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Purpose

(1) The objective of this standard operating procedure is to provide guidance to the Charles Sturt University staff on:

a. Collection of blood from a bird/chicken

Scope

- (2) This procedure applies to any person who is involved in AEC approved projects involving the collection of blood samples from a bird/chicken.
- (3) All researchers and teaching staff using animals for scientific purposes must be competent. For definition of competency refer to Charles Sturt University's Policy on 'Animal Care Competency Training and Assessment'

Details of procedure

Safety measures:

- (4) Inadequate or inappropriate restraint can result in injuries to a bird. Care must be taken with the wings and legs of chickens when handling and restraining.
- (5) Injuries can be sustained by the operator by a bird kicking (scratching), pecking as well as wing movements. Chickens can carry pathogens and care should be taken to observe appropriate hygiene measures. Wash hands and exposed body parts thoroughly with soap and water after handling. For your own safety, make sure that all OH&S conditions are met.
- (6) At all times a chicken must be handled gently; they have fragile bones, lack a diaphragm and therefore must be able to move their keel bone (sternum) to breathe. When holding a chicken their legs should not be held together but rather the operator should have a finger in between the legs to provide support and prevent injuries.

Pre-collection preparation:

(7) Prior to collection of blood samples, it is to be ensured that all required equipment, including sterile collection tubes, needles, and appropriate personal protective equipment (PPE), are available. A suitable site for blood collection is to be identified, in order to minimize stress on the bird and ensure accurate sample identification with proper labelling and documentation. Suitable collection sites for collecting blood from birds include the wing vein (brachial vein) (Figure 1), medial metatarsal vein (Figure 2) and the jugular vein (Figure 3) (Kelly & Alworth, 2013). These sites are commonly used for blood sampling in birds and selection of a site, in



each instance, is based on accessibility and the species of the bird being sampled.



Figure 1. Brachial wing vein collection (Kelly & Alworth, 2013)



Figure 2. Medial metatarsal vein location (Kelly & Alworth, 2013)





Figure 3. Jugular vein location (Kelly & Alworth, 2013)

Blood collection procedure:

(9) The method for bleeding wings in chickens, Turkeys and adult birds, is similar, with the only variation being the needle size that is used. Chicks, poults, and pet birds are generally small in size, and consequently should be bled using a ½ - ¾ inch needle with a gauge of 20 or 22. For adult chickens and larger sized birds like turkeys, a ½ - 1 inch needle with a gauge of 20 should be used. It is important to note that using smaller gauge needles (22 gauge and smaller) increases the likelihood of haemolysed samples. The maximum amount of blood that should be withdrawn from any bird should not exceed 1% of their total body weight or 10% of their total blood volume.

When collecting blood from small birds like chickens, it is important to hold the bird securely by firmly grasping both legs (with a finger in between) while keeping the wing that won't be bled positioned close to the bird's body. On the other hand, when collecting blood from larger



birds such as turkeys and pheasants, the same method of holding can be applied, but the procedure can be conducted while the bird is placed on the floor.

Pluck a minimal number of feathers over the elbow of the restrained wing to permit visualisation of the wing vein. Syringe plungers may initially stick, so it is recommended to break the seal of the syringe (1 - 5 ml syringe) by pulling back and returning the plunger to the needle hub. The injection site should be disinfected using 70% ethanol.

Remove the needle cap and, with the bevel of the needle facing up, enter the vein through the skin and vein wall in one stroke, pull back gently on the plunger to create a slight vacuum and when a splash of blood is visible in the needle hub, gently pull back further on the plunger to collect blood sample. If no blood is observed, adjust the positioning slightly. When the required volume of blood is collected, remove the needle from the skin and apply gentle pressure to the site.

If a hematoma (a collection of blood under the skin) appears during the blood collection process, the vein being used can no longer be utilized. The other wing must be used if blood collection is required from the same bird. Remove the needle from the syringe and transfer the blood from the syringe into the collection tube.

Used needles should be properly disposed of in a sharps container to ensure safe disposal.

Drugs, chemicals, or biological agents

(10) Pain relief is not usually required for this procedure.

Optional: If needed, the bird can be sedated to calm it down or for ease of restraint, under the direction of a veterinarian, using an intramuscular injection of Tiletamine/Zolazepam (4-10mg/kg).

Impact of procedure on wellbeing of animals

(11) This procedure causes minimal, or no impact on animal well-being unless excessive volume of blood is collected.

Animal care

(12) The use of low stress handling and restraint techniques are necessary for this procedure (SOP-066).

Pain relief

(13) Not required since the process generally causes minimal discomfort.

Reuse and repeated use

(14) Each bird/chicken will be used once.



Qualifications, experience or training necessary to perform this procedure

- (15) Demonstrator: Operators should be trained in the use of correct techniques and should also be knowledgeable about the anatomy of chicken/bird before attempting this procedure.
- (16) Students: Procedures should be clearly demonstrated before students attempt them. If students are required to collect blood sample, the bird must be sedated with intramuscular injection of Tiletamine/Zolazepam before procedure to limit the bird's movement. Students should be aware of the requirements for sterile technique.

Record requirements

(17) None required.

Associated documentation (including pictures if available)

(18) None required.

Glossary

(19) None required.

References and relevant links

(20) Kelly, L. M., & Alworth, L. C. (2013) Techniques for collecting blood from the domestic chicken. Lab Animal, 42(10), 359-361. https://doi.org/10.1038/laban.394