

****Failure to follow these guidelines will result in a delay. ****

Histology Request Slips

- Request slips must accompany all submitted tissues, slides or tissue blocks.
- Include detailed instructions on how tissues should be oriented, sectioned and stained.
- Remember to bring your own labeled slide boxes along with your request slip; otherwise there will be an additional cost to cover the slide boxes.

Biosafety Issues

- BSL-1 tissues from experimental rodents can be accepted for paraffin or frozen sections.
- Our current policy is that **we will only accept tissues from “humanized” animals or animals carrying xenografts of human tissue if the tissue has been fixed in 10% NBF (neutral buffered formalin) for at least 48 hours and transferred to 70% ethanol.**
- **Wet human tissue is not accepted. We do accept blocks of paraffin embedded human tissue for sectioning.**
- **We accept fixed BSL-2 tissues if well fixed in 10% NBF (48 hours minimum followed by transfer to 70% ethanol).**
- **We can-not receive non-human old world primate tissue.**
- For frozen sectioning only BSL-1 tissue can be submitted; **that means no human tissue, no animals transplanted with any sort of human tissue, and no animals exposed to any human or other BSL-2 pathogens.**
- **No BSL-2 samples can be accepted for frozen sectioning.**
- **If you have tissues you need sectioned that we can-not accept, we can assist you to find an appropriate lab to process your samples.**

Containers

- Please label containers and include the solution type in the jar, on the side, not the lid.
- Include lab’s name and phone number if containers are to be returned.
- Tissues/cassettes for submission must be placed in a **leak-proof screw top container and the jar placed within a sealed plastic bag.**
- **Refrain from** using conical-bottomed centrifugal tubes, because tissues tend to sink to the bottom where the fixative solution does not reach them.
- **Do not** put tissues in narrow mouth containers. Tissues harden and stiffen in fixative and containers may have to be broken to retrieve the specimen.
- **No eppendorf tubes** – these are too small and too hard to get tissue out of!!

Specimen Container/Transport Bag Vendors

- <http://www.fishersci.com> (Biohazard Specimen Transport Bags and Starpiex Scientific LeakBuster® Specimen Containers)
- <http://www.surgipath.com> (Empty/Prefilled Formalin Specimen Jars)

Collecting and Fixing Tissues

- Cut tissue slices about **2-3mm, or the thickness of a nickel**, for proper penetration of fixatives in the times indicated below.
- For **skin samples**, make sure the **fur is shaved off**. Skin samples should be **rectangular in shape** and no bigger than 1 cm in length and .5cm in width.
- Please **put skin samples and hollow organ tissues on paper** before putting them in a fixative to prevent curling, and place a biopsy pad over the tissue.
- Tissues should be placed with the area of interest down in the cassettes.
- Refrain from overcrowding the cassettes because not all of the tissues will be able to be positioned properly in a block.
- If the tissue touches the sides of the cassette, the tissue is too large.
- If the tissue touches the lid it is too thick.
- If the tissue is quite small, biopsy pads will be needed to protect the tissue and keep it in place during processing. Alternately, you can embed the tissue in agar prior to processing.
- **Do stick oviducts and uterus to filter paper**, cut to fit the cassette. This helps them lay flat and retain their shapes for embedding. When tissues are flat, a more complete cross-section can be obtained. The oviducts and uterus should be processed and embedded together; because of the tissue's nature it expands at the same rate on the water bath. Any tissue associated with the tubes and ovaries, e.g. tumors, should go in its own cassette.
- Kidney, adrenal, and pancreas may go together in the same cassette, if there is enough room (they expand at the same rate on the water bath). Liver should be in a separate cassette.
- Intestine can be **included** with the liver. It serves as a good control for many stains. **Do not** cut longer than 4mm; it is embedded on end vertically (like a chimney), not horizontally.

Bone Decalcification

- This may be done by either you, or the histology lab. Fixed tissue containing bone is placed in Surgipath Decalcifier II for 4-6 hours, depending on size. Unfixed tissue containing bone is placed in Surgipath Decalcifier I for 4-6 hours, depending on size. If decalcification is done by investigator, it should be noted on the request slip.

Decalcifier Vendor

- <http://www.surgipath.com> (Surgipath Decalcifier I # 004400 or Surgipath Decalcifier II # 00460)

Tissue Fixation:

- **NOTE: Use plenty of fixative!!** Cover tissue with **20** times its own volume of fixative. Dense (skin, bone) and bloody (liver, spleen) tissues are hardest to fix. If the amount of fixative is inadequate, or the sample is too large or too thick, the interior of the specimens will not be properly fixed. Inadequate fixation will inhibit proper sectioning and staining. Bloody fixative should be replaced with fresh clean fixative to ensure proper fixation.
- Fresh tissues tend to stick together and to the bottom of containers, which inhibits fixation. Therefore, it is vital that one swirls the containers every once in awhile to separate the tissues. If there is a lot of blood in the fixative after tissue collection, replace it with fresh fixative.
- Tissues must be placed in fixative promptly to prevent autolysis. Refrigerating the specimen in fixative slows autolysis.

10% Buffered Formalin Fixative (NBF)

- Fixed tissue(s) in 10% buffered formalin for 24 hours. Drain and add fresh 10% buffered formalin for another 24 hours for a **total of 48 hours**. After fixation, the tissues should be placed in 70% alcohol and taken to the Histology lab for processing.
- **Prompt processing is optimal!**

Carnoy's Fixative

- Glacial acetic acid 10 ml
- Absolute ethyl alcohol 60 ml
- Chloroform 30 ml
- This is a very rapid fixative and should not be allowed to act for longer than 2-3 hours on tissue that in 3-5mm - - 12-19 hours for tissue that is 10-15mm. Transfer to 100% ethyl alcohol - - - change alcohol 3x - 1hour for each. Place in 70% alcohol and take to the Histology lab for processing.

Bouin's Fixative

- Picric acid, saturated aqueous solution (21gm/1 liter DH₂O) 750.0ml
- 37-40% formalin 250.0ml
- Glacial acetic acid 50.0ml
- Fix tissues for 4-12 hours depending on the size. It is important to wash in several changes of 50% alcohol for 4-6 hours, changing the alcohol every hour, agitating constantly, to insure proper removal of the picric acid. After fixation, the tissues should be placed in 70% alcohol and taken to the Histology lab for processing.

NOTE: The removal of picric acid from tissues is essential in order to insure proper staining of the tissue sections.

Cassette Numbering

- If labeling cassettes without an automatic cassette labeler, please write clearly with a chemically resistant marker. **Do not use a Sharpie.** If markings are illegible or the ink washes off during processing, the histology lab will inform you of the situation but clients will still be responsible for paying for the processing.
- Cassettes should be labeled with alphanumeric characters.
- **Do not** write over numbering mistakes. **Start over!!**
- If the cassette is completely full, divide the contents making two cassettes instead of one.
- **Keep cassettes in order and rubber band them into small groups.**

Chemically Resistant Marker Vendors

- <http://www.fishersci.com> (Securline Marker II Pens # 14-905-30)
- <http://www.surgipath.com> (Marking Pens # 01880)
- <http://www.vwrsp.com> (VWR Chemical Resistant Pens # 95042-566)

Processing support of small and/or fragile tissues:

Embed in 2% Agar

Preparation:

1. Combine: 900 ml ddH₂O
20 gm agar (Difco #0140-01)
2. Boil gently until thoroughly dissolved. Use a stir bar.
3. Add 100 ml of 10% formalin or 100 ml DDH₂O – mix well.
Make sure to prepare and use formalin in a **chemical safety hood!**

Note: If tissue is already fixed substitute 100 ml DDH₂O for the 10% formalin.

4. Put the quantity to be used immediately into a small bottle with a dropper top. Store this in either a 60°C oven (keeping the bottle tightly stoppered), or while in use in a 60°C water bath in a **chemical safety hood if formalin was used.**
5. Pour the rest of the agar into a sterile 100 ml bottle. Allow to solidify. Store at room temp.
(Expiration 2 mo.)

Method for use:

1. Working on a clean piece of glass, place a small amount of the agar down. Observe the change in its consistency. When it begins to solidify, place the tissue in it. Orient the tissue as you would if you were embedding into paraffin block. Drop more agar over the tissue. Continue to do this until the tissue is surrounded by the agar and a small mound of agar is formed. If the agar is allowed to solidify too much between applications the layers will peel away from each other. Large bubbles should also be avoided during this process.
2. When the mounded agar is solid, trim the excess away with a scalpel to form a roughly square shape. Slide the scalpel under the agar/tissue mound and gently lift it into a processing cassette. Close the cassette and placed in 70% alcohol. Take to the Histology lab for processing.