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White Paper

Minimizing Pathogenic Bacteria, Including Spores, in Indoor Air

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ABSTRACT

Five experiments were conducted to access whether aerosolized bacteria, including spores, respond like particulate contaminants to the primary (electrical) forces that control the distribution of small particulate contaminants in indoor air space. Such response would suggest an approach to minimizing infections in offices, hospitals, nursing homes and other facilities. It would also have implications for the protection against intentionally introduced pathogenic bacteria, including spores. The experiments used two different genera and five different strains of bacteria including spores. Micrococcus luteus was used as a surrogate for Gram-positive cocci, because M luteus is similar in size, shape and cell-wall composition to staphylococci, streptococci and enterococci. Similarly, spore-forming and vegetative bacillus subtilis were used as surrogates for Gram-positive bacilli such as Bacillus anthracis. The experiments were conducted in a dedicated aerosol physics test facility with culture-based measurements made at timed intervals. The results indicate that the organisms do respond like particulate contaminant to typical electrical forces in a room.

INTRODUCTION

Airborne bacteria are the cause of many nosocomial and community-acquired infections in humans; approximately two million hospital-acquired infections occur in the United States each year. An estimated 20,000 deaths are directly attributable to nosocomial pneumonias each year. Among the most prevalent of these infections are those caused by airborne Gram-positive cocci (e.g., Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, coagulase-negative staphylococci, Enterococcus fecewn, and Enterococcus faecalis). Some of the most serious non-nosocomial infections are those caused by airborne Gram-positive bacilli (e.g., Bacillus anthracis). Airborne bacteria cause many types of respiratory, skin, hair, and other infections. They also cause bacteremia, endocarditis, meningitis, osteomyelitis, and postoperative wound infections. In addition, recent bioterrorism events have made bacteria control in indoor air a significant matter.

Bacterial infections are of even greater concern in recent years because some are caused by organisms that have resistance to many antimicrobial agents, which makes treatment difficult or impossible. For example, antimicrobial agents that once were used to treat infections caused by staphylococci are now of questionable value. For this reason, the treatment of choice for many hospital-acquired infections became systemic vancomycin. Recently, however, there have been reports of S. aureus with reduced susceptibility and resistance to vancomycin (Schentag, 2001; Tenover, Biddle, & Lancaster, 2001). Even with the recently approved antimicrobial agents linezolid and quinupristin-dalfo-pristin, resistance has occurred in S. allreus (Tsiodras et al., 2001; Werner, Cuny, Schmitz, & Witte, 2001). Another example is the marked increase in penicillin-resistant and multi-drug-resistant S. pneumoniae in the United States (Doern et al., 2001). An alternative to trying to treat infections caused by airborne bacteria, including spores, would be to prevent them. It would be more effective in terms of human health, medical treatment cost, loss of productivity, and quality of life to minimize the number of bacteria, including spores, in indoor air so that they are not available to cause infections.

Five experiments are reported here. The authors used two different genera and five different strains of bacteria, including spores. They assessed whether aerosolized bacteria, including spores, respond like particulate contaminants to the primary (electrical) forces that control the distribution of most contaminants in indoor air. M. luteus was used as a surrogate for Gram-positive cocci, because M. lutcus is similar in size, shape, and cell wall composition to staphy-lococci, streptococci, and enterococci. Similarly, spore-forming and vegetative Bacillus subtilis were used as surrogates for the Gram-positive bacilli such as B. anthracis. The B. subtilis are larger in size, and the vegetative forms have a different shape, than the M. luteus. The experiments were conducted in a dedicated aerosol physics test facility with culture-based measurements made at timed intervals.

The earth has a natural background electrical field, which varies with time and place, but is generally about 150 to 200 volts per meter (Hicks, 1999). In indoor spaces the natural background field is distorted by the electrical characteristics of the materials in equipment, furniture, walls, clothing, and carpets. The electrical fields generated by computers and other electrical and electronic equipment, and the electrical fields generated by power lines in the walls, also distort the ever-present background field. These factors create in a room what could be envisioned as a complex three-dimensional web of voltage gradients often referred to as electrical field lines. Adding to the complexity is that humans frequently carry a substantial electric charge that also influences the background electrical field. Several thousand volts on a man wearing woollen trousers in dry. air are not unusual. This phenomenon is what sometimes causes an electric shock when one touches a doorknob.

Aerosols vary in size, concentration, and settling time. More than 98 percent of particulates in room air are small, less than 2 microns (μm) in size, and essentially do not settle out of the air by gravity, as indicated in Table 1. Air currents in a room entrain and move large particulates, carrying· them into the ducts and thence into the filters. Most small particulates are not entrained and moved by air currents because their cross-sectional area is so small. Small particulate motion is primarily determined by the typical electrical fields that exist in all rooms. The small particulates tend to move along the electrical field lines and be

deposited in and on people and objects. Thus, relatively few small particulates are returned to the ducts, and, as a consequence, relatively few reach the filters.

As small particulates in the air age, many collide, forming larger particulates. This natural process is called coagulation. The coagulated particulates are more readily moved by air currents because they have a large enough cross-sectional area to be entrained by air currents. The coagulated particulates thus tend to be returned to the ducts. Once they are in the duct system, they are carried to and trapped in the filters as a function of factors such as filter efficiency; static pressure loss, and so forth.

In summary, the typical electrical fields in indoor spaces the electrical characteristics of particulates, and the electrical characteristics of people, objects, and surfaces in the space are some of the primary determinants of contamination. Their interactions in large part determine the deposition of contaminants in and on people, objects, and walls in a space. A large body of information is available on electrical interactions involving airborne particulates in indoor spaces.

If airborne bacteria in a room act as particulate contaminants act, then one can apply the knowledge available about the behaviour of particulate contaminants to better understand the distribution, inhalation, and body deposition of bacteria in an indoor space. Means also are available to enhance filtration and minimize airborne bacteria in spaces such as offices; hospitals and nursing homes.

Because coagulation is one of the most important phenomena in the interactions of aerosols, an assessment of whether bacteria respond as particulates to a well-established coagulation acceleration procedure can give fundamental information on bacteria behaviour in indoor air and on the control of airborne bacteria, including spores. Theory and much laboratory data indicate that acceleration of coagulation can enhance the effectiveness of filters in removing particulates (Frey1996). Thus, coagulation acceleration is a clear-cut experimental mechanism and procedure that can be, used to determine whether bacteria can be influenced and controlled in the same manner as particulates in room air.

One way to accelerate coagulation is to use equipment to generate a specific non-homogeneous electrical field within a section of duct, downstream of the filter. In a non-homogeneous electrical field within a duct, coagulation is accelerated. The coagulated particulates then enter into the room, where, somewhat like snowballs rolling down a hill, they coagulate, or sweep up, additional small particulates. The air currents then carry the now large particles from the room into the duct, and the particles air trapped in the filters.

This article reports data indicating that several surrogates of airborne pathogenic bacteria, including spores, respond in a manner similar to airborne particulates.

MATERIALS AND METHODS

Test Room

All testing was done in a room 2.75 meters wide X 4.25 meters long x 2.50 meters high (9 x 14 x 8 feet) with a floor of vinyl tile. The walls and ceiling were constructed of drywall that had been coated with three applications of polyurethane. The room had its own closed-circuit air-handling system. The volume of the room was 62,6 cubic meters (1,008 cubic feet); and the duct volume (inside and outside the room) was 4.7 cubic meters (76 cubic feet).

Table 1

A. Characteristics of Aerosols

Particle Size in Microns	Percentage of Particles by Count
10-30	<1
6-10	<1
3-5	<1
1-3	1
0.6-1	6
<0.5	92

B. Particle Settling Time in Still Air

Particle Size in Microns	Time Required to Settle 8 Feet
100	8 seconds
10	13 minutes
1	19 hours
0.1	79 days
0.01	infinite

Air entered the room through supply diffusers on one side, as shown in Figure 1, passed across the room, and exited through return grills into a duct at a rate of 21 room air changes per hour with a velocity of 225 feet per minute at the diffusers. With this system, similar results are obtained at air change rates of five to 21 per hour; 21 was used in accordance with standard engineering practice to minimize test time. In the duct, the air passed sequentially through a 55 percent bag filter, two electrical field screens and a blower; then it re-entered the room through the supply diffusers. The 55 percent efficient filters were 55 percent per the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) dust-spot Standard 52.1, and their fractional size efficiency carried a minimum efficiency rating value (MERV) of 10 per ASHRAE Standard 52.2 (ASHRAE, Atlanta, Georgia). This is a 20-inch-deep non-supported bag filter with glass media. The relative humidity in the room, measured with a sling psychrometer, was approximately 76 percent, which minimized the desiccation that the relatively high air change rate could have caused. Between test runs, the air in the room was irradiated with UV light by a ceiling-mounted model xx-40s UV lamp (UVP Co., Upland, California), purged to the outside for 30 minutes, and replaced by air-conditioned office air. Separate tests were done to determine if viable bacteria remained in the room at the end of the purge period. In these tests, impingers were used to sample, as was done in the experiments. The results indicated that no viable bacteria remained at the end of the purge.

A non-commercial system to accelerate coagulation was installed with its screens in the duct. The upstream (HV) electrical field screen was 50 X 50 centimetres (cm) (20 x 20 inches), and the downstream one (HF) was 60 x 60 cm (24 x 24 inches). They were spaced 7.6 cm (3 inches) apart, as shown in Figure 2. The HF screen was constructed of .041-inch wire mesh (Wire cloth Manufacturing, Largo, Florida) with 1.3-cm (1/2-inch) openings, mounted on a steel frame. The HV screen was constructed of 4. 7-mm (3/16-inch) flat-tinned copper braid straps (Alpha Wire Co., Elizabeth, New Jersey) mounted vertically, on 7-cm (2.75-inch) centers, on a steel frame. A Model 600 electrical source (Cosatron, Tampa, Florida) supplied a 25-kilovolt (25-kV) DC signal to the HV screen and a 700-volt root-mean-square 177 kilohertz (177 -kHz) signal to the HF screen to create an electrical excitation field. The current is trivial, less than 3 milliamperes (mA). Separate tests with a Sensidyne ozone detector system (Sensidyne, Clearwater, Florida), before and after the screens, showed that no ozone is produced by this system (unpublished data).

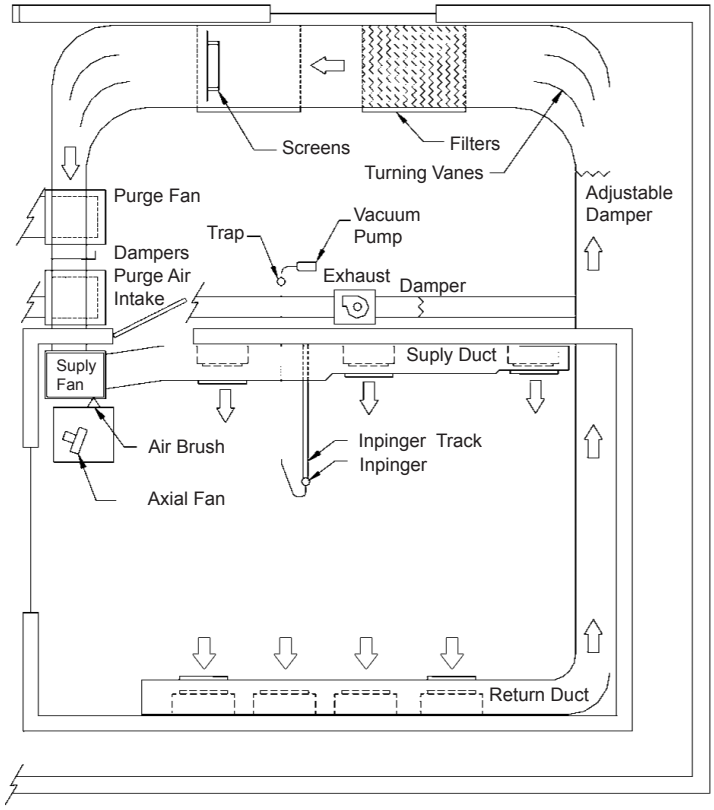

Figure 1. View from Above the Test Room and Ductwork Inside and Outside of the Test Room

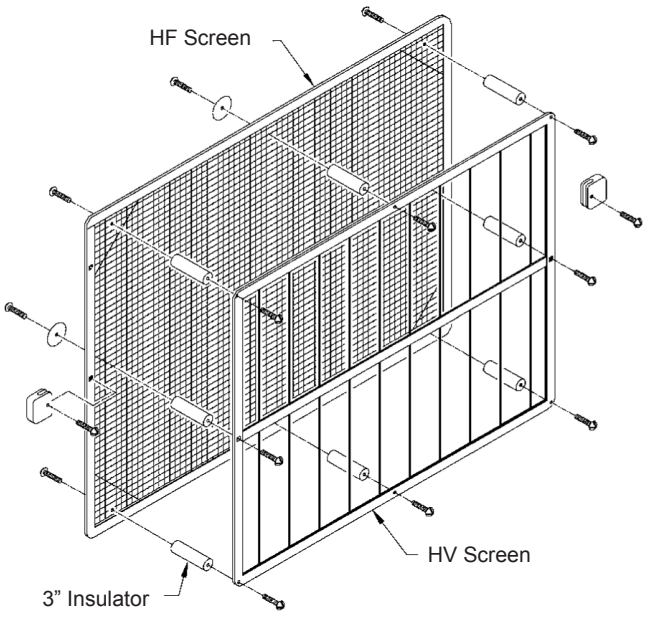

Figure 2. Construction of the In-duct Electrical Field Screens

The experimentation was conducted in a dedicated aerosol physics test facility that has yielded highly reliable data with particulates and chemicals. Extensive pilot studies were carried out with bacteria to determine the parameters that were used in the data reported below and to determine if the authors would have comparable reliability with bacteria. The reliability was comparable, as can be seen in the statistical Significance of the data obtained in the main experimentation carried out later and reported below.

Experiments 1 and 2

Two strains of M. luteus were used. They were supplied by American Type Culture Collection. (ATCC 4698) (Manassas, Virginia) and Carolina Biological Supply (Burlington, North Carolina). One strain was used in each experiment. The bacteria were streaked for isolation on trypticase soy agar plates (BD Biosciences, Sparks, Maryland) and incubated at 309C for 24 hours. Isolated colonies were suspended in 0.1 percent peptone water to a turbidity equivalent to a 0.5 McFarland standard, approximately 108 colony-forming units per milliliters (CFUs/ mL). The inoculum was used for all four runs done each day and was refrigerated between runs. Ten mL of inoculum was put into a l-ounce airbrush bottle. The bottle was then inserted through a wall access panel and mounted to a Paasche single-action, external-mix airbrush (Paasche Co., Harwood Heights, Illinois). The airbrush was located in the test room as shown in Figure 1. 3 feet above the floor and pointed toward the center of the room. A 100-cubic-foot-per-minute (lOO-cfm) axial fan was mounted 1 foot below the air stream from the airbrush and was pointed toward the center of the room.

A set of four runs was completed each day- Run 1 was done with the in-duct electric field (EF) system off (control). Runs 2 and 3 were done with the EF system on (test); and the fourth run was done with the EF system off (control).

The XXlOO-3750 impinger that was used (Millipore, Bedford, Massachusetts) is comparable in shape, configuration, and size to the AGI-30 (ACE Glass, Vineland, New Jersey) according to the manufacturers published specifications. Both draw air at approximately 12.5 L per minute at the same pressure drop. Three glass impingers were each filled with 30 mL of brain heart infusion broth (BD Biosciences). The impingers sampled the air at five, 25, and 45 minutes after the bacteria spray stopped. Each impinger was clamped onto the end of a wooden bar and wrapped with 20-gauge grounded copper wire. This assembly could be slid in and out of the room on a wooden track. Vacuum tubing was fitted onto the outlet on the side of the impinger. The impinger was then inserted, through an access panel, into the center of the room, 3 feet above the floor. The distal end of the impinger tubing was attached, outside the room, to an Erlenmeyer flask; it was used as a trap. Another piece of vacuum tubing connected the flask to a 3.5-cfm vacuum pump.

The procedure for a run was to turn on the room air system, the small axial fan located adjacent to the airbrush nozzle, and then the airbrush compressor air, set at 20 pounds per square inch. The small axial fan optimized the dispersion of the bacteria into the room. The airbrush was operated until the bacterial suspension was sprayed into the room, which took about one minute. The bacteria were allowed to circulate for five minutes so that they were distributed throughout the room and duct system. The vacuum pump was turned on, and room air was drawn through the impinger for five minutes. Then the room air system was turned briefly off while the impinger was pulled back through the wall access panel. One mL of liquid from the impinger was evenly spread onto a 150x15 mm trypticase soy agar plate (BO Biosciences). At 25 minutes and 45 minutes, the procedure was repeated. To complete the run, the room air was UV-irradiated and purged to the outside.

The EF was turned on, and runs 2 and 3 were completed in the same way. Then the EF was turned off and Run 4 was completed.

All sample plates were incubated at 30°C for 48 hours. The number of CFUs was counted and the data were recorded as CFUs per plate.

Experiments 3 and 4
All conditions were the same as above, with the following exceptions: Vegetative forms of two B. subtilis strains, ATCC 6051 and a strain from Carolina Biological Supply; were used; 55 mL of the inoculum was dispersed into the room; a 30 percent filter was used in the duct; and the air flow in the room was made turbulent by two floor fans put into operation in the room. The 30 percent filters were 30 percent per ASHRAE dust-spot Standard 52.1 and carried a fractional size efficiency MERV value of 8 per Standard 52.2. They were 2-inch thick, pleated-panel filters with glass media.

Experiment 5
All conditions were the same as in experiments 3 and 4, with the following exceptions: The spore form of B. subtilis, ATCC 6633 (BO Biosciences 212901), was used; 1 mL or approximately 1.4 X 108 spores were dispersed into the room; and the UV lamp exposure at purge was 12 minutes longer.

RESULTS

Experiment 1
The M. luteus strain from Carolina Biology Supply was used. There were 44 experimental runs, four each day The run pattern, designed to eliminate possible trend effects, assigned the first and fourth runs to EF-off as controls; the second and third runs were EF- on. In the beginning of the analysis, each run was considered individually. The five-minute data point was considered the start point. Because data would vary slightly from run to run, the authors normalized the data for each run by taking 25-and 45-minute data points as percentage of bacteria remaining Compared with the five-minute data point for each run. Thus, the start point for each of the runs was made equivalent.

The normalized data from the first and fourth runs were averaged, and the data from the second and third runs were averaged. Then a percentage reduction or increase in CFUs was calculated for the EF-on plate averages relative to the EF-off plate averages. The derivative 11 data points for the 25-minute samplings and for the 45-minute samplings were analyzed individually by use of the binomial test. The differences between EF-on and EF-off at the 25-minute sampling were significant at the .03 level, and there was a median reduction in CFUs with EF-on of 28 percent. The differences between EF-on and EF-off at the 45-minute sampling were significant at the .006 level, and there was a median reduction in CFUs with EF-on of 29 percent.

Experiment 2
M. luteus ATCC 4698 was used. There were 48 experimental runs, four each day. The run pattern used was as above, and the data were normalized. A percentage reduction or increase in CFUs was calculated for the EF-on plate averages relative to the EF-off plates. The derivative 12 data points for the 25-minute sampling and for the 45-minute sampling were analyzed individually by use of the binomial test The differences between EF-on and EF-off at the 25-minute sampling were significant at the .003 level, and there was a median reduction in CFUs with EF-on of 29 percent. The differences between EF-on and EF-off at the 45- minute sampling were significant at the .003 level, and there was a median' reduction in CFUs with EF-on of 35 percent.

Experiment 3
Vegetative cells of B. subtilis from Carolina Biological Supply were used. There were 20 experimental runs, four each day. The run pattern used was as above, and the data were normalized. A percentage reduction or increase in CFUs was calculated for the EF-on plate averages relative to the EF-off plates. The derivative five data points for the 25-minute samplings and for the 45-minute samplings were analyzed individually by use of the binomial test. The differences between EF-on and EF- off at the 25-minute sampling were significant at the .03 level, and there was a: median reduction in CFUs with EF-on of 57 percent. The differences between EF-on and EF-off at the 45-minute sampling were significant at the .03 level, and there was a median reduction in CFUs with EF-on of 76 percent.

Experiment 4
Vegetative cells of B. subtilis, ATCC6051, were used. There were 28 experimental runs, four each day. The run pattern used was as above and the data were normalized. A percentage reduction or increase in CFUs was calculated for the EF-on plate averages relative to the EF-off plates. The derivative seven data points for the 25-minute sampling and far the 45-minute sampling were analyzed individually by use of the binomial test. The differences between EF-on and EF-off at the 25-minutes sampling were significant at the .008 level, and there was a median reduction in CFUs with EP-on of 46 percent. The differences between EF-on and EF-off at the 45- minute sampling were significant at the .008 level, and there was a median reduction in CFUs with EF-on of 78 percent.

Experiment 5
The spore form of B. subtilis ATCC 6633 was used. There were 32 experimental runs, four each day. The run pattern used was as above, and the data were normalized. A percentage reduction or increase in CFUs was calculated for the EF-on plate averages relative to the EF-off plates. The derivative eight data points for the 25-minute samplings and for the 45-minute samplings were analyzed individually by use of the binomial test. The differences between EF-on and EF-off at the 25- minute sampling were significant at the .004 level and there was a median reduction in CFUs with EF-on of 46 percent. The differences between EF-on and EF-off at the 45- minute sampling were significant at the .004 level and there was a median reduction in CFUs with EF-on of 52 percent.

The comparisons between test ana control groups for each of the strains of spore-forming and vegetative bacteria are summarized in Table 2.

Table 2. Percentage Reduction in Bacteria,Includihg Spores, for Test Groups Relative to Control Groups

Bacteria	Time	Carolina Strain Reduction	Statistical Significance of Difference	ATCC Strain Reduction	Statistical Significance of Difference
M.luteus	25 minutes	28%	.03	29%	.003
	45 minutes	29%	.006	35%	.003
B. subtilis vegetative cells	25 minutes	57%	.03	46%	.008
	45 minutes	76%	.03	78%	.008
B. subtilis spores	25 minutes	-	-	46%	.004
	45 minutes	-	-	52%	.004

DISCUSSION

These experiments were carried out to determine whether aerosolized bacteria, including spores, respond like particulate contaminants to the primary (electrical) forces that control the distribution of small particulate contaminants in indoor air. Such response would suggest an approach to minimizing infection in offices, hospitals, nursing homes, and other facilities. There are also implications for protection against intentionally introduced pathogenic bacteria, including spores. M. luteus was used as a surrogate for Gram-positive cocci, because M. lutcus is similar in size, shape, and cell wall composition to staphylococci, streptococci, and enterococci. Similarly, spore-forming and vegetative B. subtilis were used as surrogates for Gram-positive bacilli such as B. anthracis. The experiments were conducted in a dedicated aerosol physics test facility with culture-based measurements made at timed intervals.

Coagulation is one of the most important phenomena in the interactions of aerosols. For this reason, the authors assessed whether bacteria, including spores, respond as particulates by using a well-established coagulation test procedure. The test procedure used equipment to generate a specific non-homogeneous electrical field within a section of duct, downstream of the filter. In this non-homogeneous electrical field within the duct, coagulation is accelerated. The coagulated particulates enter into the room with the supply air, where, somewhat like snowballs rolling down a hill, they further coagulate or sweep up additional small particulates. The air currents then carry the now large particles from the room into the duct and they are trapped in the filters.

In prior experimentation in which particulates were put into a comparable test room and the test procedure was used to accelerate coagulation, there was a substantial reduction in small particulates. In addition, the physical mechanism was confirmed; there was a modification of the particulate size spectrum. Figure 3 shows the effect of coagulation, shifting the particulate-size spectrum in a room from smaller to larger particulates.

The results reported, here are consistent with what would be expected from the particulate data obtained in prior experiments. Specifically, the data reported here show that bacteria of

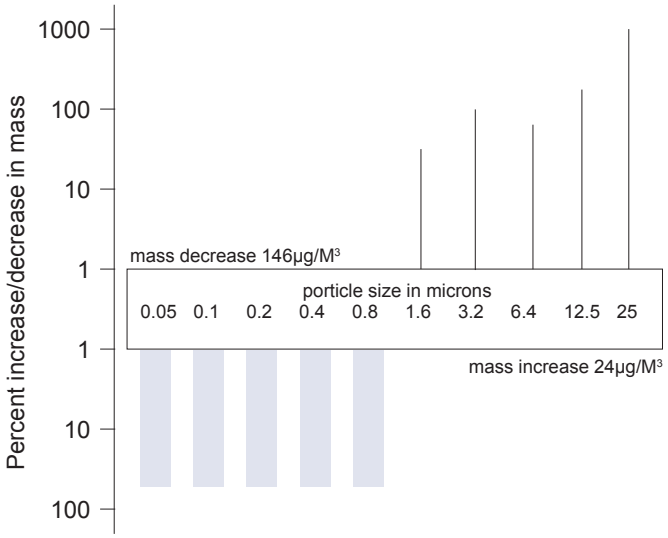

Figure 3. Percentage Decrement or Increment in Particulate Mass in a Room with In-duct Electrical Field On*

* From a baseline of 100 percent The baseline is the in-duct field-off data at each particulate size. The difference in mass between in-duct on and off conditions at each particulate size was statistically significant, except at the 1.6-µm size. The mass. of the particulates is represented by the thickness of the bars.

different sizes and shapes-vegetive cells and spores-respond like particulate contaminants to the primary forces that control the distribution of small particulates in a room. These cocci and spore-forming and vegetative bacilli also respond like particulate contaminants to equipment designed to control airborne particulate contaminants. The reductions in spore-forming and vegetative bacteria concentrations were substantial and statistically robust. This finding indicates that the body of knowledge developed for particulate control can be used for control of bacteria, including spores, It also suggests an approach to minimizing infection in offices, hospitals, nursing homes, and other facilities where airborne bacteria, including spores, are of concern; Finally, the data indicate means of enhancing protection against intentionally introduced pathogenic bacteria, including spores.

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