

Points of interest:-

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The Art of Histopathology

Despite efforts to introduce automation into histopathology it is still the most skill driven of all the pathology disciplines. Exceptional experience and knowledge is required at every step of the process. One of the most crucial steps in preparing tissues for histological examination is "Fixation". For routine purposes the excised tissue sample is placed in 10% Formalin solution. This is prepared from reagent Formaldehyde 40% and water. There is no particular benefit from using saline. Fixation preserves the microanatomy of the tissue. To avoid autolysis and other artefacts it is important to ensure full and rapid penetration of the tissue by the fixative. If possible the maximum thickness of the tissue should not be more than 10 mm. Larger pieces of tissue should be incised at 10 mm intervals to aid fixation and place in an appropriate volume of fixative.

On receipt, the tissue is carefully examined and where necessary a portion or portions of suitable size for further processing will be dissected from the main specimen. The tissue(s) are placed in a perforated cassette together with an identification label. With fine biopsy samples or curettings the specimen(s) may be placed in a fine wire gauze cassette or occasionally folded into a tissue paper parcel to ensure that fragments are not lost during processing. A macroscopic de-

scription of the specimen together with dimensions, the cutting plane and orientation is recorded. Specimens containing bone or other hard tissue require decalcification with formic acid or treatment with a softening agent. Unfortunately this step may take from 2 to 14 days to accomplish dependant upon the size and density of the specimen.

The ultimate objective of the processing stage is to place the fragments of tissue in a supporting wax block to enable extremely thin sections of the tissue to be cut. To achieve this the water based fixative has to be removed by dehydrating the tissue using increasing concentrations of ethyl alcohol (industrial spirit). This is then replaced by a petroleum based solvent (clearing agent) usually Xylene which is miscible with both the alcohol and molten wax based embedding medium. This is basically paraffin wax containing modifying polymers to reduce the natural crystalline structure and aid the cutting process. The final embedding process is accelerated by the use of a vacuum. The process is completely automatic undertaken in an enclosed computer controlled tissue processor. The process usually takes overnight but can in certain circumstances be completed in less than 4 hours. The tissues are removed from the cassette and placed in a stainless steel mould of appropriate size containing



**Shandon Excelsior
Tissue Processor**

hot molten wax. The mould is placed on a refrigerated surface to start the solidifying process and the tissue is carefully orientated before the wax becomes completely solid. Ingeniously, the plastic cassette is incorporated into the wax mould to provide a solid base for fixing the wax block into the chuck of the Microtome, the instrument used to cut the tissue sections.

Each block must then be 'trimmed on the microtome to remove excess wax from the surface and expose the tissue. A representative section of the tissue, 5µm thick, is then cut and placed on a microscope slide. This section is floated onto a glass microscope slide using a warm water bath to remove any wrinkles before finally heating and drying the slide on a hot plate or in an oven to ensure the tissue ad-



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continued **The Art of Histopathology**

heres to the slide.

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

Sections are routinely stained with haematoxylin and eosin (H&E). Frequently additional special stains may be requested by the pathologist. Such stains

may help to identify pathogenic organisms or aid in the differentiation of cell types. Commonly used stains include periodic acid Schiff (PAS), Ziehl Nielson (ZN), Giemsa, Gram stain and toluidine blue. After staining the section is protected with a thin glass coverslip. The quality of the section and the staining are carefully quality controlled be-

fore presentation to the pathologists.

Pathologists examine the sections and dictate their findings directly into the computer using voice recognition software. The report is authorized and printed. At this point the report may be automatically faxed to the recipient or increasingly will be emailed automatically.

"Pathologists dictate their findings directly into the computer using voice recognition software"

The PET Scheme In Action

Approximately 5% of animals fail the Rabies blood test

Dr Paul Burr BVMA PhD MRCVS of Biobest Laboratories made a presentation to the North West Clin Path Club at the Swallow Hotel, Preston, Lancashire about the PET scheme. Over 60 Veterinary Surgeons and Veterinary nurses attended.

Paul explained the pitfalls of the complex paper chase required to enable Cats and Dogs to re-enter the UK after a visit abroad. He warned that the identification Microchip is one of the major stumbling blocks. Because the Microchip ID numbers are long and complex it is all too easy to introduce transcription errors into the paperwork when filling in the forms. He warned also of the importance of checking that the microchip was still readable before setting off as there had been a number of embarrassing microchip failures. It was also important to ensure that the microchip complied with the current ISO standard to ensure that it could be read by any microchip reader. Owners have been known to carry their own reader with them just in case.

As many as 5% of animals are refused re-entry into the UK due to non-compliance with the requirements of PETS. Many of these are due to a failure to comply with the tick treatment requirements but 4% of these are due to paperwork or microchip irregularities.

Since the introduction of the scheme Biobest has tested many thousands of rabies samples. Paul commented on the vast amount of data that had been accumulated in this time. Through analysis of the data Paul had some interesting observations to offer: -



- **Approximately 5% of tested animals fail the test**
- **It is important to keep as close to the 30-day test interval as possible.**
- **Titres in single vaccinated animals can fall quite rapidly after 59 days**
- **Large breed dogs are more likely to fail the test**
- **Vaccination is less reliable in dogs, less than 6 months old.**
- **Two doses of vaccine reduces risk of failure**
- **Cats have fewer failures than dogs**
- **Older cats, >14 years, respond poorly to vaccination**
- **The vaccines on the market all produce different antibody responses**
- **Some dogs do not respond to vaccination at all**

Paul mentioned the importance of sticking to the letter of the scheme particularly with regard to booster vaccinations. Being as little as a day late with your re-vaccination will result in the whole vaccination process having to be started from scratch, including a repeat blood test.

In the second part of his talk Paul emphasised the risk of pets acquiring arthropod borne infections and tapeworms whilst abroad. He remarked, it is noticeable that despite the requirement to treat animals with parasiticide and insecticides before re-entry into the UK there has been a noticeable increase in the incidence of diseases such as Babesiosis, Ehrlichiosis, Leishmaniasis and Heartworm.

Paul pointed out that the treatment regimes required by PETS were more to protect the resident animals of the UK from returning travellers rather than to protect the pet whilst travelling. Paul suggested that practices could provide pre-travel clinics where advice about the PETS could be provided at the same time, offering guidance and advice as to how best to avoid acquiring these "foreign" diseases.

NWL Accredited for Opinions and Interpretation

As part of our continuing drive to provide clients with the highest level of professional excellence in quality assurance, NWL has been awarded an extension of their UKAS accreditation to include the provision of Opinion and Interpretation. The international accreditation standard for testing laboratories, ISO/IEC 17025 includes not only the provision for accrediting the results generated by a laboratory but also any opinion and interpretation relating to them.

NWL is the only commercial veterinary pathology laboratory to be able to

offer this level of assurance to their clients. Only laboratories accredited to ISO/IEC 17025 can offer clients an assurance of technical competence and tests that are validated and "fit for purpose".

Nationwide Laboratories is a UKAS accredited testing laboratory No 1733

Further information about UKAS accreditation is available on the NWL website www.nwllabs.co.uk/ukas/



Anti-Platelet Antibody Test

In future, the Anti-Platelet Antibody test will be carried out using a highly sensitive flow cytometry technique. This will replace the less sensitive visual fluorescent techniques. The test has been developed by Elizabeth Villiers at the Cambridge Vet School and currently samples are being referred to her.

Because platelets have sensitive labile receptors for immunoglobulins on their surface, ageing of the sample can cause difficulties in interpretation of the results once the sample is over 36 hours old. To ensure the best possible result from this test it is important to send the samples directly to the Cambridge Laboratory.

Samples: 5ml whole blood in EDTA and 2x Blood smears

Samples should be sent by recorded

delivery/first class post and arrive within 36 hours to be useful. Samples should not be sent on Fridays or over the weekend.

Send samples to:-

**Clinical Pathology Laboratory
University of Cambridge
Department of Clinical Veterinary Medicine
Madingley Road
Cambridge.
CB3 0ES.**

If you require further information please contact Mrs Elizabeth Villiers on:- 01223 337635.

Same Day Courier

NWL is the only laboratory to offer an extensive Sameday courier service. The service currently collects samples throughout the North West 5 days a week. The collection area currently extends throughout Lancashire, Merseyside, Wirral, Cheshire, North Staffordshire and Greater Manchester. Plans are in hand to further extend the service.

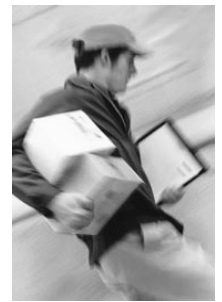
The same day service ensures safe handling and reliable delivery of the samples to the laboratory. Where possible results are processed and reported the same day as received.

To discover if the Sameday

Courier service is available to your practice please contact Client Services Manager, Joanne Kenyon.

PS. If you are an NVS customer enquire about the free sample collection service, contact your NVS contact.

PPS. A national nextday courier service is also available contact Joanne Kenyon



Innovative Integrated E-mail Reporting

To meet the ever increasing demand for faster reporting we have recently introduced a major upgrade to our E-mail Reporting option. You can now elect to receive your reports by E-Mail. There are a number of potential benefits to using this route of delivery. Reports are released from our system more quickly because there is effectively no queuing bottleneck as can occur with Faxes at peak times. Reports are delivered to your E-mail client as plain text messages. If you have an "always-on" Internet connection such as Broadband or BT SurfTime you can set up an E-mail client such as Outlook, to print reports the moment they hit your In Box. This gives you the instant response of a Fax machine. The advantage of plain text E-mail reports is you can integrate the information directly into your patient files. This can be done by cutting and past-

ing the text or depending upon which practice computer system you have the report can be imported directly into your patient files. Systems which can do this includes Veterinary Solutions systems such as PremVet, Robo-vet, Mid Shires, Mosaic Vet-Tec and RX Works .

E-mail reporting can be quicker and more convenient than fax with the major additional benefit of being able to cut and past text into your patient records or automatic integration with your practice computer system.

If you wish to have your reports sent by E-mail please contact client services. For information about importing results into your practice computer system you will need to contact your supplier.

BSAVA Congress Memories

It is BSAVA congress time again and our 20th visit to the event. It seems such a long time since those early days in the basement (Atomic Bunker) of the Hammersmith Novotel. A recent visit to Harrogate brought back hazy, inebriated memories and the challenge of a free meal if you could finish off a 14 lb steak. I didn't even try. How Congress has changed in that time. From small acorns grow strong trees. In addition to being an important business opportunity the unquestionable attraction of the annual visit to Congress is the social side of the event. An opportunity to meet up with old friends and business colleagues and of course the chance to make new friends. It is so good to see the same old faces year after year, old unfortunately being the operative word, as the years pass by. As I retire this year

this will be officially my last Congress, although that may remain to be seen!

It has traditionally been a highly stressful time particularly in the early days, building the stand bundling it all into the car and heading off after the finish of work to screw the screws and stick the Sticky Fixers. How envious we were of those who could afford to pay for someone else to do it all for them. It doesn't matter how organised you try to be there always seems to be a last minute crisis, usually because of tight deadlines with the printers. No doubt we shall have yet another record Congress I just hope I can manage to pick the Grand National winner, always one of the highlights of being at Congress.

Alistair Parker



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Professor Donald F Kelly Appointed as Consultant

We are delighted to announce that Professor Donald F Kelly MA, PhD, BVSc, MRCVS FRCPath, DipliECVP has been appointed as a consultant histopathologist.

Professor Kelly recently retired after 21 years as head of Veterinary Pathology at Liverpool University Veterinary School. He is a recognised expert in the field of histopathology and enjoys an international reputation. Professor Kelly's appointment will add considerably to the strength of the NWL pathology team



Clin Path Club

The March meeting was addressed by Dr Paul Burr BVM&S BSc (VetSci) PhD MRCVS of Biobest Laboratories with a presentation about the PET scheme. Over 60 veterinary surgeons and veterinary nurses enjoyed a very informative talk. See report on page 2.

The next meeting is Thursday 13th May at the Swallow Hotel Salmesbury, Preston.

Speaker : Robert Eustace BVSc CertEO CertEP FRCVS—Practical tips on dealing with Laminitis

Further information is available from the link on the NWL web site www.nwllabs.co.uk

The Clin Path Club is hosted by Nationwide Laboratories and is open to all Veterinary Surgeons and Veterinary Nurses

Tail End

OFFICIAL PETS

The White House has seen its share of official pets. Nixon's dog inspired his famous "Checkers" speech.

Bill Clinton's cat (Socks) inspired many jokes on late night television. John Quincy Adams kept an alligator in the East Room. Teddy Roosevelt had a lion and two bear cubs. Woodrow Wilson had a tobacco-chewing ram named Ike...

And Andrew Jackson? He had a parrot, Polly, who was taught to curse in English and Spanish – and had to be removed during Jackson's funeral in 1845 when it wouldn't stop 'practicing'!

More recently, Sunny, an African Grey parrot, was taken ashore when the Queen arrived aboard the HMS Lancaster for an official visit. Among Sunny's talents? Telling top brass to "---- off" and shouting "Show us your t---s" at the women on board.]

Quote

"Dogs look up to us. Cats look down on us. Pigs treat us as equals"

Winston Churchill

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