



Submission of whole organs, large and challenging samples for histopathology

We realise that submitting some samples for histopathology can be really challenging, for example mammary strips, amputated limbs, whole organs and large tumours – this fact sheet aims to give some guidance for these difficult cases. Fact Sheet 6 also provides more general information regarding the submission of histopathology samples and Fact Sheet 3 provides information specifically regarding splenic samples, so these should also be referred to.

Don't forget, if you are unsure, you can always speak to us first!

Please do not hesitate to get in contact with us for further assistance on submission of your samples, and either our diagnostic support team or a duty pathologist will be able to give advice.

Question 1: Does the sample fit comfortably within one of our routine biopsy pots?

Please do not squeeze large samples into too small a pot as:

- a). this will not allow for adequate fixation - ideal tissue to formalin ratio should be 1:10; 1:5 or less is inadequate.
- b). the sample will take the form of the pot! Such samples are difficult or impossible to orient and to relate to the original specimen / *in situ* lesion, making accurate assessment of surgical margins in particular a real challenge!
- c). please do not be tempted to use inappropriate containers to submit samples, and under no circumstances should a sharps bin, glass jar, Tupperware container or narrow-necked pot be used in replacement of a biopsy pot.

If the answer to Q1 is yes, please follow our normal packaging guidelines (see Fact sheet 6 for further details).

If no, see below for question 2:



**A suitable volume of formalin:
tissue in a suitable sample pot.**



Examples of unsuitable containers, too small and with an inadequate volume of formalin – inadequately fixed tissue specimens which have taken on the shape of the pot.

Question 2: Can the sample be cut in half and submitted in two pots?

Or in quarters and into four pots?

Please do not section into smaller proportions than this, as it becomes impractical to re-orient and process

If the answer to Q2 is yes:

- Section the sample and submit in separate, clearly labelled biopsy pots.
- These should be accompanied by a sketch or digital image of the original specimen to depict sectioning and orientation of the sample.
- If margin evaluation is required, the surgical margins including the deep margin should be marked appropriately (e.g. with surgical sutures or tissue ink).

If no, see below for question 3.

Question 3: Does the whole sample really need to be submitted?

If the answer is no, then submit one (or more) relevant sections in one or more of our biopsy pots (see Fact sheet 6 for further details).

Retain the remainder of the sample in fixative within the practice until the histopathology report is received – if the pathologist requires further tissues, further tissues can then be submitted.

Some samples are best submitted whole, for example hearts, brains and sometimes spleens



If the answer to Q3 is yes, the whole sample does need to be submitted, then the sample will require fixation at the practice first prior to submission:

- **First fix the sample in a large container with a lid at the practice**
- 10% neutral buffered formalin should be replaced with fresh solution after 24 hours if the specimen requires longer fixation
- A ratio of less than 5:1 formalin:tissue is considered inadequate, optimal is 10:1
- Consider slicing the sample, removing unnecessary muscle, fascia etc. to allow for penetration of formalin into the tissues - a series of partial incisions 1cm apart into the centre of the tissue will aid tissue permeation and fixation (see Fact sheet 6 for further details)
- Consider de-bulking limbs of muscle and skin that will not be required for assessment
- The length of time the samples should be left to fix at the surgery will depend on the size/type of tissue and method of transportation to the laboratory; formalin penetrates tissues very slowly at approximately 1mm per hour, so specimens need to be left to fix for an adequate length of time.
- Specimens should be fixed for approximately 6-72 hours, preferably for a minimum of 8 hours or overnight, especially for larger specimens, at room temperature
- Anatomical barriers to fixation should be removed or incised where possible (e.g. fascia, bone, faeces, thick tissues); large samples should be sectioned or opened and gently cleaned (e.g. gastrointestinal tract) to allow penetration of fixative. Do not scrape mucosal surfaces.
- *Adequate fixation should not be compromised in the interests of minimalizing turnaround times*
- **Once fixed**, package as follows:
 - Wrap the sample in formalin soaked gauze/tissue to keep the sample moist in transit
 - Place inside two sealed leak-proof bags (clinical waste/cadaver bags recommended)
 - Wrap in absorbent material (newspaper/tissue)
 - Place inside a rigid container or box (please note a sharps bin is not a suitable container for this purpose)

Hearts

Submission of the whole heart, uncut, fixed in formalin is often the preferred sample, greatly increasing the likelihood of a diagnosis for conditions such as cardiomyopathy and congenital defects.

- remove the heart whole, and strip back the pericardium.
- flush any blood and clots out from the chambers using water
- record the fresh weight of the heart (with any blood and clots flushed out of the chambers)
- fix whole in formalin and submit.



Fixed specimen, whole heart from a cat with cardiomyopathy, examined and sectioned by the pathologist.

Spleens

– see separate Fact sheet number 3

Mammary tumours or strips

- Does the entire mammary strip need to be submitted, or is there a discrete mass of clinical concern? If the latter, then the mass and regional lymph nodes can be submitted separately; the remainder of the tissue can be retained in fixative in the practice until the final report is received.
- If necessary, the mass can be cut into sections and placed into individual labelled pots, and submitted with an accompanying trimming diagram or photographs as detailed above. Consider labelling of margins. Areas of interest / suspected masses should be clearly noted on the submission form / diagram.
- Some mammary masses may need to be cut with a saw if mineralized but hard portions need to be submitted as they are representative portions of the lesion. Some mammary samples may require decalcification once received by the laboratory and may have a longer turnaround time as a result (your practice will be informed in this situation).
- If the whole strip requires assessment, there is an additional charge for larger samples such as this (test code = LHIST). Fix the sample in the practice first, and consider slicing to improve fixation. Treat as a large sample, see above for further information.



Amputated limbs

- Does the whole limb need to be submitted? E.g. if a bone tumour is suspected, then submit the portion of bone with the associated mass / bone for assessment in the first instance.
- Larger samples will take longer to fix and then to decalcify, meaning a delayed report, possibly with poor tissue fixation hampering the histological assessment. Poor fixation and decalcification will also greatly hamper any additional testing such as immunohistochemistry.
- Consider de-bulking limbs of muscle and skin that will not be required for assessment.
- Retain the remainder of the sample, in formalin, in the practice until the final histopathology report is received.
- If examination of draining lymph nodes associated with limb amputations is required the surgeon should consider dissecting the node and submitting it in a separate biopsy pot, as the lymph node can be difficult to identify following fixation whilst still within the limb tissue.
- If assessment of margins is required, these can also be submitted separately, either clearly marked for orientation, or as samples to determine the presence or absence of neoplastic tissue in the sample.

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