

# Finn Pathologists

The Veterinary Laboratory

## Clinical Pathology Protocols

### Chemistry tests

#### ***Bile acid stimulation test***

The bile acid stimulation test is used to evaluate hepatic function, and is used in the diagnosis of portosystemic shunts. Bile acids are synthesised in the liver, excreted in the bile, reabsorbed in the distal small intestine, and removed from the portal circulation by the liver. Basal bile acid levels in the peripheral circulation therefore reflect the overspill from this enterohepatic circulation. Ingestion of a fatty meal, together with the action of swallowing, stimulate gall bladder contraction and a surge of bile acids reaching the intestine and returning to the liver, and spilling over into the peripheral circulation. Thus, post-prandial levels are slightly higher than basal levels in normal animals. Post-prandial bile acids are elevated beyond normal where liver function is reduced or where there is portosystemic shunting (this can reflect alterations in enterohepatic function secondary to chronic disease sufficient to be detected histologically).

#### **Limitations:**

- The bile acid stimulation test cannot distinguish between pathological processes in the liver, and cannot provide assessment as to their reversibility.
- Failure to achieve elevations in the post-prandial sample can be seen where there has been insufficient food intake, malabsorption, premature contraction of the gall bladder prior to starting the test, failure to stimulate gall bladder emptying (individual variation), or bacterial intestinal metabolism.

#### **Protocol:**

1. Starve the patient for 12 hours.
2. Collect 1-2 ml blood into a serum gel or plain tube. Label the tube with patient details and time of sampling.
3. Feed a high fat meal – suggested meals include puppy/kitten food, Hill's a/d, n/d or p/d. The addition of oil is not usually necessary and reduces palatability.
4. Collect a second sample 2 hours post feeding, into a serum gel or plain tube. Label the tube with patient details and time of sampling.

## ***Diabetes mellitus diagnosis and monitoring***

A marked **hyperglycaemia** at a single point in time can be sufficient evidence of diabetes mellitus, if there are other compatible clinical signs (such as polyuria and polydipsia), and there is no evidence of other disease. However, severe acute stressful illness can cause a marked hyperglycaemia to levels that overlap with diabetic patients. **Glucosuria** is also most often associated with diabetes mellitus. But it also can be seen with other conditions causing hyperglycaemia, and with renal tubular disorders such as the Fanconi syndrome. Consequently, hyperglycaemia and glucosuria alone do not definitively diagnose diabetes mellitus.

**Fructosamine** represents serum proteins (particularly albumin) strongly bound to glucose because of persistent exposure over the lifetime of the proteins. A high fructosamine reflects persistent hyperglycaemia over a period of 1 to 3 weeks. This is much better evidence of diabetes mellitus. However, it will not detect very recent onset of diabetes mellitus, and can be low in hypoproteinaemic patients.

Fructosamine is also very useful for **monitoring** diabetes mellitus, as it avoids the problems of stress induced transient hyperglycaemia at the time of sampling that could give a false impression of poor control of the condition. It also gets around the problems of collecting and testing urine samples.

### **Protocol:**

Collect 1-2 ml blood into a serum gel tube or plain tube. Label the tube with the patient details. The timing of the sample is not critical for this assay as it is assessing average glucose levels over a period of time. However, a fasted sample is preferable, and the sample should be separated (or a gel tube spun) as lipaemia predisposes samples to haemolysis that can have a significant effect on the result.

## **Therapeutic Drug monitoring**

### ***Phenobarbitone***

Phenobarbitone levels stabilise in the serum within 7 to 14 days of starting or changing therapy. The timing of the sample will depend on the aim of sampling. Peak levels (4-6 hours post pill) are used for general monitoring and for checking that toxic levels are not being reached. Average levels occur approximately 8 hours post pill. Trough levels are measured (just before a pill is due) when peak levels appear adequate but clinical signs do not appear to be controlled. This can occur if the drug is being metabolised rapidly and the plasma levels are varying too widely. Phenobarbitone can induce the production of liver enzymes in dogs but not in cats. It can also cause hepatic damage. Monitoring hepatic function during therapy is recommended (ideally with a bile acid stimulation test). Serum gel tubes should not be used for the phenobarbitone assay.

## ***Potassium bromide***

KBr levels do not stabilise until the patient has been on therapy for at least 2 to 3 months, by which time plasma levels do not vary much during the day. Most patients are also receiving phenobarbitone. The sample taken for monitoring phenobarbitone levels is acceptable for monitoring KBr levels.

## ***Digoxin***

The therapeutic range for digoxin is narrow, and toxicity occurs at levels barely above the therapeutic range. Serum levels tend to stabilise 5 to 7 days after starting or changing therapy, and close monitoring is recommended. Peak levels occur at 4-6 hours post pill, with trough levels just before a pill is due.

## **Endocrinology**

### ***Hypothyroidism in dogs***

Canine hypothyroidism is notoriously difficult to diagnose definitively. Total T4 within the normal range almost excludes hypothyroidism. However, it can be depressed by many non-thyroidal illnesses, drug therapies, and anaesthesia, as well as hypothyroidism. Because of the negative feedback loop, low T4 should trigger an elevation in cTSH production from the pituitary gland. This can also be elevated by some non-thyroidal illnesses (in particular Cushing's syndrome), and is not always elevated in hypothyroid dogs. Free T4 is the active fraction of T4, and is less affected by non-thyroidal illness. Testing for free T4 should be by equilibrium dialysis as other methods are not sufficiently accurate for diagnosis. Approximately 50% of hypothyroid dogs are immune mediated, and these patients may have detectable levels of anti-thyroglobulin antibodies. Anti-T4 antibodies interfere with the T4 assay causing results to be higher than the true value. Dynamic testing with TRH or TSH (when available) theoretically improves the accuracy of diagnosis, but many patients fall within the equivocal bracket.

#### **Protocol:**

Basic screening: Total **T4 and cTSH together** on a serum sample.

Further testing where results are equivocal: Free T4 by equilibrium dialysis, anti-thyroglobulin antibodies, and anti-T4 antibodies.

Dynamic testing: TRH and TSH stimulation tests – protocols available on request.

### **Monitoring treatment for hypothyroidism**

The recommended initial treatment for hypothyroidism is the administration of levothyroxine (Soloxine, Arnolds) at a dose of 11-22 µg/kg twice daily. Treatment may be reduced to once daily following resolution of the clinical signs. Some patients appear to require continued twice daily therapy to control clinical signs.

The total T4 concentration should be monitored regularly during treatment, particularly during the initial stages. Measurement of cTSH may also be helpful when monitoring thyroid therapy in dogs as it may reflect the adequacy of the therapy in the preceding days. The total T4 concentration only provides information pertaining to the day of sampling. However, normal/low cTSH levels do not indicate that therapy is adequate, as patients do not necessarily have an elevated cTSH level when T4 levels are inadequate.

**Protocol:**

Samples for monitoring treatment should be taken **4 to 6 hours post pill**, at which point the concentration should be in the upper half of the reference interval or slightly higher. Where peak levels are adequate, a trough level is also recommended (just before a pill is due), and this should be at similar levels to peak samples. Serum samples are preferred, although heparinised plasma may also be used.

## ***Feline thyroid disease***

Total T4 is the screening test of choice for assessing thyroid function in cats. T4 fluctuates during the day, and some hyperthyroid cats have only marginally elevated T4 at the time of sampling. As in dogs, T4 can be depressed by concurrent non-thyroidal illness (chronic renal insufficiency is common, but almost any disease is a possibility). Hyperthyroid cats with concurrent disease may have T4 levels in the upper half of the reference interval. Measurement of free T4 (by equilibrium dialysis) can be of use where total T4 is within the reference range but clinical signs are strongly suggestive of hyperthyroidism. Care should be taken in its interpretation, as it can be elevated by non-thyroidal illness. Dynamic testing can also be useful in cats, with the T3 suppression test or a TRH stimulation test. The latter frequently causes nausea and vomiting and is not recommended.

### **T3 suppression test**

In healthy individuals, T3 has a suppressive effect on pituitary TSH secretion and subsequently on T4 production by the thyroid gland. This suppressive effect is lost in hyperthyroidism due to the autonomous production of thyroid hormones and chronic suppression of TSH. The serum concentrations of total T4 therefore show minimal decrease in hyperthyroid cats following T3 administration. However, concurrent measurement of T3 is advised to ensure adequate administration and absorption of the T3, avoiding false positive results.

**Protocol:**

1. Collect a baseline serum sample for total T4 and T3. Label the sample with patient details and time and date of sampling. Store the serum frozen until the

protocol has been completed and submit together with the post-Tertroxin sample.

2. Administer Tertroxin orally every 8 hours for 7 doses.  
Cats <5kg: 20 µg of Tertroxin  
Cats >5kg: 30 µg of Tertroxin
3. Collect a serum sample 2-6 hours after the last dose. Label with patient details and time and date of sampling.
4. Submit the samples for measurement of total T4 and T3.

## **Monitoring treatment for hyperthyroidism in cats**

T4 should be measured, with the aim of obtaining levels in the lower half of the reference interval. With regular tablet giving, the serum levels should not vary much, so the timing of the sample is not critical. However, if therapy is interrupted for 24-48 hours, T4 levels will rise significantly.

## ***Canine hyperadrenocorticism***

Spontaneous hyperadrenocorticism can develop as a result of a pituitary tumour (usually an adenoma) producing excessive ACTH that stimulates excessive production of glucocorticoids from the adrenal medulla, or a productive adrenal tumour. Approximately 80% of canine Cushing's cases are pituitary dependent. A single cortisol level is not adequate for diagnosis as it is often within normal limits in cushingoid dogs and can be elevated significantly during stress. Iatrogenic hyperadrenocorticism can also be seen, even when patients are on apparently low levels of glucocorticoid therapy. Glucocorticoid therapy (by any route including topical preparations) in the month prior to testing can interfere with adrenal function. Four tests are commonly used:

### **Urine Cortisol:Creatinine ratio**

This test is carried out on a plain urine sample, preferably collected at home to avoid stress during sampling. Cortisol is excreted by the kidneys as well as being removed by hepatocytes. Urine creatinine is used as a comparator as renal excretion is assumed to be constant. A normal result excludes hyperadrenocorticism. A positive result can reflect hyperadrenocorticism, but can also be seen in many stressful illnesses. A positive result should also be confirmed with a dynamic test (LDDS or ACTH stimulation tests).

### **LDDS test**

This test is more sensitive than the ACTH stimulation test, and can in many cases distinguish between adrenal dependent and pituitary dependent disease, but it has some **limitations**:

- The LDDST is not suitable for the detection of iatrogenic hyperadrenocorticism.

- The LDDST is not suitable for monitoring animals on treatment for hyperadrenocorticism.
- False positive results may occur, especially in animals with significant non-adrenal illness, such as diabetes mellitus or chronic renal insufficiency.
- It is very important to avoid any stress to the animal during the test period as far as possible as this may interfere with the results. Stress may cause animals without hyperadrenocorticism to break the suppressive effect of the dexamethasone.

**Protocol:**

1. Collect a baseline serum sample (1-2ml). Label all tubes with patient details and the times of the samples.
2. Inject 0.01mg/kg dexamethasone intravenously.
3. Collect a second serum sample 3 hours post-dexamethasone.
4. Collect a third serum sample 8 hours post-dexamethasone

**ACTH stimulation test**

This test is less sensitive than the LDDST, but less prone to false positive results. It is used in the following situations:

- To detect hyperadrenocorticism in cases where there is stress or concurrent non-adrenal illness.
- To distinguish spontaneous hyperadrenocorticism from iatrogenic disease.
- To monitor patients on therapy for hyperadrenocorticism.

It also has **limitations**:

- It does not allow differentiation between adrenal dependent and pituitary dependent disease. This can be done with a high dose dexamethasone suppression test. This test should only be carried out once a diagnosis of hyperadrenocorticism has been reached. The protocol is as for the LDDST, but using 0.1mg/kg dexamethasone.
- It may not be positive in cases of adrenal dependent hyperadrenocorticism.
- False positives may occur with chronic stressful illness such as diabetes mellitus. However, they occur less commonly than with the LDDST, and the ACTH stimulation test is the test of choice in these cases, preferably once the patient is stabilised.
- False positive results can be seen in some cases of severe gastrointestinal disease where the test may be used to investigate possible Addison's.

**Protocol:**

1. Collect a baseline serum sample (1-2ml). Label the sample with patient details and the time of sampling.
2. Inject 0.25mg synthetic ACTH (Synacthen) intravenously.
3. Collect a second sample 60 to 120 minutes later. Label the sample with patient details and the time of sampling.
4. Submit samples together for cortisol assays.

## **SHAP (sex hormone alopecia profile)**

Some patients that are clinically cushingoid fail to provide positive results with a LDDST or an ACTH stimulation test. If other differentials are excluded, the SHAP test can be useful. This is based on an ACTH stimulation test, with samples taken at 0 and 60 minutes, and tested for **17-hydroxyprogesterone** as well as cortisol.

## **Monitoring of therapy for Canine hyperadrenocorticism**

The test of choice is the ACTH stimulation test (see protocol above). For patients on trilostane (Veteryl), the timing of the test relative to the most recent tablet is critical for interpretation of the results. The test should be started 4-6 hours post-pill. If the results at this time suggest adequate control, but the patient appears out of control, a test carried out just before the next dose is due is indicated. Some patients require twice daily therapy. Patients on mitotane (Lysodren) can be tested at any time of day.

## ***Addison's disease***

In hypoadrenocorticism, the adrenal glands are incapable of responding to ACTH, and cortisol levels remain low in the basal and post-ACTH sample. The **ACTH stimulation test** is therefore the test of choice for diagnosis. The LDDST is not appropriate in these cases, as adrenal cortisol production cannot be suppressed any further than the basal level. The **limitations** include:

- Patients receiving glucocorticoid therapy will have results resembling Addison's cases. Glucocorticoid therapy can suppress adrenal function for up to a month after administration.
- Patients receiving Tardak (delmadinone acetate) can be suppressed for at least 3 months after administration.

## **Monitoring Addison's patients on therapy**

Once a diagnosis has been obtained and therapy is in progress, adrenal function will be further suppressed by the therapy. An ACTH stimulation test will not provide useful information. These cases should be monitored on the basis of electrolyte levels and clinical signs.

## ***Feline hyperadrenocorticism***

This is a generally considered a rare condition, with testing most often carried out in diabetic patients not responding to therapy. Interpretation of test results in these cases is complicated by the concurrent chronic stress of the diabetes. Both an ACTH stimulation test and a dexamethasone suppression test can be carried out, but the protocols are different from those in the dog. Both tests have high false positive rates, and should be interpreted carefully.

## **ACTH stimulation test protocol:**

1. Obtain a basal serum sample, label with patient details and time of sampling.
2. Administer 0.125mg synthetic ACTH (Synacthen) intravenously.  
Intramuscular injections can be used, but injection between muscle bellies in the fascial planes prevents absorption.
3. Collect second and third serum samples at 60 and 120 minutes after the Synacthen injection. Label the samples with patient details and the times of sampling.
4. Submit the three samples for cortisol assays.

**Dexamethasone suppression test protocol:**

1. Obtain a basal serum sample, label with patient details and time of sampling.
2. Administer dexamethasone intravenously at a dose rate of **0.1mg/kg**.
3. Collect second, third and fourth serum samples at 4, 6 and 8 hours after the dexamethasone injection. Label the samples with patient details and the times of sampling.
4. Submit the four samples for cortisol assays.

## ***Cryptorchid/testicular function***

**Basal Testosterone:**

Testosterone fluctuates during the day. A basal sample can demonstrate the presence of testicular tissue. In most cases where there is no testicular tissue present, testosterone levels are undetectable. For cases where an equivocal result is obtained, or where an absolute diagnosis is essential, the hCG stimulation test is recommended. Basal testosterone should be measured in a serum sample.

**HCG stimulation test:**

1. Collect a baseline blood sample (serum). Label with patient details and time of sampling.
2. Inject hCG intravenously (Chorulon, Intervet) according to species – see the data sheet.
3. Collect a second serum sample 30-120 minutes later, and label with patient details and time of sampling.
4. Submit samples for testosterone assays.

## ***Detection of ovarian tissue***

This test may be carried out in bitches and queens that have been spayed but still display oestrus behaviour. The aim is to detect ovarian tissue remaining after surgery.

**Protocol:**

1. The test should be started during behavioural oestrus (preferably within the first 3 days).
2. Collect a basal serum sample. Label it with patient details and date of sampling, and store refrigerated or frozen.
3. Inject hCG (Chorulon, Intervet) intramuscularly at a dose rate of 44IU/kg.
4. Collect a second sample 7 to 14 days later, labelled with patient details and date of sampling.

5. Submit the samples together for progesterone assay.