

## **From Formalin to Fax - A Brief Guide to Processing**

Before we can cut a histological section of your tissue sample, the tissue must be processed and embedded in a block of hard wax.

Representative sections are cut from the sample (unless the sample is too small to warrant such treatment) and are placed in a labeled plastic cassette.

The cassettes are loaded into one of our two processors where they are subjected to alcohol solutions of increasing concentration to dehydrate the tissue. Next they are immersed in a 'clearing agent'. This is a solvent which is miscible with both the alcohol and the wax, to ensure that the tissues will not repel the wax. This process can take 18 hours or more but, by investing in 'state of the art' processors which use pressure and vacuum infiltration, the time for this phase of processing has been cut to 4 hours in our laboratory.

After the cassettes are removed from the molten wax, the tissues, which will have shrunk considerably, are embedded in stainless steel cassette moulds to ensure appropriate orientation of each sample. The plastic cassette cover is incorporated into the solid wax mould to allow identification and archival of the block.

Each block must then be 'trimmed in' on a microtome to remove excess wax from the surface and expose the tissue. A section 5µm thick is then cut and placed on a microscope slide. This section must be baked onto the slide in a low temperature oven.

As the tissue stains have an aqueous base, the section must now be de-waxed and rehydrated prior to staining!

We routinely stain all sections with haematoxylin and eosin (H&E), but have the capacity to perform many other special stains at the request of the pathologist. Such stains may help to identify pathogenic organisms or aid in the differentiation of cell types. The special stains which we use most regularly include periodic acid Schiff (PAS), Ziehl Nielson (ZN), Giemsa, Gram and toluidine blue.

After staining the section must be protected with a coverslip and its quality checked. The prepared slides are then delivered to the pathologists.

Having examined the slide and considered the history, the pathologists enter their diagnosis, prognosis and commentary directly into the computer. Once

satisfied with their report, they then 'sign off' the case, at which point the computer will automatically fax the report to the submitting veterinary practice.

Not all tissues are suitable for processing immediately on receipt; some require further fixation or gross examination by the pathologist prior to tissue trimming, other samples, such as bone, may be mineralized and have to be decalcified in formic acid prior to sectioning. Tissues such as tendon, nail, beak and hoof are soaked in a phenol solution to soften them for sectioning. The time taken for adequate decalcification is variable and large bony specimens may take several weeks to be ready; all samples in 'decal' are checked twice weekly and are processed as soon as possible.