

## 10 STEPS TO BETTER PATHOLOGY RESULTS

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“Diffuse lymphoplasmacytic and eosinophilic enteritis”, “multifocal lymphocytic portal hepatitis”, “diffuse cortical reactive hyperplasia with atypia” – not very helpful to many clinicians I know. Getting that pathology report can be a very disappointing experience, leading you to wonder why you spent all that time (and your client’s money) taking those biopsies. Or worse yet, leaving you with knowledge that the cause of death will never be found.

While not every pathology report will yield the answers that you want, here are ten ways that will increase your likelihood of getting a diagnosis that you can work with.

#1. Choose your pathologist with care. Not all pathologists have the same interests, experience, or most importantly, caseload. Large commercial labs in the U.S. have a primarily small animal caseload; state diagnostic labs or universities are a better bet for samples from large animals. A limited number of pathologists in the U.S. specialize in exotic pets and birds, and are well worth the additional effort of mailing out your samples.

#2. Sample appropriately (and logically). When biopsying a lesion, sample from the visible interface between normal and abnormal tissue. This gives the pathologist the ability to compare diseased and normal tissue side by side. Additionally, at the edges of a long-standing lesion or neoplasm, the pathologic process is more active, and less likely to be obscured by necrosis or long-standing inflammation.

When performing an autopsy, submit the widest range of tissues possible. Far too often, pathologists are asked to make a definitive diagnosis on complex cases with

only a few samples to evaluate. The ancillary data that is recorded in all of the other tissues of the body, such as metastatic sites, paraneoplastic syndromes, and functional changes such as atrophy or hyperplasia helps to round out a case and firm up a diagnosis.

Choose your samples for logic, not convenience. Some tissues like heart, lung, liver, and kidney are large, easy to find, and quick to harvest. However, these tissues would not be expected to yield much significant information in an animal with severe neurologic deficit (but I often am faced with this dilemma, thank you very much!) The brain and spinal cord are not easy or quick to remove, especially when you have a number of clients in the waiting room - but there is no shortcut to a definitive diagnosis.



**Proper fixation requires 10 parts formalin to 1 part tissue. Ratios less than this result in delayed fixation and delayed results.**

#3. Do your autopsies first thing in the morning, rather waiting for a slow period during the day. Even in the refrigerator, tissues continue to decompose, at rates largely determined by their contents. The pancreas and the gallbladder often race to see which may break down the fastest – the pancreas with its component of prepackaged digestive enzymes, or the gallbladder, with its corrosive cargo of bile. (You get about 6 hours with each before start to lose microscopic definition.) Likewise, the intestine contains a hearty mix of digestive juices and bacteria – if you don’t get it into formalin within 12 hours, you are likely to lose the epithelial lining and a tremendous amount of diagnostic information. Most importantly, NEVER freeze your tissues – freezing converts all of the water in the cells to ice crystals – when

the tissue is thawed, the normal cell structure is so distorted that accurate microscopic diagnosis is almost impossible.

**#4. Fix your tissues properly.** The standard formula is 10 parts formalin to one part tissue for proper fixation. Formalin stops autolysis by crosslinking tissue proteins; they physically can't break down any further. If tissue sections are so thick that the formalin diffuse throughout it, then the tissue will continue to break down, and areas may be completely autolyzed before it ever reaches the lab. Optimal tissue samples are no wider than 1 cm to ensure proper fixation. Autolysis will also occur if you put too much tissue in a small vial – pieces pushed up against the wall of the vial will not receive adequate exposure to the formalin, and will be poorly fixed. If you are submitting brain tissue, remove the brain from the skull – over the years I have received many unopened skulls for examination of the brain. Formalin circulation through the foramen magnum is poor at best. Don't submit excess tissue – trim muscle off of submitted bone samples, etc. Excess tissue serves no purpose, except to increase the amount of formalin required for proper fixation.



**An impression smear can improve turnaround time in many cases, especially skin tumors and lymph nodes.**

**#5. Don't chop up your tissues prior to submission.** We all want instant gratification –each time we remove a tumor, we have to take a peek. By cutting up a small piece of tissue to see for yourself, you may be disrupting important architecture. Additionally, some tissues may not cut cleanly before fixation (once again the brain or spinal cord is an excellent example.) If you must peek, then bisecting the lesion along its longest axis with a fresh scalpel blade will provide you the best chance of

satisfying your curiosity without overly irritating your pathologist.

**#6. Don't forget the cytology.** Fixation and processing of tissue takes time – a minimum of 24 hours. If time is of the essence, then a good cytologic preparation can give you that "head start" on instituting definitive therapy. Fine needle aspirates are extremely helpful, or if you have cut into that lesion for any reason, try a tissue impression. Make several – you can look at one yourself (no clinic should be without a rapid Wright's staining kit) and send a couple off to the lab. Ask for an immediate read on the cytology while the tissue sample is being processed.

**#7. Send a complete history and all available clinicopathologic data.** There are often significant pieces of information here that will help your pathologist nail the diagnosis. Also, don't be afraid to ask specific questions on the submission form – if there is something you need to know, then ask. (Even the oracle at Delphi required specific questions from time to time.) If your question is not answered in the report, then don't be afraid to pick up the phone. Most pathologists appreciate the human contact, and certainly enjoy the extra detail and followup

**#8. Take care in packaging your tissue.** Nobody wants to either transport a box that drips formalin, or open an envelope of glass shards. While most large labs now provide tyrofoam and cardboard mailers as well as formalin, there are many times when you may need to package your samples the old fashioned way – by hand. Moreover, necropsy specimens rarely fit into a 2-ounce jar (at least not those coming from a good necropsy!)

Make sure that your materials are protected from breakage. For glass slides, small cardboard mailers or slotted plastic boxes, and adequate shockproof packaging is a must. I have oft received a two-slide cardboard mailer in a plain white envelope – and the slides within generally in very small pieces. If you value your diagnosis, take the time to pack your slides in a larger box with tyrofoam peanuts.

Besides being messy and damaging to skin, spilled formalin will also quickly render your paperwork unreadable. Be sure to label everything in the submission – not only with the patient and owner's name, but the date and sample collected. You don't have to label everything – most pathologists can tell liver from spleen, but telling which is the left eye and which is the right is far more difficult.



**Specimens should be properly labeled and carefully packaged to prevent leakage. Leaking formalin may result in smeared or unreadable labels.**

#9. Wrap small pieces of tissue in gauze or paper. I often receive tiny pieces of tissue, such as pituitaries or pancreatic tumors, and these tiny fragments get lost in the bottle, especially if thrown in as an afterthought with eight or ten other pieces of tissue. If you wrap them in gauze before putting them in the bottle, or send them in formalin-soaked gauze in a sealed plastic bag, the pathologist won't miss it. Also make sure to note its presence on the submission form in nice big block letters. Most large labs only keep tissues up to 3 days after the slides are processed, so there is a relatively short fuse to reclaim a "lost" piece of tissue.

#10. Set up a good fax routing system. While this will not improve your chances of diagnosing a particular case, it often will improve the speed with which you receive your results. In most busy practices, the person who passes the fax machine will grab an incoming fax and route it to the appropriate clinician. While this sounds simple, faxes are occasionally misplaced, and the diagnosis is delayed. Shortly after I began faxing results to clients, I also began keep records of each faxed case, what time it was faxed, to what number etc. It really

helped disarm clinicians that were convinced I was dragging my feet!

A truly system that works well is to hang a corkboard above the fax machine and to post every incoming fax there. Not only does this method satisfy everyone's curiosity, but it also ensures that well-meaning, but often forgetful individuals do not accidentally carry off faxes.

There will always be cases in which a diagnosis is not made – all vets understand this fact of life (and death). However, by following these simple steps, you can ensure that in your practice, the undiagnosed case is the rare exception, and not the tragic rule.