## 12.8. Vein Tissue Collection, Preparation, Preservation and Shipping Procedure Instructions- Timeline

1. Complete and attach the participant I.D. labels provided by the DCC to the set of tubes:
    a. Formalin (GREEN top, with tube labeled “F”)
    b. RNAlater with solution (RED top, with tube labeled “R”)
    c. Proteomic/ Mass spec buffer (BLUE top, with tube labeled “M” )
    d. Frozen tissue (CLEAR top, with tube labeled “Z”)

2. Check the source of the liquid nitrogen (LN2) at your institution. Most of the gross rooms are equipped with LN2 tanks. Fill the portable liquid nitrogen container ½ half up with liquid nitrogen (LN2 from the storage tank by the hose, or by pour in). Use appropriate gloves and safety glasses.

3. Prepare the Styrofoam box with 1- 2 pounds, or 2-inch thick layer of dry ice. Keep it covered with the lid.

4. Gather your instruments and additional supplies:
    a. Petri dish
    b. Long (steel) and short forceps (disposable autoclavable)
    c. 2 cutting blades
    d. Gloves
    e. Permanent Histopen
    f. Ruler

5. Proceed to the Operation Room area to obtain the vein sample.
    a. Collect the participant ID information
    b. Ask surgeon or assisting nurse to hand the vein sample to you on the saline soaked gauze; DO NOT submerge the tissue in any solution (saline, Ringer…)
    c. Place the sample on the gauze into the Petri dish; cover it with Petri dish top.
    d. Walk to the preparation area.
    e. Keep the tissue sample on the soaked gauze at all times; do not let it dry out.

6. Proceed with tissue preparation within 15 minutes from the excision time.

7. Before sectioning the vein sample, determine its length using the ruler in order to judge how to proceed.

a. If the vein sample is 5 mm or longer, the tissue will be divided into four equal pieces.

- to cut the vein sample, use the Petri dish as a cutting board, and obtain two clean razor blades (or scalpel blades); keep the tissue moist on the piece of gauze soaked in saline. Do not let it dry out, but do not soak it, either.
- place the middle of the first blade edge on the sample where you wish to cut. Then take the second blade edge and place it as close as possible to the first blade so that the edges are touching. Gently slide the blades away from each other to make the cut. DO NOT push down on the tissue sample, and avoid crushing the tissue during the slice.
- make an additional two cuts to get 4 equal pieces of the vein sample.

b. If the vein sample is less than 5 mm, divide the tissue into slices that are at least 1.25 mm thick.

- to cut the vein sample, use the Petri dish as a cutting board, and obtain two clean razor blades (or scalpel blades); keep the tissue moist on the piece of gauze soaked in saline. Do not let it dry out, but do not soak it, either.
- place the middle of the first blade edge on the sample where you wish to cut. Then take the second blade edge and place it as close as possible to the first blade so that the edges are touching. Gently slide the blades away from each other to make the cut. DO NOT push down on the tissue sample, and avoid crushing the tissue during the slice.

8. Tissue preservation.

a. Place the first segment, or the thickest one (if the slices are not even) into the formalin tube (GREEN top, with tube labeled “F”); use short forceps (disposable),

avoid crushing the tissue. Make sure the tissue sample is submerged into the formalin solution. Set aside and keep it at room temperature at all times.

b. Place the second segment in the RNAlater buffer tube (RED top, with tube labeled "R"). Make sure the tissue sample is submerged into the solution. Set aside and proceed with next sample.

c. Place the third segment in the frozen tissue cryotube (CLEAR top, with tube labeled "Z"). Drop the tube into the liquid nitrogen container. Do not touch the liquid.

d. Place the fourth segment in the proteomic buffer tube (BLUE top, with tube labeled "M"). Make sure the tissue sample is submerged into the solution. Drop the tube into the liquid nitrogen container. Do not touch the liquid.

e. Wait 10 minutes and then remove the frozen tissue cryotube and proteomic buffer tube (use long forceps) from the liquid nitrogen container and immediately (1-2 seconds) transfer the tubes and bury them in the dry ice in the Styrofoam box. Cover the box with the lid. DO NOT touch the liquid with your fingers.

f. dispose of the Petri dish, disposable short forceps (these forceps are autoclavable and they could be reused if necessary), and gauze into the biohazard waste container.

9. Tissue preservation. Day 1 (collection day).

a. Move to the storage area as soon as possible. Formalin (GREEN top, with tube labeled "F") tissue should be held in designated box **at room temperature at all times**.

b. RNAlater with solution (RED top, with tube labeled "R") must be transferred to $4^{o}$C refrigerator. **Do not freeze that sample**.

c. Proteomic/ Mass spec buffer (blue top, with tube labeled "M" ) tube and Frozen tissue (CLEAR top, with tube labeled "Z") tube must be transferred directly from the

Dry ice containing styrofoam box to designated cryoboxes in -80$^{o}$ C freezer (a final long term storage for these samples until the scheduled shipment).

- Avoid any delay during the transfer. The transfer of the sample should not last longer than few seconds. Use gloves at all times.

10. Tissue preservation. Day 2-3.

a. After 24-72 hours, transfer the formalin-fixed tissue sample (GREEN top, with tube labeled "F") to the 70% ethanol tube (YELLOW top, with tube labeled "Y"); use forceps, avoid crushing the tissue. Make sure that the yellow top cap is secured tightly.

- Complete and attach the participant I.D. labels provided by the DCC.
- Place the tube in designated box and **keep it at room temperature**. Never freeze that sample. The sample can be stored in 70% ethanol at room temperature for extended periods of time or until the scheduled shipment.
- Dispose of the used formalin solution and tube. Empty the tube into the designated formalin waste container. Throw away the empty tube into the biohazard waste container.

c. After 24 hours, take the RNAlater sample (RED top, with tube labeled "R") tube out of the 4$^{o}$C refrigerator and pipette out the solution (use sterile transfer pipette). Be sure to remove all the solution, recap the tube and transfer the tube to the -80 $^{o}$C freezer (a final, long term storage for that sample until the scheduled shipment) as soon as possible. The used RNAlater solution can be disposed of into the sink.

11. Prepare the shipments for the Histology Core at UW of Seattle <u>(two shipments, once a month)</u>

Contact the Histology Core one day before sending the sample.

University of Washington
HSB Room E-506
Alpers Lab

1959 NE Pacific St
Seattle, WA 98195
Phone: (206)-543-6746
Fax: (206)-221-6678
Cell: (425)-681-0275 (Tomasz)
Email: tomaszw@u.washington.edu (Tomasz Wietecha)
kellylee@u.washington.edu (Kelly Hudkins)

1. First package/envelope shipment for Formalin fixed tissue in 70 % ethanol at room temperature (YELLOW tube labeled "Y")

The sample in ethanol yellow top, with tube labeled "Y" (formalin-fixed), must be sent at room temperature (separate from frozen sample) in the boxes provided by the Histology Core inside of the FedEx bubble wrap envelope. Do not send it together with a frozen tissue; this will cause the formalin fixed tissue to freeze inadvertently.

a. Double check the subject ID, and verify that ID information on the tubes match that on the Form 610; date and identify person competing the mailing Form 610.

b. Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.

c. Assemble the shipment.

- Make sure the yellow tube caps are screwed tightly.
- Place the tubes in paper box (provided by Histology Core).
- Put a lid on and tape it.
- Place the box into the leak proof zip-lock bag, and insert it into the bubble envelope together with Form 610. Seal the envelope.
- Use the preprinted FedEx air bill to ship it to the Histology Core in Seattle, Washington. Fill in the date, your address, and phone number. Check "NO'' in section indicating no dangerous goods are contained in the shipment.
- Attach the air bill to the envelope.

Call FedEx 1-800-Go-Fedex. Give them the account number of the preprinted FedEx Air bill, and your pickup address. FedEx will dispatch the courier to pick up your package.

2. Second package shipment for Frozen tissue (clear top, with tube labeled "Z") on DRY ICE

Frozen tissue must be sent by overnight shipping (FedEx) Monday-Wednesday ONLY! (Samples must be received by Thursday, at the latest). Make sure that the sample is BURIED in dry ice inside the styrofoam box (supplied by Alpers Lab).

Contact Alpers Lab one day before sending the sample.

a. Keep the tubes on the DRY ICE all the time while preparing the shipment. NEVER let the samples sit outside of the freezer or styrofoam box with dry ice (do not let it thaw out).

b. Double check the subject ID, and verify that ID information on the tubes match that on the Form 609.

c. Date and identify person competing the mailing Form 609.

d. Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.

e. Assemble the package

- fill the styrofoam box half way up with DRY ICE
- place the frozen tissue tubes in the middle of the dry ice
- add more dry ice on the top of the tubes to fully fill the box
- place the lid on the styrofoam box and tape it
- put the Styrofoam box into the cardboard box
- place the Form 609 and close the box

- tape the box and attach FedEx air bill sticker. Use the preprinted FedEx air bill to ship it to the Histology Core in Seattle,Washington. Fill in the date, your address, and phone number. Check "NO'' in section indicating no dangerous goods are contained in the shipment.
- attach the DRY ICE sticker and put the amount of DRY ICE on the sticker

f. Call FedEx 1-800-Go-Fedex. Give them the account number of the preprinted FedEx Air bill, and your pickup address. FedEx will dispatch the courier to pick up your package.

Frozen tissue must be sent by overnight shipping (FedEx) Monday-Wednesday ONLY!

12. Prepare the shipments for NIDDK Repository (Fisher Lab) (one shipment, once a month).

Contact the NIDDK Repository one day before sending the sample.

Attn: Heather Higgins
NIDDK Repository
20301 Century Blvd.
Building 6, Suite 400
Germantown, MD 20874

Fax: (301) 515-4049
Phone: (240) 793-0353 (Heather Higgins)
Phone: (240)-686-4702 (Sandra Ke)
Email: BIO-NIDDKRepository@thermofisher.com

The RNAlater and proteomics frozen tissue must be sent by overnight shipping (FedEx) Monday-Wednesday ONLY! (Samples must be received by Thursday, at the latest). Make sure that the sample is BURIED in dry ice inside the Styrofoam box.

a. Double check the subject ID, and verify that ID information on the tubes match that on the Form 608;

b. Keep the tubes on the DRY ICE all the time, while preparing the shipment. NEVER let the samples to sit outside of the freezer or Styrofoam box with dry ice (do not let it thaw out).

c. Date and identify person competing the mailing Form 608.

d. Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.

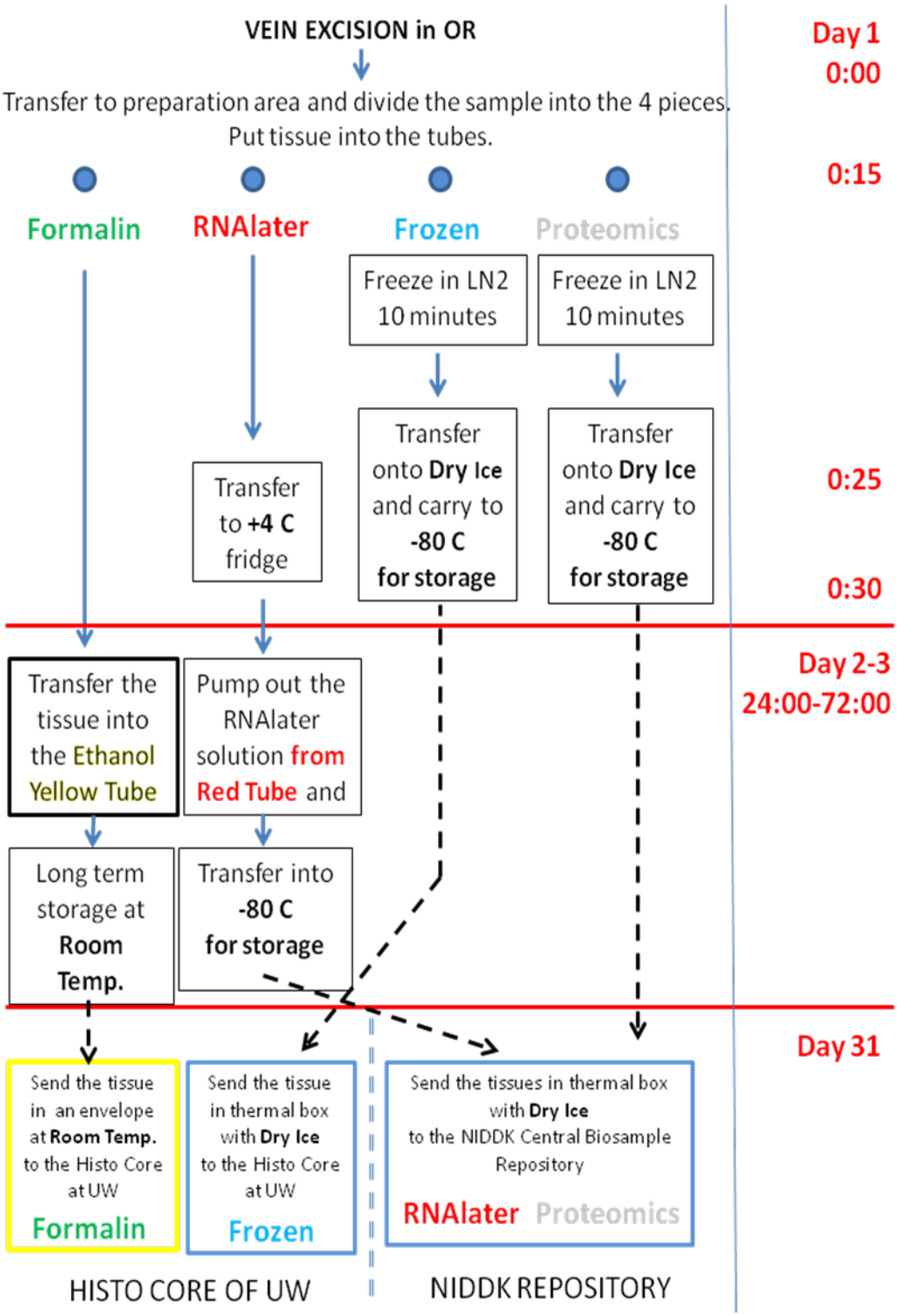
e. Assemble the package

- fill the styrofoam box half way up with DRY ICE
- place the frozen tissue tubes in the middle of the dry ice
- add more dry ice on the top of the tubes to fully fill the box
- place the lid on the Styrofoam box and tape it
- put the styrofoam box into the cardboard box
- place the Form 608 and close the box
- tape the box and attach FedEx air bill sticker. Use the preprinted FedEx air bill to ship it to the NIDDK Biosample Repository. Fill in the date, your address, and phone number. Check "NO'' in section indicating no dangerous goods are contained in the shipment.
- attach the DRY ICE sticker and put the amount of DRY ICE on the sticker

f. Call FedEx 1-800-Go-Fedex. Give them the account number of the preprinted FedEx Air bill, and your pickup address. FedEx will dispatch the courier to pick up your package.

Frozen tissue must be sent by overnight shipping (FedEx) Monday-Wednesday ONLY!

13. Summary.


VEIN EXCISION in OR
Day 1
0:00
Transfer to preparation area and divide the sample into the 4 pieces.
Put tissue into the tubes.
0:15
Formalin
RNAlater
Frozen
Proteomics
Freeze in LN2
10 minutes
Freeze in LN2
10 minutes
Transfer
to +4 C
fridge
Transfer
onto Dry Ice
and carry to
-80 C
for storage
Transfer
onto Dry Ice
and carry to
-80 C
for storage
0:25
0:30
Day 2-3
24:00-72:00
Transfer the
tissue into
the Ethanol
Yellow Tube
Pump out the
RNAlater
solution from
Red Tube and
Long term
storage at
Room
Temp.
Transfer into
-80 C
for storage
Day 31
Send the tissue
in an envelope
at Room Temp.
to the Histo Core
at UW
Formalin
Send the tissue
in thermal box
with Dry Ice
to the Histo Core
at UW
Frozen
Send the tissues in thermal box
with Dry Ice
to the NIDDK Central Biosample
Repository
RNAlater
Proteomics
HISTO CORE OF UW
NIDDK REPOSITORY