# CHAPTER 12 HISTOLOGY

## 12.1 Supplies for Tissue Collection and Shipment

1. The Histology Core (Alpers Lab, University of Washington) provides the following materials for sample collection and shipment:

   2ml cryovials (color coded, prelabeled)

   - Green, labeled "F", 10% Formalin

   - Yellow, labeled "Y", 70% Ethanol

   - Red, labeled "R", RNAlater solution

   - Blue labeled "M", Proteomic/Mass Spec solution

   - Clear labeled "Z", Frozen tissue

- The Center Coordinators will put the patient specific labels over the color coded labels

**ID# 460(Center #)-01or 02 (Coordinator #) TEST (Patient #); for example:**
ID # 460-02-0001 means: Boston Center - coordinator 02 patient # 0001

| ID # | What it is |
|---|---|
| 460-01TEST,<br>460-02TEST | F, Y, R, M, or Z |
| 461-01TEST<br>**461-02TEST** | F, Y, R, M, or Z |
| 462… | F, Y, R, M, or Z |
| 463…. | F, Y, R, M, or Z |
| 464… | F, Y, R, M, or Z |
| 465… | F, Y, R, M, or Z |

- Disposable blades, forceps, permanent histopen (StatmarkPen), cutting board (or disposable Petri dishes)
- Shipping kits for the frozen and room temperature stored samples include:
  - 5"L x 5"W x 2"H paper specimen box
  - 5"L x 2.5"W x 2" H plastic boxes (2x)
  - Absorbent sheets
  - Zip lock bags
  - Fed-ex envelopes

- Styrofoam box
- Insulated Styrofoam shipper
- Dangerous goods in limited quantities label
- UW "To" address label
- UN 3373 Category B label
- Class 9 miscellaneous dangerous goods label
- Preprinted FedEx air bill

2. The participating sites provide the following supplies and equipment for sample collection and shipment:
    - -70C freezer
    - 4C fridge
    - Liquid nitrogen
    - Laboratory supplies such as biohazard bags, gloves, pipettes, and pipette tips, alcohol pads
    - Dry ice pellets
    - Packing tape for shipping boxes
    - Participating site return address labels

### 12.1.1 RNAlater Solution

The shelf life of RNAlater is at least 1 year at room temperature.

Storage and stability

- Store RNAlater Solution at room temperature.
- If any precipitation of RNAlater Solution is seen, heat it to 37°C and agitate to redissolve it. Let it cool down to room temperature before use.

Disposal of RNAlater Solution.

- RNAlater Solution can be safely discarded down the sink and flushed with water.
- RNAlater Product Description at: http://www.ambion.com/techlib/prot/bp_7020.pdf

## 12.2 Tissue Transfer

**(See Section 12.8 for detailed procedures and timeline)**

1. Follow the rules of the local OR facility.
2. Process the tissue as soon as possible after harvesting from patient; start preparing the vein samples within 15 minutes of collection.
3. Transfer the vein from the OR to the tissue preparation room in a Petri dish containing a piece of gauze soaked in saline.

4. DO NOT submerge the tissue in saline or any other solution during transfer!

### 12.3 Tissue Preparation

1. Prepare the set of marked tubes (supplied by Alpers Lab) containing:
    a. Formalin (green top, with tube labeled “F”).
    b. RNAlater (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”).

The Center Coordinators put the patient specific labels over the color coded labels.

**ID# 460(Center #)-01or 02 (Coordinator #) TEST (Patient #); for example:**
ID # 460-02-0001 means: Boston Center - coordinator 02 patient # 0001

2. Before sectioning the vein sample, determine its length 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.
    b. If the vein sample is less than 5 mm, divide the tissue into slices that are at least 1.25 mm thick.
        - If the vein sample is less than 2.5mm, place it in formalin (green).
        - If the vein sample is from 2.5 to 3.75 and you are only able to obtain two at least 1.25 mm segments, place one in formalin (green) and one in the RNAlater (red).
        - If the vein sample is from 3.75 to 4.9, and you are only able to obtain three at least 1.25 mm segments, place one in formalin (green), one in RNAlater (red) and the third in frozen tissue cryotube (clear).
        - If the vein sample is more than 5mm, the tissue will be divided into four equal pieces.
        - If the segments obtained are uneven in thickness, prioritize the samples in a similar way (i.e., place the thickest sample in formalin, second thickest in RNAlater, etc).
3. 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.
    a. 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 (see web site link in Section 12.5).
    b. DO NOT push down on the tissue sample, and avoid crushing the tissue during the slice.

## 12.4 Tissue Preservation

**Day 1**

1. Place the first segment into the formalin tube (GREEN top, with tube labeled "F"). Allow the tissue in the formalin tube to fix for 24-72 hours at room temperature. DO NOT refrigerate or freeze this sample.

   - wipe the forceps tips with alcohol pad!!!

2. Put the second segment in the RNAlater buffer tube (RED top, with tube labeled "R"). Place the RNAlater tube in a 4 degree refrigerator for 24 hours. DO NOT freeze this sample.

   - wipe the forceps tips with alcohol pad!!!

3. Place the third segment in the frozen tissue cryotube (CLEAR top, with tube labeled "Z") and freeze it (drop in) in liquid nitrogen container (LN2). After at least 10 minutes take the tube out with forceps, place on dry ice and transfer to -80 degree freezer (do not let it thaw out). Do not touch liquid nitrogen with your fingers.

   - wipe the forceps tips with alcohol pad!!!

4. Place the fourth segment in the proteomic buffer tube (BLUE top, with tube labeled "M") and freeze it (drop in) in liquid nitrogen container (LN2). After at least 10 minutes take the tube out with forceps, place on dry ice and transfer to -80 degree freezer (do not let it thaw out). Do not touch liquid nitrogen with your fingers.

   - wipe the forceps tips with alcohol pad!!!

5. Dispose of all remaining materials (Petri dish, blades) according to safety regulations.

**Day 2**

1. 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"). The sample can be stored in 70% ethanol at room temperature for extended periods of time.

2. After 24-72 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.

## 12.5 Additional Information Available Online

PowerPoint presentation with embedded video is located at website link:
http://www.pathology.washington.edu/research/labs/alpers

**or at www.youtube.com**

**The vein collection on youtube is located at:**
**part 1 http://www.youtube.com/watch?v=Z0gp9bdfY3w**
**part 2 http://www.youtube.com/watch?v=xlNjvoTBGqM**
**or search for "vein collection" in youtube search box**

## 12.6 Shipping to UW Histology Core (Alpers Lab)

Two kinds of samples:

**a. Previously formalin-fixed tissue in ethanol (YELLOW top, with tube labeled "Y")**

**b. Frozen tissue (CLEAR top, with tube labeled "Z") and**

1. The sample in ethanol yellow top, with tube labeled "Y" (previously formalin-fixed), must be sent at room temperature in the box provided by Histology Core.
2. The frozen tissue (CLEAR top, with tube labeled "Z") must be sent by overnight shipping ( FedEx) Monday-Wednesday ONLY! (Samples should be received by Thursday, but we allow one day for potential shipping error). Make sure that the sample is BURIED in dry ice inside the styrofoam box (supplied by the Histology Core).
3. Contact Histology Core one day before sending the sample.
4. Attach Forms 609 and 610 for the University of Washington when sending samples.

   University of Washington
   Attn: Kelly Hudkins
   Room E 147
   Alpers Lab
   750 Republican St.
   Seattle, WA 98109
   Phone: (206)-543-6746
   Fax: (206)-221-6678
   kellylee@u.washington.edu (Kelly Hudkins)

## 12.7 Shipping to NIDDK Repository (Fisher Lab)

Two kinds of samples:
a. RNAlater tissue (RED top, with tube labeled "R").
b. Proteomics tissue (BLUE top, with tube labeled "M")

1. The RNAlater and proteomics tissue must be sent by overnight shipping (UPS, FedEx) Monday-Wednesday ONLY! (Samples must be received by Thursday, at the latest). All tissue, serum and plasma specimens going to the NIDDK should be batched and shipped together in the large shipping kits provided by the NIDDK. All of the vials (vein tissue, serum and plasma) should be placed in the slotted cardboard boxes and grouped by patient. See Chapter 7, section 7.6 of the MOP for information on shipping all specimens to the NIDDK.
2. Contact the NIDDK Repository one day before sending the sample.
3. Attach Form 608 for the NIDDK Repository when sending samples.

Fisher BioServices
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

### 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 pathology 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 tools 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.
        - cut the vein samples as indicated above in Section 7.a
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.

        - wipe the forceps tips with alcohol pad!!!

    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.

        -wipe the forceps tips with alcohol pad!!!

    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.

        - wipe the forceps tips with alcohol pad!!!

    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.

        - wipe the forceps tips with alcohol pad!!!

    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 the Petri dish, pippets and gauze into the biohazard waste container. Blades should be placed in biohazard sharps only 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°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° 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.

    1. 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.

    2. After 24-72 hours, take the RNAlater sample (RED top, with tube labeled "R") tube out of the 4°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° 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 (once a month)

Two kinds of samples:

**a. Previously formalin-fixed tissue in ethanol (YELLOW top, with tube labeled "Y") at room temperature**

b. Frozen tissue (CLEAR top, with tube labeled "Z") on dry ice

Contact the Histology Core one day before sending the sample.

University of Washington
Room E 147
Alpers Lab
Attn: Kelly Hudkins
750 Republican St.
Seattle, WA 98109
Phone: (206)-543-6746

Fax: (206)-221-6678
kellylee@u.washington.edu (Kelly Hudkins)

a. Formalin fixed tissue in 70 % ethanol at room temperature (YELLOW tube labeled "Y")

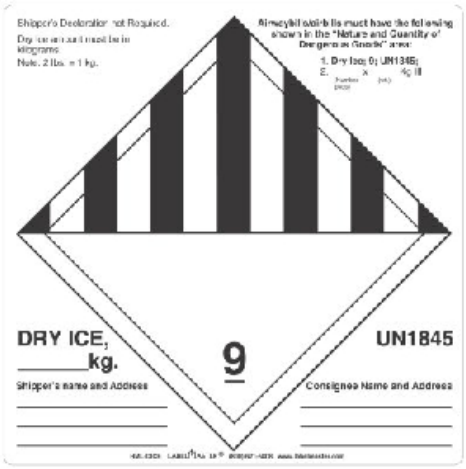
The sample in ethanol yellow top, with tube labeled "Y" (formalin-fixed), must be sent at room temperature (separate from the frozen tissue) in the plastic box provided by the Histology Core.

- 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.
- Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.
- Assemble the shipment.
  - Make sure the yellow tube caps are screwed tightly.
  - Place the tubes in plastic box (provided by Histology Core).
  - Put a lid with the absorbing material on and tape it.
  - Add more of the absorbing material around the box
  - Place the box into the leak proof zip-lock bag, and insert it into the bubble envelope.
  - 
  - Put the assemble shipment in to the insulated shipper.

b. Frozen tissue (CLEAR top, with tube labeled "Z") on DRY ICE

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

- Date and identify person completing the mailing Form 609.
- Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.
- Assemble the package
  - fill the styrofoam box provided by Histology Core, half way up with DRY ICE
  - place the frozen tissue tubes in the small zipper bag and then 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 next to the formalin fixed tissue shipment assembly into the insulated shipper box
  - 
  - add some packing materials and put the lid
  - place the Forms 609 and 610
  - tape the box
  - attach preprinted Fedex sticker or you have the option of generating the shipping labels via the online Fedex Ship Manager . You also can use Fedex Air Bill.
  - enter the information: senders name, the adress, phone number. Check the “Fedex Standard Overnight”, “Other” for the packaging, “Yes” for “Does box shipment contain dangerous goods?” check “ Dry Ice” and put the weight. Check payment method “third party” and enter account number 151716687
  - attach the DRY ICE sticker and put the amount of DRY ICE on the sticker (see Appendix A).




- attach the UN 3373 sticker (see Appendix A).


attach the dangerous goods shipping label (see Appendix A or you can use the following link): http://www.shippinglabels.com/img/lg/D/Excepted-Quantities-Paper-Label-D1779.gif

The vein tissue shipments contain tubes with ethanol, which is flammable, so on the first line, write "Class 3".


- If Air Bill is used, retain top copy of airbill for clinical center records and attach remaining copies on the outside of the shipper via the US Domestic Air Bill Sleeve. Bring to designated Fed-Ex drop-off or call Fed-Ex.

- Example of completed Fed-Ex US Air Bill for Histology Core:


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

<u>The tissue must be sent by overnight shipping (Fed-Ex) Monday-Wednesday ONLY!</u>

12. Prepare the shipments for NIDDK Repository- (Fisher Lab) (one shipment, once a month). The vein tissue should be batched along with serum and plasma being sent to the NIDDK.

Two kinds of samples:

a. RNAlater tissue (RED top, with tube labeled "R") on dry ice
b. Proteomics tissue (BLUE top, with tube labeled "M") on dry ice

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 tissue frozen tissue must be sent by overnight shipping (Fed-Ex) Monday-Wednesday ONLY! (Samples must be received by Thursday, at the latest).

- Double check the subject ID, and verify that ID information on the tubes match that on the Form 608;
- 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).
- Date and identify person completing the mailing Form 608.
- Make a copy of the form. Keep the copy for data entry and (later filing); send the original with the shipment.
    - Follow the instructions in Section 7.6 of the MOP for packing samples,
    - attach preprinted Fed-Ex sticker or you have the option of generating the shipping labels via the online Fedex Ship Manager . You also can use Fedex Air Bill.
    - enter the information: senders name, the adress, phone number. Check the "Fedex Standard Overnight", "Other" for the packaging, "No" for " Does box shipment contain dangerous goods?" , check " Dry Ice" and put the weight. Check payment method "Recipient" and enter account number 151716687
    - attach the DRY ICE sticker and put the amount of DRY ICE on the sticker
    - attach the UN 3373 sticker
    - If Air Bill is used, retain top copy of airbill for clinical center records and attach remaining copies on the outside of the shipper via the US Domestic Air Bill Sleeve. Bring to designated FedEx drop-off or call FedEx.

- Example of completed FedEx US Air Bill for NIDDK:


- 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.

<u>Tissue must be sent by overnight shipping (FedEx) Monday-Wednesday ONLY!</u>

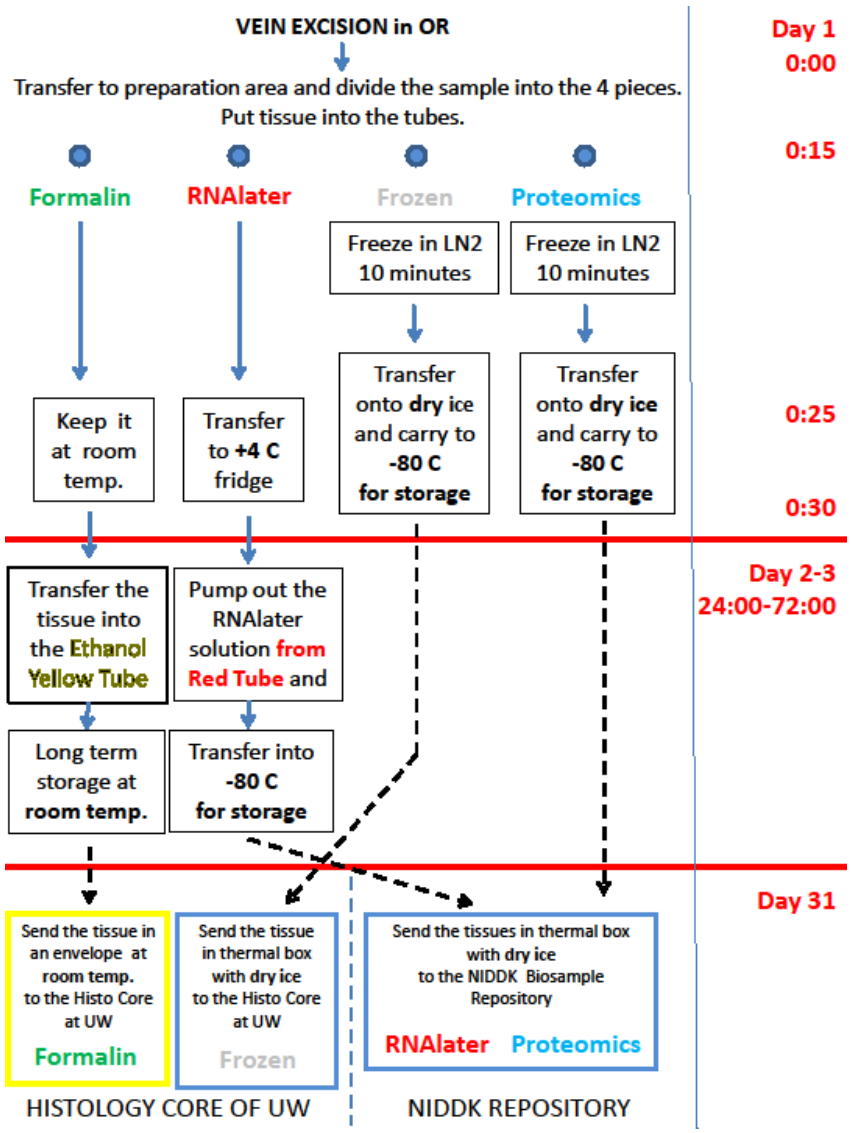
Recipient information:

University of Washington
Attn: Kelly Hudkins
Room E 147
Alpers Lab
750 Republican St.
Seattle, WA 98109
Phone: (206)-543-6746
<u>kellylee@u.washington.edu</u> (Kelly Hudkins)

or

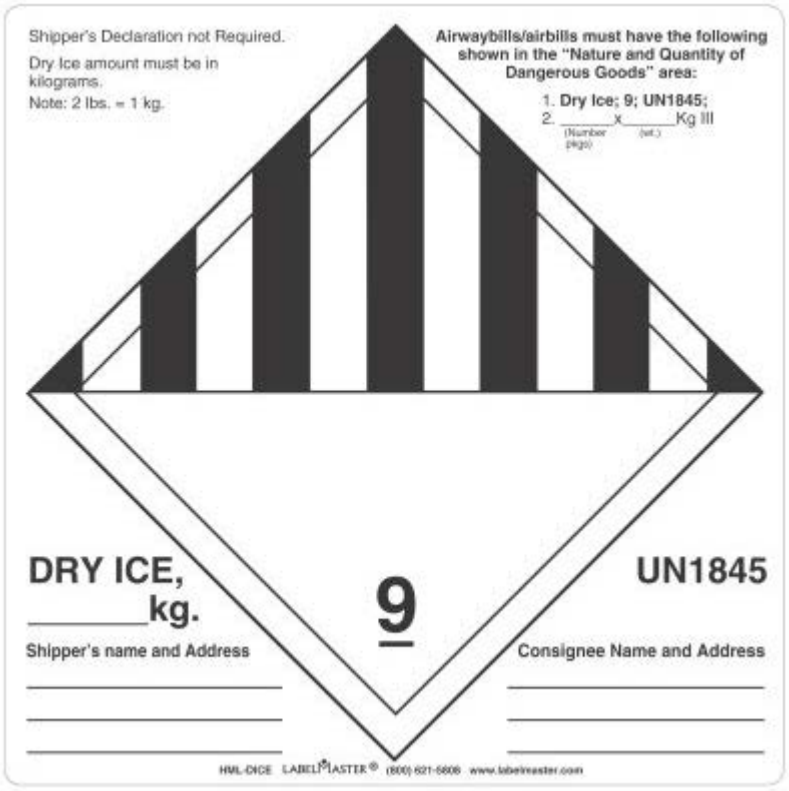
Attn: Heather Higgins
NIDDK Repository
20301 Century Blvd.
Building 6, Suite 400
Germantown, MD 20874
Phone: (240) 793-0353 (Heather Higgins)
Phone: (240)-686-4702 (Sandra Ke)

## 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
onto dry ice
and carry to
-80 C
for storage
Transfer
onto dry ice
and carry to
-80 C
for storage
Keep it
at room
temp.
Transfer
to +4 C
fridge
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 Biosample
Repository
RNAlater
Proteomics
HISTOLOGY CORE OF UW
NIDDK REPOSITORY

**Appendix A – Dangerous Goods and Dry Ice Labels**


UN3373


Shipper's Declaration not Required.
Dry Ice amount must be in kilograms.
Note: 2 lbs. = 1 kg.
Airwaybills/airbills must have the following shown in the "Nature and Quantity of Dangerous Goods" area:
1. Dry Ice; 9; UN1845;
2. _____x_____Kg III
(Number of pkgs) (wt.)
DRY ICE, _____kg.
9
UN1845
Shipper's name and Address
Consignee Name and Address
HML-DICE LABELMASTER® (800) 621-6808 www.labelmaster.com