# Charles Darwin University Animal Ethics Committee

## Standard Operating Procedure:

# Collection of blood from wildlife policy (SA DEW)

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| Version No: | Version 1 |
| Date of Approval by the CDU AEC: | 05/03/2025 |
| Date for Review by the CDU AEC: | 05/03/2028 |

NOTE:

- Crocodile blood collection techniques are not referenced in this SOP. If this is to occur, procedures should be outlined in your project application.

WILDLIFE ETHICS COMMITTEE

# Collection of blood from wildlife policy

## Introduction[1]

The purpose of this document is to provide researchers with information that will assist in designing research proposals that use techniques generally approved by the Wildlife Ethics Committee (WEC). These are the guidelines against which your application will be reviewed. This document includes suitable methods of blood collection procedures for a selection of animal species (native and exotic) that could be used in wildlife studies. The emphasis is not on practical detail, as researchers must be able to demonstrate a suitable level of knowledge, training and experience.

The most appropriate methods of blood collection will vary according to the species, individual animal characteristics (e.g. age, sex), the volume of blood that is required, and the requirements of the research. A number of recommended methods are suggested but this does not preclude the use of other techniques, which may be justified on a case-by-case basis. However, if you apply to use methods that are not recommended by the WEC, your application may take longer to assess.

Methods which may have been in common use in the past but are now considered controversial, undesirable or unsatisfactory on an animal welfare basis are mentioned.

## Blood volume [2,3,4]

The recommended maximum volume of blood collected as a single sample is 10% of the circulating blood volume. As a general guide, the circulating blood volume of most animals is approximately 5-10% of the animal's bodyweight. Thus a maximum of 1% of the total body weight is generally the maximum recommended volume of blood to be collected.

Only small amounts of blood are needed for most DNA studies. The following are the maximum allowable volumes for DNA studies[5]. Applicants will need to clearly justify why amounts larger than this are needed.

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| Animals up to 7g | - 1/3 standard capillary tube |
| Animals 7-15g | - 1/2 standard capillary tube |
| Animals over 15g | - 1 standard capillary tube |

(A standard capillary tube is defined as a tube that holds 60 microlitres)

## Three R's – Refine, Reduce, Replace

Blood collection impacts upon the pain and distress experienced by the animal, therefore **refinements** to the methodology must be considered in order to meet the requirements of the *Australian Code of Practice for the care and use of animals for scientific purposes* (2004)[6].

Arrangements should be made to ensure that any surplus materials can be shared with other researchers by lodgement at a suitable Institution (such as the Evolutionary Biology Unit of the South Australian Museum) – this can **reduce** future sampling. Blood sampling should only be an option where other tissues, that can be collected less invasively (e.g. feathers, fur, scale clippings), are not a suitable **replacement**.

These points should be addressed in your application.

**Haemostasis**
In all cases, haemostasis and arrangements for monitoring of bleeding post-procedure must be carefully planned. Pressure should be applied immediately following withdrawal of the needle, or when sufficient blood has been obtained from a needle-prick. Aids to control excessive bleeding should also be readily available (e.g. antiseptic powders, cornstarch or tissue glue). Silver nitrate sticks (styptic pencils) are caustic and should not be used on skin.

**Preparation of the puncture site**
Diluted chlorhexidine solution (1:40) can be used to prepare the puncture site. Alcohol is generally suitable, but as it is an irritant, should not be used on frogs. Geckoes and very small animals may absorb the alcohol through the skin so it must be used with care. Alcohol should be allowed to air-dry, or it may contaminate the sample.

**Unacceptable methods**
The WEC will not give approval for retro-orbital bleeding (with recovery) except under tightly argued and exceptional circumstances, as collection from the orbital sinus may cause haematomas and optic nerve damage.

The use of the footpad for obtaining blood is unacceptable because of the sensitivity of the area and the risk of infection.

## Recommended Species Specific Sites for Blood Collection

## MAMMALS

**Platypus** [7,8]
**Site** The venous sinus of the dorsal bill or the jugular vein.
**Restraint** Anaesthesia is advised to reduce the risk of laceration of the bill.
**Needle** 23-25G needle or butterfly catheter
**Notes** The jugular vein may be difficult to locate due to loose skin. A butterfly catheter can be used as an alternative to a syringe and needle, to reduce the risk of laceration of the bill.

**Echidna** [7,8]
**Site** The venous sinus, or the jugular, cephalic or femoral veins.
**Restraint** Anaesthesia is advised.
**Needle** 23-25G needle or butterfly catheter
**Notes** The venous sinus is smaller than in the platypus

**Kangaroos and Wallabies** [8]

**Site** Veins accessible for venipuncture include the lateral caudal vein, the recurrent tarsal vein, the cephalic vein and the jugular vein. The medial saphenous artery can be used to collect arterial blood samples.

**Restraint** Manual restraint is generally sufficient in small macropods. The head must be covered. Small animals should be enclosed in a bag, and only the blood collection site (e.g. tail) exposed. Anaesthesia should be used when collecting blood from a limb site, because a struggling animal may fracture bones.

**Needle** 20-25G, appropriate to the size of the animal. A butterfly catheter may be used.

**Notes** Many macropods have a short neck which makes blood collection from the jugular vein awkward in conscious animals but is the preferred site for anaesthetised animals. The parotid salivary gland may be inadvertently sampled during venipuncture.

The application of manual pressure on the needle site for up to 5 minutes can prevent haematoma formation. If other procedures are being conducted in addition to the blood sampling, anaesthesia is recommended as the cumulative stresses may induce capture myopathy.

**Large possums and large gliders** [8,9]

**Site** Potential sites for venipuncture include the jugular, cephalic, lateral caudal, ventral caudal, ear and femoral veins and the tibial artery.

**Restraint** Anaesthesia is recommended.

**Needle** 22-26G

**Notes** Possums can inflict painful wounds with their sharp claws and teeth and should be handled with care. Use of forceful restraint causes stress to animals and handlers and should be avoided.

**Pygmy possums and small gliders** [8]

**Site** The tibial artery, and the jugular and lateral caudal veins.

**Restraint** Anaesthesia is advised**.**

**Needle** 29G needle and tuberculin syringe are appropriate for the tibial artery. The lateral caudal vein can be pricked with a 25G needle and the blood collected into a microcapillary tube. The vascular plexus located in the heel of small possums may be pricked with a 26G needle and blood collected.

**Notes** If collecting from the tibial artery, take particular care to minimise haematoma formation by diligent application of pressure following withdrawal of the needle.

**Wombats** [8]

**Site** The femoral, radial, cephalic or median metatarsal vein

**Restraint** Anaesthesia is required.

**Needle** 20-22G

**Notes** The thick skin may hamper visualisation and palpation of blood vessels

**Koala** [8,10]

**Site** The cephalic vein is the most common site for venipuncture in koalas. Larger volumes of blood can be collected from the femoral or jugular veins.

**Restraint** Anaesthesia is required when collecting blood from the femoral or jugular veins.

**Needle** 22-25G

**Notes** For some koalas that are regularly handled, careful collection from the cephalic vein of a conscious animal may be accomplished with little sign of distress.

**Quolls and Tasmanian Devil** [8,11]

**Site** The femoral, cephalic or jugular vein or recurrent tarsal. Smaller volumes of blood may be obtained from the ventral caudal vein.

**Restraint** Tasmanian devils and the four species of quolls require anaesthesia to facilitate collection of blood samples

**Needle** 22-25G

**Small dasyurids** [8,9]

**Site** The lateral caudal, recurrent tarsal or jugular vein.

**Restraint** Anaesthesia is required when collecting blood from the jugular vein

**Needle** 25G needle to prick the vein and collect blood into a microcapillary tube.

**Notes** Collection from the orbital sinus may cause haematomas and optic nerve damage, and is not a recommended method.

**Bilby** [8]

**Site** The lateral caudal and the jugular veins are the best sites for blood collection for bilbies.

**Restraint** Anaesthesia is required for blood collection from the jugular.

**Needle** 22-25G

**Notes** In heavier animals, loose skin over the neck region makes the jugular vein difficult to locate

**Bandicoots** [8]

**Site** The femoral, cephalic, jugular veins and, in species or individuals with a substantial tail, the lateral caudal vein.

**Restraint** Anaesthesia is required for blood collection from the jugular, cephalic or femoral veins.

**Needle** 22-25G

**Bats** [8,12,13]

**Site** In bats larger than 100g, blood can be collected from the median vein or artery. In small bats, less than 100g, blood samples can be taken from the propatagial (cephalic) vein which runs along the leading edge of the patagium. In large microchiropterans, the external jugular vein may be used.

**Restraint** For small species, when collecting blood from the wing or tail veins, it is simple to restrain a bat with two skilled operators. However, venipuncture in all chiropterans is facilitated by anaesthesia.

**Needle** A 25G needle and syringe, or butterfly catheter, is suitable for large bats (over 100g). In small bats, a lance or a 25-27G needle is used to puncture the vein and the blood is collected using a heparinised microcapillary tube.

**Notes** Some anaesthetics block a peripheral vasoconstrictor response and facilitate a more rapid collection of blood. Care must taken to ensure adequate haemostasis as large haematomas can develop which can be life threatening in small bats. Only personnel who have been vaccinated against rabies should handle bats, and all samples should be treated as potentially infected.

**Rats and Mice** [8,14]

**Site** The lateral caudal vein is the site of choice for the blood collection from small rodents. The lateral saphenous vein may also be used. Larger volumes of blood can be collected from the jugular vein.

**Restraint** Anaesthesia is required for blood collection from the jugular.
**Needle** 25G needle and a microcapillary tube.
**Notes** Tail veins can be dilated by warming the tail first in warm water. A tourniquet can be placed at the base of the tail. Collection from the orbital sinus may cause haematomas and optic nerve damage, and is not a recommended method.

**Dolphins** [8]
**Site** Suitable sites for blood collection in dolphins are the dorsal fin vein, the pectoral flipper vein or the tail fluke veins.
**Restraint** Physical restraint is required, although captive animals may be trained to present for blood sampling.
**Needle** 18-20G, 1-1.5 inch needle or butterfly set for adults.
**Notes** For a stranded animal, sampling from the dorsal fin is less risky for the sampler.

**Sea-lion and Fur seals** [8]
**Site** Blood can be collected from the brachial vein or the caudal gluteal vein. The interdigital veins of the hind flipper are also suitable. The external jugular vein may be used as a collection site but can be hard to locate in animals with a thick blubber layer.
**Restraint** Physical restraint is sufficient for small animals, but larger animals should be anaesthetised or sedated.
**Needle** 18-21 G depending on the size of the animal. Where a 20-21 G needle is too short for blood collection (for example, in heavier animals), an 18 G 3.8cm needle may need to be used.

**Rabbit** [8,15]
**Site** Blood can be collected under from the lateral saphenous vein, cephalic or jugular vein. In some breeds with large ears, blood can also be collected from the central ear artery using a 25-27G needle
**Restraint** Anaesthesia is required, unless collecting from the ear, where venipuncture can be performed by wrapping the animal in a towel and covering the eyes
**Needle** 22-27G needles depending on the size of the vein

**Fox and Dingo** [8]
**Site** The cephalic, saphenous and jugular veins are suitable sites.
**Restraint** Anaesthesia is recommended as the use of forceful restraint causes stress to animals and handlers and should be avoided.
**Needle** 21-24 G

**Feral Cat** [8,16]
**Site** Preferred sites include the medial saphenous vein, cephalic vein or jugular vein.
**Restraint** Anaesthesia is recommended as the use of forceful restraint causes stress to animals and handlers and should be avoided.
**Needle** 22-25 G

**Wild Horse** [8]
**Site** Jugular vein. Arterial blood can be collected from the facial artery
**Restraint** Anaesthesia is essential.
**Needle** 19-21 G

**Pig** [8,17]
**Site** The cephalic, saphenous and femoral veins are suitable sites. The external auricular vein or the ventral caudal vein can also be used. The jugular can be difficult to locate.
**Restraint** Anaesthesia is essential.
**Needle** 20-23 G
**Notes** Caution – the jugular vein lies close to the carotid artery just below the angle of the jaw.

**Camel** [8,18]
**Site** Blood can be collected from the jugular vein or the lateral thoracic vein.
**Restraint** Head restraint is a minimum requirement in conditioned animals; wild camels will require anaesthesia or sedation.
**Needle** 19-21G

**Deer** [8,19]
**Site** Blood can be collected from the jugular vein and ventral caudal vein
**Restraint** Sedation or anaesthesia is normally advised.
**Needle** 19-21 G

**Cattle, goats and sheep** [8,20]
**Site** The jugular vein is the most common site for blood collection, but the auricular, ventral caudal, cephalic, and lateral or medial saphenous veins can also be used. Arterial blood can be collected from the auricular and medial tarsal vein.
**Restraint** Sedation is often required, but good physical restraint may be acceptable.
**Needle** 19-22 G

**Birds** [3]
The common sites for blood collection in most species are the right-sided jugular vein, the medial metatarsal vein and the brachial vein. The right-sided jugular is preferred in most species, due to its accessibility and size. The left-sided jugular is not suitable due to its relatively small size.

Feathers should not be plucked to locate the vein as this may tear the skin – dampening the feathers with alcohol solution is sufficient to expose the skin.

Great care must be taken to avoid haematoma and bleeding in very small birds, as the loss of a couple of extra drops of blood can represent a significant proportion of the circulating blood volume and hence prove fatal.

To reduce the risk of haematoma formation:

- Ensure that the bird is carefully restrained so it cannot struggle, causing the vein to tear.
- Use a fine needle (25-27 G).

- Apply gentle pressure with cotton wool to stop bleeding effectively.
- Handle the bird carefully so the clot is not disrupted.
- Monitor the bird for subsequent bleeding.

Toe-clipping must be the last choice for blood collection, after all avenues for venipuncture have been considered. It is suitable only in extremely small birds where blood samples cannot be collected in any other way. Haemostatic agents to control excessive bleeding must be readily available. Silver nitrate sticks or potassium permanganate are suitable for use on claws.

**Wrens, finches, honeyeaters** [22]

**Site** The suitable location for blood collection in most species is the right-sided jugular vein. Smaller amounts can be collected by puncturing the brachial or median metatarsal vein and collecting blood directly into a microcapillary tube

**Restraint** Birds must be restrained adequately if anaesthesia is not used.

**Needle** A 29-30G insulin needle and syringe to collect blood from the jugular; 25-27G needle to puncture the vein and collect blood using a heparinised microcapillary tube

**Penguins**

**Site** The anterior digital vein

**Restraint** Manual restraint with head covered or anaesthesia if blood is to be taken from the jugular vein

**Needle** 27G needle and capillary tube

***Notes*** *Thick plumage and constant movement makes access to the jugular vein very difficult. Warming the foot can raise the veins and increase the blood flow making blood collection easier.*

**Swans, ducks and geese**

**Site** The medial metatarsal or right jugular vein. In larger species, it may be possible to access the superficial veins of the inter-digital skin.

**Restraint** Manual restraint with head covered, or anaesthesia

**Needle** 23-27G appropriate to the size of the bird

**Raptors**

**Site** The medial metatarsal vein is readily accessed in most species. The right jugular vein is also suitable. Smaller amounts of blood can be collected from the ulnar or median veins.

**Restraint** Anaesthesia is recommended, but careful manual restraint may suffice.

**Needle** 23-27G appropriate to the size of the bird

**Emu**

**Site** The medial metatarsal vein is recommended. The ulnar or brachial veins can be used.

**Restraint** Manual restraint in a standing position may suffice, but anaesthesia is recommended for safety reasons.

**Needle** 23-27G appropriate to the age of the bird

**Notes** Care must be taken if restraining the wings, as they are easily fractured.

**Pigeons and doves**

**Site** The ulnar or brachial veins are suitable. The right jugular vein may be suitable.

**Restraint** Manual restraint may suffice.

**Needle** 23-27G appropriate to the size of the bird

**Notes** The jugular may be obscured by engorged skin in reproductively active birds.

**Herons, ibis, stilts, stone-curlews gulls, terns**
**Site** The medial metatarsal vein is readily accessed in most long-legged species. The ulnar, median and right jugular veins are also suitable.
**Restraint** Anaesthesia is recommended, but careful manual restraint may suffice.
**Needle** 23-27G appropriate to the size of the bird

**Pelican and cormorants**
**Site** Branches of the dorsal metatarsal vein are the best sites.
**Restraint** Anaesthesia is recommended, but careful manual restraint may suffice.
**Needle** 23-27G appropriate to the size of the bird
**Notes** Thick plumage makes access to the jugular and brachial veins difficult.

**Cockatoos and parrots**
**Site** The most suitable location for blood collection is the right-sided jugular vein. The medial metatarsal vein can be hard to access due to the short legs. Smaller amounts of blood can be collected by puncturing the brachial or median metatarsal vein and collecting blood directly into a microcapillary tube
**Restraint** Birds must be restrained adequately if anaesthesia is not used.
**Needle** 23-27G appropriate to the size of the bird

## REPTILES [4]

Cardiac sampling is not recommended in tortoises or lizards.

**Freshwater tortoises**
**Site** The right jugular vein is commonly used as in many species this is the larger of the two jugular veins. The dorsal coccygeal vein can be used. The subcarapacial venous sinus is also a useful site.
**Restraint** Sedation may be required.
**Needle** A 23-25G needle is generally suitable. Sampling from the sinus can be done with a 23G 2.5-5cm needle with syringe attached.
**Notes** Haemorrhage is uncommon but pressure at the site is recommended for several seconds after withdrawing the needle. Lymph dilution can often occur when sampling from the tail vein or the subcarapacial venous sinus.

**Lizards**
**Site** The ventral caudal vein is recommended for blood collection in most lizards. For those prone to tail loss, the abdominal vein can be used, but the vein is fragile. Jugular venipuncture is possible in monitor lizards.
**Restraint** Chemical restraint may facilitate blood collection. Sampling from the abdominal vein should be conducted under anaesthetic.
**Needle** A 23-27G needle depending on the size of the animal

**Notes** Holding the animal in a vertical position rather than dorsal recumbency can make the lizard more comfortable.  Alternatively, blood can be taken from the lizard when restrained in ventral recumbency on a table with the tail held over the edge of the table. Toe clipping is not recommended, but if it has been accepted as a form of identification, blood may be collected at this time.

**Snakes**

**Site** The ventral caudal vein is recommended. Cardiocentesis normally produces the best sample in terms of size and quality, and may be suitable in animals over 300g.

**Restraint** Good physical restraint is essential and sedation is recommended if significant cardiac trauma is to be avoided. Anaesthesia is recommended for venomous species.

**Needle** A 23-25G needle is generally suitable

**Notes** In cardiocentesis, repeated insertion of the needle can lead to haemorrhage into the pericardial sac and must be avoided. Digital pressure should be maintained for 30-60 seconds after needle withdrawal. The paired hemipenes may extend 14-16 subcaudal scales down the tail and must be avoided when collecting blood from the tail vein.

**Frogs** [23,24]

**Site** Blood samples from frogs as small as 25g maybe obtained from the lingual venous plexus that lies immediately beneath the tongue. Large frogs can be bled from the ventral abdominal vein which runs subcutaneously over the linea alba. Other sites that can be used include the femoral vein and the heart. Toe clipping is not recommended, but if it has been accepted as a form of identification, blood may be collected at this time.

**Restraint** In the case of an active frog, blood collection will be facilitated by sedating the animal with MS 222.

**Needle** The mouth vein can be punctured with a 26-27G needle and a heparinised microhaematocrit tube can be used to collect the blood that oozes from the vein. For other sites, a 27-30G needle should be used to withdraw blood into a syringe - insulin syringes are ideal.  In very small frogs, including tadpoles, heart blood can be obtained with a small gauge needle (i.e. 28G) and blood can be collected in the hub of the needle with a microhaematocrit tube.

**Notes** Because amphibians have an extensive lymphatic system, blood samples may become diluted with lymph which may affect the cell counts and some biochemical values. Haemostasis can be achieved by applying pressure to the puncture site with a cotton tipped swab. Larger gauge needles are not recommended as laceration of the vein may occur.

It is prudent to have heparinised microhaematocrit tubes available at all times as blood availability maybe limited.

**Fish**[25,26]

**Site** Blood may be collected from the caudal vein in animals over 40g in weight

**Restraint** Fish must be anaesthetised.

**Needle** 22G needle

**Notes** Blood sample size is recommended to be up to 1ml/kg body weight

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