



Testing & Diagnostic Protocols



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Laboratory Diagnosis of Canine Thyroid Disease

Canine Hypothyroidism

Hypothyroidism occurs when the thyroid glands fail to produce adequate amounts of thyroxine (T4) and triiodothyronine (T3). Primary hypothyroidism results from idiopathic thyroid gland atrophy or immune mediated (lymphocytic) thyroiditis. Uncommon causes of canine hypothyroidism are congenital disease from dysmorphogenesis of thyroid hormone, abnormal thyroid stimulating hormone production (as seen with congenital malformations in GSD), or abnormal thyroid gland development. Hypothyroidism results in myriad clinical signs because thyroid hormones are involved in a wide variety of physiological processes. Typically, young to middle aged dogs are affected, but it has been reported in both young and old dogs. Any breed can be affected although some appear predisposed, such as Doberman Pinschers, Golden Retrievers, Great Danes, Poodles and Beagles. Clinical signs are nonspecific and include lethargy, weight gain, and hair coat changes. Routine hematologic and biochemical assays are helpful in diagnostic assessment of dogs suspect for hypothyroidism and, although abnormalities seen are not specific for hypothyroidism, the results may help rule out concurrent disease processes. Laboratory abnormalities associated with hypothyroidism include the following:

Hematology

Nonregenerative anemia
RBC leptocytes (target cells)

Biochemistry

Hypercholesterolemia
Hypertriglyceridemia

Screening Assays for Hypothyroidism in Dogs

NOTE: Certain drugs can alter T4, TSH and free T4 assays including prednisone, phenobarbital, trimethoprim sulfamethoxazole, aspirin, clomipramine and thyroxine supplementation. Screening for hypothyroidism is not recommended in dogs that are systemically ill due to the euthyroid sick syndrome effect.

Thyroxine (total T4)

Reference Interval

total T4: 1.2–4.3 µg/dL

Interpretation

total T4 <1.2 µg/dL result supportive of diagnosis of hypothyroidism or euthyroid sick syndrome.

Additional assays (TSH, free T4) can help confirm the diagnosis.

Thyroid Stimulating Hormone (TSH)

Reference Interval

TSH: 0.0–0.75 ng/mL

Interpretation

TSH >0.75 ng/mL Consistent with hypothyroidism

TSH 0.0–0.75 ng/mL (normal range): Consistent with normal or hypothyroid dog.

Many hypothyroid dogs (estimated up to 40%) may have TSH concentration within the reference range. Perform free T4 assay.

Free T4

Interpretation

free T4 concentration below reference range: Supports diagnosis of hypothyroidism

free T4 concentration within reference range: Hypothyroidism unlikely

Monitoring Thyroxine Replacement Therapy

Total T4

NOTE: Assay of T4 concentration is recommended for monitoring thyroxine replacement therapy. Assay of free T4 does not appear to offer additional information except in dogs with T4 auto-antibodies.

Obtain sample for monitoring T4 4–6 hours after administration of thyroxine.

Interpretation

T4 should be in the reference range. Dosing should be adjusted based upon the results of T4 testing together with the clinical signs.

Laboratory Diagnosis of Canine Hyperthyroidism

Hyperthyroidism is rare in dogs. Although thyroid tumors (carcinomas) are not uncommon in dogs, functional tumors occur rarely. Hyperthyroidism has been induced from adulteration of some dog food with thyroid gland tissue. Inadvertent sampling of thyroid tissue during blood draw will also result in unanticipated increased T4 concentrations.

Total T4

Interpretation

Increased T4 concentration in the absence of supplementation warrant assessment for thyroid area masses, investigation of possible dog food adulteration or possible aspiration of thyroid tissue at the time of blood draw.

References

- Keller N. How to diagnose hypothyroidism. Presented at World Small Animal Veterinary Association World Congress Proceedings, 2014.
<https://www.vin.com/apputil/content/defaultadv1.aspx?pId=12886&catId=57088&id=7054887>
- Heseltine J. Canine hypothyroidism: Diagnosis and treatment. *Today's Vet Pract.*
<https://todaysveterinarypractice.com/canine-hypothyroidism-diagnosis-and-treatment/>
- Feldman ED, Nelson RW. Canine Hypothyroidism in Feldman EC, Nelson RW (eds). *Canine and Feline Endocrinology and Reproduction*. 3rd ed St. Louis, 2004, pp 88–142.
- Nelson RW. How I treat: Canine hypothyroidism. Presented at the World Small Animal Veterinary Association World Congress, 2013.
<https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11372&catId=35307&id=5709803>
- Feldman ED, Nelson RW. Canine Thyroid Tumors and Hyperthyroidism in Feldman EC, Nelson RW (eds). *Canine and Feline Endocrinology and Reproduction*. 3rd ed St. Louis, 2004, pp 219–221.
- Exogenous Hyperthyroidism and Thyroid Hormones in Pet Food- Veterinarians. *US FDA product safety information*. March 2017.
<https://www.fda.gov/animal-veterinary/product-safety-information/exogenous-hyperthyroidism-and-thyroid-hormones-pet-food-veterinarians>.

Laboratory Diagnosis of Feline Thyroid Disease

Feline Hyperthyroidism

Hyperthyroidism is the most common endocrinopathy of older cats, but the pathogenesis remains unclear. Common clinical signs are weight loss, poor hair coat, behavior changes, vomiting, polyuria, polydipsia, increased appetite and activity, restlessness, increased heart rate, occasional dyspnea, weakness and depression. Evaluation for palpable thyroid nodule is indicated in cats presenting with these clinical signs. A number of abnormalities can be manifest in routine laboratory testing in hyperthyroid cats, although some of these abnormalities may be the result of concurrent disease processes. The following laboratory abnormalities have been documented in hyperthyroid cats:

Hematology

Erythrocytosis
RBC Macrocytosis (Increased MCV)
Leukocytosis
Lymphopenia
Eosinopenia

Biochemistry

Increased ALT
Increased SAP
Increased BUN
Increased Creatinine
Hyperphosphatemia

Tests for Diagnosis of Feline Hyperthyroidism

NOTE: Results of tests below should be correlated with clinical signs, evidence of thyroid mass and results of other laboratory tests.

Total T4

Reference Interval

total T4: 0.8–4.7 µg/dL

Interpretation

total T4 >4.7 µg/dL: Supportive of diagnosis of feline hyperthyroidism. Up to 10% of hyperthyroid cats have T4 concentration in the reference range. If cat has appropriate clinical sign and palpable thyroid nodule, a T4 should be repeated and, if concentration remains in the reference range, assay for free T4 is indicated.

Free T4

Interpretation

Increased concentration of free T4 is supportive of diagnosis of feline hyperthyroidism.

Feline Hypothyroidism

Naturally occurring hypothyroidism is rare in cats and is usually a transient consequence of treatment for hyperthyroidism. Rarely, congenital hypothyroidism may occur in cats and is often associated with other dwarfism and associated abnormalities.

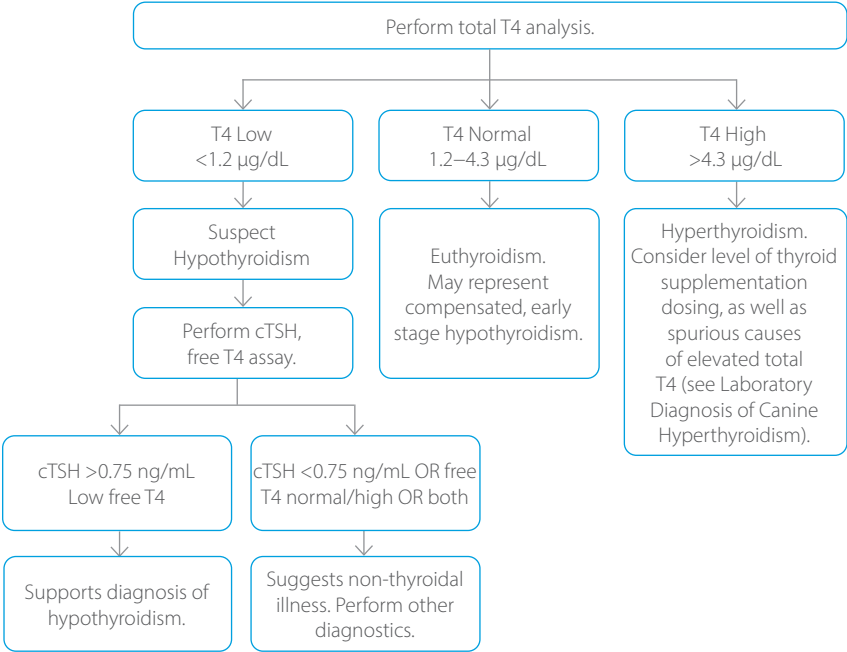
Spontaneous hypothyroidism has been reported rarely in cats and has been associated with a variety of thyroid lesions, including lymphocytic thyroiditis, thyroid atrophy and thyroid goiter. Clinical findings included polyuria, polydipsia, hair coat changes and weight gain. The most common routine laboratory abnormality associated with hypothyroidism is increased creatinine associated with urine specific gravity <1.035. Total T4 and free T4 concentrations were decreased in most of the reported cases and thyroid stimulating hormone concentration was increased.

References

- Feldman EC, Nelson RW. Feline Hyperthyroidism. In *Canine and Feline Endocrinology*, EC Feldman, RW Nelson eds., St. Louis, Elsevier, 2004, 152–218.
- Bruyette D. Feline hyperthyroidism: Diagnosis and therapeutic modalities. *Today's Vet Pract* Jan/Feb: 25–30, 2013.
- <https://todaysveterinarypractice.com/feline-hyperthyroidism-diagnosis-therapeutic-modalities/>.
- Hui TY, Bruyette DS, Moore GE, et al. Effect of feeding an iodine-restricted diet in cats with spontaneous hyperthyroidism. *J Vet Intern Med* 29: 1063–1068, 2015.
- Peterson ME, Carothers MA, Gamble DA, et al. Spontaneous primary hypothyroidism in 7 adult cats. *J Vet Intern Med* 32: 1864–1873, 2018.
- Feldman EC, Nelson RW. Feline Hypothyroidism. In *Canine and Feline Endocrinology*, EC Feldman, RW Nelson eds., St. Louis, Elsevier, 2004, 143–51.

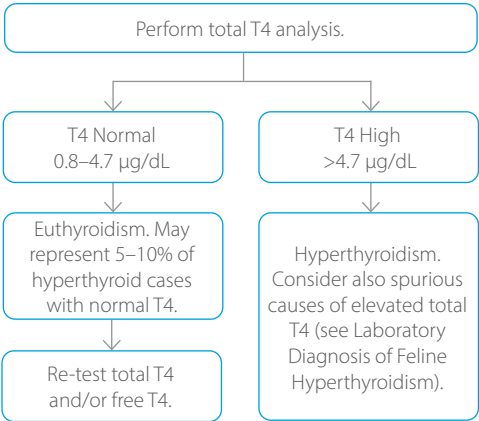
Canine Thyroid Disease Testing Protocols

If a thorough patient history and clinical signs lead to suspicion of hypothyroidism, first assess a chemistry, electrolytes, hematology and urinalysis. If non-thyroidal illness (NTI) is suspected, address that illness first.



Feline Thyroid Disease Testing Protocols

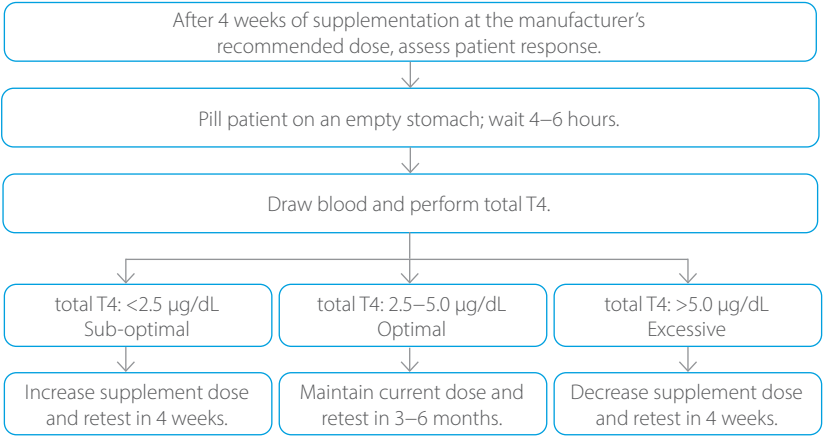
If a thorough patient history and clinical signs lead to suspicion of hyperthyroidism, first assess a chemistry, electrolytes, hematology and urinalysis.



Canine Thyroxine Supplementation Monitoring

To ensure the hypothyroid dog is receiving therapeutic supplementation, follow these recommended steps in total T4 monitoring.

NOTE: These are general guidelines and should be considered secondarily to those provided on the insert accompanying the specific supplement in use. All post pill total T4 levels should be interpreted in light of clinical signs.



Laboratory Diagnosis of Canine Hypoadrenocorticism

Hypoadrenocorticism (Addison's disease) occurs relatively uncommonly in dogs. Although many breeds can be affected, there is a possible familial predisposition in Portuguese Water Dogs, Leonbergers, Standard Poodles, Bearded Collies, Great Danes, Rottweilers, Wheaton Terriers, West Highland White Terriers and others. Clinical signs can be vague, episodic or progressive with variable rapidity of progression. In dogs that have acute disease, earlier, more chronic signs of illness may have eluded detection. Acute exacerbation may result from stressors such as boarding/grooming, lifestyle changes, moving, or a trip to the veterinarian. Common clinical signs include anorexia or poor appetite, lethargy/depression and vomiting/regurgitation. Weakness, weight loss, diarrhea, polyuria, polydipsia tremors, collapse or painful abdomen may also occur.

Primary hypoadrenocorticism is most commonly the result of immune mediated destruction of the adrenal cortex. The resultant destruction of all 3 adrenal cortical layers leads to inadequate secretion of both mineralocorticoids and glucocorticoids. Adrenal insufficiency can also occur secondary to fungal infection, adrenal neoplasia, amyloidosis, trauma or coagulopathy. Some dogs with autoimmune adrenalitis may manifest other, concurrent endocrinopathies such as hypothyroidism, diabetes mellitus or hypoparathyroidism. Rarely, dogs may manifest atypical hypoadrenocorticism with loss of glucocorticoid secreting portions of the adrenal gland and preservation of mineralocorticoids. Mineralocorticoid loss may occur later in the adrenal gland and preservation course of disease, of these atypical cases.

Secondary hypoadrenocorticism is due to a deficiency of ACTH from the anterior pituitary, resulting in glucocorticoid insufficiency (ACTH does not directly influence mineralocorticoid release, so mineralocorticoids are spared). This condition is most commonly associated with abrupt discontinuation of long-term administration of corticosteroids or progesterone analogs. Rarely, congenital defects of the pituitary gland, neoplasia or trauma can result in secondary hypoadrenocorticism.

Relative adrenal insufficiency can result from critical illnesses and is referred to as critical illness related corticosteroid insufficiency (CIRCI). CIRCI is associated with severe illnesses such as septic shock or trauma. The syndrome is transient and adrenal function returns to normal when the associated illness resolves.

Commonly Observed Abnormalities

Hematology

Anemia
Eosinophilia
Lymphocytosis

Urinalysis

Urine specific gravity <1.030

Biochemistry

Azotemia
Hypercalcemia (not seen in atypical form)
Hyperkalemia (not seen in atypical form)
Hyponatremia (not seen in atypical form)
Hypoalbuminemia
Hypocholesterolemia (only in atypical form)
Hypoglycemia

Tests to Confirm Diagnosis of Hypoadrenocorticism

Baseline Cortisol

Baseline cortisol >2 µg/dL can be used to help rule out hypoadrenocorticism
Baseline cortisol <2 µg/dL necessitates ACTH stimulation test

ACTH Stimulation Test

ACTH stimulation test is the gold standard for diagnosis of all forms of hypoadrenocorticism.

Recommended in all animals suspected of having hypoadrenocorticism.

1. Collect baseline sample for cortisol assay.

2. Administer 5 µg/kg cosyntropin or 250 µg/dog cosyntropin IV or IM

NOTE: In severely dehydrated patients, cosyntropin should be administered IV.

3. Collect post sample for cortisol assay 1 hour after administration of cosyntropin.
OR

1. Collect baseline sample for cortisol assay.

2. Administer 2.2 IU/kg ACTH gel IM or IV.

3. Collect post sample for cortisol assay 2 hours after administration of ACTH gel.

Interpretation

Post cortisol – <2 µg/dL supports diagnosis of hypoadrenocorticism

Post cortisol – <5 µg/dL may also support hypoadrenocorticism in some animals

References

Klein SC, Peterson ME. Canine hypoadrenocorticism: Part I. Can Vet J 51: 63-69, 2010.

Klein SC, Peterson ME. Canine hypoadrenocorticism: Part II. Can Vet J 51: 179-184, 2010.

Lottati M, Bruyette D. Canine hypoadrenocorticism. Overview, diagnosis and treatment. Todays Vet Pract Sept/Oct 32-37, 2014.

Laboratory Diagnosis of Feline Hypoadrenocorticism

Hypoadrenocorticism is rare in cats compared to the incidence in dogs. The disease may occur at any age but tends to affect middle aged cats. The most common presenting problems are weight loss, lethargy, anorexia, vomiting and polyuria with waxing and waning signs. Physical examination most often reveals depression, weakness, dehydration and hypothermia. Severe shock, weak pulses and extreme weakness or collapse can be seen in up to 40% of affected cats.

Laboratory Findings Associated with Hypoadrenocorticism

Hematology

Anemia
Eosinophilia
Lymphocytosis

Biochemistry

Azotemia
Hyperphosphatemia
Hyperkalemia
Hyponatremia
Hypochloremia
Hypercalcemia (rare)

Urinalysis

Urine specific gravity varies depending on extent of renal medullary washout.

Tests for Diagnosis of Feline Hypoadrenocorticism

Feline ACTH Stimulation Test

1. Collect baseline sample for cortisol assay.
2. Administer 0.125 mg synthetic ACTH IM or 5 µg/kg synthetic ACTH IV.
3. Collect post samples for cortisol assay 30 and 60 minutes after cosyntropin administration.

Interpretation of Feline ACTH Stimulation Test

Reference Interval

Baseline cortisol: 1–6 µg/dL
Post cortisol at 30 or 60 minutes: 18–20 µg/dL

Interpretation

Baseline cortisol <2 µg/dL and post cortisol <2 µg/dL supports diagnosis of hypoadrenocorticism.

Endogenous ACTH

Increased endogenous ACTH concentration (1–70 times normal in one study) supports the diagnosis of hypoadrenocorticism.

References

Bruyette DS. Feline hypoadrenocorticism: Yes cats get Addison's disease too.

<https://www.dvm360.com/view/feline-hypoadrenocorticism-yes-cats-get-addison-s-disease-too>

Peterson ME, Greco DS, Orth DN. Primary hypoadrenocorticism in ten cats. J Vet Intern Med 3: 55–58, 1989.

Laboratory Diagnosis of Canine Hyperadrenocorticism (HAC)

The diagnosis of canine hyperadrenocorticism (Cushing's syndrome) is made on the basis of appropriate clinical signs and laboratory findings. Typical clinical signs include polydipsia, polyphagia, polyuria, abdominal distention, muscle weakness, and alopecia. Some dogs exhibit lethargy and panting, thin skin, comedones, insulin resistant diabetes, urinary incontinence and pituitary macrotumor syndrome (*i.e.*, clinical signs related to an expanding pituitary mass). Uncommon signs of hyperadrenocorticism are thromboemboli, torn ligaments, myotonia, persistent anestrus, testicular atrophy and facial nerve palsy.

Laboratory abnormalities in routine hematology, chemistry and urinalysis assays which can be found in Cushingoid dogs:

Hematology

Neutrophilic leukocytosis
Lymphopenia
Eosinopenia

Urinalysis

Low urine specific gravity (<1.018)
Proteinuria
Increased WBCs

Chemistry

Increased alkaline phosphatase (ALP)
Increased alanine aminotransferase (ALT)
Hypercholesterolemia
Hypertriglyceridemia
Hyperglycemia

Screening Assays for Canine Hyperadrenocorticism

Urine Cortisol to Creatinine Assay (UCCR)

The UCCR is best performed on urine taken in the home environment when the animal is not stressed, on two consecutive days, preferably in the morning. The UCCR is a good test to rule out HAC, but an increased UCCR is a nonspecific result.

Although uncommon, some dogs with HAC will have a normal UCCR assay. Dogs with increased UCCR and clinical signs suggesting HAC should be further evaluated with one of the following tests. Dogs with signs highly suggestive of HAC but normal UCCR should also be further evaluated and screened with one of the following tests.

Canine Low Dose Dexamethasone Suppression Test (CLDDS)

The CLDDS is a screening test for the diagnosis of hyperadrenocorticism (HAC). Sensitivity and specificity are approximately 90%. Results may be affected by anticonvulsant drugs, stress, excitement, exogenous glucocorticoids and nonadrenal disease.

1. Collect baseline sample for cortisol assay.
2. Administer 0.01 to 0.015 mg/kg dexamethasone IV.
3. Collect post samples for cortisol assay at 4 and at 8 hours after dexamethasone administration.

Interpretation of CLDDS Results

The 8 hour post LDDS sample is used to confirm hyperadrenocorticism (HAC).

Reference Interval

Baseline cortisol = 1–6.0 µg/dL

8 hour post LDDS cortisol = <1.0–1.5 µg/dL

Interpretation

8 hour post LDDS is >1.5 µg/dL; supportive of HAC diagnosis (either pituitary or adrenal origin). Results between 1.0 µg/dL–1.5 µg/dL are “gray zone” and warrant further evaluation.

If 8 hour post LDDS cortisol >1.5 µg/dL; evaluate 4 hour post LDDS cortisol and look more closely at the 8 hour result to see if results support pituitary origin.

If 4 hour post CLDDS cortisol = 1.5 µg/dL or < 50% baseline or the 8 hr post cortisol is < 50% baseline, findings support pituitary dependent HAC (PDH). If neither is true, the type of HAC remains undetermined.

Canine ACTH Stimulation Test

The ACTH stimulation test is used both as a screening test to support diagnosis of HAC and to monitor therapeutic response in dogs treated for HAC with trilostane or mitotane. ACTH Stimulation tests are used for the diagnosis of hypoadrenocorticism (Addison's disease).

1. Collect baseline sample for cortisol assay.
2. Administer 5 µg/kg cosyntropin or 0.25 mg cosyntropin IV or IM.
3. Collect post sample for cortisol assay 1 hour after administration of cosyntropin.

OR

1. Collect baseline sample for cortisol assay.
2. Administer 2.2 IU/kg ACTH gel IM.
3. Collect post sample for cortisol assay 2 hours after administration of ACTH gel.

Laboratory Diagnosis of Canine Hyperadrenocorticism (HAC) *continued*

Interpretation of the Canine ACTH Stimulation Test

Reference Interval

Baseline cortisol = 0.5–6.0 µg/dL

Post cortisol = 8–18 µg/dL

Interpretation

Post cortisol is 18–24 µg/dL; inconclusive, but supportive of HAC. Perform CLDDS or other diagnostic tests if ACTH sample quality and analysis are deemed reliable.

Post cortisol is >20–24 µg/dL; supportive of HAC.

Baseline cortisol is <2 µg/dL and post cortisol is < 2 µg/dL; consistent with hypoadrenocorticism.

Tests for Distinguishing Pituitary from Adrenal Origin HAC

These following tests are performed **after** a diagnosis of HAC has been made.

These assays are not used to diagnose HAC, only to help distinguish pituitary from adrenal origin. Note that some results of CLDDS can support diagnosis of PDH as indicated above.

Canine High Dose Dexamethasone Suppression Test (CHDDS)

The CHDDS is used to aid in distinguishing pituitary from adrenal origin HAC. This test is performed **AFTER** a diagnosis of HAC (Cushing's Syndrome) has been made

1. Collect baseline sample for cortisol assay.
2. Administer 0.1 mg/kg of dexamethasone IM or IV.
3. Collect post samples for cortisol assay at 4 and at 8 hours after dexamethasone administration.

Interpretation of CHDDS

If 4 or 8 hour post cortisol is <50% baseline or <1.5 µg/dL; support pituitary HAC.

If 4 or 8 hour post cortisol is >50% baseline; does not distinguish adrenal from pituitary HAC. Only 80% of pituitary origin HAC have 4 or 8 hour cortisols <50% baseline.

Endogenous ACTH

Collect an EDTA blood sample. Mix well. Separate plasma from cells, and transfer plasma to a plastic tube for freezing. Ship overnight to reference laboratory for endogenous ACTH determination.

Interpretation of Endogenous ACTH Concentration

ACTH concentration low or undetectable: Supportive of adrenocortical tumor or iatrogenic HAC.

ACTH concentration in reference range or increased: Supportive of pituitary origin HAC.

Abdominal Ultrasound

Perform abdominal ultrasound to evaluate adrenal glands for evidence of adrenal neoplasia.

Advanced Cranial Imaging (CT/MRI)

Assess for pituitary mass.

References

Nelson RW. Diagnosing Hyperadrenocorticism. Presented at World Small Animal Veterinary Association World Congress, 2013.
<https://www.vin.com/apputil/content/defaultadv1.aspx?pld=11372&id=570980>

Laboratory Diagnosis of Feline Hyperadrenocorticism (HAC)

Hyperadrenocorticism (HAC) is rare in cats. The most common abnormalities seen in cats with HAC are unregulated diabetes mellitus and thin, fragile skin. Concurrent bacterial infections may be found in some affected animals. Hematologic and biochemical findings are nonspecific except for hyperglycemia associated with concurrent diabetes mellitus.

A number of laboratory tests can help confirm a diagnosis of HAC in cats presenting with these signs. The response of cats to ACTH necessitates obtaining two samples after administration of ACTH, at 30 and 60 minutes, to improve the sensitivity of the assay. However, In a retrospective study of 30 cats with HAC, the ACTH stimulation test was relatively insensitive (56%) with a specificity of 89%. The dexamethasone suppression test is the recommended screening test for feline HAC.

Urine Cortisol to Creatinine Assay (UCCR)

The UCCR is best performed on urine taken in the home environment when the animal is not stressed on two consecutive days, preferably in the morning. The UCCR is a good test to rule out HAC but an increased UCCR is a nonspecific result.

Although uncommon, some cats with HAC will have a normal UCCR assay. Cats with increased UCCR and clinical signs suggesting HAC should be further evaluated with one of the following tests. Cats with signs highly suggestive of HAC but normal UCCR should also be further evaluated and screen with one of the following tests.

Feline Dexamethasone Suppression Test (FDS)

The FDS is the recommended screening test for feline HAC.

1. Collect baseline sample for cortisol assay.
2. Administer 0.1 mg/kg dexamethasone.
3. Collect post samples for cortisol assay at 4 and 8 hours after administration of dexamethasone.

Interpretation of FDDS

Reference Interval

Baseline cortisol: 1–6 µg/dL

Reduction of cortisol concentration of 50% or greater than baseline in samples taken at 4 and 8 hours after dexamethasone administration.

Interpretation

Cortisol concentration >50% baseline in samples taken at 4 and 8 hours post dexamethasone administration is supportive of a diagnosis of feline HAC.

Feline ACTH Stimulation Test

1. Collect baseline sample for cortisol assay.
2. Administer 0.125 mg synthetic ACTH IM or 5 µg/kg synthetic ACTH IV.
3. Collect post sample for cortisol assay 30 and 60 minutes after cortrosyn administration.

Interpretation of Feline ACTH Stimulation Test

A retrospective study of 30 cats with HAC found the ACTH stimulation test was relatively insensitive (56%) with a specificity of 89%. The dexamethasone suppression test is the recommended screening test for feline HAC.

Reference Interval

Baseline cortisol: 1–6 µg/dL

Post cortisol at 30 or 60 minutes: 18–20 µg/dL

Interpretation

Post cortisol concentration >20–22 µg/dL supports a diagnosis of feline HAC.

Differentiation of Pituitary Dependent HAC (PDH) from Adrenal Dependent HAC (ADH)

Endogenous ACTH Assay

Collect an EDTA blood sample. Mix well. Separate plasma from cells. Transfer plasma to a plastic tube and freeze. Ship overnight to reference laboratory for endogenous ACTH determination.

Interpretation of Endogenous ACTH concentration

ACTH concentration low or undetectable: Supportive of adrenocortical tumor or iatrogenic HAC.

Abdominal Ultrasound

Evaluate for bilateral versus unilateral adrenal enlargement.

Advanced Cranial Imaging (CT/MRI)

Assess for pituitary mass.

References

Bruyette D. Feline adrenal disease. Presented at World Small Animal Veterinary Association World Congress Proceedings, 2001.

<https://www.vin.com/apputil/content/defaultadv1.aspx?pld=8708&catId=18051&id=3843751>

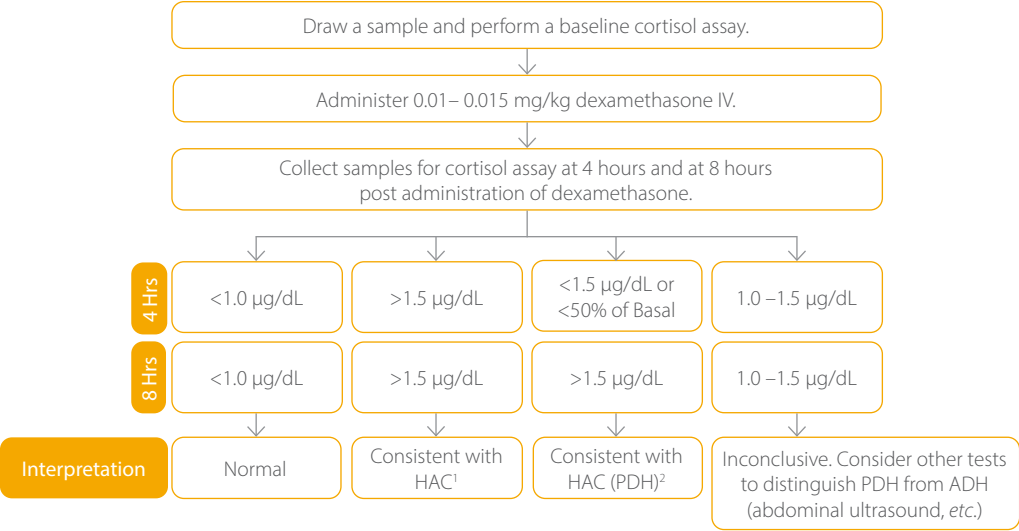
Valentin SY, Cortright CC, Nelson BM, et al. Clinical findings, diagnostic test results and treatment outcome in cats with spontaneous hyperadrenocorticism: 30 cats. *J Vet Intern Med* 28:481–487, 2014.

Feline adrenal and pituitary function tests. Cornell University Animal Health Diagnostic Center.

<https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols/feline-adrenal>.

Canine Low-Dose Dexamethasone Suppression Test (CLDDS)

NOTE: All sample types must be the same (*i.e.*, plasma or serum).

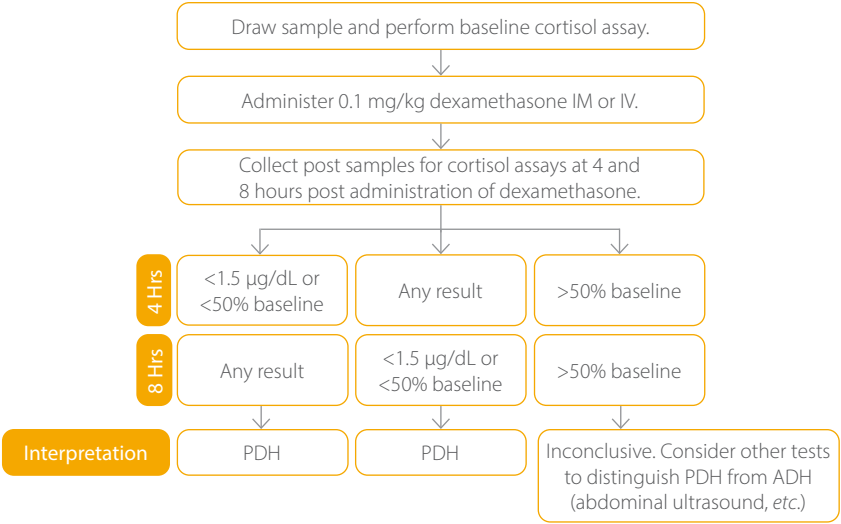


¹ HAC = Hyperadrenocorticism

² To differentiate between PDH (pituitary dependent HAC) and adrenal origin HAC, additional testing recommended (such as CHDDS, ACTH level, abdominal ultrasound).

Canine High-Dose Dexamethasone Suppression Test (CHDDS)

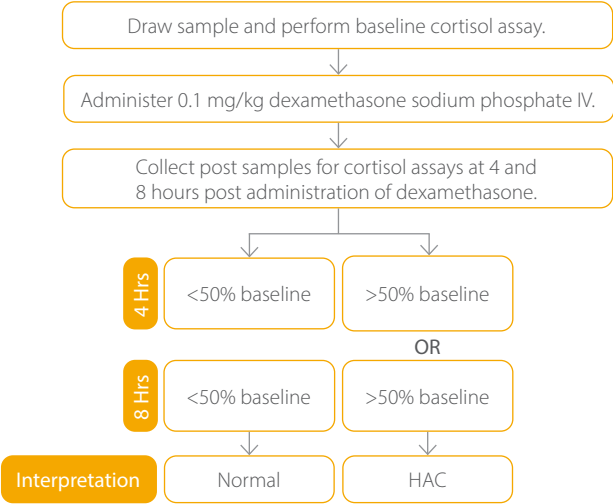
Use to differentiate Pituitary-Dependent from Adrenal Tumor Hyperadrenocorticism.
Results from Low-Dose Dexamethasone Suppression consistent with Hyperadrenocorticism, but further testing is required to distinguish between PDH¹ and ADH².
NOTE: Perform this assay only after diagnosis of HAC has been made.



¹ Pituitary dependent hyperadrenocorticism
² Adrenal dependent hyperadrenocorticism

Feline Dexamethasone Suppression Test (FDS)

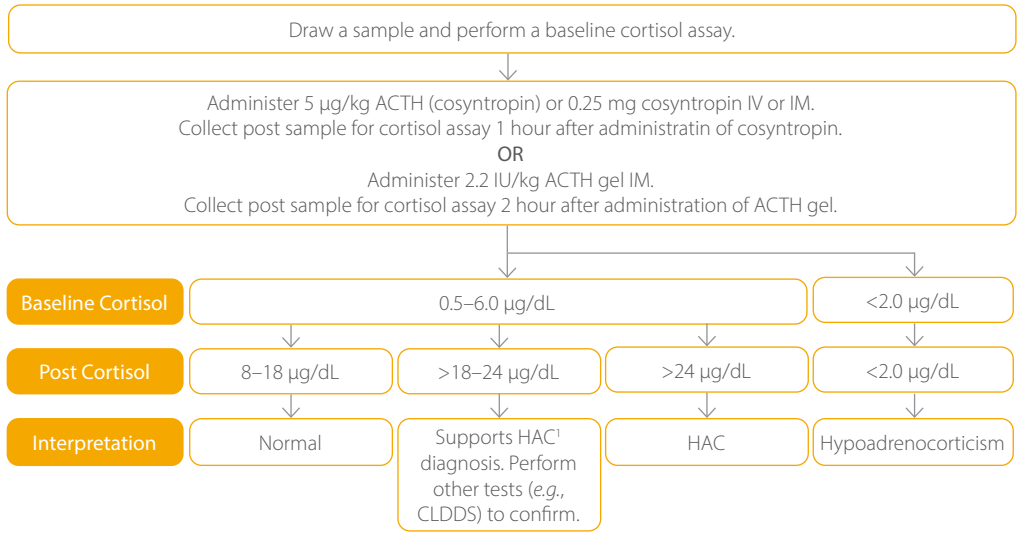
NOTE: This is the recommended screening assay for feline HAC¹.



¹ HAC = Hyperadrenocorticism

Canine ACTH Stimulation Test

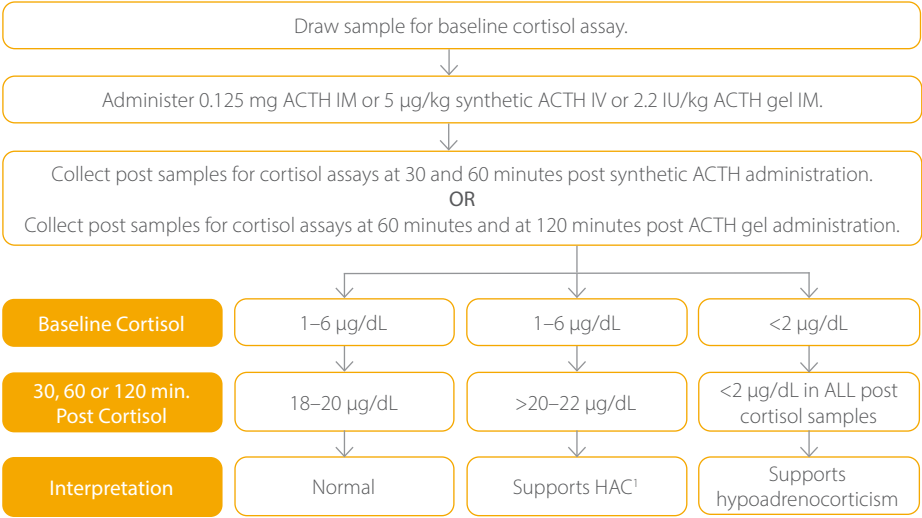
NOTE: All sample types must be the same (*i.e.*, plasma or serum).



¹ HAC = Hyperadrenocorticism

Feline ACTH Stimulation Test

NOTE: This is the recommended screening assay for feline HAC¹.



¹ HAC = Hyperadrenocorticism

Laboratory Estimation of Canine Ovulation and Parturition

Dogs are classified as monoestrus, spontaneous ovulators that occasionally exhibit seasonality in cycling. As in other animals, estrus in dogs relies on several hormonal messengers to progress through each cycle.

Progesterone is a ubiquitous reproductive hormone in mammals, though site of primary production can vary between species. In dogs, the time between the rise in concentration of progesterone and that of luteinizing hormone (LH) aids in the prediction of ovulation. Additionally, the decrease in progesterone concentration near the time of parturition in dogs facilitates timing for cesarean section. Even with the aid of progesterone assays, predicting ovulation and parturition can be challenging. Dogs tend to ovulate when blood progesterone levels are around 5–6 ng/mL. However, ovulation can occur later, when progesterone levels are closer to 9–10 ng/mL. The importance of serial progesterone measurements to aid in predicting ovulation and fertile breeding period cannot be overstated.

NOTE: The following are clinical signs associated with respective stages of estrus. Not all findings will be evident in every individual.

Proestrus

Vulvar bleeding
Aggression towards males
Listlessness, anxiety
Progesterone <1 ng/mL
Estrogen rises rapidly

Estrus

Vulvar discharge – blood tinged or straw colored
Vulvar swelling
Receptiveness to males
Progesterone rises rapidly
Estrogen falls

Tests to Confirm Ovulation and Fertile Period

NOTE: Results of tests below should be correlated with clinical signs and other diagnostic testing performed.

Blood LH Immunochromatographic Assay

Progesterone Assay

Vaginal Cytology

Progesterone Assay

1. Whole blood sample collected at first signs of estrus*.
2. Progesterone measured point-of-care via immunoassay.
3. Analyze progesterone concentrations to plan next steps (re-testing, breeding, etc.).

Interpreting Results

Results of progesterone assays should be interpreted alongside clinical picture and other diagnostic tests results, if performed. Theriogenologists and seasoned dog breeders tend to focus on the slope of the rise and fall of progesterone more than any single point. Serial measurements of progesterone are recommended for predicting ovulation and parturition. Ideally for parturition, pre-pregnancy progesterone concentrations were obtained to accurately estimate when the LH surge took place. Parturition will occur 65 +/-2 days post LH surge.

Progesterone Concentrations and Ovulation Timing

<1 ng/mL – Wait 3-4 days and test again
2–4 ng/mL – LH surge and Post LH Surge; Test every 24 hours
5–16 ng/mL – Fertile period; Breed in this range, confirming estrus via vaginal cytology at higher levels of progesterone (>12 ng/mL)

Progesterone Concentration and Parturition Timing

NOTE: Progesterone assay to determine appropriateness of surgical intervention during pregnancy should be used in combination with clinical signs, history, and signalment. Progesterone ranges below are guidelines and not meant to be diagnostic indicators of surgical safety or neonatal viability.

4-6 ng/mL – Re-test in 12–24 hours while monitoring body temperature, other clinical signs
<4 ng/mL – Surgery acceptable based on serial progesterone measurements and associated clinical signs
<2 ng/mL – Generally considered safe for cesarean section

**Avoid use of serum separator tubes (tiger top) as contact with separator gel can affect certain hormonal assay results.*

References

- Concannon PW. Reproductive cycles of the domestic bitch. *Anim Repro Sci.* 2011;124:200–210.
- Kim Y, Travis AJ, Meyers-Wallen VN. Parturition prediction and timing of canine pregnancy. *Theriogenology.* 2007;68(8):1177–1182. doi:10.1016/j.theriogenology.2007.08.018. http://therio.vetmed.lsu.edu/the_normal_canine.htm
- De Cramer KGM, Nöthling JO. The precision of peri-oestrous predictors of the date of onset of parturition in the bitch. *Theriogenology.* 2017 Jul 1;96:153–157. doi: 10.1016/j.theriogenology.2017.04.018. Epub 2017 Apr 14. PMID: 28532832.

Canine Progesterone Testing Protocols

Used to predict ovulation, parturition and identify reproductive abnormalities. The following table provides guidelines for using progesterone to optimize breeding timing.

NOTE: All sample types must be the same (*i.e.*, plasma or serum).

Result	Phase	Recommendation
<1.0 ng/mL	Anestrus or Proestrus	Retest in 3–4 days
1.0–1.9 ng/mL	Pre LH Surge	Retest in 2–3 days
2.0–3.9 ng/mL	LH Surge	Ovulation will generally occur in the next 24–48 hours.
4.0–5.9 ng/mL	Post LH Surge	Test every 24 hours; estimated breeding window should begin within 24–48 hours.
6.0–9.9 ng/mL	Ovulation	Breed in the next 1–4 days depending on method of insemination, semen quality, and number of breedings.
10.0–15.9 ng/mL	Post-ovulation, Mature Ova	Normal breeding window, timing based on LH surge and estimated ovulation.
>16.0 ng/mL	Diestrus	Likely past opportunity to breed if initial progesterone reading >16 ng/mL, confirm via vaginal cytology, <i>etc.</i>

Laboratory Evaluation of Canine C—Reactive Protein

Acute phase proteins (APP) are a group of proteins produced by the liver whose blood concentrations change rapidly, and in some cases significantly, in response to inflammation. Almost immediately after an inciting cause of inflammation, various pro-inflammatory cytokines are produced and released into the bloodstream. Some of these cytokines, most notably IL-1, IL-6, IL-8 and TNF-alpha, are transported from the region of inflammation to the liver where APP production is either increased (positive APP) or decreased (negative APP). Examples of negative APP's include albumin and transferrin. Most positive APP's are used in both human and veterinary medicine to measure acute systemic inflammation. C—reactive protein, a positive APP otherwise known as CRP, is considered one of the most sensitive measurable markers of acute systemic inflammation in the dog.

In dogs, CRP will rise within 6 hours of the inciting cause and peak around 12–24 hours post-insult. CRP also decreases in response to a reduction in tissue inflammation, usually as a steady decline over days. Therefore, it is not only a sensitive marker for the presence of systemic inflammation, but also a good tool for monitoring healing and/or response to therapy. The rate at which it declines depends on many factors—severity of inflammation, type of tissue involved, *etc.*, but return to normal levels usually occurs within days after the initiation of healing and/or treatment. It is important to note that although CRP is particularly sensitive in detecting systemic inflammation, it is not specific for the type or location of inflammation. Furthermore, studies have shown that the magnitude of increase in a single measurement in time is not useful as a prognostic indicator.

Using CRP as a screening tool for inflammation can be valuable in many settings, especially in subclinical and post-operative cases. Because of the nature of APP in response to inflammation, rises in CRP can manifest prior to clinical signs and/or other diagnostic results becoming abnormal. Various studies have demonstrated the value of measuring CRP as a post-operative monitoring tool by analyzing prior to the surgical procedure for a baseline, then conducting serial monitoring following the procedure. This provides clinicians the opportunity to monitor for post-operative complications, *e.g.*, infection. In uncomplicated surgical post-operative healing, serial monitoring shows marked increase first day post-surgery, then a steady continual decrease.

Evaluation of Canine CRP is most commonly used as a tool to evaluate underlying and acute-onset systemic inflammation, to assess post-operative inflammation, and to monitor healing and response to therapy.

Laboratory Findings Associated with Acute Inflammation

Hematology

- +/- Leukocytosis
- +/- Neutrophilia
- +/- Band Neutrophils

Biochemistry

- +/- Hyperglobulinemia—(can be mild)
- +/- Hypoalbuminemia

Other Tests

- +/- Decreased total T4—(euthyroid sick)

Inflammatory Marker Testing

C-Reactive Protein, Fibrinogen, Serum Amyloid A, and Haptoglobin

C—Reactive Protein

Reference Interval

cCRP: 0.0 mg/dL–1.0 mg/dL

**Evidence exists that pregnant individuals can have “high” cCRP levels during normal gestation. Serial measurements of cCRP are recommended to assess this inflammatory marker in pregnant individuals.*

Interpretation (Screening)

Canine CRP between 0.0 mg/dL–1.0 mg/dL indicate a normal cCRP concentration. Care should be taken to fully rule out pathologic condition(s).

Canine CRP levels between 1.0 mg/dL–3.0 mg/dL indicate a mildly elevated cCRP concentration but can represent early or resolving systemic inflammation in certain individuals. If clinical signs indicate active disease, further diagnostics should be performed to elucidate cause of cCRP elevation. If clinical signs conflict or are not present, serial measurement of cCRP is recommended to further determine whether pathologic processes are present.

Canine CRP >3.0 mg/dL indicates clinically significant systemic inflammation is present. Further diagnostics should be performed to elucidate cause of cCRP elevation. This may involve repeating a thorough physical exam, including rectal palpation and oral cavity evaluation, imaging studies such as thoracic/abdominal radiographs, and/or specialized blood diagnostics such as infectious disease panels, etc.

Interpretation (Monitoring)

Serial measurement of cCRP can also be used to monitor patient recovery and response to treatment. Numerical values will vary depending on the patient and the cause of cCRP elevation. But predictable falls in cCRP concentration over the course of hours and days post-insult can give clinicians more information regarding recovery and current inflammatory status.

If cCRP concentrations remain level or elevate further while performing serial monitoring, care should be taken to ensure inflammation is subsiding and patient recovery is occurring as expected. This may include re-testing cCRP or performing other diagnostics such as CBC, radiographs, etc.

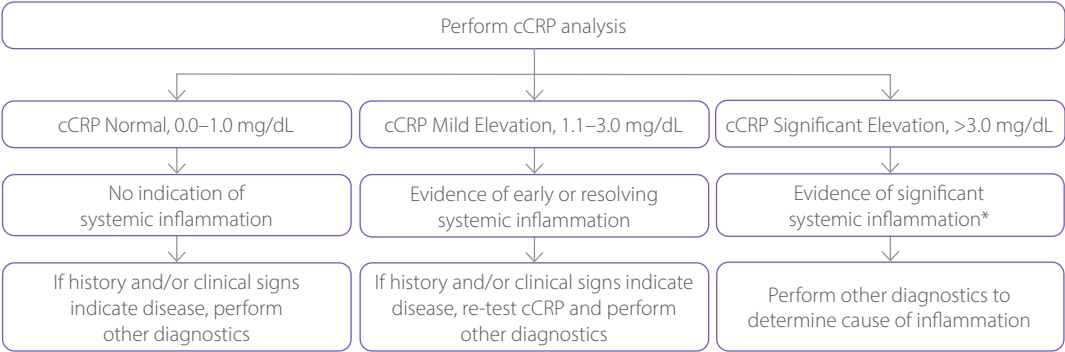
Similar to other inflammatory markers, changes within an individual's cCRP concentration are more informative than single time point measurements. Whether used for screening or monitoring inflammation, serial cCRP measurements can provide additional and sometimes vital information on patient status.

References

- Karlsson L, Hagman R, Johannisson A, Wang L, Karlstam E and Wernersson S. Cytokines as Immunological Markers for Systemic Inflammation in Dogs with Pyometra. *Reprod Dom Anim* (2012) 47 (Suppl. 6), 337–341.
- Thrall, M. A. (2012). *Veterinary Hematology and Clinical Chemistry*, Wiley-Blackwell. Nakamura, Masaaki & Takahashi, Masashi & Ohno, Koichi & Koshino, Akiko & Nakashima, Ko & Setoguchi, Asuka & Fujino, Yasuhito & Tsujimoto, Hajime. (2008). C–Reactive Protein Concentration in Dogs with Various Diseases. *The Journal of veterinary medical science/the Japanese Society of Veterinary Science*. 70. 127–31. 10.1292/jvms.70.127.
- Saellström, S., Sharif, H., Jagarlamudi, K. K., Rönnerberg, H., Wang, L., & Eriksson, S. (2022). Serum TK1 protein and C-reactive protein correlate to treatment response and predict survival in dogs with hematologic malignancies. *Research in Veterinary Science*, 145, 213–221. <https://doi.org/10.1016/j.rvsc.2022.02.019>.
- Löfqvist K, Kjølgaard-Hansen M, Nielsen MBM. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau leveling osteotomy in dogs. *Acta Vet Scand*. 2018 May 21;60(1):30. doi: 10.1186/s13028–018–0385–5. PMID: 29784055; PMCID: PMC5963089.
- Viitanen, S., Laurila, H., Lilja-Maula, L., Melamies, M., Rantala, M. and Rajamäki, M. (2014), Serum C-Reactive Protein as a Diagnostic Biomarker in Dogs with Bacterial Respiratory Diseases. *J Vet Intern Med*, 28: 84–91. <https://doi.org/10.1111/jvim.12262>.
- Israeli, I., Steiner, J., Segev, G., Kass, P., Suchodolski, J., Sattasathuchana, P., Bruchim, Y., Yudelevitch, S. and Aroch, I. (2012), Serum Pepsinogen-A, Canine Pancreatic Lipase Immunoreactivity, and C-Reactive Protein as Prognostic Markers in Dogs with Gastric Dilatation-Volvulus. *J Vet Intern Med*, 26: 920–928. <https://doi.org/10.1111/j.1939-1676.2012.00940>.
- Gebhardt, C., Hirschberger, J., Rau, S., Arndt, G., Krainer, K., Schweigert, F.J., Brunnberg, L., Kaspers, B. and Kohn, B. (2009), Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. *Journal of Veterinary Emergency and Critical Care*, 19: 450–458. <https://doi.org/10.1111/j.1476-4431.2009.00462>.
- Mitchell, K., Kruth, S., Wood, R. and Jefferson, B. (2009), Serum Acute Phase Protein Concentrations in Dogs with Autoimmune Hemolytic Anemia. *Journal of Veterinary Internal Medicine*, 23: 585–591. <https://doi.org/10.1111/j.1939-1676.2009.0282>.
- Kuribayashi, T., Shimada, T., Matsumoto, M., Kawato, K., Honjo, T., Fukuyama, M., Yamamoto, Y., & Yamamoto, S. (2003). Determination of serum C-reactive protein (CRP) in healthy Beagle Dogs of various ages and pregnant Beagle Dogs. *Experimental Animals*, 52(5), 387–390. <https://doi.org/10.1538/expanim.52.387>.
- Carney PC, Ruaux CG, Suchodolski JS, Steiner JM. Biological variability of C-reactive protein and specific canine pancreatic lipase immunoreactivity in apparently healthy dogs. *J Vet Intern Med*. 2011 Jul–Aug; 25(4):825–30. doi: 10.1111/j.1939-1676.2011.0729.x. Epub 2011 May 12. PMID: 21564294.
- Martínez-Subiela S, Tecles F, Cerón JJ. Critical differences of acute phase proteins in canine serum samples. *Vet J*. 2003 Nov; 166(3):233–7. doi: 10.1016/s1090-0233(02)00315–5. PMID: 14550733.

Canine CRP Testing Protocols

If a thorough patient history and clinical signs lead to the suspicion of injury or disease, first assess chemistry, electrolytes, hematology, and urinalysis.



**Pregnant individuals can have cCRP concentrations >30 mg/L during normal gestation.*



For further assistance, please call Heska's Medical & Technical Consulting at 800.464.3752, option 5.