- Histology -
Tissues To Slides
Tissues are fixed before being processed for histology, they should be in the fixative for a minimum of 12 hours for the process to become irreversible. There are many different types of fixative but the most commonly used is neutral buffered formalin or NBF.
After fixation tissues are cut up so that the areas of interest can fit into standard histological processing cassettes.
These are then snap closed ready for processing.
The automatic tissue processor has a series of pots containing alcohol of increasing strengths to remove the water content of the tissue. These are followed by pots containing a solvent which acts as a link between the alcohol and the final molten wax baths.
The aim is to infiltrate the tissue with a special wax in order to embed it into a solid matrix so that slices can be cut from it. The process takes a minimum of 18 hours to complete and can be up to 3 days for large or dense samples.
Once the tissues are processed they must be placed into moulds containing molten wax which is then allowed to set.
This slide shows the metal mould and the processing cassette which will be used in the finished wax block to allow it to be clamped into a microtome for cutting.
The tap supplies molten wax at about 65⁰C which is used to fill the mould. The tissue is then placed centrally in the mould and the cassette is placed on top. The whole thing is then allowed to set hard on a cold plate.
This is the wax block, you can see the tissue embedded in the wax and the cassette which acts as a firm base.
The wax blocks are clamped into a microtome, this is a rotary type and is used to cut thin slices or sections from the block.
This is a sledge microtome. It does the same job but has a moving base that runs backwards and forwards rather than a pivoting holder that goes up and down. It can cut harder and larger tissue blocks than the rotary version. You can see a ribbon of them in the photo on the right. They are just 4-5µm thick.
Sections are then floated onto a water bath containing water at around 40°C. This softens the wax and allows any creases in the sections to smooth out.
The smoothed sections are carefully lifted from the water surface using a suitably sized microscope slide that is usually coated with one of several types of adhesive. They are then drained of excess water and dried on a hotplate set to around 50-60°C. The drying firmly sticks the tissue to the slide so it can be stained at the next stage.
The slides are passed through baths of solvent which remove the wax, then through graded alcohols and finally into water. They then pass through baths of haematoxylin and eosin to stain them.
The stained sections are mounted in a mounting medium (often DPX but there are many types) under a glass coverslip. The medium sets hard and keeps the sections safe and allows them to be clearly seen under the microscope.
This is a section stained with H&E being mounted in DPX
This is a photo of an H&E stained section from the microscope.
There are many different staining techniques that can be used to identify different tissue components but every tissue section receives a haematoxylin and eosin (H&E) stain first. Other sections from the same block may later receive other stains to help the pathologist in their diagnosis.

The stains shown above are (from left to right) Grocott for fungi, Periodic acid/Schiff for carbohydrates and Warthin/Starry for spirochaetes
For more information see the Veterinary Pathology web site:
http://www.bristol.ac.uk/vetpath/cpl/cpl.htm
under Laboratory Protocols