Recommendations for Fixing Techniques for Terrestial Invertebrate Histopathology

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Histology can be a valuable tool in determining cause of disease in veterinary medicine where post mortem examination or gross examination alone can be limited. There has been some work establishing some reference histology in various invertebrate species, but this is relatively small compared to vertebrates (Cunningham, 1997). There may well be diseases and pathology present in invertebrate collections that we are unaware of because histopathology isn't commonly applied. Clinical pathology can guide future procedures and medicine thus decreasing levels of disease and pathology in collections. This in turn could result in animals with a longer and healthier life span, and in more fruitful captive breeding programs.

Euthanasia and fixation guidelines

Invertebrates should be fixed as quickly as possible as they are prone to desiccation and autolysis. Any invertebrates that are found dead should be fixed and/or sampled immediately if good histology is to be attained. In the Author's experience fixation within 12 hours of death is ideal. The level of autolysis will be variable depending on the humidity and temperature of the vivarium. Even if an animal has been deceased for more than 24 hours, viable histology can still be attained (Berzins et al., 2011).

Having a set protocol and plan in place for specific exhibits/animals (those of high conservational and economic value or those exhibits that are having significant problems with disease) can reduce the time needed to decide to fix and in turn improve the quality of the information gained from sampling.

Ideally, if an invertebrate has significant disease with clear lesions (and treatment has either been attempted or isn't possible), then euthanasia and immediate fixation will result in better and more viable histology. Euthanasia via freezing is not conducive to attaining good histology. Barbiturates or potassium chloride under general anaesthesia work well as euthaniasia methods (Bennie et al., 2012; Cooper, 2011).

When fixing an animal post-euthanasia, ensure that volume of fixative is at least 8-12 times the volume of the animal. Making an incision with a needle or scalpel blade through the dorsal or ventral midline of the exoskeleton will result in faster uptake of the fixative. In addition to this, separating the opisthosoma and prosoma (abdomen and thorax) will further improve fixation. Alternatively, if a member of the team is confident with pericardial injections, then injecting fixative directly into the pericardial sac following euthanasia can be another way of ensuring rapid fixation.

In a nutshell, the more immediate the fixation following death, the better the histology and the better the conclusions that can be gained.

Fixation mediums

There are multiple different choices of fixation mediums available. The following examples mentioned here are: 70% isopropanol, 10% formalin and Kahle's fixative. Exchanging water components with saline or sea water can improve quality of histology in crustaceans and other saltwater based invertebrates. (Berzins et al., 2011; Donald V. Lightner, 2017; Howard et al., 2004)

70% isopropanol fixation can harden tissue and so it can be more time intensive and challenging to generate slides from invertebrates as the exoskeleton can become difficult to manipulate and this can result in a loss of detail on histology. Other diagnostic tests can be performed on isopropanol fixed samples beyond histology. Isopropanol is less toxic than alternatives but is very flammable so it should still be handled and stored carefully. As isopropanol is not as caustic or toxic as formalin there are less concerns about its use.

Formalin fixation is a well-established fixation medium and will be available from the majority of veterinary surgeries. The exoskeleton is still prone to harden but less so than isopropanol. Histology tends to be better with formalin but further diagnostics are limitted (i.e. genetic and biochemical testing). Formalin is toxic and thus care should be taken when handling and disposing of it. This can make it a difficult choice for zoological collections due to concerns with its use by non-clinical personnel.

Kahle's fixative is a mixture of fixatives with acetic acid. The acetic acid works well to soften the chitinous exoskeleton of invertebrates. It isn't appropriate for long term storage. Fixation in Kahle's for 24-48 hours (depending on cuticle thickness) is usually sufficient before transfering to another fixative. The Kahle's recipe could be made up by any good pathology lab should anyone wish to have this on hand.

References

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