
Tissue preparation

1. Following excision of vein, divide into 4 even transverse sections using clean blades.
2. Move the blades away from each other gently cuting through the vein. Avoid pushing down and crushing the tissue.

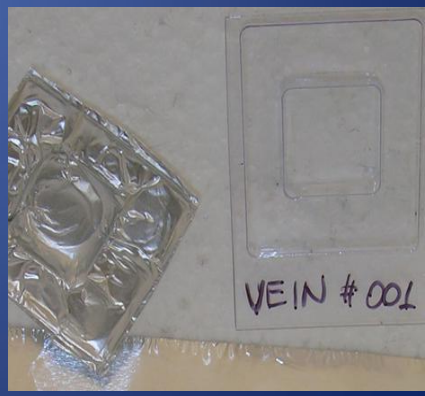
Tissue preparation – video 1

1. Following excision of vein, divide into 4 even transverse sections using clean blades.
2. Move the blades away from each other gently cuting through the vein. Avoid pushing down and crushing the tissue. Keep the blades as close to each other as possible.



Tissue preparation for OCT Frozen – video 2

Prepare aluminium foil custom mold using cryomold.



Tissue preparation for OCT Frozen – video 3

Precool all parts of the prep on dry ice for at least 1 minute before placing the tissue slice.

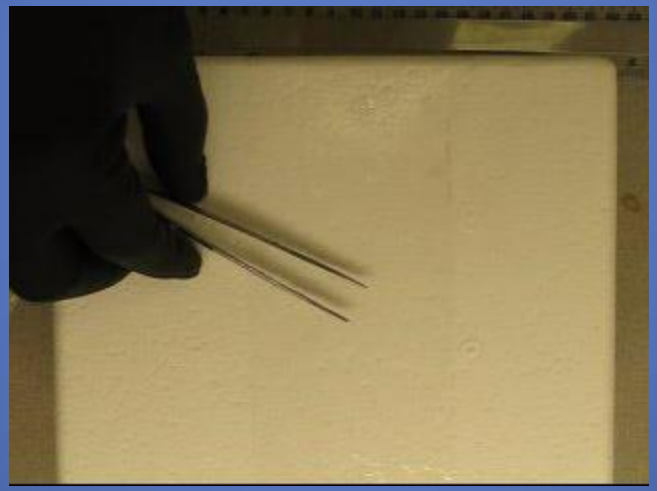
Tissue preparation for OCT Frozen – video 4

Place the tissue in the middle of the aluminum mold, transfer it into vinyl cryomold and gently pack with second piece of foil.

Keep the cryomold up at all times. Try to use forceps as often as possible to avoid heat transfer from fingers.

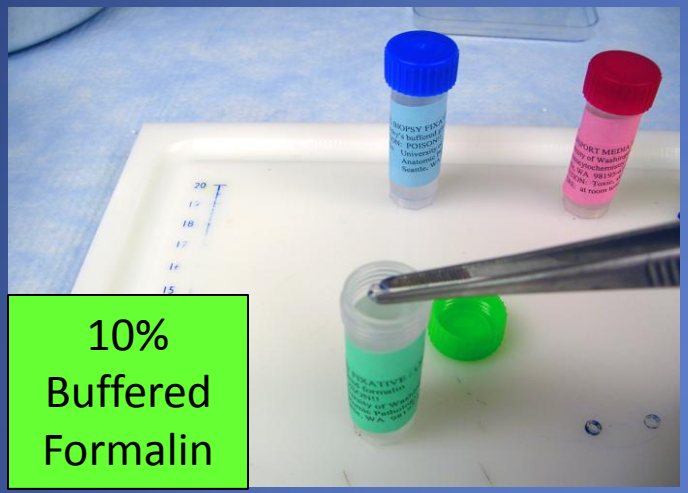
Write necessery identifying information on the foil to match the info on the cryomold.

Leave on dry ice and transfer to -20, or -80 for storage.

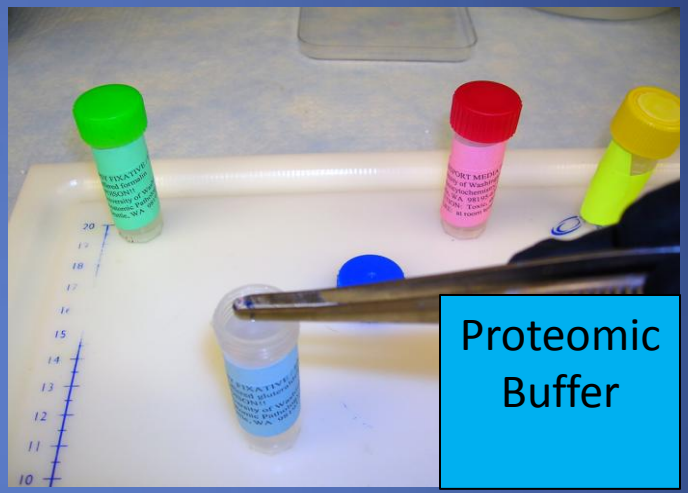


Tissue preparation

2. Place one segment into each of 3 different tubes.



10% Buffered Formalin

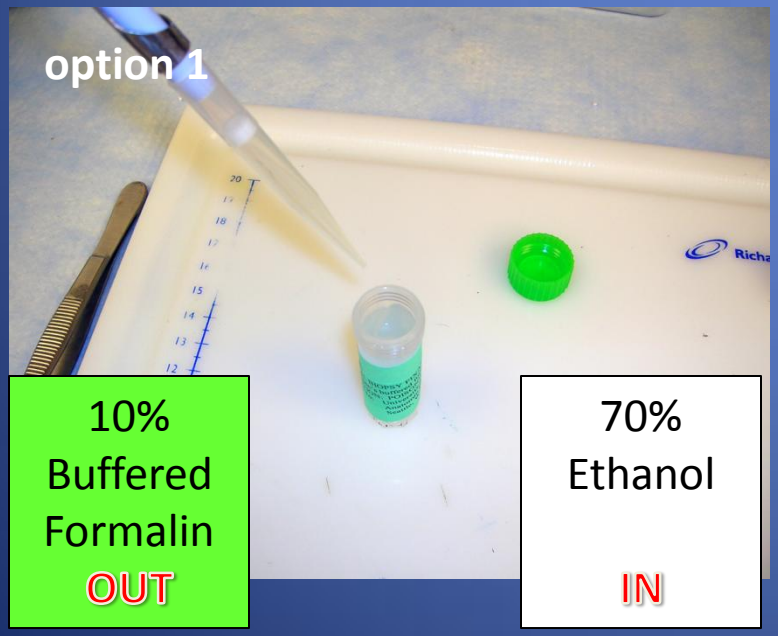


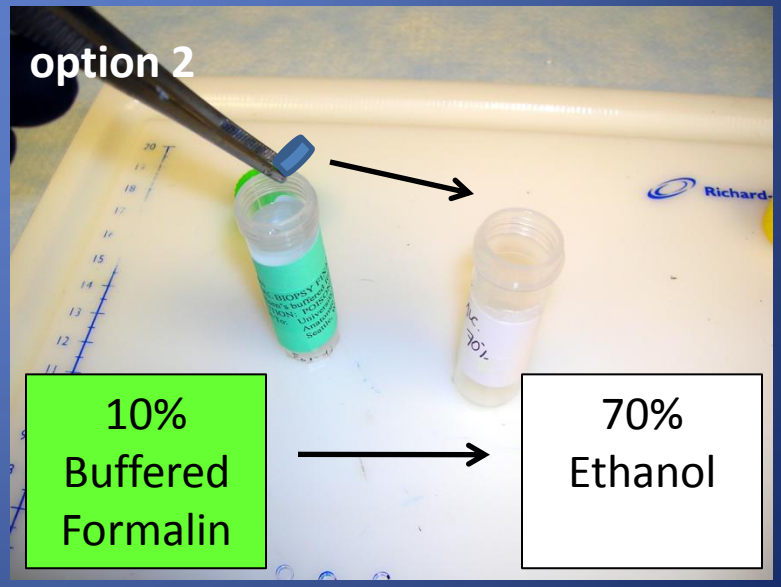
Proteomic Buffer

RNAlater Media

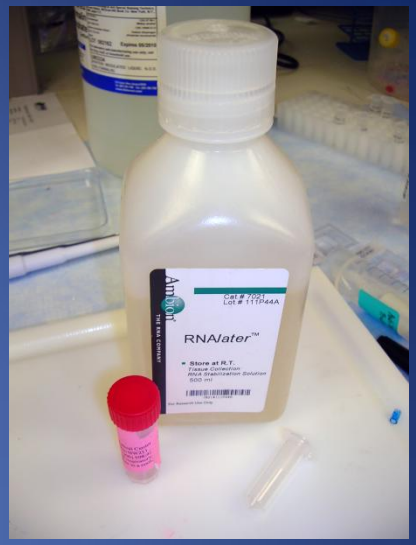
Formalin fixation

1. Allow to fix for 24 to 72 hours at room temperature and then replace the formalin with 70% ethanol.
   - option 1: Pipet the formalin out and pipet 70% ethanol in. Mark the tube using a permanent marker, or a new label ("70% ethanol, [date]"). Store at room temperature (RT).
   - option 2: Transfer tissue using forceps to a new tube with 70% ethanol. Store at RT.



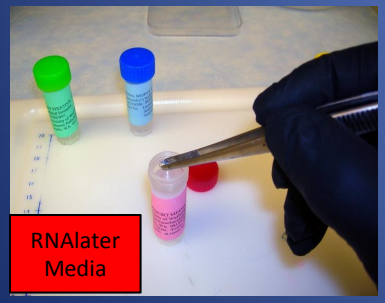


RNAlater media



1. Allow to sit at least overnight at 4^0 C (tissue RNA is stable at 4^0 C up to 1 week).
2. Move the tissue to a cryotube <u>without solution</u> and place at -20 or -80^0 C for longterm storage.
3. 500ml of RNAlater costs around $300. 2ml used per sample, cost of RNAlater per sample/tube ~ $ 1.2

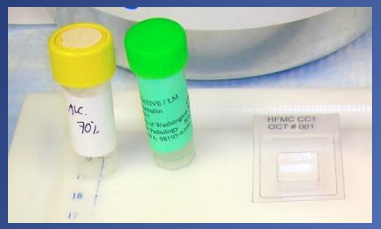
RNA tissue collection



24h at fridge
4^0 C

Long term storage
-20^0 C or -80^0 C,
a cryotube <u>without solution</u>

Where and when to send?

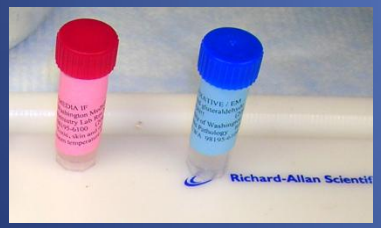


Formalin fix tissue in Ethanol in RT

OCT on DRY ICE

To UW of Seattle, Histology Core

1. Green (white) tube formalin fixed tissue in 70% ethanol. Send in room temterature (RT).
2. OCT frozen tissue in cryomold. Send on dry ice. Samples are sent by the Clinical Center once a month. Frozen OCT tissue must be sent overnight on **DRY ICE** only, Monday to Thursday only, with contact information forwarded to UW at least a day before. UW will confirm the readiness to receive that shipment (email or call UW before you send tissue).



Proteomic ?

RNA later tissue on DRY ICE

To Fisher (NIDDK Repository)

1. RNAlater tissue. Send overnight on **DRY ICE** only, Monday to Thursday only, with contact information forwarded to Fisher at least a day before . Fisher will confirm the readiness to receive that shipment (email or call Fisher first before you send tissue).
2. Proteomic tissue. To be determined and TBA.

If you need to write on tubes (especially cryotubes) use StatmarkPen, or any Histopen. Do not use regular permanent marker (Sharpie)!!!

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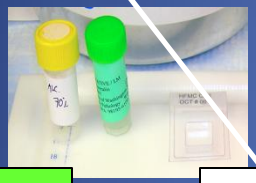

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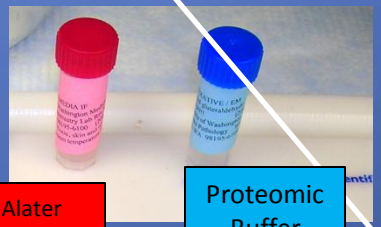

RNAlater Media

Proteomic Buffer

Processed tissue

NIDDK Central Biosample Repository

Materials for 600 pts.

				2539			
	vol., case of	will need	$ per	total			
Formalin	4 l	1x	40	40		vwr	
70 %Ethanol	4 l	1x	103	103		vwr	
RNAlater	0.5 l	2x	300	600		vwr	
Proteomics	1.2 l		?	?			
OCT		12x	9.33	95		tissue-tek	
2-methylbutane	0.5 l	6x	61	366		vwr	
Materials							
2ml tubes green	500	2x	46.5	93		us scientific	
2ml tubes blue	500	2x	46.5	93		us scientific	
2ml tubes red	500	2x	46.5	93		us scientific	
cryomolds	100	6x	19.5	117		tissue-tek	
cassettes	600	1x	100	100		vwr	
histopen	1 pack of 10	2x	30	60		vwr	
blades	1 pack of 100	12x	20	240		vwr	
labels laser	3120	2x	53	106		small cryo-babies by tough	
forceps	1	6x	3	18		vwr #25601-008	
petri dishes non-vented	480	1 x	63	63		vwr #82050-570	
shipping							
boxes thermosafe	4	2x	64	128		vwr #14100-432	25.4x25.4x17.8
ups soft			free			ups	
cardboard box	1	64	3.5	224		us scientific	

dry ice

overnight	5 lb	8lb
ups		
fedex		

10lb

	envelope
ups	
fedex	

Questions?

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