

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, 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 OF MICE AND RATS

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

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1. ASSOCIATED STANDARDS

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

- Balanced analgesia
- Anaesthesia
- Blood collection
- Clipping for Marking and DNA Analysis

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

- Post-operative infections in rodents 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.
- 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

- 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.

- Initial training of surgeons should cover generic techniques of asepsis and surgery. All new surgeons must undergo Office for Research Ethics & Integrity (OREI) online surgery and anaesthetic training and undertake practical training with the Animal Welfare Officer (AWO) or their delegate. Where a delegate is the trainer they must have completed online training within the last two years and they should have been observed delivering training to an appropriate standard as determined by the AWO or a registered veterinarian.
- After completion of initial training, surgeons 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

- Surgical procedures must be carried out under appropriate local or general anaesthesia with appropriate analgesia. Where asepsis can be maintained in immunocompetent animals, antibiotics should be avoided if possible.
- Animal preparation should take place in a different location to surgery to avoid contamination of surgical sites with hair and debris.
- A rodent surgical area can be any room or portion of a room that is easily cleaned and disinfected. The immediate surgical area should be positioned to avoid air flow from contaminated areas and should not be used for other purposes during the time of surgery. The area used for surgery should be clean and uncluttered, and large enough to allow room for the surgeon and their assistant to work without impediment as well as for the equipment necessary (eg. anaesthetic machine, animal, surgical pack, surgical light). Prior to surgeries, clean and disinfect the surface upon which surgery will be performed.
- Sterilised instruments and materials must be used for survival surgery in rodents where sterile sites (e.g. body cavities, blood vessels or the skin) will be penetrated. Scissors and scalpels must be sharp to enable effective use. Any implants or single use material should be in sterile packaging. Braided materials should not be used for external skin sutures. Silk and catgut should not be used for intestinal, bladder or external skin sutures. Intradermal sutures are preferred in areas where animals can chew. The preferred methods of sterilising instruments and materials are autoclaving, dry heat or gas vapour. An indicator strip should be used to assure appropriate temperature has been achieved.
- Surgical instruments may be used on more than one animal provided they have not become contaminated by entering non-sterile areas such as the gastrointestinal tract. Organic matter such as blood should be washed off with disinfectant between animals e.g. chlorhexidine or povidone iodine and then the instruments dipped in 80% ethanol and allowed to dry before reuse. Alternatively, sterile water or saline can be used to remove debris followed by hot bead sterilisation of instrument tips taking care to ensure that instruments have cooled sufficiently before reuse. Instruments must be left in disinfectant or heat for an appropriate time to destroy target microbes between animals. Even with the use of these methods between animals, a new sterile instrument pack should be used after every 5 major surgical procedures.

4.3 Preparation of animal and surgeon

- An assessment of animal health should be performed before surgery to ensure no unexpected underlying illness.
- In the preparation area away from the surgical area, remove hair from the surgical site using clippers or depilatory cream in preference to razors or scalpel blades which are more likely to damage the skin. Clean the skin using 4% chlorhexidine in a scrub to remove clippings and other organic debris. Chlorhexidine scrub is alternated with plain 80% ethanol and repeated for a total of at least 3 times. Then perform the surgical clean 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. Less ideally 10% povidone-iodine antiseptic can be used. Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal.
- Hypothermia prolongs recovery time and can be fatal. Steps to prevent hypothermia include not wetting the animal any more than necessary during the scrubbing process, providing an insulating layer between

the animal and the surgical surface and provision of an external heat source, and subcutaneous warm fluids during the procedure.

- The surgeon must wear clean protective covering (eg. gown, lab coat) and hair must be covered or tied back. Hands must be scrubbed using a detergent or alcohol based antiseptic. 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 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.
- If an assistant is present who can anaesthetise the animals and prepare them for surgery, it may be possible for the surgeon 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 surgeon should keep their gloves on while preparing the next animal and then change the gloves for a new pair when the animal is ready. The sterile drape may be moved from one animal to the next by the surgeon provided it is not obviously contaminated.

4.4 Surgical procedures

- 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. An exception requiring reasoned justification is the use of the 'tips only' method where only the instrument tips can come in contact with sterile surfaces whilst the surgeons gloves and instrument handles must not come in contact with sterile surfaces. 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.
- Antiseptics can delay healing and should not be placed in open wounds or surgical sites.
- 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 should be provided to the AEC.
- Where potential complications may require further surgical procedures such as re-suturing, these should be listed in the initial animal ethics application. Where surgery which is not listed on an animal ethics application is required, a registered veterinarian must perform the procedure. The AWO must also be notified of any surgery or other procedure, including the application of products (ie. bandages, creams or disinfectants) to surgery sites, that are not listed in the ethics application.

4.5 Post-operative care

- Animals must be placed in clean cages after surgery and a substrate of tissues can prevent collection of debris on the surgical site.
- 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, tranquillising and antibiotic 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 that conditions are such that they are not disturbed, attacked or killed by other animals in the same enclosure.
- Clinical records of an animal's state must be kept, including observations and administration of any drugs, fluids or other treatments, and made accessible to all investigators involved in the post-operative care of the animal.
- Investigators must ensure that adequate monitoring, treatment and care of post-operative animals is provided and that they are fully informed of each animal's state.
- 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.
- 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.
- 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. Do not place the rodent directly on bedding material until fully awake in order to prevent

aspiration of bedding. A tissue can be used on top of the bedding during the recovery period for this purpose. Animals should not be returned to the animal holding rooms until they are stable and able to assume a normal posture.

- Dehydration can be ameliorated by the administration of appropriate fluid therapy. 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. Post-operative pain or distress must be monitored and treated. If not already removed by the animal, external wound clips and sutures should be removed 10-14 days after the surgery.

4.6 Non-survival Rodent Surgeries

- While it is not necessary to follow aseptic technique when performing non-survival surgeries in rodents, at a minimum the surgical site should be clipped, the surgeon should wear gloves, and the instruments should be clean.

5. MONITORING & INTERVENTION

- The animal must be monitored closely during the surgical procedure. Surgeons should pay close attention to the animal's level of anaesthesia.
- During the immediate postoperative period, the animal should be observed constantly until it has regained the righting reflex.
- Post-operative monitoring records should 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, any therapeutics given including drugs, doses, and routes of administration.
- Assessment of the surgical site and post-operative pain must be performed at least twice daily, and weighing plus body condition scoring performed daily, for the first 3 days after surgery.

6. ADDITIONAL INFORMATION

- Access to OREI online surgery and anaesthetic training is available on the website:
<http://orei.unimelb.edu.au/content/animal-welfare-training-half-day-0>

7. ENFORCEABLE REQUIREMENTS

- Performance of the procedure by competent investigators or trainees under the direct supervision of competent investigators
- Use of sterilised instruments, drape and gloves
- Hair removal and patient preparation using disinfectants as described above
- Ensuring aseptic technique where gloves and instruments only come in contact with sterile surfaces during sterile procedures. Nonsterile gloves from a clean box may only be used if: they do not come in contact with the surgical site or material that contacts the surgical site, and the surgical instrument handles are not placed on the sterile drape (tips only method).
- Disinfection of equipment between surgeries and a maximum of 5 surgeries per kit before resterilisation is required.
- 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.
- Competency must be demonstrated on more than two consecutive occasions before investigators are permitted to perform surgery without supervision.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's Animal Ethics Committees 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 University's AWO.

9. UNEXPECTED ADVERSE INCIDENTS

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 University's AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

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.
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 Institutes of Health (2005). Guidelines for survival rodent surgery (**video**)
- 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**)

The following resources may provide additional or supplementary information:

- Newcastle University. Aseptic technique in rodent surgery (**video**)
- The University of Melbourne. Anaesthesia & aseptic surgery of mice and rats.