

Hygiene Protocols for the Prevention  
and Control of Diseases  
(Particularly Beak and Feather Disease)  
in Australian Birds

Sample Collection



**Australian Government**

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**Department of the Environment and Heritage**

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# Collection of Samples for Submission to a Laboratory

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## Specimen Collection

### i. *Collection of Tissue Samples*

#### *Microbiology*

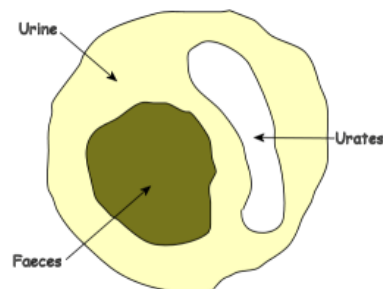
Tissues should be collected using clean forceps and a scalpel. Collect about 5 g of tissue and place it in a sterile 25 ml container, place in a small plastic bag, seal and label the bag using an indelible marker. You may wish to place the bag and seal it in a second bag. Always collect tissue for refrigeration or freezing (bacteriology or virology) as early as possible during the necropsy to avoid contamination by gut contents, feathers or other organic matter. If necessary, collected tissues may be placed in a refrigerator (4-8°C) or freezer (-20°C or colder is best) and maintained at that temperature until shipped to the laboratory.

#### *Histopathology*

Specimens for histopathology should be sufficiently small to allow for adequate fixation. A piece of tissue should generally be no thicker than 5 mm. If there is a lesion, ensure that there is a portion of "normal tissue" adjacent to the lesion, since many diseases are diagnosed based on microscopic examination of the "margin" between a normal and abnormal tissue. If you have not shipped the specimen after 24 hours, change the formalin to ensure that thorough fixation is obtained. Note that in most instances, all tissues from the one bird may be placed in the one container.

### ii. *Collection of faecal samples*

Birds' droppings are a combination of faeces (dark), uric acid crystals or urates (white) and urine (colourless and rarely seen in the field) (Figure 1). The uric acid portion may be dissolved in the urine portion. The consistency and colour of droppings vary with diet, water consumption and species of bird. Blood may arise from the cloaca, digestive or urogenital tracts. Yellow or green-stained uric acid may indicate a liver problem. Undigested seed should be considered abnormal and indicates a primary mal-digestion problem and/or rapid passage of ingesta through the digestive tract.



**Figure 1:** Diagram of a typical avian dropping. The urine is coloured yellow for clarity. Mostly it is colourless and clear.

- Swabs intended to detect the presence of an organism in faeces should be covered with readily visible quantities of faeces.
- A cloacal swab is most likely to detect what is present within a host compared to a fresh faecal sample that is more likely to detect what is present within a host as well as what is present on and around the sampled area.
- When collecting a fresh faecal sample, ensure that it is not contaminated with urates or urine, and that you do not force the swab beneath the faecal material. If for bacterial culture, place the sample (fresh) in a sterile container.
- Worm eggs in faeces may be identifiable up to 7 days after being passed by the bird.
- For motile protozoa, faecal samples must be examined microscopically within 10-15 minutes of being passed.

#### *Faecal smear*

A faecal smear may be made by placing a drop of faeces or a solution of faeces in saline, on to a slide and spreading it thinly. Allow to dry or dry with a hair dryer for shipment to the laboratory. The smear may be fixed by heating gently for a few seconds in the flame of a Bunsen burner or the heat from a hair dryer. Advise the laboratory that you have done this.

### iii. *Collection of blood samples*

- **Anticoagulated blood samples**
  - ▶ Blood samples are collected into different types of tubes according to what test is desired.
  - ▶ Blood must be drawn with a minimum of negative pressure or trauma and be gently mixed (inverted but never shaken) with the anticoagulant. Partially clotted samples are of no value either to you or the laboratory.
  - ▶ Venous blood is preferred since samples are less likely to be clotted or haemolysed.
  - ▶ Test requiring whole blood should be collected in the correct anticoagulant with the correct blood to anticoagulant ratio (1-2 mg EDTA per ml).
  - ▶ Store samples at 4 °C. Air-dried blood smears are stored at room temperature and should never be refrigerated (condensation may destroy cell morphology).
  - ▶ Do not pack the blood in direct contact with dry ice, wet ice, pre-frozen ice-packs or other frozen specimens, even if the container in which you place the blood is insulated.
  - ▶ For serum or plasma samples, time, heat and vibration can haemolyse red blood cells, releasing mainly potassium and lactic dehydrogenase into the serum. After separation from the clot, storage at room temperature and rapid transportation to the laboratory ensure accurate results.
  - ▶ Blood should be collected in the supplied tubes to the “fill line” indicated. If you fill the tube with too much blood, the sample may clot. If filled with too little blood, the erythrocytes may contract due to osmosis. Paediatric tubes are used for collection of avian blood.

Anticoagulants most commonly used are as follows:

**Purple top:** This contains potassium - ethylene diamine tetra acetic acid (EDTA) and is used for complete blood counts and lead estimation. EDTA binds calcium in the blood and so prevents coagulation. Always submit two air-dried and fixed blood smears as well.

**Green top:** This contains lithium heparin, which prevents coagulation by interfering with the conversion of prothrombin to thrombin and the action of thrombin on fibrinogen. Blood for avian CBCs is best collected in heparin.

- **Clotted blood samples**

Serum is preferred to plasma for the majority of biochemical tests, since the anticoagulants used to obtain plasma may either contain or bind with the item being measured.

Blood is collected into a red top (plain) tube that contains no anticoagulant and allowed to clot. For serologic assays, serum should be separated from the clotted blood as soon as possible to prevent deteriorating red blood cells from contaminating the serum. After about 1 hour at room temperature the clot should be gently separated from the side of the tube and allowed to retract. After the clot has retracted (after 2-3 hours at room temperature), centrifuge the sample and transfer the serum using a Pasteur pipette into a clean plain tube. If a centrifuge is not available, wait 4-6 hours, do not agitate the tube, and gently transfer as much serum as possible with a Pasteur pipette, being careful not to transfer blood cells.

If blood is collected in a tube with polymer gel and clot activator (SST - serum separator tube), the clot will form more rapidly.

- **Blood sample on filter paper**

- ▶ Place several drops of blood from each bird directly onto a piece of Whatman No. 3 filter-paper (Brugh and Beard 1980; Park *et al* (1987).
- ▶ Allow the blood-soaked filter-paper to dry in a vertical position at room temperature at 37°C for 2 hours.
- ▶ Keep the sample at room temperature - there is no need to refrigerate.
- ▶ Submit the dried sample to the laboratory for analysis.

iv. ***Collection of Samples for Microbiology (bacteriology or virology)***

- Tissue swabs may be collected for virology, bacteriology or serology. Always observe aseptic technique.
- Always collect samples for microbiology in sterile containers.
- Biopsy material can be aseptically wrapped in sterile gauze soaked in sterile saline and placed in a sterile container.
- Swabs for bacteriology are supplied with transport medium incorporated in the tube into which the swab is placed for transportation to the laboratory. This medium prevents the swab from drying out and preserves the bacteria.
- If a bacteriological sample is collected with a swab, ensure that the swab is inserted into the transport medium at the bottom of the tube into which the swab is inserted. Swabs for bacteriology do not require refrigeration if shipped within 24 hours.
- Viral transport medium needs to be maintained at 4°C until the sample is placed into it. Swabs or tissues for virology need to be frozen until shipped and during shipping. Some viruses cannot withstand freezing, particularly the enveloped RNA viruses. It is better to contact the laboratory before shipping a sample, especially if you know what type of virus you are interested in isolating.
- Always ensure that the swab is within the expiry date period.
- Swabs of the oral or choanal areas should be slightly to moderately moist following

sample collection.

- It is advantageous to submit an air-dried smear, prepared prior to plunging the swab into the transport medium - air-dried smears for microbiology should not be fixed. These smears should be left at room temperature. Smears should be made using another swab so the swab submitted for microbiological investigations is not contaminated from touching the slide.
- The immunoassay for chlamydial antigen requires a conjunctival swab that HAS NOT been placed into transport medium.
- For blood cultures, overnight incubation at 37°C is optimum for growth of any organisms present, but subsequent transportation in an esky (ie with an ice brick) will not harm the organisms, merely inhibit further growth. The organisms grown overnight will be ample for laboratory identification and further investigation.
- Sterile, sealable swabs, not cotton-tipped applicators, should be used for collecting samples for culture, cytology, or PCR-based assay.
- The type of swab chosen will vary based on the type of culture submitted (ie, aerobic or anaerobic, viral, bacterial).
- Swabs containing a liquid transport medium (not gel), should be used for collecting samples for virology. It is better to submit a dry swab for PCR and not place it in liquid.

v. **Blood Collection Sites**

**The blood volume**

The blood volume of birds is 6-12% of body weight. Up to 10% of the blood can safely be collected from a normal healthy bird (ie 1% of its body weight).

SCC	800 g	8.0 ml
galah	360 g	3.6 ml
rosella	200 g	2.0 ml
budgerigar	40 g	0.4 ml

A minimum of 0.5 ml is needed for packed cell volume (PCV), red blood cell count (RBCC) and white blood cell count (WBCC) and differential. In the smallest birds, however, only 2-3 drops can be collected.

One person can restrain a bird (up to 2 kg) for bleeding from the jugular vein. However, considerable training is required to become adept at bleeding from the jugular vein, and this is why most people opt to bleed from the wing vein. An assistant will be needed when bleeding from the wing or medial metatarsal vein. The medial metatarsal vein in some species (penguins, waterfowl, raptors and pigeons) is more reliable than the wing vein.

**Venepuncture technique**

- The choice of site for venepuncture in birds depends on the species, the level of assistance and personal preference. As with other species adequate restraint is the most important factor.
- A fine needle (25 - 30 gauge), depending on the size of the bird, and a tuberculin or 2 mL syringe are best.
- Ensure that the plunger of the syringe is free before you start!
- Drawing back too hard on the plunger of th/e syringe can cause the vein to collapse, only slight negative pressure is required.
- Slightly withdrawing or turning the needle sometimes starts blood flow.
- Squeezing the foot when bleeding from the medial metatarsal vein helps blood flow. Wild birds are generally easier to bleed than tame birds due to the greater blood pressure from stress.

- Do not use heparinised syringes and then transfer blood samples into EDTA as the cell morphology is irreversibly altered.
- *Always be ready to apply pressure to the venepuncture site after removing the needle to minimise haematoma formation.*

### **Jugular vein**

- The right jugular is usually much larger than the left and is located on the right side of the neck adjacent to the trachea. This is great for right handed, but not so good for left-handed, people. The jugular is the vein of choice for collecting large volumes of blood. It is impossible to accidentally stick the carotid. The site is recommended for all birds up to 2 kg body weight, and assistance is usually not needed.
- It is readily seen because most birds (except pigeons and some waterfowl) have a featherless tract in this area. The jugular vein is best not used in birds that lack a featherless tract in this area.
- Wrapping the bird in a towel often prevents the bird from flapping and scratching. However, more control is obtained by using bare hands, but this requires much confidence in restraint of birds.
- Restrain the bird on your lap, with your right elbow leaning gently on the bird's right side.
- Small birds may be held in the left hand, ensuring that the abdomen is not compressed (so as not to compromise respiration).
- Do not apply pressure on the bird's abdomen, since the bird needs the abdomen to breathe. Mild pressure can be applied to the thoracic area, however.
- Separate the feathers on the right side of the neck and expose the jugular vein.
- Stretch the neck between the index finger and thumb because the vein and other structures are very mobile. The vein is occluded with the left thumb, and the neck slightly flexed to the bird's left.
- The feathers of the neck can be flattened with a very small amount of 70% alcohol, allowing the area to be seen without obstructing feathers.
- Do not pluck the feathers from this area as you may tear the skin.
- The vein should be entered with the bevel side up in the direction of the head.
- Left-handed people prefer to enter the vein in the direction of the tail.
- Obtain the desired amount of blood by gently and slowly withdrawing the plunger.
- Apply gentle pressure to the entry site until there is no evidence of bleeding.
- Release the bird's head.

### **Brachial (wing) vein**

- The brachial vein is suitable for all birds but is generally used on larger caged birds . This is the vein of choice for gallinaceous birds and pigeons.
- An assistant restrains the bird on its side on a flat surface, and pulls the lower wing out.
- The vein can be seen on the ventral surface of the humerus (a superficial branch runs across the elbow) directly beneath the skin.
- The vein is very mobile.
- Swab the area with 70% alcohol and introduce the needle into the vein.
- Place pressure over the vein until bleeding stops.
- A subcutaneous haematoma frequently forms. This can be minimized by:
  - ▶ directing the needle distally;
  - ▶ sliding overlying skin to one side during sampling and then allowing it to move back on removal of the needle, prior to applying pressure on needle removal; and
  - ▶ collecting directly into a micro haematocrit tube
- The skin over the vein of small birds may be sterilised with 70% alcohol, allowed to dry, and the vein gently pricked with the tip of a hypodermic needle and blood collected with a capillary tube. Smearing the skin with a small amount of petroleum jelly makes the

blood form a large drop which can be more easily collected.

### **Medial metatarsal (leg) vein**

This is a useful site for larger species including waterfowl, penguins, gallinaceous birds, raptors and pigeons. The vein is located on the medial side of the lower leg running dorso-medially over the tibiotarsal-tarsometatarsal joint and crosses the tibiotarsal joint at its flexor aspect.

The vein is visible in most species although it may be difficult to locate in wrinkled or pigmented skin. Adequate assistance with restraint is important, especially with raptors. The advantages of this technique are that there is a very low risk of haematoma formation and it is useful for rapid multiple collection of smaller samples. Multiple attempts at blood collection will result in tissue thromboplastin contamination of the sample and clot formation. In small birds, the area may be sterilised with 70% alcohol, allowed to dry, and the vein gently pricked with the tip of a hypodermic needle and blood collected with a capillary tube.

### **Blood Smearing technique**

A blood smear is made with blood containing no heparin or EDTA. The slide to slide technique commonly causes cellular rupture, especially of white cells (smudge cells). Using bevel-edged microscope slides will reduce this effect.

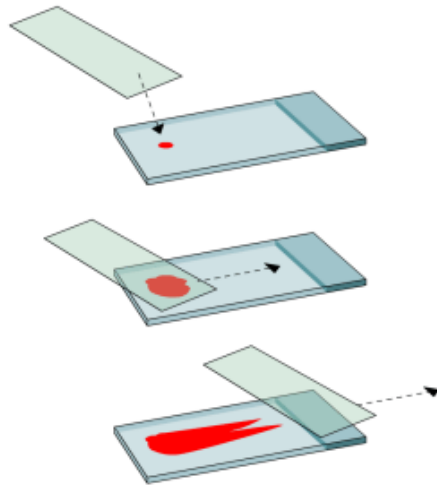
The coverslip to slide (see Figure 2) or coverslip to coverslip methods are considered the most likely method to avoid damage to the fragile avian WBCs and hence will minimize smudge cells.

- Place one drop of fresh unclotted blood in the centre of a microscope slide near the frosted end.
- Gently drop a coverslip (24 mm x 50 mm) on top of the blood at right angles to the slide.
- As blood begins to spread, pull the slide and coverslip apart horizontally.
- Do not allow the blood to spread to the sides of the coverslip before producing the smear.
- Do not pull the coverslip away from the slide's surface when making the smear.
- Rapidly air dry or use an air conditioner or hair dryer gently on low setting and do not refrigerate (condensation will destroy cell morphology).
- Identify each slide with date and the animal's identification.

The coverslip to coverslip technique is similar to the slide to coverslip method.

**Be aware that during transportation, cover slips with smears are more prone to damage than glass slides.**

Blood smears should not be refrigerated and must be kept dry (condensation may destroy cell morphology). They must reach the laboratory within 3-4 days. If fixed with absolute methanol for 5-10 seconds and then dried, they may be kept much longer. Advise the laboratory that the smears have been fixed with absolute methanol.



**Figure 2.** Method of preparing an avian blood smear by the coverslip to slide technique.