

James Cook University Animal Ethics Committee

Aseptic Technique for Survival Animal Surgery Procedure

Intent

This procedure describes 'aseptic surgical technique' and so outlines the measures the AEC expects investigators to take in order to minimise the chance of infections in animals undergoing surgical or other invasive techniques. The use of aseptic technique for animal survival surgery is prescribed in the *Australian code for the care and use of animals for scientific purposes* Sections 3.3.7 and 3.3.16.

Scope

This procedure applies to:

- Investigators conducting surgical and other invasive procedures on animals
- The AEC when assessing retrospective amendments to projects

All investigators planning to conduct survival surgery on animals must have undertaken the surgical training program, of which this procedure is a part, before AEC approval can be granted to start work.

Definitions

Antiseptic	means a chemical agent that can be applied to living skin to inhibit the growth of microbial agents. Antiseptics can either kill microbial agents or stop their growth, however application of an antiseptic to an object does not make it sterile. Eg iodine, ethanol, hydrogen peroxide, F10
Asepsis	means state with an absence of microbial (bacterial, fungal, viral) contamination.
Aseptic technique	means the procedures taken to minimise the risk of microbial contamination, or reach and maintain asepsis. It involves the use of a methodical set of work practices, and is most commonly employed in medical/veterinary (surgical) settings.
Disinfectant	means a chemical agent that inhibits the growth of microbial agents that can only be applied to non-living objects. Eg ethanol, hypochlorite (bleach), F10 SC. Disinfectants reduce the amount of contamination, but do not sterilise.
Haemostasis	means the control of bleeding.
Sterile	means the complete absence of microbial contamination.
Sterilisation	means a process that removes or destroys all microorganisms from an object. Eg autoclaving, ethylene oxide, irradiation

Introduction

While overt infections are seen in research animal surgery, their occurrence is considered uncommon. Low-grade or subclinical infections are much more common and mostly go unnoticed, with the only resulting clinical signs often missed or attributed to other causes such as pain. Researchers often underestimate the effects of these subclinical infections and their associated clinical signs on their animals and the flow on effect these infections have on their research results. It's therefore important to do everything possible to prevent infection when carrying out invasive procedures on animals.

Common clinical signs of low-grade or subclinical infections include:

- Reddening of the suture line and surrounding tissues
- Swelling of the suture line and underlying tissues
- Discharge from the wound or crusting around the wound or sutures
- Delayed healing
- Weight loss or failure to gain weight
- Inappetence or reduced appetite
- Inactivity or reduced activity
- Porphyria (red staining around eyes or nose in rodents)
- Removal of sutures, biting or scratching at incision and/or operative area

If the infections progress, the clinical signs may progress to:

- Wound breakdown
- Abscessation
- Peritonitis
- Pluritis
- Arthritis
- Septicaemia
- Other infections depending on the site/type of surgery

To prevent infections, surgeons have developed a ritualistic practice, called aseptic surgical technique that governs their behaviour from the preparation stage to completion of the surgery.

This procedure outlines aseptic technique that must be followed when conducting survival animal surgery in projects approved by the JCU AEC.

While the administration of prophylactic antibiotics peri-operatively is sometimes considered necessary, antibiotics should not be used instead of or to cover up incorrect or inappropriate surgical practices. If correct aseptic technique and surgical procedures are being used, antibiotics shouldn't be necessary.

Procedure

1. Preparation of the instruments

1.1 Sterile instruments and consumables must be used for all animal survival surgeries.

1.2 When autoclaving, autoclave indicator tape must be used to ensure process has led to sterility. Consumables should not be used after their expiry date.

1.2 When carrying out several procedures in a row or batched surgery, instruments can be sterilised in a hot bead steriliser in between animals as long as they are cleaned of blood and tissue beforehand and a new scalpel blade is used for each animal.

The use of sterile surgical instruments, consumables and other equipment is the most important aspect of aseptic technique. Instruments must be sterilised before they are used for any invasive survival surgery. **Note: placing instruments in, or spraying them with a disinfectant (such as ethanol) does not sterilise them and so must not be used as an alternative to sterilisation.** Also, most disinfectants (ethanol and hydrogen peroxide in particular) are extremely toxic to tissues and so should be kept away from instruments and any open incisions.

The most common and easiest way to sterilise surgical instruments is by autoclaving. For instruments or equipment that might be heat labile, other methods of sterilisation can be used including: ethylene oxide fumigation, glutaraldehyde immersion or irradiation, however these are generally only available to hospitals and can be expensive. A disinfectant product called F10 Sterilent can also be used in some cases but is not as effective as sterilising agents.

In order for any sterilisation method to be fully effective the instruments need to be clean and free of corrosion, so it's important to clean instruments immediately after use, dry them effectively and store them in a place where they will stay clean and dry.

2. Instrument Use and Care

2.1 Surgical instruments must be used for the purposes they are intended only, as inappropriate use can lead to damage that will affect the surgical outcomes.

2.2 Used instruments are considered dirty and must be kept separate to clean unused instruments.

2.3 A fresh scalpel blade must be used for every animal.

Surgical instruments are designed for use on animal tissues only and so should never be used elsewhere in the lab (to cut paper, plastic, fabric etc). Inappropriate use will cause instruments to become blunt or malaligned and to function ineffectively, which in turn will lead to increased tissue trauma, post-operative pain and infection (as well as frustration for the surgeon). Therefore, it's important to maintain a dedicated set of surgical instruments.

Ideally, a separate set of sterilised instruments should be available for each animal; however, in batched rodent surgery this may not always be possible. In these cases, the instruments should be washed with sterile water/saline between animals to remove any dry blood or tissue. Any used instruments are still considered contaminated and so must be placed on a new sterile drape and kept away from any unused (clean) instruments.

Alternatively, an acceptable compromise is to place the instrument tips in a hot bead steriliser after cleaning them of any blood or tissue.

A fresh scalpel blade must be used for every animal.

In case instruments are dropped or accidentally contaminated during surgeries, it's a good idea to have spare sterile replacements available.

3. Prepare the procedure room for your specific procedure

3.1 Invasive procedures that have the propensity for the development of infection must always be conducted in an appropriate facility that will minimise the risk of contamination or infection of the surgical site.

All surgical procedures must take place in the designated procedure rooms and areas of those rooms. These areas must be cleaned thoroughly with disinfectant before and after use. Air flow should be such that it minimises the risk of dust and air-borne contamination of the surgical area, while allowing for adequate ventilation for effective removal or dispersal of anaesthetic gases.

The room should be uncluttered with no storage of excess equipment or consumables that may be difficult to clean and so act as a reservoir for infectious agents or vermin.

The surgical facility should be prepared and stocked with all necessary equipment before anaesthesia is induced and surgery is commenced.

4. Preparation of the animal and procedure/surgical site

4.1 The animal and procedure site must be prepared to minimise the risk of contamination with microorganisms.

Surgical preparation of the animal follows the following steps:

a) The removal of fur/hair overlying the surgical site

A site much larger than the planned surgical area (at least twice as large) should be prepared to reduce the chance of hair or fur being touched by the surgeon or falling into the incision.

Hair or fur is best removed with clippers. Shaving can be done using a scalpel blade, but this can cause micro-abrasions which increase the risk of infection and may cause irritation which could lead to scratching or removal of the sutures. Depilatory cream can be used, as long as the skin is washed after to remove excess cream.

b) Cleaning and disinfection of the surgical site.

The initial scrub is usually done using a combination of detergent/soap and disinfectant such as chlorhexidine surgical scrub to remove larger organic matter and fur that could enter the incision and lead to inflammation, infection or abscessation. This clipping and pre-scrub wash must be done at a separate site to where the surgery will take place but at the same time (not the day before as if there are micro-abrasions, they can become infected in the interceding time increasing the chance of deep infections after the incision is made). A rinse using disinfectant should follow to remove the detergent residue.

This would be followed by a wash and wipe with a 70% ethanol swab or spray, starting at the incision site (cleanest area) and working outwards. This ensures that the incision site remains the cleanest as at the start the swab is fresh. Povidone iodine or chlorhexidine /ethanol mixture can then be applied to the area in the same way as the ethanol, and allowed to dry, making sure the iodine has dried before making the incision. NB chlorhexidine can inactivate iodine so should not be used after a chlorhexidine soapy scrub.

Preparation of the animal is best done at a site distant from the surgical facility or surgical area, and then once the animal is cleaned and prepared, it can be moved.

c) Positioning and attachment of monitoring equipment:

After the initial scrub and before the final scrub, the supportive and monitoring equipment can be put in place and attached as required (if applicable). Hypothermia is a risk in rodents and so supplementary heating must be provided to these animals in the form of a heat pad or other system. Heat lamps are not to be used as they can result in hyperthermia, burning or dehydration (especially dehydration of eyes and other mucous membranes). Sterile eye ointment (moisturising or antibiotic) must be applied to the eyes of all animals undergoing anaesthesia at the preparation stage and then again at recovery.

The position of the animal can be fixed using tape or sting/rope, but care must be taken not to interfere with respiratory movements, to fix the animal's limbs in an abnormal position, cut off circulation or prevent movement that would indicate the animal is reacting to the surgery and more anaesthetic is required.

d) Draping

Sterile drapes (cloth drapes, paper towelling, clear plastic adhesive drapes) can be placed over the unprepared, 'dirty' areas of the animal by the surgeon. This allows the surgeon to touch or manipulate the animal without contaminating their gloves or the surgical site. Care should be taken when draping not to obscure the animal so that effective monitoring cannot take place. In rodents, sterile surgical gloves or other improvised versions of drapes can be used to provide coverage of the 'dirty' areas of the animal.

5. Preparation of the surgeon

5.1 The surgeon must wear a clean gown, mask and cap to minimise the risk of contamination of the surgical site.

5.2 Before putting on surgical gloves, hands should be thoroughly scrubbed and dried.

5.3 Sterile surgical gloves must be worn for surgery and changed between every animal if conducting batched surgery.

The use of appropriate protective clothing that minimises the risk of contamination of the surgical site must be worn. This includes a fresh clean gown, although this doesn't need to be sterile for rodent surgeries.

A head cover and mask must be worn and put on prior to scrubbing. The most important step is for the surgeon to perform effective disinfection of their hands (after gowning), by "scrubbing" and then putting on sterile surgical gloves. Scrubbing of the hands is best done with an effective disinfectant agent such as an iodophore, chlorhexidine or F10 for several minutes and the hands dried with a sterile towel. The process of hand washing aims to remove surface dirt and grease from your skin, and allow sufficient contact time with the disinfectant soap to kill or inhibit bacteria in the outer layer of the skin.

The only time a scrubbing brush should be used is to clean under your nails, the remainder of the process is just careful and repeated hand washing. Total contact time varies with the disinfectant used but about 5 minutes is adequate for most skin antiseptics.

For a correct scrub, every surface/each side of the fingers and palms and wrists up to the elbows must be washed/scrubbed: all around each finger, the top surface of the hand, the palm, both sides of the hands, around the wrists all the way up to elbows. (see diagram in Appendix 1).

Wearing a pair of tight-fitting sterile surgical gloves is very important. **Note: standard laboratory gloves are not sterile, and must not be used for surgery, even after they have**

been sprayed with ethanol. Lab gloves are often not even clean and may contain residues, such as latex dust that are irritants when in contact with exposed tissue.

Once the surgeon has scrubbed up and is wearing sterile gloves, they must take care not to touch anything other than sterile surgical instruments/drapes/swabs or the aseptically prepared surgical site of the animal. Contact with anything non-sterile would require rescrubbing and re-gloving.

Towels (fabric or paper towelling) can be sterilised by autoclaving them with the instruments or drapes, so they are ready for the surgeon when the pack is opened.

6. Preparation for surgery

6.1 Before surgery can start the animal needs to be prepared, instruments readied and animal draped.

Either the surgical pack and consumables can be prepared before the surgeon is scrubbed and gloved, or an assistant can open the outer wrapping of instrument packs, sutures, and scalpel blades.

Care needs to be taken when unwrapping the pack to prevent contamination of the contents. The outside of the pack is considered dirty and the inside sterile, so the pack should be opened either before scrubbing takes place or if after, by an assistant.

The surgeon drapes the animal taking care not to touch any non-sterile surface. Using a drape prevents sterilised items touching the animal's fur causing it to become contaminated. When using a paper, plastic or rubber drape, a suitable-sized hole can be cut to access the surgical site. The sterile field can be extended by using an additional paper or cloth drape. The drapes should still allow the animal's anaesthesia depth and physiological signs to be monitored. If the surgery is to be batched, a fresh sterile set of drapes must be used for every animal.

7. Aseptic surgical technique

7.1 Aseptic surgical principles must be used during surgery.

Once the animal and surgeon are prepared, the surgical area and the equipment can be approximately divided into:

- Sterile – including the prepared surgical field, the surgeon's hands/gloves, any sterile gown the surgeon is wearing, the top side of the drapes and the instruments and the top side of the drape on which they are placed.
- Dirty – including the rest of the animal, the underside of the drapes, anaesthetic and monitoring equipment, lights, note paper and pens, the surgeons face or other parts of the body (including the gown if this is not sterile at the beginning).

The principles of aseptic surgical technique can be summarised as: nothing sterile can touch anything that is dirty from the start until the last suture has been placed. If something sterile touches something dirty, then it is considered dirty and must be disposed of, cleaned and resterilised, or replaced. This means that if the surgeon touches their face, then the surgeon must then re-glove or else they will not be able to continue surgery.

8. Surgical techniques that reduce infection and complications

8.1 Correct surgical technique must be used.

Other special techniques that must be employed during surgery to help reduce post-procedure infection include:

- Minimising incision size and unnecessary tissue dissection but don't compromise access or view by making the incision too small, as struggling through a small incision can do more damage than making a larger clean incision
- Minimising the duration of surgery
- Preventing desiccation (drying out) if tissues are to be left exposed to air for any longer than a few minutes (while measurements are taken for example); cover the surgical site with sterile gauze that has been soaked in sterile Hartman Ringers solution, 0.9% saline or sterile PBS
- Handle tissues gently to reduce tissue trauma and post-surgical necrosis. Use only rat-toothed Adson-Brown forceps to handle tissue as flat forceps will crush tissue and cause necrosis
- The appropriate use of well-maintained instruments
- Maintain effective haemostasis by placing slight digital pressure on tissue that is bleeding with a PBS/saline-soaked gauze to encourage clotting and soak up any blood that has escaped. If the vessel is large enough, it may be ligated (tied off) with an absorbable suture material or coagulated with an appropriate instrument or cautery
- Use appropriate methods of wound closure, including the correct technique, the correct suture materials (absorbable sutures internally and braided materials like silk never used on skin), needle size and shape (cutting needle for skin and round needle for all other soft tissues) for the tissues, suture pressure and spacing. Don't pull the sutures too tight as the wound will swell considerably after surgery resulting in pain, itching and infection which in turn make it more likely the animal will chew out the stitch before healing. Placing a subcutaneous suture layer will help relieve tension on the skin sutures
- Ensuring effective planning, organisation and preparation to ensure the surgical procedure(s) run smoothly and consistently
- If there are problems with wound infection, post-operative clinical signs, suture removal by the animals or the occurrence of unexpected or aberrant results then a review of the surgical technique must be conducted with a veterinarian to determine where the problems may lie or improvements may be made

Acknowledgments

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Other Resources

1. Correct handling of surgical instruments slideshow:
<https://www.slideshare.net/ahmadfsulong/handling-of-surgical-instruments>

References

1. Principles of Rodent Surgery for the New Surgeon. Pritchett-Corning, K.R., Mulder, G.B., Luo, Y., White, W.J., J. Vis. Exp. (47), e2586, doi:10.3791/2586 (2011).
<https://www.jove.com/video/2586/principles-of-rodent-surgery-for-the-new-surgeon>
Procedures with Care: <http://www.procedureswithcare.org.uk/?s=surgery> (training videos on aseptic and surgical technique)
2. Aseptic technique in Rodent Surgery: Why Should I Pay Attention? Mendenhall V, Baran S, Johnson E, Perret-Gentil M. <https://www.alnmag.com/article/2010/03/aseptic-technique-rodent-surgery-why-should-i-pay-attention>

Administration

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Appendix 1: 3 Minute Surgical Scrub

3 Minute Scrub With Brush

First Scrub Of The Day

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1 •Remove all Jewellery
•Apply PPE (Face mask & eyeshield)
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2 •Open brush
- 

3 •Turn on tap
•Rinse hands and arms
•Select cleanser
•Cleanser needs contact with skin for 2 minutes
•As per manufacturer's instructions, apply:
- 2 Pumps *Betadine QR*
2 Pumps *Chlorhexidine*
- 

4 •Cover hands and arms with cleanser 2.5 cm beyond elbows
- 

5 •Use pick for cleaning fingernails
- 

6 •Use brush for fingernails only
•Discard brush after use -do not rinse off

Thereafter 2 Minutes No-brush Scrub

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7 •Wash between fingers.
- 

8 •Wash front and backs of hands
- 

9 •Use circular downward movement from wrists to forearm
•Hands must remain higher than elbows at all times
- 

10 •Rinse from fingertips down to elbows
- 

11 •Reapply cleanser to hands
•Repeat steps 7,8
•Use circular motion from hands to wrists to finish
•Rinse from fingertips down to elbows
- 

12 •Turn off tap with elbow
•Let water drip from elbows

1min

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