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Experimental Design for a Sponge-Wipe Study to Relate the Recovery Efficiency and False Negative Rate to the Concentration of a *Bacillus anthracis* Surrogate for Six Surface Materials

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Executive Summary

This report describes the experimental design for a laboratory study planned under the auspices of the Validated Sampling Plan Working Group (VSPWG). The experimental design was jointly developed by Pacific Northwest National Laboratory (PNNL) and Sandia National Laboratories (SNL). The testing specified by the experimental design will be performed by SNL.

The high-level drivers for this work were two main concerns raised by the Government Accountability Office (GAO) following the 2001 contaminations of the Hart Senate office building and postal facilities via letters containing *Bacillus anthracis* (BA). These concerns involved 1) the lack of validated sample collection, processing, and analytical methods and 2) insufficient use of statistical sampling to provide for quantifying the confidence that an area or building can be cleared when all samples have negative results (GAO 2005a, 2005b). Critical to addressing both of these areas of concern is quantifying, for a single sample, the probability of correct detection (PCD) or the false-negative rate (FNR), where $FNR = 1 - PCD$. The PCD/FNR for a single sample may depend on the 1) method of contaminant deposition, 2) surface concentration of the contaminant, 3) surface material being sampled, 4) sample collection method, 5) sample storage and transportation method and conditions, 6) sample processing method, and 7) sample analytical (assay) method.

A review of the literature (Piepel et al. 2010) found 17 chamber and laboratory studies that focused on swab, wipe, or vacuum samples collected from a variety of surface materials contaminated by BA or a surrogate, and used culture methods to determine the surface concentration of colony-forming units (CFUs). These studies generally quantified the performance of the sampling and analysis methods in terms of recovery efficiency (RE) and not PCD/FNR. Only one study (Estill et al. 2009) developed equations for calculating PCD as a function of surface concentration. However, the data were insufficient in some regards, and the equation results were not published in the journal article. Hence, Piepel et al.^(a) noted that previous chamber and laboratory studies failed to quantify the PCD/FNR for a single sample as a function of affecting variables, and this left a major “gap” in available information. Quantifying the PCD/FNR for a single sample under a variety of conditions is a key aspect of validating sample and analysis methods, and also of calculating the confidence in characterization or clearance decisions based on a statistical sampling plan. These are the two main areas of concern expressed by the GAO.

The VSPWG decided to plan a laboratory study to partially fill the gap in PCD/FNR results from previous chamber and laboratory studies. This report documents the experimental design developed for a VSPWG laboratory study for a sponge-wipe method. It also discusses the types of data analyses and results that can be obtained from the study data. The study will investigate the effects on key response variables of sponge-wipe samples taken from six surface materials (stainless steel, vinyl tile, ceramic tile, plastic, painted wood paneling, and faux leather) contaminated with eight surface concentrations of a BA surrogate (*Bacillus atrophaeus*). The eight concentrations to be tested will be slightly different for the second block of eight tests on the last three materials, compared to the first block of eight tests on the first three materials. The key response variables include measures of the actual contamination on test coupons of surface materials tested, contamination recovered from coupons by sponge-wipe samples, RE, and PCD/FNR.

Ideally, swab and vacuum sample collection methods would also be investigated in the laboratory study. However, available funding and several challenging aspects of the study led to the decision to

focus on a sponge-wipe sampling and analysis method recommended by the Centers for Disease Control and Prevention (CDC). Similar studies can be performed in the future for swab and vacuum sampling methods.

The original plan was to perform a chamber study with dry aerosol deposition of the contaminant on test coupons inside a test chamber. However, preliminary testing by SNL showed it was not possible to reliably achieve low enough concentrations on coupons to realize false negatives with the sponge-wipe method recommended by the CDC. Hence, concentrations achievable by dry aerosol deposition would not provide useful information to address the information gap on false negatives. Therefore, the plan was changed to perform a laboratory (bench-top) study using a liquid deposition method. This approach can reliably achieve lower concentrations of the contaminant on test coupons so that false negatives can be achieved. Data for overlapping concentrations (on the high end of wet deposition tests, and the low end of preliminary dry aerosol deposition tests) will be collected to assess the impact on results of wet versus dry deposition of the contaminant.

The experimental design for the laboratory study involves 16 test runs, to be performed in two blocks of eight runs. When this report was finished, the first block of testing was completed, and the second block had begun. Three surface materials (stainless steel, vinyl tile, and ceramic tile) were tested in the first block, while three other surface materials (plastic, painted wood paneling, and faux leather) will be tested in the second block. The original plan was to replicate in Block 2 the set of eight runs from Block 1 using the same three surface materials (stainless steel, vinyl tile, and ceramic tile). The purpose of that plan was to obtain increased accuracy and precision in results through replication. However, the sponge-wipe method obtained fewer false negatives than expected in Block 1 for the first three surface materials, and the results had better precision than expected. So, it was decided to test three different surface materials in Block 2. The eight surface concentrations to be tested in Block 2 will differ slightly from the eight concentrations tested in Block 1. The eight surface concentrations of the surrogate were randomly assigned to test runs within each block (with some changes at the start of the first block). It is expected that some of the concentrations will be very low and may present challenges for deposition, sampling, and analysis. However, such tests are needed to investigate RE and PCD/FNR over the full range of concentrations of interest.

For each of the 16 test runs, three surface materials will be investigated, with one set of three surface materials for Block 1 (Tests 1–8), and another set for Block 2 (Tests 9–16). Hence, the experimental design is a split-plot experiment (Steel and Torrie 1960, Chapter 12; Myers and Montgomery 1995, Section 9.6) in which “concentration” is the whole-plot (WP) factor and “surface material” is the sub-plot (SP) factor. In each of the 16 test runs, there will be 10 test coupons of each of the three surface materials. This will provide a good basis for calculating empirical PCD values (i.e., $PCD = x/10$, where x = number of correct detections). A positive control (reference) sample will be generated immediately before each test sample, which will consist of placing the same number of drops of contaminant solution on a growth plate as are placed on the test coupon. This will provide a basis for estimating the actual contaminant concentration deposited on each test coupon (within the variation of the deposition process). For each of the six surface materials, these estimated contaminant concentrations will be used to 1) calculate RE values for the wipe sampling and analysis method and 2) fit RE- and PCD-concentration equations.

Data analyses will support 1) estimating the PCD for each combination of contaminant concentration and surface material, 2) estimating the surface concentrations (e.g., CFU/cm²) and their uncertainties

(e.g., percent relative standard deviation [%RSD]) of the contaminant for each combination of concentration and surface material, 3) estimating RE (%) and their uncertainties (% or %RSD) for each combination of contaminant concentration and surface material, 4) fitting PCD-concentration and RE-concentration equations for each of the six surface materials, 5) assessing goodness-of-fit of the equations, and 6) quantifying the uncertainty in PCD and RE predictions made with the fitted equations. From these equations, it will be possible to calculate quantities such as the lower limit of detection, the method detection limit, and concentrations with high PCD (e.g., 0.90 or 0.95).

In summary, the data resulting from the VSPWG laboratory study discussed in this report will support addressing both of the main concerns of the GAO, namely 1) contributing information toward validating the sponge-wipe method and 2) providing for calculating numbers of samples and confidence in characterization and clearance decisions based on statistical sampling plans when the FNR > 0.

Acronyms

%RSD	percent relative standard deviation
BA	<i>Bacillus anthracis</i>
CDC	Centers for Disease Control and Prevention
CFU	colony forming unit
DHS	U. S. Department of Homeland Security
DoD	U. S. Department of Defense
DOE	U. S. Department of Energy
EPA	Environmental Protection Agency
FBI	Federal Bureau of Investigation
FNR	false-negative rate
FPR	false-positive rate
GAO	Government Accountability Office
LLD	lower limit of detection
LOD	limit of detection
MDL	method detection limit
NIST	National Institute of Standards and Technology
PCD	probability of correct detection
PNNL	Pacific Northwest National Laboratory
QC	quality control
RE	recovery efficiency
S&T	Science and Technology Directorate
SNL	Sandia National Laboratories
SP	sub-plot
U.S.	United States
VSPWG	Validated Sampling Plan Working Group
WP	whole-plot

Acknowledgments

The Pacific Northwest National Laboratory (PNNL) work summarized in this report was funded by the Standards Office of the Test and Evaluation/Standards Division and the Biological Research and Development Branch of the Chemical and Biological Division in the Science and Technology Directorate of the U.S. Department of Homeland Security (DHS). The interest and FY07-FY09 funding support of Bert Coursey (Standards Portfolio Executive) for experimental and sampling design and other statistical capabilities in the Applied Statistics and Computational Modeling group at PNNL is gratefully acknowledged. The FY10-11 funding support of Lance Brooks and Chris Russell is also gratefully acknowledged. PNNL is a multiprogram national laboratory operated for the U.S. Department of Energy by Battelle under Contract DE-AC05-76RL01830.

The Sandia National Laboratories (SNL) work included in this report was funded by the Chemical and Biological Research and Development Branch of the Chemical and Biological Division in the S&T Directorate of DHS. The interest and support of Lance Brooks and Chris Russell (DHS) for the SNL work is gratefully acknowledged. SNL is a multiprogram national laboratory operated by Sandia Corporation, a Lockheed Martin company, for the U.S. Department of Energy's National Nuclear Security Administration under Contract DE-AC04-AL8500.

The work in this report was also influenced by telecon discussions and comments from Validated Sampling Plan Working Group (VSPWG) members during the planning and performance stages of this work. The authors would also like to thank Randy Long (DHS) for his leadership of the VSPWG and general guidance on this work. We also acknowledge and thank the following PNNL staff members: 1) Brett Matzke for reviewing and providing comments on a draft of this report and 2) Wayne Cosby for editing, formatting, and preparing the report for publication.

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1.0 Introduction

This report discusses the experimental design for a laboratory study planned under the auspices of the Validated Sampling Plan Working Group (VSPWG). The laboratory study is needed to address gaps identified by Piepel et al. (2010) in a review of 17 previous chamber and laboratory studies. The experimental design was developed by Pacific Northwest National Laboratory (PNNL) and Sandia National Laboratories (SNL), and the laboratory study will be performed at SNL.

The laboratory study will collect data to characterize the performance of sponge-wipe sample collection, extraction, and analysis procedures developed by the Centers for Disease Control and Prevention (CDC). The sponge-wipe sampling method is also recommended in the VSPWG sampling strategy document.^(a) The sponge-wipe study will investigate the effects of selected independent variables on several response (dependent) variables. For several reasons, it was necessary to limit the number of independent variables investigated in the sponge-wipe study. The reasons for selecting a wipe sample collection method over swab and vacuum sample collection methods are discussed in Section 2. The two independent variables that will be varied in the study are the surface concentration of a *Bacillus anthracis* (BA) surrogate (*Bacillus atrophaeus*) and surface material. Six non-porous surface materials (stainless steel, vinyl tiles, ceramic tile, plastic, painted wood paneling, and faux leather) having a range of surface roughness values will be investigated. Three materials (stainless steel, vinyl tiles, and ceramic tile) will be investigated in the first block of eight test runs, while the remaining three materials will be investigated in a second block of eight test runs. Eight surface concentrations of surrogate spores (including some very low concentrations) will be investigated, with a slightly different set of concentrations for the two blocks of tests. The variables that will be held constant and varied in the sponge-wipe study are discussed further in Sections 2 and 3, respectively. The response variables will include measures of the 1) actual contamination on coupons of surface materials tested, 2) contamination recovered from coupons by wipe samples, 3) recovery efficiency (RE), 4) probability of correct detection (PCD), and 5) false negative rate (FNR), where $FNR = 1 - PCD$.

The tests in the sponge-wipe study will be conducted according to the experimental design presented in Section 6. To limit sources of bias that might impact the experimental data, the experimental design randomizes some aspects of testing and data collection.

Concentrations of BA necessary to cause anthrax may include values low enough that the $PCD < 1$, and equivalently, the $FNR > 0$. Hence, estimates of PCD/FNR, RE, and other quantities (e.g., limits of detection) for single samples taken under various sampling conditions are needed as inputs to calculate the confidence in detection or clearance decisions based on sampling and analysis results after a contamination event. The sponge-wipe study will generate data to support developing RE and PCD/FNR equations as functions of contaminant surface concentration for each of the three surface materials. These RE-concentration and PCD/FNR-concentration equations will then be available to provide inputs for calculating 1) the numbers of samples in statistically based sampling plans to provide the desired confidence for detection and clearance decisions and 2) the confidence in detection and clearance decisions given the numbers of samples in specific sampling plans.

(a) *Environmental Sampling Strategy for Bacillus anthracis during Crisis and Consequence Management* (draft—pre-decisional), Validated Sampling Plan Working Group, December 2, 2009.

Sections 1.1 and 1.2 present, respectively, the background and justification for the work in this report. Section 1.3 discusses the organizations that performed work and their funding sources. Section 1.4 outlines the organization of the rest of the report.

1.1 Background

In 2001, letters containing BA contaminated the Hart Senate office building in Washington, DC and postal facilities that processed the letters. This experience demonstrated areas of concern in the procedures and methods used to characterize and clear buildings contaminated by BA. A congressional inquiry and the Government Accountability Office (GAO) identified two main concerns (GAO 2005a, 2005b). One main concern was the reliance on sampling specific areas where it was thought BA would be found. This type of sampling approach is referred to as *targeted sampling* or *judgmental sampling*. The GAO reports identified the need to use *statistical (probabilistic) sampling* so that when all results are negative, a building (or area within a building) can be cleared with a known level of statistical confidence. The second main concern was that the sample collection, processing, and analytical methods used were not validated. The lack of validated methods raised questions about the reliability of the negative sampling results.

The VSPWG was formed in July 2006 in response to the congressional inquiry and GAO reports. The VSPWG is headed by the U.S. Department of Homeland Security (DHS) Science and Technology Directorate (S&T) and includes experts from the Department of Defense (DoD), the Environmental Protection Agency (EPA), CDC, the National Institute of Standards and Technology (NIST), the Federal Bureau of Investigation (FBI), and U.S. national laboratories (e.g., PNNL and SNL). The VSPWG is developing a sampling strategy^(a) and working towards validating methods for steps in the sampling process. These steps include 1) sampling approach (e.g., appropriate uses of judgmental and probabilistic sampling), 2) sample collection methods, 3) handling of samples (e.g., handling, storage, and transportation), 4) sample processing and extraction methods (i.e., extraction of the contaminant from samples), and 5) sample analysis (i.e., analytical methods).

1.2 Justification

The results of the sponge-wipe study that will be conducted using the experimental design in this report will contribute to addressing both of the GAO's main concerns. Specifically, for sponge-wipe samples collected, extracted, and analyzed using the specified methods, the study will yield equations relating the PCD/FNR to the surface concentration of a BA surrogate for each of six surface materials. The six surface materials to be tested in the sponge-wipe study span the range of surface roughness for non-porous surfaces, providing the basis for assessing the PCD (or FNR) as a function of the BA surrogate concentration for other non-porous surface materials

The PCD/FNR equations will be developed from data generated by the study whose experimental design is documented in this report. These equations (and the underlying data) will make a significant contribution to the validation of sponge-wipe sample collection, extraction, and analysis by quantifying the performance of the CDC-recommended methods over a range of BA surrogate concentrations and surface types. Hence, this work addresses one of the two main concerns of the GAO—method validation.

(a) *Environmental Sampling Strategy for Bacillus anthracis during Crisis and Consequence Management* (draft—pre-decisional), Validated Sampling Plan Working Group, December 2, 2009.

The PCD/FNR equations will also serve as inputs to equations for calculating the 1) numbers of samples required to make characterization and clearance decisions with specified levels of confidence and 2) confidence in characterization and clearance decisions for specific sampling plans implemented in BA contamination events. The work to develop these equations will occur as part of another task in the PNNL project supporting the VSPWG and will be documented in a separate report. However, the experimental design for the sponge-wipe study discussed in this report will generate key data on the PCD/FNR for a single sponge-wipe sample. Clearly, the PCD/FNR for a single sponge-wipe sample will depend on the concentration of the contaminant and the surface material being wiped. The PCD/FNR for a single sample is a key input to calculations for the confidence in characterization and clearance decisions based on statistical sampling. Hence, this work contributes to addressing the other main GAO concern—having confidence in decisions based on statistical sampling.

The need for the sponge-wipe study discussed in this report was determined based on a review of the literature on previous chamber and laboratory studies by PNNL and NIST (Piepel et al. 2010). The literature review focused mainly on studies that collected swab, wipe, or vacuum samples from surfaces contaminated by BA or a surrogate and used culture methods to determine the surface concentration of the contaminant. A total of 17 studies were identified, with 14 studies having swab data, 9 studies having wipe data, and 4 studies having vacuum data. Only one of the studies used their data to develop equations relating PCD to the concentration of the contaminant, although that aspect of the study did not get reported in the published journal article (Estill et al. 2009). Only one other study tested a sufficient number of contaminant concentrations to potentially develop PCD/FNR-concentration equations. Hence, Piepel et al. (2010) noted that quantifying the PCD/FNR in a single sample as a function of contaminant concentration and surface material was a major gap in the previous chamber and laboratory studies. The study and experimental design presented in this report fills this major gap for the CDC-recommended sponge-wipe collection, extraction, and analysis methods.

1.3 Performing Organizations and Funding

The work in this report was performed by staff in the Applied Statistics and Computational Modeling group at PNNL and by staff in the Chemical and Biological Systems group at SNL. The specific contributors are listed as authors of this report. Discussions with, and inputs by, members of the VSPWG (see Section 1.1) also guided the work.

The PNNL work was funded by two divisions within DHS S&T: 1) the Standards Office of the Test and Evaluation/Standards Division and 2) the Chemical and Biological Research and Development Branch of the Chemical and Biological Division. The work was funded under the prime contract DE-AC05-76RL01830 between the U.S. Department of Energy (DOE) and the operator of PNNL for research, testing, evaluation, and/or development activities and pursuant to Section 309(a)(1)(c) of the *Homeland Security Act of 2002* (Public Law 107-296), which authorizes DHS to task the DOE national laboratories on a “work for others” basis.

The SNL work was funded by the Chemical and Biological Research and Development Branch of the Chemical and Biological Division in DHS S&T. SNL is a multiprogram national laboratory operated by Sandia Corporation, a Lockheed Martin company, for the U.S. Department of Energy’s National Nuclear Security Administration under Contract DE-AC04-AL8500.

1.4 Report Organization

The remainder of this report describes the experimental design for the VSPWG sponge-wipe study and the basis for its development. The report is organized as follows. The factors that will be held constant in the experimental design are discussed in Section 2. The factors that will be varied in the experimental design are discussed in Section 3. The quality control samples that will be included in the sponge-wipe study are discussed in Section 4. Preliminary tests that will be used to determine the surface concentration levels of the surrogate contaminant are discussed in Section 5. The experimental design for the VSPWG sponge-wipe study is presented and discussed in Section 6. All of the gaps identified in previous chamber and laboratory studies could not be filled in this sponge-wipe study, so the advantages and disadvantages of the study are given in Section 7. The data analyses that will be supported by the chosen experimental design are described in Section 8. Recommendations for future studies are presented in Section 9. The references cited in the report are listed in Section 10.

2.0 Factors to Be Held Constant in the Sponge-Wipe Study

Table 2.1 lists the factors that will be held constant during the sponge-wipe study. Based on the funding available to SNL for this study as well as several challenges in performing the testing, it was decided to focus on samples collected using wipes. The sponge-wipe sampling method was selected for this study over the swab sampling method because swabs 1) had the most information available in previously conducted chamber and laboratory studies (Piepel et al. 2010), and 2) are the least frequently used sample collection method. There were several reasons for selecting wipe sampling over vacuum sampling methods. Although vacuum sampling methods have the least information available in the literature, they were considered to be too inconsistent and difficult to work with given the goals and objectives of this study. Also, while the vacuum method offers much appeal for reducing the limits of detection for surface sampling, currently used methods involve the use of a “leaky” dust collection sock with demonstrated low overall spore RE. Finally, the vacuum method is further complicated by the need for considerable media manipulation and handling in the laboratory. For all of these reasons, this study will focus on sponge-wipe samples, collected per methods developed by the CDC.

Table 2.1. Factors to Be Held Constant in the VSPWG Sponge-Wipe Study

Factor	Test Level
Sample collection media	Wipe ^(a)
Sample/Coupon Size	10-inch by 10-inch
Wetting agent	Per method ^(a)
BA surrogate	<i>Bacillus atrophaeus</i>
Interfering material	None (clean surfaces)
Deposition	Wet deposition
Storage/transportation	None
Laboratories	One (SNL) ^(b)
Preparation/extraction	Per method ^(a)
Analytical method	Culture

(a) The wipe sample collection and analysis methods are based on methods developed by CDC and will be documented separately by SNL.

(b) Lab-to-lab variation is typically a major contributor to reproducibility uncertainty, which ideally would be estimated via tests at several laboratories. However, the available funding is not sufficient to use different laboratories as part of the study.

The original plan was to perform a chamber study with dry aerosol deposition of the contaminant on test coupons inside a test chamber. However, preliminary testing by SNL showed it was not possible to reliably achieve low enough concentrations on coupons to realize false negatives with the CDC-recommended, sponge-wipe method (which has improved efficiency compared to other wipe methods). Hence, the plan was changed to perform a laboratory (bench-top) study using a liquid deposition method,

which can reliably achieve lower concentrations of the contaminant on test coupons, so that false negatives can be achieved.

Preparation/extraction methods, storage/transportation factors, and analytical laboratories will not be varied in this sponge-wipe study. The summary of previous chamber and laboratory studies (Piepel et al. 2010) identified 1) preparation/extraction factors to be very important to sampling efficiency and 2) a near absence in the literature of information about storage/transportation and laboratory effects. However, varying these factors in addition to the factors selected for varying in this test (discussed in Section 2) would comprise a very large study and be beyond the scope of this effort. Hence, it is proposed that these factors be investigated in future chamber or laboratory studies.

3.0 Factors to Be Varied in the Sponge-Wipe Study

Table 3.1 lists the factors that will be varied in the sponge-wipe study. Six surface materials will be tested: stainless steel, vinyl tiles, ceramic tiles, plastic, painted wood paneling, and faux leather. These materials were selected to span the range of surface roughness of non-porous materials (Shellenberger and Logan 2002, Hallas and Shaw 2006).

Eight different surface concentration levels will be used to span the range of PCD/FNR values (0 to 1) for each set of three surface materials. How the eight concentration levels will be determined is discussed in Section 5. The number of concentrations (eight) was chosen to protect against the possibility that the lowest two concentrations yield a PCD \sim 0 (FNR \sim 1), and the highest two concentrations yield a PCD \sim 1 (FNR \sim 0). In such a case, there would still be four concentrations to span the interior of the 0 to 1 range for PCD/FNR.

Table 3.1. Factors to Be Varied in the VSPWG Sponge-Wipe Study

Factor	Test Levels
Surface materials	Stainless steel, vinyl tile, ceramic tile, plastic, painted wood paneling, and faux leather.
Contaminant surface concentrations	Eight different concentrations (determined from preliminary tests—see Section 5). ^(a)
Number of test runs	16—Eight runs at eight nominal contaminant concentrations will be performed twice, in each of two blocks (sets), resulting in 16 runs total. Block 1 will test stainless steel, vinyl tile, and ceramic tile, while Block 2 will test plastic, painted wood paneling, and faux leather. ^(a)
Number of test coupons in a test run	30—Each set of three surface materials will have 10 coupons within a run. Positive and negative controls will increase the total number of samples in a run.
Personnel for sample collection, processing, and analysis	A minimum of three personnel per test run will be available to perform the sample collection, extraction, and analytical steps of the work.

(a) The eight concentrations for each of Blocks 1 and 2 will be determined by preliminary, range-finding tests (see Section 5).

It was originally planned to test a set of eight concentrations on the first three surface materials (stainless steel, vinyl tile, and ceramic tile) twice (in two blocks), which would have provided for 1) quantifying the uncertainty in test results for the three surface materials, 2) increasing the accuracy and precision of results, and 3) assessing the goodness-of-fit of the equations that will be developed from the data to relate response variables (e.g., RE and PCD) to contaminant concentrations for the three surface materials. However, results from the Block 1 tests had better precision than expected, and occurrences of false negatives at the lowest concentrations were fewer than expected. Thus, it was decided that a better use of the Block 2 tests would be to study the performance of the sponge-wipe method for three additional non-porous materials, with at least one or two of those materials providing a more difficult challenge for the sponge-wipe method.

There will be 30 test coupons within each test run (not counting positive and negative controls, which are discussed in Section 4). These 30 test coupons will consist of 10 test coupons from each of the three surface materials tested in Block 1 or Block 2. Having 10 coupons for each surface material will provide a good basis for fitting PCD/FNR-concentration equations. Each test coupon will have an associated reference sample, which will be used to determine the actual contaminant concentration on each test coupon (within deposition uncertainties). The reference samples will consist of putting the same number of drops of contaminant solution on a growth media plate as will be put on a test coupon. A reference sample will be done immediately before each test coupon, so that there is a one-to-one correspondence. These contaminant concentrations from the reference samples will be used to fit PCD/FNR-concentration equations and to calculate RE values (which will be used in fitting RE-concentration equations). See Section 8 for further discussion of data analyses that can be performed using data from the experiment design presented in this report.

Three technicians are planned to perform the sample collection, extraction, and analysis (i.e., plating and counting) steps of the sampling and analysis work. The personnel performing each task in the process will be varied as part of the experimental design, as discussed in Section 6.

4.0 Quality Control Samples in the Sponge-Wipe Study

Each test run in the sponge-wipe study will include a complement of quality-control (QC) samples, which will be thoroughly documented in the SNL test plan and/or test procedures. We provide a brief overview here because the numbers and placements of positive control (reference) samples are an important part of the experimental design for the study.

The positive controls will consist of one reference sample per test coupon, with the reference sample produced immediately before the corresponding spore-seeded test coupon. The reference samples will involve putting the same number of drops of contaminant solution directly on a growth media plate as will be put on the corresponding test coupon. The negative controls will consist of 1) blank coupons processed the same as spore-seeded coupons and 2) blank wipes taken out of their package, placed in the appropriate specimen container, and processed the same as wipe samples of contaminated coupons. Additional details regarding the positive and negative controls will be provided in the SNL test plan for the experimental work.

The positive controls will provide for quantifying, for each combination of eight concentrations and six surface materials, the PCD/FNRs and REs associated with the wipe sampling and analysis method. The negative controls are process controls; hence, a positive result on any negative control sample will invalidate the corresponding test. However, positive results on negative control samples will be documented because they provide for estimating the false positive rate (FPR) of the method.

5.0 Preliminary Tests to Determine Contaminant Concentrations

Preliminary “range finding” tests will be performed before Tests 1–8 (Block 1) and Tests 9–16 (Block 2) in the sponge-wipe study to determine the eight surface concentration levels that will be used for testing in Block 1 and Block 2, respectively. Because different surface materials will be tested in Blocks 1 and 2, the eight concentrations may or may not be the same. Each of the two sets of eight concentrations of the surrogate (*Bacillus atrophaeus*) will be selected with the goal that 1) the lowest concentration is rarely detected ($PCD \approx 0$), 2) the highest concentration is nearly always detected ($PCD \approx 1$), and 3) intermediate concentrations have PCD values spanning the range of 0 to 1. The data from Tests 1–8 (Block 1) and Tests 9–16 (Block 2) will be used to fit a PCD/FNR -concentration curve and RE -concentration curve for each of the six surface materials. The eight concentration levels for each block need to be determined across all three surface materials to be tested in that block. Figure 5.1 shows an example of how the concentration levels should be chosen, taking into account the three surface materials in each block: 1) stainless steel, vinyl tile, ceramic tile in Block 1 and 2) plastic, painted wood paneling, and faux leather in Block 2. The three colored curves in Figure 5.1 represent a hypothetical relationship between PCD and concentration for each of three surface materials.

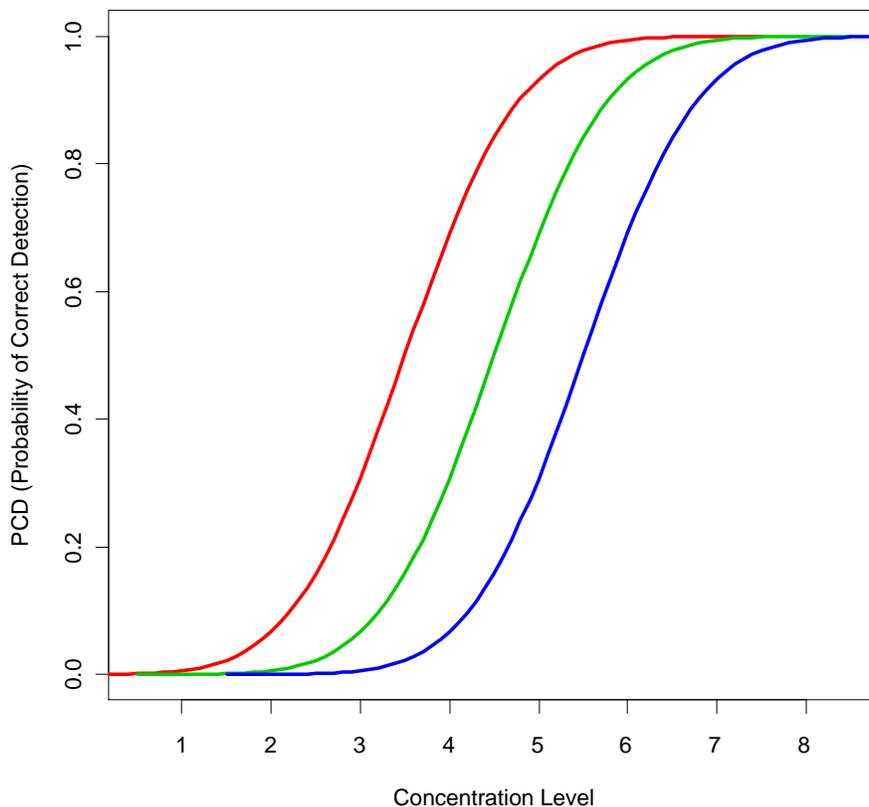


Figure 5.1. An Example of Eight Concentration Levels Selected to Span the Range of Probability-of-Correct-Detection (PCD) Values for Three Surface Materials Tested Within a Block. The three curves represent hypothetical relationships between PCD and concentration.

The set of eight concentrations for each block of tests should be chosen so that no more than two of them (for any of the set of three surface materials) would be expected to yield PCD values equal or very close to 0 or to 1. For example, suppose for a given surface material that concentrations #1 and #2 yield $PCD = 0$, while concentrations #7 and #8 yield $PCD = 1$. That would leave four concentrations to yield PCD values with intermediate values of $0 < PCD < 1$. Three to four concentrations with intermediate PCD values would be the minimally acceptable basis (during the data analysis phase of work described in Section 8) to fit sigmoidal equations to the PCD/FNR-concentration data.

As discussed in Section 2, the original plan to perform a chamber study with dry aerosol deposition of the contaminant on test coupons inside a test chamber was changed when preliminary aerosol test results demonstrated that concentrations low enough to characterize the FNR could not be accomplished using the aerosol method. The plan is now to perform a laboratory (bench-top) study using a liquid deposition method, which can reliably achieve lower concentrations of the contaminant on test coupons (so that some false negatives can be achieved). Because of this change in plans, a secondary objective was chosen for selecting the eight surface concentrations for the test runs. The primary objective is still to select the eight concentrations to span the range of PCD values with no more than two concentrations yielding $PCD = 0$ or $PCD = 1$ for any of the three surface materials tested within a block. A secondary objective is needed to select concentrations for liquid deposition that overlap with the concentrations for dry aerosol deposition investigated by SNL in preliminary tests. It would be desirable to have at least two concentrations in the liquid-deposition study that were also tested in preliminary dry aerosol deposition tests. This would provide a basis for quantifying any difference in results for liquid deposition versus dry aerosol deposition. However, it is already known that the lowest contaminant concentrations that could be achieved by dry aerosol deposition yielded $PCD = 1$. Hence, it may not be possible to achieve the secondary goal without partially violating the primary goal (i.e., having no more than two liquid-deposition concentrations with $PCD = 1$). The specific concentrations to be tested will be decided on by SNL and PNNL, based on additional preliminary liquid-deposition tests.

6.0 Experimental Design for the Sponge-Wipe Study

The experimental design for the 16 test runs is a *split-plot experiment* (Steel and Torrie 1960, Chapter 12; Myers and Montgomery 1995, Section 9.6) run in two *blocks*. Originally, it was planned to test the same three surface materials in Block 1 and Block 2 with the same eight surface concentrations (although in a different randomized order in each block). However, when Block 1 results showed 1) lower-than-expected FNRs for the lowest concentrations and 2) higher-than-expected precision in the sampling method for the original three surface materials, it was decided to test three different surface materials in Block 2. Hence, Block 1 contains Tests 1–8 with one set of eight concentrations, using stainless steel, vinyl tile, and ceramic tile as the three surface materials. Block 2 contains Tests 9–16 of a slightly different set of eight concentrations, using plastic, painted wood paneling, and faux leather. The block, split-plot, and randomization aspects of the experimental design are further described in the following paragraphs.

The eight concentrations to be tested in each block were randomly assigned to protect against confounding any possible time-trend effects with concentration effects over the eight runs within each block. A *split-plot experiment* results because one concentration must be applied to all coupons/tests in a given test run. In the terminology of split-plot experiments, the test runs are the *whole-plot (WP) units*, and concentration is the *WP treatment factor* (with eight concentration levels of the factor). Within a test run, the three non-porous surface materials will be investigated with 10 test coupons for each material. Hence, the *sub-plot (SP) units* are the coupons and the *SP treatment factor* is the surface material type.^(a) The assignment of surface materials to the 30 test coupons (3 materials \times 10 coupons each) is discussed subsequently. The basic concept and terminology of split-plot experiments is introduced because it must be accounted for in analyzing the resulting data.

(a) The split-plot, whole-plot, and sub-plot terminologies come from the original application of split-plot designs in agricultural field trials. The terminology has been retained, even though split-plot experiments are widely used in many application areas.

Table 6.1 lists the general aspects of the split-plot experimental design for the VSPWG sponge-wipe study. The test numbers listed in the first column of

Table 6.1 represent the run-order of the test runs as well as the test numbers. In Block 1, the first two test runs were assigned concentrations #3 (third lowest) and #6 (third highest) to provide a basis for modifying subsequent concentrations, if needed. This presumes that results for Tests 1 and 2 will be ready for assessment before beginning Test 3. If concentration #3 yields detections on 0 of the 10 (0/10) coupons of a given surface material, presumably the same result will occur for concentrations #1 and #2. In that case, too many low concentrations were initially selected, and there would be a need to modify the concentration levels. Similarly, if concentration #6 yields detections on all 10 of 10 (10/10) test coupons of a given surface material, presumably the same result will occur for concentrations #7 and #8. In that case, too many high concentrations were initially selected, and it may be necessary to modify the concentration levels. An exception may occur, however, if it is necessary to select the two highest concentrations (#7 and #8) for liquid deposition that match the two lowest concentrations investigated in preliminary dry aerosol tests. In that case, it may be expected that concentrations #6, #7, and #8 will yield PCD = 1 results. That would be acceptable to partially violate the primary criterion so as to meet the secondary criterion, as discussed in Section 5. However, selecting concentrations #5, #6, #7, and #8 such that all four are expected to yield PCD = 1 should definitely be avoided, because there would not be a sufficient distribution of PCD values

Table 6.1. Test Matrix for Randomized Complete Block, Split-Plot Experimental Design of the VSPWG Sponge-Wipe Study to be Performed at SNL^(a)

Test Run	Block	Concentration ^(b)	# Test Coupons	# Test Coupons	# Test Coupons	# Positive Controls ^(c)	# Negative Controls ^(d)	Sample Collection Bench 1 & 3 ^(e)	Sample Collection Bench 2 & 4 ^(e)	Sample Processing ^(e)	Sample Enumeration ^(e)
1	1	3 ^(f)	10	10	10	30	10%	1	2	2	3
2	1	6 ^(f)	10	10	10	30	10%	2	3	3	1
3	1	1	10	10	10	30	10%	3	1	1	2
4	1	5	10	10	10	30	10%	1	2	1	3
5	1	2	10	10	10	30	10%	3	1	3	2
6	1	7	10	10	10	30	10%	2	3	2	1
7	1	4	10	10	10	30	10%	1	3	1	2
8	1	8	10	10	10	30	10%	2	1	2	3
			Faux Leather	Plastic	Painted Wood						
9	2	7	10	10	10	30	10%	3	2	3	1
10	2	2	10	10	10	30	10%	2	3	2	1
11	2	4	10	10	10	30	10%	1	2	1	3
12	2	5	10	10	10	30	10%	3	1	3	2
13	2	1	10	10	10	30	10%	3	2	2	1
14	2	8	10	10	10	30	10%	2	1	1	3
15	2	3	10	10	10	30	10%	1	3	3	2
16	2	6	10	10	10	30	10%	2	1	1	3

- (a) Specifics of the test matrix for each test run not shown in this table are given in Table 6.2 and Table 6.3.
- (b) The target surface concentrations are represented by numbers, where 1 = lowest concentration, 2 = next lowest concentration, ... , 8 = highest concentration. The target concentrations are listed in Table 6.2.
- (c) There will be one positive control (reference) sample for each of the 30 test coupons (10 coupons each of the three surface materials) in a test run. This is a critical aspect of the experimental design because it provides for capturing any effects of coupon location, time trend, or other “nuisance variables.”
- (d) There will be two kinds of negative controls: blank (uncontaminated) coupons and blank wipes (removed from their packages and immediately placed in the same kind of sample containers to be used for contaminated sample coupons). It is anticipated that the number of such samples will be approximately 10% of the number of test samples, but the final determination of the numbers will be determined by SNL and documented in their test plan.
- (e) There will be three technicians available to perform the four testing tasks for a given test run. These tasks are (a) sample collection Benches 1 and 3, (b) sample collection Benches 2 and 4, (c) sample processing, and (d) sample enumeration. The numbers 1, 2, and 3 represent the specific technician assigned to perform each task for each test run. For logistics reasons, the technician who performs task (c) is one of the technicians who performed tasks (a) or (b).
- (f) The concentrations are tested in a random order within each block with the exception of concentrations 3 and 6 testing in the first two runs as discussed in the body of the report.

to develop PCD-concentration equations from the data. After the first two test runs, the remaining concentration levels for Tests 3 to 8 were randomly assigned within Block 1.

The eight concentration levels were separately randomly assigned to runs within Block 2. A process similar to the one discussed above for evaluating the upper and lower concentrations in the first two test runs applies to the Block 2 tests, although they begin with concentrations #7 and #2. The eight concentrations to be tested in each of Blocks 1 and 2 are summarized in Table 6.2.

Table 6.2. Surface Concentrations to be Tested in Blocks 1 and 2 of the Sponge-Wipe Study

Block	Concentration # and Value (spores/coupon)							
	1	2	3	4	5	6	7	8
1	2	5	10	15	20	25	100	1200
2	2	5	10	15	20	25	35	100

Because the eight concentrations will be used for all three surface materials (in each block) and detection results may vary by surface material, a decision to change some of the concentration values after Tests 1 and 2 will depend on the results for the three materials collectively. Some illustrative examples for Block 1 are now given.

- Example 1: Suppose for concentration #3 (Test 1) that two or three of the surface materials yield 0/10 detections. Similarly, suppose for concentration #6 (Test 2) that two or three of the surface materials yield 10/10 detections. In this example, concentrations #3 and #6 would become the new lowest and highest concentrations, respectively, and the remaining six concentrations would be spread over the range between the concentration values for concentrations #3 and #6.
- Example 2: Suppose concentration #3 yields 2/10 detections for two or three of the surface materials, while concentration #6 yields 10/10 detections for two or three of the surface materials. In this case, concentrations #1, #2, and #3 would remain as originally determined, and concentrations #4, #5, #7, and #8 would be re-distributed between the values for concentrations #3 and #6.
- Example 3: Suppose concentration #3 yields at least 1/10 detections and concentration #6 yields 9/10 detections for two or three surface materials. Then, the originally selected concentration levels would be used with no adjustments.

Similar examples could be written for Block 2. The need to adjust concentration levels during testing in Blocks 1 and 2 should be minimized based on the range-finding tests that will be performed to select the concentrations to be tested in each block (see Section 5). However, it is good to have in place a back-up plan in case adjustments to the concentration levels are needed after testing has begun.

The last four columns of Table 6.1 show assignments of four tasks to the three technicians who will perform the testing. These tasks consist of (a) sample collection on Benches 1 and 3, (b) sample collection on Benches 2 and 4, (c) sample processing, and (d) sample enumeration. The four test benches are represented in Figure 6.1. Technicians were assigned to tasks (a), (b), and (d) in a nearly balanced way, with the technician performing task (c) being the same as one of those who performed tasks (a) or (b). The nearly balanced assignment of technicians to the testing jobs protects against technician effects

(if any) possibly becoming confounded with the effects of concentration and surface material (the main test factors).

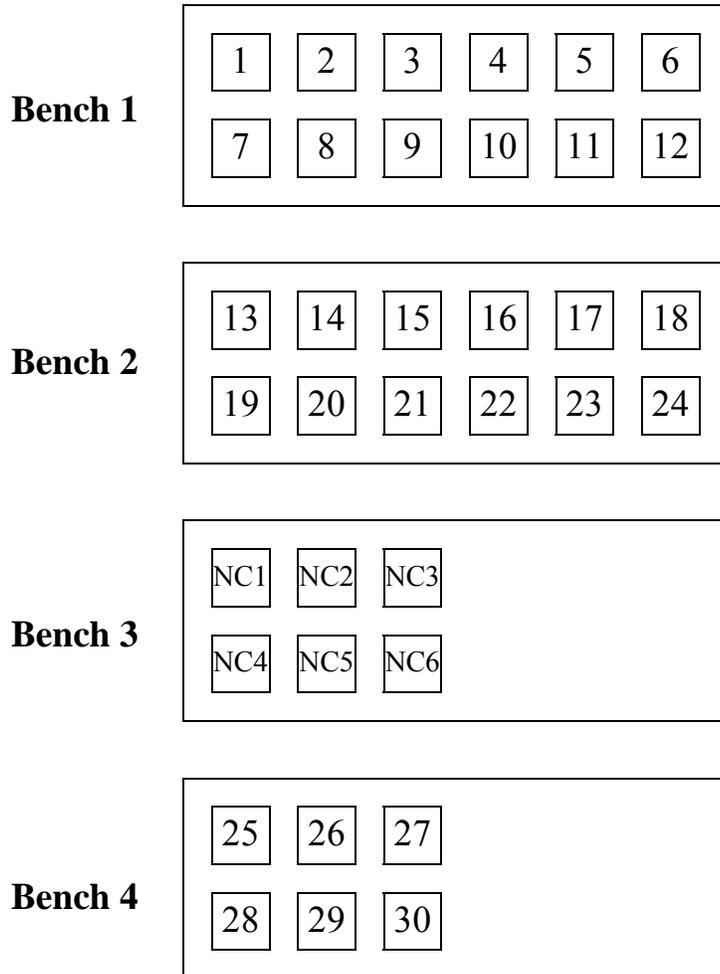


Figure 6.1. Bench Testing Configuration Showing the Locations of the 30 Test Coupons (1, 2, ... , 30) and Six Negative Controls (NC1, ... , NC6) for a Given Test Run. Note that the bench-tops and coupon locations are not drawn to scale and are a pictorial representation only.

The contaminant deposition and sample collection aspects of the testing will be performed on benches (each 72 inches long and 36 inches wide) located in the center of a room. Up to 18 of the 10-inch × 10-inch test coupons will fit on each bench. Originally only 24 (instead of 30) coupons were to be tested in each test run, so it was decided to put 12 coupons per bench on two benches. However, subsequently the decision was made to have 30 coupons (10 each of the three surface materials) per test run. This necessitated two benches each with 12 sample coupon locations (Benches 1 and 2), and another bench with 6 sample coupon locations (Bench 4). Table 6.3 lists (for each of the 16 test runs) the assignments of the 10 coupons for each set of three surface materials to the 30 bench positions (shown in Figure 6.1). The assignment of surface materials to bench positions 1 to 24 was performed using an *optimal experimental design* approach (Atkinson et al. 2007) implemented using SAS (2008) so that the split-plot

structure of the experiment, as well as the effects of the block variable, concentrations (runs) within blocks, and bench positions could be accounted for during data modeling and analysis.

Table 6.3. Assignment of Surface Material Test Coupons^(a) to Bench Positions for Each Test Run

Bench ^(b) Position	Test Run															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Concentration, Block 1 ^(c)								Concentration, Block 2 ^(c)							
	3	6	1	5	2	7	4	8	2	7	4	5	1	8	3	6
1	V	S	C	V	C	V	S	C	P	L	P	W	L	L	W	W
2	S	C	C	V	C	V	S	V	W	L	P	W	P	L	W	L
3	V	S	S	V	C	V	C	V	W	L	L	W	W	P	L	W
4	V	C	S	V	C	V	S	C	P	L	W	P	W	L	P	L
5	C	S	C	V	V	V	C	S	L	P	W	P	L	W	L	W
6	C	V	S	S	S	C	S	V	P	W	W	P	P	W	L	W
7	S	V	C	C	V	S	S	C	L	P	L	W	L	W	P	P
8	V	S	V	C	S	S	C	V	L	P	W	L	W	P	W	L
9	S	V	S	C	V	S	C	C	P	L	W	L	P	P	W	L
10	S	C	V	S	V	C	V	V	W	P	L	W	W	L	P	L
11	C	V	S	C	S	S	V	C	W	P	L	L	W	P	P	P
12	C	C	V	S	S	C	V	S	P	P	W	L	P	W	L	P
13	C	V	S	S	C	V	V	C	L	W	L	P	P	L	W	P
14	C	S	V	C	S	V	C	S	L	W	P	L	P	W	L	P
15	C	V	S	C	V	C	S	S	W	P	W	L	P	L	P	W
16	V	S	C	S	S	C	V	S	W	W	P	L	L	W	P	P
17	C	S	V	S	C	V	S	C	L	W	P	P	L	P	W	P
18	S	C	V	S	V	S	C	V	L	W	P	W	L	P	W	L
19	S	V	C	V	C	S	V	S	W	L	P	W	L	W	P	L
20	S	C	S	C	V	S	C	V	P	L	W	P	L	W	W	P
21	V	V	C	S	S	C	V	C	P	L	L	P	W	P	L	W
22	S	C	V	V	C	S	C	S	P	W	P	L	W	L	P	L
23	V	C	V	C	S	C	V	S	W	P	L	P	W	L	L	W
24	V	S	C	V	V	C	S	V	L	W	L	W	P	P	L	W
25	C	C	C	C	C	C	C	C	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)
26	V	V	V	V	V	V	V	V	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)
27	S	S	S	S	S	S	S	S	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)
28	C	C	C	C	C	C	C	C	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)
29	V	V	V	V	V	V	V	V	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)
30	S	S	S	S	S	S	S	S	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)

(a) S = stainless steel, V = vinyl tile, C = ceramic tile, P = plastic, W = painted wood, and L = faux leather.

(b) The bench position numbers are shown in Figure 6.1.

(c) The concentration numbers 1 to 8 represent the eight concentrations from smallest to largest, as listed in Table 6.2 for each of Block 1 and Block 2 tests.

(d) The two coupons of each of the three surface materials (P, W, L) for these locations will be assigned during Block 2 testing.

Although the bench, row on a bench, and column on a bench are not variables of direct interest for the testing, it is a good idea to account for possible effects of such nuisance variables in the experimental design. Doing so protects against the possibility that there may be position effects (e.g., in spore deposition or in sample collection) over the benches and positions. Such bench or position effects would otherwise be confounded with the effects of surface material (a test factor).

As previously noted, it was decided to use 10 (rather than 8) coupons of each surface material in each run after the assignment of 24 coupons to test positions 1 to 24 was completed for Block 1. Hence, the extra two coupons of each surface material were arranged in test positions 25 to 30 on Bench 4 (see Figure 6.1) as shown in Table 6.3. The assignments of these “extra” coupons for Block 2 will be determined before testing.

Figure 6.1 also displays the six locations on Bench 3 that will be used for negative control tests. These locations will have two uncontaminated samples of each of the three surface materials in Block 1 (stainless steel, vinyl tile, and ceramic tile) and Block 2 (plastic, painted wood paneling, and faux leather). The assignments of these materials to the sample locations on Bench 3 are listed in Table 6.4 for Block 1. These assignments for Block 2 will be made before testing and hence are not shown in Table 6.4.

In summary, the experimental design for the VSPWG sponge-wipe study is documented in Table 6.1 through Table 6.4.

Table 6.4. Assignments of Surface Materials^(a) to the Negative Control Sample Locations on Bench 3

Block	Negative Control Sample Location on Bench 3					
	NC1	NC2	NC3	NC4	NC5	NC6
1	C	V	S	C	V	S
2	(b)	(b)	(b)	(b)	(b)	(b)

(a) S = stainless steel, V = vinyl tile, C = ceramic tile, P = plastic, W = painted wood, and L = faux leather.

(b) The assignments of negative controls to Bench 3 positions will be made before Block 2 testing.

7.0 Gap Coverage, Advantages, and Disadvantages of this Study

Many previous chamber and laboratory studies have been performed and were summarized by Piepel et al. (2010). Several gaps were identified in these previous studies, and the current sponge-wipe study was planned to fill some of the gaps. The main gaps identified were 1) lack of results quantifying PCD/FNR and RE as functions of contaminant concentration, surface material, sample collection and analysis methods, and other factors, 2) lack of studies to investigate and quantify the effects of sample storage and transportation conditions on the performance results of sampling and analysis methods, and 3) failure of previous studies to capture all of the sources of uncertainty affecting performance results of sampling and analysis methods under various conditions. Regarding the last gap, many of the studies investigated only short-term, within-test uncertainties (repeatability), and did not investigate run-to-run or lab-to-lab uncertainties (reproducibility). Hence, the estimated uncertainties of performance measures reported in those studies can be expected to underestimate the total uncertainty.

Given the resources available for the sponge-wipe study, it was not possible to fill all of the gaps identified by Piepel et al. (2010). The advantages and disadvantages of the sponge-wipe-study experimental design presented in this report are discussed in the following bullets.

Advantages

- The study will provide for developing PCD/FNR-concentration and RE-concentration equations and corresponding uncertainty expressions for each of six surface materials to be tested in Block 1 (stainless steel, vinyl tile, and ceramic tile) and in Block 2 (plastic, painted wood paneling, and faux leather). These equations and expressions will provide for predicting the PCD/FNR, RE, and their uncertainties for any concentration within the range tested. Failure to develop PCD/FNR-concentration and RE-concentration equations was the largest gap identified by Piepel et al. (2010) in their summary of previous chamber and laboratory studies for BA (or surrogates) contamination.
- Tests will be conducted using sponge-wipe sample collection, preparation, extraction, and analysis methods according to procedures developed by the CDC. Thus, what are expected to be validated methods associated with sponge-wipe surface sampling will be used to achieve the goals of the study.
- Positive-control (reference) samples will provide for quantifying the contaminant concentration level on each test coupon. The concentration in the positive-control sample associated with each test coupon will be used to calculate REs and will also serve as the concentration value in fitting PCD/FNR-concentration and RE-concentration equations. The control sample concentration values will also be used to identify any possible variations in concentrations across test coupons within a test run because of time trend, bench, bench position, and/or other effects.
- Technicians who perform the sample collection, processing, and counting stages of testing will be varied in a balanced, randomized way during testing. This will avoid confounding their effects, if any, with the effects of surface material and contaminant concentration. It will also provide some capability to assess any technician effects on the results.
- Performance data for the sponge-wipe sample collection, extraction, and analysis methods recommended by CDC will be obtained for a BA surrogate (*Bacillus atrophaeus*) using wet deposition on test coupons. The performance data for the concentrations used in this study will be

compared to the lowest concentration data from preliminary dry aerosol tests. The goal of this comparison is to provide a basis for predicting the performance of the sponge-wipe method for lower concentrations of dry-aerosol-deposited contaminant than were able to be tested.

Disadvantages

- Only the sponge-wipe sample collection method will be tested in this laboratory study. It is envisioned that swab and vacuum sampling methods would be addressed in future similar studies.
- Preparation and extraction methods can have a significant effect on sampling performance (e.g., RE and PCD/FNR), but those factors will not be varied in this study. In this study, the preparation and extraction methods recommended by CDC and used in Laboratory Response Network laboratories will be used. Other preparation and extraction methods have been investigated and documented in the literature, and additional studies varying these factors could be performed in the future if necessary.
- The study will not consider the effects of storage and transportation factors on sponge-wipe sampling performance. Insufficient information on these factors was identified as a gap by Piepel et al. (2010). CDC has recently completed a storage/transportation study, and it is recommended that the results of that study be evaluated to decide whether additional studies are needed.
- Only the SNL laboratory will be used, and therefore lab-to-lab variation will not be investigated or quantified. Three SNL technicians will perform the test steps in the 16 test runs. However, given the small number of technicians, their schedules, and the number of steps in the work, it was not possible to assign technicians so as to capture personnel-related sources of reproducibility uncertainty. Instead, technicians were assigned in a balanced, randomized way to the test steps to avoid confounding any possible technician effects with effects of the primary test variables (concentration and surface material).
- Run-to-run uncertainty will not be quantifiable because of the decision to test three different materials in Block 2 rather than replicating the Block 1 tests in Block 2. The advantage of gathering performance data on six surface materials with a wider range of surface characteristics than for the initial three materials was considered to out-weigh the disadvantage of not being able to quantify run-to-run uncertainty.
- The study was initially planned with dry aerosol deposition of the contaminant. However, preliminary tests showed that the sponge-wipe method was efficient enough that no false negatives were obtained at the lowest surface concentrations of contaminant that could be achieved by dry-aerosol deposition. Hence, it was necessary to change the study to using wet deposition of the contaminant, which will allow achieving the lower surface concentrations of the contaminant needed to obtain some false negatives with the sponge-wipe method.

These advantages and disadvantages should be kept in mind when 1) assessing and drawing conclusions from the results of this sponge-wipe study and 2) planning any future chamber or laboratory studies.

8.0 Planned Data Analyses

An important part of designing an experiment is planning the data analyses to be performed so that the experimental data will adequately support those analyses. In general, it is envisioned that the sponge-wipe study will support completing all of the entries in Table 1 of Piepel et al. (2010), with the exceptions of lab-to-lab uncertainty (since only one laboratory will be used) and run-to-run uncertainty (since it was decided to test three different materials in Block 2). For convenience, that table is reproduced in this report as Table 8.1.

Focusing on the most important items, the experimental design for the sponge-wipe study presented in this report will support:

- Estimating the PCD/FNR and the corresponding uncertainty for each combination of contaminant concentration and surface material.
- Estimating the surface concentrations (e.g., colony forming units [CFU]/cm²) and their uncertainties (e.g., percent relative standard deviation [%RSD]) of the contaminant for each combination of concentration and surface material.
- Estimating RE (%) and their uncertainties (% or %RSD) for each combination of contaminant concentration and surface material.
- Fitting PCD/FNR-concentration and RE-concentration equations for each of the three surface materials, assessing goodness-of-fit of the equations, and quantifying the uncertainty in PCD/FNR and RE predictions made with the fitted equations.

Using results of the sponge-wipe study, it will be possible to calculate various kinds of “limits of detection” for the six non-porous materials using the CDC-recommended, sponge-wipe sampling and analysis methods. The *limit of detection* (LOD) is controversial because different researchers define and calculate it in different ways, which is often inadequately discussed. We define the LOD as the smallest amount or concentration that can be detected above the noise in a procedure and within a stated confidence level (e.g., 95%). The *lower limit of detection* (LLD) is the surface concentration of spores that will produce a detectable count of CFUs from a culture analysis of samples. The sponge-wipe study will also provide data for determining the *method detection limit* (MDL), where samples are processed through the complete analytical method. Finally, surface concentrations that will yield specified values of the PCD (e.g., 0.90 or 0.95) can be calculated from the equations mentioned in the last bullet above.

Section 6 of this document describes the experimental design with a split-plot structure in which contaminant concentrations are the WP treatments and surface materials are the SP treatments. Because it was decided to test three different surface materials in Block 2 than were tested in Block 1, the experimental design no longer involves testing all eight of the nominal contaminant concentrations twice. Such “whole-plot” replicates provide for estimating the WP uncertainty, although fitting PCD/FNR-concentration equations with a few less parameters than the number of concentration levels will also provide for estimating the WP uncertainty. Replicate coupons of each surface material within each test run will provide for estimating SP uncertainty. Some data analyses (such as fitting RE-concentration and PCD/FNR-concentration equations) will not require more complicated split-plot data-analysis methods because those data analyses will be performed separately for each surface material. Hence, the lack of WP replicates will not affect those data analyses. However, if attempts are made to develop RE-

concentration and PCD/FNR-concentration equations applicable to a range of surface materials (e.g., possibly by using surface characteristics as predictor variables), then split-plot data-analysis methods will be needed.

Table 8.1. Information Summarized by Piepel et al. (2010) in Their Literature Review of Previous Chamber and Laboratory Studies for Sampling and Analyzing Contaminated Surfaces

Study Characteristics	
Row Number	Continuous number to aid in referring to specific rows of a table
Study	Publication used to summarize the study
Test #	A number of the form x.y where x is 1, 2, 3, ... for each study, and y = 1, 2, 3, ... for the tests within a study
Swab/Wipe/Vacuum Characteristics	Characteristics or material of sampling method
Wetting Agent	Liquid, if any, used to wet or pre-moisten the sampling material (e.g., swab or wipe)
Surface Tested	Material type of surface sampled
Nature of Contaminant	<i>Bacillus anthracis</i> or related surrogate
Contaminant Amount	Amount or concentration of contaminant
Contaminant Deposition	How contaminant was deposited on the test materials
Transport / Storage Conditions	Transportation or storage conditions for samples before analysis
Preparation/Extraction Method	Method used to prepare a sample and extract the contaminant
Analytical Method	Method used to analyze prepared samples
Study Results	
Study Results - Mean and SDs	
# Test Runs	The number of test runs (set up and performed separately at different times). These may be thought of as “blocks” in statistical experimental design terminology.
Total # Test Samples	Total number of samples used to calculate mean and standard deviations, as well as recovery efficiency
Mean (CFU/cm ²)	Average contamination over the number of samples
Lab %RSD	Lab-to-lab percent relative standard deviation, which includes the variation from preparing the samples, extraction, and analysis
Run %RSD	Percent relative standard deviation from replicate runs of a test performed at different times
Sample Within Run %RSD	Percent relative standard deviation from replicate tests performed at the same time (in one run)
Within Sample %RSD	Percent relative standard deviation from split preparations and/or analyses from a given sample
Total %RSD	Total percent relative standard deviation including all of the preceding sources of variation
Recovery Efficiency (RE) – Mean and %RSDs	
RE Mean (%)	Mean recovery efficiency over the number of test samples
RE Lab %RSD	Lab-to-lab percent relative standard deviation of RE
RE Run %RSD	Run percent relative standard deviation of RE
RE Sample Within Run %RSD	Sample-within-run percent relative standard deviation of RE
RE Total %RSD	Total percent relative standard deviation of RE
LOD (CFU/cm²) & Rates	
Definition of LOD	How the limit of detection (LOD) is defined
# of Data Points	Number of data points used to calculate the LOD
Positive Result Definition (CFU)	How a positive result (detection) is defined (e.g., CFU ≥ 1)
LOD (CFU/cm ²)	Value of the limit of detection
LOD SD or 95% CI (CFU/cm ²)	Standard deviation or 95% confidence interval for the LOD
Probability Curve for Detecting a Positive	Method used to develop a probability curve for correctly detecting contamination
False Negative Rate	False negative rate based on controlled tests where the sampled surface was known to be contaminated but yielded a negative result
False Positive Rate	False positive rate based on controlled tests where the sampled surface was known to be uncontaminated but yielded a positive result
Comments	Explanatory comments about the study or results

9.0 Recommendations for Future Studies

As discussed previously, the sponge-wipe study in this report only addresses some of the gaps in previous chamber and laboratory studies identified by Piepel et al. (2010). Hence, additional evaluations of previous studies and new experimental studies are recommended to address the following topics.

- Quantifying False Negative Rates for Swab and Vacuum Sampling Methods: Studies similar to the sponge-wipe study discussed in this report should be performed for swab and vacuum sampling and analysis methods recommended by the CDC. Other methods that are likely to be used should also be investigated (see the discussion in the following bullet). Piepel et al. (2010) summarized literature results from 4 vacuum studies (noting that more were needed) and 14 swab studies. However, none of these studies investigated a sufficient number of concentrations at low enough concentration levels to quantify the PCD/FNR as a function of concentration and any other affecting variables. Quantifying the FNR is critical to being able to calculate the confidence in detection and clearance decisions when all samples yield negative results.
- Quantifying Performance of Different Methods: The CDC has performed “validation studies” for the sponge-wipe method, a specific swab method, and corresponding recommended extraction and analysis methods. A similar study for CDC-recommended vacuum sampling, extraction, and analysis methods is planned. However, it is reasonable to expect that biological contamination events in various parts of the United States may result in different (than recommended by the CDC) wipe, swab, and vacuum sample collection methods being used, as well as different storage/transportation, extraction, and analysis methods. Previous studies summarized by Piepel et al. (2010) showed that different sample collection methods and different extraction methods can have significantly different performance results. Hence, it would be prudent to quantify the PCD/FNR performance of the two or three most likely to be used swab, wipe, and vacuum sample collection methods, transportation/storage methods, extraction methods, and analysis methods. We recommend that 1) the two or three most likely to be used methods of each type be identified and 2) the existing literature data summarized by Piepel et al. (2010) be evaluated to see how well the performance of those most likely methods has been investigated. If there are gaps, then additional experimental studies may be needed.
- Reproducibility Uncertainty: The majority of previous chamber and laboratory studies only quantified within-laboratory uncertainty (Piepel et al. 2010), and sometimes only short-term within-laboratory uncertainty. Lab-to-lab uncertainty and other sources of reproducibility uncertainty (e.g., test personnel, instruments, etc.) need to be quantified as functions of any affecting variables (e.g., contaminant concentration). It was not possible to include these features in the sponge-wipe study discussed in this report, and hence it is recommended that reproducibility uncertainty be quantified in future experimental studies for the sponge-wipe method as well as in any future swab and vacuum studies.
- Transportation and Storage Effects: The Almeida et al. (2008) study and the recent CDC study of storage/transportation effects on sample results (journal article being written) should be evaluated to determine if additional studies are needed to quantify storage and transportation effects as functions of any affecting variables (e.g., contaminant concentration).

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