Vetnostics

Spring 2011

Partners in Practice

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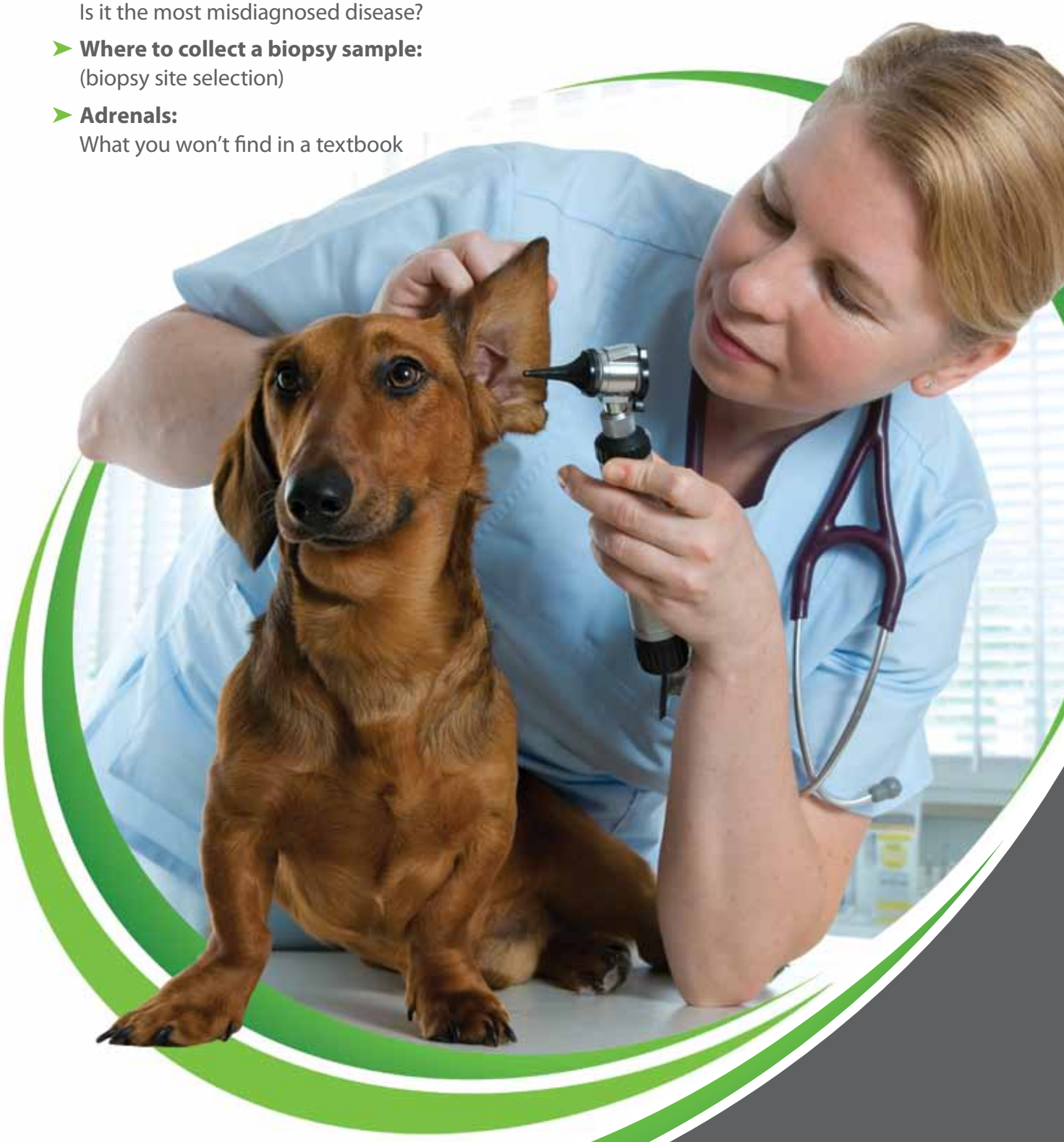

Vetnostics

Welcome...

Welcome to the Spring edition of the Vetnostics newsletter.

This edition has an endocrinology emphasis, focusing on diagnosis of hypothyroidism and useful facts/hints regarding adrencortical disease specifically. Additionally, advice regarding biopsy of skin and sampling for von Willebrand factor is covered. I hope you find the information useful. As always, please contact Dr. Doug Hayward on 02 9005 7272 or doug.hayward@vetnostics.com.au if you have any feedback.

HYPOTHYROIDISM:
Is it the most misdiagnosed disease?

Dr Sue Foster BVSc, MVetClinStud, FACVSc
Vetnostics Small Animal Medical Consultant

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 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.

THYROID FUNCTION TESTING

Serum 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 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. carprofen.

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.

Serum 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 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 total T4 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 (Immulyte) offered at IDEXX (and other laboratories in Australia) in addition to their equilibrium dialysis fT4 only has a reported sensitivity of 80% in hypothyroid dogs (IDEXX data sheet). Vetnostics can also perform this test but at this stage, we are unwilling to offer a test which would appear to have much lower sensitivity than the fT4 by equilibrium dialysis.

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)*.

Scintigraphy

Check with your referral institutions re availability and cost. The cost at University of Melbourne, as discussed at Science Week, is possibly cheaper than performing thorough laboratory testing (depending on the practice mark-ups for tests) and definitely cheaper than performing a TSH stimulation test. Scintigraphy is not likely to be affected by breed, drugs or non-thyroidal illness.

GREYHOUNDS

There is no proven relationship between basal serum thyroid hormone concentrations and bald thigh syndrome, poor racing or reproductive performance *(Graham 2005; Greyhound Racing Board Great Britain website)*. Despite this, TT4 testing is exceedingly common in this breed. This is a real issue as greyhounds have a lower reference range for TT4 and fT4 and the reference ranges for TT4 and fT4 in this breed extend below the limit of detection for currently available assays.

If hypothyroidism is really likely in a greyhound, then the single best test would be scintigraphy *(Rob Shiel pers comm.)* Normal serum fT4 concentration would EXCLUDE the diagnosis of hypothyroidism and increased cTSH would be suspicious for hypothyroidism *(Rob Shiel pers comm.)*.

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.

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Where to collect a biopsy sample:
(biopsy site selection)

Dr David Taylor BVSc, Dip ACVP

Veterinary pathologist

Skin biopsy is a useful diagnostic tool; however, too often results are "non conclusive" and this may be the result of sampling error.

The lack of a specific diagnosis in part rests with sampling error, which can be prevented by adhering to basic principles of sample selection and collection.

Select biopsy sites based on:

- Types of lesions
- The differential diagnosis list
- Knowledge of skin histology at various body sites

Remembering that skin histology varies in different anatomic sites is important when selecting biopsy sites.

Glabrous or non-haired skin has fewer follicles and smaller sebaceous glands so collecting skin from these areas for exclusion of an endocrinopathy makes evaluation more difficult than samples collected from sites with many follicles such as the shoulder.

Most useful clinical lesions to biopsy are fully developed non-treated primary lesions such as: macules, papules, pustules, nodules, vesicles, wheals.

Secondary lesions may be useful, especially crusts, ulcers, comedones etc.

Be aware that special types of lesions require special procedures.

Depigmentation: Biopsy an active area of depigmentation (grey) over a late-stage (white) area. Once the pigment has been lost from the epidermis, the process causing the pigment loss is over, and the microscopic examination may only reveal that pigment is gone, but not the cause of the pigment loss.

Alopecia: Select samples from the centre of the most alopecic area. Samples from junctional and non-affected areas should be collected into different pots to avoid confusion.

ADRENALS:
What you won't find in a textbook

Dr Sue Foster BVSc, MVetClinStud, FACVSc
Vetnostics Small Animal Medical Consultant

PART 1: SIGNALMENT

Hyperadrenocorticism (hyperA)

1. *"If you see a Maltese >10 y.o. which does not have hyperA then adrenal function testing must not have been performed!!!"*

   Whilst this is my somewhat facetious comment, it may actually be true. If you do have an 11 y.o Maltese which has no clinical signs of hyperA, has normal ALP concentration (on a commercially performed laboratory assay) and has at least one normal adrenal function test, then I would be interested to hear from you (as I want to know if they do exist!). Vetnostics would consider running cortisols at no charge (ring me to organise).

2. It was long believed that Scottish Terriers had a breed-related increase in ALP with age.[1] That always seemed unlikely given the very high prevalence of hyperA in aged Scottish Terriers (they may well be like Maltese…i.e. any Scottie over 10 y.o. requires adrenal function tests!). Finally, there has been a thorough paper investigating clinically healthy, aged Scottish Terriers with increased ALP and investigated their adrenal function. Not surprisingly, the increased ALP was found to be associated with sub-clinical hyperadrenocorticism when rigorous testing was performed.[2]

3. Hypertriglyceridaemia can occur as a breed phenomenon in Miniature Schnauzers. There are now many papers on hypertriglyceridaemia in Miniature Schnauzers including papers on the association between cPLI increase, possible pancreatitis and hypertriglyceridaemia in this breed.[3] To the best of my knowledge, none of these recent papers have studied adrenal function tests concurrently to check whether clinical or sub-clinical hyperA is another cause of hypertriglyceridaemia or pancreatitis in this breed (or whether hyperA could interfere with cPLI testing!). Middle to old-aged Miniature Schnauzers definitely get hyperadrenocorticism (quite commonly diagnosed through Vetnostics with adrenal function testing) and their triglycerides improve after treatment suggesting that, in addition to breed-related hypertriglyceridaemia, hyperA must be on the DDx list for any Miniature Schnauzer of appropriate age with hypertriglyceridaemia, Hopefully, someone will do a careful study similar to that performed in Scottish terriers and expose yet more myths and under-diagnosed hyperA!

What is your diagnosis?

Blood film (Wright's stain) from a 6 year old intact female working Kelpie that presented with acute onset seizures and no known access to toxins. No further significant findings identified on clinical examination.

Stress leucogram, red cell mass within the reference range, moderate normoblastaemia and hypokalaemia identified on routine FBC and serum biochemistry.

What are the inclusions identified within the erythrocytes (polychromatophils particularly?).

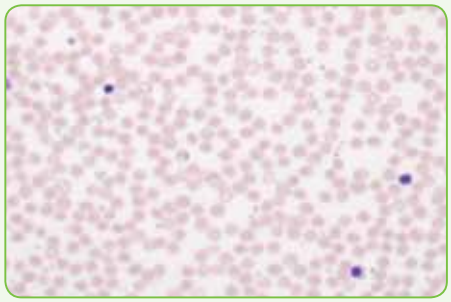

Figure 1: Wright's stained blood film, x40

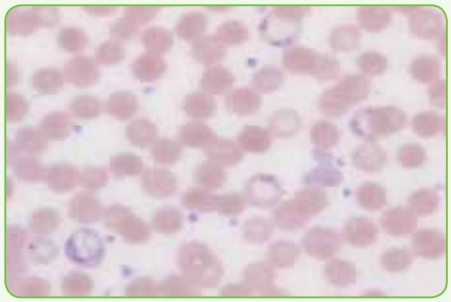

Figure 2: Wright's stained blood film, x100 (oil)

4. In Australia, I see many late middle-aged to old Australian Cattle dogs with hyperA (both clinically and through Vetnostics). The number diagnosed through Vetnostics would seem to be much higher than expected for breed prevalence suggesting that the number of cases in this medium breed dog may be due to genuine predisposition rather than overall breed prevalence in Australia. In this breed, signs of hyperA can be very subtle and include slight roughening and colour change in the hair coat (can look as though coat just singed slightly, especially at the base of the neck), panting at rest on cool days, altered body shape and **ruptured cruciate ligaments**. Polydipsia and polyuria are not always present, ALP is not always increased and alopecia is never present (though some may be slow to regrow after a clip…watch after cruciate surgery!). HyperA in this breed is nearly always pituitary dependent. I have had one vet report adrenal-dependent hyperA in this breed (based, from memory, on ultrasound findings only, not endogenous ACTH concentration).

5. Similar to the published literature, other breeds diagnosed include Poodles, Pomeranians, Beagles, Boxers and Dachshunds.

6. The commonly cited figures for hyperA are that pituitary dependent hyperadrenocorticism (PDH) comprises 85% with adrenal tumours (ATs) comprising 15%.[4] I am often asked whether these figures are accurate in Australia as so many clinics diagnosing hyperA regularly struggle to even find a single adrenal tumour amongst their cases. Similarly, Vetnostics has very few endogenous ACTH assay results confirming adrenal tumours. It would be interesting to check with a well designed study but I suspect over 95% over hyperA cases in Australia are pituitary. Could it be related to dog size? A recent North American paper evaluating trilostane in dogs found that the mean weight of dogs with hyperA to be 20.7 kg (PDH 20.42 kg). I don't think many Australian vet clinics would have 20.7kg as the mean weight of their hyperA patients. I suspect this different breed population affects the ratio of PDH to AT and also dosing for trilostane (to be discussed in a future 'Adrenals' segment).

Hypoadrenocorticism (hypoA)

1. The most common breed category diagnosed with hypoA at Vetnostics has to be the Jack Russell/Fox Terrier group. Not all of these dogs will have electrolyte abnormalities as some will have a pure glucocorticoid deficiency. So if there are vague or suggestive signs in a 3-7 y.o. JRT or Fox terrier, especially if there is no stress leucogram (or a "reverse stress leucogram" i.e. lymphocytes and eosinophils high normal or increased in a sick dog), consider doing an ACTH stimulation test.

2. The second most common breed would probably be Maltese. So yes, they get hyperA and hypoA. Whether the fact that they are a common breed influences disease incidence in this breed is unknown.

3. Similar to the published literature, other breeds diagnosed with hypoA at Vetnostics include poodles (all sizes), German Shepherds, German short-haired pointers, Great Danes and Portuguese Water Dogs.

References

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3. Xenoulis PG et al. Serum triglyceride concentrations in Miniature Schnauzers with and without a history of probable pancreatitis. J Vet Intern Med 2011 25:20-25
4. Feldman EC. Evaluation of twice-daily lower-dose trilostane treatment administered orally in dogs with naturally occurring hyperadrenocorticism. J Am Vet Med Assoc 2011; 238: 1441-1451

Answer...

The identified erythrocyte inclusions (evident in polychromatophilic erythrocytes predominantly) are consistent in appearance with basophilic stippling. The stippling effect is due to the presence of aggregated ribosomes and polyribosomes, typically only produced using reticulocyte stains. However, such a finding may be prominent on routine blood films in any species with lead poisoning and was considered a differential in this case given the reported clinical history and presence of normoblastaemia despite the absence of anaemia (another common feature of lead poisoning).

A blood lead level was subsequently assayed on this sample giving an increased result (4 x the top of the 'normal' reference interval) confirming the diagnosis. The pathogenesis of this finding in lead toxicity is the inhibition by lead of pyrimidine 5-nucleotidase that helps degrade nucleotides in RNA. Further differentials for such inclusions include stain deposit, siderotic inclusions and possibly some infectious agents.

Meet our Veterinary Medical Consultants and Consultant Histopathologists

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Submission of samples for von Willebrand factor testing:

Protocol for submission of samples for von Willebrand factor testing in dogs:

1. Animal to be fasted prior to sampling and should be clinically well, rested and calm
2. Make sure sodium citrate tube has not expired
3. Make sure citrate tube filled to mark or adequately (according to volume stated on tube)
4. 'Clean' venipuncture (i.e. minimally traumatic) is essential for accurate results
5. Send Citrate tube in chilled (i.e. NOT FROZEN) and as soon as possible post-collection so collection centre/ laboratory can check suitability of sample for accurate testing
6. NOTE: In the case of non-metropolitan veterinary practices, submitting veterinarian should please contact the local collection centre/peripheral laboratory prior to blood collection to advise sample to be arriving in order to allow appropriate processing