La Jolla Institute FOR ALLERGY AND IMMUNOLOGY — Life Without Disease.®	Microscopy and Histology Core Facility Guidelines, Policies, and Protocols		
	Document No:	MH 001	
	Version:	1.0	2024-02-29
FFPE SAMPLE SUBMISSION	Approval:	ZM	2024-02-29

Introduction:
The number one factor that affects the quality of downstream analytical testing of formalin-fixed paraffin-embedded (FFPE) tissue is the fixation of your sample. Autolysis of tissue, staining problems, poor nuclear detail, and lack of contrast between cell nucleus and cytoplasm are some of the problems that can result from poorly fixed tissue samples. We want you to have satisfactory results from the samples that you submit to LJI's Histology Core so please take a moment to review this guideline.

FIXATIVE:
The fixative of choice for routine histology at the La Jolla Institute is **buffered 10% zinc formalin** (Anatech Z-fix cat# 170) since it yields crisp morphological detail and preserves tissue antigenicity even after extended fixation times. We keep a fairly large volume of zinc formalin on hand which you are welcome to use for your experiments. Ten percent neutral buffered formalin (NBF) and 4% paraformaldehyde (PFA) are also acceptable as are several other fixatives (such as Carnoy's solution) that may be better suited to special projects. Bouin's solution is not accepted by our core due to cross-contamination of other samples with picric acid. *While we do everything we can to salvage samples we can't take responsibility for improperly fixed tissues delivered to us.* Regardless of the type of fixative that you select, the following is paramount to successful results and obtaining good data.

RAPID FIXATION:
Immerse tissue into fixative immediately after collecting. Use a flat bottom container for specimen fixation.

Urine cups work great for this purpose (eg. Fisher Scientific cat# 13-712-50). Agitation is beneficial for the fixation process. We recommend using a rocker set at about 100 motions/minute. **PLEASE AVOID CONICAL TUBES FOR FIXATION.** In conical tubes, the specimens fall to the bottom and are mostly in contact with the plastic instead of the fixative. During the fixation process, the reagent is used up and slowly replenished by diffusion; samples are fixed more efficiently in flat bottom containers with agitation.

The recommended method for trimming gut samples promotes proper tissue orientation. Strips of the colon were placed serosal side down on a wetted biopsy pad.

VOLUME:
Use an adequate volume of fixative in a *minimum ratio* of 20 parts fixative to 1 part tissue (**20:1**). Consider increasing the ratio up to 50:1 for fatty or larger specimens.

TIME:
Fix your samples for at least 24 hours, but no longer than 72 hours. Murine bones should be fixed for 72 hours with a change to fresh fixative each morning.

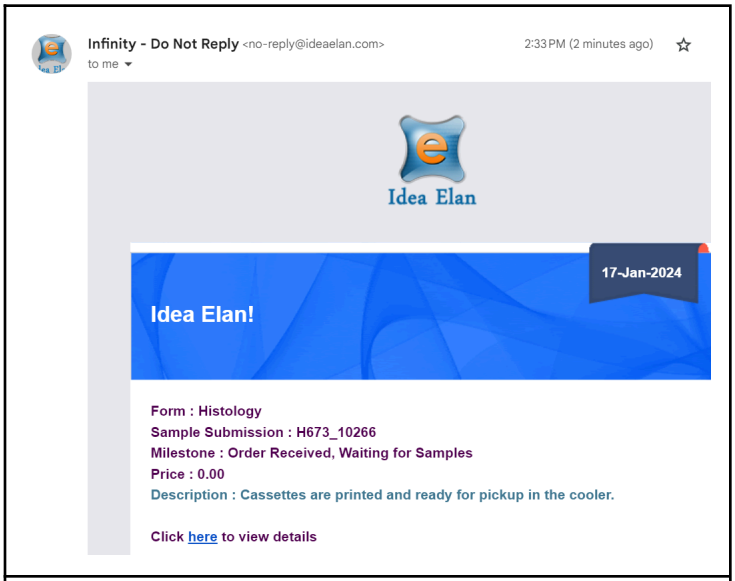
TEMPERATURE:
We recommend formalin fixation at room temperature for routine histology. Cold fixation at 4° is fine too however the rate of fixation is directly proportional to the temperature hence it becomes imperative not to take shortcuts with the time in fixative. Do not fix at elevated temperatures.

FIXATIVE PENETRATION:

Consider the size and type of tissue you are working with. Tissue should be less than 15 x 5 mm for optimal fixation. For livers, separate the lobes at harvest. Organs with strong capsules like kidneys should be gently nicked with a razor blade. The hearts and kidneys process better as whole tissues, just please make sure that they are not crushed by the cassettes. For lungs tie off the trachea and gently fill lungs with 1 mL of fixative then separate the lobes before immersion in fixative.

For the gut place 1 biopsy pad wetted with fixative in a cassette. Using fine sharp scissors, cut along the gut's antimesenteric border. Remove ingesta. Cut into 35 mm segments and place in histology cassette with serosal side on a wetted biopsy pad (the mucosal side away from the pad). Close the cassette and immerse in fixative. To allow for better penetration of the processing fluids, and not to damage the mucosal surface, we only recommend a single biopsy pad (sponge) for the gut. For most other specimens, the sponges do more harm than good. We recommend less dense sponges such as Simport M476-1. We will trim the edges of your gut strips or skin after they are hardened by tissue processing. For any samples that are small enough that they might fall through the cassette openings, we use Redi-fold biopsy paper when necessary (IMEB, cat# RF-150). Alternatively, organoids or other small samples can be prepared using HistoGel (Fisher Scientific cat# 22-110-678). Please email us for additional recommendations.

Large or dense specimens such as the liver should be trimmed. Breadloaf tissues to the proper size (15 x 5 mm) after 3-4 hour fixation, and return them to the fixative to allow for better penetration. This initial fixation time will make specimens more rigid and easier to work with. If you have large tumors that don't fit into a cassette, either bisect them before starting the fixation or do this after a few hours of fixation, and then return them to the fixative to continue the process. Work inside a fume hood and wear appropriate PPE.



Make sure to pay attention to automated emails sent by no-reply@ideaelan.com Add this email address to your contact list as this will lessen the chances that they will be classified as spam.

CASSETTES:

To allow for better sample tracking and to avoid human errors due to smudged ink we will only accept samples in our printed histological cassettes. These are provided free of charge, and we print them based on the information in Infinity sample submission forms. You will receive an automated email informing you they are ready for pickup. Samples that are provided to us in hand-written cassettes will be returned to the user. This policy allows us to track your sample efficiently, reduce errors, and make it easy for you to identify old blocks. Say goodbye to mystery blocks from an ex-postdoc! Our sample tracking will allow you to relate all slides and blocks back to the work order, which contains all critical information about the experimental protocol and sample fixation. **Please place the area of interest towards the bottom of the cassette.** Please note that very small pieces might move during the processing. We will try to orient them to obtain the largest surface area on the slide. If tissue orientation is very important to you please attach a diagram to the work order.

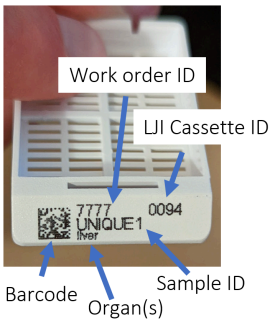
SAMPLE CODING:

Each of your samples should have a unique identifier (8 characters or less). Please don't use underscore _ and special characters %$#@!*][since our cassette printer doesn't handle these symbols. Naming your samples Mouse 1, Mouse 2…Mouse 10 etc. is ill-advised. While we do barcode our cassettes and slides and

each one is uniquely identifiable, we recommend you do not use the same IDs across projects because it can lead to confusion on everyone's part. Below are some recommendations for unique identifiers:

- A combination of letters and numbers is best (hyphen sings "-" are OK), for example
  - E12M18A – experiment 12, mouse 18, cassette A (lungs)
  - E12M19B – experiment 12, mouse 19, cassette B (liver)

- Sequential mouse/sample/block number – 00000001, ..
- Cage card/animal number
- Anonymized identifier from your sample library



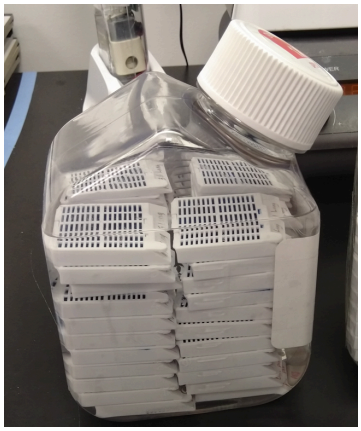
Labels on histological cassette

Please remember that all human samples must be completely de-identified! We cannot accept tissue blocks or other specimens with patient-identifying information (name, medical record number, date of birth, etc).

ADDITIONAL SAMPLE TRIMMING:

Ideally, the samples will be already trimmed at the fixation step, but if this cannot be done right away please trim the tissues so they properly fit into cassettes. This is a great opportunity to inspect the samples and see if they are fully fixed inside. Underfixed tissues tend to have pinkish centers (this happens often for large liver samples). If you notice this, please trim the samples and continue the fixation for an additional 24 hours. As a general rule, the tissue should never be squished by the cassette and needs to be able to move freely. Failing to ensure this will lead to artifacts and will result in poor sample processing. Following sample trimming, move your specimens to histological cassettes. Place the cassettes in 70% IPA, and bring them to the core in a secondary container as outlined below.

CLEAR FLUID POLICY: We cannot accept samples that discolor the alcohol. Please wash samples so they don't leach anything into the fluid covering your cassettes. We introduced this policy to protect samples of other users that can be contaminated with material leaching from your samples during tissue processing. If the alcohol looks bloody, that indicates inadequate fixation of your samples (erythrocytes are not stabilized sufficiently and are getting lysed). Please trim some of your tissues to evaluate if they are fully fixed and if necessary, continue the fixation for an additional 24 hours.
You can use special histological tissue dyes to mark regions of interest (you need a very small amount of the dye, they are very effective), but please indicate that on your work order. Generally, no other dyes are accepted. If you need to use a different dye or colorant please contact us before submitting your samples.



Please use wide-mouth containers for transport.
While the above arrangement is an impressive art piece, imagine getting the samples out.

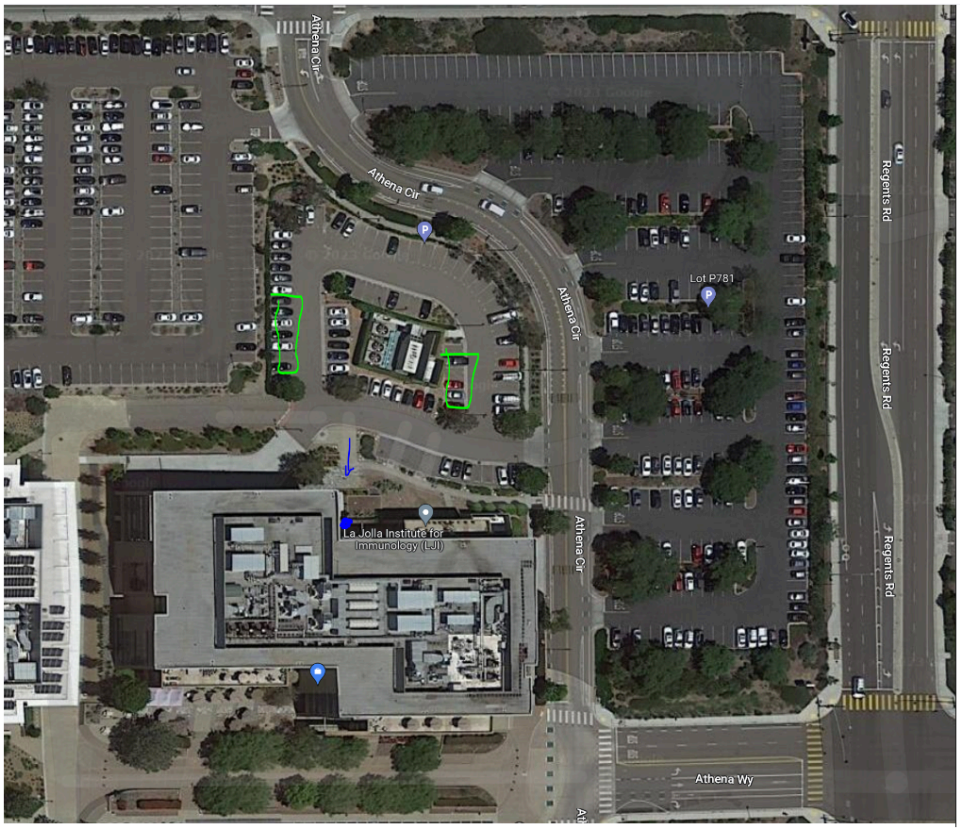
TRANSPORT: Once the fixation of your sample is complete, transfer your cassetted specimens to 70% alcohol for transport to the LJI histology lab. Isopropanol is preferred as it tends to dehydrate tissues less aggressively than ethanol. Use a leak-proof container and a secondary containment (styrofoam box or plastic bag). Write 70% EtOH or IPA on the container along with your contact information and work order number. Do not use media bottles, as it takes us extra time to remove the cassettes and creates a potential splash hazard. We recommend screw-top containers from Nalgene (Fisher Scientific cat# 22-026-314).

Please submit brains, white adipose tissue, and bones; samples smaller than 2 x 2 x 2 mm; and any samples fixed in Carnoy's in separate containers. These specimens will need to be processed using different automated tissue processing protocols. Please label the containers with the organ or indicate it's a small tissue. This not only reduces the chances of errors but it will also save us a lot of time if we receive the cassettes already separated.

SAMPLE DROP-OFF AND PICK-UP:

We have a large cooler outside LJI where you can deposit your samples and pick up your completed orders. This area is under video surveillance, and we never had any unwanted visitors. The cooler is thermally stabilized, so your samples can stay there without the risk of thermal damage. *However, we prefer to promptly pick up your samples and keep them in the lab.* **We can retrieve your samples between 9 am and 5 pm on business days**. Later drop-off is often possible, but please contact us for availability. Please email us at histology@lji.org to let us know that you are planning to drop off your materials.

We will email you as soon as your work order has been completed using the automated system as shown above, so *please don't ignore emails from no-reply@ideaelan.com* While FFPE blocks are very stable over time, unstained FFPE sections degrade quickly so we want you to have the best material for your downstream analysis. We appreciate your prompt pickup, as we don't have much space in the lab to hold completed projects. **We will discard any materials if we don't hear back from you within 1 month after finishing the work.** We understand that you might be traveling or otherwise unable to retrieve your samples, so we will send you a few reminders. Just let us know about your situation and we will make appropriate arrangements.



Sample drop-off and pick-up location

Visitor parking is marked green, and the location of our big blue histology cooler is marked by a blue dot, just in front of the main entrance to LJI. There is a biohazard sign on the cooler, but there are no biohazards inside, it's just to deter unwanted visitors.