Vetnostics

Partners in Practice

Winter 2013

- **ADRENALS: What you won't find in a text book**
- **What's your diagnosis**
- **The most difficult and frustrating histopathological diagnoses**
- **Ionised calcium and parathyroid hormone sampling instructions**

Vetnostics

Welcome...

Herewith is the Vetnostics Winter 2013 newsletter.

In this edition of the newsletter, we continue with Dr. Sue Foster's series on 'ADRENALS: What you won't find in a textbook' as well as the regular histopathology instalment.

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

ADRENALS: What you won't find in a textbook

PART 4c: ADRENAL FUNCTION TESTS: ACTH STIMULATION TEST vs LDDST

The most common question I get asked is "Which test do I do? ACTH stim test or LDDST?" So we need to look at the two with the textbook and non-textbook literature and consider the practicalities.

ACTH Stimulation Test

Uses
- diagnosis of hyperadrenocorticism (hyperA)
- differentiating spontaneous from iatrogenic hyperA
- monitoring treatment efficacy for hyperA.
- diagnosis of hypoadrenocorticism (hypoA)

Advantages:
- only requires dog to be in clinic for 1h
- only requires two blood samples (or possibly one post-stimulation, if monitoring treatment)
- detects iatrogenic hyperA
- can also be used to evaluate 17-OH progesterone response (sometimes necessary for diagnosis in atypical hyperA patients)

Disadvantages:
- does not discriminate between pituitary dependent hyperA (PDH) and adrenal tumour
- ACTH in the form of cosyntropin (Synacthen®) is expensive and not always available

LDDST

Uses
- diagnosis of hyperA
- sometimes discriminates PDH from adrenal tumours (only if classic PDH pattern present)

Advantages:
- dexamethasone is cheap and always available

Disadvantages:
- need dog for 8h
- three blood samples required

Sensitivity and Specificity of the two tests

Sensitivity

It is a widely held belief that the ACTH stimulation test is less sensitive than the LDDST but this needs to be carefully evaluated as sensitivity in the literature reports vary considerably between studies and textbook comments tend to be influenced by data generated from the Feldman group.

Reported comparative sensitivities are as follows:

1. Feldman (1983)[1], 64 dogs, 83% sensitivity for ACTH stimulation compared to 92% for LDDST); 3/7 (43%) of dogs with adrenal tumours were negative.
2. Kaplan et al (1995)[2], 21 dogs with PDH, 70% sensitivity for ACTH stimulation compared to 100% with LDDST.
3. Van Liew et al (1997)[3], 40 dogs (30 PDH and 10 AT), sensitivity of 95% (compared to 96% for LDDST) and only 9/10 (90%)dogs in dogs with adrenal tumours
4. Rijnberk et al (1988)[4], 129 dogs, the only study not from North America, had the lowest reported test sensitivity for LDDST (85%) but did not compare it with ACTH stimulation testing.

Subjectively, I feel that the ACTH stimulation test and LDDST have similar sensitivity in Australia, although the ACTH stimulation test seems a little more sensitive in the early cases, picked up from pre-anaesthetic testing.

Specificity

Unlike sensitivity, there is little debate about specificity. In dogs with non-adrenal illness, there are more false positives with the LDDST than with the ACTH stimulation test. In one study,[2] specificity of ACTH stimulation testing in dogs with non-adrenal illness was 86% compared to 44% with LDDST.

Positive predictive value

1. Van Liew et al 1996[3]: ACTH stimulation test 91% compared to 76% for the LDDST
2. Rijnberk et al 1988[4]: 92% for LDDST

So, which test to do?

Dogs unlikely to have non-adrenal illness

I think the bottom line is that the sensitivity and specificity in this population are similar. There are certainly dogs with a normal LDDST that have extremely an exaggerated ACTH stimulation test. Equally, dogs that fail to suppress on a LDDST may have a normal ACTH stimulation test. Essentially, clients should be told this is a "two-test disease" so that they are prepared for more than one test if necessary.

Thus, a decision can be made on convenience (1h vs. 8h test; dexamethasone availability vs. Synacthen® etc). However, if the signs are very subtle or not evident and testing is being performed due to increased serum alkaline phosphatase activity detected in pre-anaesthetic testing, then I would do an ACTH stimulation test. I would also do an ACTH stimulation test in a Pomeranian as 17-OH progesterone testing, which can be helpful in this breed, can be added if post-stimulation cortisol is normal.

Dogs with non-adrenal illness
ACTH stimulation test is preferable if testing has to be performed in an ill dog e.g. in a dog with ongoing congestive heart failure or renal insufficiency.

Dogs with a history of corticosteroid use
The ACTH stimulation test differentiates iatrogenic from spontaneous hyperA so is useful when dogs on exogenous glucocorticoids develop signs of hyperA (e.g. unexpectedly after chronic alternate day therapy).

Treatment monitoring
LDDST is not of use for treatment monitoring so ACTH stimulation testing is required for monitoring hyperA. It is not necessary to monitor the adrenal response in spontaneous hypoA.

In order for any of these tests to be interpreted accurately, a full history (both clinical and laboratory tests) must be provided and all tubes must be correctly and clearly labelled.

ACTH Stimulation Test Protocol

Vetnostics Protocol:
- take a 0h blood into serum tube
- inject 5µg/kg Synacthen® IV
- take another blood sample 1h later into a serum tube
- Vetnostics' post-stimulation reference range and thus interpretation is based on this protocol

Other protocols:
- another protocol frequently used is 250µg Synacthen® IV or IM with testing 1h later. This protocol is potentially more expensive for the client.

Some textbooks still recommend that the ACTH stimulation test be performed in the morning. As dogs do not have a circadian rhythm for cortisol secretion, there is no scientific justification for this recommendation. This test can be run at any time. As fasted blood samples are preferable for elective testing with chemiluminescence assays (radioimmunoassays are unaffected by haemolysis or lipaemia), this means that when performing an ACTH stimulation test in a diabetic dog with hyperA (e.g. usually fed before coming to the clinic), the test can be run late in the day with no problems.

ACTH Stimulation Test Reference Ranges FAQ

Why don't Vetnostics include a reference range for their ACTH stimulation test?

Answer: it is a test used for multiple purposes so the relevant reference ranges change depending on situation (diagnosis versus treatment monitoring; spontaneous hypoA mimicking iatrogenic hyperA) and submission history is often lacking! Each ACTH stimulation test for Vetnostics is thus interpreted relative to the age, breed, history, clinical signs and any other available blood results to give both a result (based on our established reference ranges) and a predictive value, not as a percentage but as an estimate taking into account the know features of the case, thus why it is important to supply them!

Why does Vetnostics have a reference range of 200-400 nmol/L for post-stimulation cortisol in normal healthy dogs?

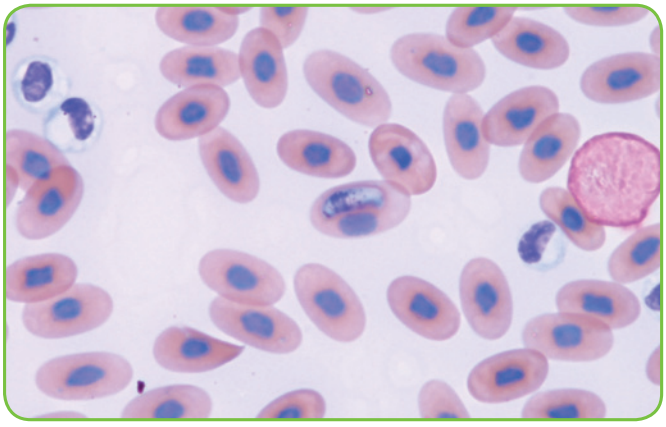
Answer: the Vetnostics assay was validated against a gold standard radioimmunoassay (RIA) run at University of Sydney. As no statistically significant difference was found between the two assays, it was appropriate to use the well-established reference range from this RIA. Unless the case almost certainly has hyperA, we usually allow for 10% interassay variation and diagnose hyperA when the test exceeds 440 nmol/L (to give an extra margin of safety).

Why are the textbook reference ranges so much higher?

Answer: most are based on data generated from the Feldman paper which had a mean of 323 ± 74 in their control dogs.[1] The upper limit of their reference interval was then calculated as being that higher than 3 standard deviations from the mean (546 nmol/L). This high cut-off for diagnosis may be why the same group reports such poor test sensitivity. These days, ranges for most endocrine tests are established by finding the cut-off that maximises sensitivity and specificity rather than a simple arithmetic calculation involving the mean.

References

1. Feldman EC. Comparison of ACTH response and dexamethasone suppression as screening tests in canine hyperadrenocorticism. J Am Vet Med Assoc 1983;182:506-510
2. Kaplan AJ, Peterson ME, Kemppainen RJ. Effects of disease on the results of diagnostic tests for use in detecting hyperadrenocorticism in dogs. J Am Vet Med Assoc 1995;207:445-451
3. Van Liew CH, Greco DS, Salman MD. Comparison of results of adrenocorticotropic hormone stimulation an low-dose dexamethasone suppression tests with necropsy findings in dogs: 81 cases (1985-1995). J Am Vet Med Assoc 1997;211:322-325
4. Rijnberk A, van Wees A, Mol JA. Assessment of two tests for the diagnosis of canine hyperadrenocorticism. Vet Rec 1988;122:178-180



What is your diagnosis?

The case in question in this edition involves a blood film from a female python. Although otherwise unwell, the abnormality identified in the image here is likely an incidental finding in this case. Can you identify the abnormality? Please see the answer later in the newsletter.

< *Figure 1:* Python blood film (x100 oil)

Vetnostics

The most difficult and frustrating histopathological diagnoses

Dr David Taylor BVSc, Dip ACVP **Veterinary pathologist**

Evaluating bone core biopsies

The diagnosis of suspected bone malignancies can be a frustrating experience for clinicians, surgeons and pathologists. Biopsies require careful planning to maximize the amount and quality of tissue available for interpretation. When you get it wrong I will recommend re-biopsy. So what are some of the factors that contribute to uninterpretable and non-diagnostic samples and how can we manage them?

1. Sampling tool. The biopsy instrument of choice is a 3mm Jamshidi needle. This tool is a good compromise between minimising the risk of pathologic fracture and getting a good tissue section without undue crush artefact. Smaller needles will compromise the tissue section through artefact and frustrate me. Wedge sections are even better for evaluation but there is a greater risk of complication.
2. Sample site. The biopsy must include the radiographically lytic centre of the tumour and the region immediately around it. You should be careful to avoid the often thick peripheral reactive fibrous and periosteal bony change that accompanies many bone tumours; sampling this tissue will result in a diagnosis of periosteal new bone and fibroplasia, frustration for you and an angry client facing re-biopsy and yet another surgery bill. The key to getting the biopsy from the right area are radiographs taken before, during and after surgery. A radiograph taken intra-operatively with the biopsy needle in-place will ensure penetration of the lesion and reduce the need for repeat surgery.
3. How many samples do I need? Taking 3 core sections from the lytic lesion will yield a diagnosis 90-95% of the time, versus 80% for one core. In this instance, more is always better!

Next newsletter… *continuing with the theme of bone disease, we will have a feature contributed by 3 experts in the areas of bone pathology, imaging and surgery.*

Ionised calcium and parathyroid hormone sampling instructions

Vetnostics is able to offer both ionised calcium and parathyroid hormone (PTH) testing on-site. PTH testing is currently available for canines and felines, with equine testing to be available as well in the near future. There are particular sampling requirements for both assays – please see below.

Vitamin D assay for these species is currently under development and is expected to be available later this year as well.

Ionised calcium

- SST (serum separation tubes which contain gel, mat. no. 646309 or 671775 as per Vetnostics stores order form) vacutainer tubes should be used into which a whole blood sample (of at least 2.5 mls) should be placed without uncapping lid (i.e. by inserting the needle through the cap of the tube).The sample should then be gently inverted 8-10 times and centrifuged 15-30 minutes after collection.
- Make sure that SST sample tube is not uncapped at any stage prior to testing – seal the tube with tape prior to submission.
- Wrap the SST sample (after having centrifuged the sample) in "glad-wrap", place in specimen collection bag and keep cool at all times.
- If a centrifuge is not available in the practice, please schedule collection of the blood sample from your patient shortly before arrival of Vetnostics courier and pre-arrange with local collection centre/laboratory for centrifugation of the sample immediately upon receipt at collection centre/laboratory.
- Please tick Ionised Calcium (code VIC) on the submission form under Biochemistry (or request under Other Tests if using an old/outdated request form).

PTH

- Plain serum tube or clot activator tube (mat. no. 654171 or 646830) should be used into which at least 2mls of whole blood is placed (either through the stopper, by uncapping the vacutainer or unscrewing the white top). The sample should then be gently inverted 8-10 times and centrifuged 20-30 minutes after collection.
- Immediately after centrifugation, the serum sample is aspirated off (with a pipette or needle and syringe) and is placed into another plain serum tube. There is no requirement for anaerobic conditions for this sample.
- The serum sample is then placed into a suitable freezer and dispatched to the lab once frozen.
- When ordering a courier, please advise the staff member that a frozen sample is available to ensure correct handling and preparation by courier staff prior to pick-up.
- Please tick Parathyroid Hormone (code VTH) under Endocrinology on the submission form (or request under Other Tests if using an old/outdated request form).

What is your diagnosis answer:

The structure in question is the intracellular parasite evident within the most central erythrocyte. This is an example of a hemogregarine parasite, a group of sporozoan haemoparasites which affect reptiles. Reptilian hemogregarines are well-adapted to their natural hosts and therefore tend not to cause clinical disease.

Apart from further erythrocytes, the further complete cells visible consist of three thrombocytes and a heterophil.