# Vetnostics

## Partners in Practice

Spring 2014

- Multiplex PCR
- Flow Cytometry
- Hendra Virus
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- Cainine Cutaneous Mast Cell Tumors

Vetnostics

# Welcome...

We at Vetnostics would like to take this opportunity to wish all of our clients a joyous festive season and a safe and prosperous New Year. We have really enjoyed being your 'Partners in Practice' this past year and look forward to another productive year of working together in 2015.

As always, please contact me (ph. 02 9005 7272 or email doug.hayward@vetnostics.com.au) if you have any requests for future newsletters, questions or any other queries.

## Multiplex PCR faecal panel

Diarrhoea in the dog and cat is a frequently encountered clinical presentation in small animal practice and this can involve the small intestine, large intestine, or both. Various disorders can lead to diarrhoea therefore a broad based diagnostic approach is useful in some cases to identify a potential cause or causes. Performing tests that allow for the identification of infectious causes of diarrhoea have traditionally involved faecal ova and parasite screens, faecal microscopy, and culture. These tests have been shown to lack sensitivity and, in some cases, specificity. The advent of molecular methods such as real time PCR (RTPCR) has provided an efficient and sensitive tool for the identification of potential enteropathogens. The Vetnostics Faecal Multiplex PCR uses multiplex tandem RTPCR methods to allow the screening of a panel of multiple infectious agents in a single faecal sample. One or more pathogens can be associated with clinical disease in both dogs and cats. For dogs, this panel includes: Campylobacter spp., Clostridium perfringens alphatoxin gene, Salmonella spp., canine parvovirus, Giardia lamblia, Cryptosporidium (parvum and hominis), canine coronavirus and canine distemper virus. For cats, this panel includes: Campylobacter spp., Clostridium perfringens alphatoxin gene, Salmonella spp., feline panleukopenia virus, Toxoplasma gondii, Tritrichomonas foetus, Giardia lamblia, Cryptosporidium (parvum and hominis), and feline coronavirus.

As with any diagnostic test, results must be interpreted in light of clinical history, clinical signs/findings, signalment, vaccination history, and other clinical data. This is particularly important for the interpretation of PCR positive results, as some enteropathogens, including many strains of non-jejuni Campylobacter spp. and C. perfringens can be excreted in healthy animals in the absence of diarrhoea. The diagnostic utility of the Vetnostics Multiplex PCR Faecal Panel may therefore be optimised by other laboratory methods such as culture, microscopy, and ELISA-based assays. The test requires 5g of fresh faeces (minimum 1g) submitted in a sterile container. Faecal samples should be kept refrigerated until submission to the laboratory. The cost of this test is $75.00 excl. GST. Please request Faecal Multiplex PCR Panel on the current submission form under Other Tests.

## Flow Cytometry is now available at Vetnostics!

Vetnostics is very pleased to announce that flow cytometry is now available for investigation and further work-up of leukaemias.

For those of you that are unfamiliar with flow cytometry, this technology simultaneously measures and then analyses multiple physical characteristics of single particles (cells) as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity and relative fluorescence intensity. A major medical application of flow cytometry is immunophenotyping leukaemias with a panel of fluorescently labelled antibodies to assess expression of cell markers, specifically to assist with definitive identification of neoplastic WBC populations as well as differentiating between reactive and neoplastic WBC populations.

Current charge is $115 (excl GST). Our turnaround time is about 2-3 working days and we prefer an EDTA blood sample sent in during a weekday for processing.

## Hendra virus Important Request

Hendra virus infection continues to be a differential diagnosis for certain veterinary cases presenting in areas of NSW and Queensland. Given the zoonotic potential of this virus and associated Health and Safety concerns in routine diagnostic laboratories, it is politely requested that Hendra virus is excluded (specific testing offered at EMAI and Queensland Government labs) where considered a clinical differential prior to submission of samples for further testing at Vetnostics. This will allow efficient processing of such samples without the requirement for phone calls, sample quarantine and subsequent sample forwarding for Hendra exclusion. Many thanks for your assistance.

## Equine Inhibin testing Amended charge

Please note that due to increased costs associated with the equine inhibin assay, the new charge for this test with immediate effect is $200 excl. GST.

Turnaround time for results and sample requirements are otherwise unchanged.

## Green Vetnostics Specimen Collection Bags

In an endeavour to further improve turnaround times and the priority which we place on the processing of veterinary specimens within our laboratory, Vetnostics have changed the colour of their specimen collection bags to green. Many of you will already be aware of this change and are already using the supplied green bags. For this of you unaware of this change, please use these bags once you have received them for **ALL** your future submissions to Vetnostics. There is no longer any requirement for submission in clear or red specimen collection bags.

## Hours of operation over the festive period

Please note that Vetnostics will continue to provide pathology services to our regular submitters over the festive period, with reduced service on the public holidays.

On the public holidays of 25th and 26th December 2014, the central (North Ryde, Sydney) courier phone line will be manned from 10am to 4pm. On the 1st January 2015, this phone line will be manned from 10am to 6pm. No scheduled pick-ups will occur on these days - telephone requests for pick-up only. Courier service will otherwise operate as normal around these dates.

Please note that specialised testing (flow cytometry, PTH assay, etc.) will not be performed between Christmas and New Year. Normal service will however resume for these tests in the new year.

# Ocular Pathology Rounds

*Canine conjunctival haemangiosarcoma with corneal involvement*
*2 interesting cases*

**Dr Karen Dunn** BVSc (Hons) **Consultant Veterinary Ocular Pathologist**

### Investigation of a case of acute onset glaucoma in an English Springer Spaniel

Primary corneal neoplasms are rare in dogs, however corneal vascular tumors are sometimes seen, usually secondarily involving the cornea by extension from the conjunctival limbus. Conjunctival vascular tumours are relatively common in dogs, and in this species, most (approximately 65%) are histologically benign (haemangioma), with lesser numbers (approximately 35%) being histologically malignant (haemangiosarcoma). The average age of affected dogs is 8.5years, and UV exposure appears to be a risk factor for tumour development; such tumours most frequently occur in non-pigmented tissue on the leading edge of the third eyelid, or in the temporal bulbar conjunctiva. Local recurrence is possible, particularly with haemangiosarcoma, but metastasis of conjunctival haemangiosarcoma to other sites is relatively uncommon where there is adequate primary excision, however the prognosis is always cautious, with monitoring recommended. Here we compare 2 cases of haemangiosarcoma with corneal involvement in dogs.

The first case is a 10year old Dalmatian living in Queensland, with a vascular mass at the right lateral limbus. En bloc tumour excision was performed by full thickness sclerectomy and keratectomy (see Fig 1) by Dr Guy Clare of PetVision at North Coast Veterinary Specialists, and submitted to FOCUS-EyePathLab at QML Vetnostics. The excision site was supported by a partial thickness autologous, peripheral corneal graft and a conjunctival pedicle graft was used to support the donor site. Histologically (see Fig 2), the mass was located largely within the superficial bulbar conjunctiva, with extension into the adjacent superficial cornea. The tumour was composed of variably sized branching vascular spaces and narrow clefts separated by minimal connective tissue and lined by plump endothelial cells with enlarged variably sized nuclei and visible nucleoli, but relatively infrequent mitoses; the mass was peripherally infiltrative but appeared fully excised in the plane of section, and was diagnosed as a well-differentiated, low-grade haemangiosarcoma, arising in the limbal conjunctiva, with peripheral corneal stromal extension. Interestingly, between the superficial mass and the non-pigmented conjunctival epithelium, the stroma contained numerous slender, angulated fibres with irregular orientation, suggestive of elastotic degeneration, or 'solar elastosis' (see Fig 3), a change I see increasingly frequently with superficially located vascular tumours, consistent with their reported UV-exposure association.

The second case is Speedy, a 10year old Shih-tzu, who presented with a melting ulcer and corneal perforation in the right eye 7 months previously, which was treated surgically by Dr Filip Nachtegaele in Belgium; as the corneal ulcer margins were already vascularised, a conjunctival island flap (taken from non-pigmented temporal bulbar conjunctiva) was used. The surgical site healed well, but 7month post-operatively, a small red mass was observed on the surface of the graft (see Fig 4). The mass was excised via keratectomy, and submitted to FOCUS-EyePathLab at QML Vetnostics in Brisbane. Histologically (see Fig 5), the mass was small and pedunculated, with open, irregularly branching vascular channels containing free erythrocytes and lined by plump active-looking endothelial cells (mitoses were rare), consistent with a low-grade haemangiosarcoma. The mass was fully excised, and there is no evidence of recurrence 12months post-operatively. Haemangiosarcoma is an unusual diagnosis at this central corneal site—the tumour may have arisen from local corneal vessels induced by the ulcer (neovascularisation), however given that the conjunctival flap came from the temporal bulbar conjunctiva, a site known to show a higher incidence of such tumours, and also the very superficial nature of the mass, conjunctival vessel origin is suspected in this case.

**For further information on this ocular pathology service, please contact Vetnostics, or visit FocusEyePathLab.com**

*Fig 1 Haemorrhagic mass at right lateral limbus, during surgery, note corneal stromal haemorrhage. Clinical image courtesy Dr Guy Clare, Petvision, QLD.*

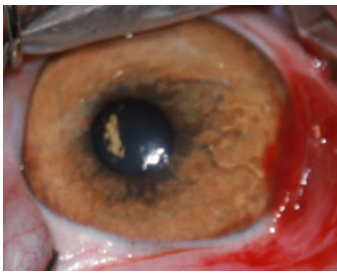


*Fig 2 Limbal conjunctival haemangiosarcoma in raised, superficial conjunctival substantia propria at left of image shows extension into dense corneal stroma at right of image, H&E, Magnification 30x.*

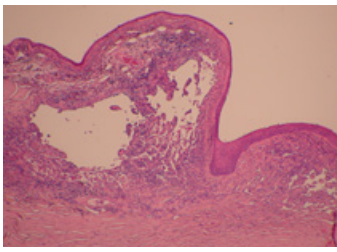


*Fig 3 Basophilic angulated fibres superficial to the mass, consistent with elastotic degeneration, H&E, Magnification 400x.*

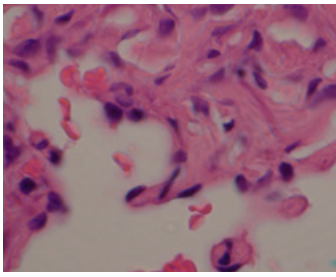


*Fig 4 Clinical image showing small red mass in paracentral cornea (with local scarring and pigmentation), courtesy Dr Filip Nachtegaele, Belgium.*

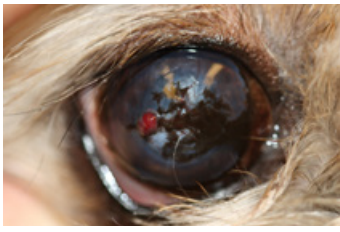


*Fig 5 Pedunculated, vascular corneal mass, H&E, 30x Magnification.*

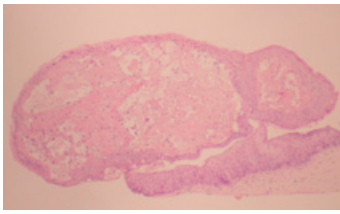
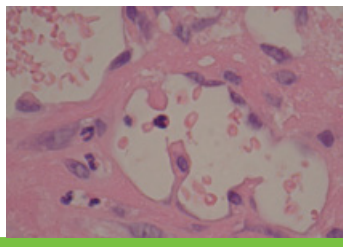


*Fig 6 Irregular vascular channels lined by plump endothelial cells, H&E, 400x Magnification.*



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# Canine Cutaneous Mast Cell Tumours:

*Vetnostics offers the complete diagnostic package*

1. **Grade:** via both Patnaik (Grades I, II or III) and Kiupel (High or Low) grading schemes.
2. **Mitotic index:** an evaluation of cellular proliferation.
3. **KIT IHC: I**mmunohistochemical evaluation of KIT protein expression pattern.
4. **c-KIT mutation status**: PCR based detection of internal tandem duplications in exon 11 and/or exon 8 of the c-KIT gene.

Mast cell tumours (MCTs) are one of the most common canine cutaneous neoplasms and they have a highly variable biological behaviour. In previous newsletters we have discussed how determination of mitotic index can help to better predict clinical outcome and that aberrant expression of KIT protein by neoplastic mast cells is a negative prognostic indicator being associated with increased incidence of local or distal recurrence, shorter disease free interval, and decreased survival times. Research indicates that together with histopathological grading and specific determinants of tumour cell proliferation, KIT protein expression, and c-KIT gene mutation status provide important prognostic information in canine cutaneous MCTs and they may also be useful in determination of therapy.

**Vetnostics also now offers canine MCT c-KIT mutational analysis** (via Colorado State University, USA) to detect the presence/absence of internal tandem duplications (ITD) of exons 8 and 11 of the c-KIT gene. There is a significant association between c-KIT ITD mutations, an increased rate of recurrent disease and mortality in dogs with canine cutaneous MCTs. ITD mutations in exons 8 or 11 of c-KIT have been detected in about 20 to 30 percent of canine cutaneous MCTs. MCTs with such mutations are highly aggressive, but respond well to tyrosine kinase inhibiting (TKI) therapies. Since tyrosine kinase inhibiting compounds are now available for the treatment of dogs, the detection of **c-KIT mutations has therapeutic as well as prognostic implications. c-KIT mutational analysis can be subsequently performed on both cytologically- and histopathologically-diagnosed canine cutaneous mast cell tumours at an additional cost of $216 (ex. GST); 10-14 day TAT.**

1. **Grade:** Histopathology is the diagnostic method of choice for grading tumours and there are several potential grading systems for use. The most widely used is the Patnaik system where lower grade lesions are grade 1 and more malignant lesions are grade 3. A predominance of intermediate grade tumours, and variation among pathologist in designation of grade are commonly mentioned weaknesses of the Patnaik histological grading system. The historical dilemma has often been associated with how to interpret the diagnosis of intermediate grade (Patnaik Grade II) tumours which comprise the majority of MCTs. Kiupel and colleagues have proposed a 2-tiered classification of either high grade or low-grade with the criteria for diagnosis of a high grade MCT based on the presence of any one of the following criteria;

   - 7 or more mitotic figures per 10 high power fields (HPF).
   - 3 or more multinucleated cells (3 or more nuclei) per 10 HPF.
   - 3 or more bizarre nuclei per 10 HPF.
   - Karyomegaly (10% of nuclei vary by 2 fold or more).

   This group reported a 96.8% consistency rate between pathologists when comparing on a 2-tiered system and high-grade MCTs were associated with significantly shorter time to metastasis or new tumour development, and with shorter survival times (median survival times of < 4 months for high-grade MCTs and > 2 years for low-grade MCTs).

   Both the Patnaik and Kiupel histological classification schemes are associated with clinical prognosis. **Vetnostics reports canine cutaneous MCTs via both the Patnaik (Grade I, II or III) and Kiupel (High or Low Grade) classification schemes.**

2. **Mitotic index:** Mitotic index (MI) is a measure of cell proliferation and MI is a strong predictor of overall survival for dogs with cutaneous MCTs.
3. **KIT IHC:** The immunohistochemical staining of KIT protein is a useful prognostic parameter in canine MCTs. There is correlation between aberrant KIT expression and increased cellular proliferation, higher histological grade, presence of c-KIT mutations, increased local tumour recurrence, and/or decreased clinical survival. The KIT staining pattern I ('normal' staining pattern) is associated with a good prognosis. An aberrant pattern of distribution of KIT protein may be present even without concurrent c-KIT mutations.
4. **c-KIT mutation status**: As discussed above. MCT grading, cell proliferation analysis, c-KIT PCR, and KIT IHC results are therefore all linked to canine cutaneous MCT-associated survival and metastasis. **Vetnostics is able to offer all of these analyses in an effort to determine the appropriate prognosis and treatment regimes for your patients.**

**References**


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