

ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice, and allow for the flexibility that is required in research and teaching activities using animals.

SURGERY AND ASEPTIC TECHNIQUE

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and approved by the University of Melbourne's Animal Ethics Committees.

V2 Date of Approval:	
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V2 Date of Review:	
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1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Analgesia
- General anaesthesia of mice and rats
- Blood collection in mice and rats

2. SUMMARY

Aseptic surgery involves the use of techniques and instruments to prevent harmful microbial and postoperative complications following incision or closure of live body tissues. Blood collection and clipping methods must follow the requirements of their specific standards and not the practices outlined below.

When an animal is to recover from an anaesthetic, surgical procedures must conform to accepted standards in medical or veterinary practice.

3. BENEFITS & RISKS

3.1 Post-operative infections in animals can and do occur. Such infections, which may not be apparent on casual observation, can cause pain and distress to the animal and may impact on research outcomes. Other complications from surgery requiring mitigation measures include hypothermia, dehydration, blood loss, tissue trauma, metabolic disturbances, poor tissue perfusion, cardiovascular and/or respiratory failure, delayed wound healing and impaired function.

3.2 The use of proper surgical technique will, among other things, minimise microbial contamination and tissue trauma and reduce the risk of post-surgical pain and distress.

4. PROCEDURE/PROTOCOL

4.1 Training

- 4.1.1 Surgery must be performed only by investigators with appropriate training and experience and who are approved as competent. Training for recovery surgeries should include an understanding of relevant anatomy, methods used for incising, tissue closure techniques, blunt dissection, gentle tissue handling, haemostasis, wound care, complications and monitoring. Training in surgical techniques must be under the direct and constant supervision of competent trainers.

- 4.1.2 Initial training of investigators should cover generic techniques of asepsis and surgery. All new investigators must undergo formal surgery and anaesthetic training and undertake practical training with the Animal Welfare Officer (AWO) or their delegates. Where a delegate is the trainer they must have completed the appropriate training within the last two years and they should have been observed delivering training to an appropriate standard as determined by the AWO or their delegate(s).
- 4.1.3 After completion of initial training, investigators must undergo further training of techniques specific to their research project. Trainers should provide the content, format and/or competency assessment sheets to the AWO prior to commencement of training. An acceptable skill level must be demonstrated on more than two consecutive occasions before investigators are permitted to perform surgery without supervision.
- 4.2 Surgical facilities, equipment and therapeutics
 - 4.2.1 Surgical procedures must be carried out under appropriate local or general anaesthesia with appropriate analgesia.
 - 4.2.2 When aseptic technique can be maintained in immunocompetent animals, antibiotics can be avoided. If antibiotics are required, perioperative use should be extended for use after the perioperative period. All antibiotics must be prescribed by a veterinarian and usage of antibiotics should be discussed with the AWO or Bioresources Veterinarian before submitting an animal ethics application. Antibiotics should not be considered a substitute for poor aseptic technique.
 - 4.2.4 A dedicated surgical area is required, and can be any room or portion of a room that is easily cleaned and disinfected and away from general traffic. The surgical area should also be positioned to avoid air flow from contaminated areas and the room/area should not be used for other purposes during the time of surgery. The surgical area should be clean and uncluttered, and large enough to allow room for the investigators and their assistant to work without impediment with the necessary equipment (eg. anaesthetic machine, animal, surgical pack, surgical light). Prior to surgery, the surgical area should be cleaned and disinfected.
 - 4.2.5 Animal preparation should take place in a different location to surgery to avoid contamination of surgical sites with hair or feathers and debris. Ideally, this is a separate room or antechamber to the surgical room, but in some circumstances can be performed in a portion of the room that is well separated from the surgery area, for example; a different bench.
 - 4.2.6 Sterilised instruments and materials must be used for survival surgery in all animals where sterile sites (e.g. body cavities, blood vessels or the skin) will be penetrated. Scissors and scalpels must be sharp for effective use. Any implants or single use material should be in sterile packaging. Any implants, catheters and equipment (such as needles, drill bits, etc.) that are reused must be cleaned and sterilised before use and sharp enough for the purpose for which they are intended. The preferred methods of sterilising instruments and materials are autoclaving, dry heat or gas vapour. An indicator strip is to be used to assure appropriate temperature has been achieved.
 - 4.2.7 Braided suture material should not be used for external skin sutures. Silk and catgut cause the most tissue inflammation of all the sutures and should not be used. Intradermal sutures are preferred in areas where animals can chew. The size of the suture should be the smallest diameter that will adequately counteract static and dynamic tension forces on the tissue. Things to consider are the holding power, time it takes for the tissue to heal (gut/bladder are faster than skin) and the size of the animal/tissue (cow skin vs mouse skin). Inappropriate suture selection (type and size) can cause increased inflammation and infection and increase the likelihood of wound breakdown and animals attempting to remove the sutures.
 - 4.2.8 Sterile surgical instruments should not be re-used on more than one occasion per animal prior to re-sterilisation. It is important instruments are cleaned before being disinfected and then sterilised. Organic matter, such as blood, should be washed off with disinfectant prior to sterilisation. Alternatively, sterile water or saline can be used to remove debris. If instruments must be used again prior to a full sterilization, they should be disinfected either by chemical or physical methods, with care taken to ensure any hot instruments have cooled sufficiently prior to next use. Even with the use of these methods between animals, a new sterile instrument pack must be used after every 5 major surgical procedures.

4.3 Preparation of animal and investigator

- 4.3.1 A physical examination and health assessment of the animal is performed before surgery to ensure no unexpected underlying illness.
- 4.3.2 Prepare the animal away from the surgical area by removing hair from the surgical site using clippers or depilatory cream (less than 3 minutes). If working with avian species, feathers must be removed from the surgery site, with care taken not to remove pin/blood feathers. To remove clippings and other organic debris, clean the skin using 4% chlorhexidine in a scrub solution, alternated with plain 80% ethanol, repeated at least 3 times. The surgical clean is then performed with 2% chlorhexidine (in 70% isopropyl alcohol) in a gradually enlarging circular pattern from the centre of the site to the periphery. Do not bring the swab back from the periphery to the clean central area. Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal.
- 4.3.3 Hypothermia prolongs recovery time, increases infection rate and can be fatal. To prevent hypothermia, provide an insulating layer between the animal and the surgical surface or bench, provide an external heat source, and use warmed fluids during the procedure.
- 4.3.4 The investigator must wear clean protective covering (eg. gown, lab coat) and hair must be covered or tied back. Hands must be scrubbed using a detergent based antiseptic such as Chlorhexidine scrub, iodine scrub or a water-free surgical hand scrub. This should be applied in a methodical manner working from finger tips to elbows. The entire scrub procedure needs to last at least 5 minutes to ensure sufficient contact time. After chlorhexidine or iodine scrubbing, hands are rinsed off, then dried with a sterile towel. Sterile gloves must be worn for all surgical procedures. Care is required to ensure gloves only come in contact with other components of the sterile field (drape, instruments, surgical site) during surgery.
- 4.3.5 If an assistant is present who can anaesthetise the animals and prepare them for surgery, it may be possible for the investigator to continue from animal to animal without re scrubbing, provided they only touch sterile areas and that a new pair of sterile gloves are used for each animal. If not, the investigator should keep their gloves on while preparing the next animal and then change the gloves for a new pair when the animal is ready. Likewise, if wearing a sterile gown, it may be worn between animals provided it is not obviously contaminated or compromised.

4.4 Surgical procedures

- 4.4.1 The surgical field must be kept sterile throughout the procedure. Surgical gloves, sterile instruments and materials must only come in contact with sterile surfaces during the procedure. In most cases, the use of sterile drapes is required for maintenance of the sterile field. The opening of the drape must not be larger than the shaved and disinfected surgical area. Clear drapes allow monitoring of respiration.
- 4.4.2 Antiseptics can delay healing and should not be placed in open wounds or surgical sites. If a wound or surgical site is suspected to be contaminated peri-operatively it can be irrigated with warmed sterile saline, taking care not to wet the whole animal or an excessively large area which may lead to hypothermia.
- 4.4.3 When more than one surgical procedure is to be performed on an individual animal, the time between each procedure must allow a recovery to good general health unless otherwise justified. Assessment and justification of the cumulative burden of multiple recoverable procedures must be provided to the AEC.
- 4.4.4 Where potential complications may require further surgical procedures such as re-suturing, the AWO/AEC must be contacted prior to the procedure. The AWO/AEC must also be contacted prior to any medical treatment or other similar procedures, including the application of products (i.e. bandages, creams or disinfectants) to surgery sites, that are not listed in the ethics application. The only exception to performing any procedures prior to AWO/AEC notification is during emergency care or treatment.

4.5 Post-operative care

- 4.5.1 Animals must be placed in clean cages after surgery and with an appropriate bedding substrate that can prevent collection of debris on the surgical site.
- 4.5.2 The comfort of animals must be promoted throughout the post-operative period. Attention should be given to warmth, hygiene, fluid and food intake, and control of infection. The use of analgesic and tranquillising agents

may be needed to minimise post-operative pain or distress. Care should be taken to ensure that animals recovering from anaesthesia do not injure themselves by uncoordinated movements, and recover in conditions that they are not disturbed, attacked or injured by other animals in the same enclosure.

- 4.5.3 Clinical records of an animal's state must be kept, including observations and administration of any drugs, fluids or other treatments. These must be made accessible to all investigators or animal care staff involved in the post-operative care of the animal.
 - 4.5.4 Investigators must ensure that adequate monitoring, treatment and care of post-operative animals is provided and all animal care staff (investigators or facility staff) are fully informed of each animal's state.
 - 4.5.5 The duties of all investigators must be clearly defined and procedures must be established for identifying and responding to post-operative emergencies, including management of pain and distress.
 - 4.5.6 Any post-operative animal observed to be in a state of severe pain or distress, which cannot be alleviated quickly, must be euthanased without delay.
 - 4.5.7 Hypothermia should be prevented by placing the animals in a warm room or cage. If necessary, the cage may be supplied with supplemental heat as required though caution should be taken to avoid over-heating. Cages can be placed halfway onto heating mats and heating mats or items should never directly contact the skin of any animal. Other methods for other species can be used in consultation with the AWO or Bioresources Veterinarian.
 - 4.5.8 Do not place the animal directly on bedding material until fully awake to prevent aspiration of bedding. Aspiration can occur in both small and large animals and particular care should be taken with fine particle bedding material. For rodents, a tissue can be used on top of the bedding during the recovery period for this purpose. For larger animals, straw or a rubber mat may be a better option. Animals should not be returned to the animal holding rooms until they are stable and able to assume a normal posture.
 - 4.5.9 Dehydration can be ameliorated by the administration of appropriate fluid therapy. In rodents, initially this may be done by giving 3 to 4 ml of warm sterile fluids (0.9% NaCl or Lactated Ringer's) per 100 gm of body weight by subcutaneous injection. Moist food or gel packs can also assist with hydration post-surgery. In larger species, perioperative fluids should be administered at a rate appropriate to the size of the animal via an intravenous catheter. This may be between 2,5-10mls/kg/hr and the AWO should be contacted to determine an appropriate fluid rate.
 - 4.5.10 Post-operative pain or distress must be monitored and treated. If the currently approved protocol does not seem to be appropriate, immediately contact the AWO for further advice. Changes in the protocols are not to be undertaken without prior consultation with the AWO unless it is an urgent or emergency event.
 - 4.5.11 Investigators should be familiar with the signs of pain and distress for the species they are working with, and recognise most research animals are prey or herd animals that do not show overt signs of pain. There are comprehensive studies and pain scales available for rodents, small animals, and large animals.
 - 4.5.12 External wound clips and sutures should be removed 10-14 days after the surgery.
- 4.6 Non-survival surgeries
 - 4.6.1 While it is not necessary to follow aseptic technique when performing non-survival surgeries, at a minimum the surgical site should be clipped and the investigators should wear clean gloves, use clean instruments and clean the incision site.

5. MONITORING & INTERVENTION

- 5.1 The animal must be monitored closely during the surgical procedure. Investigators should pay close attention to the animal's level of anaesthesia. A second investigator or staff member should be present to monitor anaesthesia.
- 5.2 During the immediate postoperative period, the animal should be observed continuously until it has regained the righting reflex and is able to move around the cage or enclosure with ease.
- 5.3 Post-operative monitoring records must be kept in the room where the animals are housed. Important information to include in the post-operative record is the animal's identification, observation date, surgical procedure summary, signs of pain/health and any therapeutics given including drugs, doses, and routes of administration.
- 5.4 Assessment of the surgical site and post-operative pain must be performed at least twice daily for the first 72 hours after surgery unless a different monitoring schedule has been approved by the AEC in the animal ethics application.

6. ADDITIONAL INFORMATION

- 6.1 Additional training in surgery and anaesthetics is available by contacting the AWO or the Melbourne Bioresources Platform Veterinarian.

7. ENFORCEABLE REQUIREMENTS

- 7.1 Performance of the procedure by competent investigators or trainees under the direct supervision of approved competent investigators.
- 7.2 Use of sterilised instruments, drapes and gloves.
- 7.3 Hair/feather removal and patient preparation using disinfectants as described above.
- 7.4 Ensuring aseptic technique where gloves and instruments only contact sterile surfaces during sterile procedures.
- 7.5 Disinfection of equipment between surgeries.
- 7.6 Weighing the animal and scoring body condition prior to surgery and daily for the first 3 days post-surgery. Monitoring the animal twice daily for the first 3 days post-surgery.
- 7.7 Competency must be demonstrated on two occasions before investigators are permitted to perform surgery without supervision.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the AWO.

9. UNEXPECTED ADVERSE EVENTS

An unexpected adverse event is any event which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

In addition, a necropsy must be performed on any animal whose illness or death constitutes an unexpected adverse event. The body of an animal found deceased, or humanely killed as a consequence of an unexpected adverse event, must be refrigerated and the necropsy performed in a timely manner to provide for accurate and reliable results. A full necropsy report as well as any relevant photographs and external laboratory results should be submitted to the AWO alongside the adverse event report.

10. GLOSSARY

Scientific Term	Lay Description
Aseptic	Minimise contamination with disease causing microorganisms.
Cardiovascular	Heart and blood vessels (arteries and veins).
Chlorhexidine	Antiseptic with a broad range of action against many bacteria, viruses and fungi. Not inactivated by organic matter. Has a residual action of up to 6 hours.
Dehydration	A reduction in body water. Can be coarsely measured by skin tenting or more accurately through blood/urine sampling.
Hypothermia	A reduction in core body temperature. Temperature is often measured by rectal probes or occasionally by telemetry implants.
Metabolic disturbances	Reduction in ability of cells to utilize energy.
Povidone iodine	Antiseptic with a broad range of action against many bacteria, viruses and fungi. Inactivated by organic matter.
Sterile	Devoid of all microorganisms (bacteria and fungus)
Tissue perfusion	Delivery of nutrients and removal of waste in the blood to/from organs.

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Australian Government (2012). Administer anaesthesia and perform surgery for scientific purposes.
- National Institute of Health (2005). Guidelines for survival rodent surgery. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_surgery.pdf
- NHMRC (2008). Guidelines to promote the wellbeing of animals used for scientific purposes.
- Pritchett-Coming K, Mulder G, Luo Y, & White W (2011). Principles of rodent surgery for the new surgeon. Journal of Visualised Experiments (video). <https://www.jove.com/video/2586/principles-of-rodent-surgery-for-the-new-surgeon>.

The following resources can provide additional or supplementary information for surgical protocols and techniques:

- National Institute of Health from the Office of Animal Care and Use https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_surgery.pdf
- Newcastle University. Aseptic technique in rodent surgery (video). <http://www.procedureswithcare.org.uk/aseptic-technique-in-rodent-surgery-tutorial/>
- The University of Melbourne Animal Care and Use Standards for Anaesthesia & Analgesia
- Penn State University (video). Surgical Scrubbing technique <https://www.youtube.com/watch?v=GsyPMFw4QJk>