**Vetpath** is a specialist veterinary laboratory dedicated to providing our clients with the finest laboratory diagnostic service. A team of veterinary pathologists and medical scientists with extensive experience in veterinary diagnostic pathology forms the core of the Vetpath team.

# VNews

VETPATH LABORATORY SERVICES

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## Avian and reptile blood samples

Collecting blood from birds and reptiles can be difficult. That's why correct storage of these precious samples is imperative.

The way in which a sample is collected, stored and submitted to a referral laboratory can greatly affect the accuracy of the results and consequently the diagnostic value of the testing. Several hints to help maximise the diagnostic usefulness of the CBC are summarized below

**Sample size**
One of the most challenging aspects of blood testing in birds and reptiles is small sample size. At least 0.25ml of anticoagulated blood is required for a complete blood count including analysis by an automated haematology analyser. However, if this is not possible, valuable data can be still obtained from packed cell volume and smear evaluation.

**Anti-coagulant**
Lithium heparin is the most commonly used anti-coagulant in birds and reptiles because one sample can be used for the CBC and biochemistry panel. However, lithium heparin can cause cell clumping which can affect the total and differential cell counts. EDTA is not recommended for reptiles as it results in cell lysis, but is a suitable for haematology in birds if sufficient blood can be collected.

**Tube size**
It is important to select the appropriate sized tube to match the volume of blood collected. The blood will be exposed to excess EDTA if the sample is too small for the tube, leading to poor staining of the cells and altered cell counts due to a dilutional effect. Choosing 1ml paediatric tubes can help prevent this phenomenon. Once collected, the sample should be processed as soon as possible to avoid prolonged anticoagulant exposure and subsequent cell smudging.

**Submission of a smear**
Preparing a well made smear is the best way to preserve cells for both counts and morphological assessment. If you have an assistant, a smear can be made using a drop of blood before placing the remaining blood sample into the tube. Alternatively, anti-coagulated blood can be used for the smear. The smear needs to have a central monolayer with evenly distributed cells and a feathered edge to correctly estimate cell count and assess morphology.

If you have any questions about sample submission from exotic (or any other!) species, please call the laboratory on (08) 9259 3600. We are happy to help.

Vetpath Laboratory Services welcomes feedback on all aspects of our service from couriers to lab results. Please feel free to contact us at 9259 3666 or email enquiries@vetpath.com.au

# Monitoring trilostane treatment

Trilostane is commonly used for treatment of hyperadrenocorticism with treatment being monitored by an ACTH stimulation test.

Trilostane is relatively short acting (compared to mitotane) and therefore the results of the ACTH stimulation test varies depending on the time of testing relative to dosing. The ACTH stimulation test should be completed **2 to 6 hours** after the administration of trilostane. Several different target ranges have been reported. The two referred to in Vetpath reports are:

1. Feldman and Nelson (2004) recommend a post ACTH-stimulation cortisol target range of 27 - 60 nmol/L.
2. Dechra recommend an optimal post-ACTH cortisol of 40-150 nmol/L.

Patients should be monitored monthly for the first three months, then every 3 months for the first year and then every 4 – 6 months thereafter.

Remember to include the patient history when submitting an ACTH stimulation test for helpful interpretation!

# What factors can affect total solids protein?

Plasma total solids protein concentration in is a routine component of a complete blood count and is usually measured using a refractometer.

The principle of refractometry is based on the bending (refraction) of light. The magnitude of light refraction as it passes through the fluid is proportional to the total amount of dissolved solids in the fluid.

Most of the light refraction is due to proteins. Other non-protein solids such as electrolytes, urea, lipids and glucose are usually present in relatively constant amounts in plasma. However, when these elements are increased the total protein concentration can be artifactually increased. This is most common with lipaemia; however severe hyperglycemia, severe haemolysis or elevated urea can also have an affect.

There is differing information in the literature about the effect of bilirubin on refractometric protein concentrations. A recent article in JAVMA found that even severe hyperbilirubinaemia **did not interfere** with protein concentration measured by refractometer. Therefore, hyperproteinaemia determined by refractometry in a jaundiced patient is not an artifactual change due to increased bilirubin.

Don't forget to check the accuracy of your refractometer daily. To calibrate or "zero" the refractometer, place a drop of distilled water on the reading surface. The reading should be at zero (1.000 on the urine SG scale). Adjustments are made using the screw on the top of the refractometer.

**Reference**: Gupta A and Stockham SL, 2014. Refactoemtric total protein concentrations in icteric serum from dogs. JAVMA 244 (1): 63 – 67.

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