Steps of a Successful Biopsy Submission

1 Collection

Each step of the biopsy submission process is important and contributes to the accuracy of the diagnosis, but the collection step is critical. While submission form information can be added to or revised at a later date and the correlation between the histologic and clinical assessments can be reassessed as new information is provided, the quality of the biopsy specimen is irrevocably determined by the collection method. One cannot make up for improper collection or fixation with a longer clinical history.

i Site

When presented with a single, small lesion, the site selection is straightforward (Figure 1), but when lesions are disseminated or the lesion is very large (Figure 2), site selection can make the difference between collection of diagnostic and non-diagnostic samples. When multiple lesions are present, take a sample from more than one as more than one disease may be present. In cases of widespread skin disease, sample several sites to get a range of the lesions. Areas of necrosis, ulceration, and secondary infection should be avoided as they often obscure the primary lesion (Figure 3). Be sure to biopsy any intact vesicles or pustules. For lesions that are too large to excise, multiple biopsies, including all grossly different appearing areas, are recommended in an attempt to submit fully representative specimens.

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ii Size

While the type of biopsy specimen collected (such as needle, punch, incisional or excisional biopsies (Figures 4–7), endoscopic or full-thickness specimens) depends on many factors, that is, size of the lesion, type and accessibility of

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Figure 1 Solitary mass, viral papilloma, on the nose of a terrier. A single excisional biopsy was diagnostic and curative. (Reproduced by permission of Bass Lake Pet Hospital, New Hope, MN 55428).

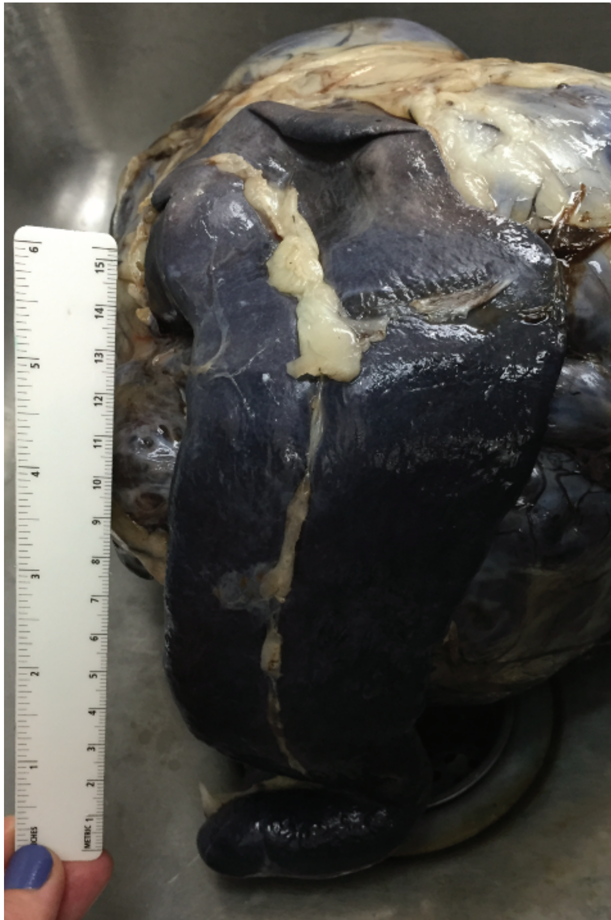

Figure 2 Spleen (in the foreground) with a large mass attached to the far side that requires sampling of all grossly different appearing areas to fully evaluate the lesion.

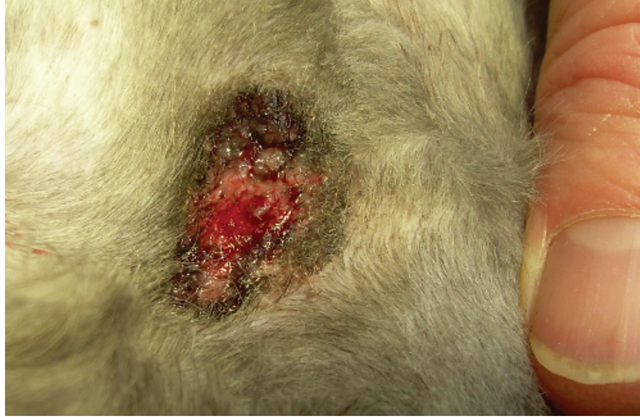

Figure 3 Ulcerated skin lesion (feline squamous cell carcinoma in situ [Bowen's-like disease] in this case). Excisional biopsy or sampling the periphery of the lesion is more likely to be diagnostic than a biopsy of the central ulcerated area. (Reproduced by permission of Whitewater Veterinary Hospital, Whitewater, WI 53190).

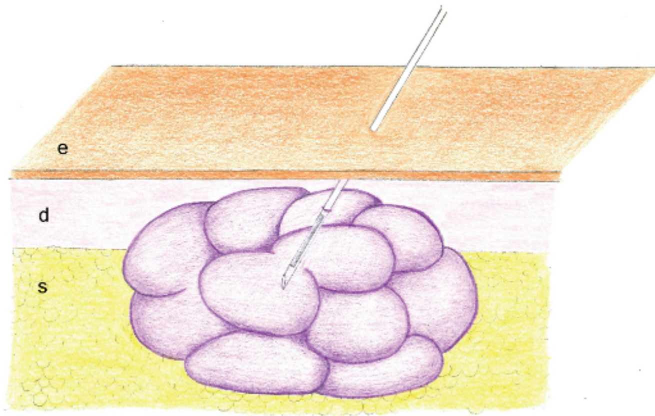

Figure 4 Drawing of a needle biopsy of a skin mass sampling a small proportion of the lesion. e = epidermis, d = dermis, and s = subcutis.

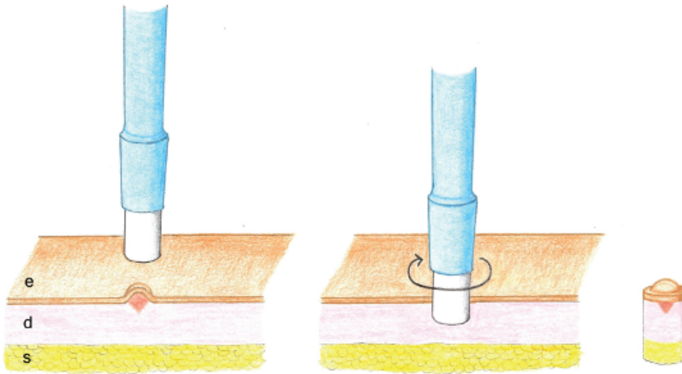

Figure 5 Drawing of the punch biopsy procedure. The punch biopsy instrument is placed over the lesion, rotated in one direction while avoiding excessive pressure and results in a cylindrical specimen. e = epidermis, d = dermis, and s = subcutis.

Figure 6 Drawing of an incisional biopsy of a skin mass sampling more than a needle biopsy, but less than an excisional biopsy. e = epidermis, d = dermis, and s = subcutis.

tissue, health of the patient, and so on, the certainty of submitting a representative specimen and, therefore, getting an accurate diagnosis, is proportional to the percentage of the lesion biopsied (Figure 8). In other words, the larger the biopsy sample, the more likely it is to be fully representative and diagnostic. When clinically reasonable, excisional biopsies are recommended. Not only are excisional biopsies fully representative of the lesion, but they can also be curative.

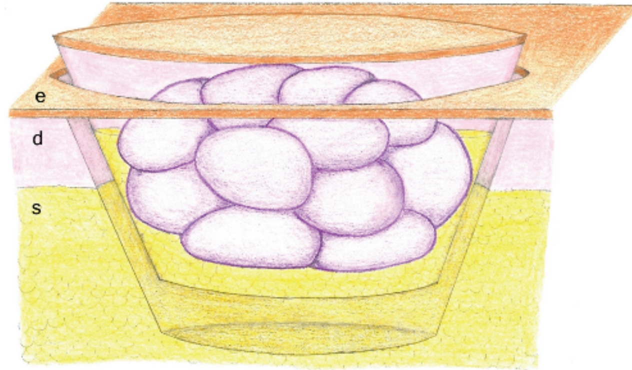

Figure 7 Drawing of an excisional biopsy of a skin lesion with removal of the entire lesion, providing (i) a fully representative specimen, (ii) the best chance at reaching an accurate diagnosis, and (iii) possibly a cure. e = epidermis, d = dermis, and s = subcutis.

Whenever possible, include some of the apparently normal surrounding tissue. The interface between the lesion and unaffected tissue often provides important information for interpreting the lesion, such as maturation of reactive tissue, well-circumscribed margin of benign neoplasms, or invasive growth of malignant tumors (Figures 9–12).

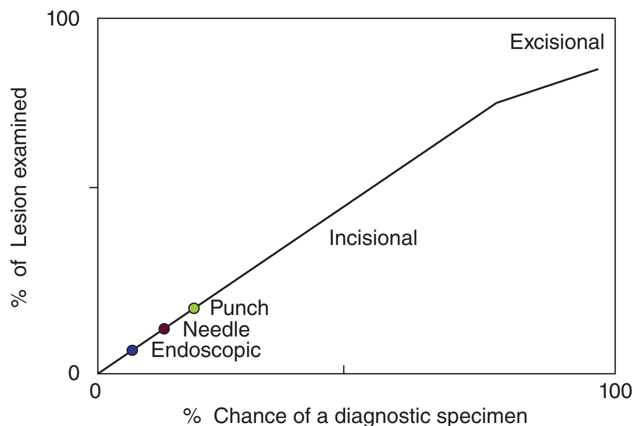

Figure 8 Graph of size of biopsy vs. likelihood of submitting a diagnostic specimen. The greater the percentage of the lesion submitted, the more likely the specimen will be fully representative and diagnostic (modified graph from Stromberg, P.C. (2009) *The principles and practice of veterinary surgical pathology*. CL Davis Foundation Workshop. ACVP Annual Meeting. Monterey, CA. Adapted with permission from Dr. Paul Stromberg.).

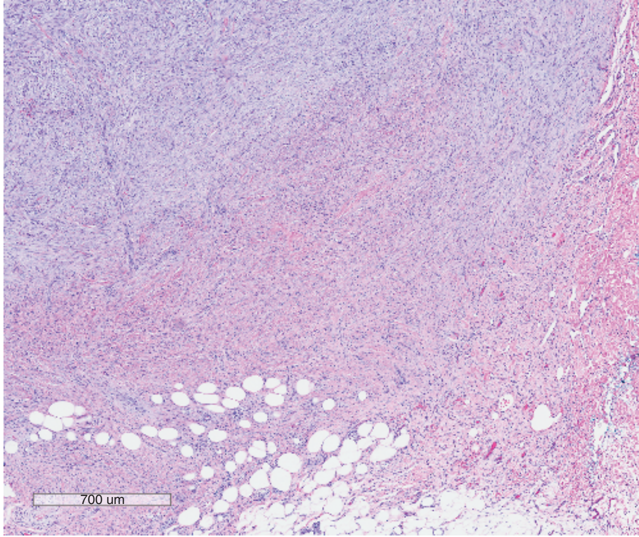

Figure 9 Photomicrograph of the peripheral maturation of granulation tissue and blending of the lesion with the adjacent connective tissue, a feature that helps distinguish this reactive lesion from a spindle cell neoplasm.

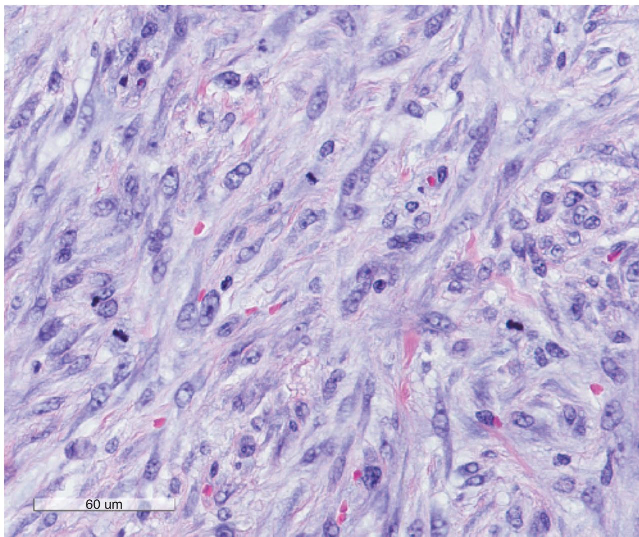

Figure 10 Photomicrograph of the center of the granulation tissue in Figure 9. These mitotically active, immature spindle cells can easily be misinterpreted as a malignant spindle cell neoplasm if the margins of the lesion cannot be evaluated.

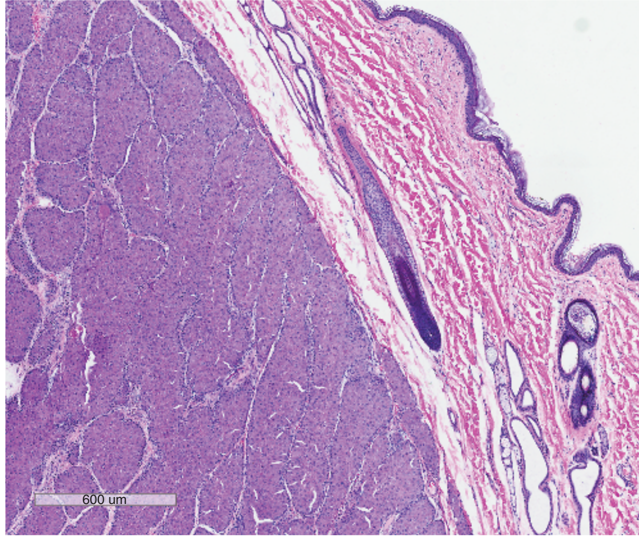

Figure 11 Photomicrograph of a portion of the smooth margin of a benign tumor (a circumanal [also called perianal or hepatoid] adenoma in this case). The tumor (left) and adjacent haired skin (right) are included in the specimen.

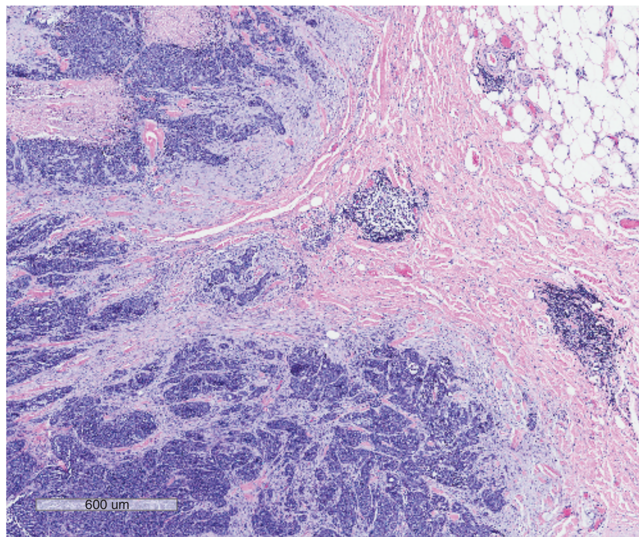

Figure 12 Photomicrograph of a portion of the irregular margin of a malignant tumor. The tumor (left) is invading the adjacent fibroadipose tissue (right). Neoplastic tissue is surrounded by a fibroblastic reaction (desmoplasia).

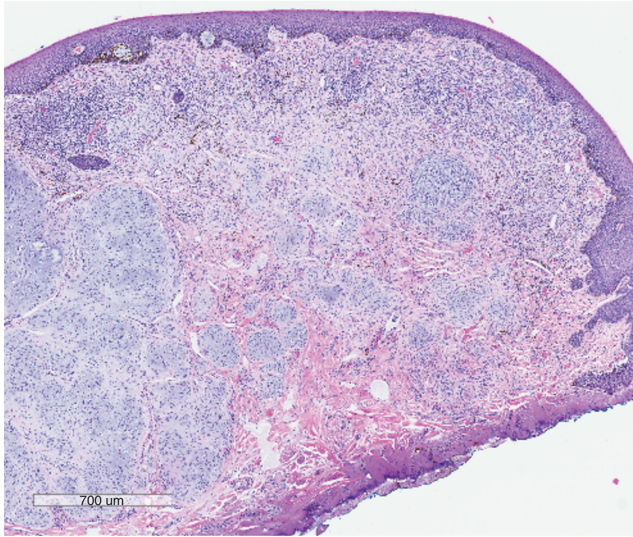

Figure 13 Photomicrograph of an oral mucosal biopsy with coagulation of the tissue margin (bottom right), consistent with electrocautery or laser-induced artifact and characterized by amorphous tissue and loss of detail. As the specimen is relatively large and only the margin was affected, the specimen is diagnostic (a sparsely pigmented oral melanocytic neoplasm in this case).

Be careful to avoid artifacts. Small specimens are especially susceptible to tissue coagulation and squeeze artifact. Electrocautery and lasers coagulate tissue, forming an amorphous residuum. In larger specimens, only the tissue margins are affected (Figure 13), leaving an area of viable, potentially diagnostic tissue, but in smaller specimens, the entire sample can be rendered non-diagnostic (Figures 14 and 15).

To avoid squeeze artifact, use sharp cutting blades and handle tissues gently, avoiding or minimizing the use of forceps. A new biopsy punch is indicated for each patient to ensure a sharp blade. Lymphoid cells rupture easily (Figures 16 and 17). If lymphoma is suspected, be particularly gentle during collection and submission of the specimens. Forceps squeeze/crush tissue, causing tissue distortion and rupturing cells (Figures 18–22). Tiny tissue fragments wrapped in gauze can be difficult to find and may be distorted during retrieval.

Some recommend the use of tissue cassettes with cassette foam sponges (Figures 23 and 24) for submission of small specimens, but when fresh tissue is placed in cassettes with cassette sponges, the sponges impale the tissue, forming triangular spaces, which distorts the tissue, rupturing cells and hampering histologic evaluation (Figures 25–28). If the specimens are so small that they will be lost through the small slits or pores in the tissue cassettes, they are usually of limited diagnostic value, and if possible, larger specimens should be obtained. When cassettes with cassette sponges are to be used, the tissue specimens should be fixed prior to placement in the cassettes, or in cases of

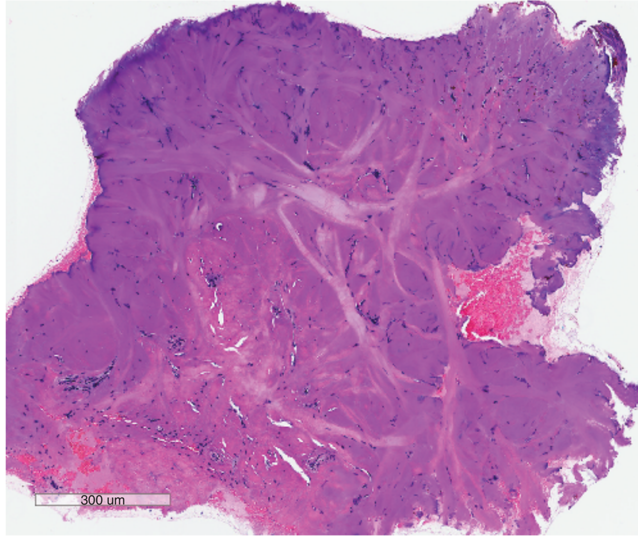

Figure 14 Photomicrograph of a diffusely coagulated specimen with loss of detail (consistent with electrocautery or laser-induced artifact), resulting in unrecognizable tissue.

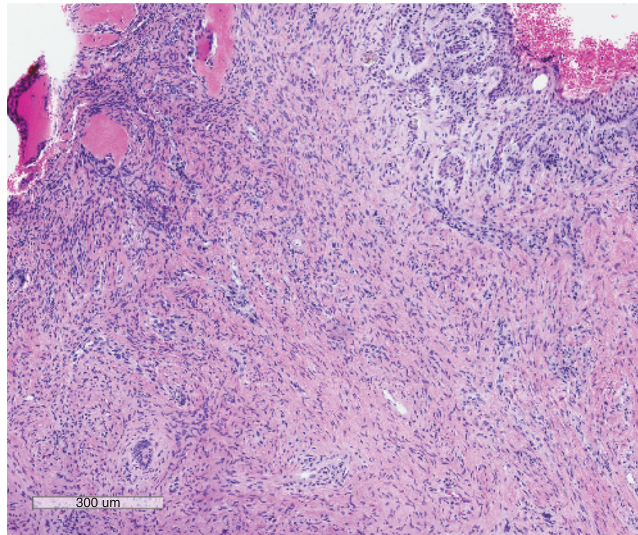

Figure 15 Photomicrograph of uncoagulated tissue from the same lesion as in Figure 14. This specimen reveals what the tissue looked like before coagulation and is diagnostic of a fibromatous epulis.

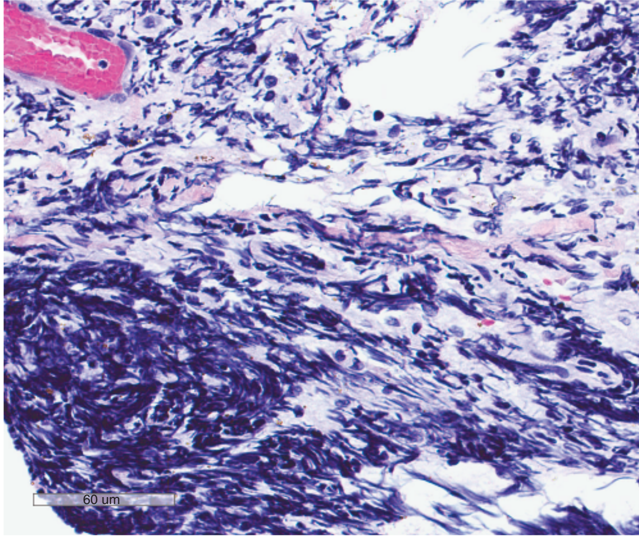

Figure 16 Photomicrograph of lymphoid tissue with squeeze artifact induced at the time of biopsy collection. The cells have ruptured, resulting in streaming chromatin (blue streaks) and precluding cell identification.

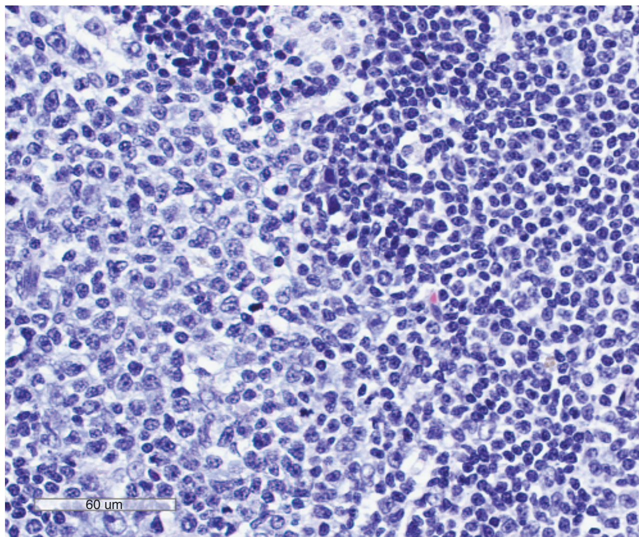

Figure 17 Photomicrograph of tissue from the same lymph node as in Figure 16 without squeeze artifact. The cells of the lymphoid follicle (left) and paracortical area (right) are intact and recognizable.

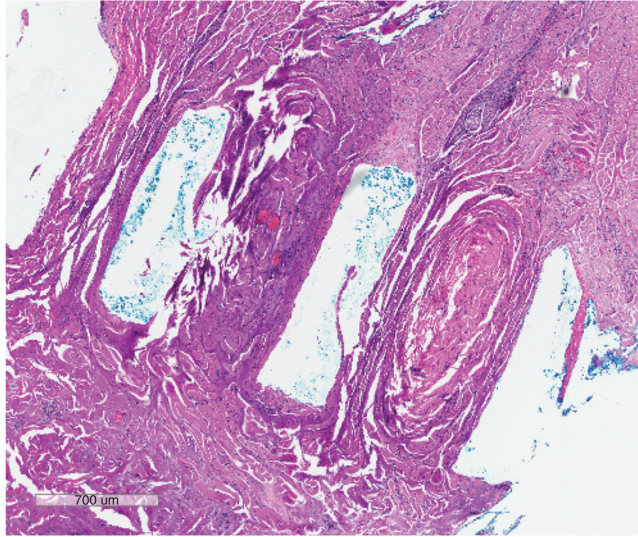

Figure 18 Photomicrograph of tissue distorted by a clamp, resulting in rectangular spaces and greatly hampering tissue identification and histologic evaluation.

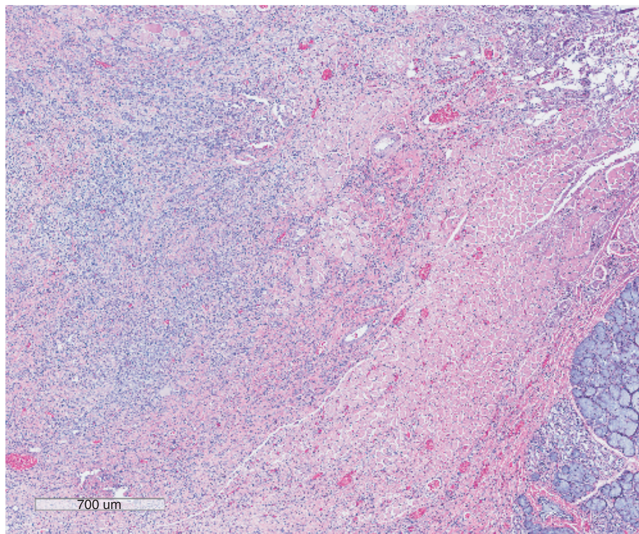

Figure 19 Photomicrograph of the same tissue as in Figure 18 without the clamp-induced artifact, revealing reactive fibroplasia involving fibrovascular tissue and skeletal muscle. Salivary gland tissue is present in the lower right.

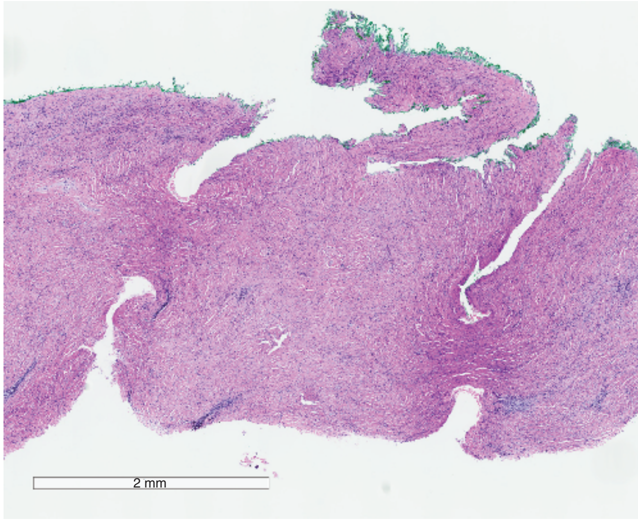

Figure 20 Photomicrograph of tissue distorted by forceps, resulting in two areas of sharp tissue constriction.

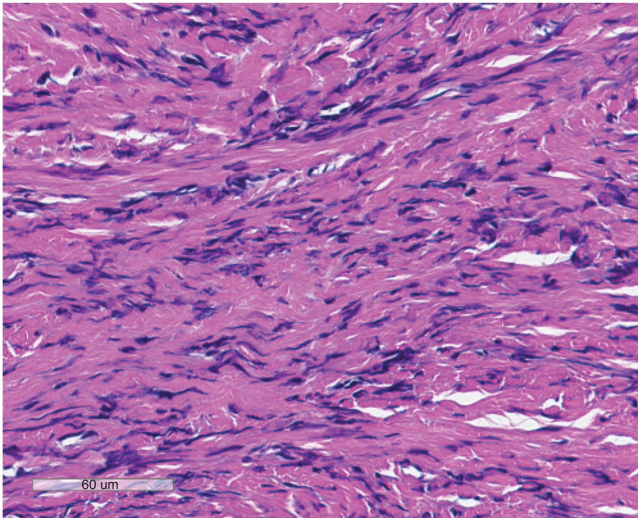

Figure 21 Higher-magnification photomicrograph of a constricted area of the tissue in Figure 20, demonstrating the squeeze artifact caused by the forceps. The cells have ruptured, resulting in streaming chromatin (blue streaks) and precluding cell identification.

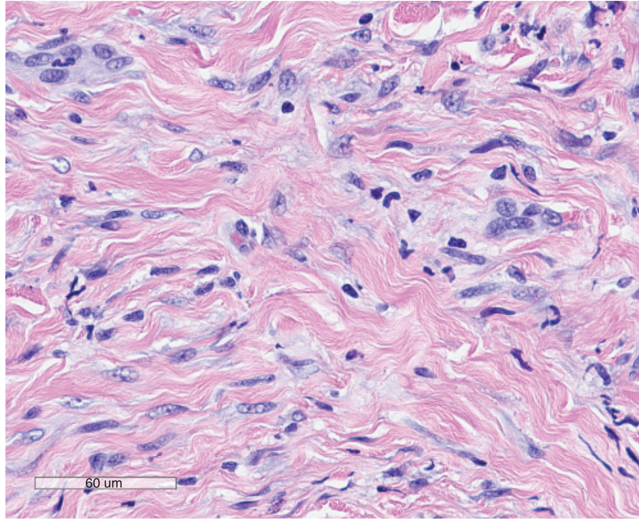

Figure 22 Higher-magnification photomicrograph of an unconstricted area of the tissue in Figure 20, demonstrating what the tissue looks like without the artifact induced by the forceps and revealing plump fibroblast separated by collagen.

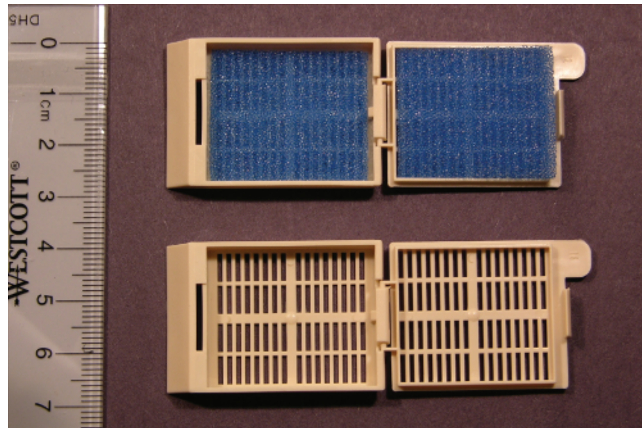

Figure 23 Open tissue cassettes with (above) and without (below) cassette sponges.

small endoscopic or needle biopsy specimens, wetting the sponges with formalin before placing the tissue in the cassette can result in good-quality samples.

iii Number

The number of biopsies taken depends on the number and distribution of the lesions, as well as the size of biopsy, with the aim of submitting representative tissue. An excisional biopsy of a single lesion requires only a single specimen; however, if punch or core biopsies are taken of a large lesion, more than one

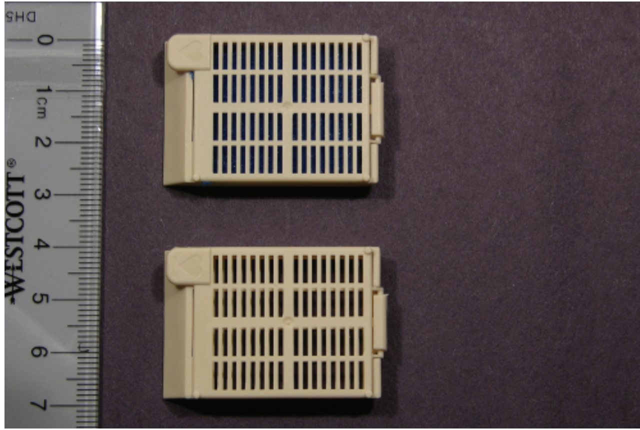

Figure 24 Closed tissue cassettes.

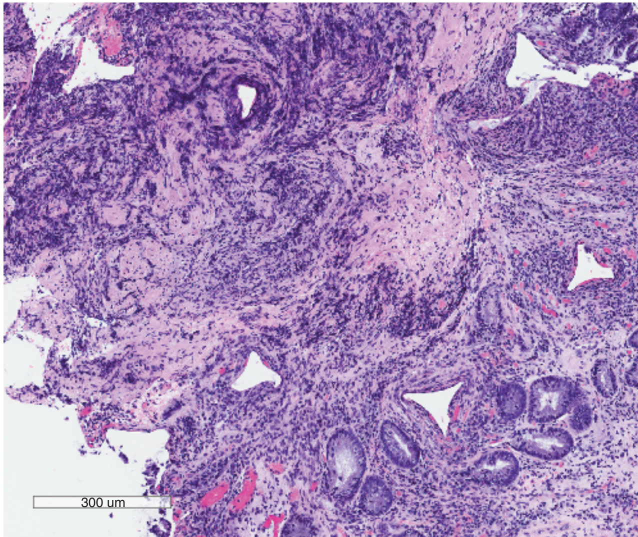

Figure 25 Photomicrograph of rectal mucosa submitted in a tissue cassette with cassette sponges. The sponges have impaled the tissue producing triangular clear spaces, distorting the tissue, rupturing cells, and greatly hampering histologic evaluation.

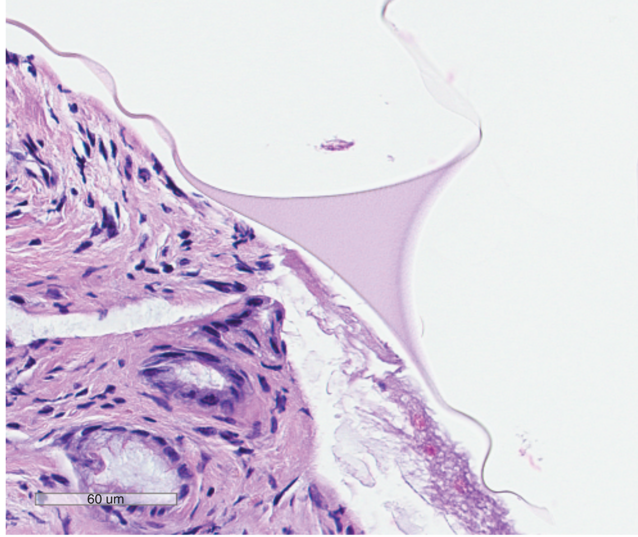

Figure 26 High-magnification photomicrograph of cassette sponge material, which demonstrates the triangular portion that causes the characteristic artifact seen in Figures 25 and 27.

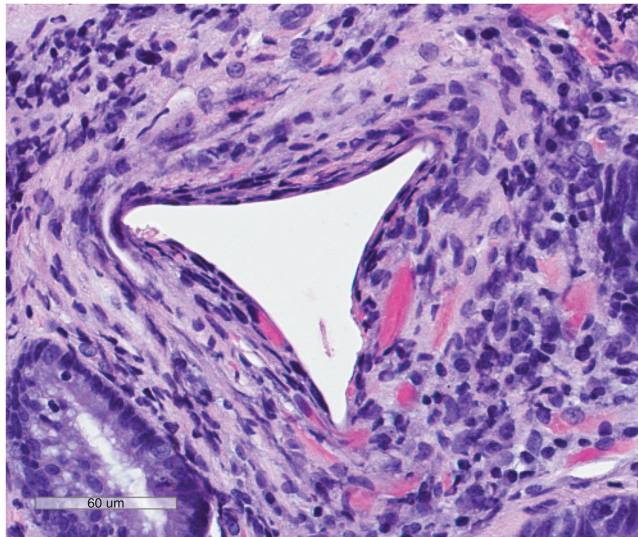

Figure 27 Higher-magnification photomicrograph of a characteristic triangular clear space in Figure 25 caused by submission of unfixed tissue in cassettes with dry cassette sponges. The tissue is distorted, hampering histologic evaluation of the tissue architecture and cell types present.

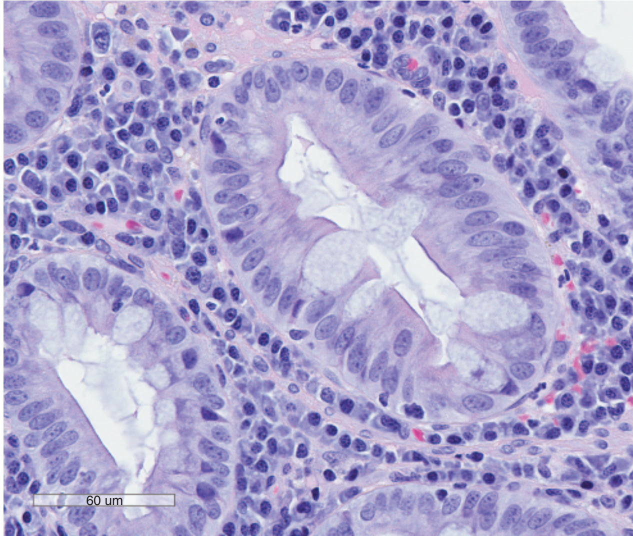

Figure 28 Photomicrograph of rectal tissue similar to that in Figure 27 without cassette sponge-induced artifact. The tissue architecture is intact, the epithelium can be properly assessed, and plasma cells and occasional lymphocytes are evident in the lamina propria.

biopsy should be taken to increase the percentage of the lesion sampled and the probability of obtaining fully representative specimens. In cases of widespread disease or multiple lesions, multiple biopsies are recommended. Lesions may be at different stages, some of which are more diagnostic than others, and all lesions may not be the result of the same disease.

Do not submit multiple specimens and ask the pathologist to choose the ones to examine. The clinician, who has the whole patient to examine, is in a better position to choose representative specimens for histologic evaluation than the pathologist or technician examining formalin-fixed tissues. Most diagnostic laboratories will process each tissue that is submitted.

iv Fixation

In general, for routine histologic evaluation, tissues should be placed in neutral buffered 10% formalin immediately after collection to prevent desiccation, autolysis (Figures 29–32) and bacterial overgrowth. Use at least 10:1 formalin to tissue volume. To avoid shipping large amounts of formalin, specimens can be shipped post-fixation in just enough formalin to keep them moist. Proper fixation requires at least 24 hours (different tissues have different rates of formalin fixation). If the specimen will be subjected to freezing temperatures, add one part 95% ethyl alcohol to nine parts formalin in order to prevent freeze artifact. Freezing causes ice crystals to form in the tissue, resulting in tissue clefts and distortion (Figures 33 and 34) and hampering histologic evaluation.

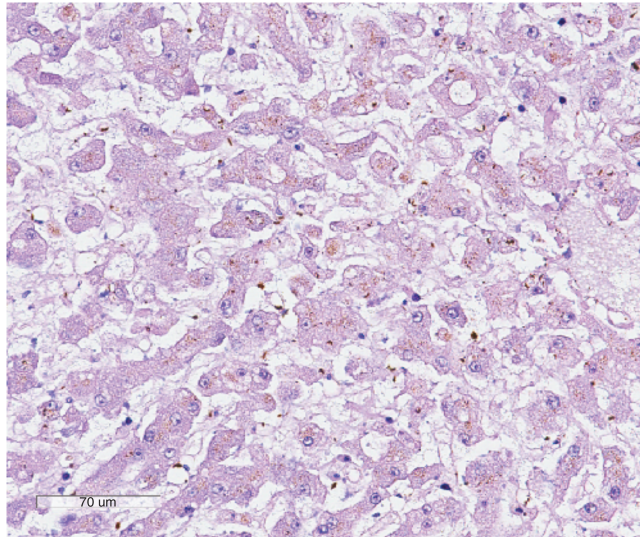

Figure 29 Photomicrograph of autolytic liver. There is loss of cellular detail with altered staining quality, cell shrinkage, and indistinct chromatin.

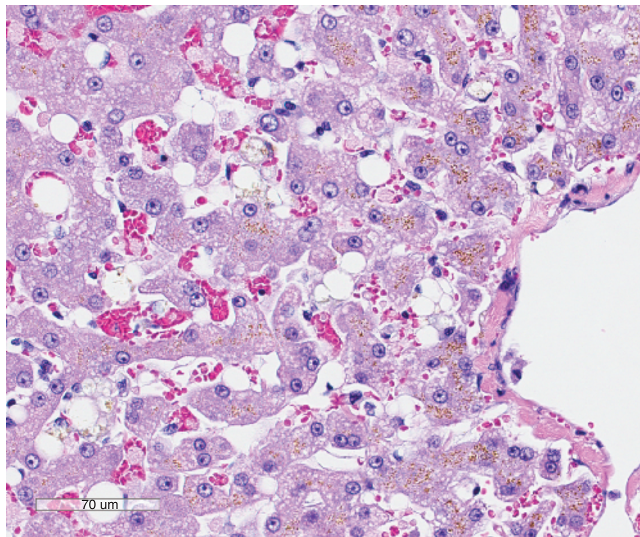

Figure 30 Photomicrograph of liver tissue that was fixed promptly and lacks significant autolytic changes. The cells are larger and exhibit more nuclear and cytoplasmic details than the autolytic tissue in Figure 29.

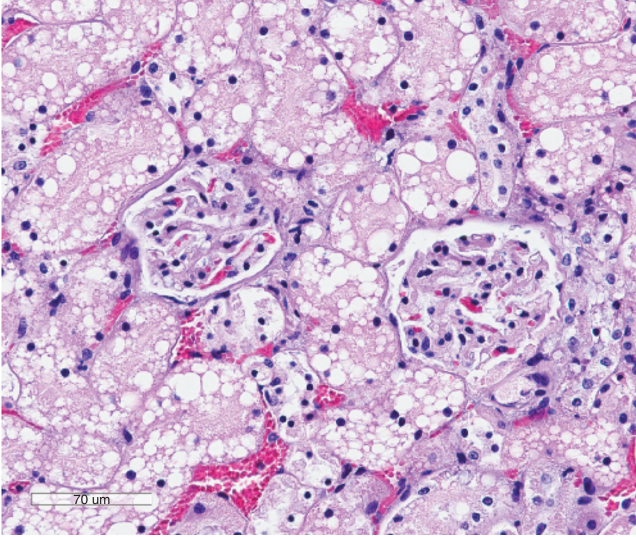

Figure 31 Photomicrograph of autolytic kidney. There is loss of cellular detail with altered staining quality, cell shrinkage, and small dark nuclei that can be misinterpreted as necrosis.

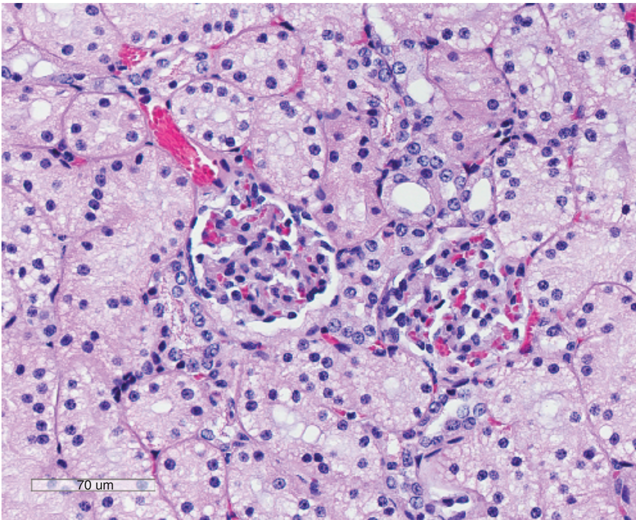

Figure 32 Photomicrograph of kidney that was fixed promptly and lacks significant autolytic changes. The cells are larger and exhibit more nuclear and cytoplasmic details than the autolytic tissue in Figure 31.

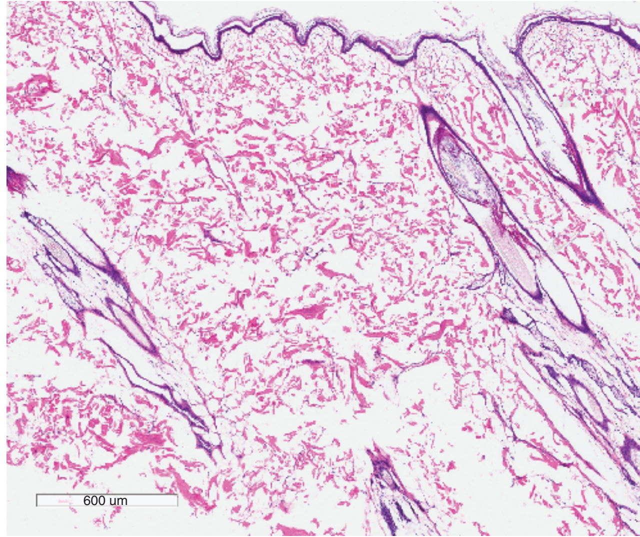

Figure 33 Photomicrograph of haired skin with freeze artifact. There are numerous tissue clefts (clear spaces) distorting the dermis.

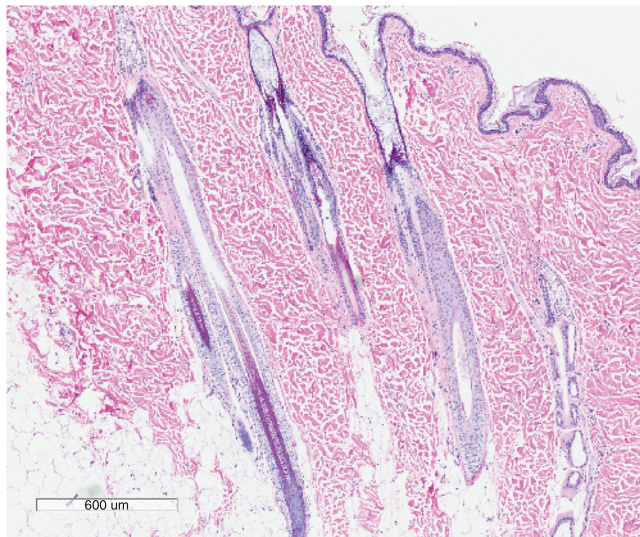

Figure 34 Photomicrograph of haired skin without freeze artifact. The dermis lacks the relatively large tissue clefts seen in the tissue that was frozen in Figure 33.

Specimens should be less than 1 cm thick to allow adequate penetration of the tissue by the formalin. The following techniques can be used to allow for adequate tissue fixation of larger specimens:

a. Incomplete serial sectioning ("breadloafing") (Figure 35) - Make incomplete (to maintain tissue orientation), parallel cuts, preferably less than 1 cm apart.

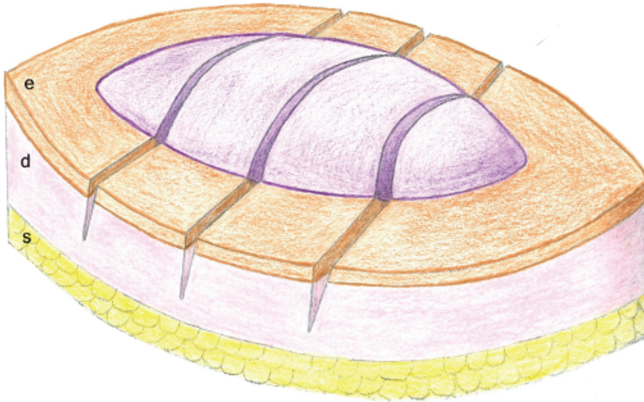

Figure 35 Drawing of an excisional biopsy specimen of a cutaneous mass that has incomplete, parallel incisions (has been "breadloafed") to allow for rapid fixation of the tissue while maintaining tissue orientation and integrity of the surgical margin. e = epidermis, d = dermis, and s = subcutis.

Figure 36 Side view of an excisional biopsy specimen of a skin mass that has been incompletely incised from the surface to allow for rapid fixation of the tissue while maintaining tissue orientation and integrity of the surgical margin.

Inking margins of interest should be done prior to breadloafing to prevent ink from adhering to non-surgical margins. When incompletely incising skin specimens, make the incision(s) through the skin surface and not the subcutis to maintain the integrity of the surgical margin (Figure 36).

b. Splitting the sample - Ink the surgical margins with India ink or commercially available tissue marking dyes prior to cutting the specimen to allow for orientation and reassembly of the specimen during histologic processing and assessment of completeness of excision.

c. Subsampling - Take representative samples of large specimens from at least three different sites to include all grossly different appearing areas.

If special testing is a consideration, additional tissue can be cultured, frozen (for toxicology, metal analysis or PCR), snap frozen (for molecular diagnostics), or placed in glutaraldehyde (for electron microscopy).

v Labeling and packaging

Submit tissue in leak proof and shatterproof, wide-mouthed containers labeled with the names of animal, owner, and veterinarian/clinic (Figures 37–39). If submitting more than one tissue, be sure to include the tissue source on the label. Glass jars can break and should not be used. The label should be affixed to the container and not the lid. Make sure the widest part of the specimen fits through the narrowest part of the container. After fixation, the specimen becomes rigid and cannot squeeze through narrow openings that it could before it was fixed.

Ideally, each specimen should be submitted in its own container. If submitting multiple specimens in one container, each specimen should be clearly differentiated by different numbers of sutures, different suture colors, different colored inks, or truly unique morphologic features. The site of endoscopic or needle biopsy samples can be indicated by submitting them in labeled cassettes; however, as indicated above, cassette sponges can impale fresh tissue, causing significant tissue artifacts. If there is a risk of the tissue escaping from the tissue cassette, cassette sponges can be used (after they have been wet with formalin or the specimens have been fixed) or Cellsafe™ biopsy capsules can be used instead of cassette sponges, but tissues this small are often of limited diagnostic use. Specimens submitted on cardboard or tongue depressors frequently become

Figure 37 An example of a leak proof and shatterproof, wide-mouthed container for biopsy specimens.

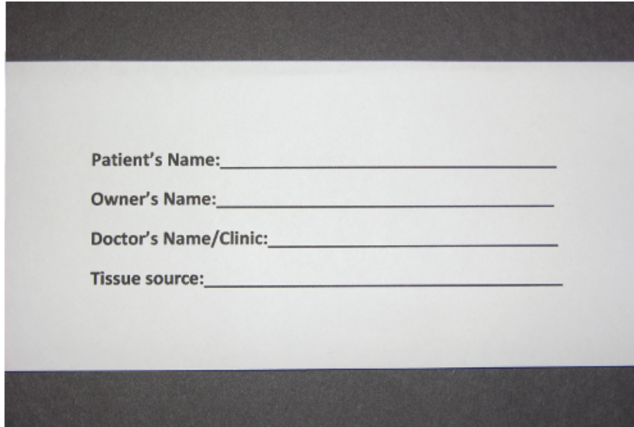

Figure 38 An example of a label template that includes prompts for the names of the animal, owner, and doctor/clinic, as well as the tissue source.

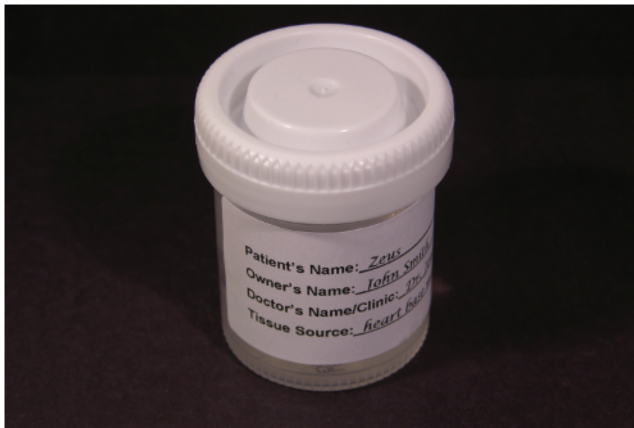

Figure 39 An example of a biopsy container with a label including the appropriate information attached to the container and not the lid.

detached, and transporting specimens this way does not provide a reliable way of labeling tissues.

Different lengths, numbers or colors of sutures, or different colors of ink can also be used to indicate orientation of the specimen (Figure 40) and different margins of interest (Figures 41, 42).

Federal shipping regulations must be observed when transporting biological specimens (U.S. Department of Transportation 2007; American Veterinary Medical Association 2015). Biopsy specimens require triple packaging with (i) a primary watertight container, (ii) watertight secondary packaging with sufficient absorbent material to absorb the entire liquid contents, and (iii) sturdy, ridged walled outer packaging. All individuals involved in the packaging and shipping

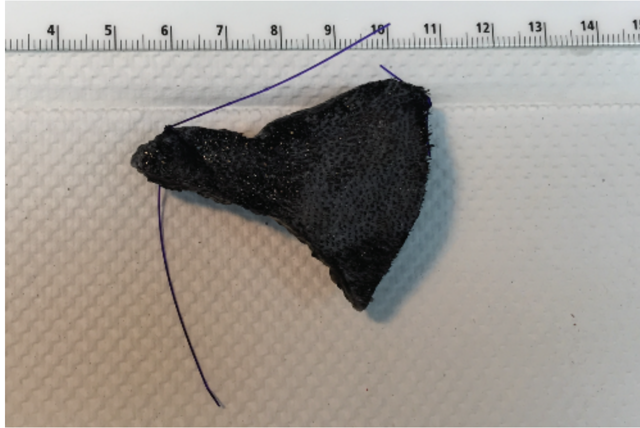

Figure 40 A skin biopsy specimen with a long suture and a short suture indicating different margins. The long suture marks the cranial margin and the short suture the dorsal margin.

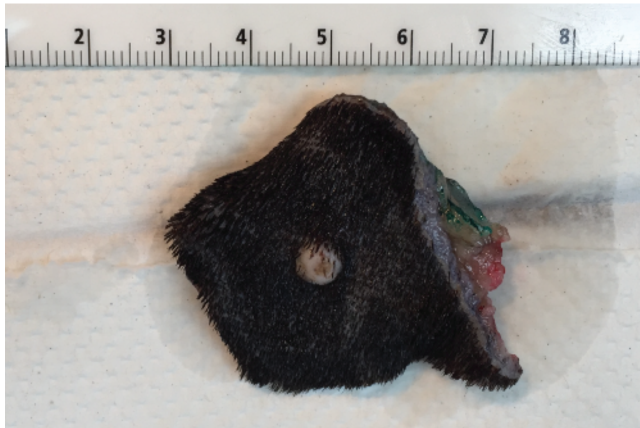

Figure 41 The superficial aspect of an excisional skin biopsy specimen marked with different colored inks to indicate different margins.

of infectious substances must have proper training, and packages must be appropriately labeled.

2 Submission form

Clinicians are busy professionals, but it is very important to take the time to provide the pathologist with the basic background information with which to interpret the histologic findings. The pathologist usually does not have access to the patient and depends on the clinician to provide the medical facts of the case. The biopsy captures a tiny percentage of the patient and often a small

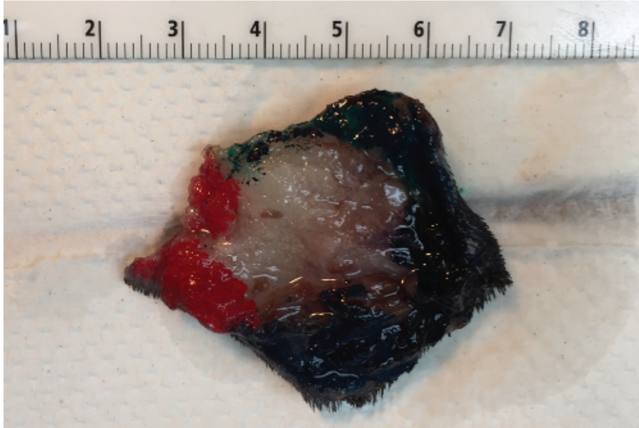

Figure 42 The deep aspect of the excisional skin biopsy specimen in Figure 41 marked with different colored inks to indicate different margins. The red ink marks the cranial margin, green ventral, black caudal, and blue dorsal.

Figure 43 A botryoid (cluster of grapes-shaped) mass from the base of the heart of a dog that was diagnosed histologically as hemangiosarcoma.

percentage of the tissue/mass being evaluated. Missing information reduces the specificity and/or certainty of the pathologist's diagnosis.

i Signalment and clinical history

Species, breed, sex (intact or neutered), and age are important. "Bird" is not a signalment. The clinical history should be succinct and include pertinent clinical signs, any previous treatment, response to treatment, and whether others in the environment are affected. Do not waste time with extraneous details, but make sure you explain why you are taking the biopsy.

ii Lesion description

Reporting that "a lesion" was biopsied does not impart any useful information. Bearing in mind that the pathologist usually cannot examine the patient, describe the lesion as if to a person who cannot see, touch or smell. The following list of attributes should be addressed in your description:

- **a.** Number, location, and distribution – Please be specific regarding location. When given "abdomen" as the location, the pathologist doesn't know if the lesion is from the skin, subcutis, mammary gland, skeletal muscle, peritoneum, lymph node, or any of the abdominal viscera.
- **b.** Size – Objective size is always best, but comparison to sizes of common items, such as dime-sized, is better than no size reference. Avoid relative terms, such as "large" and "small."
- **c.** Color
- **d.** Shape – ovoid, spherical, conical, flat, nodular, discoid, botryoid (Figure 43), and so on.
- **e.** Consistency – soft, firm, hard, resilient, friable, viscous, mucoid, gritty, homogeneous, and so on.
- **f.** Other – discrete or ill-defined, fixed or movable, ulcerated or not, malodorous and so on.

When possible, photographs of the lesion(s) in situ can be a very helpful addition to the biopsy submission material.

iii Differential diagnosis and specific questions

When provided with your differential diagnosis, the pathologist can specifically address whether or not the histologic findings support the conditions on the list. Asking specific questions on the submission form is the most efficient way to get answers to questions the pathologist might not anticipate and address in his/her report.

3 Clinicopathologic correlation

In most cases, the pathologist has no direct contact with the patient and depends on the information you provide on the submission form. Your pathologist will attempt to correlate that information with the histologic findings to reach a diagnosis, differential diagnosis, and/or offer suggestions for additional steps/testing to reach a more specific diagnosis. The histologic diagnosis should be reviewed in light of the clinical signs, physical exam, other laboratory tests, and response to treatment. If the pathologist's conclusions do not correlate well with your clinical assessment, please contact the pathologist to discuss the discrepancy. Additional biopsies should be considered if the lesion appears to recur, if the condition does not respond appropriately to the recommended treatment, or to track response to treatment.