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Evaluation of Surface Sampling for *Bacillus* Spores Using Commercially Available Cleaning Robots

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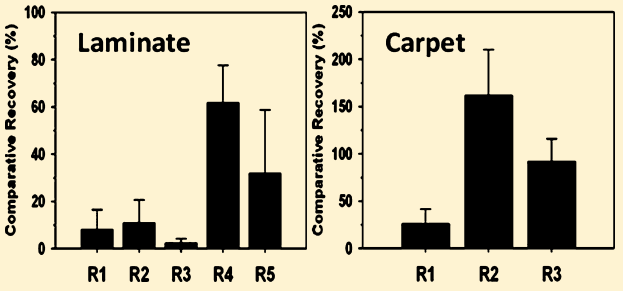
ABSTRACT: Five commercially available domestic cleaning robots were evaluated on their effectiveness for sampling aerosol-deposited *Bacillus atrophaeus* spores on different indoor material surfaces. The five robots tested include three vacuum types (R1, R2, and R3), one wet wipe (R4), and one wet vacuum (R5). Tests were conducted on two different surface types (carpet and laminate) with 10^6 colony forming units of *B. atrophaeus* spores deposited per coupon (35.5 cm ×35.5 cm). Spores were deposited on the center surface (30.5 × 30.5 cm) of the coupon's total surface area (71.5 × 71.5 cm), and the surfaces were sampled with an individual robot in an isolation chamber. Chamber air was sampled using a biofilter sampler to determine the potential for resuspension of spores during sampling. Robot test results were compared to currently used surface sampling methods (vacuum sock for carpet and sponge wipe for laminate). The test results showed that the average sampling efficacies for R1, R2, and R3 on carpet were 26, 162, and 92% of vacuum sock sampling efficacy, respectively. On laminate, R1, R2, R3, R4, and R5 average sampling efficacies were 8, 11, 2, 62, and 32% of sponge wipe sampling efficacy, respectively. We conclude that some robotic cleaners were as efficacious as the currently used surface sampling methods for *B. atrophaeus* spores on these surfaces.



INTRODUCTION

After the 2001 intentional *Bacillus anthracis* spore contamination incidents in the U.S., many studies have been conducted to develop and improve the process of remediation of contaminated buildings.[1−8] Since 2001, surface sampling studies have been emphasized especially because of their direct input into decision making during site remediation.[9,10] Sound and defensible protocols and implementation plans for surface sampling are needed, but have not yet been adequately developed.[11] Numerous studies have tested surface sampling methods to evaluate and/or validate their efficacy on various surface types under numerous environmental conditions.[12−20] As a result, surface sampling methods have been improved and optimized for real world application. However, there are still significant gaps in the sampling and analysis approaches following a release of a biological agent impacting a large urban area.[7,21,22] A *B. anthracis* release over a wide and highly populated area would tremendously increase the time, cost, and complexity of restoration of the contaminated area compared to the individual building cases in 2001 incidents. Currently used surface sampling methods are limited to small areas (10 cm^2 to 1 m^2) and would require the collection of a large number of discrete samples to be representative if deployed over a large spatial scale. Such a sample burden would strain sample processing laboratories during remediation and delay the overall recovery. Although efforts have been made to increase laboratory capability and capacity for processing biological agent samples, under the present sampling approach the laboratories will quickly be overwhelmed following a wide-area attack.[11,23,24]

The surface sampling methods recommended currently include wet wipe (sponge wipe or gauze wipe) and vacuum.[25] The existing spore sampling strategy requires the use of a variety of methods depending on the surface types, for example, vacuuming for rough and porous surfaces and wet wipes for smooth nonporous surfaces.[25,26] These methods have disadvantages such as extensive human labor, potential cross contamination, and potential exposure of the sampling personnel to pathogenic organisms. Commercially available

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domestic cleaning robots could be an alternative for *B. anthracis* spore surface sampling. The cleaning robots have been introduced and commercialized since the early 1980s for home and industrial use.[27] The cleaning mechanisms of these robots are similar to the current surface sampling methods such as vacuuming, sweeping, and scrubbing. Robots have been developed with various functions and sensors to improve cleaning performance [28] and can clean approximately 2−4 rooms (100−400 m^2) per charge, according to the manufacturers' instructions. Therefore, collection of composite samples from wide areas using such cleaning robots for *B. anthracis* spore surface sampling would reduce the number of required personnel and samples needed compared to the currently used surface sampling methods, consequently reducing the burden on laboratories. Currently, the adequacy of the collection efficiency of these robots for *B. anthracis* spore sampling is unknown.

In this study, commercially available domestic cleaning robots were evaluated for spore surface sampling efficiency on common indoor surfaces. The current study determined the sampling efficiency of each robot, without modifying the sensors, algorithms, or logics set by the manufacturers. Aerosol-deposited *B. atrophaeus* spores were used as a surrogate for *B. anthracis* spores. Test results were compared to currently used surface sampling methods (vacuum sock and sponge wipe) recommended for each surface type. The potential spore resuspension due to the cleaning robots during sampling was monitored, and the results are discussed.

MATERIALS AND METHODS

Cleaning Robots. Five commercially available cleaning robots were purchased from an Internet retail store and are described in Table 1. R1, R2, and R3 are vacuum-based cleaning robots and were tested on both carpet and laminate surfaces. R4 and R5 are wet wipe- and wet vacuum-based robots, respectively, and were tested only on laminate surfaces (not on carpet) as instructed by the factory manuals (i.e., for use on hard surfaces). All robots were sterilized inside the Consequence Management and Decontamination Evaluation Room (COMMANDER) by exposure to 250 ppm of hydrogen peroxide (H_2O_2) vapor (VHP, 1000ED, STERIS, Mentor, OH) for four hours before testing. H_2O_2 concentration in COMMANDER was monitored using a gas detector (B12 wet gas detector, Analytical Technology, Inc., Collegeville, PA), and the robots were retrieved after the H_2O_2 levels in COMMANDER were below the safe entry level (<1 ppm). Further, the sterilized robots were degassed for at least one day to remove residual fumigants. The robots were visually inspected after fumigation for any defects from the fumigation. All robots showed no difference before and after fumigation. All robots retained their factory settings during testing, and each robot was used only once before being discarded. All robots, except R3, possess internal logic that allows the robot to sample a discrete or predefined space and then subsequently deactivate itself. For these robots, the sampling duration was therefore determined by the robot itself. R3 units were manually operated for the equivalent sampling durations of R1 for carpet and R5 for laminate tests because these units had the longest sampling durations.

Table 1. List of Commercially-Available Cleaning Robots

robot	model	manufacturer	cleaning type	applicable surfaces
R1	Roomba 760	iRobot Bedford, MA	vacuum with HEPA filter and rotating bristle brush	all surfaces
R2	XV-11	Neato robotics Newark, CA	vacuum with air filter and rotating silicone flat beater	all surfaces
R3	P3 P4920	P3 International New York, NY	vacuum with air filter (no surface agitation tool)	all surfaces
R4	Mint 4200	Evolution Robotics, Inc. Pasadena, CA	sweep and mop	hard floors
R5	Scooba 380	iRobot Bedford, MA	wet vacuum	hard floors

When operating manuals required liquid inputs, sterile phosphate buffered saline with 0.05 wt % Tween 20 (PBST) was used rather than water or soapy water for R4 and R5. The surface-contacting side of a mopping cloth for R4 was soaked by evenly distributing 50 mL of sterile PBST onto it. R5 was equipped with two tanks (clean and dirty). The clean tank contained liquid cleaning agent prior to dispensing onto surfaces and the dirty tank was used to store liquid retrieved from cleaning. R5 was filled with 60 mL of sterile PBST in the clean tank before testing. Spore recovery efficiencies for individual robot types were evaluated separately. A predetermined amount of *B. atrophaeus* spores were spiked onto the filters and collection bins of each robot and allowed to dry.

Test Materials and Deposition. Robot sampling tests were conducted with two floor surface types: laminate (Pergo Estate Oak, PE-191113) and carpet (Beaulieu Laredo Sagebrush loop carpet, model 6666−01−1200-AB). These materials were purchased from a local retail store (Home Depot, Durham, NC). Coupons were fabricated into 71.5 × 71.5 cm size pieces (robot sampling test coupons) for robot sampling tests and 35.5 × 35.5 cm (surface sampling coupons) for vacuum or sponge wipe (3M Sponge-Stick, SSL-10NB, 3M, St. Paul, Minnesota) sampling tests. Both coupon types were backed with an equal-sized piece of 1.1 cm thick oriented strand board (OSB) plywood. Prior to use in tests, carpet coupons were vacuumed to remove the detachable foreign debris and particles, while laminate coupons were cleaned with a dry wipe (SIMWyPE tack cloth, Babcock & Wilcox Technical Services Y-12 L.L.C., Oak Ridge, TN). After surface cleaning, carpet and laminate coupons were sterilized using VHP by exposure to 250 ppm of H_2O_2 for four hours inside the COMMANDER. The sterilized coupons were stored in sterilization bags (General Econopak, Inc., Philadelphia, PA, P/N 63636TW) until tested. After sterilization, coupons and deposition apparatus were degassed for a minimum of three days before testing. The sterility of the coupons, deposition apparatus, robots, and other equipment needed for the test were confirmed by sampling at least one coupon and one deposition apparatus per sterilization batch by swabbing (Remel Products, Lenexa, KS, P/N R12100) their respective surfaces. The swabs were subsequently streaked onto tryptic soy agar (TSA) (BD, Franklin Lakes, NJ) plates, and the plates were incubated at 35 ± 2 °C for at least 18 h before being inspected visually to determine if bacterial growth (i.e., contamination) was present.

A powdered spore preparation of *B. atrophaeus* (ATCC 9372, Manassas, VA) and silicon dioxide particles, developed specifically for use as a surrogate for weaponized *B. anthracis* spores, was used in the current study.[12] The sterilized coupons were inoculated using the methods described previously.[29−31] A metered dose of ~1 × 10^6 spores was aerosolized into each

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dosing chamber and allowed to gravitationally settle onto the 30.5 × 30.5 cm center portion of the surface sampling coupons (35.5 × 35.5 cm) and robot sampling test coupons (71.5 × 71.5 cm).

The consistency and loading levels of inoculums were verified using four stainless steel control coupons during each inoculation event. Stainless steel surfaces were used to verify spore inoculation because stainless steel surfaces provide highly repeatable recoveries.[12,14,32] Stainless steel coupons were sterilized prior to use via a steam autoclave (STERIS Amsco century SV-120 Prevacuum Sterilizer, Mentor, OH) at 121 °C and 103 kPa for one hour. As with test coupons, each coupon was inoculated independently using separate dosing chambers originally designed for inoculation of 35.5 × 35.5 cm coupons of any thickness. Following a metered dissemination, spores were allowed to settle onto the coupons for a minimum period of 18 h. Test coupons used for robot sampling tests were inoculated in the center of the coupon with the area of 30.5 × 30.5 cm. The same size area was inoculated on the surface sampling coupons. After inoculation, the inoculation control coupons were inoculated, sampled with the sponge wipe method, and analyzed with the same methods used for test coupons.

Robot Testing Procedure. Robot sampling tests were conducted inside the COMMANDER. The COMMANDER was controlled for temperature (22 ± 0.7 °C) and relative humidity (57 ± 5%). More detailed information about the COMMANDER can be found elsewhere.[33−3435] Robot sampling tests were conducted with carpet and laminate surfaces inside robot testing chambers located inside the COMMANDER. A robot testing chamber (91 × 91 × 46 cm) was constructed of clear acrylic material (5 mm thickness) and the inside surface was coated with antistatic film (chemical-resistant PVC (Type I) antistatic Film, McMaster-Carr, Princeton, NJ). The diagram of the chamber is shown in Figure 1. The chamber had one port located on the top lid and was used for air sampling. Another port, located on the front of the chamber, was outfitted with a HEPA filter and passively supplied makeup air during sampling. Chamber air was sampled (15 LPM for 20 min) using a bio filter sampler (Via-Cell Bioaerosol Sampling Cassette, p/n VIA010, Zefon International, Inc., Ocala, FL) to determine the potential for resuspension of spores during robot sampling. Each test (one robot and one surface type) was conducted with four robot testing chambers (one blank and three replicates) inside the COMMANDER. First, the test started with a blank robot sampling. A sterilized surface coupon was placed in the middle of a robot testing chamber and a sterilized robot was placed on the bottom left corner of the coupon. The cleaning start button was pressed and the lid to the robot testing chamber was closed. Air sampling was initiated simultaneously with the onset of robot sampling and the sampling duration was monitored. After the blank sampling, *B. atrophaeus* spore-inoculated test coupons (one coupon per robot testing chamber) were sampled with a robot in each testing chamber. The inoculated coupons were placed in an individual robot testing chamber, taking care not to touch the contaminated surface. After the completion of sampling, the robots were powered off, removed from the testing chamber, and disassembled for retrieval of the filters and the particle bins for R1, R2, and R3, the mopping cloths for R4 and the clean and dirty tanks for R5. The collection parts were placed in sterilized plastic bags (one per robot). Each bag was then secondarily contained in another bag and transported to the microbiology laboratory for processing. Filters from air sampling units were aseptically removed from the robot testing chambers and placed individually in sterile plastic bags or sterile specimen cups for analysis.

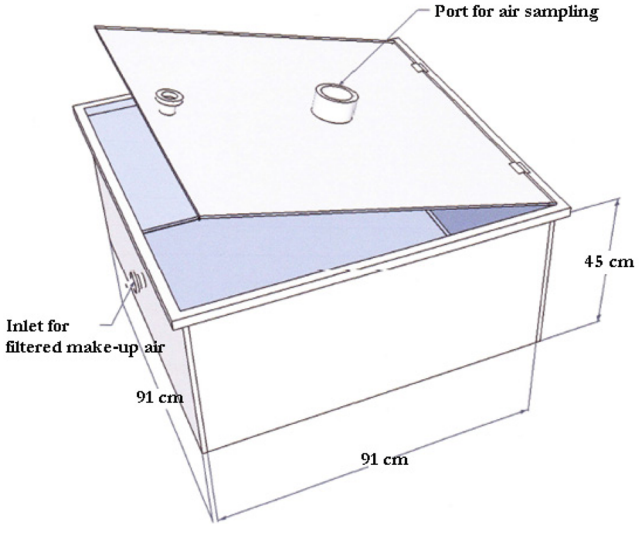

Figure 1. Robot sampling test chamber.

Surface Sampling Methods. The carpet and laminate coupons were sampled using currently used surface sampling methods.[11,13,17,18,23] Laminate surfaces were sampled with a sponge wipe sampling method and carpet surfaces with a vacuum sock method. An area of 30.5 × 30.5 cm was sampled with the sponge wipe, using a sterile stainless steel template. Samples were collected using the following four patterns: using the flat side of the sponge wipe, the surface was sampled using side ways S-strokes, covering the entire template area; the sponge wipe was then flipped over to the opposite side to sample the surface in S-strokes at 90 degrees to the previous pattern, covering the entire template area; using the narrow edges of the sponge wipe, the surface was sampled using the same S-strokes applied diagonally across the template, 45 degrees out of phase with the previous two S-stroke patterns; and finally the tip of the sponge wipe was then used to sample the perimeter of the sampling area. The sampling method is described in detail in Rose et al.[18]

Vacuum socks were used to collect spores on the carpet surface. Vacuum sampling used a 30.5 × 30.5 cm sterile stainless steel template and a sterile sock/nozzle attachment to collect the sample. Holding the nozzle at a 45 degree angle to the horizontal surface and moving over the sample area, samples were taken using the same S-stroke horizontal, 90 degrees and 45 degree out of phase to the first S-stroke pattern, and followed by the perimeter sample.

Sample Extraction and Spore Recovery. Sponge wipe samples were extracted by stomaching (1 min, 260 rpm) in 90 mL of PBST using a Seward model 400 circulator (Seward Laboratory Systems, Inc., Port Saint Lucie, FL). Vacuum sock samples were extracted by first wetting the collection portion of the filter by dipping in PBST, then cutting it with sterile scissors (vertically then horizontally) into small pieces (approximately 1 × 4 cm).[13] As the filter was fractioned, the resulting pieces were allowed to fall into a 120 mL sterile specimen cup (Starplex Scientific LeakBuster Specimen Containers, Fisher Scientific, Pittsburgh, PA) containing 20 mL sterile PBST.[13] The cups

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were then agitated (30 min, 300 rpm, ambient temperature) using an orbital platform shaker incubator (model 3625, Lab-line Instruments, Inc., Melrose Park, IL). Spores collected by R4 were recovered from the mopping cloth by stomaching the cloth (2 min, 230 rpm) in 133 mL PBST using a Seward model 400 circulator (Seward Laboratory Systems, Inc., Bohemia, NY). Two extraction procedures were required for R1, R2, and R3, as collected spores could have partitioned to either the particle bin or the filter. Recovery from the filters of R1 proceeded by placing both filters into a 120 mL specimen cup (PN 3008−3TN, Starplex Scientific Inc., Cleveland, TN,) covered with 90 mL PBST, and then agitating (30 min, 300 rpm) on an orbital platform shaker (Lab-line, model 3625). Recovery from the filters of R2 and R3 proceeded by placing each filter(s) into two 14 × 23 cm sterile sample bags (P/N 01−002−53, Fisher Scientific, Pittsburgh, PA), one inside the other for double containment, adding 180 mL of sterile PBST to the innermost bag, and then agitating (30 min, 300 rpm) the samples on an orbital platform shaker (Lab-line, model 3625). Spore recovery from the particle bins of R1, R2, and R3 was accomplished by placing the bins individually into double 25 × 38 cm sterile sample bags, aseptically adding 180 mL of PBST to each bag containing the bin, and then agitating (30 min, 300 rpm, ambient temperature) on an orbital platform shaker incubator (Lab-line, model 3625).

The wet vacuum robot (R5) also required two extraction procedures, one procedure for the liquid fraction and liquid collection reservoir and one procedure for the filter. First, the original 60 mL sterile PBST R5-collected liquid was retrieved from the dirty tank using a 100 mL sterile serological pipet. The clean and dirty tanks were then rinsed twice with 60 mL PBST per tank, and the three fractions were combined (for a total of 180 mL). The filters from R5 were extracted with the same procedures used for filters from Robots R2 and R3.

The resulting liquid extracts from all robots and all fractions were then concentrated by centrifugation. Briefly, each sample was retrieved from its respective extraction bag or cup and dispensed equally into four 50 mL conical tubes (∼45 mL for each tube). The samples were then centrifuged (3500*g*, 15 min, 4 °C) to sediment the collected spores. All but 5 mL of the supernatant was carefully removed via 50 mL sterile serological pipet. Each spore pellet was resuspended in the remaining 5 mL by three cycles of alternating vortex mixing (30 s) and sonication (30 s, 40 kHz, Branson Model 8510, Rochester, NY). Following resuspension, the four fractions per sample were recombined into one ∼20 mL sample extract. All sample extracts (robot, vacuum sock, and sponge wipe) were then subjected to 10-fold serial dilutions, as necessary, by adding 0.1 mL of the sample to 0.9 mL of PBST using a micropipet. Appropriate dilutions were spread in triplicate (0.1 mL each) onto trypticase soy agar (BD; Becton, Dickinson, and Company; Franklin Lakes, NJ) plates and incubated at 35 ± 2 °C. Resulting colony forming units (CFUs) were counted manually after approximately 18 h. Only plates with between 30 and 300 CFUs were utilized for data calculations. When CFUs abundance for undiluted extracts was below 30 CFUs per plate, larger volumes of the sample extract were subjected to filter-plating to reduce the quantitation limit. The recovery for each sample was determined by averaging the results from triplicate subsamples. The total recovered CFUs were then determined by multiplying mean CFUs by the dilution factor and extract volume.

RESULTS AND DISCUSSION

Inoculation and Recovery. Stainless steel coupons were used to verify the level of spore loadings for every inoculation event. A total of 32 stainless steel coupons were inoculated and sampled using the sponge wipe method throughout this study. The recovery results showed the average loading level per coupon of each inoculation event ranged from 9.12×10^5 through 1.61×10^7 CFUs. The coefficients of variation of spore inoculation within each test were between 8 and 63%. The sampling efficiency from stainless steel using the sponge wipe method is approximately 48% according to the study by Krauter et al.[17] Therefore, using this crude assessment, the inoculated spores can be assumed to be between 10^6 and 10^7 CFUs per coupon. Recoveries from blank coupon sampling using robots were all negative except R1 and R5 from laminate. For these samples, viable spores were recovered; however, recoveries were below the quantification limit (less than 30 CFUs per plate) and therefore had minimal impact on study results. (The contamination levels from blank tests were minimal compared to the test spore loadings ($10^6 - 10^7$ CFUs) and no further action was required.) The air sampling results from blank coupons were negative for all blank tests.

Extraction Efficiency. The extraction efficiency was determined for each robot prior to conducting surface recovery tests because extraction efficiency may affect the overall sampling efficacy. For these tests, new robot filters and robot dust bins were spiked with $\sim 1 \times 10^6$ spores of *B. atrophaeus*, delivered in 0.1 mL PBST. Extraction and recovery procedures, described above in sample extraction and spore recovery section, were conducted to determine the recovery efficiency. These data are summarized in Table 2. The efficiency was calculated by normalizing recovery from robots by the number of spores spiked onto the parts. The results showed the extraction efficiency was approximately 50−90%. The average extraction efficiencies for vacuum units (R1, R2, and R3) were within 10% of each other. The maximum difference was between R4 and R5 at approximately 40%. These results were collected with clean, unused robots using a liquid inoculum. How the extraction efficiencies determined by this method may represent the extraction efficiencies achieved during surface sampling tests is unknown because the filters and collection bins from surface testing samples showed noticeable amounts of debris after use.

Table 2. Average Robot Extraction Efficiency (±Standard Deviation, Triplicates) Test Results

robots	extracted parts	extraction method	average extraction efficiency (%)
R1	filter, dust bin	orbital shaking	64.9 ± 14.1
R2	filter, dust bin	orbital shaking	56.7 ± 7.6
R3	filter, dust bin	orbital shaking	67.2 ± 4.1
R4	wipe cloth	stomaching	49.1 ± 7.1
R5	tank	rinsing	89.6 ± 6.9

Sampling Efficiency. The sampling efficiencies of cleaning robots were compared to the sponge wipe method for laminate coupons and the vacuum sock method for carpet coupons. The robot sampling comparative recovery (CR) was calculated using eq 1.

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$$\mathrm{CR}(\%) = \frac{\text{average recovery from robot test(CFUs)}}{\text{average recovery from surface sampling method(CFUs)}} \times 100 \quad (1)$$

eq

Laminate Surfaces. Laminate coupons were tested for surface spore sampling using five cleaning robots. The test results from laminate surfaces are summarized in Table 3 and

Table 3. Summary of Laminate Surface Sampling Comparative Recoveries Using Cleaning Robots

robot	average sampling duration (sec)	mean recovery from air filter (CFUs)	mean recovery from robots[a] (CFUs ×10^5)	mean recovery from sponge wipe[a] (CFUs ×10^5)	CR (%)
R1	424	67	1.7 ± 1.5	21.0 ± 11.1	8.1
R2	76	81	0.2 ± 0.1	1.4 ± 1.1	10.9
R3	540	4	1.3[b] ± 0.7	53.4 ± 30.0	2.4
R4	106	127	18.9 ± 4.6	30.7 ± 6.3	61.7
R5	545	not available[c]	20.6 ± 17.3	64.5 ± 1.9	31.9

[a]Mean recovery ± standard deviation. [b]One R3 unit stopped after approximately 10 s of operation. Data are calculated with duplicate sample results. [c]These data were collected from ViaCell cassettes which were past the expiration date.

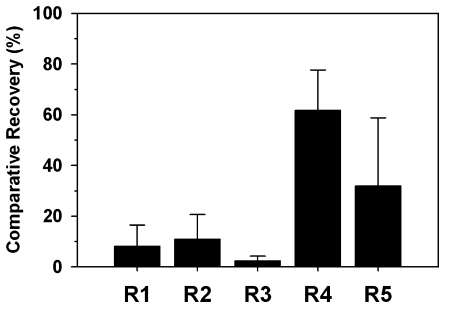

Figure 2. Cleaning robot sampling efficacy compared to sponge wipe sampling method on laminate surface.

Figure 2. The highest CR was achieved by R4 (62%) among the five robots tested. The second highest CR was achieved by R5 (32%). The vacuum units (R1, R2, and R3) demonstrated CRs close to or less than 10%. Low CRs from vacuum units agree with previous sampling studies that have shown the wet wipe or the sponge wipe method on nonporous surfaces has higher recovery efficiency than vacuum-based methods.[19] The sampling process used by R4 was similar to the wet wipe or the sponge wipe method because R4 used a PBST-wetted cloth in conjunction with a rubbing action on the surface. The size of cloth used by R4 for cleaning was approximately 25 × 10 cm and, therefore, resulted in a 10 times larger contact area than the sponge wipe. However, the sampling efficiency of R4 was lower than the sampling efficiency of the sponge wipe method. One possible explanation is the disparity in actual sampling coverage area of the two methods. Another explanation can be that the spores are collected by R4 from the center inoculated area and then redistributed to the surrounding areas where the spores were not initially inoculated. The R4 units sampled the surface by passing over the surface one time only, per the manufacturer's default. The sponge wipe method required passage over the same surface three times. The R5 units sample surfaces by first releasing clean PBST onto the surface followed by scrubbing and wiping the surface. The dispensed PBST liquid, along with any particles contained in the liquid, is recollected by the unit from the surface and stored in the dirty tank. The spore recovery by R5 is dependent mainly on recollection of the dispensed liquid. The recollection of liquid is expected to vary significantly depending on the morphology of the surface sampled. Low sampling efficiencies would be expected when coarse or irregular surfaces are sampled.

The sampling duration was shortest for R2 (mean of 76 s) and longest for R5 (mean of 545 s). R4 was also the most efficient unit in terms of the number of spores per unit area and time.

Aerosol concentrations of spores observed during sampling on laminate surface are listed in Table 3. The results showed the lowest spore resuspension from R3. Minimal spore resuspension from R3 was likely due to its lack of a surface agitation tool. Data from R5 tests were not provided due to the potential contamination from the expired ViaCell cassettes.

Carpet Surfaces. Three cleaning robots (R1, R2, and R3) were tested for sampling efficiency on carpet coupon surfaces. R4 and R5 were not tested on carpet surfaces because of their recommended usage only on hard surfaces, according to the instruction manuals. Similar to the laminate surface tests, recovery efficiencies for carpet sampling were determined by comparison of robot recoveries to that of the currently used sampling method (vacuum sock sampling method). The test results are summarized in Table 4 and Figure 3. The highest

Table 4. Summary of Carpet Surface Sampling Comparative Recovery Using Cleaning Robots

robot	average sampling duration (sec)	mean recovery from air filter (CFUs)	mean recovery from robots (CFUs ×10^5)	mean recovery from sponge wipe (CFUs ×10^5)	CR (%)
R1	423	507	1.3 ± 0.4	5.2 ± 2.7	25.8
R2	81	273	1.0 ± 0.2	0.6 ± 0.2	161.5
R3	422	760	2.4[a] ± 0.4	2.6 ± 0.6	91.9

[a]One of R3 tests was conducted with a replaced unit due to the initial malfunction.

average CR was achieved with R2 (162%). This unit was also the most effective robot per surface area and time. R3, a vacuum only unit, demonstrated recoveries similar to the

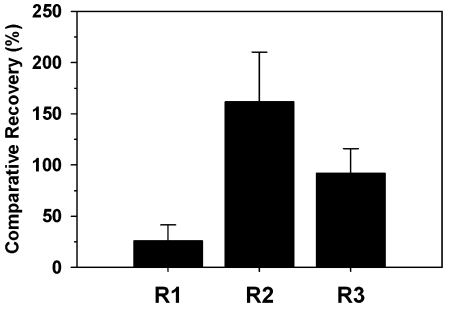

Figure 3. Cleaning robot sampling efficacy compared to vacuum sock sampling method on carpet surface.

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recoveries of the vacuum sock method. R1 showed the lowest CR among the three robots. It is not clear why R1, which was equipped with an agitating brush bar, showed a lower CR than R2 or R3. There may be various reasons to explain the low CR from R1, such as vacuum power, surface agitation method, sampling speed, sampling area coverage, etc. However, determining the effect of these variables was not part of the study objective. Two of the R3 units failed during testing (one from the laminate surface test and another unit from the carpet surface test). For one of these failures the robot failed to begin sampling; this robot was replaced with a backup R3 robot. In the other instance, the robot failure occurred 10 s after sampling was initiated. Replacement of this robot with a functioning backup was not possible in this instance, as an unknown number of spores were removed from the surface during the 10 s of sampling with the faulty robot. Data from this sample were therefore excluded from the analyses. The reliability of R3 may not be sufficient for field sampling following an actual incident. The data from the air filter analyses indicate that spore resuspension during carpet sampling tests was higher than during laminate tests for all three robots (Table 4). This result implies a higher likelihood of spore suspension from carpet surfaces than from laminate surfaces, even with the same robot activity levels.

The current study evaluated the application of commercially available cleaning robots for use in *B. anthracis* spore sampling of surfaces. The sampling efficiency of robots was assessed by comparing the recoveries achieved by the robots to recoveries obtained by the surface sampling methods in current use. The results showed that the average sampling efficacies for R1, R2, and R3 on carpet were 26, 162, and 92%, respectively, of vacuum sock sampling efficacy. On laminate, R1, R2, R3, R4, and R5 average sampling efficacies were 8, 11, 2, 62, and 32%, respectively, of sponge wipe sampling efficacy. Some cleaning robots (R2 and R3 on carpet and R4 on laminate) were as efficacious as the respective surface spore sampling methods in current use. The current test method focused only on the sampling mechanism of the individual robots by limiting sampling surface area. Varying the area cleaning logics or algorithms of individual robots was not part of this study. However, varying the area cleaning logics or algorithms of individual robots could be an important factor that could impact sampling in a wide area.

Recent cleaning robots have various convenient functions such as self-recharging, mapping, navigation, etc. These functions will allow large contaminated areas to be sampled systematically. Some benefits of using cleaning robots for wide area sampling rather than the currently used sampling methods include fewer samples because one sample is generated per deployment and less risk of personnel exposure to *B. anthracis* spores, etc. However, for real world application, these robots need further evaluation with various surfaces, deposition types, surface loadings, and environmental conditions (RH variation, exposure duration, etc.). Previous studies have shown the impact of various factors on the surface sampling efficacies.[16,17,36] The current study evaluated the robots under limited conditions (10^6 CFUs per coupon (35.5 × 35.5 cm) with two surface types), suggesting the utility of this approach. Further, the evaluation of these robots is warranted in order to optimize the use of these robots in real world scenarios.

Robotic cleaners may be beneficial following a wide-area attack, as large areas of numerous building interiors could be sampled rapidly and with reduced personnel. In addition to wide area sampling, these cleaning robots can be deployed to the areas where human sampling is difficult such as inside HVAC units and highly contaminated areas (hot zone).

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Rogers, J. V.; Sabourin, C. L.; Choi, Y. W.; Richter, W. R.; Rudnicki, D. C.; Riggs, K. B.; Taylor, M. L.; Chang, J. Decontamination assessment of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surfaces using a hydrogen peroxide gas generator. *J. Appl. Microbiol.* **2005**, *99* (4), 739−48.

(2) Beecher, D. J. Forensic application of microbiological culture analysis to identify mail intentionally contaminated with *Bacillus anthracis* spores. *Appl. Environ. Microbiol.* **2006**, *72* (8), 5304−10.

(3) Weis, C. P.; Intrepido, A. J.; Miller, A. K.; Cowin, P. G.; Durno, M. A.; Gebhardt, J. S.; Bull, R. Secondary aerosolization of viable *Bacillus anthracis* spores in a contaminated US Senate office. *JAMA, J. Am. Med. Assoc.* **2002**, *288* (22), 2853−8.

(4) Teshale, E. H.; Painter, J.; Burr, G. A.; Mead, P.; Wright, S. V.; Cseh, L. F.; Zabrocki, R.; Collins, R.; Kelley, K. A.; Hadler, J. L.; Swerdlow, D. L. Environmental sampling for spores of *Bacillus anthracis*. *Emerg. Infect. Dis* **2002**, *8* (10), 1083−1087.

(5) Raber, E.; Jin, A.; Noonan, K.; McGuire, R.; Kirvel, R. D. Decontamination issues for chemical and biological warfare agents: How clean is clean enough? *Int. J. Environ. Health Res.* **2001**, *11* (2), 128−148.

(6) Raber, E.; Hibbard, W. J.; Greenwalt, R. The national framework and consequence management guidance following a biological attack. *Biosecurity and bioterrorism: biodefense strategy, practice, and science* **2011**, *9* (3), 271−9.

(7) Raber, E. The challenge of determining the need for remediation following a wide-area biological release. *Biosecur. Bioterror.* **2011**, *9* (3), 257−61.

(8) Schmitt, K.; Zacchia, N. A. Total decontamination cost of the anthrax letter attacks. *Biosecur. Bioterror.* **2012**, *10* (1), 98−107.

(9) Price, P. N.; Sohn, M. D.; Lacommare, K. S.; McWilliams, J. A. Framework for evaluating anthrax risk in buildings. *Environ. Sci. Technol.* **2009**, *43* (6), 1783−7.

(10) Bresnitz, E. A. Indoor anthrax Decontamination: How Clean is Clean? *J. Public Health Manage. Pract.* **2010**, *16* (3), 185−8.

(11) GAO. *Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results*, GAO-05-251; U.S. Government Accountability Office: Washington, DC, March, 2005.

(12) Brown, G. S.; Betty, R. G.; Brockmann, J. E.; Lucero, D. A.; Souza, C. A.; Walsh, K. S.; Boucher, R. M.; Tezak, M.; Wilson, M. C.; Rudolph, T. Evaluation of a wipe surface sample method for collection of *Bacillus* spores from nonporous surfaces. *Appl. Environ. Microbiol.* **2007**, *73* (3), 706−710.
(13) Brown, G. S.; Betty, R. G.; Brockmann, J. E.; Lucero, D. A.; Souza, C. A.; Walsh, K. S.; Boucher, R. M.; tezak, m. s.; wilson, m. c. evaluation of vacuum filter sock surface sample collection method for *Bacillus* spores from porous and non-porous surfaces. *J. Environ. Monit.* **2007**, *9* (7), 666−671.
(14) Brown, G. S.; Betty, R. G.; Brockmann, J. E.; Lucero, D. A.; Souza, C. A.; Walsh, K. S.; Boucher, R. M.; Tezak, M. S.; Wilson, M. C.; Rudolph, T.; Lindquist, H. D. A.; Martinez, K. F. Evaluation of rayon swab surface sample collection method for *Bacillus* spores from nonporous surfaces. *J. Appl. Microbiol.* **2007**, *103* (4), 1074−1080.
(15) Buttner, M. P.; Cruz, P.; Stetzenbach, L. D.; Klima-Comba, A. K.; Stevens, V. L.; Emanuel, P. A. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. *Appl. Environ. Microbiol.* **2004**, *70* (12), 7040−7045.
(16) Edmonds, J. M.; Collett, P. J.; Valdes, E. R.; Skowronski, E. W.; Pellar, G. J.; Emanuel, P. A. Surface sampling of spores in dry-deposition aerosols. *Appl. Environ. Microbiol.* **2009**, *75* (1), 39−44.
(17) Krauter, P. A.; Piepel, G. F.; Boucher, R.; Tezak, M.; Amidan, B. G.; Einfeld, W. False-negative rate and recovery efficiency performance of a validated sponge wipe sampling method. *Appl. Environ. Microbiol.* **2012**, *78* (3), 846−854.
(18) Rose, L. J.; Hodges, L.; O'Connell, H.; Noble-Wang, J. National validation study of a cellulose sponge wipe-processing method for use after sampling *Bacillus anthracis* spores from surfaces. *Appl. Environ. Microbiol.* **2011**, *77* (23), 8355−8359.
(19) Estill, C. F.; Baron, P. A.; Beard, J. K.; Hein, M. J.; Larsen, L. D.; Rose, L.; Schaefer, F. W.; Noble-Wang, J.; Hodges, L.; Lindquist, H. D. A.; Deye, G. J.; Arduino, M. J. Recovery efficiency and limit of detection of aerosolized *Bacillus anthracis* sterne from environmental surface samples. *Appl. Environ. Microbiol.* **2009**, *75* (13), 4297−4306.
(20) Hodges, L. R.; Rose, L. J.; O'Connell, H.; Arduino, M. J. National validation study of a swab protocol for the recovery of *Bacillus anthracis* spores from surfaces. *J. Microbiol. Methods* **2010**, *81* (2), 141−146.
(21) Edmonds, J. M. Efficient methods for large-area surface sampling of sites contaminated with pathogenic microorganisms and other hazardous agents: Current state, needs, and perspectives. *Appl. Microbiol. Biotechnol.* **2009**, *84* (5), 811−816.
(22) Franco, C.; Bouri, N. Environmental decontamination following a large-scale bioterrorism attack: Federal progress and remaining gaps. *Biosecur. Bioterror.* **2010**, *8* (2), 107−17.
(23) Valiante, D. J.; Schill, D. P.; Bresnitz, E. A.; Burr, G. A.; Mead, K. R. Responding to a bioterrorist attack: Environmental investigation of anthrax in New Jersey. *Appl. Occup. Environ. Hyg.* **2003**, *18* (10), 780−5.
(24) Letant, S. E.; Murphy, G. A.; Alfaro, T. M.; Avila, J. R.; Kane, S. R.; Raber, E.; Bunt, T. M.; Shah, S. R. Rapid-viability PCR method for detection of live, virulent *Bacillus anthracis* in environmental samples. *Appl. Environ. Microbiol.* **2011**, *77* (18), 6570−8.
(25) CDC Anthrax Environmental Sampling, http://www.cdc.gov/niosh/topics/anthrax/sampling.html. (accessed November 2, 2012).
(26) *Federal On-scene Coordinator's Report for the Capitol Hill Site*, GS-10F-0076K; U.S. Environmental Protection Agency: Washington, DC, 2002.
(27) Prassler, E.; Ritter, A.; Schaeffer, C.; Fiorini, P.; Short, A. History of cleaning robots. *Auton. Robot.* **2000**, *9* (3), 211−226.
(28) Ulrich, I.; Mondada, F.; Nicoud, J. D. Autonomous vacuum cleaner. *Robot. Auton. Syst.* **1997**, *19* (3−4), 233−245.
(29) Calfee, M. W.; Ryan, S. P.; Wood, J. P.; Mickelsen, L.; Kempter, C.; Miller, L.; Colby, M.; Touati, A.; Clayton, M.; Griffin-Gatchalian, N.; McDonald, S.; Delafield, R. Laboratory evaluation of large-scale decontamination approaches. *J. Appl. Microbiol.* **2012**, *112* (5), 874−82.
(30) Calfee, M. W.; Lee, S. D.; Ryan, S. P., A rapid and repeatable method to deposit bioaerosols on material surfaces. *J. Microbiol. Methods* **2013**, http://dx.doi.org/10.1016/j.mimet.2013.01.015.
(31) Lee, S. D.; Ryan, S. P.; Snyder, E. G. Development of an aerosol surface inoculation method for *Bacillus* spores. *Appl. Environ. Microbiol.* **2011**, *77* (5), 1638−1645.
(32) Probst, A.; Facius, R.; Wirth, R.; Wolf, M.; Moissl-Eichinger, C. Recovery of *Bacillus* spore contaminants from rough surfaces: A challenge to space mission cleanliness control. *Appl. Environ. Microbiol.* **2011**, *77* (5), 1628−37.
(33) *Assessment of Liquid and Physical Decontamination Methods for Environmental Surfaces Contaminated with Bacterial Spores: Development and Evaluation of the Decontamination Procedural Steps*, EPA/600/R-12/025; U.S. Environmental Protection Agency: Washington, DC, 2012.
(34) *Effectiveness of Physical and Chemical Cleaning and Disinfection Methods for Removing, Reducing or Inactivating Agricultural Biological Threat Agents*, EPA/600/R-11/092, 2011; U.S. Environmental Protection Agency: Washington, DC, 2011.
(35) Wood, J. P.; Calfee, M. W.; Clayton, M.; Griffin-Gatchalian, N.; Touati, A.; Egler, K. Evaluation of peracetic acid fog for the inactivation of *Bacillus anthracis* spore surrogates in a large decontamination chamber. *J. Hazard. Mater.* **2013**, *250−251*, 61−67.
(36) Da Silva, S. M.; Filliben, J. J.; Morrow, J. B. Parameters affecting spore recovery from wipes used in biological surface sampling. *Appl. Environ. Microbiol.* **2011**, *77* (7), 2374−2380.