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Efficacy of Soaking in 70% Isopropyl Alcohol on Aerobic Bacterial Decontamination of Surgical Instruments and Gloves for Serial Mouse Laparotomies

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Rodent surgeries in biomedical research facilities are often performed in series. This practice presents many challenges to maintaining aseptic technique between animals. Here, we examined using soaking in 70% isopropyl alcohol for aerobic bacterial decontamination of surgical instruments and gloves used in a series of as many as 10 mouse laparotomy surgeries. These surgeries were performed on mice that were euthanized immediately prior to the procedure. Instruments and gloves were cultured before and after each procedure to determine the presence of aerobic bacterial contamination. To assess the efficacy of the decontamination protocol, culture results were grouped by procedure and then paired (before soak and after soak) for analysis using McNemar test at an α level of 0.05. In addition, by using the Fisher exact test, this modified aseptic method was compared with strict aseptic technique, for which autoclaved instruments and sterile surgical gloves were used for each procedure. In this study, we observed that the modified aseptic technique using 70% isopropyl alcohol soaks prevented aerobic bacterial contamination of instruments and gloves for as many as 5 mice.

Many of the most common surgical procedures performed on rodents in a biomedical setting (for example, embryo transfer, ovariectomy, adrenalectomy) use small incisions typically entered only by surgical instrument tips, are performed by 1 or 2 people, and usually are completed in less than 20 min per animal. These characteristics make rodents useful for high throughput surgery but are challenging in terms of maintaining aseptic technique to meet regulatory requirements. Aseptic surgical technique is designed to reduce the microbiologic contamination to the lowest possible level, thereby reducing the potential for infection. Multiple factors are involved in surgical asepsis including, but not limited to, the preparation of surgeon, patient, and instruments; surgical suite design; organization, cleanliness, and maintenance; surgeon experience; length and type of surgery; and tissue handling.

The *Animal Welfare Act Regulations* state, "all surgery on rodents…must be performed using aseptic procedures".[2] Aseptic technique for survival rodent surgery is required by the *Guide for the Care and Use of Laboratory Animals*.[20] The *Guide* recognizes that modifications to standard techniques may be "desirable or even required (for instance, in rodent or field surgery), but it should not compromise the well-being of the animals."[20] The American College of Laboratory Animal Medicine recommends that all survival surgical procedures in rodents should incorporate aseptic technique.[1] Strict aseptic technique dictates sterile surgical instruments and gloves dedicated to each animal, but this requirement may not be feasible in batch rodent surgeries, during which the same set of instruments and gloves typically are used between animals after some form of disinfection. Although the regulatory requirements for aseptic surgical technique are clear, its practical application and acceptable modifications are less so.

The idea of using isopropyl alcohol to disinfect surgical instruments and gloves used during serial rodent surgeries is not new.[7,11,13,17,18] Alcohols are not accepted for sterilizing medical and surgical instruments because these compounds are not sporicidal and are unable to penetrate protein-rich material.[17,30] For these reasons, alcohols are not classified as either sterilants or high-level disinfectants. Because all surgical instruments must be sterilized through an approved method before surgery, they are assumed to be free from all forms of microbial life, including spores. Alcohols are rapidly bactericidal, especially in the presence of water,[30] and aqueous solutions of alcohols do not leave residues. In addition to being rapidly bactericidal, alcohols are tuberculocidal, fungicidal, and virucidal at optimal concentrations of 60% to 90% (v/v) solutions in water.[30,39] Despite the positive properties of alcohols, they have several drawbacks. Alcohol damages rubber, plastics, and lensed instruments.[30,34] Instrument disinfection failure can occur and should be considered as a potential source of contamination if infection occurs.[30,34]

Currently no peer-reviewed published studies examine the practical application of using 70% isopropyl alcohol to decontaminate surgical instruments and gloves between animals in serial surgeries. The present study aims to examine the practical use of 70% isopropyl alcohol for decontamination of surgical instruments and glove of the vegetative bacteria that may occur during a series of 10 mouse laparatomies.

Materials and Methods

Animals and housing. All mice were maintained in accordance with the recommendations set forth in the *Guide* at AAALAC-

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accredited facilities (University of Rochester, Rochester, NY). All experimental procedures were approved by the university's animal care and use committee. Male (n = 16; age, 9 wk; average weight, 36.3 g) and female (n = 19; age, 9 wk; average weight, 29.5 g) Crl:CD1 (ICR) mice (*Mus musculus*) born at the University of Rochester (Rochester, NY) to parents originating from Charles River (Kingston, NY), were used in this study. Sentinel mice maintained on dirty bedding were screened quarterly for mouse hepatitis virus, mouse parvovirus, mouse minute virus, and endoparasites. Sentinels were monitored annually by using an expanded panel of agents to include cilia-associated respiratory bacillus, epizootic diarrhea of infant mice, *Ectromelia* virus, lymphocytic choriomeningitis virus, Theiler murine encephalomyelitis virus, mouse adenovirus, *Mycoplasma pulmonis*, Sendai virus, polyoma virus, pneumonia virus of mice, murine cytomegalovirus, and ectoparasites. Our institution does not routinely screen for other bacterial organisms or mouse norovirus. Mice were housed in an animal room with a negative pressure differential relative to the corridor. The rooms were maintained on a 12:12-h light:dark cycle, with humidity of 30% to 70% and temperature range of 20 to 25.5 °C (68 to 78 °F) with an average daily setpoint of 23.3 °C (74 °F). Mice were housed in groups of 5 animals per cage in individually ventilated polycarbonate cages (Allentown, Allentown, NJ) on autoclaved corncob bedding and were fed autoclaved rodent chow (LabDiet 5010, PMI Nutrition International, St Louis, MO) and autoclaved water ad libitum. Cages were changed every 14 d in a laminar flow hood by using microisolation technology.

Experimental groups. Mice were allocated into 4 groups, controls (n = 8) and experimental groups 1 through 3 (n = 10 mice each). Nonsurvival surgery was performed in series on a total of 5 mice for the control group. Each of the 5 surgeries in the series used a new set of autoclaved instruments (forceps, scissors, and needle holders) and sterile surgical gloves. Forceps, scissors, and glove fingertips were cultured prior to surgery and immediately afterward. In addition, because the first mouse in each series in the experimental groups below experienced autoclaved instruments and sterile surgical gloves, 3 additional groups of cultures bring the total number of mice for the control group to 8.

For the 3 experimental groups, nonsurvival surgery was performed in series on 10 mice per group. We wanted to know whether a single set of instruments and gloves could be reused for a reasonable number of animals experiencing serial surgeries typical for a laboratory animal setting. The procedure for all 3 experimental groups was identical. Each group of 10 mice began with one set of autoclaved instruments (forceps, scissors, and needle holders) and one set of sterile surgical gloves. Forceps, scissors, and gloves were cultured immediately prior to each surgery and immediately at the conclusion of surgery for each mouse in the group. Once the postsurgical cultures were obtained, the instruments and fingertips of the gloves were placed into a sterile bowl containing 70% isopropyl alcohol for a total contact time of at least 30 s for gloves and 2 min for instruments. Forceps, scissors, and gloves were cultured after the alcohol soak just before use in surgery (presurgical cultures) on the next animal in the group. For analysis, samples were paired as follows: the postsurgical samples (before alcohol soak) for mouse no. 1 in the series were paired with the presurgical samples (after alcohol soak) for mouse no. 2, and so forth. If any implement in the set of samples was positive for aerobic bacteria, the entire set was classified as contaminated.

Surgical suite. All procedures were performed on a clean lab bench in a dedicated animal procedure room. The lab bench was divided into 2 separate functional areas: surgical preparation and surgical area. Each area was covered with a clean absorbent pad. In addition, a sterile drape was placed over the absorbent pad to provide a sterile surgical field in the surgical area. Absorbent pads and sterile drapes were changed between surgeries for the control group and between experimental groups. Traffic flow into and out of the room was halted during all procedures.

Surgical procedure. The nonsurvival surgical procedure was adapted from a published technique.[6] Each mouse was euthanized by using CO_2 gas immediately prior to undergoing the surgical procedure, because we did not intend to allow the mice to recover and the question posed in this study was related solely to bacterial contamination of instruments contacting external and internal tissues. The surgical site was clipped and the skin was aseptically prepared by an assistant using an alternating pattern of povidone iodine surgical scrub and solution for a total contact time of 2 min. Then, the assistant placed the mouse onto the sterile drape in ventral recumbency in front of the surgeon. A dorsal left paralumbar incision was made by using scissors. The skin was separated bluntly from the body wall, and a small incision was made through the body wall perpendicular to the previous incision by using scissors. The left kidney was exteriorized. Approximately 0.05 mL sterile saline was injected under the renal capsule. The kidney was replaced into the body. The incision was closed in 2 layers. The surgical procedure from skin incision to closure lasted approximately 5 to 7 min per mouse.

Culture method. All cultures were obtained in the same manner by using separate swabs (BBL Culture Swab Collection and Transport System, Becton–Dickinson, Sparks, MD). Thumb forceps, scissors, and the fingertips of the gloves were cultured immediately after removing them from their sterile package and at the conclusion of the nonrecovery procedure for each animal in the series for each group examined. In the experimental groups, cultures also were obtained after soaking the instruments and gloves in 70% isopropyl alcohol (Hydrox Laboratories, Elgin, IL) for a minimum contact time of either 30 s (gloves) or 2 min (instruments). The fingertips of the gloves were allowed to air-dry and were cultured first in each group. The fingertips of the gloves were cultured by rolling one swab across the proximal phalanx of each hand in the following order: left hand digits 1 to 5 followed by right hand digits 5 to 1. The thumb forceps and scissors were cultured in a similar manner by using one swab for each instrument. Each instrument was removed from the alcohol soak, allowing the excess to drip away. Instruments were then held in a vertical position with jaws and blades open, facing upward, and allowed to air-dry for approximately 15 s before swabbing and proceeding with the serial surgeries. All swabs were submitted to the University of Rochester's Strong Health Clinical Laboratories for aerobic culture. Swabs were inoculated on tryptic soy agar with 5% sheep blood and incubated in ambient air at 35 °C for 24 h to determine the presence of aerobic bacterial growth.

Statistical analysis. To evaluate the efficacy of 70% isopropyl alcohol for decontamination of aerobic bacteria, contamination rates at the end of each procedure (postsurgical category) were paired with contamination rates after the instruments were soaked in alcohol (presurgical category) and analyzed by using the McNemar test. Contamination of any implement in its respective category caused all implements in the sample set to be classified collectively as contaminated. If all 3 implements were free from contamination, the respective category was classified collectively as not contaminated.

The Fisher exact test was performed to compare the contamination rates between the 2 sterilization techniques over the mice tested. Odds ratios and contamination rates are reported with exact confidence limits.

All analyses were carried out by using SAS version 9.2 (SAS Institute, Cary, NC) on a Windows XP Pro (Microsoft, Redmond, WA) platform.

Results

Across all control and experimental groups, no bacterial growth was found on autoclaved instruments and sterile surgical gloves prior to each procedure. For every group, samples were obtained immediately before and immediately after each procedure for each mouse in the series but not intraoperatively. The procedure for experimental groups 1 through 3 was identical and provided a repeated measure of alcohol decontamination. Individual culture results for all groups are presented in Table 1. Fifteen sets of implements that were contaminated after surgery were not contaminated after soaking in 70% isopropyl alcohol, whereas 2 sets remained contaminated after soaking (Table 1). No sets that were free of contamination after surgery developed contamination, and 10 sets that were free of contamination after surgery remained contamination-free after soaking (Table 1). The McNemar test rejected the hypothesis that the proportions of contamination after surgery and after soaking were equal ($P = 0.0001$; Table 2).

The difference between 0 contaminations for the sterile surgical instruments and gloves used in a total of 8 mice (5 mice in the control group plus the first mouse in each experimental group) representing our control population (proportion, 0.00; 95% confidence interval, 0.000 to 0.312) and 2 contaminations for the instruments and gloves used for the 27 mice representing our experimental groups (proportion, 0.074; 95% confidence interval, 0.0091 to 0.243) was not statistically significant ($P = 1.00$, 2-sided Fisher Exact test). Accordingly, the odds ratio for contamination in the autoclaved–sterile group compared with the experimental groups was 0.00 (95% exact confidence interval, 0.00 to 12.051).[21,35,36]

Discussion

Maintaining asepsis in a series of rodent surgeries presents many challenges not only for the investigator but also for the facility's IACUC and attending veterinarian. The investigator is challenged to maintain aseptic technique throughout the procedures even when she or he has limited help or must perform multiple tasks, including preoperative preparation, surgery, postoperative monitoring, and so on. The IACUC has an obligation to ensure regulatory requirements and that conditions of funding and accreditation are being followed.[2,20] The attending veterinarian has both legal and ethical obligations to ensure that adequate veterinary care is provided.[2,20] The requirements for aseptic surgical technique in rodents is clear, but specific details for maintaining asepsis in serial rodent surgeries requires professional judgment and performance outcome metrics that are scientifically justified and do not compromise the animal's wellbeing.[20] Our IACUC requires the use of sterile instruments and sterile surgical gloves for all survival surgeries of both regulated and nonregulated species. For multiple rodent surgeries, our IACUC allows instruments to be disinfected between surgeries by placing them into a sterile tray containing 70% to 90% ethyl or isopropyl alcohol, with replacement of the alcohol if it becomes contaminated with blood or other fluids.[37] However, no peer-reviewed scientific publication documents the number of surgeries that can be performed appropriately by using a single set of instruments and gloves.

The grouping and pairing of culture results gave us 9 pairs in each of the 3 groups of 10 mice for a total of 27 collective pairs of culture results. Based on the collective pairing of cultures, there were 4 possible outcomes. Implements could be: 1) contaminated with aerobic bacteria after both surgery and soaking in alcohol; 2) contaminated after surgery but microbe-free after soaking; 3) contamination-free after both surgery and soaking; or 4) free of contamination after surgery but contaminated after alcohol soaking. Previous data regarding contamination rates on surgical instruments and gloves used between serial rodent surgeries are nonexistent. We hypothesized, however, that at least 85% (23 of 27) of the sets of implements would be free from aerobic bacterial contamination after alcohol soaking independent of their status after surgery. Of these 23 contamination-free sets of implements, we assumed that 3 sets would be contaminated after both surgery and soaking (failure of alcohol), 19 sets that were contaminated after surgery but microbe-free after soaking (alcohol effective), 1 set that was contamination-free after surgery but contaminated after soaking, and 4 sets that were free from aerobic bacterial contamination after both surgery and soaking. We felt that these hypothesized results provided a very conservative estimate of power; we, in fact, were anticipating a higher rate of contamination-free implements after soaking. These aforementioned estimates reflected a 0.741 proportion for discordant pairs and achieved 99.8% power to detect an odds ratio of 19 (ratio of the discordant pairs) by using a 2-sided McNemar test with a significance level of 0.05.

In this study we observed that the modified aseptic technique using 70% isopropyl alcohol prevented aerobic bacterial contamination of instruments and gloves used in mouse laparotomies when performed in series of as many as 5 mice (Table 1). McNemar testing confirmed that contamination rates before and after surgery differed for the alcohol-soaking groups, thereby demonstrating alcohol's effectiveness to decontaminate instruments and gloves in a series of 10 procedures (Table 2). In the full series, only 2 contaminations occurred, and this rate was not significantly different than the autoclaved rate, although we make this statement cautiously, given the large confidence limits associated with the odds ratio for the Fisher exact test. From our present study, we have learned that the contamination rate after alcohol soaking is small. In addition, we observed no contamination for as many as 5 mice over 3 repeated measures.

We chose the particular surgical procedure performed because it is a common major invasive surgery, is relatively quick to perform, requires a small incision, and is reasonable to perform in a series. All mice in this study were euthanized immediately before surgery. Because we were interested only in detecting aerobic bacterial contamination of instruments contacting external and internal tissues throughout serial mouse surgeries, we found it acceptable to perform the surgeries on mice immediately after euthanasia. A weakness of this approach is the potential reduction of blood contamination of instruments throughout the surgical procedures. However, this surgical procedure results in minimal, if any, bleeding and gross contamination with tissues.[33] In addition, the majority of postsurgical infections in humans (and presumably animals) result from contamination by the skin of the surgeon or patient.[25,26] Therefore, circulating blood would be expected to be a minor variable. For these reasons, we believed the surgical procedure performed 2 min after euthanasia was sufficiently representative and applicable to our goal.

Table 1. Individual culture results for all groups

		No. of gram-positive colonies obtained					
		Before surgery (after soaking in alcohol)			After surgery		
Group	Mouse no.	Forceps	Scissors	Gloves	Forceps	Scissors	Gloves
Control	1	0	0	0	0	0	1
	2	0	0	0	0	0	0
	3	0	0	0	0	1	3[a]
	4	0	0	0	0	0	3
	5	0	0	0	0	0	1
Experimental group 1	1	0	0	0	0	0	1
	2	0	0	0	0	0	1
	4	0	0	0	0	0	1
	3	0	0	0	0	0	0
	5	0	0	0	0	0	1
	6	0	0	0	0	0	1
	7	0	0	0	0	0	0
	8	0	0	0	0	0	1
	9	0	0	0	0	0	1
	10	1	0	0	0	0	1
Experimental group 2	1	0	0	0	0	1	1
	2	0	0	0	0	0	1
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	7	0	0	0	0	0	0
	8	0	0	0	0	0	1
	9	0	0	0	0	0	1
	10	0	0	0	0	0	4
Experimental group 3	1	0	0	0	0	0	1
	2	0	0	0	0	0	1
	3	0	0	0	0	0	4
	4	0	0	0	0	0	0
	5	0	0	0	0	0	1
	6	0	1	0	0	0	2
	7	0	0	0	0	0	1
	8	0	0	0	0	0	0
	9	0	0	0	0	0	0
	10	0	0	0	0	0	0

Various samples were paired for analysis: for example, postsurgical culture results from mouse no. 1 were paired with the presurgical (that is, after soaking in alcohol) culture results of the next mouse in the series, mouse no. 2. Representing samples from autoclaved instruments and sterile gloves, presurgical samples from mouse 1 of each experimental group were included as controls samples with the presurgical samples from control mice.

[a]1 colony of one type of gram-positive organism; 2 colonies of another type

In this study, surgical gloves had the highest rate of contamination (23 of 35, 65.7%) overall for all groups (Table 1). One explanation for this result is that we did not use a sterile drape to cover the animal prior to surgery; doing so may have reduced the rate. However, gloves used during surgeries on humans become contaminated during a considerable proportion (52%) of the procedures, and this rate increases with surgical time.[8] Even in relatively clean, simple procedures, 61% of the gloves became contaminated.[8] The sources of the organisms identified in the cited study were likely commensals that originated from either the surgeons' hands themselves or possibly from the patient's skin.[8] These contamination values are not very different from what we observed in the present study. The relationship between contamination of surgical instruments and gloves and postoperative infection in mice has not been examined. In the current study, we assumed that bacterial contamination of surgical instruments and gloves contributes to postoperative infection and is not in compliance with aseptic technique.

Cultures were obtained by using sterile swabs to determine whether aerobic bacteria were present. We chose to use a sterile

Table 2. Contamination rates contingency table and McNemar test analysis

	Before surgery		
After surgery	Contaminated	Not contaminated	Total
Contaminated	2	15	17
	7.41	55.56	62.96
	11.76	88.24	
	100.00	60.00	
Not contaminated	0	10	10
	0.00	37.04	37.04
	0.00	100.00	
	0.00	40.00	
Total	2	25	27
	7.41	92.59	100.00

For McNemar analysis: statistic (S), 15; degrees of freedom, 1; $P = 0.0001$

swab collection system on the basis of convenience, ease of use, portability, and lack of interference with the items being cultured. Sterile culture swabs have also been accepted as tools to determine instrument and patient contamination.[5,9,12,14,22,24,27] More elaborate culture methods involving broth soaks, agitation or ultrasonication, or direct impression of instruments onto agar surfaces are available but were not chosen.[8,10,25,29] These methods require more time to prepare (broth soaks, agitation, and ultrasonication methods) and leave residues (broth, agar) that must be removed completely before proceeding to the next animal. We felt that these types of culture methods would have compromised the clinical translational relevance of our study greatly. Studies have been performed whereby gloves, instruments, or surfaces have been inoculated with a known number of bacteria and then subjected to a decontamination process to examine decreases in bacterial counts.[4,32] We similarly felt that inoculation of instruments and gloves with a known number of bacteria prior to the surgical procedures would not accurately represent a practical laboratory animal surgical setting.

During the course of the surgical series, there was no obvious, gross contamination of our instruments with tissues that would necessarily prevent disinfection of instruments by soaking in 70% isopropyl alcohol. In one case, a small amount of fatty tissue was attached to the thumb forceps and was removed using sterile gauze prior to placing into alcohol. Only visual, rather than microscopic, inspection of organic load of the surgical instruments and gloves was performed in this study. Microscopic inspection of instruments has shown that current decontamination standards may not always effectively remove biologic material, thereby posing a source of cross-contamination, and should be considered.[23,31] Each surgical series began with autoclaved instruments and sterile gloves, ensuring the absence of all microorganisms. Surgical instruments were not likely to be contaminated with fungi or spores because the procedure was a nongastrointestinal surgery. Mice and rats have been shown to be at a low risk of infection from bacterial spores from either spontaneous or iatrogenic sources.[15,18] However, rodents can and do develop postprocedural infections. For a more comprehensive discussion of potential causes and effects of postsurgical infections in rodents, we refer the reader to other publications.[3,11,13,19,28,38]

Other methods of maintaining aseptic technique between animals have been suggested, such as glass bead sterilizers and multiple sets of sterile instruments.[11,13,16] Glass bead sterilizers have been used for many years in the dental profession but are not currently cleared by the US Food and Drug Administration because of the potential risk of sterilization failure.[30] In addition, glass bead sterilization may introduce the risk of damage to the delicate, often expensive, surgical instruments used in certain rodent surgical procedures.

We recognize that alcohols are neither sterilants nor high-level disinfectants, but we nonetheless believe that they have a place in serial rodent surgeries that initially use sterile instruments and gloves. Alcohols are rapidly bactericidal, often in less than 30 s, and do not leave a residue.[30] These attributes provide a rapid, cost-effective means of disinfection between high-throughput rodent surgical procedures. In light of our study, caution is necessary when using the same instruments and gloves for 6 or more mice (up to 10) during a single surgery session similar to the one we have described. For longer, more invasive, highly specialized surgical procedures, such as gastrointestinal, cardiac, and neurologic procedures, the risks associated with contamination may exceed the benefits of rapid instrument decontamination with 70% isopropyl alcohol. Exceptions to the *Guide* are allowed, if evaluated and approved by the IACUC, and may be necessary for certain types of serial rodent surgeries. However, the basic principles of aseptic surgical technique using sterilized instruments and gloves should be followed as a best practice.

References

1. **American College of Laboratory Animal Medicine.** [Internet]. Guidelines—position statement: rodent surgery. [Cited 2 Feb 2009]. Available at: http://www.aclam.org/education/guidelines/position_rodentsurgery.html.
2. **Animal Welfare Regulations.** 2008. 9 CFR §1-4.
3. **Badia JM, Torres JM, Tur C, Sitges-Serra A.** 1996. Saline wound irrigation reduces the postoperative infection in guinea pigs. J Surg Res **63:**457–459.
4. **Best M, Kennedy ME.** 1992. Effectiveness of handwashing agents in eliminating *Staphylococcus aureus* from gloved hands. J Appl Bacteriol **73:**63–66.
5. **Bhattacharyya N, Kepnes LJ.** 2004. The effectiveness of immersion disinfection for flexible fiberoptic laryngoscopes. Otolaryngol Head Neck Surg **130:**681–685.
6. **Biology of the Mammary Gland.** [Internet]. Renal capsule grafting. [Cited 2 Feb 2009]. Available at: http://mammary.nih.gov/tools/mousework/cunha001/index.html.
7. **Brown MJ.** 2005. Aseptic surgery for rodents. Sci Cent Anim Welfare Newslett **27:**9–14.
8. **Bukhari SS, Harrison RA, Sanderson PJ.** 1993. Contamination of surgeons' glove fingertips during surgical operations. J Hosp Infect **24:**117–121.
9. **Call TR, Auerbach FJ, Riddell SW, Kiska DL, Thongrod SC, Tham SW, Nussmeier NA.** 2009. Nosocomial contamination of laryngoscope handles: challenging current guidelines. Anesth Analg **109:**479–483.
10. **Chu NS, Chan-Myers H, Ghazanfari N, Antonoplos P.** 1999. Levels of naturally occurring microorganisms on surgical instruments after clinical use and after washing. Am J Infect Control **27:**315–319.
11. **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:**27–32.
12. **Cullan DB 2nd, Wongworawat MD.** 2007. Sterility of the surgical site marking between the ink and the epidermis. J Am Coll Surg **205:**319–321.
13. **Cunliffe-Beamer TL.** 1993. Applying principles of aseptic surgery to rodents. Anim Welfare Info Center Newslett **4:**3–6.

14. **Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CRC, Wilkins EGL, Worsley MA, Kay PR.** 1999. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br **81:**886–889.
15. **Ganaway JR.** 1982. Bacterial and mycotic diseases of the digestive system, p 12. In: Foster HL, Small JD, Fox JG, editors. The mouse in biomedical research, vol 2. New York (NY): Academic Press.
16. **Héon H, Rousseau N, Montgomery J, Beauregard G, Choinèire M.** 2006. Establishment of an operating room committee and a training program to improve aseptic techniques for rodent and large animal surgery. J Am Assoc Lab Anim Sci **45:**58–62.
17. **Howard HL, Haywood JR, Laber K**. 2001. Alcohol as a disinfectant. AAALAC International Connection Winter/Spring 2001:16.
18. **Huerkamp MJ.** 2002. Alcohol as a disinfectant for aseptic surgery of rodents: crossing the thin blue line? Contemp Top Lab Anim Sci **41:**10–12.
19. **Institute for Laboratory Animal Research.** 1991. Infectious diseases of mice and rats. Washington (DC): National Academies Press.
20. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
21. **Jovanovic BD, Levy PS.** 1997. A look at the rule of three. Am Stat **51:**137–139.
22. **Kibria SMG, Kerr KG, Gough MJ, Homer-Vanniasinkam S, Mavor AID.** 2002. Bacterial colonization of Doppler probes in vascular surgical wards. Eur J Vasc Endovasc Surg **23:** 241–243.
23. **Lipscomb IP, Sihota AK, Keevil CW.** 2008. Comparison between visual analysis and microscope assessment for surgical instrument cleanliness from sterile service departments. J Hosp Infect **68:**52–58.
24. **Murdoch H, Taylor D, Dickinson J, Walker JT, Perrett D, Raven NDH, Sutton JM.** 2006. Surface decontamination of surgical instruments: an ongoing dilemma. J Hosp Infect **63:**432–438.
25. **Obasi C, Agwu A, Akinpelu W, Hammons R, Clark C, Etiennecummings R, Hill P, Rothman R, Babalola S, Ross T, Carroll K, Asiyanbola B.** 2009. Contamination of equipment in emergency settings: an exploratory study with a targeted automated intervention. Ann Surg Innov Res **3:**8.
26. **Owens CD, Stoessel K.** 2008. Surgical site infections: epidemiology, microbiology, and prevention. J Hosp Infect **70 S2:**3–10.
27. **Pinto FMG, Queiroz de Souza R, Barreto da Silva C, Mimica LMJ, Graziano KU.** 2010. Analysis of the microbial load in instruments used in orthopedic surgeries. Am J Infect Control **38:**229–233.
28. **Popp MB, Brennan MF.** 1981. Long-term vascular access in the rat: importance of asepsis. Am J Physiol **241:**H606–H612.
29. **Rutala WA, Gergen MF, Jones JF, Weber DJ.** 1998. Levels of microbial contamination on surgical instruments. Am J Infect Control **26:**143–145.
30. **Rutala, WA, Weber, DJ, Healthcare Infection Control Practices Advisory Committee.** 2008. Guideline for disinfection and sterilization in healthcare facilities, 2008. Atlanta (GA): Centers for Disease Control.
31. **Smith A, Dickson M, Aitken J, Bagg J.** 2002. Contaminated dental instruments. J Hosp Infect **51:**233–235.
32. **Sullivan T, Stethem C, Shomer N.** 2006. Examination gloves used for survival rodent surgery: assessment of various sterilization methods. 57th AALAS National Meeting, 15–19 October 2006. Salt Lake City, UT
33. **Szot GL, Koudria P, Bluestone JA.** 2007. Transplantation of pancreatic islets into the kidney capsule of diabetic mice. J Vis Exp **9:**404.
34. **Talbot GH, Skros M, Provencher M.** 1985. 70% alcohol disinfection of transducer heads: experimental trials. Infect Control **6:**237–239.
35. **Tu XM, Litvak E Pagano M.** 1994. Screening tests: can we bet more by doing less. Stat Med **13:**1905–1919.
36. **Tu XM, Litvak E, Pagano M.** 1995. On the informativeness and accuracy of pooled testing in estimating prevalence of a rare disease: application to HIV screening. Biometrika **82:**287–297.
37. **University of Rochester Medical Center.** [Internet]. University Committee on Animal Resources: guidelines for aseptic recovery surgery on nonregulated animals. [Cited 5 Feb 2010]. Available at: http://www.urmc.rochester.edu/ucar/manual/recsurg2.htm.
38. **Waynforth HB, Flecknell PA.** 1992. Experimental and surgical technique in the rat. San Diego (CA): Academic Press.
39. World Health Organization. 2004. Laboratory biosafety manual, 3rd ed. Geneva (Switzerland): World Health Organization.