

<b>SOP NUMBER:</b>	W4 V2
<b>TITLE:</b>	Blood Sampling Birds (Ulnar or Brachial)
<b>VERSION NUMBER:</b>	V2
<b>PREPARED BY:</b>	Prof. Paul McDonald
<b>REVIEWED BY:</b>	Dr Lauren Hurley
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### Purpose

The purpose of the procedure is to collect sufficient but relatively small amounts of blood (<1% body mass at any one time, and no more than 2% of the body weight in any 14 day period) from live birds for analysis of genetics (e.g. DNA or RNA isolation), circulating hormones (e.g. corticosterone and testosterone), oxidative stress, antibodies, carotenoids, blood cell counts, stable isotope analyses, blood parasites, and other purposes.

### Definitions

N/A

### Precautions: Work Health and Safety

Standard protocols for using sharps should be followed as per required Risk Assessments for field or laboratory work. This includes disposing of used sharps in a sharps container, not re-sheathing needles and following hygiene requirements. Care in the handling of birds, particularly those with the potential to cause harm (e.g. through use of talons, bill) should also be practised and applied for each species (e.g., raptors will grasp with their feet, herons/egrets will lunge at the eyes/face with their bill and so on). Normal Risk Assessment requirements to undertake field or laboratory work should also be followed.

### Training & Competency

The procedure will only be carried out by, or under the direct supervision of, competent investigators who are experienced with handling and blood collection of wild birds. This competence is species-specific. Trainees must work under supervision until deemed competent by an experienced trainer

### Equipment

Needles, blood collection materials as required for sampling protocol, cotton wool, sharps container, Vaseline/ethanol/water for easier viewing of the vein if desired.

#### Substances to be administered, if relevant:

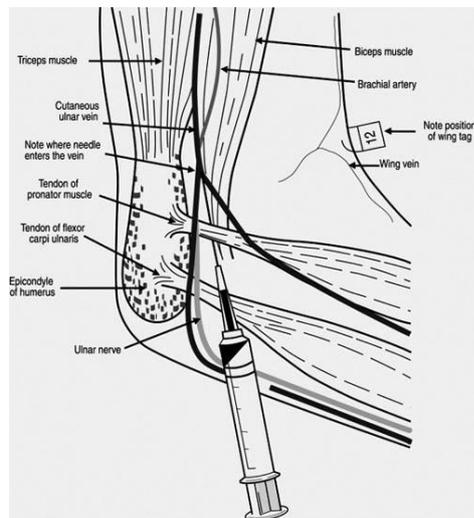
Drug name (generic name, not trade name)	Dose rate (mg/kg body weight)	Route IV/IM/SC/PO	Timing of administration and frequency (eg. 90 minutes pre- operative, to induce anaesthesia, during procedure, at specific intervals during the procedures)	Purpose

### Methods

### General description and methodology

The volume of blood collected should always be the minimum required for intended analyses and must not exceed 1% of body mass for a single sample, or 2% of body mass for any 14 day period, without additional justification and further monitoring (Gaunt et al. 1999; Whitworth et al. 2007). One percent of body mass is equivalent to approximately 10% of the bird's blood volume. This volume is generally considered safe to be taken from wild birds, allowing the birds to be released shortly after the blood sampling without any ill effects (Gaunt et al. 1997; Sheldon et al. 2008, but see Vos et al. 2010). Larger amounts require additional authorisation and are not covered by this SOP. Blood will be collected by puncturing the ulnar or brachial wing veins (fig. 1) with a sterile needle (adjust size for species, but typically 23-26G) and collecting blood in one or more capillary tubes or a microvette. For storage, the blood collected in capillary tubes may be transferred to vials or Whatman cards.

Figure 1



This following is a widely used and recommended procedure for blood sampling birds of all sizes (Gaunt et al. 1997).

1. Birds will be restrained carefully but firmly by hand throughout the procedure so the inner wing is easily accessible with out straining wing from natural range of movement. Throughout the procedure, individuals will be in direct view of the researcher(s) and therefore monitored continuously for signs of injury or dangerous stress (lethargy, heavily laboured or slowed breathing, loss of motor control, loss of consciousness). Once successfully completed, no adverse reactions are expected, so the animal is not further monitored for this procedure but placed in a quiet place (e.g. holding bag) until site has clotted and bird can be released.
2. Blood collection typically takes less than 5 minutes to complete, with a skilled operator often able to complete collection in less than a minute. Birds will only be restrained for the minimum required period, unless excessive bleeding occurs, in which case the birds will be held so that pressure can be applied to the sample site until bleeding has stopped (typically less than 5 minutes).
3. Gentle pressure may be applied to the vein proximal to the site of venipuncture, to prevent the vein from moving as well as allowing it to swell slightly to increase visibility and blood flow through the punctured vein wall. A little vaseline, water, or ethanol can be used to move feathers aside to clearly visualise the vein as needed, being mindful not to wet birds in cold weather.
4. The appropriately-sized needle (usually 23-26G), held with bevelled edge up will be inserted through the skin at an acute angle into the vein, then extracted. This orientation, angle, and minimal insertion into the vein reduces chance of causing a hematoma. Insertion can be parallel or perpendicular to vein. A drop of blood will form on the wound, which will be collected in one or more micro capillary tubes,

microvettes or blood cards. The flow of blood can be manipulated by carefully manipulating the pressure on the vein proximal to the site of venipuncture until the required sample has been collected.

5. After the blood sample has been collected, the pressure on the vein should be removed. If bleeding has stopped on its own (which it will do more readily in some species, but not in others), wing should be released and allowed normal movement before being rechecked to see if movement has restarted bleeding. If required, a small amount of cotton wool is applied to the venipuncture site and light pressure applied for approximately 30 seconds. This can be done by pressing cotton to puncture and refolding wing to body into natural position if a slight bleed (common situation), or by holding cotton and the joint gently together if bleeding is more robust (uncommon). This will prevent further bleeding and the formation of hematoma (Dawson 2004; Whitworth et al. 2007). The sampling site should always be rechecked after some normal movement to ensure bleeding has not restarted, and cotton wool/pressure reapplied as needed if this is the case.
6. Blood will be transferred and stored according to the requirements of reason for sampling. Needles and capillary tubes used in collection will be disposed of in appropriate sharps containers. Needles should not be resheathed as this poses a risk to the operator.

Procedural success will be determined when a sufficient quantity of blood (up to the maximums outlined above) for the intended analyses has been collected, bleeding has stopped, and the animal is ready for release.

#### **Any other protective measures to safeguard the animal's wellbeing**

No additional measures required.

#### **Care of the animal after the procedure**

Wing checked after normal movement to ensure bleeding has not restarted, then released shortly after near location captured.

### **Animal Welfare Considerations**

The procedure should cause only minor, short-term pain at the sampling site and does not affect the flight or survival of the birds.

If injury or signs of significant illness are detected, then urgent veterinary attention should be sought or, if this is not possible, the bird euthanized .

If an animal is showing signs of unusually high stress while in captivity or being handled (heavily laboured or slowed breathing, loss of balance or coordination, severe listlessness or loss of consciousness), such that its potential to be successfully released may be compromised, it will be placed in a cotton bird-bag in a dark and quiet place at an appropriate temperature. These birds will be monitored for up to one hour, then released. If recovery does not occur after one hour, we will seek urgent veterinary attention if possible. Alternatively, the bird will be euthanized.

#### **Pain Relief Measures:**

Pain should be short-term and minor. If injury or signs of significant illness are detected, we will seek urgent veterinary attention if possible. Alternatively, the bird will be euthanized. Under normal procedures, pain relief should be unnecessary.

### **Appendices**

N/A

### **Linked SOPs**

N/A

**References.**

- Dawson A. (2004) Techniques in physiology and genetics. In 'Bird ecology and conservation: A handbook of techniques'. (Eds WJ Sutherland, I Newton and R Green). (Oxford University Press: Oxford U.K.)
- Gaunt, A. S., L. W. Oring, K. P. Able, D. W. Anderson, L. F. Baptista, J. C. Barlow, and J. C. Wingfield, (eds), (1997) Guidelines to the use of wild birds in research. The Ornithological Council, Washington, D.C.
- Sheldon, L. D., E. H. Chin, S. A. Gill, G. Schmaltz, A. E. M. Newman, and K. K. Soma. 2008. Effects of blood collection on wild birds: An update. *Journal of Avian Biology* 39: 369–378.
- Voss, M., D. Shutler, and J. Werner. 2010. A Hard Look at Blood Sampling of Birds. *The Auk* 127: 704–708.
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**Document Control & Record of Revisions**

*Summarise revisions from previous versions*

Version Number	Description of Revision/Amendment	Approved by	Revision Date/Date effective
V2	Three yearly review and updated onto new SOP template	Animal Ethics Committee	30/04/2021