



Subject: Aseptic surgery in rodents	Number: C-1
Scope: A directive from the Direction des services vétérinaires to users and staff of Université Laval animal facilities (campus and affiliated research centres).	
Prepared by Stéphanie Caron <i>Animal Health Compliance Technician, Direction des services vétérinaires</i>	Date: February 17, 2012
Modified by Jessie Tremblay <i>Animal Health Compliance Technician, Direction des services vétérinaires</i>	Date: August 28, 2018
Revised by Geneviève Fortin Simard, Anne-Marie Catudal <i>Clinical veterinarians, Direction des services vétérinaires</i>	Date: March 14, 2019
Purpose: Describe aseptic surgery procedures in rodents	Version 6

General Considerations

- Perform aseptic surgery under conditions free from microorganisms that can lead to infection.
- Perform surgery in an easy-to-disinfect, clutter-free area with little traffic. This area must be separate from the animal preparation area.
- Ideally, perform surgery in the morning so animals can be observed postoperatively throughout the afternoon and analgesia can be administered at the designated frequency.
- All animals awaiting surgery must have access to water at all times.
- All materials used in aseptic surgery must be sterile (gauze, scalpels, forceps, cannulas, etc.). Surgical instruments must be autoclaved.
- For multiple surgeries, autoclave instruments for the first animal and use the bead sterilizer between subsequent animals. However, we recommend autoclaving instruments again:
 - after 5 surgeries,
 - between each contaminated surgery (e.g., GI surgery), and
 - when the surgeon leaves the room for a break.

- Do not use soaking solutions such as glutaraldehyde (Cidex) for multiple surgeries since these solutions are only effective with extended contact time.
- During multiple surgeries, the surgeon's gloves must be changed or cleaned with a 70% isopropyl alcohol solution between each animal.
- Three distinct areas must be prepared: preoperative, intraoperative, and postoperative. Cover the areas with a heating mat and a clean drape.
- Electric heating mats can be used under cages only. The following equipment is safe to use for maintaining an animal's temperature during surgery: circulating water mat, Deltaphase®, far-infrared mat, or an electric hot plate with a rectal thermometer for precise heat adjustment (limit the device's temperature to a maximum of 41° C and ensure the animal's body temperature does not exceed 39 °C).
- Use a heating mat preoperatively, intraoperatively, and postoperatively to reduce the risk of hypothermia.
- An animal may only undergo one major survival surgery. If a second major surgery is performed, it must be terminal.
- If multiple minor surgeries must be performed on the same animal for experimental reasons, the frequency and time between surgeries should be adjusted to minimize the impacts on the animal's welfare.

Definitions

- Asepsis: measures taken to prevent contamination of an area or surface by microorganisms
- Disinfectant: a solution used to destroy pathogens on surfaces and inert objects
- Sterilization: the destruction of all microorganisms
- Sterile area: a working area that includes the surgical site and the area in front of the surgeon
- Minor surgery: a surgical procedure that involves minor tissue removal and/or no exposure of major cavities or functional structures (e.g., peripheral blood vessel cannulation, percutaneous biopsy, castration, skin sutures, etc.).
- Major surgery: a surgical procedure that involves major tissue removal and/or direct visual access to a cavity and/or significant exposure of vascular, muscular, nerve, lymphatic, or glandular structures (e.g. stereotaxic surgery, laparotomy, ovariectomy, nephrectomy, etc.).

Animal preparation

- Perform a physical examination and record the animal's weight so as not to overdose it.
- Induce anesthesia as per *SOP A-1 Anesthesia and analgesia in rodents (PNF A-1 Anesthésie et analgésie des rongeurs)*. Administer the analgesic prescribed in the protocol and the local block at the incision site.
- Upon loss of consciousness, apply ophthalmic ointment.
- Administer warmed lactated Ringer's solution (LRS) by subcutaneous injection for all surgical procedures (see Table 4 in *SOP A-1 Anesthesia and analgesia in rodents*).
- Administer an antibiotic if prescribed in the protocol (e.g., for intestinal surgery involving high risk for bacterial contamination).
- Shave the incision site (shave as little as possible to avoid heat loss).
Note: Depilatory cream is recommended for sites where shaving is difficult or dangerous. Apply the cream with a cotton swab and use circular motions on the site to be depilated for up to 35 seconds, taking care to avoid the eyes and genitals. Carefully remove all cream with wet gauze and, if necessary, repeat. Prolonged contact time or failure to remove all traces of the cream may severely burn the skin.
- Remove hair with wet gauze and clean the site with chlorhexidine, taking care not to wet more of the animal than necessary.
- Transfer the animal to the surgery area.
- Alternating, apply 70% isopropyl alcohol/0.5% chlorhexidine three times to the shaved area, always ending with chlorhexidine. If using Baxedin Preop® (a mixture of 70% isopropyl alcohol and 0.5% chlorhexidine), apply only three times. Using one gauze pad or cotton swab per application, apply from the center of the site to the periphery (see Figure 1).



Figure 1 - Preparation of the surgical site (asepsis)

Surgeon preparation

- For surgeries where hands will not manipulate tissue, wash hands and put on a cap, mask, clean gown, and clean gloves disinfected with 70% isopropyl alcohol.
- For surgeries where hands will manipulate tissue, put on a mask and cap, scrub hands for 5 minutes with chlorhexidine soap, and put on a clean gown and sterile gloves.

Surgery

- Using the withdrawal reflex, check the depth of anesthesia.
- Verify the equipment is appropriately sterilized and lay it on a sterile surface.
- Avoid any break in sterility throughout the surgical procedure:
 - If you wear sterile gloves, touch only sterile objects and avoid touching your mask, glasses, or any non-sterile object. Put on a new pair of gloves if you break sterility.
 - If you wear non-sterile gloves, touch only non-sterile objects (or parts of objects) that will not come in contact with animal tissue.
- Cut a window out of a sterile drape and place it over the animal. The window must be no larger than the prepared site.
To more easily monitor the animal, use a transparent sticky drape such as Glad Press'n Seal®.
- Grasp instruments only by their handles and avoid touching the surgical site with your hands (mandatory if wearing non-sterile gloves).
- Do not use blunt, broken, or rusty instruments.
- Make the smallest possible incision to minimize heat loss and wound problems.
- Handle organs and skin gently and atraumatically.
- If necessary, cover the organs with sterile moist gauze or keep them moist with sterile irrigation solution.
- Administer subcutaneous fluids hourly if surgery lasts more than one hour.
- For abdominal surgeries, close the wound in multiple layers (one suture for each tissue layer). Suture using the designated material (absorbable or non-absorbable, staples, etc.). The suture material must be sterile.
- Use 3-0 to 6-0 sutures depending on the species and organ to be sutured. Consult the tables in *SOP C-10 Suture techniques and materials (PNF C-10 Matériel et techniques de suture)*.

Postoperative period

- Trim the animal's hind claws.
- If the surgery involves placing an implant, weigh the animal and record its weight on the DSV postoperative follow-up card in the space indicated (see Appendix I).
- Transfer the animal to the recovery area.
- Administer an additional dose of warmed LRS if more than 50 minutes have elapsed since the last dose or if there was significant blood loss during surgery. Administer analgesics according to the protocol.
- Return the animal to its cage when it is in sternal recumbency and breathing is regular.
- Complete the postoperative follow-up card and affix it to the cage of the animal cage (see Appendix I).
- Examine the wound each day over the next 5 to 7 days. If you see any signs of concern (discharge, redness, swelling, etc.), note them on the card and alert the animal care staff (animal health technician or veterinarian).
- At a minimum, weigh the animal on the fifth postoperative day and note its weight on the card. Contact the animal care staff if there is still weight loss on day 5.
- In the event of wound dehiscence (a surgical wound reopens) due to the loss of skin staples or stitches, promptly notify the animal care staff so they can treat the condition.
- Continue postoperative analgesia as per the protocol.
- Remove staples or skin sutures (absorbable or not) 7–10 days after surgery.

References

CCAC, *Guide to the Care and Use of Experimental Animals*, 1993.

CCAC, *Guidelines on: Procurement of Animals Used in Science*, 2007.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, *Guide for the Care and Use of Laboratory Animals*, 8th edition, 2011.

Pritchett-Corning, K. R., Mulder, G. B., Luo, Y., White, W. J., *Principles of Rodent Surgery for the New Surgeon*, 2011.

Sidhwa F., Itani, K. M., Skin preparation before surgery: options and evidence, *Surg Infect* (Larchmt). 2015.

Zarndt, B. S. et al., Use of a Far-Infrared Active Warming Device in Guinea Pigs (*Cavia porcellus*), *J Am Assoc Lab Anim Sci.*, 2015.

SOP Revision History		
Version 2	August 6, 2012	Clarifications added on wearing gloves during surgery. Various clarifications added to the procedure.
Version 3	January 11, 2016	Added Appendix I
Version 4	December 7, 2016	Clarification added on suture removal at 7 to 10 days post-op for absorbable and non-absorbable thread. Added thread type (braided or monofilament) to Table 1.
Version 5	November 17, 2017	Clarified acceptable heat sources. Added definitions of minor and major surgeries. Moved information from Table 1 to <i>SOP C- 10 Suture techniques and materials</i> . Added wound monitoring to the postoperative follow-up card
Version 6	March 14, 2019	Added guidelines for the sterilization of instruments during multiple surgeries. Clarifications added on hourly fluid (LRS) administration. Removed iodine for use as surgical scrub. Added use of depilatory cream. Added Figure 1. Added postoperative claw trimming, Added postoperative weighing if an implant was done and mandatory weighing on postoperative day 5. Clarified treatment of wound dehiscence.

Appendix I

A quick reference guide to the postoperative follow-up card - front

CHIRURGIE :		DATE :		HEURE :	
Fait par :		POSTop			
# Protocole :		Cochez lorsque le Tx et/ou le suivi de plaie est fait, indiquez l'heure et vos initiales			
Responsable du suivi <u>POSTop</u> : <input type="checkbox"/> TSA animalerie <input type="checkbox"/> ÉR		DATE			
Taille de griffes effectuée avant le réveil <input type="checkbox"/>		Suivi de la PLAIE		<input type="checkbox"/>	<input type="checkbox"/>
Analgésie	PRÉop	Bloc local	POSTop <	<input type="checkbox"/>	<input type="checkbox"/>
	Meloxicam <input type="checkbox"/> Carprofen <input type="checkbox"/> Bupré <input type="checkbox"/> Bupré SR <input type="checkbox"/> Autre <input type="checkbox"/> :	Lidocaïne <input type="checkbox"/> Bupivacaïne <input type="checkbox"/>	24h <input type="checkbox"/> 48h <input type="checkbox"/> 72h <input type="checkbox"/> Bupré <input type="checkbox"/> BID <input type="checkbox"/> TID <input type="checkbox"/> SR <input type="checkbox"/> Meloxicam (SID) <input type="checkbox"/> Carprofen (SID) <input type="checkbox"/> Autre <input type="checkbox"/> : <	<input type="checkbox"/>	<input type="checkbox"/>
Fluido	PRÉop	PERop	POSTop	<input type="checkbox"/>	<input type="checkbox"/>
	LRS <input type="checkbox"/> NaCl 0,9% <input type="checkbox"/> DW <input type="checkbox"/>	(ml et ID)	LRS <input type="checkbox"/> NaCl 0,9% <input type="checkbox"/> DW <input type="checkbox"/> SID <input type="checkbox"/> BID <input type="checkbox"/> TID <input type="checkbox"/> _____ jrs <	<input type="checkbox"/>	<input type="checkbox"/>
Supplément diète <input type="checkbox"/> : _____ SID x _____ jrs		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
INSCRIRE L'IDENTIFICATION DES ANIMAUX, LES VOLUMES À ADMINISTRER, LES POIDS ET LES PROBLÈMES DE PLAIE AU VERSO					

Indicate postoperative treatment dates.

Daily wound follow-up is required for at least 5 days post-surgery. Check off and initial. If you see any anomalies, note them on the back of the card and notify an animal health technician (AHT).

Post-op treatments: complete the applicable sections (analgesia, fluids, diet) based on the expected pain level and the protocol approved by the animal protection committee.

Analgésie: if buprenorphine is used, check off the duration of anesthesia and the frequency of administration (BID = 2x/day, TID = 3x/day). If buprenorphine SR is administered POSTop, check off the SR box. If meloxicam or carprofen is used, select the drug and check off the duration of analgesia. NSAIDs are only given 1x/day (SID) unless directed by a veterinarian.

Fluids: check off the box if fluids are administered POSTop and indicate treatment duration and frequency (LRS = lactated Ringer's sol., DW = dextrose saline, SID = 1x/day, BID = 2x/day, TID = 3x/day).

Check off each time you do a treatment. Enter your initials and the time you administered the treatment.

If a special diet is provided as supportive care, check off the box and specify the type (e.g. DietGel Recovery) and the duration of the supplement. It is best to start giving the new diet 24–48 h **before** surgery, so the animal gets used to it.

Indicate the fluids administered PREop. If fluids are administered during the procedure (INTRAop), indicate the total volume administered and the animal's identification if more than one animal underwent surgery.

Indicate the PREop analgesic administered. If the drug used is not on the list, check off "OTHER" and specify. If buprenorphine SR (slow release) is administered PREop, there is no need to reselect it for POSTop, since a single injection provides 72 hours of analgesic coverage.

Check off the local block used PREop. A combination of drugs is recommended.

Fill in the header with the requested information: the surgical procedure performed, the date and hour, the name(s) of the person(s), the name of the operator(s), and the protocol number authorized by the animal protection committee. Check off whether the person responsible for postoperative follow-up is an animal health technician or a research team member. Don't forget to trim the animal's claws before it recovers and check off the box once trimming is completed.

A quick reference guide to the postoperative follow-up card - back

Retirer broches/points de suture (7 à 10 jrs post-chirurgie)						
DATE prévue : _____						<input type="checkbox"/>
ID animal						
Volume (ml)	Lido + bupi					<input type="checkbox"/>
	Bupré					
	Autre: → _____					<input type="checkbox"/>
	AINS					
	Fluido					
DATE		POIDS (g) individuel des animaux Le poids préopératoire est obligatoire. Si applicable, le poids postopératoire avec implant est obligatoire. Le poids au 5 ^e jour postop est obligatoire.				
→ PRÉop						
→ POSTop avec implant						
						<input type="checkbox"/>
Commentaires sur le suivi de plaie						
→						

Indicate the date when stitches or staples are to be removed.

Check off after stitches or staples are removed.

Indicate the identification numbers for all animals from a cage that underwent surgery in a single session.

If another drug is used for analgesia, indicate that here.

For each animal, indicate the volume to inject for each substance to be administered PREop and POSTop.

Weigh each animal **before** the procedure and record here. Also note the date.

Immediately after surgery, weigh all animals that were given implants and record their weights here.

On day 5 POSTop, weigh the animal and record here. If you notice continued weight loss on day 5, notify an animal health technician.

If there is a problem with a wound, record your observations here and notify an animal service technician to follow-up with the veterinarian. If a specific follow-up or treatment is required, the animal service technician will open a health record.

Each time a postoperative weight is measured, indicate here and record the date. If necessary, fill out a weight tracking form.