



Effective Inking For Tumour Margin Assessment

Margin assessment is a crucial part of reporting surgically excised tumours. To improve the accuracy of reported histological margins, samples should ideally be inked. There is growing recognition of the importance of inking yet there is limited practical information about the technique. This fact sheet aims to provide easy-to-follow guidance to allow practices to apply the ink before submitting the sample to the laboratory – as margins are best identified by ink applied immediately after tumour excision.

“you do not need to be a Picasso to paint margins” - Pepe Ramos, Purdue¹

What equipment do I need?

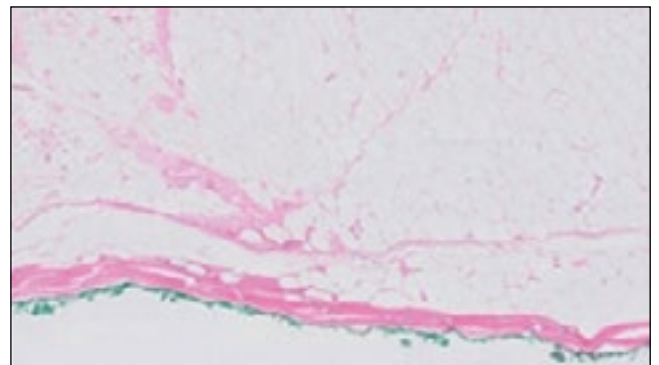
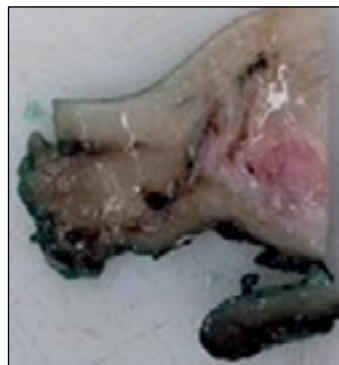
- **Suitable ink** – The Davidson Marking System Tissue Marking Dyes available from suppliers such as Solmedia:
<https://www.solmedialtd.com/product-category/histology/histology-products/sample-preparation-and-autopsy/tissue-marking-dye/>

*Please make sure the correct ink is used; using the wrong type of ink may potentially damage our laboratory equipment, as well as not being visible in the histological sections.

- **Space, gloves, swabs, cotton buds**

Choice of colours – not all colours are equally good.

- If the mass is pigmented, for example a melanoma (and oral and digits in particular), then avoid black ink and other dark colours.
- Avoid pale colours (e.g. yellow) which do not show well in the histological sections, or pink and purple as these do not always contrast well with HE-staining.
- As a general rule, green and blue tend to be the colours which show most clearly in the histological sections.
- If using multiple colours, ensure you choose colours which are clearly different to one another, and indicate on the submission form what each colour indicates.





What do I do?

1. Blot the sample so that the surface you want to ink is dry, using gauze swabs (Fig.1).
2. Apply drops of the ink onto a cotton bud. Using a rolling motion, use the cotton bud to paint the margins (i.e. the cut surfaces you wish to be histologically assessed) (Figs.2 & 3).

This might be all of the deep and lateral margins, or a particular area the surgeon is concerned about and wishes to highlight to the pathologist. Remember to indicate what/where you have inked on the submission form.

A wooden applicator stick can also be used. Do not pour the dye on to the surface, just apply as if "painting".

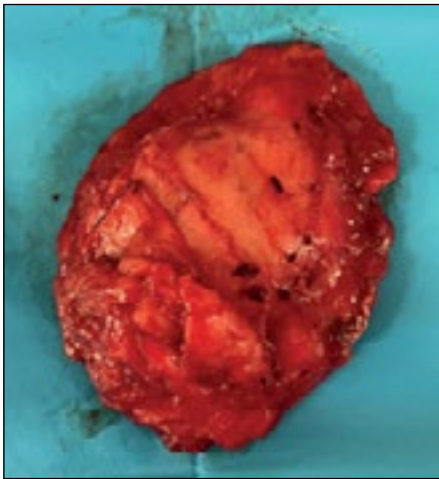


Fig.1

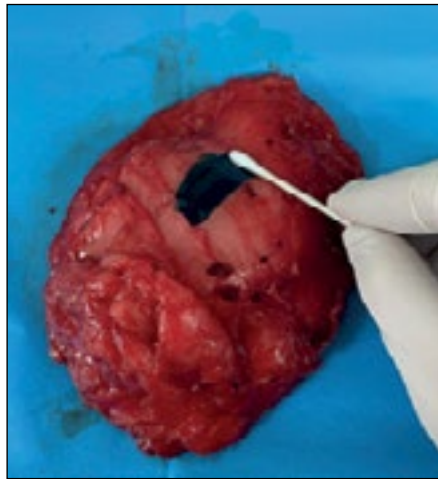


Fig.2



Fig.3

3. Once inking is complete, **leave the sample to dry for 15-20 minutes** before placing into formalin as per normal (Figs. 4 & 5).

* Don't worry, some of the ink will come off and colour the formalin!



Fig.4



Fig.5



Things to note:

- Margin reports are only needed if the surgery is intended to be curative (complete excision).
- The relationship between the tumour and surrounding tissues is altered after the tissue is placed in formalin and transported to the laboratory, therefore inking before this happens will give more accurate results.
- Avoid incising into margins before or after ink is applied.
- No tissue should be trimmed from the margins before inking and fixation.

Please remember that the tissue margin seen in histological sections is not equivalent to the margin the clinician observes at surgery, it is only an approximation. Tissue shrinkage occurs as a result of the surgical process (retraction), from formalin-fixation and processing and can vary by a factor as much as 50%. Therefore the “real margin” in the patient is larger than the reported histological tumour-free margins (by approximately 33%).

Photographs of inking the sample courtesy of Oliver Gilman, Highcroft Veterinary Referrals and Manchester Veterinary Specialists.

1. <https://www.addl.purdue.edu/newsletters/2004/winter/tubiop.asp>

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