Chapter

Implementing Good Practice in Aseptic Technique for Surgery in Laboratory Animals: Recommendations by the European Academy of Laboratory Animal Surgery (EALAS)

Bertrand Lussier, Jaco Bakker, Delphine Bouard, Kate Read and Lucy Whitfield

Abstract

Surgery in laboratory animals is an orphan field of veterinary medicine. Nevertheless, it is of the utmost importance in the development of new drugs, including their pharmacokinetic/pharmacodynamic properties, their toxicity, and their efficacy. It is also an inherent part of animal studies for the evaluation of medical devices for regulatory submission, thus improving public health. Application of the 3Rs (Replacement, Reduction, Refinement of animal use) allows surgeons to ensure that animal welfare is optimal and that unnecessary suffering is reduced to an absolute minimum while still achieving scientific objectives. In addition, surgery as a field has been subjected to stringent reviews by both ethical committees and peer review of scientific research, which has obliged us to greatly refine our surgical techniques, resulting in improved and more reproducible outcomes. This chapter will discuss the implementation of good practice in aseptic technique for surgery in laboratory animals including small (mouse, rat) and large animal models (rabbit, sheep, pig, and non-human primates).

Keywords: asepsis, surgery, laboratory animals, recommendations, European Academy of Laboratory Animal Surgery

1. Introduction

The Canadian Institutes of Health Research [1] defines biomedical research as the following: research with the goal of understanding normal and abnormal human functioning, at molecular, cellular, organ system, and whole-body level. This includes the development of tools and techniques that can be applied for this purpose: developing

new therapies or devices that improve health or the quality of life of individuals, up to the point where they are tested on human subjects. To reach these objectives, investigative teams must rely on the responsible use of animal models, which is regulated by national and supranational agencies. As well understood, we must follow and adhere to the principles of the 3Rs as stated by Russel and Burch [2]: Refinement, Reduction, and Replacement. The literature is sparse on implementation of good practice of aseptic techniques in laboratory animals. In addition, published guidelines on good surgical practices are few [3, 4] and often rely on individual institution guidance. Therefore, the European Academy of Laboratory Animal Surgery (EALAS) has drawn upon the expertise and extensive practical experience of their working group in the production of this chapter. The EALAS consists of forward-thinking professionals who promote the advancement of professional and academic standards and the education and research in the arts and sciences of surgery in laboratory animals. Additionally, EALAS promotes the humane use and treatment of animals in research and to prevent their use when other means can meet the scientific objectives. This chapter will describe the implementation of good practice of aseptic techniques in surgery in laboratory animals.

2. Why should we optimize our techniques?

When subjecting animals to experimental protocols, one must try to optimize animal welfare, along with the scientific results, by using strategies that ensure that the most robust and reproducible data are obtained. Such processes must include good laboratory practice (GLP). This quality system of management controls for experimental settings is to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of the data so that they can be analyzed and conclusions drawn.

When implementing good practice in aseptic technique for surgery, one minimizes pain or distress and optimizes animal welfare. In surgery, when strictly adhering to aseptic techniques, one minimizes complications. This decreases pain or distress, potentially decreasing the size of the cohorts utilized by minimizing attrition of groups secondary to infections and other surgical complications. By refining surgical protocols, one also increases the uniformity of groups by increasing reproducibility, hence decreasing the number of outliers and increasing 'signal to noise' ratio in data. Most importantly, aseptic technique is required for survival surgery since animals can develop either gross or inapparent surgical site infection that may affect both research outcomes and animal welfare [5]. The success is not measured merely by survival of the animals but by survival and the absence of alteration of normal physiology, immune responses, and behavioral changes. The presence of bacterial contamination activates macrophages and increases circulating levels of cytokine and B lymphocytes. This contamination may result in sub-clinical infections, which will result in the alteration of normal physiology and immune responses [5, 6]. Furthermore, subclinical infections and their consequences, including animal suffering and variable effect on scientific data, seem to be overlooked by researchers.

3. How should we optimize our surgical techniques?

The classification of surgery often focusses on the procedure itself, whereas it is the whole peri-operative process that should be considered. These can be divided into direct and indirect measures:

3.1 Direct measures

Besides the surgical procedure, it comprises both pre- and post-operative measures. Pre-operative measures consist of the preparation of the patient, of the equipment and material, of the surgical team, and of the surgery room. Post-operative measures consist of the post-operative care and follow-up.

3.2 Indirect measures

These comprise strict asepsis, atraumatic tissue manipulation, precise and meticulous hemostasis, accurate and tension-free tissue apposition while preserving vascularization, judicious use of suture materials, and obliteration of dead spaces.

These principles were endorsed by William Stewart Halsted, an American surgeon and founding professor of the US residency training system, as early as 1880 [7]. Halsted's contributions to surgery were numerous, but specifically he emphasized the importance of strict aseptic surgical technique in a successful surgical outcome. Surgical asepsis is defined as all the measures that are taken to reduce bacterial contamination at the surgical site, resulting in a reduction in sub-clinical and clinical infections. The essential strategies in different species that can be used to refine surgical techniques to meet the aim of ensuring robust science and good animal welfare will be outlined below.

4. The surgical facility

The requirements for the implementation of aseptic technique and the standards for a surgical facility are set out in guidelines [3, 5, 7] . Surgical theaters should be a dedicated space, whose layout allows for physical separation of animal preparation, sterile procedures, and recovery.

- Surfaces should be constructed of a material that can be adequately cleaned and disinfected on a regular basis.
- Open shelving and non-essential equipment should be removed to prevent accumulation of dust and debris.
- The workspace should be large enough, and arranged appropriately, to allow for proper aseptic technique to be implemented. It is recommended that the animal preparation area and surgeon preparation area are separated in two distinct zones.
- Presence of non-essential personnel should be avoided.

In addition to the physical space utilized for sterile procedures, thorough planning will also contribute to the successful application of aseptic technique. Aseptic technique requires co-operation from all personnel; as such, it is imperative that those involved understand their roles and that training requirements are identified and addressed prior to start of the surgery. The utilization of a non-scrubbed assistant is considered good practice for surgeries. A well-developed surgical plan will ensure that all relevant equipment and consumables are available and sterile, according to surgical need.

5. Implementing good practice in aseptic technique for surgery in small animal models

EALAS has developed the following guidelines, according to evidence-based good clinical practice, including a modification of Halsted's principles [7]. The guidelines are simple, efficient, and cost-effective to apply. The same principles must be applied for small and large animals. However, in small-animal models, because of their size, some techniques (like four-corner draping) cannot be applied (see sub header 6 below).

5.1 Strict asepsis

5.1.1 Surgeon preparation

- The surgeon should wear appropriate attire (scrub top and bottom, dedicated shoes, or shoe covers).
- A cap and a mask must be worn for all surgical procedures, starting at skin preparation, or when a sterile kit is opened in the surgery suite.
- For most rodent surgeries, as the forearms come in contact with the sterile field, we recommend using a sterile gown as shown in **Figure 1**. Sterile sleeves can be used as an alternative.
- Hand preparation
    - Even if sterile gloves are worn, hand preparation must be performed because evidence shows that although the incidence of glove perforation is high (30–50%) [8, 9], perforation detection by surgeons is poor (approximately

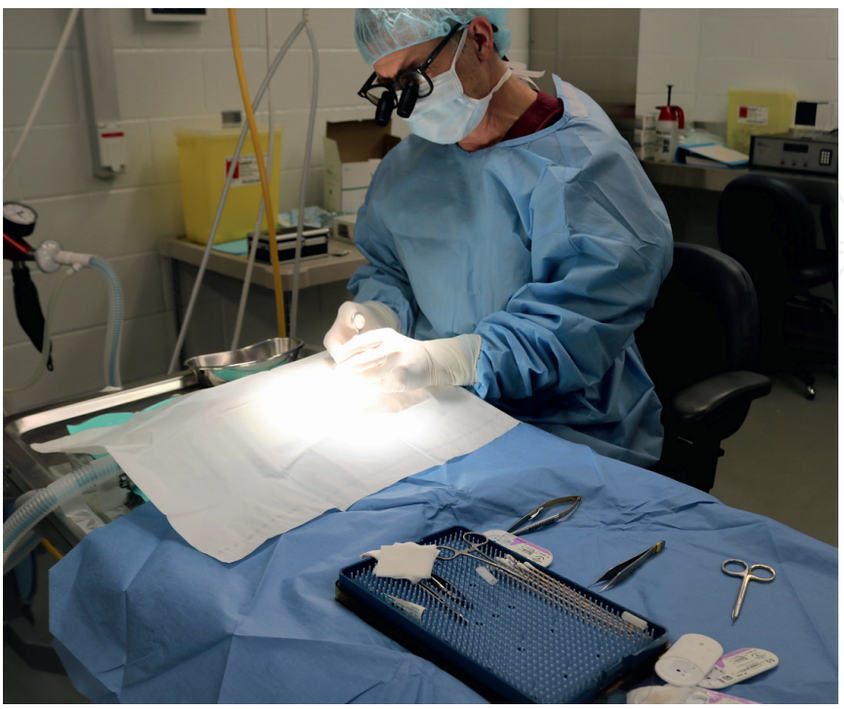


Figure 1.
Surgeon wearing proper attire: cap, mask, sterile gown and sterile gloves.

10%). Glove perforation seems to correlate with the duration of wear [9]. The use of a hand cream that contains a suspension of cornstarch and ethanol has no influence on the rate of microperforation [9].

- Two accepted methods of hand preparation are:

  1. Three- to five-minute scrub with an agent with a residual effect: either 4% chlorhexidine gluconate or 10–13% povidone-iodine

  2. Proceed with a good hand wash with soap, rinsing with water, and drying hands. Subsequently, the surgeon applies an alcohol-based hand rub (ABHR) (either 70% alcohol solution or a 70% alcohol and 1% chlorhexidine solution). Application on dry hands is important: applying gel to wet hands dilutes the ABHR and decreases its efficacy.

- Sterile surgical gloves must be worn. EALAS does not recommend wearing non-sterile gloves (nitrile or latex) and using a "tips-only" technique, as in our experience, it is extremely difficult to maintain asepsis using this technique. In addition, the use of ABHR or alcohol wipes on non-sterile gloves is not sufficient to sterilize them and is therefore not recommended. It also damages the integrity of gloves [10, 11].

- Where serial ('batch') surgeries are carried out, the surgeon must change gloves between each animal: the used gloves are taken off, ABHR is applied on the hands, and subsequently a new pair of sterile gloves is put on.

5.1.2 Patient preparation

- Two distinct zones should be established as shown in **Figure 2**. The animal is prepared in a zone, separate from where the surgical procedures will be performed. The first zone is the preparation zone, and the second zone is the surgery zone. A water-circulating heating pad or similar warming device should be used in both zones to prevent hypothermia in the animal. Non-medical electrical heating pads should be avoided, as they can result in thermal burns and injury to the animal, because of uncontrolled temperature fluctuations or 'hot-spots' in the pad. Appropriate thermoregulatory methods include:

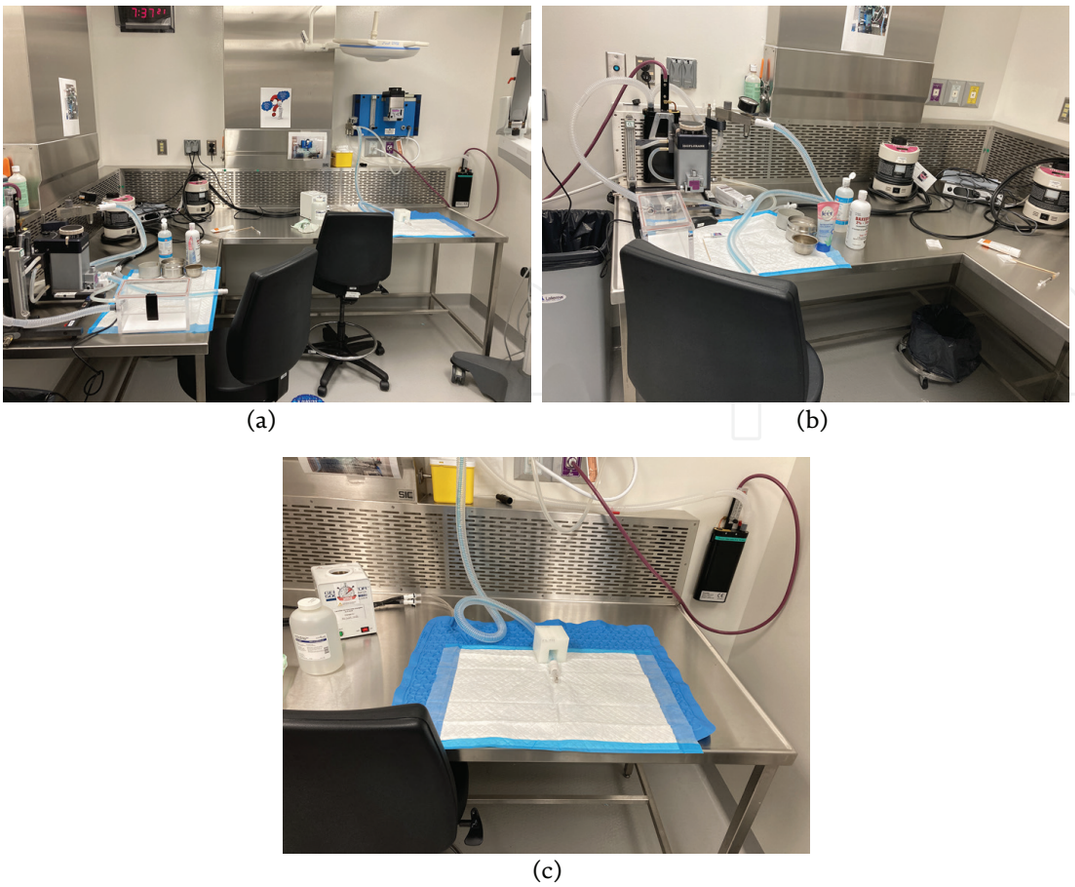
  - Equipment designed for use with animals (heats to no more than 38°C; heat pad temperatures of 40°C and above will result in burns).

  - Thermostatic feedback heat mat system.

  - Insulating material placed between the animal and the heat pad (e.g., fleece) to standardize the temperature and prevent contact with hot-spots.

  - Thermometer probe placed between the animal and the heat pad to measure the actual temperature that is in contact with the animal.

- The surgical team should consist of a minimum of two people: one person prepares the animals and acts as non-sterile assistant, and the other person performs the surgical procedures. This strategy decreases the risk of contamination.



(a) (b)

(c)

Figure 2.
Recommended set-up for preparation and surgery using 2 zones: a) general overview; b) preparation zone and c) surgical zone.

- Prepare a large surgical field. Hair must be clipped just before surgery using a clipper with a #40 clean, cool, and sharp blade. Clipping hair the day before surgery is not recommended as it causes microtrauma to the skin and results in bacterial overgrowth, thus increasing the risk of surgical site infections [12].

- A depilatory agent can be used if deemed necessary; use a non-irritant (e.g., "sensitive skin") brand for a short time and remove using sterile or demineralized water. We recommend using a timer to ensure that application time is a maximum of 30 seconds, to prevent over-long skin contact. Depilation can be repeated with care if the first application is not satisfactory.

- Once the surgical area is removed of its hair, several protocols can be used for skin disinfection. We propose that one of the following should be used:

  1. One to three alternate passages of antiseptic solutions, such as 70% alcohol alternating with either a 10% povidone iodine solution or a 2% chlorhexidine solution.

  2. Using a combination solution of alcohol (70%) and chlorhexidine (1–2%) in a single passage, which is left in place to dry. This technique is efficient and cost-effective and can reduce chilling of the animal due to application of skin preparation [13].

- Hypothermia associated with skin preparation can also be avoided by placing the antiseptic solutions on the heating pad. This allows them to warm to body temperature prior to use.

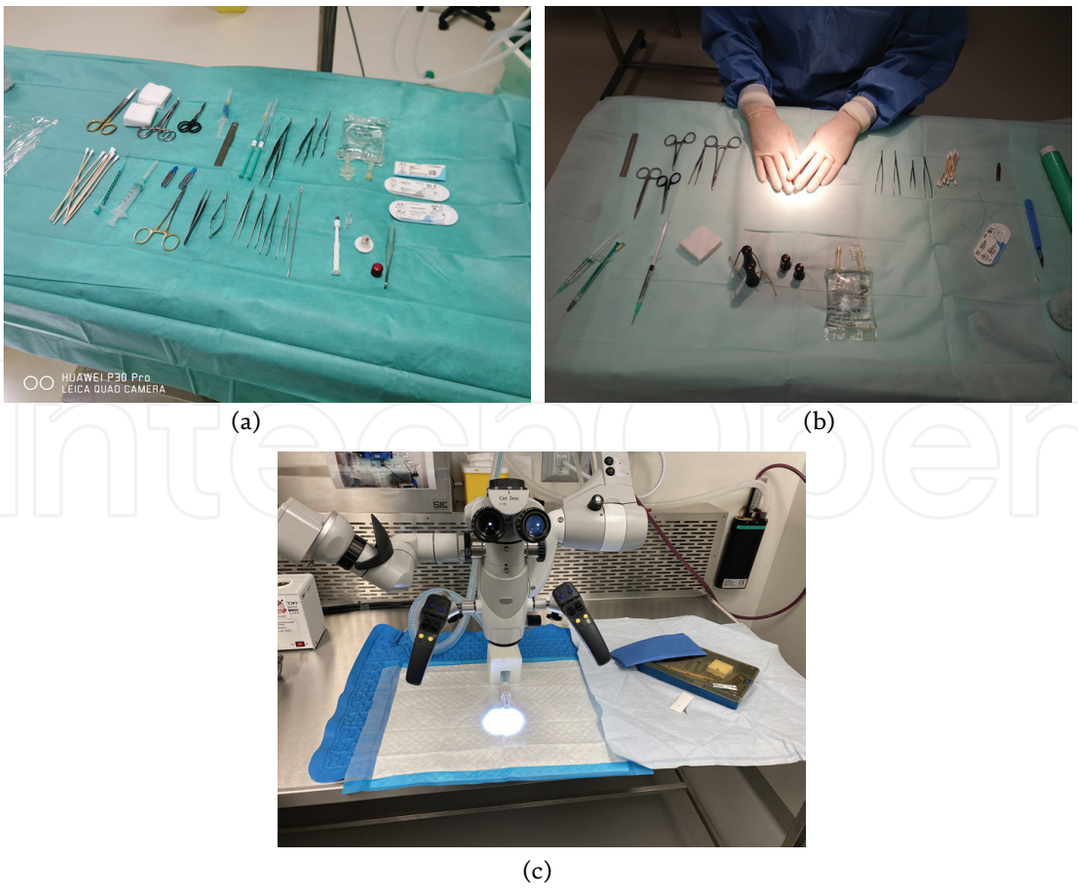
- The prepared patient is then transferred by the assistant to the surgical zone and appropriately positioned; a final application of antiseptic solution can be made to the surgical site.

5.1.3 Establishment of the surgical field

- The surgical assistant passes the sterile instruments to the surgeon, who places them onto a sterile surgical field or drape (**Figure 3**). The large wrapping used for sterilization (autoclave) of surgical kits can be used as a sterile surface.

- Establish continuity between the surgical field and the instruments, so that there's no gap between the operating field and the instruments as shown in **Figure 4**. Discontinuity of the surgical field is a source of contamination.

- A sterile field is used to cover the patient. Several strategies can be used:



(a) (b)

(c)

Figure 3.
Instruments on a sterile field: a) and b) instruments and material laid out on a large sterile disposable drape; c) microsurgical kit sterile drape opened to act as a sterile field for microsurgery on mice.

- A large commercial disposable field that is at least twice the dimension of the length of the suture is used. An opening is cut into the drape to create a fenestrated drape (see **Figure 5**).

- Sterile (fenestrated) drapes.

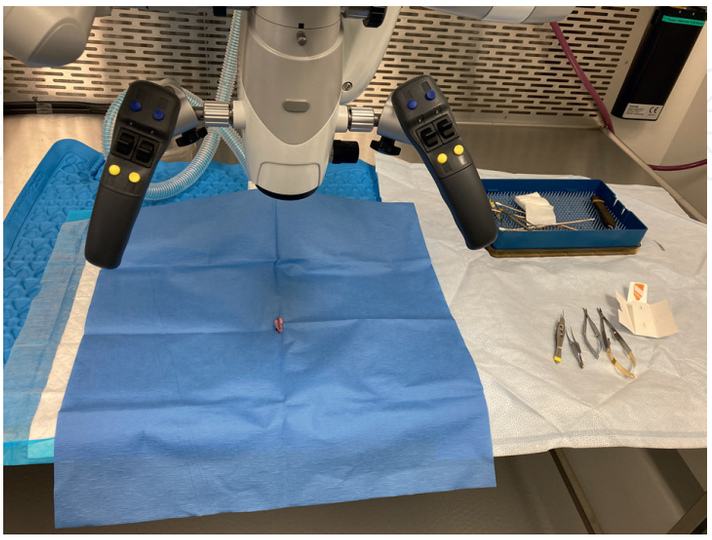


Figure 4.
Recommended layout illustrating continuity between the surgical site and the instruments.

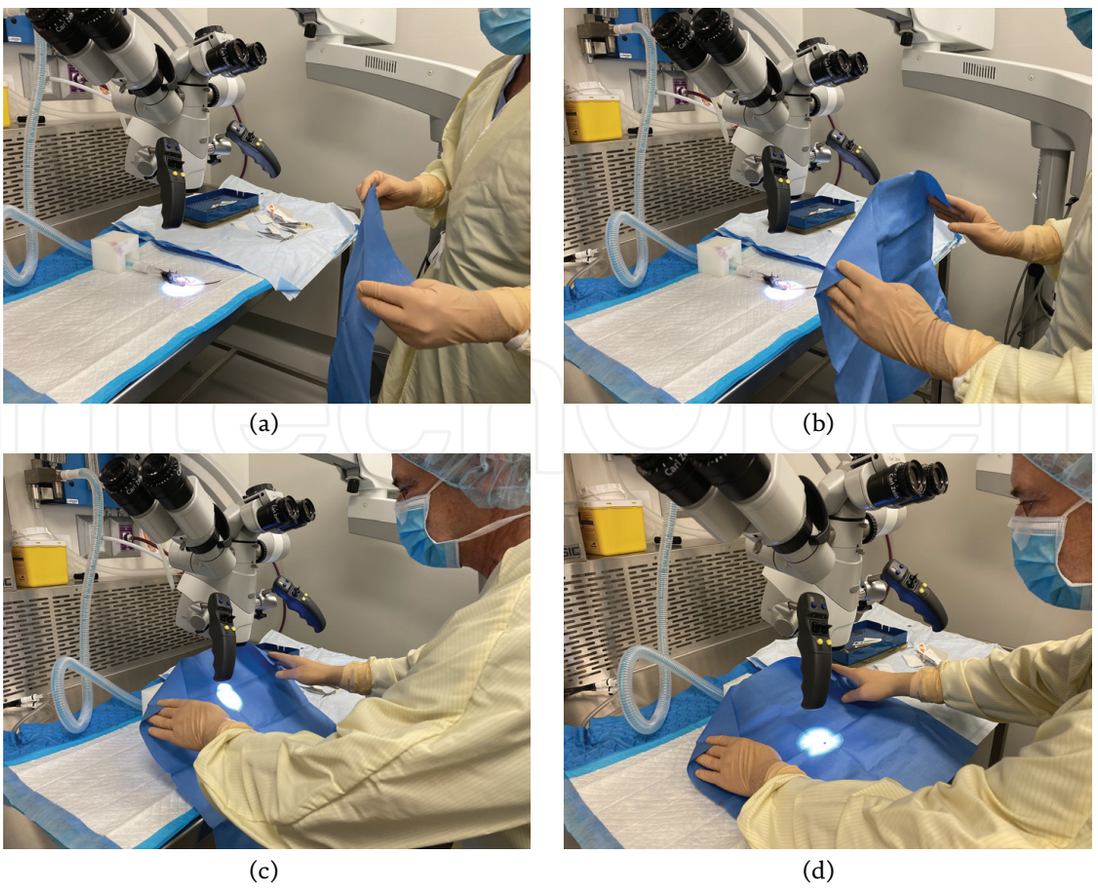


Figure 5.
(a-d) Different steps to establish a sterile field by using a sterile drape through which an opening has been cut.

  - All alimentary films can be sterilized by individually wrapping each roll in self-sealing envelopes, such as autoclave pouches. Where a number of rolls (e.g., 80–100 rolls) are irradiated together in a validated center, it can present an affordable and economical drape material [14, 15].

- Challenging surgery procedures: access to more than one incision site, for example, when putting in place a vascular access button in rodents. In this example, access to the ventral cervical area is followed by access to the dorsal interscapular area. The sterile field can be maintained by wrapping the drape or alimentary film completely around the rodent, enabling manipulation (**Figure 6**).

Figure 6.
Whole body wrapping of a rat with sterile alimentary film.

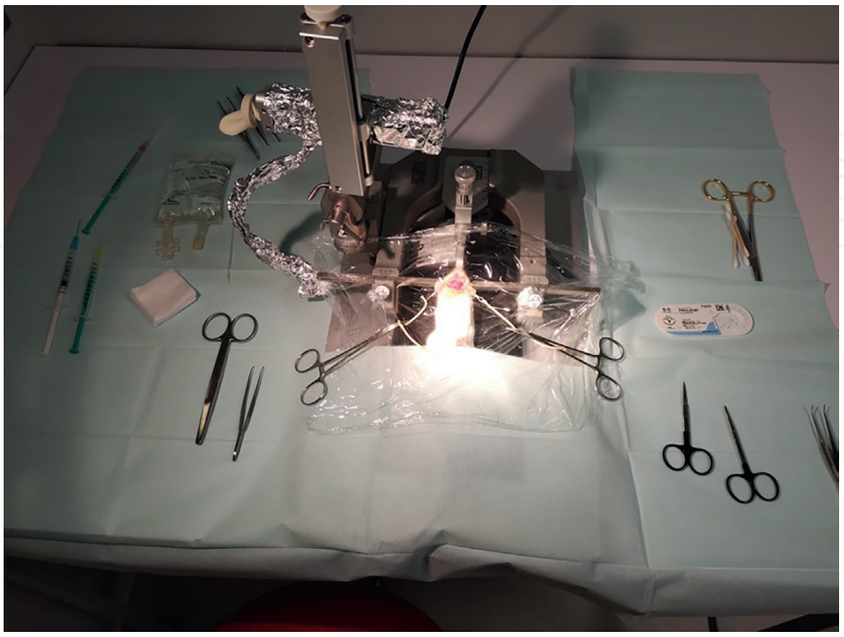


Figure 7.
Combination of sterile drape, sterile alimentary film and sterile aluminium foil to establish a sterile field for stereotaxic surgery.

- Sterilized aluminum foil [16] and other sterilizable materials can be used to cover parts of anesthetic machines, knobs, buttons, hand drills, and other material that cannot be sterilized (**Figure** 7).

5.1.4 Instruments

- Sterile instruments must be used.
- EALAS recommends using validated protocols for sterilization of instruments and materials. These include steam sterilization in an autoclave or chemical sterilization using ethylene oxide (ETO). These processes are rigorously defined by their cycle parameters tailored to the type and nature of the load, including factors such as material porosity and bio-burden level.
- It is essential to use defined cycle parameters and validate the sterility of each sterilization cycle (process) through rigorous quality assurance procedures. Verification techniques often involve the use of biological indicators, such as spore strips, and chemical indicators, like color-changing integrators or enzyme-based tests, to confirm the adequacy of the sterilization cycle (process). Moreover, adequate packing of the items is also critical to safeguard their sterile state until they are used in surgery.
- Autoclaving entails the exposure of clean and dry instruments to pressurized steam at regulated temperatures, commonly 121°C at 120 KPa for 30–45 minutes or 132°C at 210 KPa for 4 minutes. Sterility indicators are mandatory for tracking the sterilization status of these instruments.
- Thermal-sensitive components, such as certain plastic materials in devices like stereotactic frames, may deteriorate under these high-temperature conditions; instead, a chemical process, ETO sterilization, is advantageous for such items. This gas has the capacity to penetrate and diffuse through a variety of equipment types such as tubes, catheters, endoscopes, cameras, or frames. However, it requires extended cycle times (around 2½ hours) due to the need for ETO gas evacuation from the material after sterilization. Furthermore, the ETO sterilization process entails significant cost and potential risks to both patients and healthcare staff.
- Hydrogen peroxide gas plasma (HPGP) sterilization may be utilized for disinfection of some thermal-sensitive material and instruments, but note that it only achieves surface sterilization; it does not diffuse through material. It is efficient against a broad spectrum of microorganisms. The cost is less than ETO, and it is relatively safe for both patients and healthcare staff.
- Peracetic acid sterilization utilizes a potent oxidizing agent that retains its biocidal effectiveness even in the presence of organic matter. It is particularly used for the reprocessing of endoscopic equipment, effectively degrading organic residues, such as proteins, and is prevalent in the reprocessing of gastrointestinal and other flexible endoscopes in the United States.
- EALAS does not recommend the use of alcohol for the sterilization of instruments. Guidelines state that the immersion (dunking) of medical instruments in alcohol is not a recommended practice for sterilization [17].

- EALAS does not recommend using the "tips-only" technique (touching the surgical site with the instrument tips only) because it is very difficult to maintain sterility using this method.

- For serial surgeries, use a different surgical kit for every patient.

- Many procedures require relatively few and basic types of instruments. Provision of several sterile kits for batch/serial surgeries is straight-forward to arrange and relatively inexpensive and will enhance workflow when operating on several animals in sequence.

- To optimize recycling of surgical kits for use in subsequent procedures, flash sterilization can be used. It takes only 4 minutes to sterilize instruments at 132°C and 210 KPa.

- When performing surgeries using implantable devices, such as catheters, or pumps, using strict aseptic technique is essential because a minor bacterial contamination during surgery can result in biofilm formation on the device and subsequent infection.

- When conditions or situations do not allow the use of a sterile kit of instruments for each patient, it might be acceptable to use the same sterile instruments for multiple consecutive surgeries. However, this should be discussed with your designated veterinarian in advance and appropriate practices and monitoring put into place to ensure the efficacy of asepsis [18]. If approved this is how it should be done:

    - Between each animal, for up to six animals total, the tips of the instruments need to be immersed in sterile deionized water, thoroughly wiped, and dried with a sterile gauze sponge. Subsequently, the clean instrument tips need to be sterilized by using a glass bead sterilizer (GBS). There are two types: 233°C (8–12 seconds) and 250°C (2–5 seconds). Allow the instruments to cool at room temperature before being reused.

- Using the tips-only technique and/or combining ethanol wipe and GBS are not recommended [19].

5.1.5 Consumables for surgery

- All consumables, that is, needles/syringes/suture material, that are required to be handled by the surgeon should be sterile. Most disposable consumables will be provided in single-use sterile packaging, while others can be packed and sterilized appropriately.

- Sterile packaging must be opened in a manner that does not contaminate the inner contents during the opening process.

5.2 Other surgical principles

If at any point it is suspected or known that the sterile field has been breached, action must be taken immediately to avoid further contamination. This may involve

replacement of sterile gloves, drapes, equipment, or consumables. If an instrument is known to be contaminated during the procedure, it can be replaced if possible or re-sterilized if replacement is not an option.

This chapter mainly concentrates on aseptic technique, but one must not forget that a successful surgical outcome also involves other principles. Briefly, here are a few:

5.2.1 Atraumatic tissue manipulation

- Use appropriate instrumentation: Small instruments may be suitable for use in rats, but microsurgical instruments are recommended for use in mice (and may also be suitable for rats).

- Use magnification, either with surgical loupes or microscopes.

- Pursue continual development of surgical skills, in order to apply the most up-to-date and refined surgical techniques.

5.2.2 Precise and meticulous hemostasis

- All bleeding eventually stops, but the use of active hemostasis is good surgical practice. Poor or absent hemostasis results in free blood within tissues, resulting in tissue dissection and seroma formation, and increases the incidence of surgical site infections. Hemostasis can be achieved by applying direct pressure, by using micro-hemostatic forceps, or by the use of cautery.

5.2.3 Accurate and tension-free tissue apposition while preserving vascularization

- Wounds should be sutured by providing precise apposition of wound edges, which permits optimal healing. Applying too much tension during wound closure causes eversion of the wound edges, which alters wound healing by decreasing vascular supply and causing indirect healing of wound edges. This is a particularly common issue in small rodents.

- EALAS does not recommend the use of metallic wound clips (Michel suture clips, non-adapted skin staples for human or veterinary use).

- Sterile, resorbable tissue glue can be used as an alternative to skin sutures.

5.2.4 Judicious use of suture materials

- Small animals require fine suture material to close wounds. The retaining force of a suture must be as strong as the tissue sutured, so large gauge sutures are not required to close fragile tissues.

- In general, users will close the wounds of rodents with sutures that can be easily seen by the surgeon, rather than applying the appropriately sized suture gauge.

- In mice, 7-0 and 8-0-gauge sutures are strong enough for all procedures. 6-0-gauge sutures might be used in large mice (>30 g).

- In rats, 5-0 and 6-0-gauge sutures are recommended.

5.2.5 Obliteration of dead spaces

- Dead space is created by excessive tissue dissection.
- Dead space, if not obliterated, will lead to the development of a seroma, which slows down wound healing, is a rich milieu for bacterial growth, and can lead to increased wound infections.
- The best way of preventing the creation of dead space is by limiting unnecessary dissection along tissue planes or layers.
- It is of the utmost importance to remove any dead space created during surgery by systematic closure of tissue planes, that is, body wall, subcutaneous, intradermal, and skin.

5.3 Specific information of stereotaxic surgery

Unfortunately, rodent stereotaxic frames cannot be easily sterilized. A simple disinfection of the frame's surface is not sufficient to prevent contamination of sterile items (e.g., gloves, consumables, and instruments) when they come into contact with the frame.

The stereotaxic frame should be draped to cover at least the horizontal bars (as shown in **Figure 7**). This is easily done using pre-sterilized drapes, alimentary films, or sterilized aluminum foil. Some parts of the frame, such as the anterior/posterior and lateral setting buttons of manual frames, that need to be regularly touched during the surgery must be covered with sterile protection to maintain the sterile field. The animal is prepared and draped as described in Sections 5.1.2 and 5.1.3.

When complex actions must be performed, such as connecting and disconnecting parts in the apparatus (mounting Hamilton syringes, for examples), the non-sterile assistant can wear a pair of sterile gloves to perform the non-aseptic actions. The surgeon can stay focused on the surgery.

Other non-sterilizable equipment, such as hand-held drills, can similarly be covered in sterilized aluminum foil so that the surgeon's gloves remain sterile while this device is used. The drill bits are sterilized in the autoclave.

6. Implementing good practice in aseptic technique for surgery in large animal models

The same precautions must be taken as in small-animal models, but in addition to the aforementioned precautions, the following must be respected:

6.1 Strict asepsis

6.1.1 Surgeon preparation

- If the surgeon needs to enter the room where animals are being anesthetized and prepared pre-operatively, then their attire (scrub suit) must be protected, for example, by a coat worn over the surgeon's scrub suit.

- A sterile gown must be worn for all surgical procedures.
- Hand preparation is to be done as previously described (Section 5.1.1)

6.1.2 Patient preparation

- The patient is prepared in a different room from the surgery suite.
- A large surgical field is prepared by clipping sufficient hair from the animal, using a #40 blade. The hair is aspirated from the patient and the table, and the clipped skin is cleaned with mild soap or 70% alcohol.
- Once the hair has been removed from the surgical area, the skin is disinfected using one of the following methods:
  - A scrub with 4% chlorhexidine or 10-13% povidone-iodine is done by short gentle brushing and leaving the antiseptic agent on site for 5 minutes. When applying this method, three passages of antiseptic solutions must be performed, alternating 70% alcohol with either povidone iodine 10% solution or 2% chlorhexidine solution.
  - A commercial preparation containing a combination of alcohol (70%) and chlorhexidine (1–2%) can be used in a single passage, which is left in place to dry.

The prepared patient is then transferred to the surgical room and appropriately positioned; a last application of antiseptic solution can be applied to the surgical site.

6.1.3 Establishment of the surgical field

- The instruments are passed to the surgeon using aseptic technique and laid out on a sterile surface, such as a surgical table or large adjacent surface covered with a sterile, non-permeable drape.
- A sterile field is created, which covers the patient. This is achieved in the following four steps:

1. Four-corner draping can be used to broadly define the surgical field. Drapes may be made of cotton, plastic-covered paper, or non-permeable material. The previous use of skin-penetrating towel clips to fix drapes has been replaced by the use of drapes with an adhesive band of various widths on one edge.

2. Then, a large non-permeable surgical drape is placed over the sterile field, covering the patient and the entire surgery table. Reusable fenestrated cotton drapes are not recommended because they allow bacteria to migrate through to the sterile surface due to enlarged pores (following repeated wash cycles). Furthermore, once cotton drapes become wet, they can increase bacterial contamination at their surface due to capillary "wicking" through the fabric.

3. Finally, a commercial adhesive plastic "incise" drape can be added to further decrease contamination.

4. Then, the instrument table is laid adjacent to the surgery table and continuity established between the surgery table and instrument table.

6.1.4 Instruments are used for a single patient only

All instruments and devices (e.g., electrocautery, suction) are changed between patients.

7. Specific information on non-human primate surgery

- All the above-mentioned processes also need to be applied when carrying out surgery on non-human primates (NHPs).

- The potential risks of exposure to zoonotic pathogens during surgery are considered high in Old World primates (which includes macaques such as rhesus and cynomolgus monkeys) compared to New World primates (such as marmosets). The increased concern with macaque exposure centers mostly around the risk of surgeon exposure to Herpes B virus (Herpesvirus simiae) in NHPs from potentially B-virus-positive colonies. Therefore, N95 facemasks in combination with eye protection devices, such as goggles or glasses with solid side shields or chin-length face shields, should be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated [20–22].

- Providing medications to NHPs is challenging because they are difficult to handle for daily injections and notorious for trying to avoid medicated food. Training to accept handling and treats can be implemented before surgery to facilitate post-operative care.

- In addition, the formal hierarchy in some NHP species, such as macaques, involves social conflicts and aggression, resulting in severe wounding. Therefore, one should keep in mind that separating an animal from a group, or its buddy, for surgery or treatment can worsen these social conflicts, particularly in debilitated individuals, when they are returned to the rest of the group. Therefore, application of long-acting antibiotics, non-steroidal anti-inflammatory drugs (NSAIDS), and other analgesics (opioids) is recommended, so that individuals can remain within their usual social groups with minimal disturbance [23–27].

- NHPs may interfere with external skin sutures, so using intradermal sutures is recommended, which reduces the risk of post-operative wound opening.

8. Summary

The use of aseptic surgical technique for surgery in small and large laboratory animals is the norm for all species and applies as much to rodents as it does to pigs or

sheep. Application of aseptic technique requires optimization of surgical techniques, but it is easily achieved by changing only a few peri-operative processes. It is at the heart of our mission to ensure good animal welfare and therefore to optimize the quality and reproducibility of research involving animal surgery.

Author details

Bertrand Lussier[1,2*†], Jaco Bakker[3†], Delphine Bouard[4†], Kate Read[5†] and Lucy Whitfield[6†]

1 Faculty of Veterinary Medicine, Department of Clinical Sciences, University of Montreal, St-Hyacinthe, Canada

2 Osteoarthritis Research Unit, Hospital Research Centre (CRCHUM), University of Montreal, Montreal, Canada

3 Animal Science Department, Biomedical Primate Research Centre, Rijswijk, The Netherlands

4 Vetsalius, Saint-Didier-au-Mont-d'Or, France

5 Labcorp Animal Welfare and Veterinary Services, UK

6 OWL Vets Ltd, UK

*Address all correspondence to: bertrand.lussier@umontreal.ca

† All co-authors are Council members of the European Academy of Laboratory Animal Surgery.

References

[1] Biomedical research. 2023. Available from: https://cihr-irsc.gc.ca/e/48804.html

[2] Russell W, Burch R. The Principles of Humane Experimental Technique. Wheathampstead (UK): Methuen: Universities Federation for Animal Welfare; 1959

[3] Berdoy M et al. Guiding Principles for Preparing for and Undertaking Aseptic Surgery. London, UK: L.A.S. Association; 2017

[4] Hoogstraten-Miller SL, Brown PA. Techniques in aseptic rodent surgery. Current Protocols in Immunology. August 2008. Chapter : Unit 1.12-14

[5] Coll I. ACLAM position statement on rodent surgery. Journal of the American Association for Laboratory Animal Science. 2016;**55**(6):822-823

[6] Bradfield JF et al. Behavioral and physiologic effects of in apparent wound infection in rats. Laboratory Animal Science. 1992;**42**(6):572-578

[7] Boothe H. Instruments and tissue handling techniques. In: Johnston SA, Tobias KM, editors. Veterinary Surgery Small Animal. St. Louis MO: Elsevier Saunders; 2012. pp. 201-213

[8] Character BJ et al. Postoperative integrity of veterinary surgical gloves. Journal of the American Animal Hospital Association. 2003;**39**(3):311-320

[9] Partecke LI et al. Incidence of microperforation for surgical gloves depends on duration of wear. Infection Control and Hospital Epidemiology. 2009;**30**(5):409-414

[10] De Souza Araujo IJ et al. Rubbing ethanol and time of use: Critical factors compromising latex gloves structure. Revista de Odontologia da UNESP. 2021;**50**:1-8

[11] Pengfei G et al. Effect of multiple alcohol-based hand rub applications on the tensile properties of thirteen brands of medical exam nitrile and latex gloves. Journal of Occupational and Environmental Hygiene. 2016;**13**(12):905-914

[12] Brown DC et al. Epidemiologic evaluation of postoperative wound infections in dogs and cats. Journal of the American Veterinary Medical Association. 1997;**210**(9):1302-1306

[13] Del Valle JM et al. Comparison of aqueous and alcohol-based agents for presurgical skin preparation methods in mice. Journal of the American Association for Laboratory Animal Science. 2018;**57**(4):401-414

[14] Celeste NA et al. Effects of cling film draping material on body temperature of mice during surgery. Journal of the American Association for Laboratory Animal Science. 2021;**60**(2):195-200

[15] Emmer KM et al. Evaluation of the sterility of press'n seal cling film for use in rodent surgery. Journal of the American Association for Laboratory Animal Science. 2019;**58**(2):235-239

[16] Nolan KE et al. Evaluation of the sterility of Reynolds wrap aluminum foil for use during rodent surgery. Journal of the American Association for Laboratory Animal Science. 2021;**60**(1):85-90

[17] Keen JN et al. Efficacy of soaking in 70% isopropyl alcohol on aerobic bacterial decontamination of surgical instruments and gloves for serial mouse

laparotomies. Journal of the American Association for Laboratory Animal Science. 2010;**49**(6):832-837

[18] Heon H et al. Establishment of an operating room committee and a training program to improve aseptic techniques for rodent and large animal surgery. Journal of the American Association for Laboratory Animal Science. 2006;**45**(6):58-62

[19] Skiles B et al. Effectiveness of the glass bead sterilizer for sterilizing surgical instruments. Journal of the American Association for Laboratory Animal Science. 2022;**61**(3):252-255

[20] Basso MA et al. Using non-invasive neuroimaging to enhance the care, well-being and experimental outcomes of laboratory non-human primates (monkeys). NeuroImage. 2021;**228**:117667

[21] Chen Y et al. Circuit-specific gene therapy reverses core symptoms in a primate Parkinson's disease model. Cell. 2023;**186**(24):5394-5410 e18

[22] Yu P et al. Protocol for MRI-guided virus injection in macaque deep brain regions. STAR Protocols. 2023;**4**(4):102768

[23] Fabian NJ et al. Evaluation and comparison of pharmacokinetic profiles and safety of two extended-release buprenorphine formulations in common marmosets (Callithrix jacchus). Scientific Reports. 2023;**13**(1):11864

[24] Fitz CB et al. Pharmacokinetics of buprenorphine and sustained-release buprenorphine in common marmosets (Callithrix jacchus). Journal of the American Association for Laboratory Animal Science. 2021;**60**(2):188-194

[25] Mackiewicz AL et al. Pharmacokinetics of a long-lasting, highly concentrated buprenorphine solution after subcutaneous administration in Rhesus Macaques (Macaca mulatta). Journal of the American Association for Laboratory Animal Science. 2019;**58**(4):501-509

[26] Nunamaker EA et al. Pharmacokinetics of 2 formulations of buprenorphine in macaques (Macaca mulatta and Macaca fascicularis). Journal of the American Association for Laboratory Animal Science. 2013;**52**(1):48-56

[27] Salyards GW et al. Pharmacokinetics of ceftiofur crystalline free acid in male rhesus macaques (Macaca mulatta) after subcutaneous administration. Journal of the American Association for Laboratory Animal Science. 2015;**54**(5):557-563