LAB_001 Aseptic Technique for Laboratory Animal Surgery (Expiry: March 2026)

I. OBJECTIVE

To describe ideal aseptic 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 sterile environment between animals
 e.g. sterilising instruments between animals, replacing table drapes; changing gloves.
- 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.
- Please note, this SOP has been written with the expectation that a "non-sterile" surgical assistance will be available to aid the "sterile surgeon"

III. EQUIPMENT

- Personal Protective Equipment (PPE) relative to facility requirements, but should at least include:
 - hair bonnet, face mask, eye protection, disposable gloves and sterile surgical gloves, sterile laboratory gown
- Disinfectants
 - Hard surface disinfectant (e.g. 70% ethanol spray)
 - Surgical hand wash (e.g. 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 (within sterilised packs)
- Drapes (within sterilised packs)
- Specific surgical instruments and equipment (within sterilised packs)
- Specific skin closure equipment and material (within sterilised packs)
- Sharps bin
- Clinical waste bin (open top)

IV. PROCEDURE

- 1. Ensure facility specific PPE is being worn.
- 2. Remove any unnecessary equipment from the work area and surrounds.
- 3. Clean and disinfect surgical area and non-sterile equipment such as heating mats and microscope controls

e.g. spray and wipe-down with 70% Ethanol

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4. Assemble and organise materials required for the procedure within the surgical area, but do not remove any of the sterile equipment from their sterile packs

e.g. skin disinfectants, heat mats, surgical instruments etc.

5. Turn on and allow heating mats time to warm.

For further information, see UQBR Guidelines 13 Rodent Heating Procedures

"Non-sterile" surgical assistant:

6. Anaesthetise the animal in a location adjacent to the surgical area/work station, as per AEC approved protocol.

This is done outside the surgical are so that hair debris does not contaminate the surgical area.

7. 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.

- 8. Move the anaesthetised animal to the surgical area/work station and position the animal appropriately for surgery.
- 9. 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.

"Sterile" surgeon:

- 10. Remove any jewellery and wash hands for at least 3 minutes with appropriate pre-surgical hand wash (e.g. chlorhexidine or iodine based solution)
- 11. With the aid of your surgical assistant, don a sterile gown and sterile surgical gloves.
- 12. With the aid of your surgical assistant, place sterile drapes around the surgical site to cover any non-sterile surfaces from potential contact with the surgeon's hands or equipment.

Draping material must not interfere with your ability to monitor the animal's anaesthetic condition (e.g. respiratory rate). The appropriateness and extent of draping will vary dependent on the procedure being performed.

- 13. With the aid of your surgical assistant, place sterile table drapes (or sterile instrument trays) within your work station to create a sterile surgical field.
- 14. With the aid of your surgical assistant, retrieve any required materials from their sterile packaging in a manner so as to not contaminate their contents and organise them within the sterile surgical field.
- 15. During surgery, when interchanging between instruments place the used instrument down within the sterile surgical field not onto the non-sterile benchtop.

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- 16. Any lapses in sterility requires correction to retain sterility of the surgical field e.g. if you touch something with a gloved hand outside the sterile field you will need to change your sterile gloves.
- 17. Before starting the procedure on another animal sterility needs to be re-established. This would require at least sterilisation of instruments, changing instrument drapes and animal drapes, and disinfecting gloves.

V. REFERENCE INFORMATION

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

AGENT	*EXAMPLES	COMMENTS		
lodophors	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:				
 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%; lodine 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 <u>Guidelines for Survival Rodent</u> <u>Surgery 2019</u>, <u>National Institute of Health</u>). 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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Levels of dioxide Alcide®) ypochlorite 10% solution) ehyde	Corrosive to instruments. Items must be clean and free of organic material. Instruments must be rinsed with sterile saline or sterile water before use.
ehvde	Soveral hours required for starilization. Corresive
Cetylcide®, ®)	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.
por-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
•	por-Klenz® examples doe ilution, exposu

Table 3. Recommended Hard Surface Disinfectants (as per <u>Guidelines for Survival Rodent Surgery 2019</u>, <u>National Institute of Health</u>). 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® Cetylcide®, 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 <u>https://animal.research.uiowa.edu/iacuc-guidelines-rodent-survival-surgery</u>.

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 <u>http://research-dev.utsa.edu/wp-content/uploads/2015/02/Rodent-Surgery-Handout-Application-of-Aseptic-Technique-and-Perioperative-Care.pdf</u>.

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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=%2F olac%2F%5Flayouts%2Fmobile%2Fview%2Easpx%3FList%3D09338985%252Df55c%252D452f%252Da884 %252D81b82597083f%26View%3D2028855b%252D155e%252D4ea8%252D8aa1%252D5fd9d5cf63a5%26">%252D81b82597083f%26View%3D2028855b%252D155e%252D4ea8%252D8aa1%252D5fd9d5cf63a5%26 CurrentPage%3D1.

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

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

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