Evaluation of Surgical Margins

Surgical margins of tumor biopsies are evaluated on every routine section in our laboratory. However, on routine samples the evaluation is limited to the extent of the neoplasm on 2 cross sections. A more complete margin evaluation is a complex process and has to be specifically requested for each biopsy submission. In human medicine neoplasms are sectioned like a “bread loaf” or a “pie” to have the most detailed information for each submission. Such procedure is time and cost intensive, especially for larger tumor samples. To avoid unnecessary costs for our clients and to provide detailed information on the extent of a neoplasm in a submitted section we have revised a specific protocol explained within the following paragraphs.

**Figure 1:** To correctly identify surgical margins during the trimming process in the histology laboratory, it is necessary to paint (ink) the surgical margins. This can be done by the submitting veterinarian on unfixed samples or in our laboratory after the samples have been fixed. The procedure is simple and does not interfere with the microscopic evaluation. Besides ink, cotton swabs and wooden applicator sticks are all that is needed to perform the procedure.

**Figure 2:** Surgical margins of a biopsy are painted with a dye that adheres to the tissue and is visible under the microscope. There are many commercial dyes available, such as the one depicted from Cancer Diagnostics, Inc. Such kits contain multiple different colors (black, blue, green, red, yellow, etc) for different aspects of mass orientation.

**Figure 3:** To save money simple waterproof drawing ink can be purchased (Wal-Mart etc.) and such bottles will last several years.

**Figure 4:** The drawing ink can even be further diluted with isopropyl alcohol (1:1). Isopropyl alcohol is also useful when submitting fixed tissue through the mail in winter months. The 10% neutral buffered formalin used for fixation of specimens is subject to freezing in very low temperatures. The addition of a small amount of isopropyl alcohol to the formalin specimen container (1 part alcohol to 10 parts formalin) will help to prevent damage to the tissue specimen related to freezing and thawing.

**Figures 5 and 6:** Biopsy margins can be painted on unfixed or fixed tissues. It is often an advantage for referring veterinarians to ink the margins on an unfixed tissue because they performed the surgery and can best identify margins of concern. The mass

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**WEBCD.SP.REF.001.02**

Issue Date: 01/27/2009
should be placed on some absorptive material and needs to be blotted dry prior to painting the margins.

**Figure 7:** Different colors may be used to mark the cutaneous surface of a mass, which will help the histotechnician to correctly identify the orientation of the neoplasm. Such inking marks are superior to using sutures of different colors in identifying proximal and lateral margins of a mass.

**Figure 8:** Using a cotton swab facilitates even color distribution over the deep tissue margins. The referring veterinarians may decide to ink only margins of concern where they suspect incomplete removal. We will only evaluate the inked margins.

**Figure 9:** The biopsy margins of the mass may be inked with multiple colors too. Do not pour dye on the surface, but use the cotton swab or wooden applicator stick. A wooden applicator stick is especially helpful to ink the lateral margins by rolling it along these tissue margins. After inking the margins the dye should dry for 5-10 minutes prior to immersing the sample in formalin. Some dye will dissolve within the fixative, but this will not affect the evaluation. For large samples (thicker than 1-2 cm) incisions should be made into the mass to improve penetration of fixative.

**Figure 10:** After receiving the tissue, our technicians will palpate the section to determine where the mass/lesion comes closest to the surgical margins.

**Figures 11 and 12:** We will bisect the specimen vertically through the mass so the section extends through the margin closest to the identified mass and the center of the mass. A 2-6 mm full thickness plane/slab/piece will be cut from the cross section surface of the mass.

**Figure 13:** In our laboratory the standard trimming method used for ellipse sections is the “Cross Method” (i.e. ½’s and ¼’s). The red line demonstrates the cross section of the mass and the associated closest specimen margin as illustrated in figures 11 and 12 and 14 to 15. The blue line demonstrates the quarter sections (1/4’s) as illustrated in figures 17 and 18.

**Figures 14 and 15:** The inked margins are easy to recognize on the half section of the mass. Depending on the size of the mass, the half section may have to be split to fit into cassettes for further processing.

**Figure 16:** Following embedding in paraffin and sectioning, the half sections of the mass will be placed on slides for microscopic evaluation.

**Figures 17 and 18:** Halves of the mass that have resulted from the cross section are vertically cut from the mass/lesion through the longest axis of the tissue and thin sections are placed into cassettes.
These pieces demonstrate the mass in a different plane, and the association of the mass with surrounding normal long axis tissue margins. **Figure 19:** The number of slides necessary to identify whether the deep margins are clean is determined by size of mass as illustrated. Evaluation of deep margins will be performed along the previously described half and quarter sections. **Figure 20:** The half and quarter sections have been placed in cassettes for further processing. **Figures 21, 22 and 23:** In addition to the deep margins, lateral margins will be evaluated. These margins are cut vertically to the cutaneous surface of the submitted section and trimmed into cassettes. Detailed evaluation of margins will only be performed following a written or oral request. The number of slides evaluating margins and the price for margin evaluation is based on the size of the excised tissue. As a general rule, sections smaller than 2 cm long will require 2 additional slides. Section of 2-4 cm length will require 6 additional slides, sections of 4-6 cm long 9 additional slides and sections of 6-8 cm long will require 11 additional slides. For each slide there will be an additional charge of $5. For larger samples, call for a quote. **Figures 24 and 25:** Inked tissue section margins (lateral margins: figure 24; deep margins: figure 25) are easily recognized on microscopic examination and will help the pathologist to determine complete surgical removal.