

Testimony of Robert G. Hamilton, Ph.D.

Subcommittee of National Security, Emerging Threats
and International Relations

Committee on Government Reform

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Introduction

Good Afternoon, Mr. Chairman and members of the Committee. My name is Robert Hamilton. I am a Professor of Medicine and Pathology at the Johns Hopkins University School of Medicine in Baltimore and the director of the Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory. Thank you for the invitation to speak to you regarding the environmental testing methods for anthrax detection, and the lessons that we can learn from Wallingford.

I am speaking to you today as an academic scientist who was not directly involved in the anthrax-associated events. However, my group at Johns Hopkins was drawn into the anthrax testing issue when this vacuum collector was used in the “rule out” detection of anthrax contamination at the Brentwood and Wallingford postal facilities. More than a decade ago, we participated in the development and application of this vacuum-collection device (sometimes called a “nozzle sock”). Our application of this device over a decade has been for surface dust collection in homes and schools. We process and analyze the collected dust for dust mite, cat, dog, cockroach, mouse, rat and mold spore aeroallergens in homes and schools of asthmatic children.

In consultation with my colleague Barry Skolnick, we have identified a number of technical issues that relate to the performance and extent of validation of environmental testing methods that were used to assess the postal facilities and congressional offices for anthrax. In my testimony, I will refer to a number of “environmental surface-testing systems”. By this term, I mean overall procedures which share *four* integrated components. First, a surface is *sampled* at a site using a swab, wipe, or vacuum-based collection method (Table 1). Then these are transferred to a laboratory where the dust or particulate specimen is *extracted* from the collector, *analyzed* for bacterial spores, and the data are *reported*. I will refer to specific issues related to a number of these components of the environmental surface-testing system.

My comments today will refer only to environmental testing systems that use culture-based analytical methods for viable anthrax detection. I will not refer to any of the newer PCR-based technologies or “rapid assay” biosensors that are in development for on-site use. However, please remember that the performance of even these newly-emerging analytical techniques rely upon the efficiency of same surface-sampling procedures at their “front end” to get the bacterial spores out the environment and into a form for analytical testing (1).

Recommendations:

I would like to begin with three recommendations to your committee. They can be summarized with the words: *leadership*, *support* and *peer-review*.

First is *leadership*. We would ask that *a single Federal agency assume the leadership role in guiding the evaluation, performance improvement and validation of standardized surface-testing systems* for anthrax detection in indoor environments. Our primary concern has been the lack of a unifying national doctrine that establishes the level of performance (sensitivity, reproducibility, accuracy, practicability) needed in surface-testing systems. This has led to the Federal agencies using environmental testing procedures and laboratory protocols that differ in their technical details. This has a direct impact on their interoperability. We need a single leading Federal agency to implement a unified, optimized and verifiable approach to environmental testing for the detection indoors of dispersed agents of bioterrorism.

Second is *support*. We feel that the surface-detection methods need optimization and validation. To improve these methods, *adequate Federal funding and resources need to be allocated* in response to “top down” requirements. We have prepared a research proposal to study these issues ourselves and have been amazed that there is no Federal program we can identify with a clear mission to support environmental surface-testing systems development. Extramural funding of research by academic and industrial laboratories is needed.

Third is *peer-review*. We need *open, scientific peer review* to allow the relevant expertise of academic and industrial specialists to assist capable government scientists in (a) evaluating existing methods (b) developing optimized consensus procedures and (c) validating these integrated testing systems. In the academic community, we are used to this open interchange of peer review. We feel it provides the best approach to minimizing turf battles among different groups while extracting the best ideas from each participant. While the interchange of ideas should be open, we also understand that some national security issues will have to be managed in this peer-review process, with engagement by the Departments of Defense and Justice.

The experiences of testing for anthrax at Wallingford, Brentwood and Capitol Hill have taught us that we need leadership, funding and resource allocation, and peer review to insure we have optimized, consensus-based environmental surface testing systems for future use.

The Importance of Surface-Testing Systems

More work is needed on *surface* detection methods, as distinguished from *air*-sampling methods. A recent methods-comparison study at the contaminated Brentwood Road postal facility by Sanderson et al. (2) has clearly taught us that bacterial spores *settle into reservoir dust and do not remain airborne*. In fact, all air samples collected in this study were negative. It was the surface-sampling methods and not air sampling (3) that provided the useful environmental data for making decisions about both the presence and amount of contamination in the building. While the support of air sampling method development has been extensive, I have been unable to identify a defined Federal mission and funding support for surface sampling method development. This has resulted in a lack of preparedness because we do not have validated, sensitive, specific, quantitative and reproducible environmental surface detection methods for bacteria, viruses and toxins ready for use.

Improvements to Existing CDC and US Postal Service Environmental Testing Procedures:

The Centers for Disease Control and Prevention (CDC) and the U.S. Postal Service (USPS) have both issued interim guidelines with procedures for environmental sampling and analysis to detect anthrax in buildings (4-6). I would like to thank the authors of these documents for a tremendous effort in their preparation during a time of national crisis. We now have the opportunity to enhance these procedures by making a number of small but significant technical improvements. Moreover, the procedures need to be validated for field collection and laboratory analysis. We really do not know the sensitivity (minimum detectable dose), reproducibility (variation), and quantitative detection capabilities of the available environmental testing systems. These need to be documented with positive “challenge” testing using suitable “surrogates” and actual bioagents of concern.

Based on our review of relevant scientific literature, we believe that a number of details in the published CDC and USPS surface-testing procedures need re-examination. They involve both procedural differences between these two agencies’ methods, and some features that they share in common. A more extensive list of these issues is provided as Appendix I to our written testimony. I would like to illustrate a few of the technical differences in the swab-based assay procedures that can lead to variable performance. The CDC and USPS swab-rinse assay procedures varied as to:

- (a) *whether dry or wet swabs are used*. As far back as the introduction of the swab-rinse assay in 1917 (7), we could identify no justification for the use of *dry* swabs in swab-rinse environmental testing. Moreover, the inter-agency Brentwood study (2) lead us to consider the dry swab data unreliable.
- (b) *whether or not any detergent was added to the sample rinse to aid spore extraction*. The USPS did not incorporate its use in their swab-rinse procedure.
- (c) *the volume of rinse used to extract the swab: (CDC: 3 ml vs. USPS, 1.5 ml)*.

- (d) *the fraction of the total extract volume inoculated onto culture plates: (CDC: 1/10 vs. USPS: 1/15).* We believe that both methods cultured too little of total extract volume for use as a “rule out” assay that should be maximally sensitive to support a “zero” tolerance policy – a policy which itself needs re-assessment (8).
- (e) *how many culture plates were inoculated per sample (CDC: 3 vs. USPS: 1).* The culturing of a single plate provides no measure of variation, and I do not consider this good laboratory practice.

I believe that these and other technical issues that both procedures share may have made all the difference between successful anthrax detection and failure. One shared characteristic among the CDC and USPS swab rinse methods that should be reviewed is the surface area covered per swab. A 100 cm² area is probably too big for a small swab. Sampling this large an area could lead to both incomplete area coverage and overloading of the swab with surface debris. Other standardized testing procedures have reported the covering of only a fifth to a quarter of this surface area per swab

The cumulative effect of all such variables on swab-rinse techniques may have led to the early negative results from surface testing at the Wallingford postal facility on November 11, 14 and 25, 2001 and subsequent positive results for anthrax on November 28. From published data at Brentwood, I can conclude that there may have been some “false negative” test results reported at Brentwood due to these technical issues associated with the sampling process. Another feature shared by CDC and USPS procedures is the lack of any provision for “positive controls”, measures designed to enable the reduction of risk of any such “false negative” testing outcomes through proficiency training and quality assurance. By contrast, “negative controls” are well provided for in both procedures, which call for numerous sample “blanks” to monitor cross-contaminations which could cause “false positive” testing results.

Another variable that deserves more careful review is the practice of reporting vacuumed sample test results in terms of “colony forming units per gram” (CFU/g) of collected dust. The utility of this unit is not intuitively obvious to me, in the context of bioterrorism. Because the amount of surface dust often varies across a confined surface area, actual differences in the levels of anthrax spores per unit area or device may be masked. This is schematically shown in (Figure 1). A more useful way of expressing assay data is as surface “loading”, which is reported as the quantity of spores per unit area (e.g. CFU/cm²). From this, the total bacterial burden on a machine, floor or desktop area can be calculated. To do this, however, the area sampled needs to be accurately recorded as the CDC procedure specifies (6).

In terms of positive test-outcomes, it appears that some wet-wipe and vacuum nozzle-sock collection techniques appeared to work better at Brentwood and Wallingford than did either the dry or wet swabs. Even so, we feel there are a number of variables in these more successful procedures which need further review and possible optimization. For instance, scientists associated with the National Aeronautics and Space Administration (NASA) developed (10) and have utilized an alternative version of wipe-rinse assay procedure for over twenty years to monitor spacecraft contamination as part of a program

now termed “planetary protection”. They have used a bonded-polyester 10 x 10-in. “clean room cloth” wipe that was folded and rubbed over surfaces in a defined manner (10, 11). The CDC’s recommended wipe-rinse assay differs from this NASA method partly because it specifies 3 in x 3 in. or smaller synthetic gauze pads. It also lumps wipes with different characteristics (gauze, sponges and Handi-Wipe^R) together as equivalent, which may have the effect of increasing variations in assay performance. There are other technical differences, so validation of the CDC’s methods in relation to NASA’s prior art would certainly be prudent.

There are also a number of improvements to the High Efficiency Particulate Air (HEPA) vacuuming procedure reported by the CDC that are suggested by the literature. Because of our interest in the vacuum nozzle sock, we have planned research studies to optimize the HEPA vacuum-rinse surface testing system.

Conclusion:

In conclusion, we have the intellectual capability in the United States and an excellent existing framework of available surface-detection procedures as published by the CDC and USPS. What we need now is for a single agency to lead our scientific body, with sufficient financial and personnel support and peer-review discussion to modify the existing environmental surface testing systems so they are maximally sensitive, reproducible and quantitative.

Mr. Chairman, this concludes my formal remarks. I look forward to working with you on these important issues, and would be happy to take any questions from the Subcommittee.

References:

1. Cf. CDC: Notice to Readers: Use of Onsite Technologies for Rapidly Assessing Environmental *Bacillus anthracis* Contamination on Surfaces in Buildings. December 7, 2001. Morb. Mortal. Wkly. Rep. 50(48):1087. Available at URL: <<http://www.cdc.gov/mmwr/PDF/wk/mm5048.pdf>>.
2. Sanderson WT, Hein MJ, Taylor L, Curwin BD, Kinnes GM, Seitz TA, Popovic T, Holmes HT, Kellum ME, McAllister SK, Whaley DN, Tupin EA, Walker T, Freed JA, Small DS, Klusaritz B, Bridges JH (2002) Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg. Infect. Dis. 8(10):1145-1151. Available at URL: <<http://www.cdc.gov/ncidod/EID/vol8no10/02-0382.htm>>.
3. An important exception is the use of so-called “aggressive sampling” techniques, such as have been recognized for use in testing decontaminated sites for residues of viable anthrax. In these methods, settled spores are mechanically resuspended from surfaces, and then air sampling is employed for detection. See Weis CP, Intrepido AJ, Miller AK, Cowin PG, Durno MA, Gebhardt JS, Bull R (2002) Secondary Aerosolization of Viable *Bacillus anthracis* Spores in a Contaminated US Senate Office. J Am Med Assoc 288(22):2853-2858, December 11, 2002. Available at URL:

<<http://jama.ama-assn.org/issues/v288n22/rfull/joc21393.html>>. See also: National Response Team: Technical Assistance for Anthrax Response, Interim Final Draft, September 2002. Available at URL:

<[http://www.nrt.org/production/nrt/home.nsf/resources/publications/\\$file/final_anthrax_TAD_10_3_02.pdf](http://www.nrt.org/production/nrt/home.nsf/resources/publications/$file/final_anthrax_TAD_10_3_02.pdf)>

4. CDC: Procedures for Collecting Surface Environmental Samples for Culturing *Bacillus anthracis*, November 13, 2001. URL (as accessed December 7, 2001): <<http://www.bt.cdc.gov/DocumentsApp/Anthrax/11132001/final42.asp>>

5. USPS: Interim guidelines for sampling, analysis, decontamination and disposal of anthrax for U.S. Postal Service facilities, December 4, 2001. Available from the American Postal Workers Union at URL:
<http://www.apwu.org/departments/ir/s&h/anthrax/Protocol/120501_uspsissued_version.doc>
6. CDC: Comprehensive Procedures for Collecting Environmental Samples for Culturing *Bacillus anthracis*, April 25, 2002 (Revised). Available at URL:
<<http://www.bt.cdc.gov/Agent/Anthrax/environmental-sampling-apr2002.asp>>
7. Manheimer WA, Ybanez T. Observations and experiments on dishwashing. Am. J. Public Health 7:614-618, 1917. In contrast to the established use of moistened swabs in the testing of environmental surfaces for microbial contamination, dry swabs are commonly used by physicians to collect moist clinical specimens from patients for what is termed the “swab-smear” assay.
8. Cf. “EPA used a standard for the remediation of ‘negative result for anthrax.’ The standard means that the tests taken after cleanup come back from the laboratory as negative for anthrax.” See: Environmental Protection Administration (EPA): “Fact Sheet for the Hart Senate Office Building Cleanup” dated 20 Nov. 2001. URL:
<http://www.epa.gov/epahome/headline2_112001.htm#24>. See also questions answered following the statement of EPA Administrator Gov. Christine Todd Whitman delivered at a hearing on “EPA and Federal Workplace Safety” of the U.S. Senate Subcommittee on HUD, VA and Independent Agencies of the Committee on Appropriations, on November 28, 2001; cf. URL: <<http://www.epa.gov/ocir/hearings/testimony/112801ctw.PDF>>. A streaming-video archive of the full hearing has been made available indefinitely for on-line viewing by C-Span.org, at URL:
<http://cspanrm.fplive.net:554/ramgen/cspan/mdrive/ter112801_epa.rm>
9. Government Accounting Office. U.S. Postal Service. Better guidance is needed to improve communication should anthrax contamination occur in the future. GAO-03-316. April 2003. Available at URL: <<http://www.gao.gov/new.items/d03316.pdf>>
10. Kirschner LE, Puleo JR. Wipe-rinse technique for quantifying microbial contamination on large surfaces. Appl. Environ. Microbiol. 38(3):466-470, 1979.
11. NASA (2000) Procedures and Guidelines NPG: 5340.1D (FINAL DRAFT). NASA Standard Procedure for the microbial examination of space hardware. January 2000. Available at URL: <http://centauri.larc.nasa.gov/discovery/FinalNPG_5340.1D.PDF>

Appendix I.

Our primary technical concerns about the details of CDC and USPS issued procedures for testing environmental surfaces in anthrax-incident response, are as follows:

1. Specification of “non-cotton” rather than cotton swabs may reduce spore-extraction success (see #9) (CDC and USPS)
2. No detergent included in sampling-media (swab, wipe) wetting agent may reduce spore removal from tested surfaces and also reduce extraction efficiency (CDC)
3. Dry swabs (no wetting agent) used for sampling surfaces lacks any known scientific foundation (USPS)
4. Surface coverage area per sample is too large in comparison to other standardized practices, or is ill-defined in terms of quality assurance (CDC and USPS)
5. Ill-defined details of manual surface-contact and vacuuming techniques are inadequate to assure uniformity and quality in actual practice (CDC and USPS)
6. Swab and wipe specimens transported dry to the assay laboratory may reduce extraction efficiency while increasing safety risks to laboratory workers (CDC and USPS)
7. No detergent included in rinse liquid formulation expected to reduce extraction efficiency (USPS)
8. Mechanical extraction by “vortexing” inadequate to “disintegrate” fibrous swabs sufficiently to disperse spores uniformly into liquid extract (CDC and USPS)
9. Sonication techniques (NASA) not employed for improved mechanical wet-extraction (CDC and USPS)
10. Concentrating extracts by centrifugation and resuspension raises particulates-binding issues which may affect accuracy of “CFU” counts (a CDC-reported (2) on-site practice)
11. Excessive sample “splitting”: using only a small fraction of the total extract volume to inoculate culture plates for each sample (USPS: 1/15, CDC: 1/10) introduces a serious statistical sampling error for a “rule out” assay at “zero” tolerance
12. Non-replicate (single) rather than triplicate plating is poor laboratory practice and introduces statistical sampling error (USPS)
13. No provision of any “positive controls” to calibrate testing procedures, support proficiency training, enable quality assurance and thereby reduce risks of “false negative” outcomes of testing (CDC and USPS). Cf. “negative controls” which are provided for in the form of numerous sample “blanks” to monitor cross-contamination (CDC and USPS).
14. No well-preserved retention of extracted media (“spent” swabs, wipes or filters) for optional enrichment culture by broth immersion, for a “fail-safe” assurance of reliability in “rule out” testing (CDC and USPS)

Figure 1

Schematic: Variabilities in HEPA Vacuum-Rinse Assays for Anthrax, When Expressed in Units of Sample Spore Concentration (CFU/g)

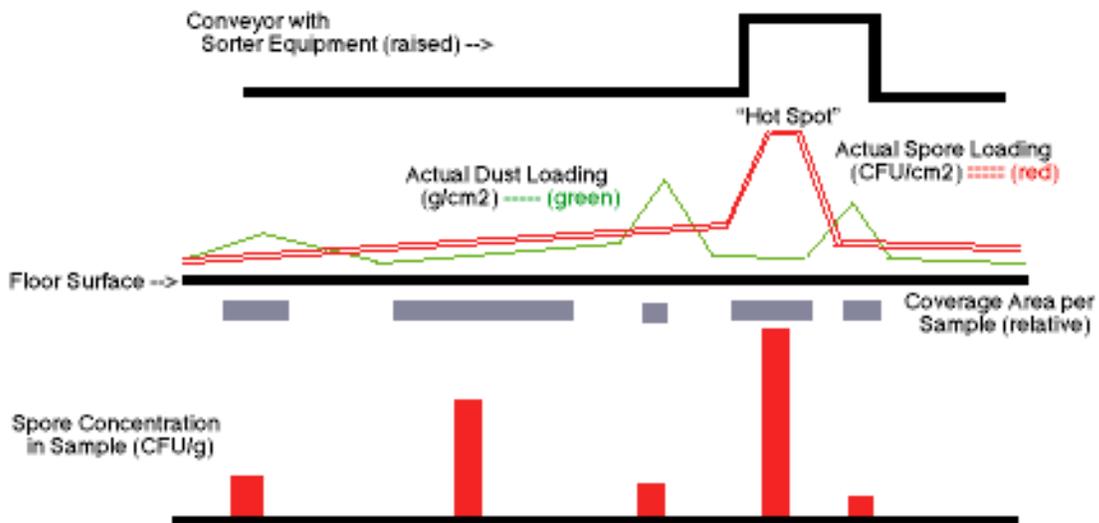


Figure 1: Schematic illustrating the possible ambiguity associated with the units (CFU/g versus CFU/cm²) used to report sample spore concentration derived from HEPA vacuum-rinse assay data. The top panel indicates a theoretical conveyor belt room in a mail distri

bution center in which the raised area indicates the sorter equipment location. If a letter with anthrax passes through the room, a "hot spot" of settled spores is created on the floor, as illustrated in the middle graph (*double-lined curve*). The spore-concentration values reported in the lower panel as CFU/g are computed as the ratios of total spores detected to the total weight of dust mass collected (*vertical bars*). The CFU/g levels can vary greatly because of large differences in sampling surface coverage areas (*horizontal gray bars*) and do not reflect actual spore loading (CFU/cm²). This results from sample collection areas being adapted to varying levels of surface dusts (*single-lined curve*) – in order to maintain consistency in total dust mass collected – rather than being held constant so that the total quantity of spores in a sample would be proportionate to any actual differences in *Bacillus anthracis* spore surface-loading levels (population density) at the floor locations tested (*double-lined curve*). Graphic prepared by: Johns Hopkins DACI Reference Laboratory, Johns Hopkins University School of Medicine, Baltimore Md. 21224.

TABLE 1

Some Environmental Surface-Sampling Methods

Specimen Type	Agency	Material	Wetting agent	Area Sampled	Collection Pattern	Ref.
Dry Swab	USPS	Dacron or Rayon (non-cotton) sterile swab	None	100 cm ² (“about the size of half a sheet of paper”)	Horizontal S strokes, <i>rotate</i> , then vertical S strokes (<i>illustrated</i>)	5
Wet Swab	CDC	Non-cotton (e.g., Rayon) sterile swab	Sterile water, saline or PBS*	<100 cm ² (“Avoid letting the swab dry completely”)	“Enough vertical S strokes to cover area completely”	6
Wet Swab (for “surface bioburden” of spacecraft hardware)	NASA	Autoclaved then dried sterile cotton	Sterile water (10 ml)	No more than 26 cm ² (2 in x 2 in)	Rotational swabbing motions in three 90-degree changes of direction, then immerse in water	11
Wet Wipe	CDC	3 in x 3 in or smaller synthetic (non-cotton) gauze pad (gauze, Handi-Wipe ^R , sterile sponge)	Sterile water, saline or PBS* (moisten)	Approximately 1 ft ² (0.0929 m ²) (“Avoid letting the gauze pad dry completely.”)	Vertical S strokes, <i>fold</i> , then horizontal S strokes	6
Wet Wipe (for “surface bioburden” of spacecraft hardware)	NASA	Autoclaved then dried 100% polyester bonded clean room wipes, 26 cm x 26 cm (~10 in x 10 in)	Sterile distilled water (15 ml)	Unspecified; routinely up to 0.74 m ² (8 ft ²), according to Kirschner and Puleo (1979)	Rotational rubbing motions in three 90-degree changes of direction w/folding	10, 11
HEPA Vacuum Dust Collection Filter (“Nozzle Sock”)	CDC	HD polyethylene filter (1 µm nom. porosity) in high volume air (28 cfm) intake device	None	No area specified	One pass at 12”/sec; 1-2 tablespoons debris/dust needed/desired	6
Microvacuum (modified personal air sampler)	EPA	Gelatin filter (3 µm nom. porosity) in low volume air (4 cfm) intake device	None	100 cm ² (defined by template)	Slow back-and-forth motion, first in one direction, than 90 degrees perpendicular	3

* PBS = phosphate buffered saline

