American Journal of Infection Control 43 (2015) 1331-5

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Ability of cleaning-disinfecting wipes to remove bacteria from medical device surfaces

Elizabeth A. Gonzalez PhD *, Poulomi Nandy PhD, Anne D. Lucas PhD, Victoria M. Hitchins PhD

Division of Biology, Chemistry, and Material Sciences, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD

Key Words:
Reusable medical devices
Cleaning
Disinfection
Nosocomial infection

Background: Nosocomial infections are a serious problem in health care facilities. Bacteria can be transferred from patient to patient via contaminated reusable medical devices and equipment.

Methods: An anesthesia machine and objects representative of smooth and ridged machine knobs were contaminated with *Staphylococcus aureus*, *Bacillus atrophaeus* spores, and *Clostridium sporogenes* spores. The ability of 5 commercially available cleaning-disinfecting wipes to remove bacteria was compared with gauze soaked with water or bleach. Gauze soaked with water was used to determine the optimal wetness for bacteria removal, which was then used to evaluate the efficacy of the wipe ingredients.

Results: All of the wipes cleaned the device surfaces significantly better than the no wipe control. Some wipes performed equally well as gauze with water, whereas others performed worse. Overall, the wipe containing sodium hypochlorite was the most effective at removing bacteria. When the wipe ingredients were re-evaluated using the determined optimal wipe wetness on gauze, their effectiveness at cleaning *S aureus*, but not spores, significantly improved.

Conclusion: Physically removing bacteria from device surfaces with water was often as effective as the cleaning-disinfecting wipes. Of the wipe active ingredients evaluated, sodium hypochlorite was the most effective overall. The wetness of the wipes may also play a role in their effectiveness.

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Hospital-acquired infections remain a serious problem, especially for critically ill or immunocompromised patients. These infections can increase the time and cost of health care and fatality rates.[1-3] As new strains of antibiotic-resistant bacteria evolve, treating these hospital-acquired infections becomes more difficult. There are numerous reports of outbreaks in hospitals of multidrug-resistant bacteria, including multidrug-resistant *Pseudomonas aeruginosa*,[4-7] *Mycobacterium tuberculosis*,[8] *Acinetobacter baumanni*,[7,9] and *Staphylococcus aureus*.[10-12]

Cleaning is the critical first step in reprocessing reusable medical devices to reduce soil and bioburden. Reducing the soil on a used device is needed to ensure subsequent effective disinfection or sterilization.[13,14] Bacteria responsible for nosocomial infections can be introduced directly by a contaminated device or indirectly via the gloved hands of health care personnel who touch a contaminated device and then a patient.[15] Therefore, it is important that noncritical reusable devices and equipment, which are not in direct contact with patients (eg, anesthesia machines), are cleaned appropriately between uses. Increasing the level of environmental disinfection has been shown to decrease the spread of both vancomycin-resistant *Enterococcus* and methicillin-resistant *S aureus* (MRSA) in health care settings.[16,17]

Currently, many hospitals use commercially available disinfecting wipes to clean and disinfect their noncritical devices between uses. These wipes make antimicrobial claims on their labels and are

* Address correspondence to Elizabeth A. Gonzalez, PhD, 10903 New Hampshire Ave, Bldg 64, Rm 4022, Silver Spring, MD 20993.
E-mail address: Elizabeth.gonzalez@fda.hhs.gov (E.A. Gonzalez).

Funding/Support: This work was supported in part by an appointment to the Research Participation Program at the Center for Devices and Radiological Health administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration and from the U.S. Food and Drug Administration's Medical Countermeasures Initiative.

Disclaimer: Because of limitations in publication length, some data were not included in the manuscript. The data are available on direct request from the corresponding author. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services. The findings and conclusions in this article have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any agency determination or policy.

Conflicts of Interest: None to report.

0196-6553/$00.00 - Published by Elsevier Inc. on behalf of the Association for Professionals in Infection Control and Epidemiology, Inc.
http://dx.doi.org/10.1016/j.ajic.2015.07.024

regulated by both the Environmental Protection Agency and the Food and Drug Administration. However, although the ingredients may be effective bactericides when their activity is measured in a test tube, it is unclear how the wipes perform on a device surface. Siani et al demonstrated that there was a discrepancy between the ability of a wipe to kill *Clostridium difficile* spores in a test tube versus on a surface.[18] Previously, our laboratory compared the ability of several cleaning and disinfecting wipes to remove *Streptococcus pneumoniae* and artificial blood test soil from a medical device surface.[19] In this study, *S aureus* (surrogate for MRSA), *C sporogenes* (surrogate for *C difficile*), and *Bacillus atrophaeus* (surrogate for *B anthracis*) were used to study the influences of device design and wipe wetness on removing bacteria from device surfaces. *C difficile* and MRSA are 2 bacteria known to cause nosocomial infections,[20,21] and *B anthracis* is a Centers for Disease Control and Prevention category A select agent pathogen.[22] *C difficile* and *B anthracis* are of particular concern because they form spores.

METHODS

Bacteria

S aureus (ATCC 6538) was purchased from American Type Culture Collection (Manassas, VA) and used as a surrogate for MRSA. Liquid cultures were grown in trypticase soy broth in a shaking incubator at 225 rpm at 37°C. Colonies were enumerated after plating on trypticase soy agar (TSA) and incubating for approximately 16 hours at 37°C. *B atrophaeus* spores (NRRL B4418), surrogate for *B anthracis* spores, were purchased from Steris (Mentor, OH). Colonies were enumerated after plating on TSA. *C sporogenes* spores (ATCC 7955), surrogate for *C difficile*, were purchased from Mesa Laboratories (Lakewood, CO). For propagation, *C sporogenes* spores were plated on blood agar plates, incubated in an anaerobic growth chamber with GasPak EZ (BD Biosciences, San Jose, CA) at 37°C for 10 days, and then scraped off the plates with a glass rod. The bacteria were then collected as a pellet through centrifugation at 11,000 × g for 15 minutes in a tabletop centrifuge. The pellet was washed with phosphate buffered saline (PBS), centrifuge as previously indicated, and then resuspended in sterile PBS. The bacteria were then incubated in an 80°C water bath for 20 minutes to kill any remaining vegetative cells. Spores were stored at 4°C.

Cleaning bacteria from the surface of an anesthesia machine

As previously reported, the surface of the Dräger Fabius GS anesthesia machine (Draeger Medical Inc., Telford PA) was taped off into 2.5- × 2.5-cm squares.[19] Then, 10 μL of bacteria was applied to each square and allowed to dry for 1 hour. *S aureus* was applied at approximately 10^{10} colony forming units (CFU)/mL. *B atrophaeus* and *C sporogenes* spores were applied at approximately 10^8 CFU/mL. Squares were cleaned by wiping in a horizontal motion 3 times, with 1 of the 5 commercially available wipes or with sterile gauze soaked in water or in 5% bleach diluted 1:10 in water. The positive control square was not cleaned, and the negative control square was not inoculated with bacteria. After 10 minutes, each square was then swabbed with a BactiSwab (Remel, Lenexa, KS) and vigorously swished in 1 mL of PBS. Serial dilutions and plating on TSA were performed to calculate CFU/mL. Percent of CFU remaining was calculated by dividing the sample CFU/mL by that of the positive control. Experiments were repeated 3-5 times.

Cleaning bacteria from smooth and ridged caps

To simulate the actual smooth and ridged knobs on the anesthesia machine, sterile flat caps and ridged caps of 15-mL conical Falcon tubes (Thermo Fisher Scientific, Waltham, MA) were used. These caps were immersed in the bacteria solution at 10^8 CFU/mL for 1 second and dried for 1 hour. Caps were then cleaned by wiping them 3 times in a circular motion with 1 of the 5 commercially available wipes or with sterile gauze soaked in water or bleach diluted 1:10 in water. The positive control cap received no cleaning, and the negative control cap was not inoculated with bacteria. After 10 minutes, the caps were placed in 5 mL of sterile PBS and vortexed for 3 seconds. The supernatant was then plated to collect residual bacteria. Plating and calculations were performed as previously described. Experiments were run 3-5 times.

Table 1
Details of the 5 commercially available wipes

Wipe no.	Active ingredient	% ingredient	Wetness (g/cm^3)
1	Diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride	0.28/17.2	0.618
2	Citric acid	0.6	0.619
3	Sodium hypochlorite	0.55	0.541
4	Hydrogen peroxide	0.5	0.667
5	o-phenylphenol/o-benzyl-p-chlorophenol	0.28/0.03	0.227

NOTE. The 5 commercially available wipes used to kill and remove bacteria are listed with their active ingredients, percent ingredient, and their wetness expressed as grams of liquid per cubic centimeter of wipe.

Neutralization

First, it was determined whether Dey/Engley (D/E) Neutralizing Broth (BD Biosciences, San Jose, CA) was capable of neutralizing the disinfectants of interest. To do this, bleach (1:10) and the 5 wipe ingredients were diluted 1:10 in either PBS or D/E Neutralizing Broth, incubated for 10 minutes at room temperature to allow for neutralization. *S aureus* was then added at approximately 10^3 CFU/mL. After another 10 minutes of incubation at room temperature, samples were serially diluted, plated, and incubated overnight. Experiments were performed in triplicate.

To determine whether there was significant death from residual disinfectant after serial dilution, bacteria were added to bleach (1:10) or liquid squeezed from 1 of the 5 wipes (Table 1) and then incubated at room temperature for 10 min. *S aureus* was added at 10^{10} CFU/mL, whereas *B atrophaeus* and *C sporogenes* spores were added at 10^8 CFU/mL. The bacteria-disinfectant solutions were then diluted 1:10 in either PBS or D/E Neutralizing Broth and incubated at room temperature for 10 minutes. Samples were then diluted serially and plated, and incubated overnight. Experiments were performed in triplicate.

Long-term survival

Bacteria were applied to several squares on the anesthesia machine surface and several smooth and ridged caps as previously described. Bacteria were used at the same concentrations as those used in the cleaning experiments. The initial time 0 sample was taken after 1 hour of drying. Subsequent samples were taken after 1 day, 3 days, 1 week, 2 weeks, and 1 month. All incubations were performed at ambient room temperature. Samples were collected, diluted, and plated as previously described. Experiments were performed in triplicate.

Statistics

All experiments were repeated 3-5 times. Data is reported as the mean ± the standard error of the mean (SEM). Unpaired Student *t* tests were performed using Microsoft Excel (Microsoft, Redmond,

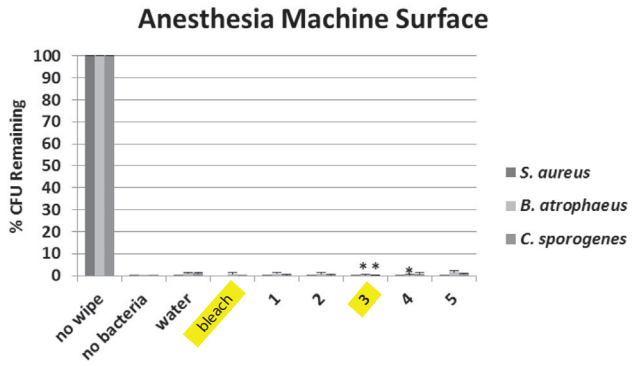

Fig 1. Cleaning bacteria from the anesthesia machine surface using cleaning-disinfecting wipes as packaged. All of the 5 commercially available wipes significantly cleaned Staphylococcus aureus, Bacillus atrophaeus spores, and Clostridium sporogenes spores from the surface of the anesthesia machine compared with the positive control. *Wipes that cleaned the surface significantly different than gauze soaked with water ($P < .05$). CFU, colony forming units.

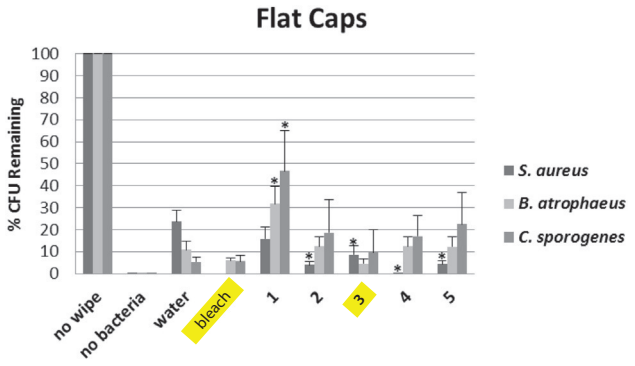

Fig 2. Cleaning bacteria from the flat caps using cleaning-disinfecting wipes as packaged. All of the 5 commercially available wipes significantly cleaned Staphylococcus aureus, Bacillus atrophaeus spores, and Clostridium sporogenes spores from the flat caps compared with the positive control. *Wipes that cleaned the surface significantly different than gauze soaked with water ($P < .05$). CFU, colony forming units.

WA) to determine if 2 data sets were significantly different from one another. A P value of $< .05$ was considered to be significant.

RESULTS

Wipe effectiveness as packaged

The effectiveness of cleaning-disinfecting wipes to clean an anesthesia machine was measured. This device is reusable, has a variety of surface types, and can be neglected between uses because it is a noncritical device with often no specific person assigned to reprocess it. The 2 device surface types that were compared for cleanability were as follows: (1) a large, flat, horizontal, relatively smooth area and (2) smaller knobs on the device. The anesthesia machine had 3 knobs with varying ridge sizes. The caps of the 15-mL Falcon tubes served as surrogates because the caps are similar to the knobs in size and material. Additionally, we investigated 2 types of caps (flat and ridged) to directly compare the effect that added texture, such as ridges, has on cleanability.

D/E Neutralizing Broth neutralized all of the wipe ingredients, and there was no difference in the number of bacteria after exposure to disinfectant whether or not neutralization was performed (data available on request). The 5 commercial wipes and gauze wetted with water and bleach significantly cleaned S aureus, B atrophaeus spores, and C sporogenes spores from both the anesthesia machine surface and the caps compared with the no wipe control. This indicates that the cleaning-disinfecting wipes tested are capable of removing and killing both vegetative and spore-forming bacteria from a variety of device designs.

Even though the commercially available wipes removed all of the S aureus from the surface of the anesthesia machine, their performance was not statistically better than gauze with water, which removed almost all (99.99%) of the bacteria (Fig 1). However, all of the commercial wipes, except wipe 1, significantly outperformed gauze with water when cleaning S aureus from the flat and ridged caps (Figs 2-3). Wipe 4, containing hydrogen peroxide as the active ingredient, was the most effective at removing S aureus from either the flat or ridged caps.

The B atrophaeus and C sporogenes spores were more difficult to clean from both the anesthesia machine surface and the caps compared with S aureus. On the anesthesia machine surface, cleaning with water and gauze reduced the number of spores by approximately 99%, which is 2 logs less than the reduction of S aureus from the anesthesia machine surface (Fig 1). The only wipe to perform significantly better than gauze with water in removing both spore types from the machine surface was wipe 3 (Fig 1). Wipe 4 significantly removed more B atrophaeus spores, but not C sporogenes spores, from the anesthesia machine surface than gauze with water (Fig 1).

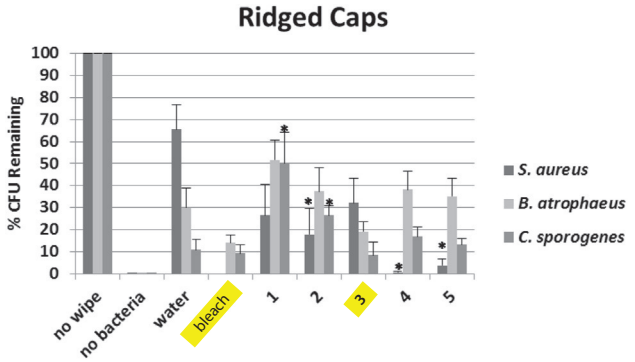

Fig 3. Cleaning bacteria from the ridged caps using cleaning-disinfecting wipes as packaged. All of the 5 commercially available wipes significantly cleaned Staphylococcus aureus, Bacillus atrophaeus spores, and Clostridium sporogenes spores from the ridged caps compared with the positive control. *Wipes that cleaned the surface significantly different than gauze soaked with water ($P < .05$). CFU, colony forming units.

Gauze with water removed approximately 70%-90% of the spores from the flat and ridged caps. When cleaning spores from caps, none of the wipes performed significantly better than gauze with water, and there were 2 wipes that performed significantly worse than the water control (Figs 2-3). Wipe 1 reduced the number of both B atrophaeus and C sporogenes spores on the flat caps by approximately 55%-70% or 0.6 log, which was consistently worse than the water control (Fig 2). Both wipes 1 and 2 were significantly worse than gauze with water at removing C sporogenes spores from ridged caps (Fig 3). The wipes reduced the number of C sporogenes spores by approximately 50%-75% or 0.5 log. Given that gauze with water was meant to represent the physical removal aspect of cleaning, it was unexpected that the cleaning-disinfecting wipes performed worse in some instances. It was noted that one variable that varied between the different wipes used was their wetness. Therefore, we investigated whether the wetness of the wipes in this study may play a role in their effectiveness.

Wipe ingredient effectiveness reapplied at optimal wipe wetness on gauze

The wipes were weighed before and after drying as previously reported[19] and were found to have a wipe wetness of 0.23-0.67 g of liquid/cm^3 of wipe (Table 1). To control for the varying amount of wetness in each wipe, the liquid was squeezed from each of the commercial wipes and reapplied on gauze at an optimal wipe wetness. The optimal wipe wetness was first determined for each of the device surfaces using gauze and water. Water was added to sterile gauze at 5 different levels of wipe wetness: 0.2, 0.4, 0.6, 0.8, and 1.0 g of liquid/cm^3 of wipe, which encompassed the range of wetness seen in the commercially available wipes. Because *B atrophaeus* spores were generally the most difficult organism to remove from the devices, they were used as a worst-case cleaning scenario in this study.

A wipe wetness of 0.6 g of water/cm^3 of gauze removed the most *B atrophaeus* spores from the anesthesia machine surface and had the smallest SEM (0.33) compared with other degrees of wipe wetness (data available on request). Additionally, the 0.6 g/cm^3 wetness performed significantly better than the wettest wipe when removing spores from the anesthesia machine surface. Therefore, 0.6 g/cm^3 was chosen as the optimal wipe wetness to clean the anesthesia machine surface. When cleaning the flat caps, 0.8 g/cm^3 was used as the optimal wipe wetness because it had both the least bacteria remaining (3.03%) and the smallest SEM (0.89). Additionally, the 0.8 g/cm^3 gauze cleaned significantly better than the driest of the wipes. None of the differing degrees of wipe wetness were significantly better than the others at cleaning the ridged caps, indicating that wetness did not play a significant role in the ability to clean the ridged caps (data available on request). To be comprehensive, we decided to include the ridged caps in the optimal wipe wetness experiments and used the flat caps' optimal wipe wetness value (0.8 g/cm^3).

The liquid from the 5 commercial wipes was collected and added to sterile gauze at 0.6 and 0.8 g/cm^3 to clean the anesthesia machine surface and caps, respectively. Because the cleaning-disinfecting wipes already removed 100% of the *S aureus* from the anesthesia machine surface when they were used as packaged, this experiment was not repeated at optimal wipe wetness. The wipe ingredients used on gauze at optimal wipe wetness behaved similarly to the packaged wipes when cleaning either *B atrophaeus* or *C sporogenes* spores from the anesthesia machine surface (data available on request). None of the wipe ingredients performed worse than water, and only sodium hypochlorite (active ingredient in bleach and wipe 3) performed significantly better than water (data available on request). The biggest difference in cleaning effectiveness was seen when the wipe ingredients were used at 0.8 g/cm^3 to clean *S aureus* from the caps. Although most wipes used as packaged removed significantly more *S aureus* from the caps compared with water, there were still approximately 10% of the bacteria remaining on the devices (Figs 2-3). However, when the caps were cleaned with the wipe ingredients on gauze at optimal wipe wetness, almost all (>99.9%) of the *S aureus* was removed (Fig 4). Data for cleaning the ridged caps with wipe ingredients at optimal wipe wetness are available on request.

When the wipe ingredients were used to clean spores from the caps at optimal wipe wetness, only ingredients from wipe 3 cleaned significantly better than water. Interestingly, when the ingredients from wipes 1 and 2 were used at 0.8 g/cm^3 on gauze, they were no longer significantly less effective than water at removing the spores from the caps (Fig 4). Because cleaning endpoints are typically normalized to the area of the device, we also calculated the bacteria remaining on the device surfaces after cleaning at optimal wipe wetness (CFU/cm^2) (data available on request). After cleaning the bacteria from the device surfaces, there was 2-3 log CFU/cm^2 remaining on the anesthesia machine surface and 2-5 log CFU/cm^2 on the caps. In all but one of the experiments, ingredients from wipe 3 left the fewest number of bacteria on the surfaces.

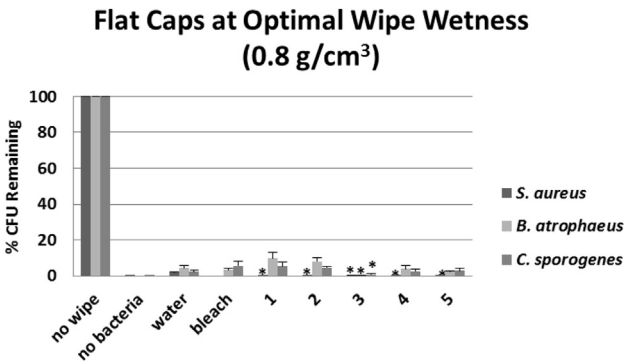

Fig 4. Cleaning bacteria from the flat caps using gauze and the ingredients from the cleaning-disinfecting wipes. Ingredients from the 5 cleaning-disinfecting wipes were applied to sterile gauze at 0.6 g/cm^3 to clean the flat caps. All of the wipes significantly cleaned the surface from all 3 bacteria types compared with the positive control. *Wipes that cleaned the surface significantly better than gauze soaked with water ($P < .05$). *CFU*, colony forming units.

Long-term survival on device surfaces

Both *B atrophaeus* and *C sporogenes* are spore formers and are resistant to environmental hazards, such as temperature and drying. *S aureus* has also been shown to survive on surfaces for several months.[23] We determined if these bacteria were capable of remaining viable after approximately 10^6-10^8 CFU were dried on the anesthesia machine surface and caps. Both spore types remained relatively constant in number over a period of 1 month on all 3 surface types. *S aureus* were reduced by approximately 1 log after 3 days at room temperature on the surfaces and 4 log after a month (data available on request). These data indicate that if bacteria were not removed or killed immediately after contamination, they could remain on the device for a long time and be a source for cross-contamination.

CONCLUSION

In general, when the wipes were used as packaged, they performed either as well or significantly better than gauze with water to clean the bacteria from the devices. When cleaning vegetative bacteria from a smooth flat surface, the wipes were successful at removing bacteria to the limit of detection. Interestingly, there were a few instances when the wipes performed significantly worse than the gauze-water control while cleaning the caps. We hypothesized that this could be caused by a difference in the wipes' wetness. Using gauze soaked with water we determined that a moderate wipe wetness appeared to be optimal for cleaning both the anesthesia machine surface and the flat caps. There did not appear to be a clear effect of wipe wetness in cleaning the ridged caps. Of the 5 commercial wipes that were used, wipes 1-4 had very similar degrees of wipe wetness, whereas wipe 5 was noticeably drier.

When the wipe ingredients were used at optimal wetness to clean the anesthesia machine surface, there were between 2 and 3 log of bacteria remaining per cm_2 of the device surface (data available on request). Currently, there is no accepted cleaning benchmark for bacteria on medical devices. However, Alfa et al found that soiled

endoscopes had approximately 2.5 log CFU/cm^2 after performing manual cleaning,[24] a number that is consistent with what we found on the anesthesia machine surface. When cleaning *S aureus* off of the caps, all of the wipe ingredients left well below 2.5 log CFU/cm^2, with most leaving no detectable bacteria. However, when cleaning spores from the caps, most of the wipe ingredients were not able to meet 2.5 log CFU/cm^2, leaving behind between 4 and 5 log of spores/cm^2. However, it is difficult to draw any conclusions based on the raw CFU values alone because the number of bacteria in the positive controls vary greatly between the anesthesia machine surface and the caps (data available on request). We have taken these differences into account by calculating the percent CFU remaining compared with the positive control to compare the data proportionally across the bacteria and surface types.

While analyzing the data as percent CFU remaining, wipes 1 and 2 cleaned the caps significantly worse than water. However, the ingredients in wipes 1 and 2 performed equally well as water when they were applied on gauze at the optimal wipe wetness. Therefore, we can conclude that the wipe ingredients themselves are not inferior to water. However, it does not appear that wipe wetness is the only factor driving the cleaning effectiveness of the wipes because the 2 wipes that cleaned significantly worse than water when used as packaged (wipes 1 and 2) were not particularly wetter or drier than wipe 3, which performed the best in those experiments. It is possible that another secondary factor may also be playing a role in a wipe's effectiveness: texture. Although we did not directly measure the effect of texture on the wipes' cleaning ability, it may be that the texture and composition of gauze are more effective at removing bacteria than wipes 1 and 2.

This is not to say that wipe wetness is an unimportant variable. We demonstrated that gauze with a moderate amount of liquid was superior in cleaning than wetter or drier gauze. Interestingly, 4 out of the 5 wipes have a wipe wetness ratio around 0.6 g/cm^3, which was determined to be the optimal wipe wetness in cleaning the anesthesia machine surface. There are other disinfecting wipes on the market that are significantly wetter than the ones we used; although we have not included them in this study for technical reasons, users should realize that, based on our studies, a wetter wipe is not necessarily a more effective wipe. Additionally, wet wipes may seep into the electronic components in reusable devices, which could lead to device failures.[25]

Finally, we have reaffirmed the importance of actively cleaning surfaces between uses by demonstrating that both spores and *S aureus* can remain viable after being dried on the surface of a reusable medical device for a month. The hardiness of *Staphylococcus* bacteria and *Clostridium* spores may contribute to them being a continual source for cross-contamination in the health care environment. If these bacteria are not successfully removed from device surfaces, they can remain viable and ready for transfer to the next patient.

Acknowledgments

We thank Drs. Elaine Mayhall and Samantha Spindel for providing comments on the manuscript.

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