

LAB_002 Clean Technique for Laboratory Animal Surgery

I. OBJECTIVE

To describe clean surgical technique for use in laboratory animal surgery.

II. COMMENTS / RECOMMENDATIONS

- Whenever it is appropriate, aseptic technique should be given preference over clean technique
 - Characteristics of the surgical model determine the appropriateness of clean vs aseptic technique e.g. immunocompromised animals, protracted surgery, and any surgery which accesses (infection) susceptible tissue such as intra-abdominal or orthopaedic surgery, should be considered for aseptic technique, rather than clean technique
- If performing 'batch surgery', planning is required to maintain a clean environment between animals
 - e.g. cleaning instruments between animals, replacing table drapes between animals if grossly contaminated; disinfecting gloves via ethanol spray between every animal, and changing gloves at regular intervals (e.g. every 30minutes) and whenever grossly contaminated.
- When applying this SOP, once a disposable item has been used it should be immediately placed into the clinical waste bin or sharps bin (as appropriate). Used disposable items should never be left on the work station surface, cluttering the surgical field.

III. EQUIPMENT

- Personal Protective Equipment (PPE) – relative to facility requirements, but should at least include:
 - hair bonnet, face mask, eye protection, clean laboratory gown, disposable gloves
- Disinfectants
 - Hard surface disinfectant (e.g. 70% ethanol spray)
 - Hand wash (ideally chlorhexidine or iodine based surgical hand wash)
 - Surgical (rodent) skin disinfectant (e.g. chlorhexidine or iodine based pre-surgical scrub)
- Anaesthetic & analgesic agents – as per AEC approved protocol
- Animal heating equipment (e.g. thermostatic heat mats)
- Hair removal equipment (electric clippers +/- depilatory cream (e.g. Veet® hair removal cream)).
- Paper towel
- Gauze swabs and cotton tips
- Specific surgical instruments and equipment (sterile)
- Specific skin closure equipment and material (sterile)
- Sharps bin
- Clinical waste bin (open top)

IV. PROCEDURE

1. Remove jewellery and wash and dry your hands.
2. Apply appropriate PPE
3. Remove any unnecessary equipment from the work area and surrounds.

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4. Clean and disinfect the surgical area and any non-sterile equipment such as heating mats and microscope controls
e.g. spray and wipe-down with 70% Ethanol
5. Assemble and organise materials required for the procedure within the surgical area
e.g. skin disinfectants, heat mats, surgical instruments etc.
6. Turn on and allow heating mats time to warm.
For further information, see UQBR Guidelines 13 Rodent Heating Procedures
7. Change into a clean gown and gloves, as required.
8. Anaesthetise the animal as per AEC approved protocol.
Ideally this is done in a location adjacent to the surgical area/work station so that hair debris does not contaminate the surgical area.
9. Remove hair from the surgery site, as close to the skin as possible, using either clippers or hair removal cream. Dispose of hair in a manner that creates minimal airborne particles.
Clipping the fur with the rodent on a sheet of disposable paper towel which is then discarded and using gentle adhesive tape to collect hair is ideal.
For further information, see UQBR Guideline 3 Rodent Hair Removal.
10. Move or position the anaesthetised animal appropriately for surgery, within your surgical area.
11. Using cotton tips or gauze swabs disinfect the skin over the surgical site. Start by cleaning from the centre of the surgical site and work your way out, towards the margins of the surgical site. Never risk of dragging fomites back across the surgical site. This should be repeated at least 3 times, and enable at least 3 minutes of “contact time”.
“Contact time” refers to the total time in which disinfectant is present and active on the skin.
See Table 1, within V. REFERENCE INFORMATION for options for pre-surgical skin disinfection
12. Consider the use of disposable draping material or sterile swabs to shield any uncleaned or haired parts of the animal that may contact the surgeon’s hands or equipment during the procedure. Draping material must not interfere with your ability to monitor the animal’s anaesthetic condition (e.g. respiratory rate).
13. Open any materials from their sterile packaging, within the surgical area, in such a way as to not contaminate their contents.
14. Spray your gloved hands with 70% ethanol, rub them together until all surfaces of the gloves are coated, then allow the gloves to dry.
Throughout the procedure, if your gloved hands touch something outside the surgical field, such as the vaporiser unit (to adjust isoflurane concentration), repeat this step before continuing with the surgery.
15. During surgery, when interchanging between instruments place the used instrument onto a disinfected or sterile surface. Do not place them onto the exposed bench top.
16. Minor lapses in sterility, such as suture material inadvertently touching the surgical table, are difficult to avoid but should be minimised.
17. More significant lapses in sterility, such as dropping a surgical instrument on the floor would require the instrument to be sterilised again before continued use.
18. Before starting the procedure on another animal evaluate the cleanliness of your work station: clean and disinfect any equipment as required, replace any disposables (e.g. gloves).

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V. REFERENCE INFORMATION

Table 1. Pre-surgical skin disinfectants (as per [Guidelines for Survival Rodent Surgery 2019](#), [National Institute of Health](#)). The compounds commonly used within UQ facilities, and generally readily available are shaded in yellow.

AGENT	*EXAMPLES	COMMENTS
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.
*The use of common brand names as examples does not indicate a product endorsement.		
Please note: <ul style="list-style-type: none"> Some studies have indicated an increased efficacy of iodine and chlorhexidine disinfectants when used in combination with alcohol based disinfectants. Chlorhexidine is generally desired at 4%; Iodine is generally desired at 10%; Tap water is appropriate for their dilutions. Other disinfectant may be appropriate (seek veterinary advice if required). 		

Table 2. Recommended Sterilant for Surgical Instruments & Equipment (as per [Guidelines for Survival Rodent Surgery 2019](#), [National Institute of Health](#)). The methods commonly used within UQ facilities, and generally readily available are shaded in yellow.

AGENT	*EXAMPLES	COMMENTS
Steam Sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time, e.g. 121°C for 15 min vs 131°C for 3 min. Appropriate sterilization indicators should be used to ensure sterility.
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.
Alcohol	Ethanol or Isopropanol	Alcohol is neither a sterilant or high-level disinfectant. May be acceptable for some procedures, if prolonged contact time are used (Keen <i>et al.</i> , 2010; Huerkamp, 2002)

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Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time. Appropriate sterilization indicators should be used to ensure sterility.
Chlorine	Sterilant Levels of Chlorine dioxide (Clidox®, Alcide®) Sodium hypochlorite (Clorox® 10% solution)	Corrosive to instruments. Items must be clean and free of organic material. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcode®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use. Product expiration dates must be adhered to as per manufacturer's instructions.
Hydrogen peroxide Acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
*The use of common brand names as examples does not indicate a product endorsement. Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods.		

Table 3. Recommended Hard Surface Disinfectants (as per [Guidelines for Survival Rodent Surgery 2019, National Institute of Health](#)). The compounds commonly used within UQ facilities, and generally readily available are shaded in yellow.

AGENT	EXAMPLES*	COMMENTS**
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
Quaternary Ammonium	Roccal®, Quatricide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehydes (Cidex® Cetylcode®, Cide Wipes®)	Rapidly disinfects surfaces.

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Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Hydrogen peroxide Peracetic acid Acetic acid	Spor Klenz	Contact time 10 minutes.
*The use of common brand names as examples does not indicate a product endorsement ** Always follow manufacturer's instructions for dilution and expiration periods		

VI. BIBLIOGRAPHY

Bernal, J., M. Baldwin, T. Gleason, S. Kuhlman, G. Moore and M. Talcott (2009). "Guidelines for Rodent Survival Surgery." *Journal of Investigative Surgery* 22(6): 445-451.

Cooper, D. M., R. McIver and R. Bianco (2000). "The thin blue line: a review and discussion of aseptic technique and postprocedural infections in rodents." *Contemp Top Lab Anim Sci* 39(6): 27-32.

Hoogstraten-Miller, S. L. and P. A. Brown (2008). "Techniques in aseptic rodent surgery." *Current protocols in immunology* Chapter 1: Unit-1.1.14.

Huerkamp, M. J. (2002). "Alcohol as a disinfectant for aseptic surgery of rodents: crossing the thin blue line?" *Contemp Top Lab Anim Sci* 41(1): 10-12.

Iowa, T. U. o. (2019). "Surgery - Rodent Survival Surgery (Guideline)." The University of Iowa (reviewed by the IACUC), from <https://animal.research.uiowa.edu/iacuc-guidelines-rodent-survival-surgery>.

Keen, J. N., M. Austin, L. S. Huang, S. Messing and J. D. Wyatt (2010). "Efficacy of soaking in 70% isopropyl alcohol on aerobic bacterial decontamination of surgical instruments and gloves for serial mouse laparotomies." *J Am Assoc Lab Anim Sci* 49(6): 832-837.

LeMoine, D. M., V. K. Bergdall and C. Freed (2015). "Performance analysis of exam gloves used for aseptic rodent surgery." *Journal of the American Association for Laboratory Animal Science : JAALAS* 54(3): 311-316.

Lilley, E. and M. Berdoy (2017). *Guiding Principles for Preparing for and Undertaking Aseptic Surgery*. . A report by the LASA Education, Training and Ethics section.

NHMRC (2008). *Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes: The Assessment and Alleviation of Pain and Distress in Research Animals*, National Health and Medical Research Council (NHMRC).

NHMRC (2013). *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

NIH. (2019). "Animal Research Advisory Committee Guidelines: Guidelines for Survival Rodent Surgery." U.S. Department of Health and Human Services, National Institutes of Health (NIH), from https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b6_survival_rodent_surgery.pdf.

Perret-Gentil, M. I. "Rodent Surgery: Application of Aseptic Technique and Perioperative Care." *Laboratory Animal Resources Center, The University of Texas at San Antonio.*, from <http://research-dev.utsa.edu/wp-content/uploads/2015/02/Rodent-Surgery-Handout-Application-of-Aseptic-Technique-and-Perioperative-Care.pdf>.

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Pritchett-Corning, K. R., Y. Luo, G. B. Mulder and W. J. White (2011). "Principles of rodent surgery for the new surgeon." *Journal of visualized experiments* : JoVE(47): 2586.

Tennessee, U. o. (2018). "Institutional Animal Care and Use Standard Operating Procedure: Guidelines for Survival Rodent Surgery." University of Tennessee (Revised and Approved by IACUC), from <https://vetmed.tennessee.edu/olac/Documents/Rodent%20surgery%20guidelines.pdf?Mobile=1&Source=%2Folac%2F%5Fflayouts%2Fmobile%2Fview%2Easpx%3FList%3D09338985%252Df55c%252D452f%252Da884%252D81b82597083f%26View%3D2028855b%252D155e%252D4ea8%252D8aa1%252D5fd9d5cf63a5%26CurrentPage%3D1>.

University, C. M. (2013). "Guidelines for Rodent Survival Surgery." Central Michigan University, from https://www.cmich.edu/office_provost/ORGS/ComplianceandResearchIntegrity/InstitutionalAnimalCareandUseCommittee/Pages/Policies.aspx.

University, O. S. (2020). "University Laboratory Animal Resources: Veterinary Guidelines." Ohio State University, from <https://ular.osu.edu/resources/veterinary-guidelines/>.

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