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Major article

# Cleaning assessment of disinfectant cleaning wipes on an external surface of a medical device contaminated with artificial blood or *Streptococcus pneumoniae*

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*Key Words:*
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***Background:*** Improperly cleaned, disinfected, or sterilized reusable medical devices are a critical cause of health care-associated infections. More effective studies are required to address the improvement of cleaning and disinfection instructions, as well as selection of cleaning and disinfecting agents, for surfaces of reusable devices and equipment.

***Methods:*** Six commercially available disinfectant cleaning wipes were evaluated for their effectiveness to remove a coagulated blood test soil or *Streptococcus pneumoniae* bacteria from the surface of a reusable medical device. Liquid aliquots of the coagulated blood or bacteria were dried onto the surface of the device and removed with the wipes. Effectiveness of the wipes was assessed by 3 methods: residual protein debris by o-phthaldialdehyde analysis, bacterial survival by adenosine triphosphate measurement, and force required to remove the dried debris by force measurement.

***Results:*** A sodium hypochlorite wipe was most effective in removing protein debris from the device surface. All tested wipes were equivalent in disinfecting bacterial contamination from the device surface.

***Conclusion:*** The active ingredient, wipe design, and wipe wetness are important factors to consider when selecting a disinfectant cleaning wipe. Additionally, achieving conditions that effectively clean, disinfect, and/or inactivate surface bacterial contamination is critical to preventing the spread of health care-associated infections.

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Medical devices have become an increasingly common source of health care-associated infections (HAIs).[1-3] A growing body of literature suggests that device-associated HAIs (DA-HAIs) are among the main causes of patient morbidity and mortality within hospital intensive care units.[3-5] Statistics released by the Centers for Disease Control and Prevention in 2002 indicate that there were over 1.7 million HAIs within the course of a single year, leading to almost 99,000 deaths that were either directly caused by or associated with HAI.[6] It has also been suggested that DA-HAIs account for an upwards of 60% to 80% of all bloodstream, urinary tract, and pneumonia-related HAIs,[3,7] signifying a need for new infection control measures to reduce the involvement of medical devices in such infections. Although many of these DA-HAIs are due to single-use devices (eg, urinary catheters, needleless connectors), reusable medical devices and equipment are also a concern because they have been documented to serve as a reservoir for pathogens if not reprocessed correctly between uses.[8,9]

One of the critical issues that need to be considered when designing new infection control measures is the influence of device design (physical design, materials used in fabrication) on the reprocessing of reusable medical devices. Reprocessing is a detailed, multistep procedure that reusable medical devices undergo to make them ready for reuse on the next patient. This procedure involves any, or a combination of, the following processes: cleaning,

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decontamination/disinfection, packaging, and sterilization.[10] Cleaning a device is the critical first step in reprocessing any device after it has been used on a patient. This process is designed to remove all gross contaminants, including biologic matter (eg, blood, tissue, pathogens) and nonbiologic materials (eg, lubricants, detergents) from the device for further processing, because their presence can potentially compromise the effectiveness of disinfection or sterilization processes.[9-11] Such contamination can occur by contact with contaminated gloved hands of a health care provider or by inadvertent contamination by aerosols and splatters. Following cleaning of the device, decontamination/disinfection generally occurs; this process kills pathogenic and other microorganisms by physical or chemical means.[10] Last, sterilization is used to render a product free of all viable microorganisms.[10]

For certain device designs such as rough surfaces, knobs, crevices, and narrow lumens, even the initial step of cleaning the device can be difficult. Ali et al noted that the surface roughness of bed rails determined the ability to clean and disinfect organic soil, *Staphylococcus aureus*, and *Acinetobacter baumannii*.[12] They observed that it was easier to clean and disinfect unabsorbed bacteria from bed rails that had fewer microscopic irregularities (ie, that were smoother) than bed rails that had higher surface roughness values.[12] Additionally, much of the interest by others has focused on cleaning and disinfecting reusable devices that are in direct contact with patients.[13,14] However, the external surfaces of reusable medical devices and equipment can also be contaminated by biologic materials (eg, blood, pathogens, and toxins) that are transmitted via skin contact and environmental surfaces. A study performed by Sui et al investigated the bacterial contamination rate on surfaces of mechanical ventilator systems and monitoring equipment.[15] Furthermore, others have found pathogenic microorganisms on monitors, blood gas analyzers, ventilator knobs, and radiant warmer control buttons.[16-18] All such pathogens and other patient materials need to be removed to protect both the patients and the health care providers during reuse.

To aid in the cleaning and disinfection of residual patient material found on reusable medical devices and increase the effectiveness of reprocessing to protect patient safety, more effective methods need to be developed that will aid in cleaning validation for the manufacturer of reusable devices and verification by the health care providers. Toward this end, the goal of this study was to examine how the exterior surface of a medical device may influence the effectiveness of disinfectant cleaning wipes to remove biologic contaminants from a surface of a reusable medical device. Specifically, the aim was to develop a method to assess the efficacy of several commercially available disinfectant cleaning wipes to remove artificial blood debris or *Streptococcus pneumoniae* bacteria. A quantitative protein assay was used to determine residual protein on the external surface of an anesthesia machine after use of a disinfectant cleaning wipe. Additionally, the force required to remove the dried debris was measured using a piezoelectric force plate. Last, an adenosine triphosphate (ATP) bioluminescence system was used to monitor the persistence of bacteria on the surface after wiping. Results from this study will provide useful information on how to improve cleaning and disinfection instructions for external surfaces of these and other reusable devices and equipment.

## METHODS

### *Test soil*

The coagulated blood test soil used within this study was prepared according to Pfeifer.[19] Two solid components A (400 mg albumin, 400 mg hemoglobin, 60 mg fibrinogen; Sigma Aldrich, St Louis, MO) and B (400 mg albumin, 400 mg hemoglobin, 12.5 National Institutes of Health units thrombin; Sigma Aldrich) were dissolved in their respective solvents A (5.0 mL 0.4% NaCl solution) and B (5.0 mL 0.4% NaCl solution + 8.0 mmol/L $CaCl_2$). To dissolve the lyophilized protein products in solution, both components were incubated at 37°C while shaking at 160 rpm for 90 minutes in a heated water bath (Shaking Heated Water Bath Model 25; GCA/Precision Scientific, Chicago, IL). Equal parts of components A and B were mixed immediately before use.

### *Bacterial strain, media, and growth conditions*

*S pneumoniae* ATCC BAA-334 was purchased from American Type Culture Collection (Manassas, VA). Liquid cultures were grown in Brain Heart Infusion media (Becton Dickinson and Company, Franklin Lakes, NJ). For the bacterial counts and relative light unit (RLU) standard curve, bacteria were plated on trypticase soy agar with 5% defibrinated sheep blood (TSA II) (BD). All growths were conducted at 37°C with greater than or equal to 13% $CO_2$ (GasPak EZ Anaerobe Container System Sachet; BD).

### *Disinfectant cleaning wipes and medical device test surface*

The names, manufacturers, Environmental Protection Agency registration numbers, active ingredients, type of packaging, and instructions for use of the disinfectant cleaning wipes can be found in Table 1. Wipes 1, 3, 4, and 6 were purchased from Henry Schein Medical (Melville, NY). Wipes 2 and 5 were purchased from Wexford Labs (Kirkwood, MO) and Detergent Solutions (Sterling Heights, MI), respectively.

A refurbished anesthesia machine was purchased from a third-party vendor. Experiments were performed on the tabletop of the machine, which had a surface roughness equal to 38.7 μm (for measurement method, see the section *Measurement of surface roughness*). This test surface was divided with laboratory tape (Fisher Scientific, Pittsburgh, PA) to include 27 separate test areas, each measuring 9.5-cm wide and 3-cm long with a total area of 28.5 $cm^2$. The tape on the test surface was changed no less than every 4 trials, during which time the surface was cleaned with both sodium hypochlorite and 70% ethanol to limit the amount of gross filth that collected underneath it.

### *Application of the test soil*

One 100-μL drop of coagulated blood test soil or one 50-μL drop consisting of an average of $7 \times 10^5$ colony-forming units (CFUs) of *S pneumoniae* bacteria was applied to the center of each test area on the anesthesia machine tabletop. For the blood test soil, using the pipette tip, the drop was spread in a circular formation to cover an area approximately 2.67 $cm^2$, with a 1.7-cm diameter. Because of the low surface tension of the bacterial drop, the bacteria spread in a rectangular fashion to cover an area approximately 6.4 $cm^2$ with a height of 0.5 cm. Both the coagulated blood and the bacteria were left on the surface to dry at room temperature (between 20°C and 22°C) for approximately 4 hours before cleaning and disinfection began.

### *Cleaning and disinfection of the surface*

To clean and disinfect the anesthesia machine test surface, the manufacturer's instructions for use of each of the 6 wipes were followed (see Table 1). For each disinfectant cleaning wipe tested, the 3 test area replicates were wiped separately with 2 wipes. The first wipe used on the test area served as the prewipe and was used to remove the entire visible blood or bacterial spot (gross filth). The second wipe was used to clean and disinfect the same test area and

**Table 1**
Disinfectant cleaning wipes: the names, manufacturers, active ingredients, type of packaging, instructions for use, and contact time for bacterial kill

| Wipe number | Disinfectant wipe | Manufacturer (EPA registration number) | Active ingredient (%) | Type of packaging | Instructions for use | Contact time (min) |
|---|---|---|---|---|---|---|
| 1 | ProSpray wipe (Cloth size: 6" × 6.75") | Certol International, LLC (46851-10) | o-phenylphenol, o-benzyl-p-chlorophenol (0.28%, 0.03%) | Canister | For surface disinfection, use the 2-step method. To clean, remove 1 or more wipe towelettes and wipe surfaces thoroughly to remove all soils. Discard used wipe towelettes. Remove 1 or more additional fresh wipe towelettes. Reapply disinfectant to previously cleaned surfaces for a 10-minute contact time. | 10 |
| 2 | CleanCide Ready-To-Use Germicidal Detergent Wipes (Cloth size: 6" × 6.75") | Wexford Labs, Inc (34810-35) | Citric acid (0.60%) | Canister | To clean: unfold premoistened cloth and wipe area to be cleaned for 30 seconds or until clean. To disinfect: unfold premoistened cloth and wipe area to be cleaned and disinfected. Use enough wipes for treated surface to remain visibly wet for allotted time. Wipe or let air-dry. For gross filth or heavy soil, use a wipe to clean and then a second wipe to disinfect the surface. | 5-10 |
| 3 | Clorox Germicidal Wipes (Cloth size: 8.75" × 9") | Clorox Professional Products Co (67619-12) | Sodium hypochlorite (0.55%) | Canister | To clean, disinfect, and deodorize hard, nonporous surfaces: wipe hard, nonporous surface to be disinfected. Use enough wipes for treated surface to remain visibly wet for the contact time listed on label. Let air-dry. Gross filth should be removed prior to disinfecting. If streaking is observed, wipe with a clean, damp cloth or paper towel after appropriate contact time has expired. | 0.5-5 |
| 4 | HypeWipe Bleach Towelette (Cloth size: 6" × 12") | Current Technology, Inc (70590-1) | Sodium hypochlorite (0.94%) | Pouch | Open pouch, remove towel. Use towel and excess liquid to wipe surface. Allow solution to remain wet on surface for a proper amount of time. Rinsing: Some manufacturers of equipment/surfaces require rinsing: follow these specific manufacturer's directions for cleaning/disinfecting/ rinsing. Remove all gross filth and heavy soil from surfaces to be disinfected. | 1-2 |
| 5 | Oxivir Tb Disinfectant Wipes (Cloth size: 6" × 7") | Johnson Diversey, Inc (70627-56) | Hydrogen peroxide (0.50%) | Canister | For use as a 1-step cleaner/ disinfectant: preclean heavily soiled areas. Pull wipe from dispenser (canister) and wipe hard, nonporous environmental surfaces. All surfaces must remain visibly wet for allotted time. Allow to air-dry or rinse with potable water if necessary. | 1-10 |
| 6 | CaviWipe (Cloth size: 6" × 6.75") | Metrex Research (46781-8) | Di-isobutylphenoxyethyl dimethyl benzyl ammonium chloride, isopropanol (0.28%, 17.20%) | Canister | Cleaning instructions: use 1 wipe to completely preclean surface of all gross debris. Use a second wipe to thoroughly wet the surface. Repeated use of the product may be required to ensure that the surface remains visibly wet for allotted time at room temperature. For use as a disinfectant: use a second wipe to thoroughly wet the surface. Repeated use of the product may be required to ensure that the surface remains visibly wet for the recommended amount of time. | 2-3 |

*EPA*, Environmental Protection Agency.

made contact with the test surface for approximately 3 seconds. To wipe the small test area, the wipe had to be folded/configured to be no wider than 3 cm. The test areas were wiped predominantly left-to-right (horizontal direction) because this was the largest dimension of the test area. The surfaces were kept wet as per manufacturer's instructions with reapplications if necessary. For each disinfectant cleaning wipe used, a new pair of disposable gloves was worn (1 pair of gloves/disinfectant wipe brand).

For the blood test soil, a total of 10 trials (each consisting of 3 replicates) were performed, whereas, for the bacterial soil, 3 trials were performed (each consisting of 3 replicates). For each separate trial, the wipes used for cleaning and disinfecting were rotated around the demarcated areas of the test surface in a counter-clockwise fashion so that no one disinfectant cleaning wipe was used on the same area more than twice. The same person performed all of the cleaning/disinfecting throughout the study to limit the variation in force applied while wiping. (Note: actual force applied was not measured or standardized during these experiments.)

*Extraction of residual protein debris*

To remove the residual protein debris from the surface of the anesthesia machine, we adapted a technique commonly used to verify cleaning of large, reusable medical devices employing swabs. For our "swab," we utilized a disposable, polystyrene cuvette (Fisher Scientific) that had been filled with epoxy and cured (Loctite Instant Mix 0.47 fluid oz; Westlake, OH) to give the top of the cuvette a flat, even surface. A 9-$cm^2$ (3 cm $\times$ 3 cm) piece of WYPALL brand X60 (Kimberly Clark Professional, Neenah, WI) paper towel was cut and attached to the top of the epoxy-filled cuvette with a small latex elastic band (Goody Products pony-tail holder 100-pk; Atlanta, GA). To extract the residual protein debris from the test surface, the paper towel square was wetted with 50 μL of 1% sodium dodecyl sulfate (SDS; Sigma Aldrich). This swab was then used to wipe and sample the entire surface of the test area. The flat test surface areas were wiped 3 times left-to-right (horizontal direction) using an average force of 5.9 $\pm$ 0.18 Newtons (N) and 8 times up-and-down (vertical direction, 90° to the horizontal direction) with an average of 7.3 $\pm$ 0.26 N. (For the force measurement method, see the section *Force measurement*.) After sampling, the paper towel square was removed from the top of the "swab" and submerged in o-phthaldialdehyde (OPA) solution to extract the residual protein debris for absorbance determination (see *OPA protein method*).

*OPA protein method*

OPA assays were performed and modified from the method previously described by Friedrich et al[20] and McCormick et al.[21] Briefly, OPA solution was prepared by dissolving 40 mg of phthaldialdehyde (Sigma Aldrich) and 100 mg 2-dimethylaminoethanethiol hydrochloride (Acros Organics, Somerville, NJ) in 1 mL methanol (Fisher Scientific). To this solution, 50 mL of a 0.1 mol/L sodium tetraborate decahydrate (Sigma Aldrich) buffer and 1.25 mL of a 20% SDS solution were added. A 1-mL aliquot of the freshly prepared OPA solution was then added to a 24-well, nontreated polystyrene cell culture plate (Fisher Scientific) followed by the 9-$cm^2$ WYPALL square from the top of the disposable cuvette. The OPA solution was pipetted up and down several times across the WYPALL square to elute the protein, and the reaction was allowed to proceed for no less than 3 minutes. A 200-μL aliquot of the OPA solution was then transferred to a 96-well plate for absorbance determination. The maximum absorbance coefficient was calculated versus a pure OPA solution at 340 nm in a calibrated Spectramax 190 absorbance microplate reader (Molecular Devices, Sunnyvale, CA).

*Cleaning efficacy calculations and analysis*

To determine the background absorbance of the disinfectant cleaning wipe's liquid active ingredients, 10 μL of each wipe's liquid extract was placed in 1 mL of the OPA solution in triplicate. The absorbance of each sample was read and averaged to give the overall disinfectant background for each trial. Additionally, the general background for the experiment was calculated by a pre-moistened 9-$cm^2$ paper towel square (using 50 μL of 1% SDS) placed in the OPA solution.

Using these 2 backgrounds, each absorbance calculated from the trial had the background (both the general and disinfectant) absorbance values subtracted from them. Each disinfectant cleaning wipe experiment was done in triplicate. These absorbance values were then averaged for each disinfectant cleaning wipe tested.

To determine the amount of residual protein debris left on the test surface by the disinfectant cleaning wipe, a standard curve was created from 0.5, 1, 2.5, 5, and 10 μL of the test soil, which was spotted on 9-$cm^2$ paper towel squares (also performed in triplicate) and allowed to dry for 4 hours. The squares were then placed and submerged in OPA solution, and the absorbance was read as previously described. By plotting the average absorbance for each test soil volume against the amount of protein that made up that specific volume, a linear regression trend line was created. From this equation, using the absorbance as input, the residual amount of protein debris was calculated in micrograms.

*Measurement of surface roughness*

Surface roughness of the anesthesia machine was measured using a Bruker Contour GT-K1 3D optical microscope (Bruker AXS, Inc, Tucson, AZ). Postmeasurement processing consisted of tilt removal and basic statistic calculations were performed through Bruker's Vision64 software (Bruker AXS, Inc).

*Force measurement*

To calculate the force associated with the cleaning efficacy of the disinfectant cleaning wipe on the tabletop of the anesthesia machine, a piezoelectric Kistler multiaxial force plate, model 9260AA (Kistler Instrument Corp, Amherst, NY) with a frame rate of 2,000 Hz was used. The anesthesia machine was placed on the center of the plate and balanced. Force was measured in the x, y, and z directions in Newtons (N) and was recorded using the Vicon Nexus version 1.7.1 program (Vicon, Los Angeles, CA). To calculate the total resultant force, the equation $r = (x^2 + y^2 + z^2)^{1/2}$ was used. For each disinfectant cleaning wipe, 5 trials were performed. Each trial consisted of removing the artificial blood spot by a wipe. Immediately upon visual removal of the spot, the force on the anesthesia machine was removed, which instantaneously stopped the time and force measurements. Data were removed from calculations once the measurements in the z direction went above zero.

*ATP bioluminescence assay*

ATP samples were collected and measured using the Ruhof ATP Complete Contamination Monitoring System (Ruhof, Mineola, NY) as per the manufacturer's instructions. The ATP samples were collected with the provided ATP Test swab within the 28.5-$cm^2$ sampling area by swabbing in a zigzag left-to-right and up-and-down pattern (left-to-right: 16 times, up-and-down: 28 times) while rotating the swab and applying slight pressure on the sampled surface. Readings were taken and recorded in RLUs.

The cleanliness guidelines provided by the manufacturer are RLU >45 = dirty, RLU <45 = clean.

*Statistical analysis*

For the blood soil, 10 trials were performed, whereas, for the bacterial soil, 3 trials were performed. Data were statistically analyzed using GraphPad Prism version 4.03 (GraphPad Software, La Jolla, CA).

## RESULTS

To detect the residual protein debris remaining on the anesthesia machine's surface after cleaning and disinfecting, several protein assay methods from AAMI TIR 30 were initially tested.[10] Several of the colorimetric reagents used for protein detection were not suitable for use in our experiment because the active ingredients in the disinfectant cleaning wipes reacted with the detection reagents, creating false positives (data not shown). For this reason, the OPA method was chosen, based on its detection limit and sensitivity[20] and lack of false positives created by the disinfectant cleaning wipes.

Using the OPA method, we obtained the average amount of residual protein debris (in micrograms) left on the surface by the 6 different disinfectant cleaning wipes (Fig 1). Whereas each wipe left a considerable amount of protein remaining on the anesthesia machine surface (Fig 1), the wipe that performed the best (wipe 3) and the wipe that performed the worst (wipe 4) have the exact same active ingredient, albeit in slightly different percentages (Table 1). Three of the remaining 4 wipes (wipes 1, 2, and 6) left similar residual levels of protein debris to one another, whereas wipe 5 performed more comparably with wipe 3 (Fig 1). Visual observation of the anesthesia machine surface after cleaning and disinfecting with the wipes concurred with the OPA results.

Another method to quantify the effectiveness of these disinfectant cleaning wipes was to calculate the force associated with cleaning the blood spot from the surface. To measure this, the anesthesia machine was placed on top of a piezoelectric force plate. The resultant force was then calculated from the force measured in the x, y, and z directions and was plotted against the time required by the wipe to clean the blood spot from the surface. As seen in Table 2, wipe 3 was once again most effective in removing the coagulated blood spot and cleaning the surface and required the least amount of time and force to do so. This wipe was followed by wipe 1 and wipe 4. Wipe 5 required the most amount of force to clean the blood spot, and wipe 6 required the most amount of time. Wipe 2 fell in between wipes 5 and 6 in regards to both time and force required to clean the blood spot from the anesthesia machine surface.

We lastly spotted the machine's surface with a known concentration of bacteria and subsequently cleaned and disinfected the surface with the wipes and measured residual bacterial debris. Using Ruhof's ATP bioluminescence swabs and assay, all 6 of the wipes tested removed more than 98% of the initial bacterial inoculums (Table 3). This was an equivalent amount to what was found on the negative control's surface, which had not been spotted with bacteria. The worst performing wipes, wipes 4 and 6, appeared to be slightly less effective (<1% difference in CFU remaining) than the top performing wipe, but this difference is negligible.

## DISCUSSION

Health care infections associated with contaminated medical devices have become an increasingly more common occurrence in the health care setting.[1-3] Because of several compounding factors, such as noncompliance with manufacturer's instructions for cleaning and disinfection, improper personnel training, increased medical design complexity, and/or new device materials,[9,10,22] research is needed to delineate how cleaning and disinfection practices of medical devices can be improved.

This report was aimed at cleaning and disinfection of an anesthesia machine surface and specifically focused on one of the more commonly contaminated areas of the machine.[23] Additionally, the machine's tabletop surface was chosen because it would be used routinely, whether the anesthetic care provided was general, regional, or monitored.[24] The purpose of this study was not to evaluate disinfectant cleaning wipes for endorsement purposes but rather to evaluate their effectiveness under conditions representative of actual use. Two different soils were used as contaminants on the surface. A coagulated blood test soil composed of purified blood proteins[19] was chosen based on the prevalence of visible and/or occult blood found on anesthesia equipment.[23,25] Additionally, *S pneumoniae* was chosen for the bacterial debris because alpha *Streptococci* are a common nosocomial pathogen and bacterial contaminant of anesthesia machine equipment.[24,26]

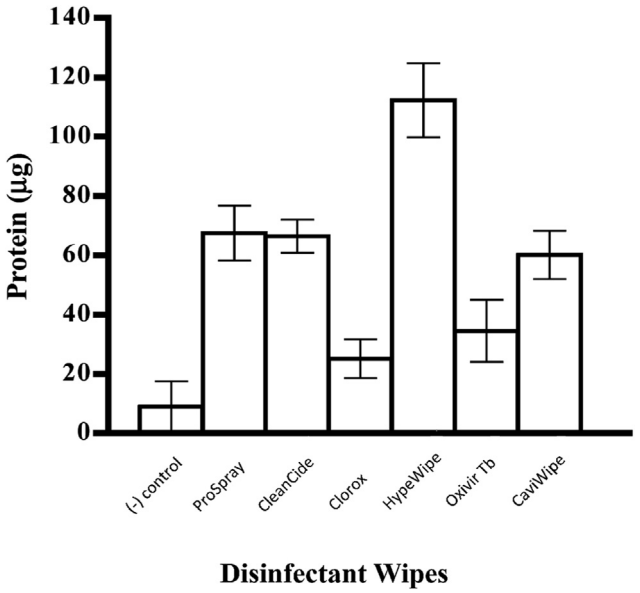


**Fig 1.** Quantification of residual protein debris. After cleaning and disinfection of the artificial blood soil on the anesthesia machine surface with the six tested wipes, residual protein debris results from the 10 trials were statistically analyzed with the average amount of residual protein debris plotted. Errors bars represent standard error of the mean.

**Table 2**
Correlation of force and time required by the 6 disinfectant cleaning wipes to remove the coagulated blood spot

| Wipe | Time (seconds) | Resultant force (Newtons) |
|---|---|---|
| 1 | 6.52 ± 0.15 | 14.27 ± 0.74 |
| 2 | 7.63 ± 0.15 | 15.71 ± 0.53 |
| 3 | 5.01 ± 0.09 | 12.58 ± 0.59 |
| 4 | 6.60 ± 0.11 | 14.14 ± 0.41 |
| 5 | 6.71 ± 0.25 | 18.65 ± 0.75 |
| 6 | 8.72 ± 0.09 | 15.70 ± 0.97 |

NOTE. Force (Newtons) and time (seconds) were recorded by the Vicon Nexus version 1.7.1 program. Using GraphPad Prism 4.0 software, the numbers were averaged, and a standard error of the mean was calculated. (One Newton [N] is the amount of net force required to accelerate a mass of 1 kg at a rate of 1 meter per second squared. On Earth's surface, a mass of 1 kg (about 2.2 pounds) exerts a force of 9.8 N.)

**Table 3**
Average results of bacterial debris disinfection by the disinfectant cleaning wipes

| Wipe | RLU | % CFU remaining |
|---|---|---|
| 1 | 4.11 ± 2.00 | 1.21 |
| 2 | 1.56 ± 1.09 | 0.98 |
| 3 | 5.22 ± 2.72 | 1.31 |
| 4 | 12.00 ± 2.59 | 1.92 |
| 5 | 9.44 ± 3.19 | 1.69 |
| 6 | 12.00 ± 3.02 | 1.92 |

NOTE. RLU = number of relative light units recorded by the Ruhof ATP Complete Contamination Monitoring System ± SEM = standard error of the mean for the RLU counts, and % CFU remaining = percent of colony forming units remaining based upon total amount of CFU spotted on the surface.

**Table 4**
Determination of average "wetness" per disinfectant cleaning wipe

| Wipe | Wet weight (g) | Dry weight (g) | Wetness of wipe (g) | Surface area ($cm^2$) | Wetness of wipe ($g/cm^2$) |
|---|---|---|---|---|---|
| 1 | 2.57 | 0.96 | 1.6 | 268.67 | 0.006 |
| 2 | 5.36 | 1.33 | 4.03 | 260.67 | 0.015 |
| 3 | 7.68 | 2.01 | 5.66 | 425.25 | 0.013 |
| 4 | 14.02 | 1.71 | 12.31 | 457.03 | 0.027 |
| 5 | 5.25 | 0.93 | 4.32 | 267.12 | 0.016 |
| 6 | 5.03 | 0.95 | 4.09 | 260.67 | 0.016 |

NOTE. Each wipe was weighed in triplicate straight from the canister (approximately 4-5 wipes were removed prior to weighing to ensure adequate wetness of the wipes from the canister). Wipes were left to dry at room temperature until a stable weight was maintained (≥24 hours). "Wetness" measurements (g) were made by subtraction of the dry weight from the wet weight, and the results were then averaged. To determine the average surface area of each wipe, 3 wipes were laid flat and measured; area was calculated using the following equation: area = width × height. For calculation of average wetness of each brand of wipe (g) per square centimeter, the average wipe "wetness" was divided by the average surface area.

The results from the cleaning experiments using artificial blood and the commercially available disinfectant cleaning wipes underscore the variability of effectiveness in these wipes. Although all of the tested wipes left less than 6.4 $\mu g/cm^2$ (0.03% of the total applied) of protein on the test surface (current acceptance criteria for flexible endoscopes outlined by AAMI TIR 30),[10] there were marked differences in how well the disinfectant cleaning wipes actually performed. Under the experimental test conditions, the wipe that performed the best, ie, leaving behind the least amount of residual protein debris as detected by the OPA method, and requiring the least mechanical effort, contained sodium hypochlorite as its active ingredient (wipe 3). In descending order, the remaining wipes ranked from best to worst (in efficacy) were as follows: a wipe containing an oxidizing agent (hydrogen peroxide, wipe 5), an alcohol (isopropanol, wipe 6), an acid (citric acid, wipe 2), a phenol (o-phenylphenol, wipe 1), and, last, a second wipe containing sodium hypochlorite (wipe 4) (Fig 1). As noted earlier, it was notable that the wipes that performed the best and worst within our study both contained sodium hypochlorite as the active ingredient. Even more surprising, wipe 4, the worst performing wipe, has a higher percentage of sodium hypochlorite than the best performing wipe, the wipe 3 (Table 1).

So why the disparity between wipes 3 and 4? We construe this variation in efficacy to be based on the differences in the packaging and wetness between the wipes because they share the same active ingredient. Wipe 4 was significantly wetter than wipe 3 ($P = .0015$) because it was saturated with more than 2 times the amount of liquid per square centimeter than the top performing wipe 3 (Table 4). In conjunction with the individual packaging nature of the wipe (instead of in a canister), we believe that this wipe was too wet to effectively remove all the debris from the surface because it appeared, instead, to push its own liquid across the surface. More importantly, when a wipe is too wet, other problems may arise, eg, corrosion of electronic circuitry,[27] leading to far greater concerns for the wipe than simply poor cleaning and disinfection efficacy.

As for the remaining wipes, their difference in efficacy may be understood by their concentration exponent, a numerical expression of a disinfectant's activity typically used to describe the dynamics of disinfection in relation to microbial kill (the larger the concentration exponent, the more rapidly the disinfectant loses activity when diluted). The results appear to align with the size of the concentration exponent with the exclusion of the wipe 4, as wipe 3 (a halogen) and wipe 5 (an oxidizing agent) have the smallest exponents, 0.5 and 0.5, respectively.[28] The remaining wipes in order of efficacy have exponents in the ranges of 6.0 to 12.7 (wipe 6, aliphatic alcohol) and 4.0 to 9.9 (wipe 1, phenolic compounds).[28] No known concentration exponent exists for citric acid (wipe 2).

We also evaluated the force and time required by the disinfectant cleaning wipes to clean the coagulated blood debris from the machine's surface and observed a similar trend in our data. Similar to the protein debris results in which the wipe 3 was considered most effective, it, too, performed in a superior manner to the other wipes, requiring the least amount of force and time to remove the coagulated blood.

To determine the effectiveness of the disinfectant cleaning wipes in the removal and disinfection of the *S pneumoniae* bacterial soil, an ATP bioluminescence assay was used. Several others have reported using an ATP bioluminescence system to monitor the effectiveness of hospital cleaning practices or assess the cleanliness of medical equipment.[29-33] Although one study has demonstrated that commonly used cleaning agents could either enhance or quench the ATP light signal[34] causing interference with the reading, we did not observe this phenomenon with the disinfectant cleaning wipes used in our experiments because their backgrounds were close to 0 RLU (no detectable light signal). We observed that all of the disinfectant cleaning wipes performed similarly to one another in terms of disinfecting the bacteria. Additionally, all surfaces wiped had an ATP reading equivalent to "clean" by the ATP manufacturer's instructions for use. Thus, the results indicate that, under the experimental conditions in this study, no specific disinfectant cleaning wipe was superior to another in removing or disinfecting the bacteria from the surface of the anesthesia machine when following the manufacturer's instructions for the wipes for cleaning and disinfecting.

There are, nevertheless, limitations within our experiments. As noted by AAMI TIR 30 document, the sensitivity of the OPA assay used to detect proteins can be unrealistic because routine handling or touching of the surface or instrument can create false-positive reactions.[10] Additionally, for the ATP bioluminescence assay, the efficiency of bacterial pick up from the medical device surface is affected by the concentrations of the organisms present, with the efficiency decreasing with increasing bacterial concentration.[35] Furthermore, the degree of wetness of the ATP test swabs (prewetted by the manufacturer) was highly variable, both within and between batches, which also may have had an effect on bacterial pick up efficiency. More generally speaking, an anesthesia machine is only one of many reusable medical devices used in the health care setting. Because of the fact that most devices have their own unique surface roughness and there is no standard surface, these results may not be applicable to all medical device surfaces. Taking these limitations into account, however, even if the amount of residual protein and/or bacteria were minimally distorted, a repeatable efficacy trend of the disinfectant cleaning wipes was still apparent through both assays.

Whereas this study was not aimed at singling out a particular active ingredient or disinfectant cleaning wipe as being superior to another, it is imperative for health care providers to always read the instructions for use provided by the manufacturers of reusable devices and medical equipment to choose the most appropriate cleaning agent. It is hoped that the results from this study will contribute to more knowledgeable and informed decisions regarding the selection of disinfectant cleaning wipes based on their efficacy on the intended surfaces on which they will be used. Nonetheless, these results will provide useful information on how to improve cleaning and disinfection instructions for external surfaces of these and other reusable devices and equipment. Although these cleaning and disinfecting studies were completed using only one such surface, the methods and assays that were employed to test the efficacy of these wipes can be extended beyond the single flat, horizontal medical device surface described and tested here and may even be adapted to more difficult-to-clean surfaces, such as knobs, grooves, crevices, rough surfaces, or vertical surfaces. Along with better techniques and methods in place to test and monitor such disinfectant cleaning wipes against their intended medical device surface, it is hoped that the number of DA-HAIs will be reduced as infection control procedures improve and manufacturers re/design devices that are easier to clean and disinfect.

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