Sorting Out the Adrenal Function Testing Landscape

Q&A with Rhett Nichols, DVM, DACVIM, ANTECH Internal Medicine and Endocrinology Consultant, Past President, Society of Comparative Endocrinology

Q: There are multiple screening tests available for suspected cases of Cushing’s disease in dogs or cats. Which one do you recommend?

A: At the 2012 ACVIM meeting, internists created a consensus statement to address many of the current confusing issues regarding Cushing’s Syndrome in dogs and cats. The consensus for the best screening test was the low dose dexamethasone suppression test (LDDST). This test should be performed after the pet has had a thorough history and physical exam, and is exhibiting at least one clinical sign of Cushing’s Syndrome. A major advantage of this test is its high sensitivity (up to 95% in some studies) -- and a positive test can differentiate pituitary-dependent disease from an adrenal tumor in about 1/3 of cases. It is important to remember not to use this test, or any other screening test for that matter, for patients with suspected Cushing’s Syndrome in the face of another serious illness, as false positive results can occur.
The most frequently used screening test is the ACTH stimulation test. It has the advantage of convenience – depending on the ACTH preparation used, it takes only 1-2 hours to perform this test versus 8 hours for the LDDST. But the ACTH stimulation test is not as sensitive as the LDDST.

Last but not least is the urine cortisol to creatinine ratio (UCCR). The UCCR is most useful as a screening test to rule out Cushing’s disease. In other words, if the UCCR is negative, it is unlikely the animal has Cushing’s Syndrome; if the test is positive, the result is consistent but not diagnostic for the disorder. However, in the situation where a Cushing’s suspect gets extremely stressed during hospitalization or is vicious, or the owner is very reluctant to leave the animal for diagnostic testing, the UCCR is the best screening test since the urine sample can be obtained in the home environment. If you go that route, I recommend having the owner take two or three urine samples – one sample daily collected first thing in the morning over a two or three day period – and then pooling the samples so you get an average UCCR to minimize the issue of day-to-day variability seen in urine cortisol concentrations.

Q: What are the other indications for the ACTH response test?

A: The ACTH response test is extremely useful for monitoring an animal being treated with Lysodren or trilostane. It’s also the “gold standard” test for ruling in or ruling out Addison’s disease (hypoadrenocorticism) in dogs and cats. In addition, it’s the only screening test recommended for diagnosing what is referred to as Atypical Cushing’s disease in dogs (see below). Finally, it’s the best test to detect iatrogenic Cushing’s due to prolonged cortisone therapy.

Q: Where does the resting serum cortisol test fit in?

A: Resting cortisol concentrations are not useful for diagnosing Cushing’s Syndrome. In fact, resting cortisol levels in dogs with known Cushing’s Syndrome overlap 50% of the time with normal dogs. However, resting cortisol levels are very useful as a diagnostic aid in ruling in or ruling out Addison’s disease. If a resting cortisol is > 2 µg/dl (>55 nmol/L), Addison’s disease is very unlikely. Conversely, a resting cortisol < 2 µg/dl (<55 nmol/L) supports the diagnosis of Addison’s disease and an ACTH stimulation test is recommended to confirm the diagnosis.

Q: How important is it to differentiate between pituitary-dependent (PDH) and adrenal-dependent hyperadrenocorticism (ADH) once Cushing’s is diagnosed?

A: The distinction is critical since ADH can be poorly responsive to medical therapy, the therapy itself can be toxic (especially with Lysodren), and malignant adrenal tumors may metastasize to the liver, lungs, or to adjacent blood vessels. Luckily we have three diagnostic tools at our disposal to make this differentiation: high-dose dexamethasone suppression testing (HDDST), the endogenous ACTH test, and diagnostic imaging. Whether you choose the HDDST or endogenous ACTH test is really a matter of personal preference, as their accuracy is comparable. The endogenous ACTH test avoids the 8-hour stay (while trying to keep the animal minimally stressed!) required for the HDDST, but a special tube and strict adherence to the submission protocol is required to ensure accurate results.
**Q:** Diagnostic imaging is indicated in every Cushing’s work-up, but it’s not affordable for all clients. When is imaging most essential?

**A:** While I strongly recommend imaging of the abdomen, paying particular attention to the adrenals, pancreas, kidneys, gall bladder and liver for every dog getting worked up for Cushing’s, I recognize it’s not always feasible. At a minimum, imaging is indicated for patients with equivocal HDDST results (i.e., they don’t suppress) or endogenous ACTH values in the “gray zone” (i.e., between 20 and 45 pg/ml). To better understand the potential value of imaging in these cases, check out the excellent article by Dr. Gross (third article) on the use of imaging to identify or rule out adrenal tumors as the cause of Cushing’s.

**Q:** There are multiple ACTH preparations besides Cortrosyn®. Do you have a strong opinion regarding which preparation should be used?

**A:** I recommend using Cortrosyn given the lack of stability studies or peer-reviewed validation of compounded ACTH gels, and uncertain lot-to-lot variability. Of course, the problem is that Cortrosyn keeps getting more expensive (over $100 per vial), but a vial can be divided into several doses and frozen up to six months, which makes it very cost competitive with the compounded products. I recommend checking out Dr. Mark Peterson’s blog entry (included below) for his simple protocol on how to aliquot and store each vial of Cortrosyn for subsequent use.

**Q:** Two recent areas of confusion around adrenal function testing are what to do in cases of so-called “Atypical Cushing’s” and whether or not it’s appropriate to perform low-dose or high-dose oral dexamethasone suppression testing using the urine cortisol-creatinine ratio rather than serum, as practiced by some European veterinarians. What are your positions on these issues?

**A:** “Atypical Cushing’s” describes an animal with clinical signs and lab abnormalities consistent with Cushing’s, but normal or equivocal adrenal function test results. In these cases, we often suggest the Atypical Cushing’s Profile (also called the Canine Adrenal Androgen Profile), interpreted in conjunction with the ACTH response test. The profile, which is run at the University of Tennessee, looks at resting and stimulated values (“pre” and “post”) of a variety of sex hormones. A great deal of work remains to clarify the diagnostic value of many of these hormones, so this is an area of research that ANTECH will continue to track closely.

Regarding performing the LDDST or HDDST using oral dexamethasone protocols and UCCR testing. All the published studies using oral dexamethasone protocols in conjunction with urine cortisol testing were performed by clinical researchers at Utrecht University using a “home-made” urine cortisol assay. This assay was developed at Utrecht over twenty years ago and was designed to eliminate cross reaction of urine cortisol with other urine metabolites. To date, no studies have been published to determine if the results of UCCR testing following a low or high dose of oral dexamethasone using commercial assays for urinary cortisol determination give comparable results to the Utrecht assay. In short, we recommend holding off on this approach until some U.S.-based validation studies are published.

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**Atypical Cushing’s Profile**

Measures pre- and post-ACTH levels of:
- Cortisol
- Progesterone
- 17-hydroxypregesterone
- Estradiol
- Androstenedione
- Aldosterone

**Test Code:** S16597

**Samples:** Two samples, pre- and post-ACTH serum (2.0mL)

**Turnaround:** 7-10 days
### Tests for Suspected Cushing’s Syndrome

<table>
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<th>Indication</th>
<th>Test</th>
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| **1** Screen for disease | LDDST  
**Pro:** Recommended; highest sensitivity  
**Con:** Requires 8 hours to perform  
**UCCR**  
**Pro:** Good rule out test  
**Pro:** Sample taken at home (ideally first thing in the morning)  
**Con:** Not highly specific  
**ACTH response**  
**Pro:** Recommended for dogs on cortisone therapy or with concurrent illness; used to diagnose Atypical Cushing’s; requires only 1-2 hours to perform  
**Con:** Lower sensitivity than LDDST |
| **2** Differentiate form of disease | HDDST  
Comparable sensitivity to endogenous ACTH for differentiating ADH/PDH; Requires 8 hours, 3 samples.  
**Endogenous ACTH**  
Comparable sensitivity to HDDST; Simple to perform, but requires special tube and sample handling  
**Adrenal imaging**  
Identifies adrenal tumors; resolves equivocal cases (HDDST or endog. ACTH results in “gray zone”) |
| **3** Monitor response to therapy | ACTH response  
Extremely useful for monitoring response to Lysodren or trilostane therapy |

### Tests for Suspected Addison's Disease

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<th>Indication</th>
<th>Tests for Suspected Addison’s disease</th>
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| **Screen** for disease | ACTH response  
Gold standard test for hypoadrenocorticism  
Resting cortisol  
Simple screening test to rule in or rule out Addison’s disease; followed by confirmatory ACTH response test |
Practical Tips:

How to Extend Your Supply of Cortrosyn® and Lower the Cost of ACTH Stimulation Testing

Mark E. Peterson, DVM, DACVIM

Reproduced with permission from Dr. Mark E. Peterson’s “Insights into veterinary endocrinology” blog, March 22, 2011, endocrinevet.blogspot.com.

Cortrosyn is expensive. And once reconstituted, it has a limited shelf life. However, by following the protocol outlined below, you can easily dilute, aliquot and store Cortrosyn after reconstitution for up to 6 months. This makes each ACTH stimulation test much less expensive, because each vial of Cortrosyn can be used to perform as many as five ACTH stimulation tests.

Cortrosyn is supplied in vials each containing 0.25 mg (250 μg) of synthetic ACTH (cosyntropin) in powder form. Because the dose of Cortrosyn used to perform an ACTH stimulation test is only 5 μg/kg, small to medium sized dogs require only a fraction of the ACTH contained in each vial. For more information on how to perform an ACTH stimulation test, see my post entitled, “What’s the best protocol for ACTH stimulation testing in dogs and cats?“.

How to aliquot and store each vial of Cortrosyn for subsequent use?
It’s simple — just follow the directions outlined here:

1. Reconstitute the Cortrosyn powder by adding exactly 2.5-ml of sterile saline solution to the vial. With this dilution, the resulting concentration of Cortrosyn solution would be 100 μg/ml.

2. Once Cortrosyn is reconstituted, aspirate 50-μg doses (0.5 ml) into 5 plastic syringes. Or, if smaller ACTH doses are desired, aspirate 25-μg doses (0.25 ml) into 10 syringes. (For more accurate dosing, I use insulin or tuberculin syringes).

   DO NOT store reconstituted Cortrosyn in glass containers or vials. The reason that the Cortrosyn needs to be stored in plastic is that ACTH will stick to glass, thereby lowering the available amount of Cortrosyn that would be injected at time of testing.

3. The syringes containing the reconstituted, diluted Cortrosyn should be labeled with the product, dose in each syringe, and the date the Cortrosyn was reconstituted.

4. Freeze each of the syringes at -20°C. Avoid storing these syringes in a frost-free freezer, which must periodically warm up to defrost. Repeated freezing and thawing cycles would compromise the integrity of the Cortrosyn. When frozen properly, aliquots can be stored for up to 6 months without loss of efficacy.

5. Alternatively, the Cortrosyn solution can be stored refrigerated (4°C) where it has been shown to be bioactive and stable for at least 4 weeks.

Diluting the Cortrosyn and using a low-dose ACTH stimulation test protocol is very cost-effective. In this way, one vial of Cortrosyn can be used to test multiple patients without compromising the quality of the test results. As an example, at the 5-μg/kg dose, five dogs weighing 10 kg (22 lbs) can be tested using a single vial, reducing the cost of the drug by 80%. By looking at the cost benefit, it is clear that the savings could be significant by using this testing protocol.
The Use of Imaging in Cushing’s Syndrome

Bill Gross DVM, MS, DACVR
Staff Radiologist, ANTECH Imaging Services

Specific imaging of the adrenal glands is warranted whenever canine patients exhibit clinical signs or blood work abnormalities consistent with adrenal related disease. The most common presentation is a primary differential diagnosis of hyperadrenocorticism (Cushing’s Syndrome) determined based on history, physical exam findings and lab work. While thin section contrast-enhanced CT scan of the retroperitoneal space provides for the best imaging and characterization of the adrenal glands and adjacent vasculature and tissues, ultrasound exam is typically preferred over CT due to lower cost, increased availability and ability to be performed without anesthesia, making it more client and patient friendly.

Once a diagnosis of hyperadrenocorticism is made, imaging of the adrenal glands is used to identify or rule out an adrenal tumor as the cause of the signs. Adrenal-dependent hyperadrenocorticism (ADH), which occurs in approximately 20% of naturally occurring canine Cushing’s cases, is important to distinguish from pituitary-dependent hyperadrenocorticism (PDH), as adrenal tumors often respond poorly to medical therapy and require surgical excision, both to alleviate clinical signs and remove the potential for local invasion or distant metastasis. In cases of PDH, the adrenal glands are typically bilaterally enlarged (caudal pole thickness > 7.4 mm in mid to large-breed dogs and > 6.0 mm in small breed dogs as guidelines), but can be normal in appearance as well.

Types of adrenal tumors include benign adenomas (functional or incidental non-functional nodules), functional adenocarcinomas and pheochromocytomas. As with other parenchymal organs, metastatic nodules from other primary tumors are also occasionally encountered.

While benign versus malignant or functional versus non-functional tumors cannot be definitely determined via ultrasound, studies have reported that an adrenal nodule larger than 2 cm or which shows any signs of adjacent tissue or vascular invasion is much more likely to be a malignant tumor. As with many aspects of diagnostic imaging, the findings at the adrenal glands on ultrasound or CT/MR must be placed into the context of the big picture of the patient: history, clinical signs, physical exam findings and blood work results, to come to a determination of confidence in diagnosis, prognosis and treatment. In cases where a small (<2 cm) adrenal nodule is identified incidentally on ultrasound, a recheck exam in 1-3 months is recommended to evaluate for static or progressive appearance.

If an adrenal tumor is identified, careful evaluation of the margins of the gland, capsule contours, and adjacent retroperitoneal tissues, kidneys and vasculature is indicated for surgical planning and prognosis. Pheochromocytomas and adenocarcinomas can invade the phrenicoabdominal vein, renal vein or caudal vena cava or result in thrombus extension into the vena cava. Additional color Doppler imaging of the mass and vessels should be used to best evaluate the blood flow and lumen characteristics of the vessels, but it remains difficult to definitely distinguish between tumor invasion and thrombus formation at the vessel lumen.

Ultrasound exam has been shown to very sensitive and specific in characterizing adrenal tumors and adjacent tissue/vessel invasion; however, if thorough and complete imaging of both glands is not possible with ultrasound exam due to patient cooperation/size, operator experience or obscuring overlying structures such as intestinal gas, then CT scan or MRI scan is warranted to best evaluate the tissues for surgical planning/prognosis.

For more information about ANTECH® Imaging Services, which offers 24/7/365 diagnostic imaging and telemedicine consultations on all modalities, please visit AntechImagingServices.com
Avian/Exotic Update

Now Available: The Elementary Body Agglutination titer test for diagnosing Avian Chlamydiosis (psittacosis) is available once again, effective October 1, 2012, through the work of Dr. Branson Ritchie & the Infectious Disease Lab at UGA.

Test Code: S16670.
Sample Requirements: 200 ul serum or heparinized plasma.
Note: Do not submit microhematocrit tubes.

New & Improved Avian Profiles! Several of ANTECH’s most popular avian profiles have been expanded to include additional tests. Our new comprehensive profiles include:

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<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE021</td>
<td>Avian comprehensive profile PLUS Bile acids</td>
</tr>
<tr>
<td>AE025</td>
<td>Avian comprehensive profile PLUS Bile acids PLUS electrophoresis</td>
</tr>
<tr>
<td>AE051</td>
<td>Avian standard profile PLUS Bile acids</td>
</tr>
<tr>
<td>AE055</td>
<td>Avian standard profile PLUS Bile acids PLUS electrophoresis</td>
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For further details, please visit [http://www.antechdiagnostics.com/Main/TestGuide.aspx](http://www.antechdiagnostics.com/Main/TestGuide.aspx) and select the “Avian & Exotics” test category.

Chlamydophila Screening Update

For general wellness screening for Chlamydophila, the Chlamydophila PCR test (Test Code: S16672) is recommended. Submit a single swab combining sites of the upper respiratory tract. Note that the recommended sites to swab are the conjunctiva and choanae, with less emphasis on cloacal swabbing.

For specialized situations when more definitive testing for Chlamydophila is required, such as the introduction of new birds into a closed aviary or potential contact with immunocompromised individuals (nursing homes, schools, etc.), the comprehensive Chlamydophila Profile is recommended (Test Code: S85206). The profile includes four tests – two PCR assays and two serology assays:

- Upper respiratory PCR test (combined choanal, conjunctival)
- Whole blood PCR test
- IFA (IgG)
- EBA (IgM)

Specimen requirements include: GT (gel), GT (unspun) plus swab in red top tube. Turnaround time is 7 to 14 days.

The Avian/Reptile Sensitivity Profile has been updated to include the most frequently used antibiotics and omit antibiotics that are no longer relevant to this profile.

The updated A&R sensitivity profile includes:

- Amikacin
- Cefadroxil
- Ceftazidime
- Ceftiophur
- Chloramphenicol
- Ciprofloxacin
- Clavamox
- Doxycycline
- Enrofloxacin
- Orbifloxacin
- Pipericillin
- TMS/Sulfa

For more information, please call the ANTECH Consult line and request one of our Avian medicine specialists.