Preparing Ocular Tissues for Histopathology

What is COPLOW?
The Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) was founded in 1983 by Dr. Richard R. Dubielzig, a board certified veterinary pathologist with a long-time research interest in ocular pathology. The COPLOW is a mail-in ocular pathology service designed to provide pathology services for clinical veterinary ophthalmologists around the country and world. We provide histopathology services for approximately 3,000 mail-in cases annually. Veterinary specialists in ophthalmology need a service that not only provides a timely and accurate diagnosis, but also is capable of going beyond the diagnosis and investigating relevant aspects of ocular pathology to better understand the pathogenesis and prognosis of ocular disease. The laboratory is also interested in disseminating information on ocular pathology to students of ophthalmology at all levels.

Clinics: Approximately 50% of ACVO Diplomats and practicing veterinary ophthalmologists around the world use the service occasionally or on a regular basis. The COPLOW goal is to provide a high quality, accurate, and timely mail-in ocular histopathology service specifically designed to fit the needs of board certified veterinary ophthalmologists around the world.

Research: The COPLOW histopathology collection contains more than 41,000 specimens housed in a searchable format with access to all paraffin blocks and microscopic slides, as well as submission reports and pathology reports available in one room. We think of the collection as a resource in veterinary ocular pathology to be used to investigate the pathogenesis, prognosis, effectiveness of treatment, and epidemiology of spontaneous ocular disease in animal species.

Educational: We try to provide a resource for disseminating information on ocular pathology and ocular disease to veterinary ophthalmology students at all levels through case presentations, scientific reports, invited lectures and distance education. We also love hosting visitors.

Facebook: With the advents of social media the COPLOW has created a Facebook page. The COPLOW updates interesting and beautiful gross pathology and histopathology images to this website on a daily basis. This website is also a good platform to find people that share similar interests. We invite you to join our website and enjoy a plethora of images and knowledge.

Why Submit Globes and Ocular Tissue?
The general rule states that if there is justification to go to surgery for the removal of diseased tissue, there is reason enough to examine it under the microscope. When
removing tissue suspected of neoplasia, the lab can comment on the margins of this tissue and on the nature of the neoplastic tissue. This way, a tumor of benign nature but dirty margins may be neglected, whereas a highly malignant tumor may require further surgery, or perhaps thorough workup for disease staging. Even in cases where the preceding event is clear, histology may be of great value. Imagine the scenario of BDLD- a big dog bites a little dog in the face, causing trauma that warrants enucleation. When the eye is examined we may notice changes that indicate systemic hypertension, dysplasia of the pectinate ligaments that may lead to lens luxation or narrowing or closure of the iridocorneal angle (goniodysgenesis) that warrants follow-up of the second eye. In other cases we encounter true surprises; it isn’t uncommon for us to find two different tumors or disease processes in one eye.

Handling Samples the Appropriate Way:
It is important to handle samples appropriately. Inappropriate handling may result in non-diagnostic tissues, distortion of the tissue under examination, etc.

Before the tissue is removed from the patient you should already have prepared a container that can be well sealed with 10% Formalin in it. Although formalin is a known carcinogenic substance, it is mostly dangerous when people are exposed to high levels of the substance over a long period of time. However, you should attempt to minimize exposure levels and time where possible. Please use gloves when handling formalin, try not to breath the fumes in, and keep the substance away from your eyes and face. Keep the container closed when not in use. Some people are more sensitive than others and may experience adverse effects such as watery eyes, burning sensations in the eyes, nose, and throat, coughing, wheezing, nausea, and skin irritation.

It Is Up To You!
When the surgeon is busy in the OR it is important to timely transfer the tissue to the fixative. This is important for several reasons. The longer the tissue remains without blood supply and away from fixative the more autolysis will occur, and the sample will be less diagnostic. Especially when removing small tissues, they may get crushed and damaged when left unhandled, or worse, they may even get lost.

Preparing Globe and Tissue:
Globes: unless extraocular tissue will assist diagnosis, please trim all the extraocular tissues without damaging the globe. This is important to prevent distortion, improve penetration and also to make the histotech’s lives easier. You should avoid severing the optic nerve. Many doctors will enucleate in the closed technique and remove the globe with the eyelids- please remove the eyelids and third eyelid before submission.

When should you leave the orbital tissue? If there is suspicion of an orbital tumor you want to include orbital tissue in the submission so we can comment about invasiveness. If there is a suspicion of VKH you may want to include the eyelids. In any case, any extra tissue left should be explained and justified on the submission
form, so we know why it’s there.

Sometimes lesions that are small and clearly seen with a slit lamp are not well visualized following fixation. Please use our drawing sheets to precisely describe the location of the lesions and if necessary mark the location of the lesion with a suture or with surgical ink. We love receiving drawings with our submissions; they are helpful and also fun. Our new form allows for accurate description of lesion location.

We are always happy if clinical photos are sent to us by email, together with patient information.

Some people recommend formalin injection into the globe for better fixation. We do not encourage this since it may create artifacts. If done, please make sure you tell us on the form, and make sure you inject only 0.1-0.3 ml with a small needle into the vitreous- avoiding lens or retina.

Tissues should be handled with care. Especially small tissues like conjunctival samples can be markedly distorted by the crush effect when using forceps. Evisceration samples should be placed in formalin directly after removal, and the lens should be included. If the evisceration includes small fragments it should be placed first in a cassette and only then in formalin.

Conjunctival and Corneal Samples:
These samples are often small and will often roll up and lose orientation when placed in formalin. You should lay these samples flat on a non-absorbent foil (like suture wrapper foil), or plastic, or even a slice of cucumber. Allow the sample to dry a bit and then place it in the fixative.

Small Samples:
Please place tiny samples in mesh cassettes and avoid small tubes. Sometimes a sample is sent over and cannot be retrieved or visualized due to size. Don’t forget, tissue may shrink up to 30% in formalin. Now imagine that small piece of clear cornea that was removed is dropped into a container for fixation. This piece is significantly smaller after it rolls up and shrinks.

Packaging:
Make sure you mark the containers appropriately if more than one tissue is submitted. Please make sure you use only clear and designated containers, and never use glass jars. Ensure the container is large enough for the sample submitted. The sample should include 1:10 buffered formalin, or at least enough to cover the tissue. Sometimes people will add formalin soaked gauze (not cotton) to the tissue before shipping. This is great to prevent leakage and improve safety, but should only be done if 48 hours were first allowed for full fixation of tissue. Once done the sample should be placed in a sealed bag. All jars leak, and if they leak too much both fixation and handler safety may be compromised. You can use paraffin tape to improve jar seal, but please do not use duct or packing tape.
Other Fixatives:
Davidson’s fixative is often used by zoos and human hospitals. Although it provides excellent fixation, tissue should really be removed from this fixative after 48 hours and then shipped in formalin.

COPLOW Submission Form
We have a new form available on our website, and we encourage you to use it. Soon we hope to make online submissions available. You can also print sticker labels for shipping off the website to avoid confusion.
When filling out the form please make sure to fill it in completely. Each portion of the form is there for a reason, and may assist us with diagnosis or research. We often get incomplete forms, and often without contact information for report return.

History
In the history questions please make sure to fill in every part. Some tumors are very similar visually but may be more likely in a young vs older dog, or for instance in a blue eyed dog. Important medical history includes any history of neoplasia, any systemic endocrine or immune-mediated disorders, hypertension etc. Less helpful history includes things like mild dental tartar.

Shipping:
After the sample and form are complete please place the form in a separate pouch or bag so it stays dry if there is any leaking from the container. In the winter it gets quite cold in Madison and samples may freeze on their way when shipped, damaging the submitted tissue. To prevent freezing one may add 1:10 alcohol to the fixative.

If you have any questions please do not hesitate to contact me personally or anyone at our lab:

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