GUIDE TO ENDOCRINOLOGY

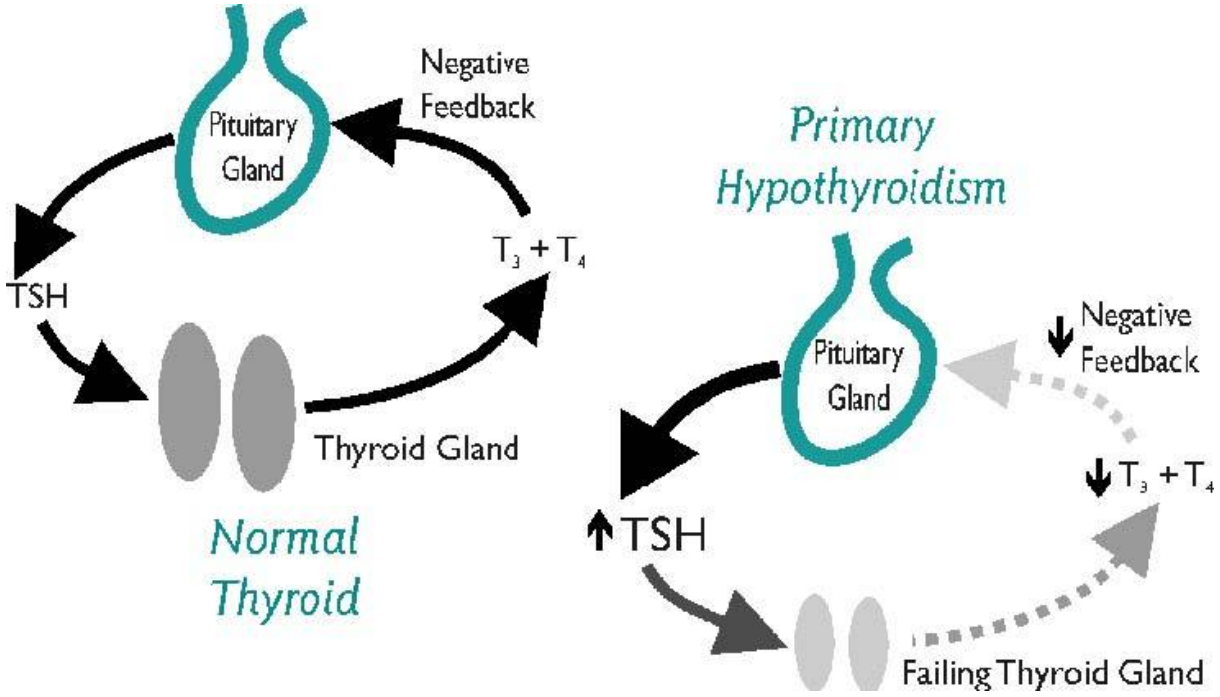
CANINE HYPOTHYROIDISM

Signalment

Hypothyroidism is a common endocrine disease of dogs that can affect both males and females of nearly any age although it is rare in dogs less than two years old.

Pathophysiology

Hypothyroidism is invariably primary *(i.e. disease of the thyroid gland itself)* and is usually caused by either lymphocytic thyroiditis or idiopathic atrophy. As the disease progresses the ability of the thyroid gland to secrete the principal thyroid hormone T_4 decreases and clinical signs develop. T_4 normally inhibits the secretion of cTSH from the pituitary gland by a negative feedback mechanism. In primary hypothyroidism this feedback is lost and cTSH concentrations increase.



Clinical Signs

The most common clinical features of hypothyroidism include:

- Lethargy
- Weight gain
- Dermatological signs including hair loss, pyoderma, scaling & scurfing
- Neurological signs e.g. facial nerve neuropathy
- Exercise intolerance and weakness

Diagnostic Tests

Combined measurement of total T_4 and cTSH concentrations *(standard profile)* provide an inexpensive and accurate method of confirming or excluding hypothyroidism. Further improvement in diagnostic accuracy is obtained by concurrent estimation of free T_4 measured by dialysis *(premium profile).* Free T_4 is the metabolically active portion of T_4 and most accurately reflects tissue thyroid status. Note that the old analogue method for free T_4 measurement is now known to offer no advantage over total T_4 measurement. If free T_4 measurement is being offered, always enquire as to whether a dialysis method is being used. Recent research has demonstrated that the presence of circulating thyroglobulin autoantibodies is very strong evidence for the presence of thyroid pathology. The comprehensive profile includes these antibodies in the test panel and therefore provides maximal information regarding thyroidal status. Dynamic tests of thyroid function are also available. If you are considering performing one of these tests, we recommend that you contact the laboratory to discuss this in advance.

Monitoring Tests

Most dogs can be well controlled with once daily thyroid hormone supplementation. Twice daily therapy is rarely required. When monitoring therapy, the time of sampling relative to thyroid hormone administration has a profound influence on the hormone concentrations that are obtained. It is therefore recommended that samples collected for monitoring profiles should be collected six hours post-pill, for total T_4 and cTSH estimation. Monitoring profiles can be performed within approximately 7-14 days of starting treatment or changing the dosage. Well treated dogs usually have peak total T_4 values approximately 50-70 nmol/L and cTSH concentration near the assay limit of *detection (0.01-0.03 nglml).*

Sample Requirements

- Standard profile *(T_4, cTSH)* 1-2 ml serum *(clotted)*
- Premium profile *(T_4, cTSH, fT4d)* 1-2 ml serum *(clotted)*
- Comprehensive profile *(T_4, cTSH, fT_4d, TgAb)* 1-2 ml serum *(clotted)*
- Monitoring profile *(T_4, cTSH) (6 hours post-pill)* 1-2 ml serum *(clotted)*

Points to Note

Various therapies will influence thyroid test results. Ideally dogs should not have received these for a minimum of six weeks prior to sampling. If testing cannot be delayed, please ensure that the relevant drug therapies are noted on the submission form as these drugs will alter the results obtained. The most important drugs to try and avoid are:

- Steroids
- Anticonvulsants
- Sulphonamides
- Thyroid hormone replacement *(unless monitoring treatment of course !)*

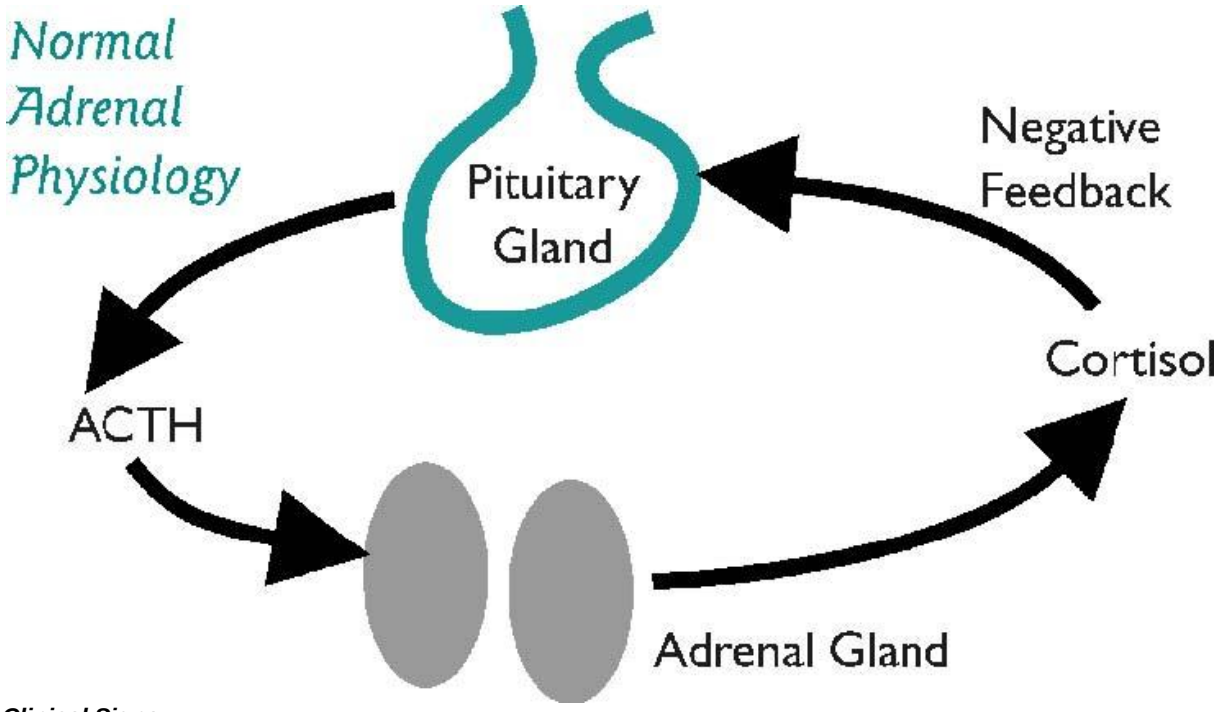
CANINE HYPERADRENOCORTICISM

Signalment

Hyperadrenocorticism *(HAC)* usually occurs in middle aged or old dogs. Predisposed breeds include poodles, small terriers *(e.g. Border and Yorkshire terriers)* and Dachshunds. Both males and females can be affected.

Pathophysiology

HAC results from excess production of glucocorticoids by the adrenal cortices. This is usually caused by excessive ACTH secretion from the pituitary gland pituitary dependant hyperadrenocorticism *(PDH).* PDH is most commonly caused by an adenoma the pars intermedia of the pituitary gland and accounts for approximately 80 % of all cases of HAC. The remaining 20 % *of* HAC cases result from excessive glucocorticoid secretion by a functioning adrenal tumour *(AT) (either an adenoma or a carcinoma, both of which occur with approximately equal frequency).*



Clinical Signs

The most common clinical features of HAC include:

- Polyuria
- Polydipsia
- Weight loss
- Abdominal enlargement
- Calcinosis cutis
- Poor exercise tolerance
- *"Endocrine"* alopecia
- Polyphagia
- Muscle weakness
- Skin thinning
- Panting and restlessness
- Neurological signs referable to a pituitary mass

Diagnostic Tests

It is important to recognise that there is no *"perfect"* test for HAC. Consequently, it is particularly important that tests of adrenal status are interpreted with a strong bearing ultimately being placed on your own clinical judgement. For this reason please provide as much clinical information as possible when submitting samples for the confirmation of HAC.

In the dexamethasone suppression test, cortisol is estimated before and after exogenous dexamethasone administration. In healthy dogs, the dexamethasone has a negative feedback effect on the pituitary gland and inhibits ACTH secretion. This reduces endogenous cortisol production by the adrenal cortices and consequently post dexamethasone cortisol concentrations are suppressed. However, in dogs with PDH, the pituitary gland is secreting large quantities of ACTH autonomously and is not responsive to the negative feedback effect of dexamethasone. Similarly, in dogs with AT, the secretion of cortisol by the neoplastic adrenocortical cells is independent of ACTH. Thus, in dogs with HAC caused by either PDH or AT, the cortisol concentration does not decrease significantly after dexamethasone administration. The dexamethasone suppression test is a good screening test for HAC, but can give false positive results. The most reliable method of minimising these false positive results, is to *(as far as possible)* avoid stressing patients during the test, and to judiciously select patients to test based on their clinical signs, routine biochemical and haematological changes, and the prior exclusion of other possible diseases.

In the ACTH stimulation test blood samples are collected for cortisol estimation before and after exogenous ACTH administration. The dose of ACTH given is supraphysiological and consequently the cortisol response to ACTH is essentially dependant upon the number of functioning adrenocortical cells. The test can therefore be considered to be

an assessment of “adrenal size”. Since there is an increase in adrenal tissue in both PDH *(which causes bilateral adrenal hyperplasia)* and AT, an exaggerated cortisol response to ACTH usually occurs in dogs with HAC irrespective of the cause. Post-ACTH cortisol values greater than 660 nmol/L support a clinical diagnosis of HAC. The ACTH stimulation test can give false negative results and so don’t completely rule out the possibility of HAC because of a normal ACTH stimulation test, if you remain strongly clinically suspicious of the disease.

Monitoring Tests
When monitoring therapy for HAC, the ACTH stimulation test is the test of choice. Dogs receiving Vetoryl therapy should be tested 4-6 hours post-pill.

The determination of the Urinary Cortisol to Creatinine Ratio *(UCCR)* can be used as a screening test for HAC. The UCCR is unable to definitively confirm HAC since non-adrenal disease can also give positive results, but the test can quite confidently rule out HAC as a possible diagnosis, helping in the overall final patient assessment. Since the secretion of cortisol *(even in dogs with HAC)* tends to be quite episodic, the estimation of *“average”* blood cortisol secretion would be a useful marker of adrenal status. Since cortisol is excreted in the urine, this can be achieved by measurement of urine cortisol concentration. Compensation for variable rates of glomerular filtration is achieved by determination of the UCCR. A UCCR less than 10 rules out the possibility of HAC. A UCCR more than 70 may be consistent with HAC and warrants more specific testing such as the dexamethasone suppression or ACTH stimulation tests.

Differentiating PDH from AT
Endogenous ACTH measurement can be used to differentiate PDH *(in which the ACTH concentration is usually elevated)* from AT *(in which the ACTH concentration is usually depressed).* Samples must be collected using a strict protocol outlined below.

Sample Requirements
In the ACTH stimulation test clotted blood samples are collected for cortisol estimation immediately before and one hour after intramuscular or intravenous administration of 250 ug soluble exogenous ACTH. In the dexamethasone suppression test clotted blood samples are collected for cortisol estimation immediately before, and three and eight hours after the intravenous administration of 0.015 mg/kg dexamethasone. For both tests 1 ml serum is required per sample. For the urinary cortisol:creatinine ratio, an overnight urine sample should be collected at approximately 9am *at home.*

Endogenous ACTH is very labile and consequently strict sample handling procedures must be followed. Collect blood into a chilled plastic *(not glass)* tube containing EDTA as the anticoagulant. Gently mix and then immediately centrifuge for 3-5 minutes. Immediately decant the plasma into a chilled plain plastic *(not glass)* tube and **freeze immediately.** Transport the sample frozen on ice for ACTH estimation *(contact us for a cool box and courier collection).*

Points to Note
Try and avoid patient stress as much as possible during these tests. In particular, do not collect urine for the UCCR in a hospital environment as the stress of hospitalisation can in itself lead to false positive results. Instead, have the sample collected from the dog by the owner in the home environment.

Steroid therapy will interfere with both normal adrenal physiology and assays for cortisol estimation. If in doubt about which test to use in this situation, please contact the laboratory for specific advice.

HYPERTHYROIDISM

Signalment
Hyperthyroidism is a very common disease of older cats. It is rare in cats less than seven years. All breeds and types of cats may be affected but Siamese are under-represented. Hyperthyroidism has been increasingly recognised in dogs over the past five years, but it remains a rare disease in this species.

Pathophysiology Feline hyperthyroidism almost always results from excessive secretion of T_4 caused by benign adenomatous hyperplasia usually affecting both thyroid lobes. Canine hyperthyroidism is usually caused by a functional thyroid carcinoma.

Clinical Signs
The most common clinical features associated with hyperthyroidism are:

- Weight loss
- Polyphagia
- Polyuria

- Polydipsia
- Tachycardia
- Vomiting
- Cardiorespiratory disease
- Poor coat condition
- Hyperactivity

Diagnostic Tests
Confirmation of hyperthyroidism is usually achieved by measurement of total T_4 in a serum sample. In equivocal cases, with borderline total T_4 values, dynamic tests such as the T_3 suppression test can be used. However, the recommended approach in such cases is usually to wait and re-test total T_4 about four weeks later or measure fT4d which is less affected by non-thyroidal illness. This usually clarifies thyroid status.

Monitoring Tests
Monitoring therapy for hyperthyroidism relies upon demonstration of a normalisation of thyroid hormone concentrations. It is generally desirable to decrease total T_4 values to approximately 15-30 nmol/L to maintain optimal clinical control. Iatrogenic hypothyroidism very rarely occurs as a complication of therapy, and certainly it is much more common for cats to be inadequately treated than to be over-treated.

Sample Requirements Serum *(clotted blood) is* required for total T_4 estimation and this is the test of choice for both confirming the diagnosis, and monitoring therapy.

DIABETES MELLITUS

Signalment
There are multiple underlying factors that may contribute to the development of diabetes mellitus *(DM)* in dogs and cats. Consequently, nearly any age or breed may be affected. However, DM occurs most commonly in middle aged dogs, and crossbreeds, cairn terriers, English setters, poodles and rottweilers have been reported to be over-represented.

Pathophysiology
The causes and contributors to a state of DM are numerous and a detailed review is beyond the scope of this manual. As a generalisation, the most common causes of DM produce a relative or absolute deficiency of insulin through combinations of reduced insulin production and/or decreased peripheral insulin sensitivity. Common specific causes include autoimmune pancreatic islet cell destruction, increased concentrations of endogenous or exogenous glucocorticoids, and increased circulating progesterone and growth hormone concentrations associated with metoestrus.

Clinical Signs
The most common clinical signs of DM include
- Polyuria
- Polydipsia
- Polyphagia
- Recurring infections
- Exercise intolerance
- Cataracts
- Weight Loss

Diagnostic Tests
Demonstration of concurrent glucosuria and persistent fasting hyperglycaemia is confirmatory of DM. Transient hyperglycaemia caused by stress and other illnesses can complicate the diagnosis of DM, particularly in cats. Broadly speaking demonstration of a fasting blood glucose concentration greater than 14 nmol/L *(dogs)* or 20 nmol/L *(cats)* is highly suspicious of DM, but if confirmation of glycaemic control is required, fructosamine estimation can be performed. Fructosamine is a glycated protein the concentration of which reflects the “average” blood glucose concentration over the previous 1-2 weeks. Markedly increased fructosamine concentrations are confirmatory of DM.

Monitoring Tests
If monitoring is being performed with blood glucose estimation, blood samples should be collected six hours after insulin administration to determine the nadir *(lowest)* blood glucose concentration. However, monitoring therapy of diabetic patients can be complicated, particularly in cats in which stress hyperglycaemia makes routine blood glucose

measurement problematic. The clinical assessment of diabetic stability is therefore of paramount importance. In addition to blood and urine glucose measurement, routine estimation of blood fructosamine concentration can be used to improve evaluation of glycaemic control. A decrease in fructosamine values towards normal is generally consistent with an improvement in glycaemic control, whilst an increase suggests a worsening of diabetic control.

Sample Requirements
Blood glucose can be performed on whole blood collected into fluoride anticoagulant tubes. Fructosamine measurement can be performed on a heparinised or clotted blood sample.

Points to Note:
Note that falsely decreased urine glucose results can be obtained if standard dip-stick methods for glucose estimation are used in patients receiving vitamin C supplements.
Blood glucose cannot be measured on heparinised or EDTA samples sent through the post. If blood is taken into these tubes by mistake, immediately centrifuge the samples and separate the plasma before sending off for analysis.
Wait 2-3 weeks after instituting or changing therapy before evaluating control with fructosamine measurements.

HYPOADRENOCORTICISM

Signalment
Hypoadrenocorticism occurs in most breeds of dogs, but a particular predisposition has been reported in bearded collies, spaniels and standard poodles. It is most commonly a disease of young adult animals, typically aged around 3-4 years. However, it has been reported in dogs as young as three months and as old as 14 years.

Pathophysiology
The normal adrenal cortex consists of three layers, the zona glomerulosa, zona fasciculata, and zona reticularis. The first of these secretes mainly mineralocorticoids, the most important of which is aldosterone, whilst the latter two layers synthesise and secrete mainly glucocorticoids, the most important of which is cortisol. Spontaneous hypoadrenocorticism is usually caused by immune mediated destruction of the adrenal cortex. This usually affects all three cortical zones and consequently there is failure of both glucocorticoid and mineralocorticoid secretion. Failure to synthesise mineralocorticoids and glucocorticoids can have profound, frequently fatal consequences, reducing the ability to maintain electrolyte homeostasis and respond to stressful stimuli.

Clinical Signs
Because the adrenal cortices are destroyed progressively over a period of months or years, the clinical signs of hypoadrenocorticism can be vague and often intermittent. However, dogs can also present acutely in an *"Addisonian crisis".*

The most common clinical features of hypoadrenocorticism include:
Chronic Presentation
- Waxing and waning history
- Vomiting
- Diarrhoea
- Polydipsia
- Intermittent anorexia
- Lethargy
- Weaknes

Acute Presentation
- Collapse
- Hypovolaemia
- Vomiting
- Diarrhoea
- Bradycardia
- Hypothermia

Diagnostic Tests
The identification of hyperkalaemia and hyponatraemia is useful in supporting a diagnosis of hypoadrenocorticism. These are routinely measured on heparinised samples. Other routine abnormalities that may be encountered in dogs with Addisons disease include hypercalcaemia, mild normochromic normocytic non-regenerative anaemia, lymphocytosis and eosinophilia. Particularly in dogs with an acute crisis, increased urea and creatinine are common due to profound hypovolaemic pre-renal azotaemia. Confirmation of hypoadrenocorticism is readily achieved with an

ACTH stimulation test. Failure of cortisol to significantly increase confirms a lack of functioning adrenocortical cells and supports a diagnosis of hypoadrenocorticism.

Monitoring Tests
Biochemical therapeutic monitoring of hypoadrenocorticism relies largely on electrolyte *(Na & K)* measurements. Once hypoadrenocorticism has been confirmed, repeat ACTH stimulation tests are of no value.

Sample Requirements
Heparinised blood is suitable for the routine electrolytes. Note that separation of the plasma prior to sending to the laboratory is recommended to help accurately determine electrolyte concentrations. For the ACTH stimulation test, whole blood *(clotted)* should be collected immediately before and one hour after the intramuscular or intravenous administration of 250µg synthetic ACTH.

Points to Note:
The ACTH stimulation test is required to confirm or exclude a diagnosis of hypoadrenocorticism. This test should be performed *prior* to administration of steroids.

Most steroids used in veterinary practice cross-react with cortisol assays and if administered prior to or during an ACTH stimulation test, will give inaccurate cortisol results. Dexamethasone does not cross react in this way and can be used in the emergency situation for patients who need urgent glucocorticoid replacement whilst the ACTH stimulation test is being performed.

Summary of Endocrine Protocols for Quick Reference

Adrenal Function

ACTH STIMULATION TEST *(Dogs)*

1. Collect fasted clotted blood sample *(0 hour)*
2. Inject 250 µg synthetic ACTH *(Synacthen)* intravenously or intramuscularly *(use 125µg Synacthen if patient <5kg)*
3. Collect second clotted blood sample one hour later.
4. Cortisol is measured on both samples

Indications & Uses: Confirming spontaneous hyperadrenocorticism

Confirming iatrogenic hyperadrenocorticism

Confirming hypoadrenocorticism

Monitoring treatment of hyperadrenocorticism *(perform test 4-6 hours post-pill)*

LOW DOSE DEXAMETHASONE SUPPRESSION TEST *(Dogs)*

1. Collect fasted clotted blood sample *(0 hour)*
2. Inject 0.015mg/kg dexamethasone intravenously
3. Collect two further clotted blood samples 3 and 8 hours after the dexamethasone
4. Ensure sample times are labelled on tubes
5. Cortisol is measured on all three samples

Indications & Uses: Screening test for spontaneous hyperadrenocorticism

URINE CORTISOL : CREATININE RATIO *(Dogs, Cats and Horses)*

1. Collect morning urine sample from patient in normal home environment

Indications & Uses: Screening test for spontaneous hyperadrenocorticism

HIGH DOSE DEXAMETHASONE SUPPRESSION TEST *(Dogs)*

1. Collect fasted clotted blood sample
2. Inject 0.1mg/kg dexamethasone intravenously
3. Collect two further clotted blood samples 3 and 8 hours after dexamethasone.
4. Ensure sample times are labelled on tubes
5. Cortisol is measured on all three samples

Indications & Uses: Can only be used AFTER hyperadrenocorticism has been confirmed by other methods

Used to help differentiate between pituitary dependent hyperadrenocorticism and adrenal tumours.

ENDOGENOUS ACTH ASSAY *(Dogs)*

1. Collect blood into chilled plastic EDTA tube (do not use glass tubes)
2. Centrifuge immediately
3. Decant plasma into chilled plain plastic tube
4. Freeze **immediately**
5. Contact lab to request a cool box
6. Sample MUST arrive at lab frozen.

Indications & Uses: Differentiating pituitary dependent HAC from an adrenal tumour.

ADREANAL FUNCTION TESTING *(Cats)*

Hyperadrencorticism is rare in cats. Urine cortisol to creatinine ratio may be used as a screening test and is performed on a morning urine sample collected by the owner in the home environment.

Dexamethasone Suppression Test:

1. Collect fasted clotted blood sample
2. Inject 0.1mg/kg dexamethasone intravenously
3. Collect further clotted blood samples at 3-4 hours and 8 hours after dexamethasone injection
4. Ensure sample times are labelled on tubes
5. Cortisol is measured on all three samples

ACTH stimulation Test:

This test has a low sensitivity and specificity for the detection of hyperadrenocorticism in cats

1. Collect clotted blood sample
2. Inject 125µg of synthetic ACTH *(Synacthen)* intravenously
3. Collect further blood samples 60 and 90 minutes later *(NB: If Synacthen given IM then collect samples at 30 and 60 minutes later)*
4. Ensure sample times are labelled on all three samples

ADREANAL FUNCTION TESTING *(Horses)*

The dexamethasone suppression test and TRH response test are used to help confirm Cushings in horses

Dexamethasone suppression test:

1. Collecting whole blood at approximately 5pm
2. Administer 40ug/ml dexamethasone intra muscularly.
3. Collect second whole blood sample 19 hours later *(approximately 11am, day 2).*

TRH response test:

1. Collect clotted blood sample *(perform test in the morning).*
2. Administer 1 mg TRH intravenously *(slowly over approximately one minute).*
3. Collect whole blood samples at 0, 30 and 60 minutes.

Thyroid Function

THYROID FUNCTION TESTING *(Dogs)*

The minimum database for the diagnosis of hypothyroidism in dogs includes TT4 and cTSH measurement. In equivocal cases measurement of free T4 and thyroglobulin antibodies may be beneficial.

Standard Profile *(TT4 and cTSH):*

- Clotted blood sample
- Used to confirm hypothyroidism
- Used to monitor thyroid hormone supplementation *(collect sample at 6 hrs post-pill)*

Premium profile *(TT4, cTSH & fT4d):*

- Clotted blood sample
- Used to confirm hypothyroidism
- Free T4 *(compared to TT4)* is less affected by non-thyroidal illness and certain drug therapies

Comprehensive profile *(TT4 cTSH fT4d & TgAb):*

- Clotted blood sample
- Used to confirm hypothyroidism
- The presence of TgAbs strongly suggests the presence of immune-mediated lymphocytic thyroiditis

TSH stimulation test *(Dogs)*

1. Collect clotted blood sample for basal TT4 measurement
2. Administer 0.1 international units of bovine TSH intravenously *(NOT licensed for this use)*.
3. Collect a second clotted blood sample 4-6 hours later
4. TT4 is measured on both samples

Indications & Uses: Used to confirm hypothyroidism in dogs where standard tests have been equivocal

TRH Stimulation Test *(Dogs)*

1. Collect clotted blood sample for basal TT4 measurement
2. Administer 200µg TRH intravenously *(for dogs <5kg use 100µg TRH)*.
3. Collect clotted blood sample 4-6 hours later
4. TT4 is measured on both samples

Indications & Uses: Used to help rule out hypothyroidism where other standard tests have been equivocal

THYROID FUNCTION TESTING *(Cats)*

TT4 is the diagnostic test of choice in cats with suspected hyperthyroidism. It is often useful to combine measurements of TT4 with renal parameters when monitoring response to therapy.

Standard *(TT4)*:

- Clotted blood sample
- Used to confirm hyperthyroidism
- Used to monitor response to treatment

Monitoring *(TT4, urea, creatinine):*

- Clotted blood sample
- Used to confirm hyperthyroidism and assess renal function
- Used to monitor response to therapy

Premium *(TT4, fT4d)*

- Clotted blood sample
- Used to confirm hyperthyroidism in equivocal cases

T3 Suppression test

1. Collect clotted blood sample for TT4 and TT3 measurement
2. Administer 20µg T3 three times daily orally for 7 doses
3. Collect clotted blood 3 hours after the final dose
4. TT4 and TT3 measured on both samples

Uses & Indications: Not routinely recommended
Used for diagnosis of equivocal hyperthyroidism

THYROID FUNCTION TESTING *(Horses)*

TSH stimulation Test

1. Collect clotted blood for basal TT4 measurement
2. Administer 5 i.u. TSH intramuscularly
3. Collect second clotted sample 6 hours later

Uses & Indications: Used to evaluate thyroid function

MISCELLANEOUS ENDOCRINE PROTOCOLS

Fructosamine *(Dogs and Cats)*

1. Collect blood into both heparin *(fructosamine)* and fluoride oxalate *(glucose)* tubes

Indications & Uses: Used in diabetic patients to monitor the adequacy of therapy
Indicates degree of glycaemic control over previous 1-2 weeks
Used to help differentiate stress hyperglycaemia from diabetes mellitus in cats

Serum Insulin *(Dogs, Cats and Horses)*

1. Collect blood into plan tube
2. Centrifuge immediately
3. Decant serum into plain tube
4. Send concurrent fluoride oxalate tube for glucose measurement

Indications & Uses: Diagnosis of insulinoma in dogs *(Must have concurrent glucose measurement for interpretation)*
Investigation of Cushings in horses

PARATHYROID FUNCTION *(Dogs & Cats)*

Parathyroid Hormone (PTH)

1. Collect blood into chilled plastic EDTA tube
2. Centrifuge immediately
3. Decant plasma into chilled plain plastic tube
4. Freeze **immediately**
5. Contact lab to request a cool box
6. Sample MUST arrive at lab frozen
7. Collect serum sample at same time for calcium measurement!!

Indications & Uses: Investigation of persistent hypercalcaemia
Investigation of persistent hypocalcaemia
Interpretation requires concurrent measurement of serum calcium

Parathyroid Hormone Related Peptide (*PTHrP)*

1. Collect blood into chilled plastic EDTA tube
2. Centrifuge immediately
3. Decant plasma into chilled plain plastic tube
4. Freeze **immediately**
5. Contact lab to request a cool box
6. Sample MUST arrive at lab frozen
7. Collect serum sample at same time for calcium measurement!!

Indications & Uses: Investigation of persistent hypercalcaemia
Increased levels usually associated with malignancy
Interpretation requires concurrent measurement of serum calcium

HAC Hyperadrenocorticism **TSH** Thyrotropin *(thyroid stimulating hormone)*

PDH Pituitary dependant hyperadrenocorticism **cTSH** Canine thyrotropin

AT Hyperadrenocorticism caused by an adrenal tumour **TgAb** Thyroglobulin autoantibodies

TT_4 Total thyroxine **T_3** Triiodothyronine

FT_4d Free thyroxine *(measured by dialysis)* **TRH** Thyrotropin releasing hormone

Reproductive Endocrinology

CRYPTORCHIDISM

This test is based on the measurement of dynamic *(pre and post HCG)* Testosterone.
If testicular tissue is present then a significant rise in testosterone is detected post stimulation.

Horses:

- Take basal serum or heparinised plasma samples
- inject 6000 iu HCG I.V.
- Collect a second sample between 30 mins-2 hours post injection, noting the time taken on the post sample.

Single sample Oestrone sulphate tests may be used in horses over 3 years of age, but are not of any value under this age and cannot be used in donkeys. This laboratory recommends the dynamic Testosterone test in most cases.

Dogs:

- Take basal serum or heparinised samples
- Inject 750 iu HCG intravenously
- Collect second sample between 30 mins – 2 hours post-injection

Cats:

- Take basal serum or heparinised plasma samples
- Inject 500 iu HCG intravenously
- Collect second sample 30 mins-2 hours post-injection

OVARIAN REMNANTS

Dogs:

- Take basal or heparinised plasma sample
- Inject 200 iu HCG intravenously (9-15kg dogs) or 300 iu HCG (>15kg dogs)
- Collect second sample 90 minutes later
- Oestadiol measured on both samples

Cats:

- Take basal serum or heparinised plasma, inject 500 iu HCG intravenously
- Collect second sample seven days later
- Measure *progesterone* on both samples

DYNAMIC TESTING FOR ADRENAL SEX HORMONE PRODUCTION

The protocols for this are undergoing constant change. As such it is advisable to telephone the laboratory prior to any testing to discuss the current protocols.

OVULATION DETECTION IN BITCHES

- This involves sequential heparinised plasma or serum samples for progesterone.
- As the values vary slightly between plasma and serum, it is advised that either sample is used, but not mixed, i.e., do not use a plasma sample, followed two days later by a serum sample.
    - Most bitches ovulate on day 13 of the cycle, it is recommended that basal levels are established on days 9-10, then the lab will advise on when the next sample should be taken, based on the following :

<6 nmol/L NOT OVULATED

6-10 nmol/L OVULATED MATE WITHIN 72 HOURS

10-20 nmol/L OVULATED MATE WITHIN 48 HOURS

20-32 nmol/L OVULATED MATE WITHIN 24 HOURS

32 nmol/L TOO LATE

- It is wise to note that these values are usually reported 24 hours post sampling and those 24 hours need to be taken off the above times, i.e. the above times relate to the time of sampling.

PREGNANCY TESTING

In equine serum PMSG levels can be measured for pregnancy between days 45-90. The production of PMSG is biologically variable and it is better to sample between these dates e.g. around 60 days, rather than at either end of the range.

Oestrone sulphate will be present at approximately day 70 and in significant amounts between days 110 & 240. It is present until the end of pregnancy but values fall in the last 4-6 weeks and interpretation can be difficult in this period. The cow and pig have raised oestrone sulphate levels at certain periods through pregnancy. It is advisable to therefore contact the lab first to discuss.

Single progesterone values have been used in the horse and cow, at the predicted return to oestrus dates, to indicate possible earlypregnancy. They are not specific indicators of pregnancy, just indicators of luteal activity, from which assumptions are made.