**Vetpath** is a specialist veterinary laboratory dedicated to providing our clients with the finest laboratory diagnostic service. A team of veterinary pathologists and medical scientists with extensive experience in veterinary diagnostic pathology forms the core of the Vetpath team.

#### **MAY 2016**

## Is it real and is it relevant?

The pathologists at Vetpath are trained to detect and interpret abnormalities in laboratory data and blood, urine and tissue samples.

But identifying an abnormality is not enough! The accuracy and relevance of the finding must also be determined and that can often be the more difficult task. This is where training and experience are invaluable.

An example of differentiating between a real or artifactual finding is the presence of stain precipitate on a blood smear (see figure 1). Stain precipitation on blood and cytology smears can be mistaken for infectious agents such as haemoparasites or bacteria. This is particularly problematic with old stains or if there has been inadequate rinsing, and the stains used at Vetpath are filtered multiple times during preparation to counteract this problem. The precipitate is often in a higher plane of focus and can usually be distinguished from true infectious agents.

NEWS



**Figure 1**: Stain precipitate on a blood smear.

Even if the pathological finding is accurate, it is not necessarily significant. An example of this is the presence of crenation of erythrocytes (echinocytes). These cells are often present due to exposure of erythrocytes to excess EDTA; this can occur when only a small amount of blood is placed into the blood tube, or if there is a significant delay in processing of the sample. *In vitro* crenation is not clinically significant and needs to be differentiated from poikilocytosis due to a vascular bed abnormality (acanthocytes or schistocytes). Submission of an air-dried, unfixed smear with the EDTA sample will help prevent artifactual changes in red cell morphology.



**Figure 2:** Echinocytes on a blood smear.

Critical assessment of laboratory data will help to reduce incorrect interpretation of abnormalities. If you have any questions about potential abnormalities in your lab data, please call to speak with one of our pathologists.

Vetpath Laboratory Services welcomes feedback on all aspects of our service from couriers to lab results. Please feel free to contact us at 9259 3666 or email enquiries@vetpath.com.au

# The facts on formalin

Adequate fixation is one of the most important factors determining the quality of histological samples. The most common fixative used is 10% neutral buffered formalin.

There are several aspects of formalin usage that can influence how successfully a tissue is fixed. Autolysis begins immediately following disruption of the blood flow, and therefore rapid fixation is imperative. Samples should be placed into formalin immediately, and then allowed to fix for at least 24 hours at room temperature before sectioning. Some tissues may require a longer fixation period, particularly if they are bloody or contain bone.

The thickness of the tissue is very important for adequate fixation. Formalin may not diffuse into the tissue if the biopsy is thicker than 1cm. Larger lesions, such as entire spleens or large skin tumours should be sectioned before submission to prevent autolysis of the central tissue (see figure 1). Care should be taken to avoid damaging the tissue with forceps during this process. Sufficient formalin should be used to create a ratio of 1:10 (see figure 2). If in doubt, use a larger pot

with more formalin, or section the tissue to reduce the size of the biopsy.



**Figure 1:** A large biopsy in a small pot with inadequate formalin.



**Figure 2:** A biopsy with the correct tissue:formalin ratio.

While formalin is essential for histopathology, the opposite is true for cytology and microbiology samples. Exposure to formalin liquid and fumes (eg in the submission bag) will render cytological samples unusable. Cytology stains cannot penetrate the cells on smears exposed to formalin. This results in the cell morphology being impossible to evaluate. Formalin will also hinder growth of infectious agents and should therefore be kept away from tissue samples intended for culture. Always submit biopsy samples in separate specimen bags to avoid contamination of other samples.

### Beware the under filled citrate tube

The ratio of blood to citrate is extremely important for accurate PT and PTT results.

Under filling the citrate tube results in overcitration of the sample and prolonged PT and PTT. It is then impossible to determine whether a true haemostatic disorder is present, or the abnormal results are artifactual.

We appreciate that it can be difficult to obtain sufficient blood from a small, sick and possibly anaemic patient. However, care should be taken to obtain the correct volume of blood required for the citrate tube. Paediatric tubes are available for our small patients to ensure the minimum volume of blood is required.



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