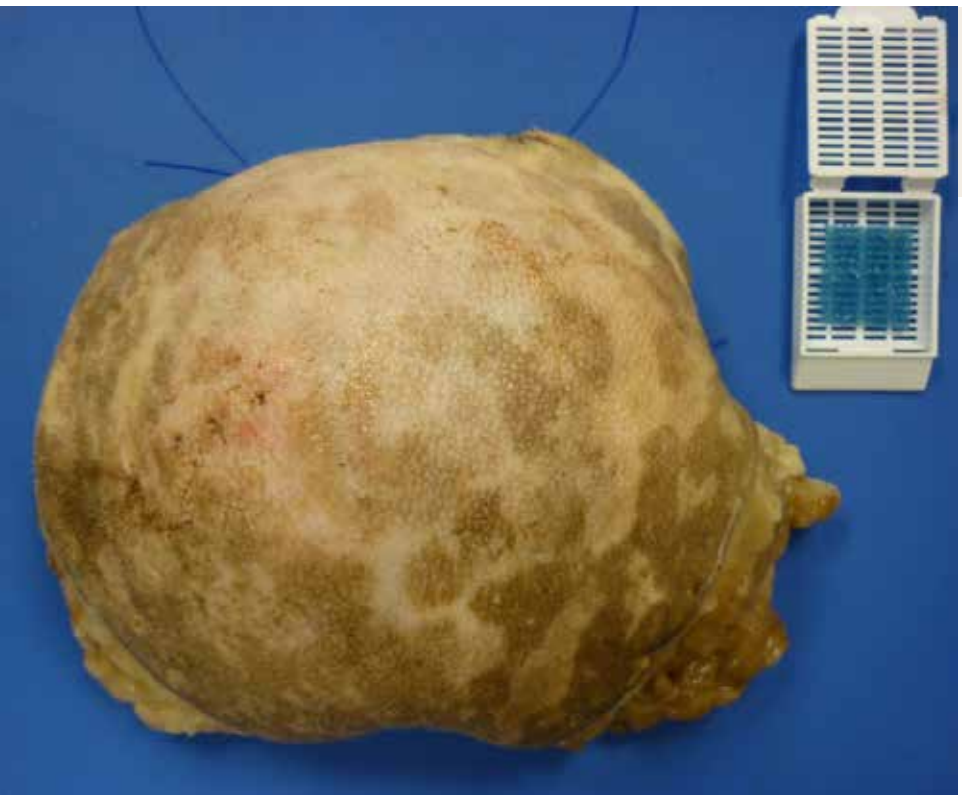

FIGURE 1: Large fixed tissue received for histology, side by side with a tissue cassette used for sub-sample processing. The blue biopsy pad indicates the typical size of a sub-sample.

Margin call

Michael Hardcastle, registered veterinary specialist anatomic pathology, Gribbles Veterinary, Auckland, explains the difficulties of assessing and reporting margins in surgical biopsies.

WHEN A TUMOUR is diagnosed by histopathology from a surgical biopsy, the submitting clinician invariably needs to know two crucial pieces of information: what it was, and whether or not it has been completely removed.

Providing these two key facts is often more difficult than it might seem, and in the case of surgical margins, I find that practitioners have widely varying levels of expectation and understanding about how margins are assessed and reported in a commercial veterinary diagnostic laboratory.

The truth is that this is a somewhat subjective process, which is not standardised in veterinary pathology (Kamstock et al., 2011). The following is a summary of my approach to margin evaluation and reporting in surgical biopsies.

PREPARING CASES FOR MARGIN ASSESSMENT

When a biopsy arrives in our laboratory, it is measured, described and sketched. The surgical margin is then inked before it is processed (see Figure 1).

The majority of samples require cutting and sub-sampling for processing, because thick tissues do not process very well through the various stages of dehydration and solvent to wax infiltration. Sub-samples are placed into tissue cassettes for processing. These typically have an interior volume of approximately 30 x 25 x 5mm, and it is not recommended to fill cassettes completely; therefore most sub-samples submitted for processing are about 2-3mm thick, and about the diameter of a 10-cent piece (Figure 1).

This creates two problems relevant to margin assessment. The first is that a large tumour such as that in Figure 1 is very difficult to cut into 2-3mm thick slices without distorting the surgical margin. Invariably, a firm tumour such as a soft tissue sarcoma will tend to slip over, stretch or squash the margin beneath. The margin may still slip away from the mass if sectioning is performed from the surgical aspect. Then the margin may fold or detach from the tumour during the transfer of the sub-sample from the cutting board to the cassette, or during processing. Therefore, the definitive in vivo margin can be hard to replicate faithfully on the microscope slide. Ink is used to help mitigate this problem, since it shows the histology technician how the sample should be embedded in paraffin, and establishes that a histological margin seen under the microscope is at least the 'true' margin, rather than a folded or distorted area.

The other problem is that cassette and sub-sample size constrains the amount of tumour margin that can be examined. It can be appreciated that a considerable number of cassettes would be needed to examine every millimetre of the margin of the mass in Figure 1, which appears

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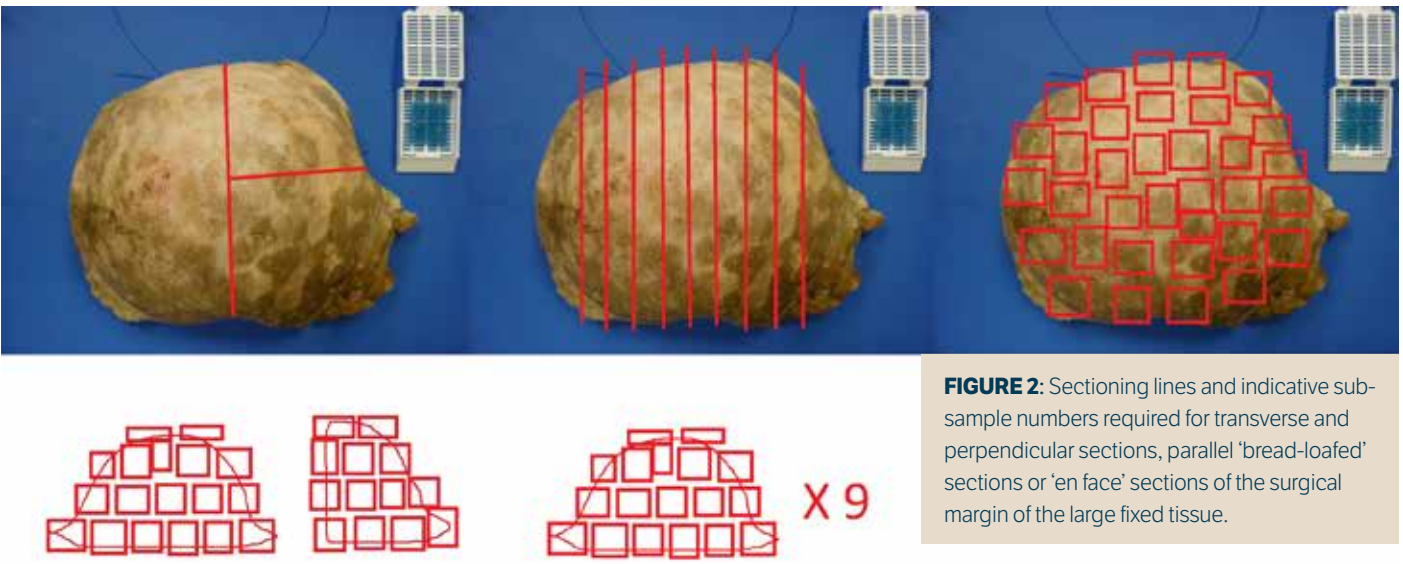


FIGURE 2: Sectioning lines and indicative sub-sample numbers required for transverse and perpendicular sections, parallel 'bread-loafed' sections or 'en face' sections of the surgical margin of the large fixed tissue.

to extend very close to all of the lateral (and probably deep) margins. This could be processed in a number of different ways, as illustrated in Figure 2, including transverse and perpendicular sections, parallel 'bread-loafed' sections, 'en face' sections taken in the same plane as the entire surgical margin, or horizontal sections taken in the same plane as the skin. Unfortunately these approaches are generally uneconomic in most veterinary histopathology submissions due to the cost of processing tissue cassettes, and the large amount of pathologist time needed to examine every slide generated. Also, while there are standardised protocols in human medicine for organ trimming (King et al., 2015), these do not exist in veterinary medicine.

Consequently, a subjective decision is made to cut sections from areas considered most likely to be the 'close' or 'dirty' margins. The mass is sometimes obviously asymmetrical, with palpable areas close to one margin, or the surgeon may have indicated a margin of concern using suture tags or ink. The margin of a special tissue such as a digit or segment of bowel might be easily assessed via an en face section of the end(s) of the organ. However, in many cases (eg a typical skin mass removed via an elliptical incision, as in Figure 3) sections are chosen based on an assumption that tumours infiltrate and expand their sites in a symmetrical manner.

This means that, generally speaking, I select areas with the grossly closest lateral and deep margins to transect, and skip those areas deemed less likely to contain tumour. There might be up to two parallel samples taken through the grossly closest margins, or a transverse and one or two perpendicular or oblique areas might be sampled to include the 'next closest' margin(s). As illustrated in Figure 3, I might only examine marginal sections from a large mass.

This approach maximises the efficiency of histopathology, but still only allows the examination of a few 4-5µm-wide sections of the histological margin. Also, the assumption of tumour symmetry will not always be correct, since neoplastic cells can infiltrate widely and unpredictably within their sites

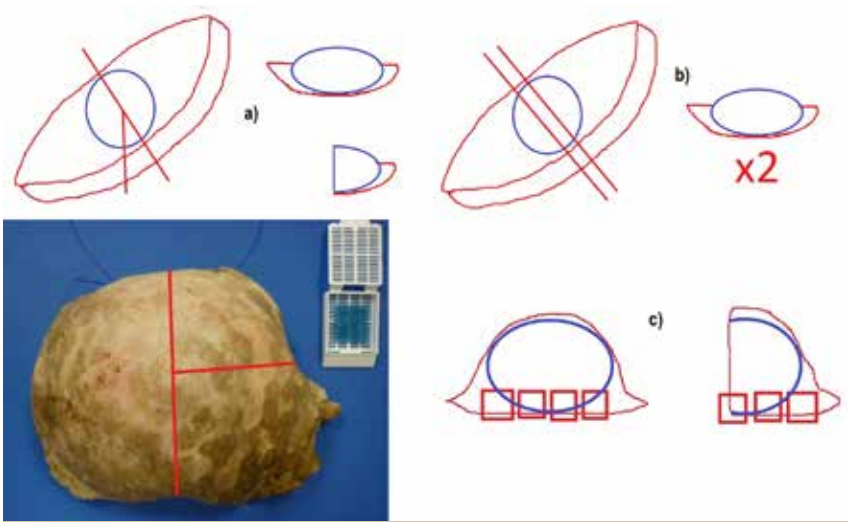
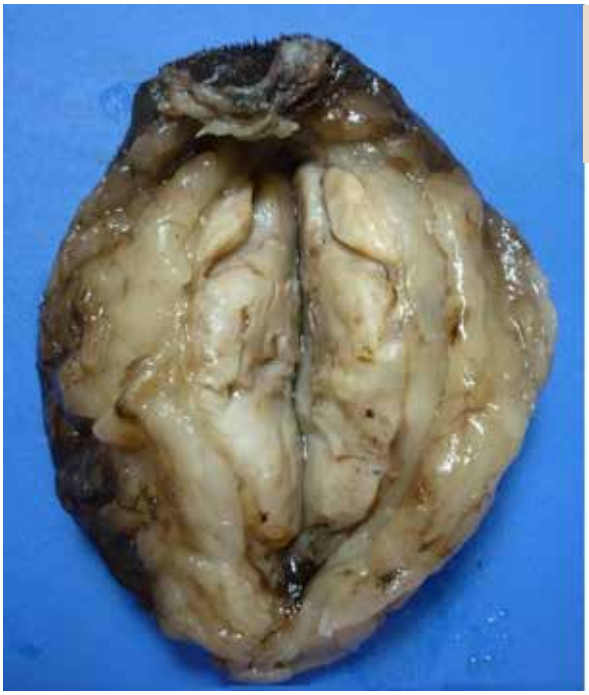


FIGURE 3: Various sub-sampling examples for a typical skin mass removed by an elliptical incision, including: (a) transverse or perpendicular/oblique areas sampled to include the grossly closest margin(s); (b) two parallel samples taken through the grossly closest margins; and (c) marginal sections from the large fixed tissue.



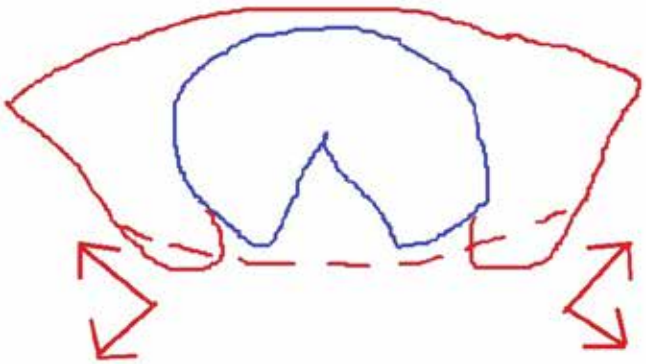


FIGURE 4: Tissue with a surgical margin incision prior to formalin fixation (left), and a graphic representation of how the margin retracts and distorts.

(especially in round cell tumours such as mast cell tumours). These facts probably help to explain why some tumours recur after reportedly 'clear' margins.

REPORTING THE MARGINS

When reporting surgical margins, I typically examine the slides in front of me and report the closest distance between the inked margins and the neoplastic cells using a millimetre-scale reticule built into my microscope. This could be anywhere along the lateral or deep margin.

I tend not to distinguish where the closest margin was located unless the surgeon has requested the examination of a specific region, since the surgical revision of a site for incomplete excision usually involves the removal of the entire wound and a margin around it. Therefore it may be irrelevant whether the 'dirty' margin was lateral or deep.

Two common issues arise when surgical margins are reported in this way. One is that there are few clear guidelines on what constitutes an acceptable histological margin in most tumours, especially since recurrence is not always predicted by dirty margins (Blackwood et al., 2012; Dennis et al., 2011).

Furthermore, tissues contract and warp after surgical removal and during fixation, sometimes by up to 40%, leading to differences between the margin identified at the time of surgery and the measurement made on histopathology. This process is complicated by margin retraction if the surgical margin was incised before fixation (Figure 4).

Generally speaking, incised margins will retract from the incision and become plumper compared with their 'in vivo' position, making it difficult to be sure how thick the margin really was.

Consequently, it needs to be understood that the true in vivo margin can only be estimated by histopathology, and that a subjective decision sometimes needs to be made on whether or not the margins are clear. The relative importance of margin size in comparison to other known prognostic factors for the tumour diagnosed should be considered.

SUMMARY

- The fixation and processing of tissues creates inevitable artefacts and can impair the assessment of margins.
- It is helpful if specimens are submitted with ink or sutures identifying areas of concern, and they should not be incised through the surgical margin.
- It is not realistic to examine the entire surgical margin in most cases.
- Clinicians need to understand that the reported margins are only an estimate of the in vivo margins, and need to be considered alongside other prognostic factors. VS

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