Journal of the American Association for Laboratory Animal Science
Copyright 2006
by the American Association for Laboratory Animal Science

Vol 45, No 6
November 2006
Pages 58-62

Establishment of an Operating Room Committee and a Training Program to Improve Aseptic Techniques for Rodent and Large Animal Surgery

Hélène Héon,[1,*] Nathalie Rousseau,[1] Jane Montgomery,[1] Gilles Beauregard,[2] and Manon Choinière[2]

Investigators of our research facility generally accept the concept of asepsis as an important component of adequate surgical care for animals. However, they experience difficulties putting it into practice, especially in the case of rodents. The reasons for this are inconvenience, cost, and lack of training. To better assist investigators in the implementation of aseptic surgical techniques in their laboratories, we have created an Operating Room (OR) Committee modeled after OR committees found in human hospitals. A reconstructive surgeon, a veterinarian, a research scientist, a nurse involved in the training of OR personnel, interns, graduate students, and an animal health technician were chosen as committee members in light of their OR and animal care expertise. The first task of the OR Committee was to establish institutional guidelines for aseptic surgery, taking into account the costs imposed on research budgets by these procedures. The OR Committee also supports a complete training program in aseptic surgery techniques, which consists of lectures, a training manual, videos, and a practical course. Furthermore, when experimental procedures require specialized equipment, the OR Committee collaborates with researchers to develop strategies to achieve asepsis. This OR Committee and the training program proved to be important tools to promote and improve the quality of animal care during surgery.

Abbreviations: CCAC, Canadian Council on Animal Care; IACUC, institutional animal care and use committee; OR, operating room

Asepsis in surgery is an important component of surgical care for animals. The Canadian Council on Animal Care (CCAC) guidelines stipulate that "good surgical techniques are essential for the welfare of experimental animals" and for success of the surgical component of research projects.[17] In the United States, the Animal Welfare Act and the Animal and Plant Health Inspection Service require "that survival surgeries be performed using aseptic techniques."[1,2]

Case Report

Although scientists at our institution generally accept the concept of asepsis, they experience difficulty putting it into practice. Research teams working with rodents encounter many difficulties as their technicians or graduate students face the challenge of performing the surgery on their own. They often lack training and experience in surgery and, in many cases, do not have the support of a trained surgeon. The task of learning the surgical procedure is complicated enough; these personnel may perceive that ensuring asepsis during surgery is too difficult. Most rodent operations imply 'multiple run' surgeries in which a large number of rodents are undergoing the same procedure, and ensuring asepsis is not an easy task. Finally, in some circumstances, the experimental procedures require a special set-up (for example, the use of a stereotaxic instrument to inject drugs in the brain of rat fetus in utero), making the implementation of aseptic techniques difficult. Despite these difficulties, solutions were necessary because our institutional animal care and use committee (IACUC) requested that all surgeries at our institution be performed according to CCAC guidelines and sound veterinary technique.

In an effort to assist investigators in the implementation of good surgical care and aseptic surgical techniques for their research projects and to ensure the development of uniform practices over all campuses, an Operating Room (OR) Committee for our research facility was created and modeled after the Clinical OR Committee at the Centre Hospitalier de l'Université de Montréal. The Chairman of the OR Committee is a reconstructive surgeon with 25 y of OR expertise. Other members are a research scientist involved in a surgical research program and a registered nurse responsible for training medical students and nursing staff working in ORs. An animal health technician with several years of experience in rodent surgeries and a veterinarian with surgical and teaching experience complete the team. The Committee reports and makes recommendations to our center's Director of Research. Two members of the OR Committee are also IACUC members, permitting communication between the committees, but the OR Committee has no responsibility or authority for protocol approval.

The mandate of the OR committee is to 1) establish guidelines for asepsis, 2) provide training and technical support, 3) evaluate compliance, and 4) report to the IACUC.

The first task of the OR Committee was to establish institutional guidelines for aseptic surgery. The need for asepsis was the source of much debate among people involved in surgery care,[5] and it was important to establish minimal standards that

Received: 27 Apr 2006. Revision requested: 14 July 2006. Accepted: 14 July 2006.
[1]Hôpital Notre Dame and [2]Hôtel-Dieu, Centre de Recherche, Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada.
[*]Corresponding author. Email: helene.heon@sympatico.ca

Table 1. Minimal standards for aseptic surgery in rodents

1. All surgeries are performed in a surgical suite located in the animal facility or in a designated area of a laboratory reserved exclusively for surgery.
2. Surgical mask and cap are mandatory. Surgeons must wear a clean labcoat or gown which are not required to be sterile.
3. Sterile gloves must be worn.
   A. The use of powder-free nonsterile gloves taken from a clean box is acceptable only under the following conditions:
      a) If there is to be no contact with the surgical site or any material destined to be inserted in the wound;
      b) If only the sterile tip of the surgical instruments will come in contact with the wound;
      c) If the surgeon is also the person who will shave and prepare the animal for surgery, gloves have to be changed to a sterile pair before beginning the actual surgery;
   B. If there is accidental contact with nonsterile material, gloves must be changed before resuming surgery.
4. All material coming in contact with the surgical site has to be sterile, including
      a) Surgical instruments;
      b) Implants or pumps, solutions filling osmotic mini-pumps, surgical drapes, gauze, solutions used during surgery to soak organs or clean the instruments;
      c) Suture material.
5. The surgical kit must be properly packed and sterilized.
6. If multiple surgeries are planned, instruments must be sterilized between animals. A maximum of 6 surgeries is allowed per kit before resterilization with heat and pressure.
7. Sutures must rest on a sterile surface at all times.
8. Shaving must be done in a separate area from where the surgery will be performed.
9. For rodents skin must be disinfected with a standard prepping method (3 strokes of isopropyl alcohol 70% and 3 strokes of a 2% chlorhexidine gluconate topical solution). For large animals skin is previously washed 5 min with a 4% chlorhexidine gluconate solution.
10. The opening in the surgical drape must not be larger than the shaved and disinfected area on the animal. There should be no hair showing through the opening.

Table 2. Policies and standard operating procedures relating to surgical techniques

Policies	Standard operating procedures
Minimal standards for rodent surgery	Working in OR rooms
Surgery principles for large animals	Preparation of the surgical site
Surgery principles for rodents	Proper use of the dry sterilizer
	Proper use of the ultrasound apparatus for cleaning surgical instruments
	Washing and care of surgical instruments
	Preparation of materials for sterilization

could be implemented by everyone. These standards were based on recommendations found in veterinary literature,[4,5,7-9,11,16,20] but compromises were necessary to accommodate the costs imposed on research budgets by these procedures.

Among these compromises was the use of the same surgical kit for several surgeries on rodents; a maximum of 6 surgeries per kit is allowed before resterilization with heat and pressure. This number is a compromise between the best practices for surgical care and the needs of the research groups, which cannot afford to buy enough instruments to have a kit for each animal. Because most groups usually perform 10 to 12 surgeries per day, 6 surgeries per surgical kit seemed reasonable. The instruments are decontaminated with a proper chemical disinfectant or dry heat[15] between each animal. To test that reuse of surgical instruments did not result in contamination, swabs were performed on the instruments after 6 surgeries and dry heat decontamination; no bacterial growth was noted. Another compromise was the use of nonsterile gloves, providing that only the sterile tip of the surgical instruments would contact the wound and that the nonsterile gloves would not touch the surgical site or any material destined to be inserted in the wound. The minimal standards for asepsis are listed in Table 1.

The OR Committee also helped to implement rules for the appropriate use of ORs. The ORs are reserved for surgery and are not used for other purposes. They must be kept clean at all times. No food and drink are allowed. Only people who need to perform surgeries are permitted to use these rooms.[11] A list of established policies and standard operating procedures appears in Table 2.

As the second part of its mandate, the OR Committee supports an extensive training program consisting of lectures, a training manual, videos, and a practical course. Over time, this training program has become the core of OR Committee efforts and has proven itself to be the best way of promoting and implementing good surgical care and practices. The program is financed by the institution. The technical and veterinary time is not charged to the investigators, but they are asked to pay for materials used.

All people performing surgeries are required to take the training program. Although this program consists of a formal course, it also lends itself to open discussion where all participants can give suggestions to facilitate the implementation of aseptic techniques. In addition, the participants are requested to fill an evaluation form so the course can be improved continually.

As our research facility is part of a university hospital complex, animal health services have access to hospital resources. Collaboration with the different departments facilitated the preparation of the aseptic surgery course. Animal health technicians have taken a 3-d course offered by the hospital's Sterilization Service. To see how to organize work and equip-

Table 3. Course contents

Day 1	
Lectures	**Animal preparation (mannequins used)**
Effects and prevention of infection	Anesthesia
Planning the surgery	Preparation of the surgical site
Protocol approval, policies, and standard operating procedures	Draping
Technical aspects	Use of the surgical microscope
Surgical instruments and suture materials	
Washing and maintenance of surgical instruments	**Day 2**
Wrapping method, pack layout, sterilization, and shelf life	**Sham surgeries (mannequins used)**
How to put on surgical gloves	Suturing techniques
Preparation of the instrument table	Intra-abdominal implants
Unwrapping drapes and surgical kits	Installation of a subcutaneous mini-pump
How to arrange surgical instruments and other sterile materials	Ligature of blood vessels.
	Postoperative care
	Monitoring animal recovery
	Analgesia
	Record keeping

Table 4. Videos available

Hand washing and how to put on surgical gloves
Wrapping and unwrapping surgical instruments
Animal preparation

ment in the OR, the animal health technicians have observed at least 1 human surgery. They also meet with the facility veterinarian on a regular basis to establish and refine the asepsis program and course work.

We have designed a 2-d training program consisting of lectures, a practical course (Table 3) and videos (Table 4). Three animal health technicians and the veterinarian teach the course. To ensure personalized attention, there are only 6 students per class. On the first day, the course begins with a short lecture on the effects and prevention of infection. In addition, experimental protocols, policies, and standard operating procedures are addressed. Participants then are divided into 3 pairs, each supervised by an instructor. The instructor teaches aseptic techniques and closely guides the students while they practice. On the second day, the participants perform all the previously learned tasks by themselves under the supervision of the instructor.

Rodent models often involve operating on several animals serially on the same day. Ensuring asepsis in these cases is not an easy task. To learn how to maintain asepsis while having to operate on several animals, the students have to perform a minimum of 3 surgeries one after the other. We work with home-made mannequins because we have found that when we use animals, the participants are sometimes overwhelmed by their first contact with surgery and concentrate too much on the surgery to be performed and not enough on the asepsis techniques. The mannequins are made of layers of sponge used to imitate the skin and the abdominal muscles; cotton wool imitates subcutaneous tissue (Figure 1). The mannequin can be used to practice animal preparation as well as the surgery: plastic film is used to protect it from alcohol and chlorhexidine solution. The mannequins can be reused, allowing the students to practice suture techniques many times, which is very cost-effective. Working with mannequins also permits the use of expired supplies when simulating surgery, thus reducing course expenses.

Once aseptic techniques are mastered, the students are permitted to work with animals that are part of their approved research protocol. The animal health technician then provides individual training, helping and guiding the students until they are able to perform their surgeries alone. We found that this method is the best way to help students, especially when their experimental procedures require an elaborate set-up (for example, the use of a stereotaxic frame or the use of a fluid percussion device to produce a brain injury of moderate severity). The animal health technician's expertise is much appreciated in these cases.

All aspects of proper surgical procedures are covered. Special emphasis is placed on the preparation of instruments. People often do not realize that organic material or other contaminants will block the sterilizing agent before it can reach the surface of the instruments.[14] Cleaning the instruments thoroughly is a crucial step before sterility can be achieved. Each instrument should be soaked in warm water or water-detergent or enzymatic solution. Soaking prevents the drying of blood and tissue on the instruments, facilitating their removal.[10,12] We recommend the use of an enzymatic solution, which removes organic matter without mechanical action.[12] This type of solution is useful for surfaces that are difficult to clean, such as lumens.[10] Alcohol should be avoided because, although it has a favorable bactericidal effect, it binds blood proteins to stainless steel.[18] After presoaking, the instruments have to be scrubbed, rinsed, and dried. The students are shown the various sterilization methods and how to properly prepare a surgical pack for sterilization. They are also taught how to put on sterile gloves properly, a difficult task for some people. Sterile gloves are mandatory if the surgeon has to touch any material that will be in contact with the surgical site.

Animal preparation is also very important, and the student is guided every step of the way. It is best to shave the surgical site with a small razor (model 8900, Harvard Apparatus, Saint-Laurent, Québec, Canada) for mice or with a #40 clipper blade for larger animals. Alternatively a depilatory cream may be used for mice. Shaving avoids hair from getting into the wound and facilitates surveillance of the surgical wound during postoperative care.[19] It also prevents the cutting instruments from becoming dull. For rodents aseptic preparation of the skin consists of 3 applications of 70% isopropyl alcohol and 3 applications of a topical solution of 2% chlorhexidine gluconate. Gauze is used for rats; cotton swabs are used for mice to prevent chilling the animal due to the evaporation of a large quantity of alcohol. Care is taken to work outwardly from the incision site.[9,20] For large animals the preparation is the same except that the skin is previously washed 5 min with a surgical sponge and a 4% chlorhexidine gluconate solution.[9]

During the course, instructors emphasize the importance of maintaining asepsis throughout the surgical procedure, giv-

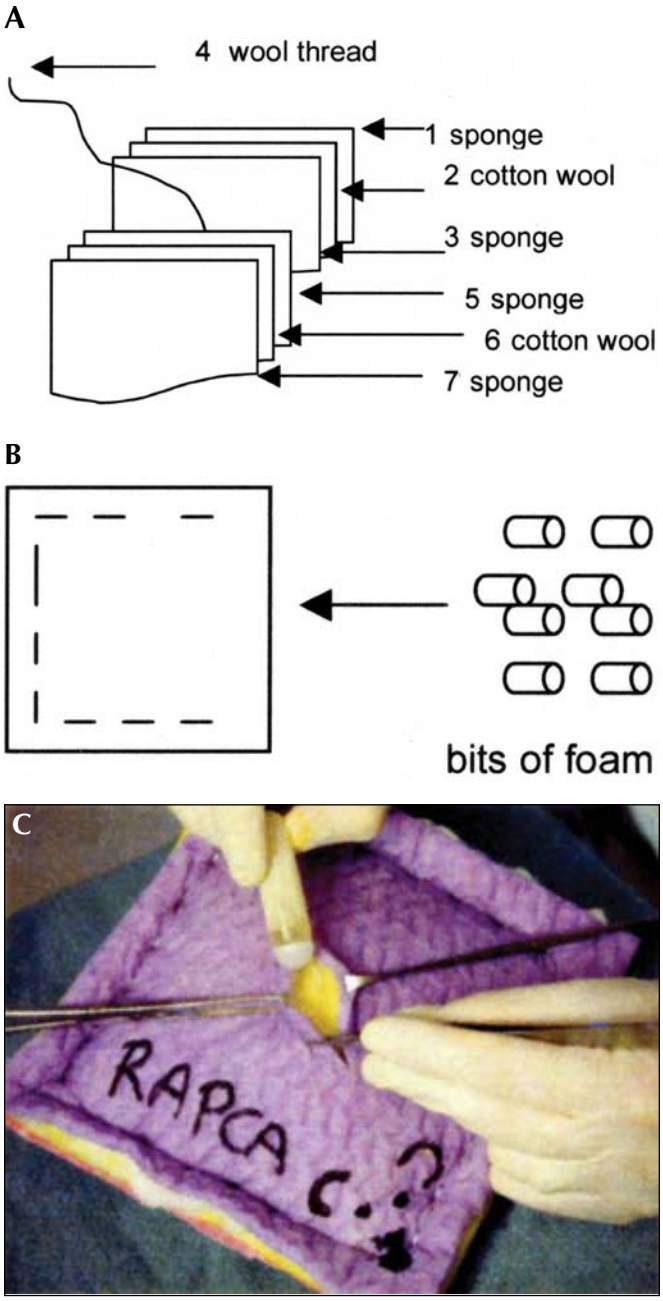

Figure 1. Home-made mannequin. (A) Mannequins are made with layers of sponge with a 5-mm thickness and cotton wool. A wool thread is used as a blood vessel. (B) The layers are stapled on 3 sides, bits of foam are introduced as filling, and then the last side is stapled. (C) The finished mannequin. Size, 20 × 20 cm.

ing helpful tips and advice. If multiple surgeries are planned, surgical kits have to include 1 paper surgical drape for each animal. A different surgical blade must be used for each animal. All materials coming in contact with the surgical site have to be sterile.[4] A single suture strand cannot be used for several animals, and sutures must rest on a sterile surface at all times. Students are taught to place a sterile surgical drape on the animal—in rodents we suggest using a surgical drape large enough to reach the surgical kit that is placed nearby, so a sterile working area is provided. The proper setting of the surgical site is demonstrated in Figure 2. The opening in the surgical drape must not be larger than the proposed incision site. There should be no hair showing through the opening.

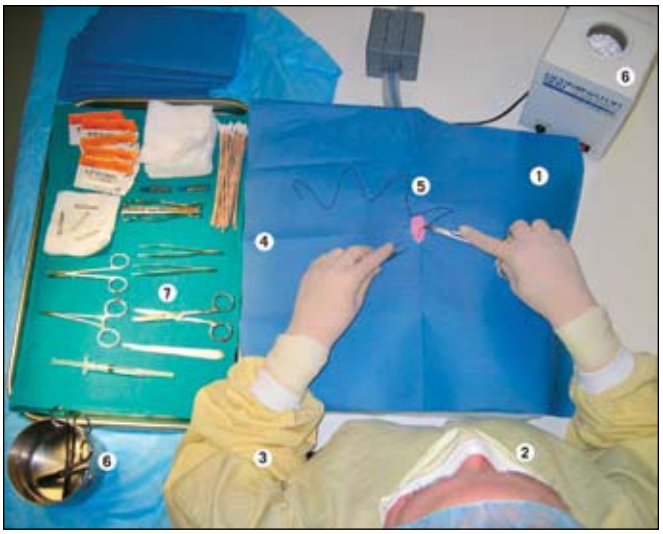

Figure 2. Surgical set-up. (1) To avoid hypothermia, animals are placed on a heating pad during surgery. (2) A surgical mask and cap are mandatory. (3) Surgeons must wear a clean (not necessarily sterile) labcoat or surgical gown. (4) A sterile surgical drape is placed on the animal to create a sterile field between the animal and the surgical kit. (5) Sutures must rest on a sterile surface at all times. (6) If multiple surgeries are planned, a sterile bowl filled with sterile water is included, to clean blood off instruments between animals. Instruments have to be dried and then resterilized by use of a dry heat sterilizing device. A maximum of 6 surgeries per kit is allowed. (7) The following items are added to the prepared sterile surgical kit using a sterile insertion technique: (a) 1 suture per animal; (b) 1 surgical blade per animal; (c) syringes and needles.

At the end of the procedure, the students are taught how to take care for their animals during recovery. The animals receive sterile isotonic fluids to prevent hypovolemia; 2.5% dextrose is administered subcutaneously to prevent hypoglycaemia if the surgical procedure is longer than 20 min.[3,13] Analgesic drugs also are given; we use buprenorphine at 0.05 mg/kg subcutaneously twice daily for mice and 0.025 mg/kg subcutaneously or 0.25 mg/kg orally twice daily for the rats. Surgical wounds are infiltrated with bupivacain at 2 mg/kg.[6] For larger animals, we follow the veterinarian's recommendation. The animals are placed in a warm environment until they recover and are able to move about. This environment can be provided by placing heating pads under animal cages, but we have found that an incubator is more efficient. Unused surplus incubators from the hospital are used for this purpose.

Finally the students are taught how to maintain records for each animal. The protocol number and type of surgery are noted. Postoperative care is described, and all drugs administered to the animal have to be indicated with dose, time, and route of administration. Investigators are encouraged to write a standard operating procedure describing the surgery they are performing, including specific forms with postoperative care instructions and spaces to indicate animal body weight, drug dosage, and comments about animal status.

As the final part of its mandate, the OR committee meets 3 or 4 times a year, but most of its efforts are in the field. Research teams having difficulties in implementing minimal standards for asepsis either contact the Committee for help or are identified by the animal health technicians or the veterinarian. Committee members meet the research group and advise them on the best approach to solve particular problems. Efforts are concentrated on finding practical and cost-effective solutions. All research

teams performing surgeries are visited annually by committee members to observe the surgical procedures and to offer advice if they find gaps in aseptic techniques. A report of the visit is sent to the principal investigator. If there are major problems, the investigator is invited to meet with the Committee so appropriate solutions can be found. The Committee reports its visits and activities to the IACUC. Although part of the Committee's mandate is to inform the IACUC if an investigator refuses to cooperate, this measure has never had to be taken.

Discussion

The OR Committee has proven to be an important tool to promote and improve the quality of animal care during surgery. Before the creation of the Committee, some cases of postoperative infections, although not frequent, were reported to the veterinarian. After the implementation of the Committee, reports of postoperative infections have disappeared completely for large animals. Postoperative infections still occur in rodents but are much less frequent with the renewed emphasis on asepsis. The interactive approach adopted by the OR Committee allows for simple supervision and cooperation instead of strict rule enforcement. Members of the different research groups know that asepsis in surgery is their responsibility, but they do not have to face the problem alone, because they can count on the support of experts and dedicated personnel. The involvement of animal health technicians in the training program has been extremely beneficial. The technicians have developed confidence in their skills and knowledge and are highly motivated to help. We also have found that the students are very receptive of the course and make many positive changes in their work methods.

The quality of surgical care in our facility has improved dramatically, as evidenced by far fewer postoperative complications. We have met our goal to have researchers perform their own surgeries under aseptic techniques. On their most recent site visit, the CCAC commended the Committee for their efforts. Among our future plans is the creation of an advanced surgery course to meet the needs of a number of research groups. Additional videos will be produced and made available on a website, which will also offer tips and advice on surgical care.

Acknowledgments

We thank Emma Dedelis, AHT, and Guylaine Gadoury, AHT, for their contribution to the asepsis course, Bruno St-Pierre for assistance with image processing, and Ovid Da Silva (Research Support Office, Research Centre, Centre Hospitalier de l'Université de Montréal, Montréal, Canada) for editorial work on this manuscript.

References

1. **Animal and the Plant Health Inspection Service.** 2006. Animal resources guide, policy number 3. Veterinary care [Internet]. Washington (DC): US Department of Agriculture; [updated 2006 Sep 13; cited 2006 June 22]. Available at: http://www.aphis.usda.gov/ac/publications/policy/policy3.pdf.
2. **Animal Welfare Act.** 2001. Additional areas of responsibility for attending veterinarians - 2) Consultation with the principal investigator to minimize pain and distress. Section 2.31(d) in general and section 2.31 (d) (1) (iv) through (xi) specifically. [Internet]. Washington (DC): US Department of Agriculture; [cited 22 June 2006]. Available at: http://www.nal.usda.gov/awic/legislat/awabrief.htm#Q1.
3. **Bennett RA, Mullen HS.** 2004. Soft tissue surgery. In: Quesenberry K, Carpenter J, Quesenberry P, editors. Ferrets, rabbits, and rodents clinical medicine and surgery. Philadelphia: WB Saunders. p 316–328.
4. **Cockshutt J.** 2002. Principles of surgical asepsis. In: Slatter D, editor. Textbook of small animal surgery. Philadelphia: WB Saunders. p 149–155.
5. **Cooper DM, McIver R, Bianco R.** 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.
6. **Dobromylskyj P, Flecknell PA, Lascelles BD, Pascoe PJ, Taylor P, Waterman-Pearson A**. 2000. Management of postoperative and other acute pain. In: Flecknell PA, Waterman-Pearson A, editors. Pain management in animals. Philadelphia: WB Saunders. p 81–145.
7. **Dupuis J, Neault MJ, Bonneau NH.** 1999. Asepsis protocols used by the small animal surgical unit of the Montréal University veterinary hospital. Part 3 : Sterilization. Med Vet Québec **29:**200–206.
8. **Dupuis J, Neault MJ, Bonneau NH, Beauregard G, Lanthier T, Carrier M.** 1996. Asepsis protocols used by the small animal surgical unit of the Montréal University veterinary hospital. Part 1: principles and generalities. Med. Vet Québec **26:**129–135.
9. **Dupuis J, Neault MJ, Bonneau NH, Beauregard G, Lanthier T, Carrier M.** 1996. Asepsis protocols used by the small animal surgical unit of the Montréal University veterinary hospital. Part 2: patient and surgical team preparation. Med Vet Québec **26:**135–145.
10. **Harrison SK, Evans JE, LeBlanc DA, Bush LW.** 1990. Cleaning and decontaminating medical instruments. J Health Mater Manage **8(1):**36–42.
11. **Hobson HP.** 2002. Surgical facilities and equipment In: Slatter D, editor. Textbook of small animal surgery. Philadelphia: WB Saunders. p 179–185.
12. **Kneedler JA, Darling MH.** 1990 Using an enzymatic detergent to prerinse instruments AORN J **51:**1326–1332.
13. **Kona-Boun JJ, Langlois I, Mercier B, Troncy E, Paré J.** 2004 New house pets anesthesiology—domestic rodents. Med Vet Québec **24:**210–219.
14. **Miller CH.** 1991 Sterilization. Disciplined microbial control. Dent Clin North Am **35:**339–355.
15. **Miller JA, Harrower KM, Costello MJ.** 1992. A novel method of sterilizing orthodontic instruments. Aust Orthod J **12:**151–152.
16. **Mitchell SL, Berg J.** 2002. Sterilization. In: Slatter D, editor. Textbook of small animal surgery. Philadelphia: WB Saunders. p 155–162.
17. **Olfert ED, Cross BM, McWilliam AA.** 1993. Standards for experimental animal surgery [Internet]. In: Canadian council on animal care guide to the care and use of experimental animals, vol 1, 2nd ed. Ottawa: Canadian Council on Animal Care; [cited 22 June 2006]. Available at: http://ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/V1_93/CHAP/CHIX.HTM#9A.
18. **Prior F, Fernie K, Renfrew A, Heneaghan G.** 2004. Alcoholic fixation of blood to surgical instruments—a possible factor in the surgical transmission of CJD? J Hosp Infect **58:**78–80.
19. **Robinson V, Morton DB, Anderson D, Carver JFA, Francis RJ, Hubrecht R, Jenkins E, Mathers KE, Raymond R, Rosewell I, Wallace J, Wells DJ.** 2003. Sixth report of BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Refinement and reduction in the production of genetically modified mice. Lab Anim **37(Suppl 1):**S1–S49.
20. **Shmon C.** 2003. Assessment and preparation of the surgical patient. In: Slatter D, editor. Textbook of small animal surgery. Philadelphia: WB Saunders. p 162–178.