Applied and Environmental Microbiology, Dec. 2011, p. 8355–8359 Vol. 77, No. 23
0099-2240/11/$12.00 doi:10.1128/AEM.05377-11

National Validation Study of a Cellulose Sponge Wipe-Processing Method for Use after Sampling *Bacillus anthracis* Spores from Surfaces[▽]

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Received 5 May 2011/Accepted 24 September 2011

This work was initiated to address the gaps identified by Congress regarding validated biothreat environmental sampling and processing methods. Nine Laboratory Response Network-affiliated laboratories participated in a validation study of a cellulose sponge wipe-processing protocol for the recovery, detection, and quantification of viable *Bacillus anthracis* Sterne spores from steel surfaces. Steel coupons (645.16 cm^2) were inoculated with 1 to 4 log_{10} spores and then sampled with cellulose sponges (Sponge-Stick; 3M, St. Paul, MN). Surrogate dust and background organisms were added to the sponges to mimic environmental conditions. Labs processed the sponges according to the provided protocol. Sensitivity, specificity, and mean percent recovery (%R), between-lab variability, within-lab variability, and total percent coefficient of variation were calculated. The mean %R (standard error) of spores from the surface was 32.4 (4.4), 24.4 (2.8), and 30.1 (2.3) for the 1-, 2-, and 4-log_{10} inoculum levels, respectively. Sensitivities for colony counts were 84.1%, 100%, and 100% for the 1-, 2-, and 4-log_{10} inocula, respectively. These data help to characterize the variability of the processing method and thereby enhance confidence in the interpretation of the results of environmental sampling conducted during a *B. anthracis* contamination investigation.

Environmental sampling was a critical component of the anthrax mail contamination investigation (9). Swab, wipe, and HEPA sock samplings were conducted without (i) adequate knowledge of the performance characteristics of each device used to recover *Bacillus anthracis* spores or (ii) consistent methods to extract the spores from the device used for the culture or detection of viable spores. The absence of standard, validated device-processing methods raised concerns about the results of the tests. In 2005, the U.S. Government Accounting Office (GAO) expressed concern that validated sampling and detection methods for biothreat agents were still not available (9). In the document, validation of a method was defined as "a formal, empirical process in which an authority determines and certifies the performance characteristics of a given method" (9).

This laboratory previously validated a method for the processing of a macrofoam swab after sampling of *B. anthracis* from a small surface area of 5.08 by 5.08 cm (25.8 cm^2). Preliminary investigations were conducted to select the optimum swab type and the optimum processing method (10, 11, 16). Others have evaluated the efficiency of various swab types for conducting surface sampling and processing methods for the swabs (1, 4, 8, 15, 19). However, using a swab to sample an area larger than 25.8 cm^2 may result in reduced recovery efficiency, so an alternative device for sampling of larger surface areas is needed. Though other researchers have evaluated the efficiency of gauze wipes (2, 7, 19), a foam spatula (14), or the Biological Sampling Kit (BiSKit; Quicksilver Analytics, Inc., Abingdon, MD) (3) for sampling of *B. anthracis* or surrogates for *B. anthracis* from larger surface areas, none of these methods were evaluated to the extent required to provide a validated method. No acceptance criteria for environmental sampling currently exist; hence, one goal of this study was to investigate the variability of the method and establish acceptable limits for environmental sampling and processing protocols. These data will help public health officials interpret the results of environmental sampling after an anthrax investigation. The device evaluated here, the Sponge-Stick (3M, St. Paul, MN), has been used for numerous epidemiological investigations in health care settings and has been found to be effective and easy to use. This work was conducted to address the GAO's concern by providing a well-characterized protocol for the processing of an easy-to-use, commercially available sponge wipe for the sampling of a nonporous surface for *B. anthracis* spores.

MATERIALS AND METHODS

Planning and oversight. Since the protocol would be posted on the Centers for Disease Control and Prevention (CDC) Laboratory Response Network (LRN) website, the plan for this study was proposed to CDC's LRN review committee. The LRN committee consisted of 10 experts in the fields of microbiology (*B. anthracis*, in particular), molecular biology, health science, regulatory affairs, industrial hygiene, risk analysis, and LRN administration. The committee provided oversight of the process and reviewed the data.

Spore preparation and reference check. *B. anthracis* Sterne spores were prepared as described previously (16) and suspended in 95% ethanol. Ethanol was chosen because of its ability to evaporate quickly, allowing a more uniform distribution of spores. The spore suspension was adjusted to one of the three target inoculum levels (1, 2, and 4 log_{10} CFU/ml), as confirmed by serial dilutions and culture on Trypticase soy agar plus 5% sheep blood (TSAB; BD). The suspension was vortexed and sonicated well and plated three times in the 3 days

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▽ Published ahead of print on 30 September 2011.

TABLE 1. Characterization of ATD[a]

Culture medium	No. of CFU/10 mg[b]	Organisms (no.) identified[c]
R2A	$4.4 \times 10^4 \pm 2.1 \times 10^3$	Yeast (1), *Micrococcus luteus* (1)
TSAB	$5.1 \times 10^4 \pm 9.6 \times 10^3$	*B. licheniformis* (4), *B. cereus* (5), *Micrococcus roseus* (2)
Sabouraud dextrose agar	$7.6 \times 10^3 \pm 1.3 \times 10^3$	*B. cereus* (4), *B. licheniformis* (3), *B. mycoides* (2), *B. endophyticus* (1), actinomycetes (9), yeast (2), *Streptomyces* sp. (1), molds (4)

[a] ATD (Powder Technology Inc., Burnsville, MN) was suspended in PBS plus 0.02% Tween 80 to a final concentration of 10 mg/ml and cultured on the media indicated to establish the composition of the existing flora.
[b] Triplicate dilutions, triplicate plating, $n = 9$.
[c] Thirty-nine morphologically distinct colony types were found during dilution plating on TSAB, R2A, and Sabouraud dextrose agar. Standard morphological testing were used to determine identity.

preceding the test to check the inoculum's number of CFU/ml. The number of CFU/ml on each plate ($n = 15$ to 30) was determined, and the mean of these suspension checks was used as the reference to determine the percent recovery (%R) at each inoculum level.

Surface inoculation, sampling, and shipping. Three milliliters of the inoculum was spread on steel coupons (10 by 10 in. [645 cm^2]) using sterile cell spreaders. The coupons were allowed to dry for 2 h in a biosafety cabinet with the airflow turned off and the front sash closed. After 2 h of drying, the coupons were sampled with premoistened (neutralizing buffer) cellulose sampling sponges (Sponge-Stick, catalog no. SSL10NB; 3M, St. Paul, MN). Sampling was performed by two individuals to keep the variability of the sampling technique to a minimum. Sampling consisted of using the sponge to wipe across the coupons in a vertical motion, turning the sponge over, wiping in a horizontal motion, and then using the sides of the sponge to swipe in a diagonal motion across the coupons. The tip of the sponge (held perpendicular to the surface) was then used to wipe around the edges of the coupon to pick up spores that may have been pushed to the edges. To mimic background dust and organisms, after sampling, each sponge was also inoculated with 200 μl of a slurry of Arizona Test Dust (ATD; Powder Technology, Inc., Burnsville, MN) at 10 mg/ml, *Bacillus atrophaeus* spores (10^4/ml), and *Staphylococcus epidermidis* (10^4/ml). The bioburden of the ATD alone was characterized and is presented in Table 1. The sponge heads were then placed into sealable stomacher bags (Seward, Bohemia, NY), and the handles were aseptically removed from the sponge heads while they were in the bags. Positive-control sponges were directly inoculated with 1 ml of the same suspension used to inoculate the steel coupons and 200 μl of the ATD slurry. One milliliter (instead of 3 ml) was placed on the sponges to avoid oversaturation of the sponges and to ensure CFU counts on the plates similar to those that sampled sponges would yield. Each shipment contained 11 sponges, i.e., 7 test sponges with background dust and organisms, 2 positive controls, 1 dirty blank (background dust and organisms only, no *B. anthracis*), and 1 sterile blank. The 11 sponges were placed into secondary shipping bags, placed into an insulated box, and then shipped with a cold pack to the participating laboratories. Each lab received 3 shipments of sponges, one inoculum level per shipment, though the participating laboratories were blind as to which inoculum level was sent in a given shipment. The participating laboratories consisted of seven state public health laboratories, one national laboratory, and one CDC laboratory. The laboratories followed the protocol provided and processed the sponges within 48 h of sampling.

Sponge processing. All handling of sponges was conducted under biosafety level 3 conditions. Ninety milliliters of sterile phosphate-buffered saline with 0.02% Tween 80 (PBST) was added to the stomacher bags containing the sponge heads (all plastic remnants of the handles removed), and the bags were sealed and processed in a Stomacher 400 Circulator (Seward, Bohemia, NY) for 1 min at 260 rpm. The excess liquid was aseptically expressed from the sponge while inside the Stomacher bag, and the sponge was then removed from the bag and placed in a 4-oz. specimen cup containing 50 ml of Trypticase soy broth (TSB; BD, Franklin Lakes, NJ) in order to enhance the growth (enrichment) of any spores that may have remained on the sponge during processing.

The eluent remaining in the Stomacher bag was transferred to two sterile 50-ml centrifuge tubes and centrifuged at 3,500 × *g* for 15 min. All but 3 ml of the supernatant was discarded from each of the two tubes, the pellets were resuspended, and the contents of the two centrifuge tubes were combined. The exact volume of the combined eluent was measured and recorded. One milliliter of the eluent was diluted 10-fold in series twice, and three 100-μl aliquots of the eluent and each dilution were spread plated onto three TSAB plates. To capture low numbers of spores, two 1-ml aliquots of spore elution suspensions were vacuum filtered through two separate 0.45-μm Microfunnel filter funnels (Pall Corp., Ann Arbor, MI) to capture spores on membranes. Membranes were placed on TSAB plates. The spread plates, filter plates, and sponge wipes in TSB were incubated at 36°C overnight, and the suspected *B. anthracis* colonies were counted the next day. This is referred to as the plate count method.

The TSB in the specimen cups was checked for turbidity (growth), and 10 μl was streaked onto TSAB for isolation. Streak plates were incubated overnight at 36°C and observed for isolation of *B. anthracis* colonies. This is referred to as the TSB enrichment method.

Quantitative PCR. Representative colonies from these cultured samples, whether isolated from the TSB enrichment, from spread plates, or from filters, were picked and analyzed using real-time PCR (at least 2 CFU per sample) to confirm *B. anthracis* identification. In addition, an aliquot taken directly from the TSB was prepared for quantitative PCR analysis. DNA was extracted from chosen suspected *B. anthracis* colonies and TSB aliquots by lysis at 95°C for 15 min, cooling on ice for 5 min, and then centrifugation of the aliquot at 12,000 × *g* for 1 min in a 0.1-μm-pore-size filter tube (Millipore, Billerica, MA) to remove any remaining spores or cells. LRN primers and probes (12) and the LightCycler-FastStart DNA Master Hybridization Probes (Roche Molecular Biochemicals, Indianapolis, IN) were used to confirm *B. anthracis*. Each participating laboratory used an LRN-supported real-time PCR platform to perform the analysis.

Data analysis. Spread plates and filter plates were inspected, and suspected *B. anthracis* colonies were counted and recorded. If colony counts were between 25 and 250 on the spread plates, then the spread plates were used to calculate recovery as follows: CFU recovered = (mean CFU on spread plates/dilution factor) × 6 ml (total volume). If colony counts were below 25 on the spread plates, then the colony counts on the filter plates were used to calculate the recovery as follows: CFU recovered = [mean CFU on filter plates/filtered volume (1.0 ml)] × 6 ml (total volume). If *B. anthracis* colonies were seen in the streaks from the TSB enrichment, then no quantification was attempted, since this could represent one or many spores reproducing over the incubation period. This test indicated only that the number of spores remaining on the sponge was ≥1.

Statistical methods. Fifteen to 30 reference plates were available as referents for each target inoculum level. Reference levels were summarized using the sample mean and coefficient of variation (CV). For each sponge wipe, the percentage of CFU recovered (%R) was calculated as follows:

$$\%R_{ij} = 100\% \frac{R_{ij}}{I_i} = 100\% \frac{R_{ij}}{\frac{1}{n_i}\sum_{k=1}^{n_i} I_{ik}}$$

where R_{ij} is the recovered level (CFU count) for inoculum i and sample j, n_i is the number of reference plates for inoculum i, and I_{ik} is the reference level (CFU count) for inoculum i and reference plate k. To account for the correlated nature of the data, a random-effect regression model was used to estimate the mean %R in addition to the between-lab, within-lab, and total variances (13). Variances were expressed as %CV by dividing the estimated between-lab and within-lab variances and total standard deviation by the mean %R. Separate regression models were fitted using the MIXED procedure in SAS (version 9.2; SAS Institute Inc., Cary, NC) for each method (sampled, direct inoculation) and target inoculum level (1, 2, or 4 $\log_{10}$).

Sensitivity (the percentage of true positive sponge wipe samples classified as positive) and specificity (the percentage of true negative sponge wipe samples classified as negative) were calculated based on both the plate count method and the TSB enrichment method at each target inoculum level (1, 2, or 4 $\log_{10}$ CFU).

TABLE 2. Spore recovery from a stainless steel surface[a] using a sponge wipe sampling and processing protocol

Type and target inoculum level (log_{10})	Reference CFU[b]			Recovered CFU		%R[c]				
	No. of plates	Mean	%CV	No. of samples	Mean	Mean	SE	CV_B[d]	CV_W[e]	CV_T[f]
Sampled wipes (sampled and processed)										
1	28	26.1	52	63	8.4	32.4	4.4	31.3	69.3	76.1
2	30	536	25	63	132.6	24.4	2.8	31.3	35.0	47.0
4	15	33,140	20	56[g]	9,984.0	30.1	2.3	19.9	19.5	27.9
Positive-control wipes (processed only)										
1	28	8.7	52	17[h]	4.1	46.1	9.6	47.7	54.0	72.0
2	30	179	25	18	112.7	66.5	7.3	29.5	20.3	35.8
4	15	11,047	20	16[g]	8,607.0	77.9	4.5	14.4	10.6	17.8

[a] Measuring 25.4 by 25.4 cm (645.16 cm^2).
[b] Reference CFU count estimated by replicate spread plate and culture of inoculating suspension. Three milliliters applied to stainless steel surface and 1 ml applied to positive-control wipes.
[c] Mean, SE, and between- and within-lab variances of %R were estimated using a random-effect regression model to account for the correlated nature of the data.
[d] $\%CV_B$, between-lab coefficient of variation defined as 100% times the square root of the estimated between-lab variance divided by the mean %R.
[e] $\%CV_W$, within-lab coefficient of variation defined as 100% times the square root of the estimated within-lab variance divided by the mean %R.
[f] $\%CV_T$, the total coefficient of variation, was estimated as 100% times the square root of the total variance (incorporating between- and within-lab variances) divided by the mean %R.
[g] The number is lower for the 10^4 inoculum because one laboratory's quantitative data were omitted due to procedural error.
[h] Results exclude a single sample that was deemed to be an outlier.

RESULTS

During the processing of the sampled sponge wipes with 4 log_{10} CFU, one laboratory failed to follow procedures properly, resulting in their data being omitted from the quantitative analysis (7 samples, 2 negative controls, 2 positive controls). In addition, one positive control from the 1-log_{10} CFU inoculum shipment was omitted from the quantitative analysis after being considered an outlier.

The percent recovery from the sampled sponge wipes at each inoculum level is found in Table 2. The %R (standard error [SE]) of the sampled sponge wipe in the presence of dust and other organisms was 32.4 (4.4), 24.4 (2.8), and 30.1 (2.3) for the 1-, 2-, and 4-log_{10} CFU inoculum levels, respectively. The mean %R varied as much as 8% between inoculum levels, and the SE was greater at the lowest inoculum level. When spores were directly inoculated onto the sponge wipes and processed, the %R was greater at all inoculum levels, indicating that 29.7%, 63.3%, and 61.4% [100% × (1 − %R sampled/%R control)] remained on the steel surface at the 1-, 2-, and 4-log_{10} CFU inoculum levels, respectively.

Using the CFU recovered formula shown above and the estimated recovery efficiency, we can determine the theoretical limits of detection of this sampling and processing protocol. If only 1 CFU is found on a filter plate, the limit of detection of the processing method is as follows: $R = (1/1) \times 6$ ml = 6 CFU per sponge wipe.

If we consider 30% recovery efficiency, the estimated number of spores on the surface would be as follows: 6 CFU per sponge wipe/0.30 = 20 spores on the total surface sampled.

If *B. anthracis* DNA is detected in the incubated TSB but no colonies are isolated, we can state that the organism is present but below the limit of detection of the protocol. It is also possible that *B. anthracis* bacteria are present in larger numbers but not viable.

The within-laboratory variability ($\%CV_W$) was lower with increasing inoculum sizes (Table 2). The $\%CV_W$ was also lower for the directly inoculated control sponge wipes than for sampled sponge wipes, indicating that removal of spores from a surface cannot be done consistently, even in a controlled laboratory environment.

As presented in Table 2, the variability between laboratories ($\%CV_B$) at each inoculum level ranged from 19.9% to 31.3%. At the lower inoculum level, the between-laboratory variability (pooled results from each lab) was less than the within-laboratory (between-sample) variability (i.e., CV_B was smaller than CV_W), suggesting that more of the variability may be inherent in the act of sampling than in the processing. The CV_T, which incorporates within- and between-lab variances together, was greater at the lower inoculum level (76.1) than at the higher inoculum level (27.9) (Table 2).

The sensitivity of the plate count method, the percentage of known positive samples identified correctly, was 84.1, 100, and 100% at the 1-, 2-, and 4-log_{10} CFU inoculum levels, respectively (Table 3). The reduced sensitivity at the 1-log_{10} CFU inoculum level indicates that the inoculum was close to the limit of detection of the method. The specificity of the protocol, the percentage of negative controls that were correctly identified as negative, was 100% at the 1- and 2-log_{10} CFU

TABLE 3. Sensitivity of plate count culture alone compared to that of TSB enrichment of sponge wipe after extraction

No. of spores inoculated (log_{10})	Sensitivity[a] (%)		Specificity[b] (%)	
	Plate count method	TSB enrichment[c]	Plate count method	TSB enrichment[c]
1	84.1	81.0	100	100
2	100	96.8	100	100
4	100	90.5	94.4[d]	100

[a] Percentage of true positive sampled sponge wipes as determined by culture and PCR confirmation of colonies (n = 63 for each inoculum level).
[b] Percentage of true negative (control) sponge wipes (n = 18 for each inoculum level).
[c] Based on positive PCR results directly from TSB or confirmed isolates from subculture of TSB.
[d] Cross contamination of one negative-control plate due to a malfunctioning biosafety cabinet was responsible for the specificity being less than 100%.

inoculum levels and 94.4% at the 4-$\log_{10}$ CFU inoculum level. The 94.4% specificity at the 4-$\log_{10}$ CFU inoculum level was due to one negative-control plate being cross-contaminated possibly because the work was performed in a faulty biosafety cabinet. If we eliminate this known error, the overall specificity of the method is 100%.

TSB enrichment of the extracted sponge wipe did not enhance the sensitivity of the method, compared to that of the plate count method, as evidenced by the lower sensitivity results (Table 3). The sensitivity of the TSB method includes positive identification of *B. anthracis* DNA from the incubated broth, as well as confirmed identity of isolated *B. anthracis* colonies obtained from streaking of the broth for isolation. Isolated colonies were difficult to obtain from the TSB due to competition from other *Bacillus* spp. Only once did the TSB enrichment provide isolated colonies when the plate count method did not. The detection of *B. anthracis* DNA in TSB without the isolation of colonies indicates the presence of *B. anthracis* spores below the detection limit of the method or the presence of nonviable spores.

DISCUSSION

The sponge wipe method has not previously been evaluated for recovery of *B. anthracis* spores from surfaces. Other researchers have evaluated the efficiency of gauze wipes to sample for *B. anthracis* spores on surfaces. Brown et al. (2) found that polyester-rayon blend gauze wipes recovered 35% (SD, 12%) of the *B. atrophaeus* spores that had been deposited on a 25-cm^2 steel surface by settling from aerosol dispersion. This gauze wipe study was conducted in one laboratory with 20 samples using a surrogate organism for *B. anthracis* at a spore concentration range of 100 to 1,000/645-cm^2 surface area. Estill et al. (7), using a rayon gauze wipe, recovered 18% of the aerosolized *B. anthracis* Sterne spores from 929-cm^2 steel surfaces when the inoculum level was 270 CFU/100 cm^2. The Estill study was conducted with *B. anthracis* spores, and processing was conducted in three independent laboratories, but the number of gauze wipes sent in each shipment was limited to three. Lewandowski et al. (14) used a foam spatula to recover aerosolized *B. atrophaeus* spores from surfaces and obtained a median efficiency of 9%. Though the authors recognize that in a *B. anthracis* contamination event, the spores would most likely be deposited on a surface by the settling of spores in air, in a laboratory setting, depositing spores on a surface in a uniform manner is difficult, especially at low concentrations (7). Edmonds et al. (6) compared the recovery of spores deposited as a liquid (in water) to the recovery of spores deposited as an aerosol and found that neither deposition resulted in consistently better or worse recovery of spores. We therefore chose the liquid deposition method for better inoculum size accuracy.

In preliminary work, limited in-house evaluations ($n = 10$) of several sampling devices and premoistening solutions were conducted. All sampling devices were inoculated and processed using the same method as the sponge wipe used in this study. The results of those in-house evaluations (Table 4) indicated that other products may yield similar results, since all of the devices recovered between 26% and 36% of the spores. In order to be confident in the data collected with these alternate sampling devices, more extensive evaluations need to be conducted to better characterize the performance of these devices before use in a contamination event. During the preliminary investigations, heat shock times and temperatures were also investigated. Even with low heat shock times and temperatures (65°C, 15 min), a significant loss of viability was observed (data not published). This reduction in the number of viable spores after heat shock treatment was also seen by Turnbull et al. (18). Considering that environmental sampling of dry, nonporous surfaces would most likely be cocontaminated with other *Bacillus* sp. spores or fungal spores that would not be reduced by a heat shock step; we eliminated heat treatment from the protocol.

TABLE 4. Evaluation of the sampling efficiency of alternate wipes and alternate premoistening fluids[a]

Wipe and moistening fluid	No. of wipes tested	%R (SD)
Rayon gauze,[b] PBST	10	30.8 (15.5)
Cellulose sponge,[c] Dey Engle broth	10	32.3 (9.7)
Cellulose sponge,[c] Butterfield buffer	10	26.8 (5.6)
Sponge-Stick,[d] PBST	10	36.3 (9.2)
Polyester foam sponge,[e] PBST	15	26.0 (10.0)

[a] All surfaces sampled were 645 cm^2 of stainless steel.
[b] Kendall 8042 Versalon sponges (2 by 2 in.; Wound Care Shop, Indianapolis, IN). Inoculum tested: 10^4 spores/coupon.
[c] SOLAR-CULT premoistened sponges (Solar Biologics, Ogdensburg, NY). Inoculum tested: 10^4 spores/coupon.
[d] Dry Sponge-Stick with PBST added in house (3M, St. Paul, MN). Inoculum tested: 10^4 spores/coupon.
[e] Dry sponge (SK711; Hardy Diagnostics, Santa Maria, CA) premoistened in house. Inocula tested: 10^1, 10^2, and 10^4 spores/coupon. Five wipes were evaluated at each inoculum level.

In order to investigate the effect of shipping conditions on environmental samples, an extensive in-house evaluation of the stability of inoculated macrofoam swabs stored at various temperatures was conducted. The results demonstrated that environmental samples maintained at 5 or 20°C would not suffer loss of viability or germinate and multiply if processed within 24 h of sampling (unpublished data). These data may be applicable to *B. anthracis* spores on cellulose sponge sticks as well but should be confirmed with additional testing.

This sponge wipe method is capable of sampling a larger surface area than a previously validated swab sampling and processing method described by Hodges et al. (11). There may be instances when a swab sample is appropriate, such as when sampling irregularly shaped or hard-to-reach areas. However, sampling of larger areas can often provide a better assessment of the contamination of a room or building (5, 17). The %R (SE) of the swab sampling (25.8 cm^2) and processing method is 55.0 (5.2), 27.9 (3.9), and 42.0 (4.6) for the 1-, 2-, and 4-$\log_{10}$ CFU inoculum levels, respectively, if dust and background organisms are present (10, 11). Though the %R from swab sampling may appear to be slightly better than that found for the sponge wipe, we cannot directly compare them since the surface areas sampled were different. Further work is needed to determine the effect of surface area on the sampling efficiency of a given sampling device.

This sponge wipe sampling study found that the sampling efficiency of the sponge wipe method varied with the inoculum level sampled and that the SE was greater at the lowest inoc-

ulum level. The between-lab, within-lab, and total variabilities, as measured by %CV also varied with the inoculum level, with the greatest variability seen at the lowest inoculum level. The sensitivity of the method was 100% when ≥ 2 $\log_{10}$ spores were present on the surface and 84% when 1 $\log_{10}$ spores were present. The specificity in the presence of a well-characterized dust containing a consortium of other organisms (10^3 CFU/10 mg) was 100% (if the one case of a malfunctioning biosafety cabinet is omitted).

Though the mean %R varied from 24.4% to 34.6% for each of the inoculum levels, it should be noted that these values represent recovery on the same order of magnitude. When sampling after the anthrax contamination of a room, building, or area, obtaining the same order of magnitude is still helpful in determining the source of the contamination or in characterizing the extent of the contamination.

Enriching the spores remaining on the sponge wipe by placing the sponge wipe into TSB and incubating it did not help lower the level of detection as we thought it might. The other *Bacillus* spp. present in the dust appeared to outcompete the low levels of *B. anthracis* remaining on the sponge, and *B. anthracis* colonies were isolated only a few times at the low inoculum level. The presence of the background consortia of organisms, the cellulose from the sponge wipe, and/or the ingredients of the TSB may have contributed to inhibition of the PCR reagents when attempting to detect *B. anthracis* DNA directly from TSB. Further work is needed to investigate whether an alternate DNA preparation method would enhance detection by blocking inhibitors.

Because this work was meant to focus on the variability of the processing method in multiple laboratories, only two individuals conducted the actual surface sampling in this study. In an actual environmental sampling event, additional variability may be introduced by multiple individuals conducting the sampling. In addition, the data do not address the potential for variability between runs (multiple shipments) at the same inoculum level since each lab processed only a single run for each inoculum level.

Conclusion. These data provide performance characteristics for a sponge wipe-processing method for use when investigating environmental *B. anthracis* contamination. If validation is defined as stated in the GAO report, as "a formal, empirical process in which an authority determines and certifies the performance characteristics of a given method" (9), we consider this method validated, with the LRN review committee as the oversight authority. This controlled study brought to light the large variability inherent in environmental sampling and processing, especially when dealing with low numbers of organisms. Nonetheless, these data help to establish acceptance criteria and characterize the variability of the method and thereby enhance confidence in the interpretation of the results of environmental sampling after a *B. anthracis* contamination incident.

ACKNOWLEDGMENTS

We thank the following participating labs: Virginia Department of General Services, Division of Consolidated Laboratory Services, Richmond, VA; Pennsylvania Department of Health, Bureau of Laboratories, Lionville, PA; New York City Department of Health, Public Health Laboratory, New York, NY; Florida Department of Health, Bureau of Laboratories, Jacksonville, FL; Minnesota Department of Health, Public Health Laboratory, St. Paul, MN; Los Angeles County Department of Public Health, Public Health Laboratory, Los Angeles, CA; Washington Department of Health, Public Health Laboratories, Shoreline, WA; and Lawrence Livermore National Laboratory, Livermore, CA.

Many thanks to Misty Hein, NIOSH, CDC, Cincinnati, OH, for her assistance with the statistical analysis of these data.

The findings and conclusions in this report are ours and do not necessarily represent the official position of the CDC.

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