STANDARD OPERATING PROCEDURE

RES005 measurement and sampling of **pest animals** used in research

Prepared by Trudy Sharp, Glen Saunders and Bruce Mitchell,
NSW Department of Primary Industries

Background

Research involving pest animals may require the taking of measurements of and samples from individual animals. Examples include body weight and size measurements, blood, hair and faecal samples.

Measurement and sampling techniques must be appropriate for the species and minimise distress and the risk of injury to the animal. Inappropriate techniques may lead to major and possibly fatal physiological disturbances.

This standard operating procedure (SOP) is a guide only; it does not replace or override the legislation that applies in the relevant State or Territory jurisdiction. The SOP should only be used subject to the applicable legal requirements (including OHS) operating in the relevant jurisdiction.

Application

- This document provides guidelines for research involving pest animals. It aims to ensure that measurement and sampling procedures are performed humanely and effectively.
- The acquisition, care and use of animals for scientific purposes in Australia must be in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and with Commonwealth, State and Territory legislation. All animal research must be approved by an Animal Ethics Committee (AEC) and covered by a valid animal research authority issued by an AEC.
- Personnel handling animals should be thoroughly trained in the planned procedure as well as in contingency methods of capture and restraint that may be required.
- The majority of measurement and sampling methods require that animals be captured and/or restrained. Proper capture techniques are essential to minimise pain, fear, distress and anxiety experienced by the animal and also for the safety of the operator. Where capture or restraint may cause significant pain, injury or anxiety to the animal or pose a danger to the operator, the prior use of sedative and/or immobilising drugs may be necessary. For more information on capture, handling and restraint techniques refer to ***RES001 Live Capture of Pest Animals used in Research*** and ***RES002 Restraint and Handling of Pest Animals used in Research***.

- For many situations physical restraint, rather than chemical will be the most appropriate. Procedures that cause more than momentary or slight distress to the animal should be performed with appropriate sedation, analgesia or anaesthesia.

Animal Welfare Considerations

- To understand and potentially reduce the impact of measurement and sampling procedures on animals, the researchers must have a thorough knowledge of the habits and behaviours of the species under study. For example details of social structure, reaction to stress and pain and defensive capabilities.
- Operators must anticipate and be prepared to deal with the range of conditions that may cause undue stress and/or injury to the animals. If an animal is injured during measuring or sampling procedures it must receive appropriate treatment. Animals that are suffering intractable pain and/or distress should be euthanased using a technique that is suitable for the species. For more information on euthanasia techniques refer to ***GEN001 Methods of Euthanasia.***
- Precautions must be taken to prevent the spread of infectious disease from one animal to another. Contaminated equipment should be disinfected between animals.
- The potential welfare implications of body measurement and sampling include:
  - Pain and injury from handling and inappropriate use of sampling/measuring equipment or techniques;
  - Pain from biopsy of skin or other tissues;
  - Entrapment of animals in hair tubes resulting in predation or dehydration;
  - Sampling of excessive amounts of hair, resulting in hypothermia/sunburn;
  - Sampling of excessive amounts of blood, resulting in hypovolaemia, weakness or shock; and
  - Infection at blood sampling or biopsy sites.
- Measurement and sampling procedures must be performed by persons competent in the methods to be used. Some methods require considerable training and experience to be used appropriately.
- The methods should be quick, result in the loss of minimal tissue and not result in infections or loss of function.

Health and Safety Considerations

Animal handling

- Operators need to be wary of the potential for serious injury when handling wild animals. Some species can be aggressive and may attack e.g. feral pigs.
- When working in the field, personnel should work in teams of at least two people.
- Protective clothing, footwear and gloves may reduce the chances of injury when handling wild animals. However, the use of heavy gloves decreases sensitivity and dexterity and may increase the risk of handling injuries to small species.

Chemical restraint

- Drugs used for chemical restraint are potentially toxic to humans and should be used with appropriate safety precautions. Operators should be familiar with the specific risks associated with each agent being used.

Zoonotic hazards

- Care must be taken when handling live animals and carcasses as they may carry diseases that can affect humans and other animals e.g. hydatidosis, sarcoptic mange, leptospirosis, Q fever, brucellosis, melioidosis, tuberculosis, psittacosis (chlamydiosis/chlamydophilosis) etc.
- Routinely wash hands and other skin surfaces after handling all animals and also carcasses or bodily fluids.
- Operators must be protected by tetanus immunisation in case of infection of wounds.
- Bite wounds from some animals (e.g. feral cats, foxes, wild dogs) can result in serious infections and should be treated by a doctor.
- Q fever can be transmitted to humans during contact with infected animals, or with infected uterine or placental tissue. A variety of animals may be infected including kangaroos, wallabies, dogs, cats, cattle, sheep and goats. Vaccination is recommended for people who come into regular contact with potentially infected animals. Blood testing of personnel is recommended to assess previous exposure, followed by vaccination for susceptible individuals.
- Zoonotic risks from birds include psittacosis (chlamydiosis), aspergillosis, erysipelas, yersiniosis and salmonellosis. Face masks, are recommended to reduce the risk of contracting disease.
- Some bird species can deliver painful bites and scratches. For example, parrots (e.g. cockatoos, galahs, corellas) have large, heavy beaks and strong jaws that are capable of inflicting serious injury. Protective gloves can be used if required for handling large birds, although these may hinder dexterity. A towel is useful to place over the birds head.

Methods of Measurement and Sampling

- **Body weight**
  - Small animals can be weighed by placing in a bag and attaching it to a spring balance. The dark environment of the bag will calm most animals. Also, the weight of an animal caught in a cage trap can be estimated after subtracting the weight of the trap.
  - Larger animals restrained on a restraint board may be weighed by weighing the board with the animal attached.
  - Weighing animals in a body sling will usually require sedation or anaesthesia.
  - An electronic scale incorporated into a race is the most suitable method for large animals such as goats and horses.

- **Body condition**

  Condition may be assessed by palpation of various muscle masses or fat storage sites, depending on species. The pectoral muscles in birds, the lumbar and tail base fat storage areas in ruminants and macropods and the deltoid and temporalis muscles in smaller animals all give an indication of body condition, especially when combined with body weight.

- **Body temperature, heart rate and respiratory rate**

  Body temperature, heart rate and respiratory rate may be significantly altered due to the stress of capture and handling. The use of sedatives and anaesthetics will also affect these parameters.

- **Hair sampling**

  A small number of hairs may be sampled by a hairtube or snare without requiring capture of the animal. If hair samples are required from captured animals, these may be taken using scissors. Plucking should only be used where the hair follicle is required.

  - *Hair tubes*
    - A hairtube is a plastic or metal tube lined with an adhesive material designed to hold hairs whilst allowing the animal to pass through the tube unharmed. Bait is used to attract animals into the tube.
    - Tubes should be set out in a similar fashion to live capture traps (e.g. in a grid formation, 5 × 5 or 4 × 20 with 10 m intervals between traps).
    - Tubes must be positioned at an angle so that water drains out and small animals cannot drown inside. They should be secured with pegs or u-shaped hooks driven into the ground to prevent them from being moved by animals.
    - The floor of the tube must be free of adhesive material to prevent small animals from becoming trapped.
    - The location of all hair tubes must be identified and recorded.
    - A small amount of bait is placed inside the tube so that it cannot be removed.
    - All tubes should be checked regularly in case any animals have become trapped.
  - *Hair snares*
    - Hair snares snag or catch hairs from a passing animal. An example is a carpet square attached to a tree and sprayed with an attractant.
    - Researchers should use methods which do not have sharp points or edges which could cause injury to animals.
    - Snares may be used for medium to large animals and are custom made to suit the target species (see Foran *et al.* 1997; Woods *et al.* 1999; and McDaniel *et al.* for examples). Lures and bait similar to those used for live capture may be used.
    - Snares must be securely positioned to prevent them from being moved by animals and to ensure adequate hair removal.
    - The location of all hair snares should be recorded and marked with flagging tape and a sequential numbering system.
    - Snares should be rechecked after approximately 10 days.

- **Faecal sampling**
  - Faeces may be sampled from the ground without having to capture the animal. Disposable gloves or plastic bags should be used and faeces should never be handled with bare hands.
  - However, where fresh samples are required directly from the animal (either to avoid contamination of the sample or to relate the sample to an individual), sampling per rectum may be necessary.
  - Rectal swabs are obtained by passing the swab through the anus into the rectum and then removing it.
  - Faecal samples may be obtained from larger species (goats, pigs, large macropods) by passing a lubricated, latex/polythene gloved hand through the anus into the distal rectum, grasping a handful of faeces then removing the hand. For smaller species a finger is used. Note that macropods have a single cloaca.
- **Blood sampling**
  - Blood should not be collected from sick, dehydrated or heat/cold stressed animals except where it is required and approved as part of the research protocol.
  - Blood samples are collected from a suitable vein using a hypodermic needle and either a syringe or an evacuated blood tube. The gauge of needle used should be the narrowest consistent with the volume and speed of collection required. Blood collected through a narrower needle is also more prone to haemolysis.
  - The most appropriate vein to use will vary between species. In mammals, the common sites for blood collection are:

    Cephalic vein – runs over the anterior (front) of the forearm;
    Saphenous vein – runs along the inside of the lower hind leg
    Jugular vein – in the neck; and
    Coccygeal veins – ventral and lateral veins located in the tail.

    In birds, the wing vein (also called the brachial vein or cutaneous ulnar vein) and the medial metatarsal vein are commonly used.
  - Physical restraint is usually adequate for most species. However, sedation or anaesthesia may be required for some species or individuals, especially where veins are small or where venipuncture causes significant pain or distress.
  - Needles and syringes/tubes must be sterile and must not be used for taking more than one sample.
  - Where the site is covered with hair, this should be clipped although the skin of some species, such as rabbits, is thin and prone to clipper damage.
  - The site should be swabbed with alcohol, chlorhexidine, povidone iodine or a similar disinfecting agent
  - With the exception of vena cava sampling in piglets, the vein must be visualised. Visibility is improved by impeding blood flow between the sampling site and the heart by applying a tourniquet or digital pressure, e.g. around the elbow for cephalic vein sampling or at the base of the jugular furrow at the thoracic inlet for jugular sampling.
  - In cold conditions, warming the sampling site (e.g. with a lamp or hot water bottle) may be required to enable venipuncture.

- The needle (attached to the syringe or tube holder) is pushed through the skin and into the vein and gentle suction applied with the syringe or the tube pushed onto the needle to aspirate the blood. If venipuncture is not achieved, the needle is pulled back *without exiting the skin*, and venipuncture reattempted.
- The required amount of blood is sampled. The volume of blood collected at a single sampling should not exceed 10% of the circulating blood volume of the animal. This is approximately 7–8 ml/kg bodyweight in mammals, depending on species.
- If a second tube of blood is required, the first is removed, leaving the needle in the vein and the second tube pushed onto the needle. After blood is collected, the needle is withdrawn and pressure applied to the site for 30–60 seconds to prevent haemorrhage or haematoma formation. This should be extended to 90 seconds for birds. The animal must only be released after any bleeding has stopped.
- More information on blood collection techniques can be found in Rose (2005) Wildlife Health Investigation Manual, Chapter 4.

- **Collection of Tissue Samples**
  - Tissue samples from live animals are usually in the form of punch biopsies for skin samples and needle biopsies for sampling of internal tissues.
  - Physical restraint is usually adequate for most species. However, sedation or anaesthesia may be required for some species or individuals, especially where the length of restraint or the procedure may cause significant distress.
  - *Punch Biopsies*
    - Select a biopsy site that is suitable for the sample required, is in a location that is unlikely to become infected, is unlikely to cause significant haemorrhage (e.g. away from major blood vessels) and will cause little subsequent pain and discomfort. For example, sites which are likely to cause lasting pain and discomfort include the digital pads and skin closely overlying bone.
    - Where the site is covered with hair, this should be clipped and preferably shaved although the skin of some species, such as rabbits, is thin and prone to clipper and razor damage.
    - Local anaesthetic (e.g. lignocaine) should be injected subcutaneously at the site (1–2 mls through a 25 or narrower gauge needle is usually adequate). The drug should be allowed to take effect over a couple of minutes.
    - The site should be swabbed with alcohol, chlorhexidine, povidone iodine or a similar disinfecting agent.
    - A sterile punch should be used for each sample.
    - The biopsy punch is pressed firmly into the skin and rotated in one direction until a sufficient depth has been reached (i.e. base of the dermis). The punch is removed. If the required tissue is captured in the punch, remove with sterile forceps. If the tissue remains attached to the subdermal tissue, the sample is lifted with sterile forceps and freed from the underlying tissue with sterile scissors or scalpel blade.
    - For larger biopsies (> 5 mm) the defect should be closed with a single suture using absorbable suture material or tissue adhesive (e.g. Vetbond, 3M)
  - *Needle biopsies*
    - Needle biopsies (aspiration or core biopsies) require specialised knowledge of the anatomy of the species under study. Anaesthesia and analgesia are usually required and strict asepsis is essential. These techniques should only be carried out by trained personnel.

Recommended Methods of Measurement and Sampling for a Range of Species

Species	Body Weight and Condition	Faeces	Blood	Comments
Rabbits	Bag	Ground/Swab	Marginal ear vein, Jugular	Blood: Leave the rabbit in the bag and expose the ears while keeping the eyes covered. Cold conditions may require local heat to aid venipuncture and blood flow. Marginal vein: Sample at $^1/_3$ of the ear length from the base of the ear.
Foxes	Bag	Ground/Swab	Cephalic, jugular	
Pigs	Race scales	Ground/Swab Per rectum	Small: Cranial Vena Cava Large: Jugular/Ear	
Dogs	Sling	Ground/Swab	Cephalic, jugular	
Cat	Bag	Ground/Swab	Cephalic, jugular	Cephalic suitable only for very small volumes (< 1 ml)
Goats	Race scales	Ground/Swab Per rectum	Jugular	Blindfold may calm goats in race
Deer	Race scales/sling	Ground/Swab Per rectum	Coccygeal	
Birds	Bag	Swab	Ulnar (wing vein or brachial vein) Medial metatarsal vein Jugular	This vein runs over the medial surface of the elbow. Haematoma formation is likely when using ulnar vein. This vein runs alongside the inside of the leg, and is usually most visible near the hock (ankle). The right jugular vein is much larger than the left in birds
Rodents	Bag	Ground/Swab	Coccygeal, saphenous	Anaesthesia recommended for blood sampling. A fine needle and 1 ml syringe or a lancet (to nick the vein) and capillary tube required Local heat may be needed to increase blood flow to facilitate blood collection from the tail vein. A tourniquet is useful to raise the vein of larger rodents.
Horses	Race scales	Ground/Swab Per rectum	Jugular	
Macropods	Bag	Ground/Swab	Cephalic, Jugular, Coccygeal	Cephalic suitable only for very small volumes (< 1 ml)
Cane toads	Bag	Swab		

References

American Society of Mammalogists (1998) Guidelines for the capture, handling and care of mammals. Animal Care and Use Committee, American Society of Mammalogists. Document available electronically from the American Society of Mammalogists website: http://www.mammalsociety.org/committees/commanimalcareuse/98acucguidelines.PDF

Anon. (2003) *The Australian Immunisation Handbook*. 8th Edition. National Health and Medical Research Council, Canberra.

Anon. (2004) Sea Turtle Life History Form and Manual. Appendix D: Biopsy Sampling Protocols. Southeast Fisheries Science Center. Document available electronically from the SEFC website: http://www.sefsc.noaa.gov/seaturtlefisheriesobservers.jsp

Brunner, H., Triggs, B., and Ecobyte Pty, L. (2002) Hair ID: an interactive tool for identifying Australian mammalian hair. CD-ROM, CSIRO Publishing, Collingwood.

Canadian Council on Animal Care (2003) *Guidelines on: the care and use of wildlife*. Canadian Council on Animal Care, Ottawa, Canada.

Carlton, C. (ed) (2001) Community Biodiversity Survey Manual. 2nd Edition. National Parks Association of NSW Inc and NSW National Parks and Wildlife Service, Sydney.

Clark, P., Holz, P., and Duignan, P.J. (2004) Collection and handling of blood samples. In *Haematology of Australian Mammals* . Clark, P. (ed). CSIRO Publishing, Collingwood, Victoria: pp. 1–20.

Foran, D.R., Minta, S.C., and Heinemeyer, K.S. (1997) DNA-based analysis of hair to identify species and individuals for population research and monitoring. *Wildlife Society Bulletin* **25**: 840–847.

Friend, M., Toweill, D.E., Brownell, R.L.Jr., Nettles, V.F., Davis, D.S., and Foreyt, W.J. (1999) Guidelines for the proper care and use of wildlife in field research. In *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds*. Friend, M. and Franson, J.C. (eds). U.S. Department of the Interior, U.S. Geological Survey: pp. 53–71.

Harden, B. and Paul, A. (2004) Wildlife Surveys. Animal Welfare Unit, NSW Department of Primary Industries. Document available electronically from the Animal Ethics Infolink website:
http://www.animalethics.org.au/reader/whatsnew/wildlife-surveys.htm

Jackson, S.M. (2003) *Australian mammals: biology and captive management*. CSIRO Publishing, Collingwood, Victoria.

Karesh, W.B., Smith, F., and Frazier-Taylor, H. (1987) A remote method for obtaining skin biopsy samples. *Conservation Biology* **1**: 261–262.

Lindenmayer, D.B., Incoll, R.D., Cunningham, R.B., Pope, M.L., Donnelly, C.F., MacGregor, C.I., Tribolet, C., and Triggs, B.E. (1999) Comparison of hairtube types for the detection of mammals. *Wildlife Research* **26**: 745–753.

Lowe, K.W. (1989) The Australian bird bander's manual. Australian National Parks and Wildlife Service, Canberra.

McDaniel, G.W., McKelvey, K.S., Aquires, J.R., and Ruggiero, L.F. (2000) Efficay of lures and hair snares to detect lynx. *Wildlife Society Bulletin* **28**: 119–123.

Mills, D.J., Harris, B., Claridge, A.W., and Barry, S.C. (2002) Efficacy of hair-sampling techniques for the detection of medium-sized terrestrial mammal. I. A comparison between hair-funnels, hair-tubes and indirect signs. *Wildlife Research* **29**: 379–387.

National Health and Medical Research Council (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. 7th edition. Australian Government Publishing Service, Canberra.

Noonan, D. (2000) Blood collection guidelines. Animal Ethics Office Monash University, Melbourne. Document available electronically from the Monash University website: http://www.monash.edu.au/resgrant/animal-ethics/guidpol/bloodcol.html

Rose, (2005). *Wildlife Health Investigation Manual*. Zoological Parks Board of New South Wales, Mosman.

Sharp, T. and Saunders, G. (2004) GEN001 Methods of euthanasia. NSW Department of Primary Industries and Department of Environment and Heritage. Document available electronically from the DEH website:
http://www.deh.gov.au/biodiversity/invasive/publications/humane-control/

Woods, J.G., Paetkau, D., Lewis, D., McLellan, B.N., Proctor, M., and Strobeck, C. (1999) Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin* **27**: 616–627.

Natural Heritage Trust
Helping Communities Helping Australia
A Commonwealth Government Initiative

NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Disclaimer