Avian and reptilian diagnostics

HAEMATOLOGY AND BIOCHEMISTRY

Recommended testing and sample requirements:

Test	Sample	Sample Volume	Components	
Haematology	• Lithium heparin (preferred) or EDTA • Air-dried smears (x2)	Minimum 300µL	• Packed cell volume • Estimated white cell count • White cell differential • Morphology of red and white cells • Thrombocyte assessment	
Biochemistry*	• Lithium heparin or plain (red top)	Minimum 400µL (or 300µL without electrolytes)	• Liver disease • Renal disease • General	AST GLDH Bile acids Uric acid Total protein Glucose Calcium Phosphorus CK

** For small volume samples please indicate biochemistry analytes in order of priority.*

Interpretation of biochemistry

As interpretation of avian chemistry results differs from mammalian chemistry, a brief explanation of the various tests is included as follows:

LIVER AND MUSCLE

1. AST and Bile Acids are the most sensitive indicators of liver disease in birds.
   - AST is not liver specific and can be increased in any septic or inflammatory condition, muscle disease and with certain antibiotics and steroids.
   - GLDH is present within hepatocyte mitochondria and is considered the most specific indicator for hepatocellular damage in birds. GLDH also has high activity in renal tissue in birds but most of the enzyme is excreted directly into urine and never reaches the blood.
   - Serum bile acid concentration is a reliable indicator of liver function.
     - Requires serum as heparin interferes with the assay.
     - Feeding can increase bile acids up to 1.6-4.5 times the normal reference range, therefore fasting samples are preferred. Pigeons, ostriches and some parrots lack a gall bladder so fasting is not needed in these species.
     - Amazon parrots normally have slightly higher bile acids than other companion avian species
     - Low bile acid concentrations are common in birds with microhepatica, poor feather formation, and an overgrown malformed beak.

   Generally, a bile acid concentration > 100 µmol/l is considered abnormal and > 75µmol/l is suspicious for hepatic insufficiency.

2. CK activity increases with muscle damage and this, along with AST and GLDH, is used to differentiate muscle from liver disease.
3. The following tests are NOT recommended as indicators of liver disease in birds:
   - ALT and GGT occurs in many different tissues and in some species enzyme activity is below the sensitivity of many analysers.

- ALP activity can be increased due to inflammation therefore is considered nonspecific.
- Bilirubin concentrations increase inconsistently in cases of liver disease and some birds (chickens) cannot form bilirubin

RENAL FUNCTION

1. Uric Acid is the most reliable test of renal disease.
    - It requires more serum or plasma than the other tests (a minimum of 50µl) so is often done last.
    - Age, diet, sex, and recent feeding may affect results especially in raptors where postprandial levels can increase to twice normal for up to 8 hours.
    - Elevations are seen with severe dehydration and renal disease.
    - It can be used as a prognostic indicator for gout - the solubility of uric acid in plasma in birds is around 600 µmol/l - levels higher than this (1500 – 2500 µmol/l) will lead to precipitation in joints.
2. Urea and creatinine are not useful tests for monitoring for renal disease.
    - Urea can be useful in assessing hydration status - concentrations of 0.4 – 0.7 mmol/L are considered normal, but up to 10-15x increase can be seen in dehydration.
    - Creatinine is not synthesised by birds - most muscle breakdown products are excreted as creatine rather than creatinine.

OTHER TESTS

1. Protein concentrations in serum are generally lower in birds than mammals.
    - In most avian species levels range between 20-40 g/L (some spp. are as low as 15 g/L)
    - Plasma concentrations will be approximately 1-2g/l higher than serum concentrations due to the presence of fibrinogen.
    - Glucose is not utilised by avian erythrocytes therefore concentrations in serum are much more stable in birds.
    - Levels will fall slowly over 24-48 hours if the serum stays on the clot.
    - If there is a delay in the sample reaching the laboratory, either spin and separate the sample or use a fluoride oxalate (grey top) tube.
2. The glucose reference range in birds (11.2-27.7 mmol/L) is generally higher than in mammals
    - Stress and postprandial levels can cause transient increases to 24.9 – 33.3 mmol/L.
    - Conditions such as egg yolk peritonitis and renal carcinoma can also cause similar increases
    - Borderline hypoglycaemia is common in cockatoos and probably of no clinical significance.
3. Calcium measured in serum includes albumin bound and free forms therefore concentration varies with albumin concentration.
    - Oestrogen induces hypercalcaemia therefore calcium concentration in serum increases approximately 4 days prior to ovulation.
    - Corticosteroids decrease total calcium.
    - As in mammals dehydration and some tumors can increase calcium concentrations.
    - African Grey Parrots have a described idiopathic hypocalcaemia (a unique form of hypoparathyroidism in which calcium is not properly released from bone).

AVIAN URINALYSIS

Urine collection can be aided by giving water by a crop tube – the bird will often urinate shortly after. Avian urine has a concentrated white to off white uric acid component and clear watery component – the watery fraction is assessed.

Test	Expected results
pH	Ranges from 6.0-8.0 (diet related)
Protein	Trace amounts normal
Glucose	Trace amounts normal in some species. Will occur if blood glucose is >33.3 mmol/L
Ketones	Negative in normal birds. Increased with diabetes and increased fat metabolism
Specific gravity	1.005-1.020
Bilirubin	Not normally present. Biliverdin is the major bile pigment which does not react with the mammalian urine dip stick.
Blood	Negative or trace
Nitrate	Unreliable in birds
Urates	White, pale yellow and pale grey are normal
Liver disease	Urates are yellow to green
Acute lead poisoning	Urates brown or blood tinged
Sediment*	RBC and WBC: normal <3 cells/HPF Epithelial cells: Normally none present Casts: Presence is associated with renal disease Bacteria: False positive with faecal contamination of urine (can proliferate in transit)

** A fresh or recently refrigerated sample is required. Prolonged storage causes lysis of cells.*

SPECIFIC AVIAN CYTOLOGY AND MICROBIOLOGY

Collection and submission of coelomic fluids and routine aspirates is as described for other species (see routine cytology section).

Crop Aspirate/Wash

Indicated if there is vomiting, regurgitation not associated with courtship, delayed crop emptying or other suspected disorders of upper alimentary tract.

- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Choana

- Indicated in cases of choanal ulceration/erosion
- Samples can be obtained by swabbing gently with pre-moistened swab (sterile saline) - avoid touching the feathers around the vent
- Make multiple air-dried direct smears

Sinus Aspirate in Psittacines

- Indicated in cases of facial swelling, nasal discharge or other upper respiratory tract disease.
- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Air Sac Samples

- Sampling is indicated in suspected chlamydial, bacterial or fungal infections of air sacs
- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Faeces

- Sampling is indicated if birds have gastrointestinal signs or are doing poorly.
- Tests available include faecal worm egg count, coccidial oocysts, Giardia parasites and faecal culture

AVIAN SEROLOGY/PCR

Test	Sample required
*Chlamydia psittaci PCR	Cloacal swab
*Psittacine Beak and Feather Disease PCR	Blood or blood feather
*Polyoma virus PCR	Blood or blood feather
*Mareks disease PCR	Blood or blood feather
Sexing	Blood spot on filter paper or blood feather

** can be done individually or as part of the Avian Panel*

Chlamydophila (Chlamydia) psittaci

Chlamydophila psittaci infection can be the cause of fever, anorexia, lethargy, diarrhoea, excretion of green to yellow urates and occasionally shock and death in birds. Infection can be associated with conjunctivitis, enteritis, pericarditis, air sacculitis, sinusitis, coelomitis, hepatitis and splenitis. The importance of this infection in birds is enhanced by its zoonotic potential. *C. psittaci* infection has been demonstrated in over 460 bird species with the highest infections rates reported in psittacine birds and pigeons. Survivors of infection can become asymptomatic carriers. Transmission is from close proximity to another infected bird. The bacteria are shed in nasal secretions and faeces – faecal shedding is intermittent and can be activated by any cause of stress. The organism can survive in the environment for several months if protected by organic debris. Predator or scavenger species can become infected through consumption of the carcass of an infected bird. Nest transmission is possible through regurgitation feeding and via biting/blood-sucking arthropods.

Human infections (psittacosis) occur most commonly from inhaling aerosolized organisms from urine, respiratory secretions or dried faeces. Beaks to mouth contact, a bite from an infected bird or handling the plumage of an infected bird are other possible sources of infection. Appropriate protective equipment should be used if performing a post-mortem on an infected bird.

Psittacine Beak and Feather disease (PBFD)

PBFD is caused by beak and feather virus which is from the family *Circoviridae*. Infection with this virus causes chronic feather dystrophy and loss, beak deformities, occasionally immunosuppression and death. Death in chronic cases is often due to secondary infections associated with immunosuppression. There are acute and peracute forms which can cause sudden death. Parrots that are known to be particularly susceptible to PBFD include, Cockatoos, Ringneck parakeets, Eclectus Parrots and Lovebirds but all parrot breeds should be considered at risk. Any psittacine bird with chronic feather loss should be tested for this infection.

Polyoma virus (Budgerigar Fledgling Disease)

Polyoma virus in birds can be seen clinically as sudden death, neurological signs, abdominal distension (hepatomegaly or ascites), petechial to widespread haemorrhage or failure of development of normal feathers. All psittacine birds can be infected however fledgling and juvenile birds are most susceptible. Chronic, subclinical infection with intermittent viral shedding is common in birds that recover from acute infection. Infection is most commonly due to contact with another infected bird – the virus is shed in feather dander and faeces. The virus can remain stable in the environment for long periods.

Marek's disease

Caused by Gallid herpesvirus-2, Marek's disease is a highly infectious, wide spread infection in chickens. The viral incubation period is 4-6 weeks and flock mortality is reported to be 10-50%. Infection is most common in young flocks. Clinical signs include hind limb or wing paralysis, dyspnoea, depression, weakness, tumors on feather follicles, blindness and a withered comb. Most clinical signs are associated with the development of T cell lymphoma and the infiltration of lymphocytes into tissues and nerves. Infection with Marek's disease also causes immunosuppression and increases susceptibility to secondary infections. The virus is shed in the feather dander and droppings or any secretions. The virus can persist for long periods in poultry yards.

AVIAN TOXICOLOGY

Heavy metal intoxication is not uncommon in caged and wild birds. Caging material and paint can be source of lead or zinc if chewed or ingested.

Test	Sample required	
	Live bird	Dead bird
Lead	Minimum of 200 µL blood in a lithium heparin tube or EDTA	Fresh liver and kidney (as much as available)
Zinc	Minimum 200 µL blood in lithium heparin or plain tube – do not use EDTA as it chelates the zinc	Fresh liver, kidney or pancreas (minimum 100g of tissue)

AVIAN VIRUS ISOLATION

Species: Avian

Specimen: Swabs, fresh tissue, serum or blood

Container: Red top tube for sera, EDTA tube for blood, virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol: Venepuncture, post mortem, swab of lesions

Special handling/shipping requirements: Ship all samples chilled

General information about the disease: This will vary depending on the disease (see table below).

Virus	Sample	Container
Adenovirus	Caecal tonsil	Sterile pottle
Avian encephalomyelitis (AE)	Serum, brain	
Egg drop 76	Serum	Red top tube
Fowl pox	Lesion, serum	Sterile pottle, red top tube
Infectious bronchitis (IB)	Trachea, caecal tonsil, kidney, serum	Sterile pottle, red top tube
Infectious bursal disease (IBD)	Brain, bursa, serum	Sterile pottle, red top tube
Infectious laryngotracheitis (ILT)	Trachea, larynx, serum	Sterile pottle, red top tube
Infectious anaemia	Liver	Sterile pottle
Tenosynovitis avian reovirus	Tendons, caecal tonsil, serum	Sterile pottle, red top tube
Viral arthritis	Synovial fluid, hock joint	Sterile pottle, red top tube

General information about when this test is indicated: This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

Comparison with other related tests: Check if an ELISA test is available to test for the virus either as an antigen or antibody

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