Efficacy of directed misting application of a peroxygen disinfectant for environmental decontamination of a veterinary hospital

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Objective—To evaluate effectiveness of 4% peroxymonosulfate disinfectant applied as a mist to surfaces in a large animal hospital as measured by recovery of *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium.

Design—Field trial.

Sample Population—Polyester transparencies inoculated with bacteria.

Procedure—Polyester transparencies were inoculated with *S aureus* or *S* Typhimurium and placed in various locations in the hospital. After mist application of the peroxygen disinfectant, viable bacterial numbers were quantified and compared with growth from control transparencies to assess reduction in bacterial count.

Results—When applied as a mist directed at environmental surfaces contaminated with a geometric mean of 4.03×10^7 CFUs of S aureus (95% confidence interval [CI], 3.95×10^7 to 4.11×10^7) or 6.17×10^6 CFUs of S Typhimurium (95% CI, 5.55×10^6 to 6.86×10^6), 4% peroxymonosulfate reduced the geometric mean number of viable S aureus by 3.04×10^7 CFUs (95% CI, 8.6×10^6 to 1.7×10^6) and S Typhimurium by 3.97×10^6 CFUs (95% CI, 8.6×10^5 to 3.5×10^6).

Conclusions and Clinical Relevance—Environmental disinfection with directed mist application of a 4% peroxymonosulfate solution was successful in reducing counts of bacterial CFUs by > 99.9999%. Directed mist application with this peroxygen disinfectant as evaluated in this study appeared to be an effective and efficient means of environmental disinfection in a large animal veterinary hospital and would be less disruptive than more traditional approaches to intensive environmental cleaning and disinfection. (*J Am Vet Med Assoc* 2005;227:597–602)

Nosocomial infections are an ever-present threat in health care settings, where transmission of infectious microorganisms is facilitated by frequent patient

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contact with health care providers and contaminated surfaces or medical instruments. 1-12 The risk of nosocomial infections is further compounded because of the congregation of sick or immunocompromised patients with others in hospital environments, which creates an increased likelihood of the spread of contagious pathogens. The magnitude of consequences associated with outbreaks of nosocomial disease was realized at the James L. Voss Veterinary Teaching Hospital (VTH) at Colorado State University (CSU) in 1996 and again in 2001 when the VTH had outbreaks associated with Salmonella enterica serovar Infantis infections.³ Fiftynine animals were infected during the 1996 epidemic, with 3 associated deaths, and 7 infections were detected in 2001, with 1 patient death.^{3,13} Both outbreaks were associated with environmental contamination with S Infantis, which likely was the source of exposure for at least some of the affected patients. These epidemics resulted in substantial financial costs, in addition to threatening public confidence in our hospital.3 Because of the need to empty, scrub, and disinfect the contaminated areas, the large animal hospital at the VTH was partially or fully closed for 3 months as a result of the first outbreak and was closed for 1 month after the second outbreak.³

Previous research clearly indicates that Salmonella spp are commonly spread in hospital environments wherever animals shedding Salmonella bacteria are housed or where fecal samples containing Salmonella spp are handled, even in the absence of epidemic salmonellosis.¹³ Experiences at the CSU-VTH and other veterinary hospitals reveal that it is very important to eliminate environmental reservoirs of Salmonella spp to effectively reduce the risk of nosocomial transmission. The primary method used to eliminate reservoirs of Salmonella spp in hospital environments after these major epidemics has been to rigorously clean and decontaminate. 35,9 Briefly, this in-depth process typically has involved removing all materials from a particular room or area, hand scrubbing all surfaces in the room as well as all materials removed from the room with detergent solutions, and application of 1 or more disinfectants to materials and the environment before items are replaced. This is obviously labor intensive and disruptive to normal operations. However, certain circumstances preclude this type of cleaning and disinfection because of facility characteristics or other limitations. For example, the need to apply copious amounts of water and disinfectant solutions to ceilings and upper walls was not considered when the large animal facilities at the CSU-VTH were designed several decades ago. As such, electrical conduits and fixtures were not sealed during construction, and it is not possible to safely use large volumes of aqueous solutions around these fixtures.

For these reasons, alternative methods of environmental decontamination, including the use of aerosol distribution of a peroxygen disinfectant, have been evaluated. 14,a It will probably remain necessary to use intensive scrubbing and disinfection when faced with extensive environmental contamination. However, the less labor-intensive and disruptive method of aerosol distribution of disinfectants may successfully decrease environmental reservoirs of potential pathogens and decrease the frequency that this type of intensive cleaning is needed. Safety should be a primary consideration when determining which methods of cleaning and disinfection to use, and electrical hazards are an important aspect of these considerations. Use of aerosols or directed misting can potentially help to minimize risks associated with electrical hazards and application of disinfectant solutions. The purpose of the study reported here was to evaluate the effectiveness of 4% peroxymonosulfate solution^a when applied as a mist to surfaces in a large animal facility as measured by recovery of Staphylococcus aureus and Salmonella enterica serovar Typhimurium.

Materials and Methods

Study overview—A 4% peroxymonosulfate solution was applied to all surfaces in the food animal ward at the VTH by use of high-volume, directed mist application. The effectiveness of this disinfection procedure was evaluated by seeding polyester transparencies with *S aureus* and *S* Typhimurium and placing these in various locations prior to misting. To evaluate disinfectant efficacy, bacterial CFUs were determined on transparencies after misting and compared with CFUs on control transparencies not exposed to disinfectant.

Bacterial inoculates—Reference strains of *S* Typhimurium^b and *S aureus*^c were inoculated into tryptic soy broth^d and incubated 8 hours at 37°C. By inoculating 10-fold dilutions of broth cultures onto tryptic soy agar plates^c with 5% sheep

blood, the bacterial concentrations of broth cultures were estimated to be 5.56×10^8 CFUs/mL for S Typhimurium and 1.97×10^8 CFUs/mL for S aureus.

Transparencies—One hundred polyester transparencies $(6 \times 6 \text{ cm})$ were prepared, and both sides were disinfected with 70% ethanol solution. After drying, 1 side of 48 transparencies was inoculated with 100 μ L of S Typhimurium broth culture and 1 side of another 48 was inoculated with S aureus. The inoculates were dispersed over approximately 5×5 cm, and the transparencies were allowed to air dry at 21° C for 14 hours in a laminar-flow biological safety cabinet. The remaining 4 transparencies were used as uninoculated control surfaces.

At least 48 hours prior to placement of transparencies, all surfaces of the food animal ward at the VTH were thoroughly cleaned and decontaminated from the floor up to a height of approximately 9 feet. Briefly, animal bedding and debris were removed, and surfaces were scrubbed with a detergent,⁸

disinfected with hypochlorite solution, h rinsed with tap water, and disinfected with a quaternary ammonium disinfectant solution that was allowed to dry in place. Transparencies inoculated with S Typhimurium and S aureus were taped to surfaces in 40 locations in the food animal ward (Figure 1); a transparency inoculated with S Typhimurium was placed adjacent to a transparency inoculated with S aureus in each location. Transparencies were placed throughout the facility on 10 high vertical surfaces (eg, tops of interior walls and sides of structural ceiling beams), 10 high horizontal surfaces (eg, on top of stall walls with seeded surfaces facing upward), 10 low vertical surfaces (eg, bottoms of walls), and 10 low horizontal (eg, floor) surfaces. The 4 uninoculated control transparencies were taped to a wall in an area adjacent to the food animal ward that had been cleaned and decontaminated but was not exposed to disinfectant misting. These uninoculated controls were used to evaluate the background concentrations of bacteria that may have been transferred to transparencies in the sampling process. Additionally, the remaining 16 inoculated transparencies (8 each inoculated with S aureus and S Typhimurium) were used as control samples. Eight of the inoculated control transparencies (4 for each organism) were processed for cultures at the same time as the transparencies that were exposed to disinfectant misting (postmisting inoculated controls). The remaining 8 inoculated control transparencies (4 for each organism) were processed just prior to misting (premisting inoculated controls), and bacterial counts were compared with results from postmisting inoculated controls to determine whether viability was reduced in the absence of disinfectant exposure during the time that misting took place.

Disinfectant misting—To prepare the 4% disinfectant solution, 760 g of peroxygen compound was added to each 19 L (5.0 gallons) of water used. Distribution was carried out by 2 individuals with backpack mist blowers. One person applied mist to high surfaces throughout the food animal ward, and the other applied mist to low surfaces. According to the manufacturer's specifications, the mist blowers used in this study were capable of distributing the peroxygen compound at a rate of approximately 0.8 L/min and produced aerosol particles that were approximately 100 to 200 μm in diameter. To cover all surfaces in the food animal ward (607 m² [6,530 ft²]; 2,178 m³ [76,918 ft³]), misting required approximately 170 L (45 gal-

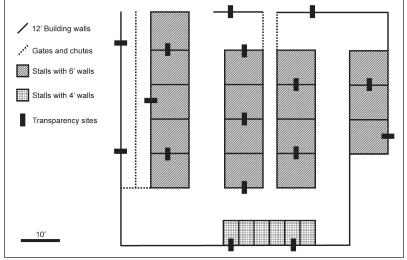


Figure 1—Schematic diagram of the food animal ward at the James L. Voss Veterinary Teaching Hospital at Colorado State University. Polyester transparencies were inoculated with bacteria and placed in various locations in a study of the efficacy of directed misting application of a peroxygen disinfectant.

lons) of disinfectant solution that was distributed during a 2-hour period.

Safety precautions—Although the diluted peroxygen disinfectant used in this study was classified as nonirritating for skin and ocular exposure according to occupational safety experts at CSU via information provided by the manufacturer, 15 use of rubber gloves and protective eyewear when handling the concentrated powder is still recommended.¹⁵ Personnel used full-face respirators, rubber gloves, disposable coveralls with hoods, and calf-height rubber boots during the mist application process. Personnel were examined by occupational health physicians and were fit-tested by occupational safety specialists prior to use of the full-face respirators. Because of concerns about electrical shock hazards, occupational safety specialists evaluated the food animal ward and approved protocols for the mist application prior to initiation of this experiment. To prevent accidental exposures to the aerosolized disinfectant, prior to initiating the misting procedures, the ventilation systems in the food animal ward were inactivated, access was limited to authorized personnel, all animals were removed from the ward, and all doors were closed.

Sample processing—Thirty minutes after misting was completed, all sample transparencies were collected, sealed in sterile containers with 10 mL of Dey-Engley broth^k that contained neutralizers for common disinfectants (neutralizing broth), briefly mixed, placed on ice, and transported to the laboratory. The samples were vortexed vigorously to remove bacteria from the transparency, and 50 µL of the vortexed sample was plated on a 100 × 15-mm tryptic soy agar plate with 5% sheep blood.6 The plates were incubated at 35°C for 18 hours. The 16 inoculated control transparencies used to measure pre- and postmisting bacterial counts were treated in an identical manner with the exception that a 1:100 dilution of the vortexed neutralizing broth was used for quantification. Bacterial CFUs were estimated by use of a spiral plater according to the manufacturer's directions. Briefly, sample solutions were progressively diluted in this apparatus as they were applied in a spiral pattern to agar plates. The bacterial concentrations of solutions were estimated by counting CFUs in different zones on plates, in accordance with the manufacturer's directions. Dilution factors were accounted for to obtain final estimates for total CFUs recovered from transparencies.

Limits of quantification—Plates with < 30 CFUs after incubation were considered to have numbers of viable bacteria that were less than could be reliably quantified with the spiral plater, and samples were therefore assigned a concentration of ≤ 500 CFUs/mL, the manufacturer's published lower limit of quantification.¹ Plates with > 300 CFUs in the most dilute counting zone were considered to have more viable bacteria than could be reliably quantified with the spiral plater, and samples were assigned a concentration of $\geq 5.0 \times 10^6$ CFUs/mL $(5.0 \times 10^7$ total bacteria in the sample), the upper limit of quantification as published by the manufacturer.¹

Statistical analyses—The estimated CFUs recovered from transparencies were transformed to log₁₀ values to permit parametric analysis. Reduction factors associated with exposure to disinfectants were calculated by subtracting the log₁₀ CFUs for transparencies exposed to disinfectant misting from the mean CFUs recovered from postmisting inoculated control transparencies. Mean and SE estimates were calculated for these reduction factors and were used to determine 95% confidence intervals (CIs) of log₁₀ values. The mean reduction factors and CIs were back-transformed to provide geometric means and associated 95% CIs. Log-transformed CFU estimates for uninoculated control samples, premisting inoculated control samples, postmisting inoculated control

samples, and disinfectant-treated transparencies were compared by use of ANOVA. $^{\rm m}$

Results

Control transparencies—The numbers of recoverable CFUs remained fairly constant during the time between initiation of disinfectant misting and initiation of bacterial recovery. The geometric mean CFUs recovered from control transparencies processed prior to misting were 4.18×10^7 CFUs (95% CI, 4.01×10^7 to 4.35×10^7 CFUs) and 6.31×10^6 CFUs (95% CI, 5.32) \times 10⁶ to 7.49 \times 10⁶ CFUs) for S aureus and S Typhimurium, respectively, which were not significantly different from the geometric mean CFUs for postmisting samples of 4.03×10^7 CFUs (95% CI, 3.95 X 10^7 to 4.11×10^7 CFUs) for S aureus and 6.17×10^6 CFUs (95% CI, 5.55 × 10⁶ to 6.86 × 10⁶ CFUs) for S Typhimurium (P = 0.98 for both organisms). No bacteria were recovered from any of the uninoculated control transparencies after removal from wall surfaces.

Effects of disinfectant misting—The CFUs for S aureus were less than the limit of quantification for 28 of 40 (70%) transparencies and thus, for purposes of analyses, were assigned bacterial counts of 5.0 X 10³ CFUs. Bacterial CFUs were greater than quantification limits for 8 of 40 (20%) S aureus transparencies and were therefore assigned a quantity of 5.0 X 10⁷ CFUs. The CFUs of S aureus that were recovered from the remaining transparencies were estimated to be 5.2×10^4 CFUs (3 transparencies) and 1.4 × 10⁶ CFUs (1 transparency). Reductions in bacterial concentrations for S aureus were fairly consistent among transparencies placed on high horizontal, high vertical, and low horizontal surfaces (P > 0.40 for all comparisons) but were markedly lower on low vertical surfaces (P < 0.001 when low vertical surfaces were compared with other locations; Table 1). Compared with geometric mean CFUs obtained from postmisting control transparencies, the mean log reduction value for all S aureus transparencies was 3.04×10^7 CFUs (95% CI, 8.62×10^5 to 1.71 × 10⁶ CFUs; Table 1). The difference between S aureus concentrations on postmisting control samples and those on test samples that were exposed to disinfectant was significant (P < 0.001).

After disinfectant misting, CFUs for S Typhimurium were less than the detection limit for 30 of 40 (75%) transparencies, irrespective of placement, and CFUs were greater than detection limits on 2 transparencies. The remaining 8 transparencies had estimated bacterial growth of 3.5×10^4 CFUs (n = 4) and 1.8×10^5 CFUs (4). Reductions were slightly lower on transparencies placed on low surfaces, compared with high surfaces, but these differences were small, and there were no significant differences among reductions in geometric mean concentrations for the 4 locations (P > 0.30; Table 1). Mean bacterial count for all S Typhimurium transparencies was 3.97×10^6 CFUs (95% CI, 8.62×10^5 to 3.49×10^6 CFUs). The difference between S Typhimurium concentrations on postmisting control samples and those on test samples that were exposed to disinfectant was significant (P < 0.001).

Table 1—Geometric mean reductions in counts of bacterial CFUs following directed mist application of peroxygen disinfectant.

Organism	Type of site	n	GMean reduction*†	95% Confidence interval
Staphylococcus aureus	High horizontal High vertical Low horizontal Low vertical‡ All sites	10 10 10 10 40	$\begin{array}{c} 4.03\times10^{7} \\ 4.04\times10^{7} \\ 7.01\times10^{6} \\ 1.91\times10^{2} \\ \textbf{1.22}\times10^{6} \end{array}$	4.02×10^7 to 4.03×10^7 4.04×10^7 to 4.04×10^7 2.50×10^6 to 1.96×10^7 3.97×10^1 to 9.23×10^2 1.35×10^9 to 1.10×10^7
Salmonella enterica serovar Typhimurium	High horizontal High vertical Low horizontal Low vertical All sites§	10 10 10 10 40	6.47×10^{6} 6.50×10^{6} 1.35×10^{6} 1.34×10^{6} 2.95 \times 10 ⁶	6.46×10^{6} to 6.48×10^{6} 6.50×10^{6} to 6.50×10^{6} 5.38×10^{5} to 3.40×10^{6} 5.34×10^{5} to 3.37×10^{6} 1.01×10^{6} to 8.64×10^{6}

*Baseline counts used for comparison were 4.03 X 10^7 CFUs of S aureus (95% confidence interval, 3.95 X 10^7 to 4.11 X 10^7 CFUs) and 6.17 X 10^6 CFUs of S Typhimurium (95% confidence interval, 5.55 X 10^6 to 6.86 X 10^6 CFUs). †Differences between mean CFUs recovered from test samples and control samples were significant (P < 0.001) for both S aureus and S Typhimurium. ‡GMean reduction of S aureus on low vertical sites was significantly (P < 0.001) less than other locations, but there were no significant differences in reductions among the other locations (P = 0.40). \$GMean reduction of S Typhimurium was not different among the different sites (P = 0.30).

GMean reduction = Geometric mean reduction of bacterial CFUs associated with disinfectant exposure as measured by estimating CFUs on polyester transparencies after misting and comparing those counts with geometric mean CFUs on control transparencies not exposed to the disinfectant.

Discussion

These results suggested that directed mist application of 4% peroxygen compound as evaluated in this study appeared to be an effective and efficient means of disinfection of animal holding facilities. Typically, a reduction factor of 3 to 5 logs is considered the minimum needed for effective disinfection. The mean reductions in this trial exceeded this value for *S aureus* and *S* Typhimurium, achieving > 6 log reductions for both bacteria. This is equivalent to > 99.9999% reduction in CFUs. Directed mist application was a very rapid and efficient method of distributing the disinfectant and could easily be applied in a variety of agricultural or veterinary settings.

Despite these reductions in viable bacteria, a few caveats must be considered when interpreting these data. The polyester transparencies used in this trial represented an ideal surface for disinfection because of their smooth, nonporous nature, whereas results from evaluating other surfaces typically found in the VTH (eg, concrete and painted cinder block) may have yielded less impressive results. The hospital environment was used for this experiment to provide a practical example of constraints that would be encountered in directed mist application of disinfectants, and typically, this would require use of different surface materials to fully evaluate the distribution in the VTH environment. However, our previous experience suggested that similar tests with porous or corrugated surface materials reduced the ability to recover seeded bacteria and impaired the ability to evaluate efficacy of the process. 14 The use of transparencies as a test surface allowed better control of the variability in recovery that is found when multiple surfaces are used as inoculation points (eg, concrete, masonry, wood, rubber, or plastic). 14 Furthermore, this allowed evaluation of the efficacy of disinfection in the VTH with less concern about residual infectious organisms that may have been left in the environment after the study. Overall, it was believed that removing variability associated with recovery from different surfaces outweighed the potential problems associated with use of a more ideal surface for disinfection for all tests. Therefore, these results should be considered to more accurately reflect the maximum achievable effects, whereas results obtained from other surfaces in animal handling facilities may be less optimal.

The impact that dirt and organic material have on efficacy of disinfection should also be considered. Although the tryptic soy broth used as a culture medium for inoculates should mimic contamination in the presence of a small amount of organic material, it is clear that it does not mimic the presence of grossly abundant quantities that would no doubt reduce efficacy of disinfection. As with any disinfection process, mechanical disruption with a suitable detergent solution will probably improve the expected efficacy of aerosol application of 4% peroxymonosulfate applied by directed misting. ¹⁶

Salmonella Typhimurium and S aureus were used in this experiment because the in vitro effects of the peroxygen disinfectant on these 2 species have been well documented, ¹⁷⁻²⁰ and both organisms pose a serious nosocomial threat in VTHs. ^{1,3-5,9-11,21-23} Use of standard reference strains is recommended when evaluating the efficacy of disinfectants because this improves the comparability of results from different trials and different laboratories.16 However, the reference strains used in this trial have been repeatedly passaged in the laboratory, which could have affected our experimental results, compared with those that may be achievable under unaltered field settings or with wild-type isolates. Repeated passage of isolates may have made them more or less susceptible to the peroxygen disinfectant, compared with wild-type strains. The culture-adapted strains were probably easier to amplify and recover in the culture systems used for this trial, which likely minimized the impact of this variable on results; use of non-culture-adapted strains may have resulted in falsely lowered estimates of bacterial concentrations in pre- and postdisinfection estimates. We attempted to minimize the impact of laboratory adaptation by use of inoculates recovered from frozen stocks that were preserved at a low passage number. Although the peroxygen compound was not evaluated with other organisms in this study, it is likely that similar effects would be seen with related bacteria (eg, other gram-positive commensals and gram-negative enteric bacteria that are culturable in aerobic conditions) as has been reported for in vitro studies. 17-20 The reactions of other bacteria that are adapted to anaerobic and microaerophilic conditions are less predictable, but experimental data provided by the manufacturer suggest that favorable results may be obtained with this aerosol distribution method.¹⁷ This study was not designed to evaluate the risk of transmitting specific pathogens, such as Salmonella spp, but the significant reductions in bacterial concentrations suggest that environmental decontamination via directed mist application would decrease the likelihood of transmission of important pathogens, such as Salmonella spp and S aureus, in animal environments, such as a veterinary hospital.

It should be noted that reductions in bacterial concentrations may have been larger than represented by the mean reduction estimates because most of the estimated CFUs were lower than quantification limits. There were large differences among the log reduction values for different test sites, which may be largely attributable to differences in uniformity of the mist application. The protocol used in this study required 1 person to apply disinfectant mist to lower surfaces, whereas the other applied disinfectant to upper surfaces. Because of the directed nature of this application process, differences in application technique, speed, and thoroughness could have contributed to differences in estimated bacterial reduction at different locations. Differences in operational efficiency of the 2 mist blowers also could have added to the variability in the process.

The peroxygen compound chosen for this experiment is a broad-spectrum disinfectant that is reportedly^{15,17-20} effective against a wide variety of viruses, bacteria, and fungi. Reports from the manufacturer and evaluation of the safety and toxicologic data also suggest that this product has a minimal impact on the environment and is nonirritating to skin, eyes, and respiratory mucosa. ^{15,17,24,25} The manufacturer has also reported^{24,25} that aerosol exposure of cattle, sheep, horses, pigs, and chickens had no adverse effects. However, as with all chemicals, it is prudent to use appropriate personal protective equipment such as gloves and eye protection and to avoid exposure to humans and animals whenever possible.

Although there are published data to support the effectiveness of this peroxygen compound when applied under controlled laboratory conditions, $^{16-19}$ little data are available on its efficacy when used under typical field settings. 14,26,27 Other research 14 suggests that small-particle (approx 50 μm) cold aerosolization by use of stationary distribution (as opposed to mobile, directed misting with larger aerosol particles) can be useful in surface disinfection, although that method is less versatile and requires longer holding times for aerosolized particles to settle. In addition, use of a 1% solution distributed by stationary small-particle aerosolization yielded at most 2 to 3 log (99% to 99.9%) reductions,

which is a reason that we chose to use a higher (4%) concentration of the peroxygen compound in an attempt to maximize the potential efficiency of the disinfection process. He Research results provided by the disinfectant manufacturer suggest that stationary, thermal, small-particle aerosolization with 4% solution with propylene glycol as a stabilizing agent yielded similar 2 to 3 log reductions in environmental bacterial concentrations. Efficacy of the peroxygen disinfectant was similar to that achieved through aerosolization of formaldehyde and superior to that achieved by aerosolization of a glutaraldehyde and quaternary ammonium compound mixture. Es

Information provided by the manufacturer of the motorized mist blower suggests that mist output from the unit used in this experiment can reach > 9 m (> 30 ft) from the nozzle, with an output diameter of > 4 m (> 13 ft). Although stationary aerosolization has an advantage in that less personnel time is required during distribution of the disinfectant because the aersolization unit can run unattended, our experience suggested that thorough distribution in an area requires substantially more time overall than was required with the directed mist application. In addition, stationary aerosolization (by use of both cold and thermal methods) requires distribution in a closed environment and generally uses smaller aerosol particle sizes (\leq 50 µm) to achieve better distribution in the airspace. In contrast, as long as inadvertent exposure to personnel and animals was prevented, directed mist application could be used in more open animal holding environments. The larger aerosol particles produced by the mist blower (100 to 200 µm) provide more efficient surface disinfection through better surface wetting, and the animal environments can be entered sooner after completion of the distribution because the larger aerosol particles require much less time to settle out of the air, compared with those generated by stationary aerosolization units.

- a. Virkon-S, Antec International, a DuPont Co, Wilmington, Del.
- Salmonella enterica serovar Typhimurium, Sarb No. 65, Salmonella Genetic Stock Centre, University of Calgary, Calgary, AB, Canada.
- Staphylococcus aureus strain 29213, American Type Culture Collection, Manassas, Va.
- d. Bacto tryptic soy broth, Becton-Dickinson, Franklin Lakes, NJ.
- e. BBL trypticase soy agar with 5% sheep red cells, Becton-Dickinson, Franklin Lakes, NJ.
- f. Apollo plain paper copier transparency film, Item No. PP100C, Acco Brands Inc, Ronkonkoma, NY.
- g. Tide with Bleach, Procter & Gamble Corp, Cincinnati, Ohio.
- Clorox Bleach, 1:32 dilution with tap water, The Clorox Co, Oakland, Calif.
- A-464-N, Airkem Professional Products, Ecolab Corp, Saint Paul, Minn.
- j. Model 430 motorized mist blower, Solo, Newport News, Va.
- k. Difco D/E Broth, Becton-Dickinson, Franklin Lakes, NJ.
- l. Model D spiral plater, Spiral Biotech Inc, Norwood, Mass.
- m. PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.

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