Newsletter August 2011

QML Pathology | Vetnostics

Now Available iPad/iPhone App

Real-Time Results... Anytime, Anywhere.

Introducing Path-Way, a new web-based application by QML Pathology, providing you with real-time access to our database.

Instant Access

As soon as the result is available at the laboratory, it is available at Path-Way - enabling you to view your clients' results quickly, efficiently and securely over the Internet.

With no paper to handle, instantaneous delivery and secure access, Path-Way ensures your clients' results are available real-time, anywhere, on time, all the time.

New Features

Increased search functionality, including new filters

Unique username and password

Update your account details online

View pending requests

Print off hard copy reports in a familiar format

View interactive charts

View cumulative results

To register, visit www.path-way.com.au

Path-Way

Non-interpreted Profiles Now Available

QML Pathology Vetnostics now offers specific non-interpreted profiles for dogs, cats and horses. These are exceptional value for money whilst still providing extensive haematological and blood biochemistry analysis together with Total T4 for cats and Fibrinogen for horses. The available non-interpreted panels are:

Feline Non-interpreted Profile:
Includes: Routine Body Function + Total T4 — **Cost: $46.00** (ex. GST)

Canine Non-interpreted Profile:
Includes: Pancreatic Body Function — **Cost: $46.00** (ex. GST)

Equine Non-interpreted Profile:
Includes: Equine Health and Fibrinogen — **Cost: $44.00** (ex. GST)

The reduced pricing available for additional tests requested with profiles (TT4, Urinalysis, Urinalysis + Urine Culture, FIV & FeLV serology and USG) will also apply for non-interpreted profiles, however results of these additional tests will not be interpreted either. (Refer to 2011 pricelist and pricelist table)

Pathologists and medical consultants **ARE NOT** available for result/case comment or discussion for non-interpreted profiles.

Non-interpreted profiles can be upgraded to an interpreted profile to include pathologist +/- medical consultant comments and case discussion at an additional fee of $50.00 (ex. GST). Panels must be upgraded **PRIOR** to phone discussion or issue of a written interpretation.

Important Information: Stickers containing the non-interpreted profile codes have been issued for use with current request forms that do not have the non-interpreted profiles listed in the profiles section. **Please place these stickers in the 'Other Tests' section of the request form and mark the appropriate box**. More stickers and new request pads can be ordered by contacting the laboratory.

It is essential that this procedure is followed correctly and that the appropriate non-interpreted box is highlighted. **Failure to do so will result in samples being processed as interpreted profiles and they will be charged accordingly.**

REMEMBER OUR CONTACT NUMBER 1300 VET QML (1300 838 765)

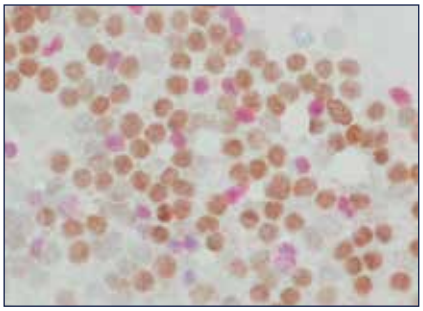
Our QML Pathology Vetnostics contact number 1300 838 765 has now been in operation for over two years. For a fast, efficient service, this number will take you directly to the following options:

1 - Results enquiries
2 - Added tests
3 - Speak to a Pathologist
4 - Order veterinary vaccines
5 - All other enquiries/Vetnostics Manager.

Calls will be charged at local rates from landlines. Mobile charges may vary.

Newsletter August 2011

QML Pathology. | Vetnostics



Contacts:

For further details regarding these tests and collection requirements, please contact:

Dr Brett Stone or Dr Michelle Dennis on 1300 838 765 (Then dial Option 3).

NEW TESTS Available at Vetnostics

1) T & B Cell Immunocytochemistry is now available on Cytology smears of Lymphoma cases:

For Lymphoma cases diagnosed by cytological examination of fine needle aspirates (FNA), we can now perform Immunocytochemistry on both stained and unstained smears of these FNA aspirates and determine T or B-cell phenotype when requested.

Cost of T&B Immunocytochemistry on submitted smears is **$75.00** (ex. GST).
Turnaround time for test is 2-3 working days.

For T/B cell Immunocytochemistry on FNA aspirates we require:

1) A minimum of 4 well made cytology smears
2) Smears can be submitted stained (with Diff-Quick) or unstained
3) Smears **must** contain a significant number of intact lymphoid cells forming a monolayer for Immunocytochemistry to succeed
4) Smears **must not** have immersion oil placed on them
5) Smears **must** be labelled with patient details.

2) Flow Cytometry:

Flow cytometry involves staining live cells with labelled antibodies that bind to proteins expressed on the cell surface. Different types of lymphocytes express different protein (e.g., T cells express protein CD3, B cells express protein CD21). The cells are analysed on a flow cytometer, which tells us how many cells of each type are present. This information allows us to determine the lineage of the cells present, and whether they are homogeneous (more consistent with neoplasia) or heterogeneous (more consistent with a reactive process).

Flow cytometry is recommended for the following indications:

PRESENTING COMPLAINT	BEST SITE	BEST SITE	COST TO SUBMITTERS (AS STANDALONE TESTS)
Lymphocytosis or Leukaemia	Peripheral Blood	Flow cytometry	$240.00 - ex. GST *(includes FBC)*
Lymphocyte rich effusion	Effusion Fluid	Flow cytometry	$200.00 - ex. GST

Turnaround time - 7-10 working days. For collection requirements and special forms, please ring Vet Pathologist.

3) PARR (PCR for antigen receptor rearrangements):

The PARR assay is a PCR assay in which we are amplifying DNA. The results tell us if the majority of cells in the sample are derived from the same original clone (most consistent with neoplasia), or from multiple clones (most consistent with a reactive process).

The PARR assay can be used in the following indications:

PRESENTING COMPLAINT	BEST SITE	BEST SITE	COST TO SUBMITTERS (AS STANDALONE TESTS)
Lymphoma suspect, equivocal histopathology/cytology	Lymph node	PARR	$200.00 - ex. GST
Rare suspicious cells in peripheral blood, no Lymphocytosis	Peripheral Blood	PARR	$200.00 - ex. GST
Splenomegaly equivocal histopathology/cytology	Spleen	PARR	$200.00 - ex. GST
Peripheral cyto paenia, suspicious cells in marrow	Bone Marrow	PARR	$200.00 - ex. GST
Lymphocyte rich in CSF	CSF	PARR	$200.00 - ex. GST

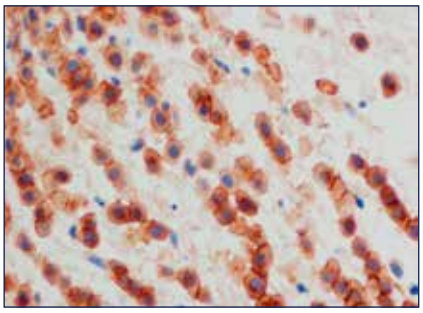
Turnaround time - 7-10 working days. For collection requirements and special forms, please ring Vet Pathologist.

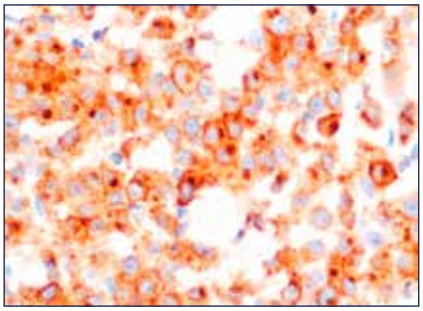
Help the environment, get the Newsletter by email

In an effort to reduce our carbon footprint, we would like to distribute this newsletter by email where possible. If you would like to receive this newsletter by email in the future, please send your email address and contact details to Sandra Rolls: **sandra.rolls@qml.com.au.**

Newsletter August 2011

QML Pathology | Vetnostics





KIT Immunohistochemistry for Canine Cutaneous Mast Cell Tumours

Mast cell tumours (MCTs) are one of the most common cutaneous neoplasms of the dog. They have highly variable biological behaviour, ranging from slowing growing nodules that are cured by simple surgical excision, to fast growing and invasive masses which rapidly progress to fatal widespread metastatic disease. When a dog is diagnosed with a cutaneous MCT, clinicians often look to histopathological grading to clarify prognosis. However, wide variation in the outcome of dogs with intermediate grade tumours, the predominance of intermediate grade tumours, and variation among pathologist in designation of grade are commonly mentioned weaknesses of histological grading systems. In a previous newsletter (http://www.qml.com.au/Files/Vet_NL_May08.pdf), we discussed how taking mitotic index into account can help to better predict clinical outcome. In addition, there has been considerable recent advancement in the understanding of the molecular basis of MCT development which appears to be promising for improving canine cutaneous MCT prognostication.

Up to 30% of dogs with cutaneous MCT have mutations of the c-KIT proto-oncogene which play an important role in oncogenesis, especially for aggressive tumours. KIT, the protein product of c-KIT, is a receptor tyrosine kinase which normally functions to stimulate the cell cycle and is necessary for proliferation and differentiation of normal mast cells. Aberrant expression of KIT protein by neoplastic MCT cells has been shown to be a negative prognostic indicator in dogs.

Using KIT immunohistochemistry, three staining patterns can be observed in neoplastic mast cells. The first comprises perimembrane KIT staining, coinciding with the normal location of the protein in the cytoplasmic membrane of well-differentiated mast cells (Figure 1). Tumours with this KIT expression pattern are more likely to exhibit benign biologic behaviour. The other staining patterns show increased expression of KIT in the cytoplasm (Figure 2), an abnormal location that suggests potential for downstream events which may lead to increased cellular proliferation. Dogs with tumours that have increased cytoplasmic KIT expression are at increased risk of poorer clinical outcome, including increased incidence of local or distal recurrence, shorter disease free interval, and decreased survival time. When such patients have been treated with surgical excision only, more aggressive therapy may be consequently elected.

QML Vetnostics has recently developed and validated a KIT immunohistochemistry test which reliably assesses the cellular localisation of KIT, and it is available for $40.00 (ex. GST), with a turnaround time of 2 - 3 days. The test is performed on formalin-fixed paraffin-embedded sections from canine cutaneous mast cell tumours following routine histopathology. Presently, we recommend this test for intermediate grade canine cutaneous MCTs to provide additional information, allowing for prompt identification of those dogs with tumours that may pose a high risk of aggressive behaviour, thus ensuring appropriate treatment strategies are utilised.

References

Hahn, K.A., G. Ogilvie, T. Rusk, et al., Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med, 22(6): p. 1301-1309, 2008.

Kiupel, M., J.D. Webster, J.B. Kaneene, et al., The use of KIT and Tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. Vet Pathol, 41(4): 371-377, 2004.

London, C.A., P.B. Malpas, S.L. Wood-Follis, et al., Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clin Cancer Res, 15(11): p. 3856-3865, 2009.

Thompson, J.J., J.A. Yager, S.J. Best, et al., Canine subcutaneous mast cell tumors: Cellular proliferation and KIT expression as prognostic indices. Vet Pathol, 48(1): 169-181, 2011.

Webster, J.D., V. Yuzbasiyan-Gurkan, R.A. Miller, et al., Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. Vet Pathol, 44(3): p. 298-308, 2007.

Webster, J.D., V. Yuzbasiyan-Gurkan, D.H. Thamm, et al., Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. BMC Vet Res, 4(32), 2008.

Newsletter August 2011

QML Pathology | Vetnostics

VET VACCINES AND CONSUMABLES

QML Pathology has expanded its vaccinations catalogue to include vet vaccines and consumables.

- A wide range of vaccines including canine, feline and equine are available for sale at competitive prices.
- Our internationally recognised integrated cold chain network ensures the integrity and quality of our vaccines during transportation.
- Next day delivery is available (excluding weekends and public holidays) for practices located within metropolitan and regional centres throughout Queensland and northern New South Wales.
- Commonly requested veterinary consumables are now also available for purchase, including **MAX-ACT™ Clotting tubes**.
- All vet vaccine purchases are eligible for Win Rewards Points.

Your Rewards, Your Way
winrewards
Pfizer Animal Health

Vaccines can be ordered by calling 1300 838 765 (1300 VET QML) or by downloading the form from our website.

'USG FOR FREE' AND DISCOUNTED COMBINATION TESTING

In an effort to allow for more comprehensive testing with our profiles, Urine Specific Gravity (USG) will be performed free of charge whenever a USG is requested with ANY of the profiles listed within the Pathology Profiles section of the request form.

To receive the 'USG for Free' all you need to do is to **ensure that you tick the specific gravity box listed within the Urine Examination section** of the request form together with your profile request.

The current complete list of tests offered at a significantly discount price when requested with ANY of the listed profiles is now as follows:

TEST	Normal Price (ex. GST)	Reduced Price (ex. GST)
Total T4	$37.00	**$17.00**
Urinalysis	$30.00	**$17.00**
Urinalysis + Urine Culture	$57.00	**$36.00**
FIV & FeLV	$67.00	**$44.00**
Urine Specific Gravity	$21.00	**FREE**

As per current price list (effective February 2011).

These reduced prices will also apply for any of these tests that are subsequently 'added on' to an initial pathology profile already performed.

REDUCED PRICE ON VACCINATION TITRE TESTING

Re-vaccination of adult pets is a complex issue requiring discussion between Veterinarians and pet owners to tailor the most appropriate vaccine and vaccination protocol for each individual animal. There is an increased concern of pet owners regarding 'over vaccination' of pets and possible adverse side effects associated with vaccination. The current recommendation is for triennial vaccination of adult cats and dogs with core vaccines where appropriate.

QML Vetnostics has reduced the price of canine and feline core vaccine antibody titre testing in an effort to help Veterinarians make a more informed decision regarding the re-vaccination of dogs and cats.

TEST	Previous Price (ex. GST)	Current Price (ex. GST)
Canine Distemper (CDV) Antibody	$48.00	**$35.00**
Canine Parvovirus (CPV) Antibody	$48.00	**$35.00**
CDV & CPV	$72.00	**$60.00**
Canine Hepatitis Antibody	$58.00	**$45.00**
Feline Calicivirus, Herpesvirus and Panleukopaneia Virus Antibody	$95.00	**$60.00**

As per current price list (effective February 2011).

Newsletter August 2011

QML Pathology | Vetnostics

Dr Sue Foster
Veterinary Medical Consultant

Utilise our Veterinary Medical Consultant Service

Dr Sue Foster (Vetnostics Medical Consultant) is available to discuss canine and feline medical cases. Sue can be contacted after 10.00am weekdays by phoning 0423 783 689. If leaving a voicemail message, please also leave the relevant QML Pathology Vetnostics laboratory number so that Sue can review the results before returning your call.

Hypothyroidism: Is it the most misdiagnosed disease?

QML Pathology Veterinary Medical Consultant
Dr Sue Foster BVSc, M Vet Clin Stud, FACVS (Feline Medicine)

Canine hypothyroidism is a relatively uncommon endocrinopathy in Australia, yet it is commonly 'diagnosed'. The big problem with the diagnosis of hypothyroidism is that no single diagnostic test confirms the diagnosis of hypothyroidism (Feldman and Nelson, 2004). Diagnosis of hypothyroidism depends on appropriate clinical signs, lack of concurrent non-thyroidal disease, consistent haematology and biochemistry results, and thyroid function testing.

So how should you approach diagnosing hypothyroidism in dogs?

1) Make sure all signs are typical for the disease. The most common signs in hypothyroidism are lethargy, weight gain, weakness, endocrine alopecia and pyoderma. If any of the signs are not consistent, then other disease(s) need to be investigated PRIOR to thyroid function testing. **Polydipsia is NOT a sign of hypothyroidism** and any disease causing polydipsia is likely to affect total T4 assays, and may affect free T4 assays also. This is particularly true for hyperadrenocorticism.

2) Check haematology and biochemistry to try and rule out non-thyroidal diseases and to see if any features associated with hypothyroidism are present. 75% of cases are reported to have hypercholesterolaemia so even though a normal cholesterol does not rule out hyperthyroidism, it lessens the chance.

3) If no other diseases are evident, or if mild non regenerative anaemia (0.28-0.36L/L) or hypercholesterolaemia is present, then consider thyroid function testing.

Total T4 Concentration

This can be used as an initial test. Whilst it is well-known that illness in euthyroid dogs can decrease serum T4 concentration, it is not as well-known that the range of serum T4 concentration overlaps between hypothyroid dogs and healthy dogs. In one study, the range of serum T4 concentration in 62 healthy dogs was 12.9 nmol/L to 42.5 nmol/L, and in 51 hypothyroid dogs was undetectable to 19.3 nmol/L. Random daily fluctuations in T4 into the hypothyroid range can occur in healthy dogs (Feldman and Nelson, 2004).

The reference range for normal T4 concentration is undoubtedly different in different breeds. Greyhounds especially have a much lower reference range, and a range of 14+/- 6 nmol/L is more appropriate in greyhounds (Gaughan and Bruyette, 2001) and probably other sighthounds. Young Labradors have also been shown to have lower mean T4 concentrations than young Beagles or mongrels (Minten et al 1985).

Factors that can cause decreased T4 concentration include:

a) age: decreased T4 in dogs > 6 y.o.
b) body size: large dogs > 30kg have lower concentrations than small dogs < 10kg
c) breed, especially sighthounds: standard reference ranges are probably inappropriate to account for all breeds
d) random daily fluctuations
e) concurrent illness especially hyperadrenocorticism
f) drugs such as phenobarbitone, frusemide, sulphonamides, non-steroidal anti-inflammatory drugs, e.g., carpofen.

In summary, a normal total T4 rules out hypothyroidism in nearly all cases and the main benefit of T4 testing is exclusion of hypothyroidism! A low total T4, unless accompanied by classical clinical features, haematology (non regenerative anaemia; <50% hypothyroid dogs) and biochemistry (hypercholesterolaemia: 75% hypothyroid dogs), is not adequate for diagnosis of hypothyroidism.

QML Pathology. | Vetnostics

References

DeRoover K, Duchateau L, Carmichael N et al. Effect of storage of reconstituted recombinant human thyroid stimulating hormone (rhTSH) on thyroid-stimulating hormone (TSH) response testing in euthyroid dogs. J Vet Intern Med 2006;20:812-817

Diaz Espineira MM, Mol JA, Peeters ME et al. Assessment of thyroid function in dogs with low plasma thyroxine concentration. J Vet Intern Med 2007:21:25-32

Dixon RM, Mooney CT. Evaluation of serum free thyroxine and thyrotopin concentrations in the diagnosis of canine hypothyroidism. J Sm Anim Pract 1999;40:72-78

Feldman EC, Nelson RW. Canine and Feline Endocrinology and Reproduction. 3rd edn. Saunders, St Louis, 2004 pp 89-143

Ferguson, DC. Testing for hypothyroidism in dogs. Vet Clin Small Anim 2007;37:647-669.

Gaughan KR, Bruyette DS. Thyroid function testing in greyhounds. Am J Vet Res 2001;62:1130-1133

IDEXX Laboratories. IDEXX reference laboratories introduces new free T4 test with proven accuracy, better precision and faster results. IDEXX Fact Sheet. November 2010.

Minten J, Kuhn ER, De Geest H. Plasma concentrations of thyroid hormones in dogs:influence of sampling hour, breed and age. Chronobiol Int 1985;2:121-129

Panciera DL. Is it possible to diagnose canine hypothyroidism? J Sm Anim Pract 1999:40:152-157

Scott-Moncrieff JS, Nelson RW, Bruner JM, Williams DA. Comparison of serum concentrations of thyroid-stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. J Am Vet Med Assoc 1998;212:387-391

Free T4

Serum free T4 (fT4) measured by equilibrium dialysis is the single most accurate test of thyroid gland function. Free T4 concentration is less affected by illness than total T4 concentration, although sick euthyroid dogs can still have fT4 results consistent with hypothyroidism. Hyperadrenocorticism nearly always suppresses fT4 in addition to total T4. A normal free T4 is unlikely to occur in hypothyroidism (sensitivity 98%).

Free T4 measured by equilibrium dialysis is available only through IDEXX in Australia and QML Vetnostics send all free T4 samples to IDEXX for testing by equilibrium dialysis. Free T4 determination by non-dialysis analogue assays do not correlate well with those obtained with equilibrium dialysis techniques. Only the dialysis methods for fT4 measurement provide the additional information needed to distinguish dogs with low TT4 concentrations attributed to non-thyroidal illness (euthyroid suppression) from those with hypothyroidism (Ferguson 2007, Panciera 1999). It is interesting to note that the new chemiluminescent fT4 assay offered at IDEXX in addition to their equilibrium dialysis fT4 only has a reported sensitivity of 80% in hypothyroid dogs (IDEXX data sheet).

Canine TSH

Serum canine TSH (cTSH) concentration may be increased in hypothyroidism but is normal in up to 38% of hypothyroid dogs. It has a high specificity when used for diagnosis of hypothyroidism, so long as it is used in conjunction with serum T4 or fT4 concentration. In general, cTSH is more likely to be within the reference range in euthyroid dogs with concurrent illness than total T4 or fT4. However, some dogs with euthyroid sick syndrome have high cTSH concentrations.

TSH Stimulation Test

This is considered the gold standard to differentiate hypothyroidism from euthyroid sick syndrome. Recombinant human TSH (rhTSH) has been validated for this test at a dose of 50-92 µg / dog IV with serum total T4 measured prior to administration and 6 hours after. Euthyroid dogs have a post-TSH T4 > 30-40 nmol/L. Dogs with primary hypothyroidism have results <20 nmol/L. The grey zone in between is a non-diagnostic area: early hypothyroidism or euthyroid sick syndrome (Scott-Moncrieff JS et al 1998). Dogs with hyperadrenocorticism and dogs receiving phenobarbitone will have decreased responsiveness to TSH, and severe systemic illness can result in post-TSH T4 concentration in the hypothyroid range. However, this test should not be performed on dogs with severe systemic illness.

rhTSH is very expensive but if you can get hold of a 'cheap' vial, a TSH stimulation test can be really useful when pursuing a diagnosis of hypothyroidism. Once opened, reconstituted rhTSH can be stored for up to 4 weeks in the fridge and up to 8 weeks in the freezer (in an insulin syringe) (DeRoover et al 2006).

What about Therapeutic Trials?

Thyroid hormone supplementation with twice daily levothyroxine should be continued for a minimum of 6 to 8 weeks before critically evaluating therapy. After 4 weeks of therapy, total T4 concentration must be measured 4-6 hours post dosing to ensure dosing is adequate. This could mean 8 weeks of 'misdiagnosis' in addition to the cost and inconvenience of twice daily medication, and cost and inconvenience of monitoring serum T4 concentrations. In addition, if there is no response to medication, 6-8 weeks are required after withdrawal of the drug prior to revaluating thyroid function. Realistically, it is easier to ensure that one has the right diagnosis before embarking on thyroxine treatment, unless you have a very high clinical suspicion and no other diseases are apparent.

In Summary

Like many other endocrine diseases, hypothyroidism requires astute clinical acumen, routine haematology and biochemistry, and specific endocrine function testing. Rarely can an accurate diagnosis be achieved without multiple thyroid function tests.