



Guiding Principles for Preparing for and Undertaking Aseptic Surgery

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Updates:

Guidelines may be updated from time to time to reflect changes in practice. If you have received a hard copy of this report, please check on the website that this is the latest version.

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SUMMARY

This document is designed as a guide to aseptic technique for both new and experienced personal licence holders who undertake surgical procedures under the Animals (Scientific Procedures) Act, 1986 (it does not deal with housing, husbandry, anaesthesia and analgesia in detail). Aseptic technique is essential when undertaking surgery on any species and the principles laid out in this document apply (equally) to both recovery surgery and lengthy procedures carried out under terminal anaesthesia.

The main aim of any surgical procedure is that it is carried out skilfully with the minimum of risk and disturbance to the animal and without infection. These guiding principles set high, aspirational standards. LASA encourages institutions to use the principles set out in this document to develop standard operating procedures (SOPs) which are appropriate for the species, the staff undertaking surgical procedures and the facility where the work is carried out. Development and periodic review of surgical SOPs and the application of high standards of aseptic technique will go a long way to eliminating infection as a source of potential research variability.

Preparation prior to Surgery

- Ensure that all legal requirements are met; Certificate of Designation, personal and project licence permissions.
- Ensure that all staff involved are trained and competent (or supervised) in the procedure, anaesthesia and post-operative care (**Note:** records of training and supervision must be kept).
- Prepare a peri-operative plan in consultation with the Named Animal Care and Welfare Officer (NACWO) and Named Veterinary Surgeon (NVS) as needed. This will include post-operative husbandry and welfare assessment.
- Book the theatre and facilities.
- Ensure that appropriate equipment, instruments and consumables (e.g. gloves, swabs, and drapes) are available and sterile. Check that there are sufficient medicines available and that they are in date.
- Liaise with support staff to make sure that they will be available and know what you plan to do.
- Check that the animal is in a fit state for the proposed surgery and has had the appropriate acclimatisation period to recover from any transport stress. Weigh it and record the bodyweight.
- Administer analgesics prior to surgery as well as post-operatively; administer antibiotics and fluids as required.
- As set out in the Home Office Codes of Practice, animal, equipment and staff preparation should be performed in a pre-operative area located outside the theatre.

Surgery

- Perform the final topical skin preparation in the operating theatre area.
- Prepare appropriately for aseptic surgery by scrubbing up and changing into a sterile gown etc. Once the surgeon has prepared for surgery, non sterile objects must not be touched and if they are, at least a new sterile pair of gloves should be used.
- Drape the animal with sterile drapes and place instruments on to a sterile instrument tray before commencing surgery.

- Ensure that the animal is sufficiently anaesthetised before performing surgery, using a method appropriate for the species. Ensure that the animal is continually monitored throughout by a competent person.
- It is beneficial to have a 'non-scrubbed' surgical assistant and/or separate anaesthetist, particularly for longer or more complicated surgeries, or those involving the use of neuromuscular blocking agents. Non-scrubbed assistants must not touch sterile areas.
- **Note:** an anaesthetised animal should **NEVER** be left unattended.

Recovery and Post-operative care

- Administer analgesics, antibiotics, fluids and supplementary heat (or cooling when there is evidence of hyperthermia) as required.
- Do not leave the recovering animal until it is conscious, able to remain upright and protect its own airway without assistance.
- Regroup the animal with its conspecifics as soon as appropriate after surgery.
- Reassess the animal's well-being at intervals after surgery, including the need for analgesia, fluids, warmth or modified diet.
- Non-absorbable skin closures must be removed at the appropriate interval post-operatively.

Monitoring Outcomes of Surgery

- Record key events of the procedure, including outcome.
- Compare success of the procedure and the surgeon to benchmarks. Any surgeons who perform poorly should be re-trained or refrain from operating on animals.
- Review literature and discuss procedure with peers, in order to identify refinements that might be applied.

1 AIMS

This document is designed as a short guide to the principles of aseptic technique for both new and experienced personal licence holders who undertake surgical procedures under the Animals (Scientific Procedures) Act, 1986.

Asepsis is the elimination of all potential sources of contamination of the surgical field, the main sources of which are, the atmosphere, the surgical team, the instruments and equipment, and the skin of the animal. It is useful to remember that surgery can only be aseptic or not – there is no middle state.

Aseptic technique is the method by which asepsis is achieved and is a variable. Therefore, the rigor with which aseptic technique is applied will influence whether or not surgery is undertaken aseptically or not and will dictate the ultimate outcome. While good practice should always be the goal, the principles applied at the time of surgery may also be influenced by the circumstances under which surgery is undertaken and the species used.

Good practice in surgical technique is clearly essential but it is also very important to ensure that good practice is followed for anaesthesia, analgesia and the development of peri-operative care programmes. The husbandry needs of animals before, during and after such procedures also require careful consideration. However, it is **not** the purpose of this document to address all of these topics in detail; guidance for these should be sought elsewhere (e.g. the NVS and NACWO for the unit).

Note: the principles outlined in Sections 3 and 4 also apply to lengthy procedures (e.g. lasting more than 1 hour) undertaken under terminal anaesthesia (whether or not the wound is closed during the non recovery monitoring period), since infection could compromise experimental data if an aseptic protocol were not followed.

2 INTRODUCTION

Aseptic technique is essential when undertaking surgery on any species, including rodents and wild animals, for scientific and animal welfare reasons. Infection may lead to wound breakdown, pain and delayed recovery, which causes avoidable suffering and can compromise scientific data. Although it is claimed that mice and rats are resistant to post-surgical infections, the literature contains numerous articles that document how subclinical infections can become clinical diseases following stress or immune suppression^{1, 2}. Rodents are used as models of surgical infection and they clearly develop localised infection, sepsis, and physiologic alterations that may, in extreme cases, progress to death^{3, 4, 5}. They have been used extensively in immunologic research and are not known to possess any remarkable features of their immune systems that would completely prevent infection from developing. Bradfield et al. (1992)⁶ reported that subclinical infection in rats had significant effects on behaviour, blood cell counts, plasma fibrinogen and serum glucose, even though lesions were not visibly identified in tissues.

Prior planning and attention to detail should be an integral part of all surgical procedures where invasive surgical techniques are to be undertaken. **Every step in the process should be considered**, from the checking of licence authorities, to the preparation of instruments, consumables, facilities, the surgeon and the animal, through to post-operative care.

3 PREPARATION FOR SURGERY

a Planning for Surgery

- Before beginning any procedure or series of procedures on a live animal,
 - ensure that the procedure(s) is/are specifically authorised in the project licence under which you are working; and
 - that the purpose for which you are doing the work is authorised; and
 - that you have the relevant technique/s on your personal licence; and
 - that the facilities are correctly specified in the Schedule to the Certificate of Designation*;
 - as and when required, arrange for training and supervision for the procedure(s) that you intend to perform⁷ and ensure that up-to-date records of this training and supervision are kept**. ‘Refresher’ training may also be necessary where a procedure has not been performed for some time. For additional practical advice see the [LASA “Guiding Principles on the Supervision Requirements for Personal Licensees”](#).⁸

- A peri-operative care plan should be agreed with the NVS and NACWO before surgery commences. This may include:
 - **Housing and husbandry:**
 - acclimatisation periods for animals moved between facilities, or for testing, to ensure that any stress caused by transport has returned to baseline levels;
 - whether any dietary restriction is required prior to surgery;
 - any requirements for special diets pre or post-surgery;
 - housing requirements e.g. when and whether animals can be regrouped after surgery;
 - requirement for husbandry refinements post-operatively, e.g. heat pads, mash, specific nesting/bedding materials, whether or not a refuge can be provided;
 - routine weighing, monitoring food and water intake^{***}. When measurements are taken accurate records should be kept.

 - **Assessment of animal well-being and recognition of pain and distress** should be carefully planned and tailored to the species, strain and procedure. This should be discussed with Named Persons and animal technologists or care staff.

 - **Analgesic requirements** of the animal. The use of anaesthesia and analgesia in painful procedures benefits the animal and is a legal requirement unless specifically excluded by the project licence^{****}. Information and training videos are available at [AHWLA](#)⁽ⁱⁱ⁾. If in doubt, ask the NVS for advice when planning your particular experiment and do not

* & ** These are legal requirements.

*** It is important to monitor food and water intake and the physical appearance of the animal because this provides essential baseline data for use in health and welfare assessment. Deviations from normal values can be used to identify an animal that is unwell and requires attention/treatment. These observations may indicate that the procedure and/or aftercare require refinement and should be used when reviewing surgical outcomes and anaesthetic and/or analgesic regimes.

**** See project licence standard condition 6, 6A, 6B, 6C ^{vi Appendix D}.

leave this until the day of surgery or during surgery. Where possible, analgesics should be administered before surgery starts as well as after surgery has finished. Pre-emptive analgesia has been shown to alleviate pain more effectively⁹.

- **Antibiotics.** In many cases antibiotics should not be necessary if surgery is done aseptically and competently. The NVS is the best person to advise on the use of antibiotics. Any potential interference with scientific data should also be considered.
 - **Fluid replacement.** Any requirement for fluid replacement to prevent dehydration and aid recovery should be considered at this stage. Consult your NVS for advice.
 - **Temperature control.** Consideration should be given to the need for supplementary heat (or cooling when there is risk of hyperthermia) and methods to prevent adverse core body temperature changes during surgery should be adopted.
- Before you start surgery, ensure you know which records you need to keep. (For additional practical advice see the [LASA “Guiding Principles on Record Keeping for Personal Licence Holders”](#)¹⁰) For example, check that appropriate animal record sheets are available for recording the procedure(s) undertaken and ensure you know how to use any recording sheets (sometimes referred to as ‘score’ or ‘clinical observation’ sheets) designed to monitor animal welfare during surgery and during the post-operative period. The NACWO will be the best person to advise you on local procedures.
 - Ensure that sufficient support staff will be available for both surgery and post-operative care. These may include animal care, veterinary staff, anaesthetist and scientists. **Note:** the primary legal responsibility for the health and welfare of the animal remains with the last personal licensee who undertook a regulated procedure on that animal. This licensee must make adequate arrangements for the welfare of the animal if they cannot, themselves, be available.
 - If necessary, book the surgical facilities. **Note:** surgical procedures should be carried out early in the day and as early in the working week as possible, to facilitate and help ensure post-operative monitoring and care. If surgery cannot be performed at these times licensees are responsible for ensuring the health and welfare of the animals during the post-operative period which might include provision for animal care overnight and at weekends.
 - Before commencing the surgical procedure, check that the operating theatre area is clean and ready for use; make sure that all unnecessary equipment and clutter has been removed. After completing surgery make sure that the room/area in which you have been working is left clean and tidy.

b Preparation of Instruments and Consumables

- All surgical and/or consumable equipment should be ordered and checked to ensure availability, suitability and functionality well in advance of surgery. It may be necessary to acquire spares of some instruments in case of breakage or malfunction.
- Check that there are sufficient medicines and gas supplies for the whole procedure. Extra gas cylinders may be required for prolonged surgeries. Remember to check expiry dates: out of date medicines should not be used; fresh dilutions of medicines should be made each day.
- All instruments must be sterilised (packed and autoclaved) before use.
- Similarly all consumables (e.g. swabs, needles, suture materials) used during the procedure must be sterile and should be of an appropriate size and packaged in suitable quantities.
- Check that the sterilisation process (e.g. autoclaving, irradiation or other suitable method) has been effective, for example by checking the indicator/mark on packaged instruments and checking that the outer packaging has not been breached.
- It is good practice to use a new set of sterile instruments for each animal to avoid spreading infection between animals. Therefore, if multiple animals are undergoing surgery during any one session, ensure that you have a sufficient number of sterile instrument and consumable kits available for use before you start. Alternatively, instruments can be sterilised between animals; if this is the preferred procedure then do not start to prepare the next animal until the instruments required have been adequately sterilised.
- A 'bead steriliser' may be used during surgery to sterilise the tips of instruments. However, it is not good practice to use a 'bead steriliser' to replace autoclaving or other means of sterilisation, as 'bead sterilisers' sterilise only the instrument's tip, so the possibility still remains that the surgeon's glove may be contaminated by the non-sterile handle of the instrument during handling.
- The use of other methods of sterilisation, such as the use of alcohol/disinfectant dipping, although currently widely used, are not considered good practice and should be discouraged.

c Dressing for Surgery

- All hair should be covered; a suitable cap should be used to cover the hair on the head (this should be applied first).
- The mask is applied before scrubbing, gowning and gloving. Bearded licensees should use masks which are large enough to cover their facial hair. A face mask is worn primarily to protect the licensee from allergy and, for some species, the animal from potential infection from the licensee. Immunocompromised animals may be more susceptible to infection via this route.

- The surgeon must remove all jewellery from hands and wrists and perform a thorough scrub of hands and nails. Finger nails should be cut short and cleaned before starting to 'scrub up' for surgery.
- Typically a commercial product, such as Hibiscrub[®], is used which recommends that users wet their hands and forearms, apply 5ml of chlorhexidine skin disinfectant and wash for one minute cleaning the fingernails with a brush or scraper. Rinse, apply a further 5ml and continue washing for a further 2 minutes. Rinse thoroughly and dry. For other products use the same principles but follow the manufacturers' instructions for volumes and contact time. During the 'scrub up', hands should be held with the fingertips uppermost and the hands held higher than the elbows so that water runs away from the fingers down the arms thereby reducing the risk of re-contaminating the fingertips. Hands should be dried using a sterile towel starting with the hands and finishing with the elbows.
- Alternatively, instead of an aqueous scrub, an alcohol rub containing additional active ingredients (e.g. chlorhexidine) could be used, as a recent report¹¹ demonstrated a comparable level of antisepsis.
- A sterile, clean, long sleeved operating gown should be worn during surgery. The gown should be put on by the 'scrubbed' surgeon and the gown ties carefully passed to an assistant to tie at the back.
- Sterile gloves should be worn. The outer packet of gloves must be opened either by the surgeon before scrubbing up or by an assistant. To prevent contamination, the internal sterile packing containing the gloves should be touched only by the 'scrubbed' surgeon and the gloves unpacked without touching their outer surface^(v).
- Once the surgeon is wearing sterile clothing and gloves, care must be taken, throughout the procedure, to avoid touching non-sterile items such as the table, animal, anaesthetic equipment or operating lights. Ideally an assistant should be available, throughout surgery, to move the animal, adjust the table and any non-sterile equipment and to assist with monitoring depth of anaesthesia and making adjustments as required. For these reasons the use of an assistant is strongly recommended but if an assistant is not available, or if the surgeon needs to make fine adjustments to equipment such as the vaporiser or operating microscope, the adjustable knobs should be covered with suitable sterile material, such as foil, or plastic covering before surgery starts. If working alone cannot be avoided, the surgeon must take particular care not to touch non-sterile materials. If this cannot be avoided or happens accidentally, the surgeon must at least change his/her contaminated gloves for a new sterile pair.
- Where it is suspected that instruments may have been accidentally contaminated, these must be replaced with sterile ones before continuing or, if the surgeon believes only the tips of the instrument have been contaminated, a 'bead sterilizer' may be used.
- 'Non-scrubbed' surgical assistants must not touch sterile instruments, drapes or consumables.

d Preparing the Animal for Surgery

- The general health and clinical condition of the animal(s) should be checked at least 24 hours prior to surgery to ensure that each animal is in a fit state for the proposed surgery (e.g. food and water intake, urine and faecal output and appearance of the animal and in the case of larger animals, this may include body temperature and auscultation of the heart and lungs).
- Weigh animal(s) immediately prior to surgery (for growing animals it is important to establish the rate of growth, 2 or 3 weighings prior to surgery should be sufficient to monitor the growth curve, which is required in order accurately to assess that all is well with an animal post-surgically).
- Where possible and when appropriate, the animal should be anaesthetised in a room which is separate from holding rooms which house conscious animals. However, there may be some species, such as pigs and sheep, which benefit from the administration of the pre-operative sedative in their home pen because they become anxious when moved to unfamiliar surroundings. Minimising stress will have a positive impact on anaesthesia. Advice should be sought from the NVS if you are unsure what is best for the welfare of the animal which you are anaesthetising.
- Following the induction of anaesthesia, sufficient hair must be clipped from the incision area to expose the surgical site, allow adequate skin preparation to prevent hair ingress into the incision during surgery and afterwards during wound healing. The size of the clipped margin around the incision site will depend on the size of the animal and hair length but, to reduce the risk of hypothermia, this should be the minimum compatible with achieving the objectives outlined above. Hair removal should be undertaken with care because inappropriate preparation of the incision area can make the skin more susceptible to infection through disturbance to the delicate skin microfloral ecosystem, grazes and other skin surface damage. For this reason shaving the surgical area is now no longer considered good practice because of the potential for damage to the skin****. Pre-operative hair removal using an electric clipper appears to have decreased the risk of deep wound infection compared with razor hair removal^{12, 13}. As recommended in the Home Office Codes of Practice, clipping should be done outside the operating theatre, or in an area from which airborne particulates cannot escape, in order to prevent contamination of the surgical area/facility with hair, dander and associated microbes. Where a separate area is not available, an alternative is the use of a small portable vacuum cleaner to capture the dander as the animal is clipped, although this is not considered good practice.
- Preliminary skin preparation should also be done in an ante-room or an area such as a containment cabinet and not in the surgical theatre/area.
- The skin must be washed then prepared with a suitable topical solution (e.g. dilute chlorhexidine or povidone-iodine). These solutions should be used

**** *Shaving refers to the removal of hair using a sharp razor blade or scalpel. Clipping refers to the removal of hair using an electrical clipper. Surgical clippers offer benefits over razors as the cutting blade is on top of the stationary blade, away from the skin, so there is minimal risk of nicks or cuts. Clippers should be cleaned after use, using a medical clipper spray, and maintained by following the manufacturer's maintenance guidelines.*

sparingly and not allowed to soak the animal. Where possible, solutions should be warmed to body temperature; this increases their effectiveness and reduces the risk of causing hypothermia. The use of warm solutions is especially important in small animals, such as rodents, or sparsely haired animals, such as pigs. Where surgery is to be performed on non-mammalian species, it is essential to discuss peri-operative preparation of the animal with the NVS or other suitably qualified person.

- If neuromuscular blocking agents (NMBs) are required, check the project and personal licence authorities and the additional standard conditions applied to these licences. Seek advice and assistance from the NVS until competent in the use of NMBs and how to monitor and maintain animals where NMBs are used. When NMBs are used an assistant, preferably a trained anaesthetist, should be present at all times.

4 SURGERY

- a** Final skin cleansing and preparation should be completed in the surgical theatre, just prior to draping. Avoid the over application of alcohol, especially to small rodents, because chilling will occur as the alcohol evaporates.
- b** Sterile drapes of a sufficient size must be used to cover unprepared parts of the animal and adjacent surfaces. Proper draping will provide sufficient space for the surgeon to use instruments and suture materials without accidentally contacting non-sterile items or surfaces. The use of transparent disposable drapes over the animal can aid anaesthetic monitoring.
- c** Instruments should either be placed on a sterile surface (e.g. plastic sterile drape) or on a sterile tray.
- d** During surgery it is preferable and highly beneficial for the surgeon to have an assistant on hand to pass him/her extra materials, to adjust equipment (e.g. operating microscope) and to help with the surgery (e.g. retraction of skin edges and organs). A 'scrubbed' surgical assistant must be surgically attired (e.g. sterile gown and gloves) if assisting with the surgery itself. If it is not possible to have a 'non-scrubbed' assistant present, sufficient consumables must be laid out on a sterile drape/tray prior to the surgeon commencing the procedure.
- e** Ensure that the animal is adequately anaesthetised before making any incision. A withdrawal response to toe web pinch (suitable to assess in most species) or brisk eyelid reflex (not appropriate for rodents) means that the animal is able to perceive pain and is not sufficiently anaesthetised for surgery. Alternatively for small rodents, the tail pinch reflex can be used (an increase in respiration rate following a sharp pinch of the tail base indicates that the animal can still feel pain). If the surgeon is making this assessment s/he must avoid contaminating gloves or instruments (e.g. carry out pinch through a sterile drape). Alternatively, the 'non-scrubbed' assistant may perform this test.
- f** Maintenance of body temperature during surgery is essential for all species. Particularly for small and/or sparsely haired animals, external heat (e.g. a warmed gel pad or thermostatically controlled electric pad) should be used during surgery to prevent heat loss; in other species, cooling may be required. Body temperature should

therefore be monitored throughout the surgical procedure and normal core body temperature maintained by the external application of an appropriate heat or cooling source and application of insulating materials (e.g. bubble wrap) as appropriate. In aquatic species it may be necessary to keep the skin moist.

- g** Physiological parameters should be monitored, recorded and, as necessary, acted upon throughout the surgical procedure; including breathing, circulation (e.g. heart rate and blood pressure), body temperature and response to surgical stimulation. Physical checks may be supplemented by monitoring instruments such as electrocardiographs, pulse oximeters, capnographs or blood pressure monitors, especially in animals over 10kgs.
- h** Always apply a corneal lubricant to the eyes to prevent drying, especially for prolonged surgical interventions unless the procedure only lasts a few minutes or surgery involves the eye.
- i** For warm blooded animals, sterile, physiologically-buffered fluids warmed to 37°C may be given (preferably by the subcutaneous, intraperitoneal and on occasion, intravenous routes) during and/or after surgery to prevent dehydration and aid recovery. This is particularly important for procedures lasting longer than 30 minutes, or where a body cavity is opened. The use of all intravenous fluid treatment should be carefully monitored to minimise the risk of over-hydration which could result in pulmonary congestion and pneumonia. Consult your NVS for advice.

5 RECOVERY AND POST-OPERATIVE CARE

- a** The animal should be allowed to recover in a separate area or room⁽ⁱⁱⁱ⁾, which is clean and quiet. It is essential to maintain the body temperature of the animal during the recovery period, as the anaesthetic agent impairs the animal's ability to thermoregulate. The ideal temperature is at the upper end of the animal's thermoneutral zone. This can be achieved by using a temperature-regulated environment; direct heat from lamps is not suitable as this may result in inadvertent thermal injury.
- b** No animal should be left unattended before it has recovered consciousness, is able to remain upright and protect its own airway without assistance.
- c** Unconscious animals should not be placed in a cage with conscious animals, although it is acceptable and recommended to re-group once the animals are fully conscious. Some establishments re-group certain species, such as rabbits, before they are fully recovered from anaesthesia because they believe that the animals benefit from minimal time away from their established groups^{14, 15}. Where this is the practice, senior care staff and the NVS are likely to be responsible for ensuring that sufficient well trained staff members are available to monitor the animals until they have fully recovered and are exhibiting normal behaviour. **Note:** this takes time and will require forward planning.
- d** During recovery, and sometimes in the first few days after surgery, soft bedding materials such as paper or lint-free fabrics should be used instead of normal bedding material, to prevent particles entering the eyes or the wound.

- e** Soaked diet, transgel, baby foods or small pieces of fruit and vegetables may be provided to promote feeding and assist adequate fluid intake. Such interventions (if different from the norm) should be planned and introduced for a short period before the surgical interventions commence so the animals have become accustomed to the post-operative diet before the surgery takes place.
- f** Check the animal at appropriate intervals post-operatively, to assess the state of the wound and any continued analgesic requirements. If a licensee has any doubt about the health or welfare of the animal they should immediately seek advice from the NVS and/ or the NACWO.
- g** Remove non-absorbable skin sutures or clips usually 7 to 10 days after surgery, or as advised by the NVS, as the interval can be dependent on the species of animal and position of the wound (e.g. if the wound is in an area of the body where the skin is especially mobile). Alternatively, consider the use of absorbable suture materials or tissue glue. **Tip:** at the time of opening a new bottle of tissue glue, the opening and discarding dates should be written on the bottle because the shelf life is limited.

6 MONITORING OUTCOMES OF SURGERY

- a** Even when surgeons are trained and competent, surgical abilities can vary widely. Therefore, it is important to monitor standards for each surgeon by keeping accurate records to facilitate review; e.g. mortality figures, infection and wound breakdown rates, requirements for antibiotics, analgesic usage and the clinical signs observed following completion of each procedure. Acceptable success rates should be benchmarked for each type of procedure and surgeons who perform poorly should either re-train or refrain from operating on animals.
- b** Licensees should keep abreast of progress in surgical techniques and models through literature review, discussion with colleagues and the Named Persons in order to apply refinements to their procedures.

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