

**VCU**

Tissue and Data Acquisition and Analysis Core Animal Histology Lab

Histology Sample Submissions protocol taken & adapted from: <http://sites.tufts.edu/histopath/animal-histology-core/>

Please note that we cannot guarantee the quality of specimens that have been improperly handled, fixed or stored by the customer.

Histology involves all processes from the collection of tissue from the animal to cover slipping the final slide product. For the final product to be high quality, all steps in the process must be performed optimally. Please refer to this document for recommendations on steps you can take before submitting fixed tissue to the TDAAC Animal Histology Lab to help ensure you get the best results.

Our services include: paraffin embedding, sectioning of paraffin blocks, unstained slides (for your staining), H&E (morphology stain) and IHC.

Sorry, we do not cryosection.

How to Fix and Prepare Tissue for Histology Submission

Basic Steps in Histology

1. Tissue collection from the animal
2. Fixation
3. Special processes such as decalcification
4. Tissue Trimming
5. Cassetting
6. Processing
7. Embedding
8. Sectioning
9. Deparaffinization and rehydration
10. Staining
11. Cover slipping

These **guidelines will cover recommendations for actions you can take in your laboratory for steps 1-4 (also step 5 if required)** that will 1) improve the quality of the final slide; 2) reduce your costs; 3) decrease turnaround time from the histology laboratory and 4) avoid the most common histology mistakes.

Step 1: Tissue Collection from the Animal

General Recommendations

- Collect tissue immediately after euthanasia to prevent postmortem autolysis and decomposition. Changes occur in tissues within minutes of death.



- Place tissue into fixative immediately after collection. **20:1 ratio of formalin to tissue.**
- Don't let tissue dry out before it gets placed in fixative. Dry edges can create artifacts in IHC.
- Don't freeze animal carcasses if you plan to do histology. Freezing results in ice crystal formation in the tissues and poor histologic morphology. Collect tissue into formalin to preserve it rather than freezing the carcass. If an animal dies and you cannot do tissue collection right away, put the carcass in the refrigerator and then do the tissue collection as soon as you are able.
- **Mouse organs:** Heart, kidney and brain must be cut in half on either the transverse, sagittal or coronal plane based on your preference in order for adequate penetration by fixative. **Remember the basic rule of thumb for proper tissue size for fixing and embedding:**
"As thick as an American nickel and no larger than a postage stamp!"
- In animals larger than a mouse (rats, rabbits, swine, etc.) many **or all organs will need to be trimmed to no thicker than 5 mm in order for the fixative to penetrate the tissue rapidly.** In a rat, kidney, liver, and testes are three tissues that would normally **need to be cut into smaller pieces before fixation.**
"As thick as an American nickel and no larger than a postage stamp!"

Containers and Labeling

- Always label the container itself and not the lid (to avoid confusing container IDs when you remove the lid)
- Use leak-proof containers so fixative does not spill in your lab or in transit to the histology lab. We recommend that you invest in containers made for histology samples, as these are usually sturdy, wide-mouthed, and large enough for an adequate volume of fixative. Our lab will return the containers to you. It is your responsibility to properly dispose tube, remaining tissue and reagents according to VCU OEHS guidelines.
- Select a large enough container to hold a **20:1 ratio of formalin to tissue.**
- Fix tissues in flat-bottomed jars, not conical tubes. If a tissue sits at the bottom of a small conical tube, it will not have adequate formalin exposure.

500 mL histology container

25 cassettes of pancreas collected from 25 different animals. All can be fixed in the same container of formalin because they are individually labeled in separate cassettes

20 mL histology container (flat bottomed) 50 mL conical (invert periodically)

1 mouse kidney, add adequate fix volume.

15 mL conical tube

2 mL micro-centrifuge tube

Do not use. Mouse organ will sink to cone bottom and not get adequate fixation. Reduced tissue surface area exposure., poor fix penetration. Volume of formalin would not be high enough.

- Avoid containers with narrow necks. Tissues may expand in formalin. Tissues that went into a jar easily may be more difficult to remove through a narrow neck after fixation.
- Label containers clearly and neatly with sample ID.

Step 2: Fixation

General Recommendations

- Most tissues can be fixed in 10% neutral buffered formalin (NBF) or 4% PFA. You can buy histology containers prefilled with formalin or you can buy formalin separately.
- Use freshly prepared 4% PFA (prepared day of use or freshly thawed aliquots, toss unused portions) or **NBF that has not expired**. This is imperative in facilitating the preservation of your sample so that it may withstand the rigors of processing and embedding!! **NEVER REUSE FIXATIVE!!!**
- Use a 20:1 ratio of fixative to tissue. The most common fixation problem we see in tissues submitted to our laboratory is tissue that is fixed in an inadequate volume of fixative. This reduces the quality of the final slide product.
- Occasional swirling of the jar or gentle agitation for the first 24 hours in fixative may increase the quality of fixation.
- The duration of exposure to the fixative must be optimized and researched for each specimen type by the investigator.

Step 3: Decalcification of Bone (as needed)

Bone or mineralized tissue must be decalcified prior to routine sectioning of paraffin embedded blocks. If you are submitting tissue for histology that contains bone or calcified tissue, it needs to be decalcified to remove the mineral before it is trimmed and processed. Mineralized tissue is very hard and may either pop out of the block when it is sectioned, or the sections may be poor quality because the microtome blade will not cut mineralized tissue smoothly. The decalcification process removes calcium mineral from fixed bone, leaving the tissue bendable and easily cut with a sharp blade.

There are several techniques and solutions to decalcify bone. Please see the following link for guidance in choosing the appropriate method for your needs.

<https://www.leicabiosystems.com/pathologyleaders/an-introduction-to-decalcification/>

Step 4: Tissue Trimming

Trimming is cutting a fixed tissue or organ to create a flat surface with correct orientation. You can do this yourself with training and a few basic supplies (cutting board, forceps and single edge razor blade) or our techs can do this for an additional fee. ***Trimming should be done after, not prior to, fixation.*** If you trim before tissue fixes, it will continue change shape and usually bulges on the cut surface, requiring trimming again after fixation. The purpose of trimming is to create an even, flat surface in the area of interest in the tissue so that the Core's techs do not have to face (cut with the microtome) into the paraffin block as deeply when trying to get the first good sections for a slide. There is less tissue loss in the block and a better visualization of the structures of interest.

- Tissues or organs are *often trimmed* for optimal examination. Trimming is recommended but may not be required: e.g. tumors (size dependent), liver, lungs, intestines
- Some tissues or organs *should not or need not be trimmed* for routine screening: e.g. rodent eyes, pancreas, ovary, rodent lymph nodes, or mouse uterus. However, there are instances in which even these organs might need to be trimmed.

Custom trimming should be done when 1) trying to locate or demonstrate a very specific area of an organ not generally captured using standard trim guidelines; 2) when an organ has lesions that might not be captured on standard trimming sections; and 3) whenever trimming is requested at custom landmarks by project design.

Trimming and cassetting are usually done at the same time.

Step 5: Cassetting

All tissues for paraffin embedding must be cassetted before processing. Cassetting is taking trimmed or untrimmed tissue and orienting it in a tissue cassette to the area of interest. You can cassette tissues yourself or the TDAAC techs can provide that service.

To trim tissues in your laboratory, you will need:

- a cutting board
- single-edged razor blades or a specialty histology trim knife and blades
- a small scissors
- a forceps preferably without teeth
- histology cassettes
- a wide-mouthed histology container of formalin to place the cassettes into after cassetting
- a #2 pencil
- A chemical hood or a hood fitted with an appropriate filter to remove formalin vapors.
- Appropriate personal safety protection, to include nitrile gloves, a lab coat, and safety goggles.

General recommendations for labeling cassettes

- **Caution! Label cassettes only in #2 pencil.** You also may be able to use certain solvent resistant histology markers, but please have our lab do a trial run with your pen in our processor first to be sure. Never use a Sharpie marker, which is not solvent resistant, to label histology cassettes or all your labels will disappear from your cassettes. The TDAAC is not responsible for loss of cassette identification from improperly labeled cassettes.

- **Each cassette must be labeled.** Our staff will label the slide with the cassette label. An electronic excel spreadsheet listing, in one column your tissue id and tissue type in the next column and must be emailed to TDAAC.

Label excel file in the following format: PI Last name and order sample drop off date, example: Smith 01012018

Jennifer.koblinski@vcuhealth.org

- Label each cassette on the top (preferably) or side of the cassette with a **unique ID number** for each cassette. When labeling, keep in mind other submissions you may make at a later date and be sure that you will be able to distinguish the slides and blocks later. A **good labeling system** will also likely designate a unique number to each individual animal as well as to each cassette. Multiple cassettes submitted for the same animal may be labeled with organ contained within the cassette. It will also be brief enough that errors are less likely to be made when transcribing the cassette label to the slide.

Poor labeling or having no planned labeling system in place can lead to blocks and slides from multiple animals labeled with the same ID over time or can be so long as to be impossible to transcribe to a slide.

General Recommendations for Placing Tissue in Cassettes

- **Use of blades.** Use a new, sharp, clean single-edged razor blade or specialty blades. At a minimum, change blades between tissues from different animals. Use a new blade if you feel that your blade is beginning to dull or a different end of the blade that may be sharper.

- **Cut down (into the cutting board) and away from you.** For your safety, always hold tissues with a forceps, not with your fingers when cutting. With a new, sharp blade, you should be able to make one smooth cut by just pressing down. Do not saw at the tissue. If you find that the tissue begins to just press down under the blade and not cut, use a new blade. Do not use a scissors to cut the tissue. Scissor cuts do not leave a flat and smooth enough surface for optimal slide quality.

- **The area of most interest to you in your tissue should face down in the cassette.** Universally, histologists know that the side facing down in a cassette is the face of the tissue you want sectioned.



- The general rule of thumb is that *tissues combined in the same cassette should be of the same density* (for example heart, liver, spleen, and kidney often cut well in a cassette together, versus decalcified bone and lung, which would not cut well together). Brain, tumors and spinal cords must be submitted in its own cassette, but there can be multiple sections of each in the same cassette. Please tell us and write on the submission form if you wish to have combined tissues embedded together in same block or separately.

- Review the list of tissues above that “must be trimmed.” *Never submit tissues in a cassette that are listed above in the “must always be trimmed” category without trimming them unless you tell the histologist that they still need to be trimmed.*

Histology labs assume that tissues submitted in cassettes are ready for processing. Once cassettes have been put on the tissue processor and the tissues are infiltrated with paraffin, it is much harder to then go back and trim and orient tissues correctly. The final slide quality may not be as good as tissue that was trimmed correctly before processing.

☐ Caution! Note that very small tissues (such as normal mouse lymph nodes, transverse sections of normal mouse spleens, endoscopic biopsies, and cell pellets) can pass through the holes of some histology cassettes during transport and processing and be lost to analysis.

For small tissues, consider using cassettes with biopsy sponges, lens paper, or Histogel to ensure that small tissues are not lost in processing. The TDAAC is not responsible for small tissues that are lost in processing due to improper cassetting if you cassette them yourself. On request, we will provide you with our recommendations for cassetting your small tissues.

What to Request from the Animal Histology Core

Above, we covered steps that may be taken in your own laboratory to prepare tissues for histology. At this point, the remaining steps are usually done by the histology core. Review our TDAAC Histology Checklist (below) and then prepare to bring your samples to the TDAAC histology lab in Massey cancer center 1-112. If you are unsure what you need to request, please email your questions to jennifer.koblinski@vcuhealth.org

The remaining steps in the histology process that are generally done in the histology laboratory are briefly described below. Please also refer to the Price list for additional definitions and information on services.

Processing: Tissue processing includes dehydration, clearing, and infiltration of the tissue with paraffin wax. This is usually accomplished on a piece of equipment called a tissue processor using programs that the lab has established for a species, tissue, or special need. Tissue processing is done by our lab unless you have access to a tissue processor in another lab. If you are submitting tissue for routine paraffin embedding and sectioning, then processing will be part of your service.

Paraffin Embedding: Embedding immediately follows tissue processing. Tissue embedding involves carefully removing processed tissue from the cassettes, placing them into a mold while maintaining their original orientation, and then filling the mold with paraffin wax. This produces a paraffin block. Embedding is done by us unless you have access to an embedding station in another lab or already have embedded blocks that were previously prepared. Tissues that have been processed and embedded can remain in a block indefinitely. Paraffin blocks can be sectioned immediately or years later. If your protocol calls for a defined period of tissue fixation, you can get the tissue processed and embedded after the defined period of fixation and can then hold the blocks until you determine your final sectioning needs later across your whole study.



Sectioning: Sectioning of a paraffin block is done using a microtome that cuts very thin sections of the paraffin-embedded tissues, which are placed on a slide. Routine thickness for paraffin sections in the TDAAC is 5 microns. If you require thicker or thinner paraffin sections, please give us special instructions in the submission form. Normally, once a block is embedded, the technician must cut (or “face”) into the block with the microtome to get their first good section. A small amount of tissue will be lost from the block in the facing process. You must notify our us if your area of interest in the tissue is so close to the start of the tissue in the block that facing into the block might be a problem.

Our standard is 2 sections/slide, please write on the order form if you need more or less

Unstained section: Slides with a paraffin section of tissue that has not been deparaffinized and has not been stained with any stains.

Serial Section: Sections of tissue that are placed sequentially on the slide one right after the next.

Levels: Sections of tissue taken at intervals, with the technician cutting further into the block. Multiple levels can be placed on one slide or several slides, usually 2-3 depending on the size of the tissue. Also called step-sectioning. For instance, a section is taken, then the block is cut deeper wasting 100-200 microns of tissue, which is discarded, then another section is taken and put on the slide.

Stains:

- The basic histology stain is Hematoxylin and Eosin (H&E). The hematoxylin is a nuclear stain and stains the nucleus blue/black/purple, depending on the hematoxylin used. Eosin is a cytoplasmic counterstain, with at least 3 different shades of pink depending on the cell part or tissue type.

- Immunohistochemistry: This procedure uses antigen-antibody binding properties with labeling by a chromogen. For any questions on IHC please email TDAAC at Jennifer.koblinski@vcuhealth.org

TDAAC Histology Submission Check List

1. Go to <https://forms.office.com/r/6Q1wVWCxWF> and COMPLETELY fill out the TDAAC Histology Service Submission Form. Print a hard copy and bring it with you at time of sample drop off. Samples will not be processed if order form is incomplete or is missing the Index Code.
2. Bring samples in leak proof, well labeled containers with your order form to Massey Cancer Center 1st Floor Room 112. For directions to the lab please email us at Jennifer.koblinski@vcuhealth.org
3. If you are bringing samples in cassettes, please label cassettes with a #2 pencil ONLY! Label leak proof cassette storage container with PI's name and order date.

Email us an electronic excel spreadsheet listing all sample ID#s in a column. Second column adjacent to each sample's ID# please identify the organ or tissue type. Label file in the following format example: PI name and submission date “Smith 05152018”

