APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2006, p. 4429–4430 Vol. 72, No. 6
0099-2240/06/$08.00+0 doi:10.1128/AEM.02923-05

Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface

L. R. Hodges,* L. J. Rose, A. Peterson, J. Noble-Wang, and M. J. Arduino

Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Healthcare Quality Promotion, Atlanta, Georgia

Received 12 December 2005/Accepted 21 March 2006

A protocol to recover *Bacillus anthracis* spores from a steel surface using macrofoam swabs was evaluated for its accuracy, precision, reproducibility, and limit of detection. Macrofoam swabs recovered 31.7 to 49.1% of spores from 10-cm^2 steel surfaces with a ≤32.7% coefficient of variation in sampling precision and reproducibility for inocula of ≥38 spores.

Thousands of swab samples of *Bacillus anthracis* spores were taken during investigations of the 2001 bioterrorist events (3, 7, 10, 11, 12, 13), but no validated protocol was available at the time (8). The swab protocol used during the incident was modified from swab methods used by the food industry (1), National and Aeronautics and Space Administration (5), and hospital epidemiologists (4), but these methods did not involve sampling for spores. A recent study found that macrofoam swabs recover ≥30% more spores than rayon and polyester swabs, premoistening the swab increases recovery by ≥30%, and vortexing the swab recovers >25% more spores than extraction by sonication (9).

We evaluated a protocol that recovers spores from steel surfaces with premoistened macrofoam swabs and vortex processing. Steel coupons with 10-cm^2 surface area, as recommended for environmental and medical-device sampling (6), were chosen for their smooth surfaces. Ethanol was used as the inoculation medium in order to spread the spores evenly over the coupon. These conditions may increase the difficulty of removing spores with a swab compared to the removal of weapons grade powder spores. Five analysts from two laboratories performed the protocol, and the data were evaluated to determine accuracy, sampling precision, reproducibility, and limit of detection (LOD).

B. anthracis Sterne 34F2 spores (Colorado Serum, Denver, CO), previously stored in 50% ethanol to prevent germination, were added to 95% ethanol to attain ~10^6 spores ml^{-1}. This suspension was serially diluted 1:10 in 95% ethanol to attain inocula (I) ranging from 4.0×10^0 to 6.0×10^4 spores ml^{-1} (Table 1).

For each experiment, 10 to 15 sterile 10-cm^2 steel coupons were placed in glass petri dishes and inoculated with 0.5 ml of the appropriate inoculum of spores, as described previously (9). Petri dish lids remained partially open as the coupons dried in a biological safety cabinet for 2 h. A macrofoam swab (Critical Swab; VWR, Suwanee, GA; catalog no. 10812-046) was dipped in phosphate-buffered saline plus 0.04% Tween-80 (PBST; pH 7.2), and excess fluid was expressed from the head. The swab was swept in horizontal, vertical, and diagonal sweeps to cover the coupon during each orientation. The swab was vortexed in 5 ml PBST for 2 min at 10-s intervals (V1), placed into a second tube of PBST, and vortexed again (V2).

Ten sampled and 10 unsampled coupons were processed to determine the number of spores left after swabbing (L) and recovered from inoculated controls (C), respectively. Control coupon tests were performed prior to swab experiments to ensure that spores remained on the coupons during drying. The coupons were sonicated in 20 ml PBST in 600-ml glass beakers for 12 min in a 42-kHz (100-W) ultrasonic bath (Branson Instruments, Danbury, CT) and scraped with a cell scraper (Fisher Scientific) for 1 min.

The number of spores for I, V1, V2, L, and C were determined by culturing suspensions on trypticase soy agar containing 5% sheep blood (SBA; Becton Dickinson, Franklin Lakes, NJ). Suspensions from 10^4 inoculum experiments were serially diluted 1:10 in Butterfield Buffer (Becton Dickinson, Franklin Lakes, NJ), and 100-μl aliquots of each dilution were plated on SBA. Five plates per dilution were counted for I ($n = 15$), and three plates per dilution were counted for V1, V2, L, and C ($n = 9$). Spores from 10^2 and ≤10^1 inocula were enumerated by filtering suspensions of 2.5 ml ($n = 2$) and 5 ml ($n = 1$), respectively, through a 47-mm mixed-cellulose-ester membrane (0.45-μm pore-size) microfunnel (Gelman Sciences, East Hills, NY) with low vacuum pressure (<10 mm Hg). The filter funnels were rinsed with 20 ml PBST, and the membranes were placed on SBA. All plates were incubated at 35°C overnight, and the CFU were counted. The mean inoculum (CFU ml^{-1}) was multiplied by the volume inoculated (0.5 ml) to determine I. For 10^4 inocula, the mean CFU ml^{-1} was calculated and multiplied by 5 and 20 ml to calculate swab V1 and V2 and coupon L and C values, respectively. The values V1 and V2 were added to calculate the total number of spores recovered (R). The value R was determined using CFU from one or two membranes when I was ≤10^1 and when I was equal to 10^2 spores, respectively.

Accuracy, defined as the percent recovery (%R) of total spores recovered (R) from inocula (I), for all analysts at an I of ≥38 spores, ranged from 38.0 to 49.1% ($n = 105$) (Table 1). A linear relationship was demonstrated between R and I for all inocula ($r = 0.999$). Our recovery results were similar to those

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Rd., Mail Stop C16, Atlanta, GA 30333. Phone: (404) 639-4865. Fax: (404) 639-3822. E-mail: lwh9@cdc.gov.

TABLE 1. Recovery of *B. anthracis* spores from steel coupons using macrofoam swabs

No. of spores inoculated (*I*) (SD[a])	No. of spores recovered (*R*) (SD)	*%R* (SD)	Precision CV[b] (%)	Reproducibility CV[c] (%)	No. of coupons sampled
4.0×10^0 (2.0×10^0)	1.0×10^0 (1.0×10^0)	31.7 (25.8)	81.5	ND	15
6.0×10^0 (3.0×10^0)	2.0×10^0 (2.0×10^0)	37.8 (34.2)	90.5	ND	15
1.2×10^1 (4.0×10^0)	4.0×10^0 (3.0×10^0)	37.2 (21.6)	57.9	ND	15
3.8×10^1 (7.0×10^0)	1.5×10^1 (4.0×10^0)	40.1 (12.8)	23.2	32.0	15
5.9×10^2 (9.9×10^1)	2.2×10^2 (6.5×10^1)	38.0 (10.7)	20.6	28.2	45
6.0×10^4 (4.8×10^3)	2.9×10^4 (8.7×10^3)	49.1 (16.0)	27.3	32.7	45

[a] Standard deviation between five analysts ($I \geq 38$ spores) or from one set of experiments by one analyst ($I \leq 12$).
[b] CV of *%R* between replicates for each experimental set averaged at each inoculum level.
[c] CV of *%R* of all sampled coupons by five analysts at each inoculum level. ND, not done.

of other studies, which recovered 43.7% (9) and 42.6 to 47.3% (2) of spores from a smooth surface with a foam swab. In contrast to our study, Buttner et al. (2) premoistened the sampling surface, not the swab. Premoistening the swab or surface may therefore be essential in swab surface sampling.

We accounted for approximately 60% of *I*: approximately 43.0 percent recovery (*% R*) and 6 to 10% each left on the swab and coupon. Mean recoveries for V2 and *L* were 6.2% (±2.9%; $n = 85$) and 6.1% (±4.3%; $n = 10$) of *I*, respectively. Similarly, another study found that one vortex extraction recovered 93.4% of spores directly inoculated onto the foam swab (9), suggesting that ~7% of spores may be lost during vortex processing. Spore recovery for control coupons was 101.2% (± 20.5% standard deviation; $n = 10$) of *I*, demonstrating that neither loss of spores nor loss of viability occurs during coupon inoculation and drying. Less than 20% of the spores were therefore left on the swab and coupon during the procedure. The remaining 40% of the spores may be lost inherently during processing or trapped inside the swab or in coupon grooves.

The sampling precision between replicates of the same inoculum was measured by determining the coefficient of variation (CV) for each experimental set for each analyst. The reproducibility of the protocol was measured by determining the CV for each test inoculum, including data from all analysts. The sampling precision and reproducibility CV values were ≤32.7% for an *I* of ≥38 spores (Table 1).

Fifteen coupons were sampled at three low inocula to determine the LOD. Fifteen 0.5-ml aliquots of the inoculum were plated directly onto SBA to determine the *I* of these low spore numbers. Spores were recovered from 11 and 13 of 15 coupons inoculated with 4 and 6 CFU 10 cm^{-2}, respectively, which is comparable to the LOD for a foam sponge kit at 42 to 100 CFU m^{-2} (2). The lowest inoculum at which *R* was ≥1 for all replicate coupons, however, was 12 CFU 10 cm^{-2}. If we define the LOD to be the lowest concentration of inoculum at which at least one spore ($R \geq 1$) is cultured from ≥90% of all experimental replicates, then the LOD for this protocol is 12 CFU cm^{-2} (Table 1). The *%R*, however, was decreased at these lower inocula, and precision and reproducibility CV values were ≥57.9% (Table 1).

The premoistened macrofoam swab protocol for recovery of *B. anthracis* spores will be used as a model for an interagency surface-sampling study. The results from this study will be used to set acceptable values for validation criteria. Low CV for recovery values when *I* is ≥38 CFU cm^{-2} suggest that this swab protocol is precise and reproducible for surface sampling at these expected spore numbers. Further evaluation of the protocol, however, is needed to optimize the recovery of lower numbers of spores, which may be expected during evaluation of surface samples after decontamination from a bioterrorism event. The protocol will also be tested to determine its efficiency in the presence of competing microorganisms and environmental inhibitors and on various surface compositions.

Funding for L. Hodges was provided by a joint fellowship from Oak Ridge Institute for Science and Education and the Centers for Disease Control and Prevention.

We thank our study participants, G. Fosheim and A. Thompson from the Centers for Disease Control and Prevention.

REFERENCES

1. **Baldock, J. D.** 1974. Microbiologic monitoring of the food plant: methods to assess bacterial contamination on surfaces. J. Milk Food Technol. **37:**361–368.
2. **Buttner, M. P., P. Cruz, L. D. Stetzenbach, A. K. Klima-Comba, V. L. Stevens, and P. A. Emanuel.** 2004. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. Appl. Environ. Microbiol. **70:**7040–7045.
3. **Dewan, P. K., A. M. Fry, K. Laserson, B. C. Tierney, C. P. Quinn, J. A. Hayslett, L. N. Broyles, A. Shane, K. L. Winthrop, I. Walks, L. Siegel, T. Hales, V. A. Semenova, S. Romero-Steiner, C. Elie, R. Khabbaz, A. S. Khan, R. A. Hajjeh, A. Schuchat, et al.** 2002. Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg. Infect. Dis. **8:**1066–1072.
4. **Favero, M. S.** 1968. Microbiological sampling of surfaces. J. Appl. Bacteriol. **31:**336–343.
5. **Favero, M. S.** 1971. Microbiologic assay of space hardware. Environ. Biol. Med. **1:**27–36.
6. **Gilchrist, M. J. R.** 1992. Microbiological culturing of environmental and medical-device surfaces, p. 11.10.4. *In* H. Eisenberg (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Higgins, J. A., M. Cooper, L. Schroeder-Tucker, S. Black, D. Miller, J. S. Karns, E. Manthey, R. Breeze, and M. L. Perdue.** 2003. A field investigation of *Bacillus anthracis* contamination of U.S. Department of Agriculture and other Washington D.C. buildings during the anthrax attack of October 2001. Appl. Environ. Microbiol. **69:**593–599.
8. **Rhodes, K. A.** 2005. Anthrax detection: agencies need to validate sampling activities in order to increase confidence in negative results. U.S. Government Accountability Office. GAO-05-493T. [Online.] http://www.gao.gov/cgi-bin/getrpt?GAO-05-493T.
9. **Rose, L., B. Jensen, A. Peterson, S. N. Banerjee, and M. J. Arduino.** 2004. Swab materials and *Bacillus anthracis* spore recovery from nonporous surfaces. Emerg. Infect. Dis. **10:**1023–1029.
10. **Sanderson, W. T., M. J. Hein, L. Taylor, B. D. Curwin, G. M. Kinnes, T. A. Seitz, T. Popovic, H. T. Holmes, M. E. Kellum, S. K. McAllister, D. N. Whaley, E. A. Tupin, T. Walker, J. A. Freed, D. S. Small, B. Klusaritz, and J. H. Bridges.** 2002. Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg. Infect. Dis. **8:**1145–1151.
11. **Sanderson, W. T., R. R. Stoddard, A. S. Echt, C. A. Piacitelli, D. Kim, J. Horan, M. M. Davies, R. E. McCleery, P. Muller, T. M. Schnorr, E. M. Ward, and T. R. Hales.** 2004. *Bacillus anthracis* contamination and inhalational anthrax in a mail processing and distribution center. J. Appl. Microbiol. **96:**1048–1056.
12. **Teshale, E. H., J. Painter, G. A. Burr, P. Mead, S. V. Wright, L. F. Cseh, R. Zabrocki, R. Collins, K. A. Kelley, J. L. Hadler, D. L. Swerdlow, and the Connecticut Anthrax Response Team.** 2002. Environmental sampling for spores of *Bacillus anthracis*. Emerg. Infect. Dis. **8:**1083–1087.
13. **Valiante, D. J., D. P. Schill, E. A. Bresnitz, G. A. Burr, and K. R. Mead.** 2003. Responding to a bioterrorist attack: environmental investigation of anthrax in New Jersey. Appl. Occup. Environ. Hyg. **18:**780–785.