

WSU Guideline for Aseptic Technique

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1. Introduction

Scope

This document is intended as a guide to aseptic technique for researchers, it does not deal with housing, husbandry, anaesthesia and analgesia in detail.

Definitions

Asepsis is the elimination (as far as is possible) of potential sources of contamination of the surgical wound by pathogenic microorganisms, the main sources of which are: the animal itself, the surgical team, the instruments and equipment, and the atmosphere (Fossum, 2018). *Aseptic technique* is the method by which asepsis is achieved. The way in which aseptic technique is applied will need to be adjusted depending on circumstances (e.g. species, facilities) but good practice remains the goal.

Sterile implies complete absence of all microorganisms and their spores.

These guiding principles set high, aspirational standards and are intended to add to the minimum requirement of the [AUSTRALIAN CODE \(2013\)](#):

3.3.16. (ii) using aseptic procedures if the animal is expected to recover from surgery

Appropriate aseptic technique is essential when undertaking recovery surgery on any species, and regardless of whether surgery is considered minor (involving skin only) or major (e.g. entering a body cavity). These principles are also relevant for non-recovery procedures carried out under terminal anaesthesia, to reduce variability which may be introduced by pathogens.

Rigorous application of aseptic technique reduces the risk of surgical site infection (SSIs). SSIs are likely under-recognised in laboratory animals, due to multiple factors (Inns, 2019).

2. Preparatory considerations

- SSIs are a welfare concern and introduce variables to your work. Rodents may not show obvious clinical signs (diagnosis may require microbial culture), however this does not mean that their welfare is not compromised (Bradfield et al, 1992; Inns, 2019).
- Longer duration of surgery is associated with higher risk of wound infection. Therefore thorough planning will minimise time wasted during a procedure.
- Wound infections may occur at any level of the surgery, e.g. from deep (the target organ) to superficial layer (skin).
- Check that the animal(s) is(are) in a fit state for the proposed surgery, and that the appropriate acclimatisation period has been observed to recover from any transport stress.
 - Stress and pre-existing infections (e.g. dermatitis) both increase the risk of post-operative wound infections.
 - Infections can spread via the blood to a surgical site, therefore infection anywhere (e.g. a fight wound) may compromise a remote surgical site
- The risk of infection changes with several factors, including: the surgical wound classification, the immune status of the animal, the presence of foreign materials.
- Surgical wound classification, in order of lowest to highest risk of wound infection, as per the Center for Disease Control (Mangram 1999):

Clean: an incision in which no inflammation is encountered in a surgical procedure, without a break in sterile technique, and during which the respiratory, alimentary and genitourinary tracts are not entered.

Clean-contaminated: an incision through which the respiratory, alimentary or genitourinary tract is entered under controlled conditions but with no contamination encountered.

Contaminated: an incision undertaken during an operation in which there is a major break in sterile technique or gross spillage from the gastrointestinal tract, or an incision in which acute, non-purulent inflammation is encountered. Open traumatic wounds that are more than 12–24 hours old also fall into this category (e.g. fight wounds, dermatitis)

Dirty or infected: an incision undertaken during an operation in which the viscera are perforated or when acute inflammation with pus is encountered during the operation (for example, repairing a broken-down wound)

Be aware that the intended class of surgery may change if there is a problem encountered intra-operatively! Consider what your plan will be if contamination happens (e.g. if a visceral organ is punctured)

- In some cases, bacteria can survive and flourish particularly well - a much lower 'dose' of bacteria can result in clinical infection. Some examples of this where asepsis must be particularly rigorous include:
 - Implants – implanted foreign materials (including non-absorbable or non-synthetic sutures) can act as surfaces for bacteria to adhere to and flourish.
 - Immunoprivileged sites – e.g. surgery in the central nervous system
 - Immunocompromised patients (such as diabetic mice)
 - Vascular surgery - infection will rapidly become systemic

3. Surgery

Room: the area chosen should be clean, well-lit, quiet, with easily-disinfected surfaces. A dedicated room is best. Ideally there should be separate areas used for preparation, surgery and recovery, which can be within the same room. A flow cabinet or downdraft table (if available) provides extra control against airborne contaminants.

- Ensure general supplies are present, including clippers, ophthalmic lubricant, heat pad, skin prep agents (e.g. chlorhexidine or iodine, and ethanol 80% w/v. Chlorhexidine is the preferred veterinary antiseptic for most sites, and iodine-based antiseptics for sites on or in proximity to the eyes).
- Ensure sterile supplies are available and that sterility is in date. This will include items sterilised in-house (e.g. autoclaved instruments, drapes, gauze swabs) and items purchased pre-sterilised (e.g. suture materials, catheters, scalpels, sterile saline). For items sterilised in-house, ensure that sterilisation was adequate (e.g. autoclave tape external to the packed instruments, or a Browne's tube packed alongside the instruments).
- If any equipment may need to be touched by the surgeon (e.g. stereotactic frame, operating microscope) ensure that sterile covers are available to provide a barrier and maintain glove sterility. Autoclaved tinfoil can be used for this purpose.
- Animals awaiting surgery should not be in the room while surgery is happening, to avoid distress.

Animal: Once anaesthetised the surgical site should be prepared by clipping with clean sharp clippers. Dirty clippers may become hot, and may damage the skin leading to local skin infection. Small 'beard trimmer' style clippers are ideal for rodents. Ensure that hair is removed with a margin around the intended surgical site (not excessive however as this may lead to excessive body heat loss in rodents both intra and post-operatively).

- Ensure that loose hair is removed so that it cannot contaminate site (e.g. lint roller, careful vacuuming).
- Perform skin prep as demonstrated (see Procedures With Care video), with a final topical skin preparation when in position immediately prior to surgery. The animal's own skin is the source of many surgical site infections. Contact time of the chemical prep agent with skin should be observed. Note that chlorhexidine requires >5 minutes for full efficacy in large or dirty animals, but is acceptably effective with a shorter contact time in small laboratory animals, who have restricted microflora and rapidly become hypothermic when wet.
- Cover the animal with sterile drapes. The 'window' in the drape should not allow any hair to protrude into the sterile prepared area.
- Place instruments onto a sterile draped area or instrument tray before commencing surgery. Animal drape should be contiguous with instrument drape, to ensure a single sterile field of work. Arrange instruments so that you can reach them in the likely order that they will be needed, to minimise reaching over other instruments
 - For skin prep, see video
 - For draping procedure, see video
 - Drapes can be reusable cloth, or single use. Transparent drapes may be useful for monitoring small rodents.

Surgeon: Prepare for aseptic surgery by putting on a facemask and hairnet, then scrubbing up and donning gown and sterile gloves. Ideally the gown should be sterile too but at least a clean covering is required.

- For hand scrubbing, gowning and gloving, see Procedures With Care video.

Once surgeon is gowned & gloved, do not touch anything. The surgeon's sterile field is limited to hands (plus arms and the front of the chest, if wearing a sterile gown) but any area can become contaminated by contact with a non-sterile surface. Surgeon must be aware of what they have touched with 'sterile' areas; non-sterile objects must not be touched and, if they are, that item considered contaminated.

- Some common situations: do not adjust glasses or facemask with sterile gloves; be careful where arms are rested if wearing sterile gown; use a sterile covering for anything that may need adjusting (e.g. autoclaved aluminium foil placed over the dials of a stereotaxic frame)

Surgery conduct:

- Ensure that the animal is sufficiently anaesthetised before performing surgery. Ensure the animal is securely positioned, especially if breathing volatile anaesthetic gas, to avoid need to reposition during procedure. Ensure that the animal is continually monitored throughout by a competent person. *Never leave an anaesthetised animal unattended*
- Only touch sterile areas with sterile objects. If an item touches a non-sterile area, it can no longer be considered sterile

- It is best practice to have a “non-scrubbed” surgical assistant and/or anaesthetist present, particularly for novel surgeries. Non-scrubbed assistants must not touch sterile areas. Their tasks may include monitoring the animal under anaesthetic, passing objects to the surgeon (e.g. instruments, suture and flush), taking notes.
- A “non-scrubbed” assistant is also helpful for consecutive surgeries, to prepare and monitor recovery of animals, or to re-autoclave instruments.
- Any areas that become wet can allow bacteria to ‘wick’ through and therefore breach sterility (e.g. wet cotton drapes)
- If drape needs to be adjusted by a non-sterile assistant (e.g. to monitor animal), this must be done from the underside, as the underside is considered non-sterile.
- Surgeon should only handle the animal from the sterile (outer) side of the drape.
- If subcutaneous tissues are exposed, these should be regularly lavaged with warm sterile saline to prevent desiccation and infection (approximately every 15 minutes, especially if under a light source which may produce radiant heat).
 - If inadvertent contamination of the surgical site happens, lavage with copious amounts of sterile saline will help reduce the infective load
- Minimise blood loss, as blood provides an ideal medium for bacteria to thrive. Swabs moistened in sterile saline can be used to apply pressure. Haemostatic agents may be useful to control ooze (various commercial products available).
 - Be aware that blood leakage is likely to increase on recovery, as blood pressure may be low under anaesthetic; additionally, some anaesthetic drugs (medetomidine, xylazine) actively constrict small blood vessels and increased bleeding may be seen when these are reversed.
- Fluid accumulation (seroma) also provides a good medium for bacteria. Aim to minimise ‘dead space’ (i.e. empty pockets of tissue) by careful dissection.

Batch surgeries: Ideally a fresh set of instruments is available for each animal. If not, it is possible to use a bead steriliser but this must be planned and used within its limitations (see Appendix). Immersing instruments in alcohol is not considered an effective sterilant (see 6.16 below).

4. Recovery

- Administer analgesics, fluids and supplementary heat (or cooling when there is evidence of hyperthermia) as required. Antibiotics should not be required if rigorous aseptic technique is used for ‘clean’ procedures, but may be necessary for ‘contaminated’ ones.
- Move animal to a clean, low-dust recovery cage. This should be warm and quiet.
- Do not leave the recovering animal until it is conscious, able to remain upright and to protect its own airway (if intubated) without assistance.
- Monitor the wound for possible bleeding, or self-trauma
- Plan to reassess the animal’s well-being at appropriate intervals after surgery, including the need for analgesia, fluids, warmth or modified diet. *Be aware of how long the analgesic you are using is likely to last in that species*
- Consider what your plan will be if there are complications with the wound in the days following surgery (e.g. self-trauma, closure breakdown).

5. Monitoring outcomes of surgery

- Record key events of the procedure, including: anaesthetic induction, procedure conduct, recovery and outcome. These should be recorded on a pre-planned surgery monitoring

sheet forming part of the ACEC application, to ensure that no aspect is overlooked. Examples are available on the Animal Ethics intranet page.

- Post-operative monitoring should also be done as per a sheet agreed with ACEC. This must include aspects such as checking for signs of pain, food and water intake, defecation, behaviour, wound condition. There must be clear actions to take if certain signs seen or thresholds reached (e.g. adjunctive analgesia, wound repair, euthanasia).
- Poorly-healing wounds should be investigated (e.g. swabbed for bacterial culture)
- External signs of wound infection: swelling, redness, heat, pain, reluctance to move area, discharge, non-healing wound.
- Internal wound infections may not present with obvious signs, instead the animal may show a variety of clinical signs such as appearing dull, lethargic, inappetent, ruffled fur, display pain behaviours, hyperthermia (or hypothermia, as sepsis advances).
- Additionally, please bear in mind that rodents are very good at 'masking' distress and clinical signs may be absent or minimal (e.g. weight loss).
- Unexpected adverse events (e.g. wound breakdown; any pain or distress that was not predicted) should be reported to facility management and AWO
- In the long term: compare the success of the procedure and the surgeon with benchmarks. Any surgeons who perform poorly should be re-trained or refrain from operating on animals.
- Review the literature and discuss the procedure with peers, in order to identify refinements that could be applied.

For a video series demonstrating practice of good aseptic practice in rodent surgery, please watch the example videos available at the website [Procedures With Care](#) (see Resources).

6. Inventory of practical tips

1. **Drugs** – analgesics, anaesthetics. The use of anaesthesia and analgesia in painful procedures benefits the animal, greatly refines the procedure, and should be discussed in the ARA. Dilutions of injectable agents should be made up fresh every day with sterile water or saline, and that expiry dates (including vial broaching dates) should be adhered to, to avoid injecting animal with a pathogenic burden. Analgesics can be local or systemic. Various types of systemic analgesics are available. You may provide best welfare by using several simultaneously. Top-up doses may be required
2. **Antibiotics** – should not be needed if aseptic technique is followed for ‘clean’ surgeries. If required consider how to administer (e.g. via drinking water).
3. **Fluids** – for longer procedures (e.g. over 20 minutes) or if blood loss likely, administering supportive fluid (e.g. subcutaneous isotonic NaCl, 10ml/kg body weight) reduces dehydration and associated physiological compromise of the animal, which may leave it less able to mount an immune response to any infections. This may be able to be incorporated into dilutions of injectable drugs for small rodents.
4. **Procedure room & set-up** – ensure you have separate areas for prepping the animal (e.g. clipping fur) and operating. Ensure that if surgeon is to operate any equipment that a sterile barrier is in place, e.g. sterile foil to be placed over knobs of stereotactic frame, lights, or operating microscope. Ensure equipment is laid out so that the surgeon can access everything without having to lean into non-aseptic areas; and ensure that the sterile field is as small as possible to minimise breaches of asepsis.
5. **Instruments** – ensure these have been cleaned, packed, and autoclaved effectively. Various indicator systems exist to demonstrate that sufficient heat & pressure was achieved during the cycle e.g. external autoclave tape, or Browne’s tubes (visible through clear instrument pouches). All autoclaved products have a shelf-life, ensure that this is adhered to.
6. **Drapes** – disposable and reusable varieties are available. The drape should encircle over the edges of the clipped area, so that a sterile field is maintained with no fur intruding. The drape should be secure, so that it cannot move and ‘track’ contamination from the unclipped fur into the sterile field. Transparent drapes are useful for small animals.
7. **Temperature** – maintaining normothermia is important aspect of physiology, cold animals are more vulnerable to opportunist peri-operative infections and recover more slowly from anaesthesia. Small animals can become very cold very quickly when anaesthetised and especially when breathing cold compressed anaesthetic gas on a non-rebreathing circuit. Placing a warming device under the animal helps mitigate the development of hypothermia (Taylor, 2007). Wrapping the hairless parts (e.g. feet and tail) in clingfilm is also useful. Having a warm area for recovery helps with a fast and smooth recovery. Conversely hyperthermia may occur in certain circumstances, e.g. a hot environment with a densely-furred animal on a rebreathing circuit. Some animals may pant which may lead to unstable anaesthesia (if on gaseous GA).
8. **Gown/ cap/ mask** – these must be worn by the surgeon to reduce contaminants from hair or clothing falling into surgical field.
9. **Scrubbing hands& sterile gloves** –Wash hands with suitable agent (e.g. chlorhexidine, povidone-iodine) for a minimum of 5minutes’ contact time. Ensure all surfaces of fingers are cleaned, and scrub the nails with a brush. Ensure water runs from hands down to elbows (i.e. clean -> dirty). Ensure that gloves available in your size. Dry hands on a sterile towel & don gloves. See the WHO guidelines on surgical hand preparation for more information; and the Procedures With Care video.

Full surgical gowning procedure involves drying hands, donning gown, then donning gloves via 'closed gloving' - Procedures With Care "Preparation of the surgeon" section video recommended (see Resources).

10. **Preparation of surgical site** – Small electric trimmers suggested to clip fur. Ensure clippers clean, sharp and lubricated – poor-functioning clippers can damage the skin. Ensure area shaved is larger than needed, to provide a margin of skin around the surgical site, although not excessive to avoid hypothermia. Remove all loose hair. Prep skin with chlorhexidine or povidone iodine, working from the centre of the wound outwards towards the fur. Repeat with a few swabs, depending on contamination levels. Using warmed solutions reduces chilling the animal. A final prep with 80% w/v alcohol can be used on the skin although do not use excess, as it can produce rapid evaporative cooling of very small animals and contribute to hypothermia.
Where possible, optimal contact time of prep agents with the skin should be observed i.e. 5 minutes (Belo et al, 2010). This is not recommended for laboratory rodents owing both to rapid hypothermia by evaporative cooling, and to their restricted range of opportunistic pathogen flora. See *Procedures with Care video for more information on rodents*.
11. **Eye lube** – corneas must be lubricated under anaesthesia, as the animal cannot blink and corneal desiccation is painful with lasting damage such as ulceration. A variety of artificial tears are available e.g. Viscotears.
12. **General surgical technique** – Surgeon must only touch what is sterile once scrubbed, gowned & gloved. Do not allow alcohol to come into contact with any body tissue other than intact skin. Accurate tissue handling; sharp clean incisions. Minimise tissue trauma: avoid using scissors to make or extend skin incisions, these tend to crush tissue and result in poorer healing – use a scalpel for sharp incisions. Minimise subcutaneous fluid accumulation (seromas). Avoid tissue desiccation – irrigate exposed tissues with warm sterile saline (do not drench the animal or drape).
13. **Haemostasis** – Minimising blood loss is important, not only for cardiovascular function but because blood is a good medium for bacterial growth. Isolate & ligate large blood vessels prior to cutting them. If your procedure produces diffuse 'ooze' you may find haemostatic powders, cautery etc useful. Blunt dissection (where possible, i.e. fatty tissue or along tissue planes, minimises bleeding). Avoid cutting through muscles where possible, it is very vascular.
14. **Suture material** – synthetic types with swaged-on needles produce the least tissue damage and are the least likely to promote bacterial growth or inflammation. Nonsynthetic types (e.g. catgut, silk) are more likely to provoke inflammation and permit bacterial growth. Absorbable suture should be used inside the body unless there is a requirement for non-absorbable. Only use monofilament suture in the skin (braided types allow more bacteria to wick into the wound). A new pack of swaged-on-needle suture per animal is recommended. Diligent handling of excess suture lengths is important – do not let them trail out of the sterile field! Have an area on your sterile instrument drape to place the needle and suture if it is necessary to set it down.
15. **Bead sterilisers** – These sterilise the tips only. They can (with care) be used successfully for batch surgeries. Preferably you would have enough autoclaved instruments to avoid their use. See Appendix for more details.
16. **Alcohol dipping of instruments** – not effective against bacterial spores, fungi etc and therefore not advised. Documented efficacy is limited to reduction in aerobic bacterial contamination by 70% isopropyl alcohol (Keen et al, 2010). It may be useful for fragile

instruments damaged by the heat of a bead steriliser; however as for bead sterilisers, having sufficient autoclaved instruments for each animal is preferable.

17. **Implants** – it is critical that any object intended to be implanted must be **sterile** (e.g. dataloggers, catheters, minipumps). The means used should be appropriate to the device, and must not leave any chemical residue behind that may produce tissue irritation or toxicity. *Presence of an implant reduces the initial 'load' of bacteria needed to produce an infection, by providing a surface that bacteria can adhere to and flourish on.*
18. **Closure of wound** – synthetic monofilament suture suggested. Medical skin glue is acceptable (but only for skin, as it is non-absorbable). Wound clips may be useful but can be more prone to dislodging or snagging on objects. Whatever method used, ensure that there are no gaping pockets which could collect contamination. Also ensure that the wound is not under tension, as this will be more likely to break down.
19. **Aftercare** – A rapid comfortable recovery avoids immunocompromise that may result from deleterious aspects of surgery (e.g. pain, hypothermia, inadequate food intake). Have a clean quiet warm low-dust cage. Do NOT put anaesthetised animals with conscious animals. Monitor animal until it can maintain posture. Consider an easily-digested recovery diet – e.g. special gel diet. Consider low-dust bedding, e.g. paper only. Consider social structure if group housed – animals will benefit from being returned to their conspecifics but conversely, fighting, or licking each other's wounds may occur. If you separate adult male mice, they may not be able to be re-introduced. Check animal frequently (as per the study's monitoring plan) in initial days for signs of pain (e.g. grimace scale; weight loss; posture; reduced activity; fur ruffling; 'red tears' in rats). Check animals especially at time when analgesia predicted to lose efficacy (e.g. buprenorphine lasts 8-12 hours in rodents) - 'top-up' analgesia may be required. Check wound for signs of healing in the days following surgery, or, conversely closure breakdown or infection (swelling, pain, heat, redness, self-trauma. Discovery of these should prompt microbiological investigation). *Absence of visible pus does not mean absence of infection.*
Remove sutures or wound clips after 7-10 days.

Resources

Procedures With Care instructional videos: <http://www.procedureswithcare.org.uk/aseptic-technique-in-rodent-surgery-tutorial/>

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<https://anzccart.adelaide.edu.au/system/files/media/documents/2019-12/2019proceedings.pdf>

Australian code for the care and use of animals for scientific purposes (8th edition, 2013)
https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes#toc_620

International statements:

USA: ACLAM Position Statement on Rodent Surgery *J Am Assoc Lab Anim Sci* 2016; 55(6):822-823

UK: Home Office Minimum Standards for Aseptic Surgery Downloaded March 20201 and available at: <http://www.procedureswithcare.org.uk/ASMS2012.pdf>

General references

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WHO guidelines for surgical hand preparation: <https://www.ncbi.nlm.nih.gov/books/NBK144036/>

Appendix. Glass Bead Sterilisers

Introduction

These can be used as part of a planned 'tips-only' surgery, where the instrument tips alone are used to handle and manipulate tissues, while conducting high-throughput 'batch' surgeries in rodents.

Only the tips of the instruments are re-sterilised using this method. All other parts of the instrument and your gloves are considered 'dirty'. The surgical area must be carefully panned and managed to avoid cross-contamination.

Repeated use of a hot bead steriliser may damage the tips of delicate instruments – be sure to regularly inspect your instruments.

It is advised to limit the consecutive uses of a bead steriliser to five, then change over to a fresh autoclaved kit. This permits conduct of up to six surgeries using one kit. This is because only the tips are sterilised and progressive contamination of other areas is likely. Changing gloves along with a new kit is recommended.

Maintenance: glass beads need to be changed regularly, and more frequently if they become contaminated or if the level drops. Refer to manufacturer's manual.

Practical use of the bead steriliser

1. Plan in advance how to manage your surgical area so that the instrument tips remain sterile, despite their handles being 'dirty'. One suggestion is to rest the instruments angled against a petri dish with handles resting on the drape and tips overhanging the dish in the air, so that the sterile tips are not touching anything.
2. Ensure the bead steriliser is turned on and adequately heated prior to use. This may take ~20 minutes. Some models have a temperature gauge.
3. Beads will be very hot: >250°C. Do not touch beads, or move the steriliser once turned on, to prevent spillage.
4. Place it away from the sterile surgical area, as the machine itself is not sterile.
5. Start with an autoclaved instrument kit, and sterile drapes, gloves etc as detailed elsewhere.
6. Stir beads occasionally to improve even heat dispersion.
7. Prior to use, clean any blood and debris from the instruments using sterile water (not saline) to avoid contamination of the beads with tissue debris or salt crystals.
8. Place instruments one at a time into the steriliser, to ensure optimal temperature achieved (some models may accommodate more). Ensure the tips are deep and well covered with beads - the topmost ~5mm of beads may not be adequately hot.
9. Instruments should be left in the beads for 15 – 60 seconds –larger/ thicker instruments may require longer.
10. After use, ensure that no beads are clinging to the instrument. Ensure the instrument is cooled prior to contacting any part of an animal.
11. Change to a fresh autoclaved kit after 5 uses of the bead steriliser.