

# Demystifying Tests for Hyperadrenocorticism

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You have asked...  
How do I test a dog for hyperadrenocorticism?



Typical pot-belly appearance, thinning hair, and hyperpigmentation (left lumbar region) in a dog with pituitary-dependant hyperadrenocorticism .

## The expert says...

**H**yperadrenocorticism (HAC) is a common differential diagnosis in dogs with polyuria/polydipsia (PU/PD), chronic skin issues (eg, alopecia, recurrent pyoderma), increased alkaline phosphatase (ALP) activity, weight gain, or hepatomegaly (**Figure 1**). Veterinarians must often decide when to test for HAC and which test to use.

## When to Test for Hyperadrenocorticism

When evaluating the utility of a diagnostic test, test sensitivity and specificity must be considered. In addition, positive predictive value (PPV), which indicates the likelihood that an individual with a positive test result truly has the disease, should be considered. PPV is influenced by disease prevalence in the population being tested. For instance, if every dog that enters the clinic (a population with low HAC prevalence) is tested for HAC, the likelihood that a positive test result represents a true positive would be low (ie, a low PPV); however, if only dogs that have PU/PD, increased ALP activity, a stress leukogram, and alopecia (a population with a higher HAC prevalence) are tested, a positive test result is more likely accurate (ie, a high PPV).

To maximize the usefulness of HAC tests, only dogs for which there is a clinical suspicion of disease based on history, physical examination, and routine laboratory findings (**Tables 1 and 2**, next page) should be tested. A diagnosis of HAC should never be made on the basis of adrenal function testing or imaging alone. Ideally, dogs should not be tested when other significant, concurrent diseases are present, as this increases the risk for false-positive results. However, finding

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ALP = alkaline phosphatase, eACTH = endogenous ACTH, HAC = hyperadrenocorticism, PD = polydipsia, PPV = positive predictive value, PU = polyuria

an unexpected adrenal mass on imaging for a different presentation should prompt testing for HAC.

### Which Test Is Best?

None of the currently available tests for HAC have 100% diagnostic accuracy (**Table 3**). Tests can be separated into screening and differentiating tests. Screening tests are used to support the clinical diagnosis of HAC and, rather than positive or negative, can be thought of as *consistent with* or *not consistent with* an HAC diagnosis. If a high suspicion for HAC exists but an initial screening test is negative, a different screening test should be performed.

Commonly used screening tests include the urine cortisol:creatinine ratio (UCCR), low-dose dexamethasone suppression test (LDDST), and the ACTH stimulation test. Differentiating tests are used to help differentiate pituitary-dependent (PDH) from adrenal-dependent hyperadrenocorticism (ADH) and should only be performed after a diagnosis of HAC is established. Differentiating tests include LDDST, high-dose dexamethasone suppression test (HDDST), endogenous ACTH (eACTH) measurement, and abdominal ultrasound.

To maximize the usefulness of HAC tests, only dogs for which there is a clinical suspicion of disease based on history, physical examination, and routine laboratory findings should be tested.

The initial screening test should be partly chosen based on the circumstances of the case. In dogs with a known adrenal tumor, the LDDST should be the initial screening test because of its higher sensitivity in ADH compared with the ACTH stimulation test. Dogs with suspected iatrogenic HAC should be screened with the ACTH stimulation test, as it is the only test that can diagnose this condition. If testing must be done with concurrent disease present, the LDDST may be best, as a negative test likely excludes the presence of HAC. A positive LDDST requires further testing to confirm the diagnosis. If there is a low likelihood of HAC result, then the UCCR can be used as an easy, inexpensive screening test. When choosing between the LDDST and the ACTH stimulation test, the author does not recommend one test over the other except for cases as previously noted. Rather, it is more important to understand the sensitivity and specificity of the tests (**Table 3**) and interpret the results in light of the clinical findings.

#### Urine cortisol:creatinine ratio

The UCCR is a simple and inexpensive screening test best performed on a urine sample from a patient's first urination of the day collected at home by the owner. The sample should not be collected within 2 days of a stressful event (eg, a clinic visit).

**Table 1** Clinical Findings in Canine Hyperadrenocorticism

<i>Common</i>	<i>Less common</i>	<i>Rare</i>
PU/PD	Lethargy	Thromboembolism
Polyphagia	Thin skin	Cruciate ligament rupture
Abdominal distension	Comedones	
Hepatomegaly	Hyperpigmentation	
Alopecia	Poorly controlled diabetes mellitus	
Panting		

**Table 2** Laboratory Test Findings in Canine Hyperadrenocorticism

<i>Chemistry</i>	<i>CBC</i>	<i>Urinalysis</i>
Increased ALP, ALT	Mature neutrophilia	Low urine specific gravity
Hyperglycemia	Lymphopenia	Proteinuria
Hypercholesterolemia	Eosinopenia	Possible pyuria/bacteriuria
Hypertriglyceridemia	Thrombocytosis	

ADH = adrenal-dependent hyperadrenocorticism, ALP = alkaline phosphatase, eACTH = endogenous ACTH, HAC = hyperadrenocorticism, HDDST = high-dose dexamethasone suppression test, LDDST = low-dose dexamethasone suppression test, PD = polydipsia, PDH = pituitary-dependent hyperadrenocorticism, PU = polyuria, UCCR = urine cortisol:creatinine ratio

**Table 3** Comparative Aspects of Screening Tests for Canine Hyperadrenocorticism

Test	Sensitivity (%)	Specificity (%)	Comments
UCCR	90–100	20–40	Good screening test when HAC unlikely
LDDS	85–100	44–73	Good initial test because of high sensitivity; best initial test for ADH; can also be a differentiating test
ACTH Stimulation	60–85	60–90	Only test that identifies iatrogenic HAC

The sensitivity of this test is good (90%–100%) with a normal ratio making HAC unlikely. However, the specificity of the UCCR is poor (20%–40%), particularly in dogs with concurrent diseases.<sup>1–3</sup> Hence, the UCCR is best used as a screening test in dogs for which the clinical suspicion of HAC is low; positive results should be investigated further with other screening tests.

#### Low-dose dexamethasone suppression test

The LDDST can be used as a screening and a differentiating test. In the recent ACVIM Consensus Statement, the LDDST was recommended as the initial screening test in dogs suspected to have HAC because of its better sensitivity compared with the ACTH stimulation test.<sup>4</sup>

#### How to Perform an LDDST

- Collect blood for a baseline plasma cortisol concentration
- Administer 0.01–0.015 mg/kg of dexamethasone IV
- Collect additional blood samples at 4 and 8 hours

The LDDST is performed by collecting blood for a baseline cortisol concentration, administering 0.01–0.015 mg/kg of dexamethasone IV, and collecting additional blood samples at 4 and 8 hours post-injection (see **How to Perform an LDDST**). As a screening test, only the 8-hour cortisol concentration is initially evaluated. If the 8-hour postdexamethasone cortisol level is  $<1.4 \mu\text{g/dL}$ , appropriate suppression has occurred and the test is not consistent with HAC. However, it has been recommended

that this cut-off value be reevaluated because of changes in assays and populations used in early studies.<sup>4</sup> A cut-off of  $1.0 \mu\text{g/dL}$  has been suggested. Using a cut-off of  $1.4 \mu\text{g/dL}$ , the LDDST has a good sensitivity (85%–100%), but the specificity is only fair

(44%–73%).<sup>3,5–8</sup> An *inverse* pattern on the LDDST has been described where the 8-hour cortisol shows suppression but the 4-hour cortisol does not.<sup>9</sup> While this pattern is suspicious for HAC, further testing is recommended for definitive diagnosis.

If there is no suppression of cortisol at 8 hours, then the test is consistent with HAC and the LDDST results are further used as a differentiating test. The baseline and 4-hour cortisol levels are evaluated; if the 4-hour cortisol is  $<1.4 \mu\text{g/dL}$  or is  $<50\%$  of the baseline cortisol, the LDDST is consistent with a diagnosis of PDH. Additionally, suppression of the 8-hour cortisol to  $<50\%$  of baseline has also been reported to be consistent with PDH, but the author prefers to verify this with an alternate differentiating test such as abdominal ultrasound. If none of these criteria is met, then either PDH or ADH may be present and further testing is needed.

#### ACTH stimulation test

The ACTH stimulation test is the only test that can diagnosis iatrogenic HAC. The test is performed using a synthetic ACTH (eg, cosyntropin, tetracosactrin) administered at a dose of  $5 \mu\text{g/kg IV}$ . Cortrosyn can also be administered IM at the same dose. After reconstitution, unused product can be separated into aliquots in plastic syringes and frozen for up to 6 months (see **How to Perform an ACTH Stimulation Test**, next page). Compounded ACTH gels are not recommended because of variability in quality and timing of maximum stimulation of the adrenals. Blood samples are collected before and 1 hour after administration of ACTH for cortisol determination. Post-ACTH cortisol concentrations above the laboratory reference range are consistent with HAC. Cortisol concentrations that are only marginally increased should be interpreted with caution, especially in patients with minimal signs of HAC or with concurrent diseases. Dogs with iatrogenic HAC typically have a normal–low normal baseline cortisol with no-to-minimal

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**The low-dose dexamethasone suppression test (LDDST) can be used as a screening and a differentiating test.**

increase post-ACTH despite having findings consistent with HAC. Test sensitivity ranges from 60% to 85% with poorer sensitivity in dogs with ADH compared to PDH. For this reason, LDDST is recommended as the initial screening test in any dog in which an adrenal mass is already known or suspected to be present. Specificity ranges from 60% to 90%, as nonadrenal illness can result in false-positive results.<sup>3,6,10-12</sup>

### High-dose dexamethasone suppression test

The HDDST is performed similarly to the LDDST with samples collected for cortisol measurement at baseline and then 4 and 8 hours after dexamethasone injection, but the dose of dexamethasone is 0.1 mg/kg IV. This test should only be performed once a diagnosis of HAC is established. Suppression occurs when either the 4- or the 8-hour cortisol is <1.4 µg/dL or <50% of the baseline. Suppression is consistent with PDH. A lack of

suppression does not exclude PDH and, in fact, there is still almost an equal likelihood of PDH or ADH.

**Endogenous ACTH measurement**  
Measurement of eACTH may be used to differentiate ADH and PDH. In ADH, eACTH concentration is typically low or unmeasurable. In PDH, eACTH may be high or normal because of the pulsatile release of ACTH. Sample handling is critical for accurate measurement. Plasma samples should be collected, frozen immediately, and shipped overnight to a laboratory on ice/dry ice so they remain frozen during transit. Poor sample handling can result in degradation of eACTH and artificially decreased measurements. For this reason, the author does not recommend routine use of eACTH measurement as a differentiating test. ■ **cb**

See **Aids & Resources**, back page, for references & suggested reading.

### How to Perform an ACTH Stimulation Test

- Use a synthetic ACTH (eg, cosyntropin, tetracosactrin)
- Reconstitute the product\*
- Administer a dose of 5 µg/kg IV. Cortrosyn can also be administered IM.
- Compounded ACTH gels are not recommended because of variability in quality and timing of maximum stimulation of the adrenals.
- Collect blood samples before and 1 hour after administration of ACTH for plasma cortisol determination

\* Unused product can be separated into aliquots in plastic syringes and kept frozen for up to 6 months.

ADH = adrenal-dependent hyperadrenocorticism, eACTH = endogenous ACTH, HDDST = high-dose dexamethasone suppression test, LDDST = low-dose dexamethasone suppression test

## VETORYL® CAPSULES (trilostane)

**10 mg, 30 mg, 60 mg and 120 mg strengths**  
Adrenocortical suppressant for oral use in dogs only

**BRIEF SUMMARY** (For Full Prescribing Information, see package insert.)

**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION:** VETORYL is an orally active synthetic steroid analogue that blocks production of hormones produced in the adrenal cortex of dogs.

**INDICATIONS:** VETORYL CAPSULES are indicated for the treatment of pituitary-dependent hyperadrenocorticism in dogs. VETORYL CAPSULES are indicated for the treatment of hyperadrenocorticism due to adrenocortical tumor in dogs.

**CONTRAINDICATIONS:** The use of VETORYL CAPSULES is contraindicated in dogs that have demonstrated hypersensitivity to trilostane. Do not use VETORYL CAPSULES in animals with primary hepatic disease or renal insufficiency. Do not use in pregnant dogs. Studies conducted with trilostane in laboratory animals have shown teratogenic effects and early pregnancy loss.

**WARNINGS:** In case of overdosage, symptomatic treatment of hypoadrenocorticism with corticosteroids, mineralocorticoids and intravenous fluids may be required. Angiotensin-converting enzyme (ACE) inhibitors should be used with caution with VETORYL Capsules, as both drugs have aldosterone-lowering effects which may be additive, impairing the patient's ability to maintain normal electrolytes, blood volume and renal perfusion. Potassium-sparing diuretics (e.g., spironolactone) should not be used with VETORYL CAPSULES as both drugs have the potential to inhibit aldosterone, increasing the likelihood of hyperkalemia.

**HUMAN WARNINGS:** Keep out of reach of children. Not for human use. Wash hands after use. Do not empty capsule contents and do not attempt to divide the capsules. Do not handle the capsules if pregnant or if trying to conceive. Trilostane is associated with teratogenic effects and early pregnancy loss in laboratory animals. In the event of accidental ingestion/overdose, seek medical advice immediately and take the labeled container with you.

**PRECAUTIONS:** Hypoadrenocorticism can develop at any dose of VETORYL CAPSULES. A small percentage of dogs may develop corticosteroid withdrawal syndrome within 10 days of starting treatment. Mitotane (o,p'-DDD) treatment will reduce adrenal function. Experience in foreign markets suggests that when mitotane therapy is stopped, an interval of at least one month should elapse before the introduction of VETORYL CAPSULES. The use of VETORYL CAPSULES will not affect the adrenal tumor itself. Adrenalectomy should be considered as an option for cases that are good surgical candidates.

**ADVERSE REACTIONS:** The most common adverse reactions reported are poor/reduced appetite, vomiting, lethargy/dullness, diarrhea, and weakness. Occasionally, more serious reactions including severe depression, hemorrhagic diarrhea, collapse, hypoadrenocortical crisis, or adrenal necrosis/rupture may occur, and may result in death.



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