

NATIONWIDE LABORATORIES AUTUMN NEWSLETTER

KINDER TO THE PLANET AND TO EACH OTHER

BLACK FRIDAY LEARNING WITH NWL: FREE CPD WEBINARS

HELP! IT'S A FERRET by Madonna
Livingstone BVMS MRCVS

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Basic cytology for practitioners by
Trevor Whitbread BSc BVSc DipECVP
MRCVS

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**Hypercalcaemia in dogs and cats: an
Overview** by Stacey A. Newton BVSc
Cert EM (Int Med) PhD FRCPath MRCVS

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**Lunchtime Basic Bitesize Pathology:
Small Animal Mammary Pathology** by
Sandra Dawson BSc BVMS FRCPath
MRCVS

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**What are you itching to know about
feline atopic skin syndrome?** by Dr
Rosario Cerundolo DVM, Cert. VD, Dipl.
ECVD, FRCVS

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**Clinical Pathology in cats and dogs -
why species, breed and age matter** by
Helen Campbell BVM&S FRCPath
MRCVS and Alina Bodnariu DVM MSc
PhD FRCPath MRCVS

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Message from David Charvill

Dear friends,

Welcome to our autumn newsletter. Having just come back from The London Vet show, it was a really great chance to actually meet up with our clients and colleagues after all this time. It was great to hear and discuss challenges facing both old and new clients in the current climate and to have the opportunity to discuss the high quality service we aim to provide to our clients. This was the first large face-to-face event we have attended in the last 2 years, so we hope there will be many more to come. This autumn we proudly supported a number of online events too, including BVDSG and BSVP autumn meetings and ESVCP/ECVCP congress and we will be welcoming you at BSAVA 2022 both in person and virtually. With all the recent events that have gone on in the world including the pandemic and more recently the COP26 meeting in Glasgow, the sustainability pledge, kindness to our planet and to each other has become even more important to talk about. NationWide Laboratories will continue donating old laboratory equipment, such as microscopes, to schools rather than disposing of them in landfill. Our non-recyclable plastics are sent to an energy recovery facility and are used for electricity generation. PPE such as vinyl, nitrile and latex gloves are recycled through the Terracycle Zero Waste box programme. We are keen on reducing travel by offering versatile online learning opportunities to veterinary professionals. Our price list 2022 will be printed with vegetable inks on Carbon Balanced Paper through the World Land Trust scheme. We are moving forward with the times and we would like to invite you to be part of this journey.

David Charvill,
Director of Laboratory Services

The grading of tumours

The grading of tumours has become an integral part of the art of diagnostic pathology. Most grading schemes have numerical grades and I have always been concerned that putting a number on a grade implies to clinicians objectivity. Pathologists do their best to be as objective as possible but there is still a varying degree of subjectivity and there are a number of published papers confirming this. Grade should therefore be considered as a guide.

A major factor in grading schemes is the mitotic rate. One could be forgiven in assuming that identification of mitotic figures is relatively straightforward and the most objective feature of a scheme. However, there are a number of papers documenting the inter pathologist variation in identifying mitotic figures from mitotic-like figures. In the published data disagreements between pathologists occurred with frequencies of 6% to 35%, 10% to 46%, 17% and 68% depending on the publication. This has been considered a significant enough problem for the journal Veterinary Pathology to publish a guide to differentiating mitotic figures from mitotic-like figures.

Although things are improving there are also a number of grading schemes with insufficient follow up studies using those schemes to make them relevant to clinicians.

Grading is probably better than nothing in informing you, the clinician, as to the likely outcome or the aggressiveness of therapy but should be considered a guide. Even after numerous follow up studies on a particular tumour, biological variation is also a factor in prognosis. Note a 15% to 20% discrepancy between grading and behaviour of mast cell tumours irrespective of the grading scheme used. In addition, there is one paper in Veterinary Pathology which concluded that the use of a grading scheme for osteosarcomas did not correlate at all with outcome.

Vet. Path. (2021) Vol. 58, 243-257.

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WHAT'S IN THE PRESS?

What do I need to know about allergy testing? by Stacey A. Newton BVSc Cert EM (Int Med) PhD FRCPath MRCVS

Allergy testing is a useful tool that should only be performed when a diagnosis of allergic disease has been made.

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Investigation of calcium disorders and sample preparation by Stacey A. Newton BVSc Cert EM (Int Med) PhD FRCPath MRCVS

Sample preparation is essential to avoid incorrect or spurious results when investigating calcium disorders using parathyroid hormone and ionised calcium measurements.

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Histology samples memo



Specimens should be no more than 10mm thick. Fix in 10% neutral buffered formalin (NBF) which is available on request. Interpretation will be aided by a relevant clinical history, description of the lesion and site.

Tissues fixed in formalin should be placed inside a well-sealed plastic histology container (please do not use narrow necked containers). The histology container must be enclosed in sealed plastic bag with enough absorbent material to absorb the formalin in the event of a leak. This should be placed in an additional outer bag or container.

Please note: all pots should be properly labelled and have the animal's and owner's name.

Large specimens

All biopsies should be fixed in 10% NBF. Maximum tissue thickness to allow optimal penetration of NBF is 10mm.

Some samples are too large, e.g. mammary strips, spleens, limbs. You can either take multiple representative samples of up to 10mm from different areas of the lesion. We will examine up to 4 specimens for the same price.

Or alternatively, if you want to send the whole sample please fix it prior to sending in a large container containing 10% NBF at the practice for 48 hours prior to submission.

You will need to make some incisions into the sample approx. 10mm apart to allow it to fix.

After 48 hours fixation, take out the specimen and then wrap in wet paper towels, place in a sealable plastic bag or container with a sealable lid. Double bag with cotton wool between the bags to absorb any fluid that might leak out. **Please make sure the sample will not leak.**

Cytology slides

Cytology slides should never be packaged in the same bag as tissue fixed in formalin. The slides should be put in slide mailers in a separate bag to avoid contamination from formalin fumes as the fumes from the formalin cause cellular changes which can affect the reading of the cytology. **Please note: all slides should be properly labelled and have the animal's and owner's name.**

WHAT'S IN THE PRESS?

Sampling and packaging techniques for histology samples, and how to deal with unusual biopsy specimens by Sandra Dawson BSc BVMS FRCPath MRCVS

Some biopsies are not straightforward, but these sampling and packaging tips will help ensure specimens arrive at the histology laboratory in good condition and can be easily examined.

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Immunochemistry at NationWide Laboratories by Sandra Dawson BSc BVMS FRCPath MRCVS

General practitioners are increasingly faced with a histology report which suggests they use immunohistochemistry to further classify the tumour at hand – but what does this entail?

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Histiocytic diseases in dogs and cats: an overview of clinical presentation and diagnosis by Alison Lee BSc MVB MRCVS DipACVP

This is an overview of histiocytic diseases in dogs (histiocytoma, cutaneous Langerhans cell histiocytosis, cutaneous histiocytosis, systemic histiocytosis, histiocytic sarcoma and dendritic cell leukaemia) and in cats (progressive histiocytosis, histiocytic sarcoma and pulmonary Langerhans cell histiocytosis), including their cellular origins and clinical presentation. An overview of their diagnosis, including the use of cytology, histopathology and immunohistochemistry is also provided.

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Getting the best from your pathologist: post-mortems and surgical biopsies - tips and tricks for vets in practice by Alison Lee BSc MVB MRCVS DipACVP and Danilo Gouveia Wasques MV MSc

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Histopathology workflow: from pot to final report

Samples from veterinary practices are collected by the courier and delivered to our Poulton laboratory. All histology samples are transported overnight to our site in Newton Abbot. The samples are received at Newton Abbot in the morning and the sample processing starts on the same day. Technical staff in the laboratory sort and log the samples. They then grossly examine the specimens and take representative samples for histologic processing (trimming). If samples are large or complex (e.g. large tumours, amputated limbs, eyes, digits, etc), a pathologist examines and trims the specimen. The samples are processed overnight, and the slides are prepared the following morning to be read by the in-house pathologists. Slides are usually ready for examination by late morning-early afternoon and a report will be issued after the slides have been examined by the pathologists.

This process may be prolonged in the following situations:

Delivery time: If histopathology samples are sent to our Poulton site, they then must be delivered to Newton Abbot. To minimise transit time, urgent histopathology samples can be posted direct to Newton Abbot (Nationwide Laboratories, 89 Queen Street, Newton Abbot, Devon, TQ12 2BG).

Unfixed samples: Samples must be fully fixed in formalin prior to processing. If, during gross examination, a tissue sample appears unfixed (i.e. still has a pink colour) the sample will be left in formalin until fully fixed. Large samples take longer to fix than small ones. Therefore, it should be borne in mind that larger samples may take longer to process.

Bony tissue: Samples including bone (e.g. digits, bone tumours, etc) are very hard and the bone portion is impossible to trim. If there is a soft tissue portion included with the bony sample, this is processed and examined histologically to generate an initial report. Sometimes, this soft tissue sample yields a full diagnosis. The remaining bony part of the sample is placed in decalcifying solution, and this can be processed for

histologic examination after the bone softens, if the initial soft tissue sample did not yield sufficient information for a diagnosis or if examination of the bone may yield further useful information. The time required for decalcification is variable, depending on the thickness of the bone. Small samples may decalcify in a short time (sometimes overnight). Other samples, such as mandibles or digits, require more time (sometimes up to several weeks). For samples where no soft tissue is available for initial processing, a "holding report" will be sent to the referring veterinarian (RV) to inform them that the sample processing time will be prolonged due to the requirement for decalcification.

Re-processing of the gross specimen: Sometimes, after examination of the H&E-stained section(s), the pathologist will examine the gross specimen and trim more tissue for histologic examination. This may be done if, for example, the section on the glass slide does not match the RV's clinical description, if the gross sample is especially large (e.g. a splenic mass in which haemangiosarcoma is a

SAME DAY PTH

NationWide Specialist Laboratories are offering a SAME DAY service for the analysis of canine, feline and equine parathyroid hormone (PTH). Parathyroid hormone-related protein (or PTHrP) is assayed once a week on Wednesday with results available on Thursday.

If you want our RAPID PTH service as well as PTHrP, please submit 2 separate frozen EDTA Plasma samples. PTHrP will continue to be analysed once a week.

For more details, please contact us at 01223 493400.

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differential), or if the pathologist needs to grossly examine the margins of a tumour. Depending on the case, the pathologist may issue a preliminary report after initial examination of the section(s).

Special stains and bleaching: In some cases, after examining the H&E slide(s), the pathologist will request special stains (e.g. Gram stain for bacteria, Giemsa stain for mast cell granules) to highlight features that are difficult to appreciate on H&E staining. In cases of melanoma, the pathologist may request the slide be bleached, in order to carry out a mitotic count (as melanin pigment may obscure the nuclei of the neoplastic cells). The pathologist receives the stained/bleached slide(s) the following day for examination. Depending on the

case, the pathologist may issue a preliminary report after initial examination of the H&E-stained section(s).

Recuts or deeper sections: Occasionally, after examination of the initial H&E-stained slide(s), the pathologist will request that recuts of the section be made, if the section quality is suboptimal. The pathologist may request deeper sections be produced if they suspect pathologic changes is present out of the plane of section (e.g. skin biopsies in which pemphigus foliaceus or interface dermatitis are suspected). The pathologist receives the recut or deeper sections the following day. Depending on the case, the pathologist may issue a preliminary report after initial examination of the original sections.

We endeavour to send preliminary reports where possible. However, if the suspected diagnosis may be altered significantly by the results of further testing carried out (e.g. special stains, recuts etc), it is preferable to wait until these results have been obtained to issue a final comprehensive report.

We hope the above information is helpful to our valued clients in understanding the workflow of our laboratory and the various extra steps that may be required to achieve an accurate diagnosis.

By Alison Lee BSc MVB MRCVS DipACVP

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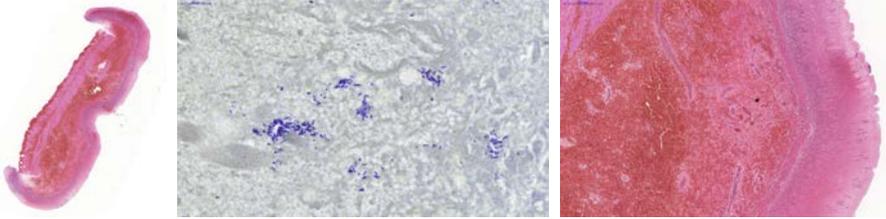
WORK EXPERIENCE WITH NATIONWIDE LABORATORIES

Evie from Sandbach High School and Sixth form college: I would just like to say how much I appreciate the work you have put in to organising and supporting my work experience. I had a brilliant week and I really enjoyed it and I have come away with a lot of knowledge as well as an understanding of how everyone in the workplace can make a difference to that environment. Your staff made me feel beyond welcome and were patient, engaging and friendly.

Thank you again for this wonderful opportunity I cannot emphasise enough how lovely everyone was, and I think it was the perfect choice of work experience for me. Next time my dog needs a sample at the vet I know it will be in safe hands!



Interesting case studies



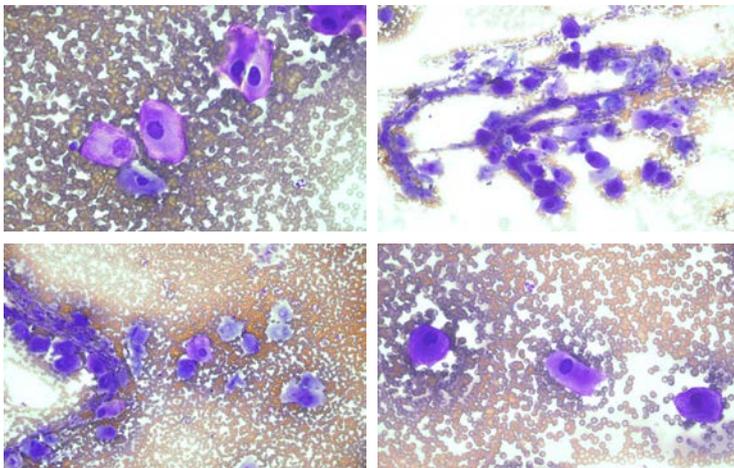
This is a case of a 5 years-old canine, Yorkshire. This patient has a one-day history of vomiting after change of diet to raw food, before dying had large volume of dark faeces.

We can see in these slides severe transmural gastric haemorrhage and necrosis, with intralesional Gram positive sporulated bacilli. Considering the bacterial morphology, location and lesions observed they most likely represent *Clostridium* sp. It has been suggested that *Clostridium perfringens* enterotoxin and *Clostridium difficile* toxins A and B may be involved in the pathogenesis of a syndrome similar to this case. A recent report also described a similar case caused by *Clostridium sordellii*. Haemorrhagic diarrhoea may reflect destruction of the protective intestinal epithelial barrier resulting in mucosal translocation of resident bacteria.

You may find reference of interest:

Fatal *Clostridium sordellii*-mediated hemorrhagic and necrotizing gastroenteropathy in a dog: case report. Paul Capewell 1, Angie Rupp 2, Manuel Fuentes 2, Michael McDonald 2, William Weir 2.

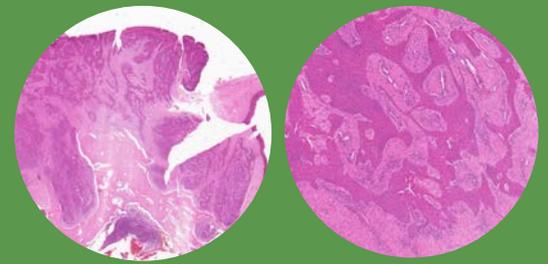
By Karina Fresneda



This is quite an unusual finding in cytology samples from dogs. This sample was taken from a skin nodule on the distal hindlimb. These are squamous cells, and some of them contain features consistent with viral cytopathic effects (pinkish fibrillary cytoplasm, dark/condensed chromatin). These cells are termed koilocytes, and are indicative of a viral papilloma.

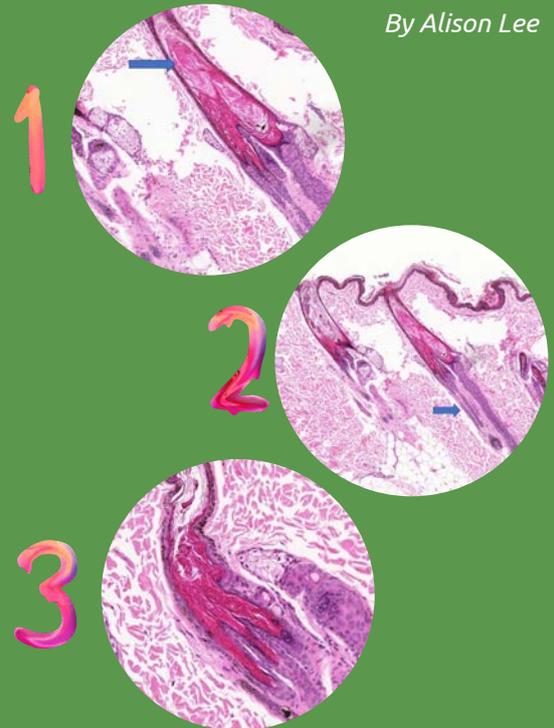
It is quite unusual to see these in cytological samples. Finding these cells in a cytological sample gives the clinician the opportunity to avoid unnecessary surgery, as viral papillomas often spontaneously regress. Even so, some of these (particularly the inverted papillomas) do not incite a very strong cellular response, and may be more resistant to regression. In these instances, excision is the treatment of choice.

By Danilo Gouveia Wasques



This is an acanthomatous ameloblastoma, a tumour of the odontogenic epithelial cells of the gum. These tumours used to be known as "acanthomatous epulis". They are locally invasive and may infiltrate the bone, but they do not metastasize around the body and full surgical removal is curative.

By Alison Lee



Seasonal, or cyclic, flank alopecia is a poorly understood condition causing hair loss in dogs, usually in the early autumn to late spring. Affected breeds include Boxers, English bulldogs, French bulldogs, and Miniature Schnauzers. Hair loss usually occurs on the flanks, and the hair coat can become dull and dry.

Image 1: Hair follicles become dilated with keratin (blue arrow) and there is hyperpigmentation of the epidermis.

Image 2: The bases of the hair follicles become distorted and narrow.

Image 3: Hair follicles can form irregular shapes resembling "crow's feet".

By Alison Lee

Quiz time: is it easy to guess the breed of Izzy?

Izzy is a rescue dog who was brought over to the UK from Greece in 2014 aged one in search of her forever home. Izzy is a loyal, loving companion to Cheryl Corral BVMS MRCV. Try to guess which breeds make up her identity.



To find an answer simply click on Izzy's picture.

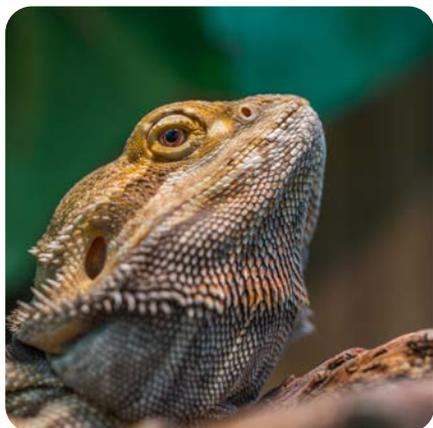
For your chance to win a Smart Touch Pet ID Tag name 10 conditions Izzy is at risk of developing based on her genetic makeup, age, sex, weight and lifestyle. Email your answers to info@nwlabs.co.uk with a subject line IZZY QUIZ. One winner will be chosen from a random draw of received entries and notified via email.



How often do you see a bearded dragon or a chameleon in your practice?

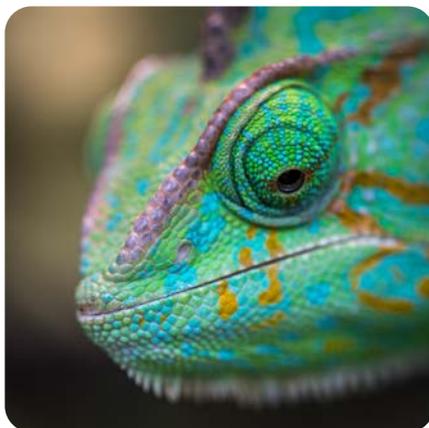
We are pleased to present to your kind attention short case study videos:

Fungal disease in bearded dragons by Karina Fresneda



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Curious chameleon case by Alison Lee



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