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Evaluation of Vacuum-based Sampling Devices for Collection of *Bacillus* Spores from Environmental Surfaces

Assessment and Evaluation Report

Office of Research and Development
National Homeland Security Research Center

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Research Triangle Park, NC 27711

Disclaimer

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List of Acronyms and Abbreviations

$\%C_v$	Percent Coefficient of Variation
ADA	aerosol deposition apparatus
ANOVA	Analysis of Variance
APPCD	Air Pollution Prevention and Control Division
ATCC	American Type Culture Collection
CBRN	Chemical, Biological, Radiological, and Nuclear
CDC	Centers for Disease Control and Prevention
CFM	cubic feet per minute
CFU	colony forming unit(s)
CM	critical measurement
cm^2	square centimeter
CMAT	Consequence Management Advisory Team
COC	chain of custody
DCMD	Decontamination and Consequence Management Division
DFWED	Division of Foodborne, Waterborne, and Environmental Diseases
DHQP	Division of Healthcare Quality Promotion
DPG	Dugway Proving Ground
DQI	Data Quality Indicator
DQO	Data Quality Objective
ECBC	Edgewood Chemical Biological Center
EPA	U. S. Environmental Protection Agency
EtO	ethylene oxide
ft^2	square feet
HAZMAT	Hazardous Materials
H_2O_2	hydrogen peroxide
HVAC	heating, ventilation, and air conditioning
ID	Internal Diameter
in.	inch(es)
INL	Idaho National Laboratory
INOC	Inoculation
ISO	International Organization for Standardization
L	liter(s)
$L\ min^{-1}$	liters per minute
LR	Linear Range
LV	Laboratory Variability
MCE	Mixed Cellulose Ester
MDI	metered dose inhaler
MERV	Minimum Efficiency Reporting Value
MOP	Miscellaneous Operating Procedure
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management
PBS	Phosphate Buffered Saline

PBST	Phosphate Buffered Saline with 0.05% TWEEN® 20
PRB	Polyester-Rayon Blend
PTFE	Polytetrafluoroethylene
PVC	Polyvinylchloride
rpm	rotations per minute
RR%	Relative Recovery Percentage
RSD	Relative Standard Deviation
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
SCFM	standard cubic feet per minute
SOP	Standard Operating Procedure
STS	Sodium Thiosulfate
TEF	Trace Evidence Filter
VHP	Vaporous Hydrogen Peroxide
WAM	Work Assignment Manager

Executive Summary

The existing surface sampling strategy for a biological incident involving *B. anthracis* spores necessitates the use of various methods depending on the surface type. Currently-recommended surface sample collection methods include pre-moistened wipes (for smooth nonporous surfaces), vacuuming (for rough and porous surfaces), and wet swabs (for small and/or hard-to-sample areas such as keyboards). The currently-used vacuum-based method utilizes woven collection socks attached to a cardboard nozzle. Some criticisms of the current method are that the vacuum socks often come from the manufacturer with visible holes in the sock seams, the method is vulnerable to cross-contamination between samples, the socks are constructed of materials with large pore-sizes (> 1µm), and the filters are cumbersome for laboratory handling and extraction during analysis. This project comparatively evaluated the vacuum sock and two additional vacuum-based collection devices (37 mm filter cassette and 3M™ Trace Evidence Filter) for their sampling efficacy. The 37 mm filter cassette was evaluated with mixed cellulose ester (MCE) filters or polytetrafluoroethylene (PTFE) filters installed, each was considered a unique device. These data were generated so appropriate sampling devices could be selected following a *B. anthracis* incident.

A known quantity of *Bacillus* atrophaeus (*B. anthracis* surrogate) spores was aerosolized and deposited onto large coupons (1 square foot (ft^2)) of various materials common to the built environment, including carpet, upholstery, unpainted (smooth finish) concrete, and two types (electrostatic and mechanical) of heating, ventilation and air conditioning (HVAC) filters. Coupons were then subjected to vacuum-based sampling and sample analysis according to protocols developed jointly by the US Centers for Disease Control and Prevention (CDC) and US Environmental Protection Agency (EPA). Recovery was determined for each sampling method according to culture-based microbiological assays following physical extraction methods developed by the CDC.

Phase 1 of this study included the evaluation of vacuum-based sampling devices. These tests were conducted with four different devices and one sampling variation (fast or slow sampling rate at which the device passed over the sampled surface) for the vacuum sock. Samples were collected from three porous surface types for each device, each with ten replicate samples. Vacuum and wipe samples were also collected from stainless steel coupons as a method to standardize results collected on different days and with different inoculation devices.

For carpet, the slow vacuum sock method had the highest spore recovery. For concrete, the 37 mm MCE had the highest spore recovery. For upholstery, the 37 mm MCE had the highest spore recovery. The vacuum sock method afforded more ease of use and may therefore be more desirable for larger sample areas. However, on concrete and upholstery, the 37 mm MCE filter method demonstrated higher recoveries per unit area than did the vacuum sock device. The significance of these differences was dependent upon the statistical test performed. There are advantages and disadvantages of each device. Changing the speed at which the vacuum sock sampling device passed over the surface did not necessarily improve the recovery of spores from the porous surfaces tested.

During Phase 2, collection of *B. atrophaeus* spores from contaminated HVAC filters with two vacuum-based devices (devices with the highest recoveries from Phase 1, vacuum sock (slow rate) and 37 mm MCE cassette) was compared to sampling by extractive methods (removing a portion of the filter and extracting spores from the filter matrix). Contamination of HVAC filters during a biological incident, unlike

most surfaces, is expected to occur under flow conditions, with HVAC blowers pulling spores to the interior of the filter. A method was developed which successfully deposited spores on (and within) HVAC filters under flow conditions.

Two types of clean (new) HVAC filters (electrostatic and mechanical) were inoculated similarly to Phase 1, yet under flow conditions. The contaminated surfaces were then evaluated using three methods: direct extraction from excised sections of the HVAC filter, vacuum sock sampling, and 37 mm MCE filter vacuum sampling. In addition, the magnitude and variability of the inoculating metered dose inhaler (MDI) was evaluated with three methods: sponge-wipe sampling of stainless steel coupons, pre-moistened (polyester-rayon blend, PRB) wipe sampling of stainless steel coupons, and direct extraction of stainless steel coupons.

Three methods (two vacuum methods and one extractive) of sampling HVAC filters performed reliably on mechanical and electrostatic filters. The data suggest that extractive methods are more efficient than vacuum-based recovery methods for electrostatic filters, but not for mechanical filters. When comparing the two vacuum-based methods, the vacuum sock method provided higher recoveries than the 37 mm MCE method from electrostatic filters, but there was no statistical difference in recoveries from the electrostatic filters. Vacuum-based methods may be more applicable to HVAC filter media that are not easily sectioned.

Phase 2 data also suggested there was no statistical difference between direct extraction, sponge-wipes, or pre-moistened PRB wipes for recovery (inoculum magnitude) from stainless steel coupons. Mean variability (%C_v) was also similar across all methods, at 28%, 22%, and 20% for the extraction-based method, sponge-wipe method, and PRB wipe method.

During Phase 3, Inoculum Variability and Level Tests (INOCs) were conducted to determine the repeatability and magnitude of inoculation from the MDI/ aerosol deposition apparatus (ADA) dosing method. Three inoculum levels (corresponding to three concentrations of spores within MDIs - 10^4, 10^6, 10^7 spores per coupon) were used to dose up to ten replicate stainless steel coupons. The MDI inoculation method was demonstrated over a broad range of surface contamination concentrations, from 1 to 1 × 10^4 CFU/square centimeter (cm^2).

Also during Phase 3, Linearity Recovery (LR) Tests and Laboratory Variability (LV) Tests were conducted to determine: (1) if vacuum-based method recoveries were linear over a range of concentrations; and (2) if recoveries varied significantly between two different technicians processing (extracting and plating) the samples. During these tests, carpet coupons were sampled using two chosen vacuum sampling methods: vacuum sock (slow) and 37 mm MCE. Following collection, all samples were extracted and plated by two technicians. All samples for both devices and all three inoculum levels were collected on a single day so that comparisons could be made across devices, inoculum levels, and technicians. One technician operated the vacuum-based collection device for all samples. Recoveries were compared between the two technicians to determine if technician-induced variability was significant. The linearity of recoveries for each device was determined graphically by plotting recovery versus the targeted inoculum. The two vacuum methods were demonstrated effective for all inoculum concentrations. Compared to sponge-wipes (from stainless steel), the vacuum sock method recovered between 3% and 22% CFU, while the 37 mm MCE method recovered between 4% and 140% CFU from carpet samples. Relative recoveries greater than 100% were possible because these values were determined by dividing vacuum method recovery by wipe-based recovery from stainless steel. Greater than 100% relative recovery

indicated that the vacuum method out-performed the wipe method. Finally, the extraction procedures for both vacuum methods were evaluated by two independent laboratory technicians with no statistical difference in the two recoveries.

These data were collected to aid in sampling device and strategy selection following a biological contamination incident.

1 Introduction

The U.S. Environmental Protection Agency (EPA) conducted a study, in collaboration with the Centers for Disease Control and Prevention (CDC), to evaluate several vacuum-based sampling devices for collection of biological agent from environmental surfaces. Methods for detection and characterization of biological agent on surfaces following a bioterrorism incident include the use of swabs, wipes, and vacuum. Vacuum-based methods are preferred when sampling porous surfaces. Currently, there are no vacuum-based methods validated for collection of *Bacillus anthracis* spores. Further, multiple vacuum-based devices, sample collection methods, and sample extraction methods were utilized to characterize the extent of contamination following the 2001 anthrax incidents. Recently, some work has been conducted to characterize the performance of vacuum-based surface sampling devices when used to collect *Bacillus* spores [1-3]. However, significant gaps remain in our understanding of vacuum-based sampler performance, efficiencies, ease of use, and applicability to various material surface types. The current study was conducted to generate data that could be used to inform selection of appropriate sampling methodologies following a *B. anthracis* incident. Scientifically tested sampling methods will provide increased confidence in the ability to characterize contamination following such an incident.

1.1 Process

Consistent with previous sampling studies [1], spores of *Bacillus atrophaeus* (formerly known as *Bacillus globigii* or *Bacillus subtilis* var. *niger*) served as surrogates for *Bacillus anthracis* spores. Collection of *B. atrophaeus* spores from multiple surface types was evaluated with four vacuum-based sampling devices. In addition, collection of biological agent from contaminated heating, ventilation, and air conditioning (HVAC) filters with the two top-performing vacuum-based devices was compared to sampling by destructive methods (removing a portion of the filter and extracting spores directly from the filter matrix).

A known quantity of aerosolized dry *B.atrophaeus* spores was gravitationally deposited onto large coupons (35.6 cm x 35.6 cm) of various materials common to the built environment, which included carpet, upholstery, unpainted (smooth finish) concrete, and two types of HVAC filters. The coupons were then subjected to vacuum-based sampling according to protocols developed jointly by the CDC and EPA. Recovery was determined for each sampling method according to culture-based microbiological assays following physical extraction methods developed by the CDC. All test parameters, such as coupon materials and sizes, sampling methods, and methods of extraction and analysis were determined by agreement among participating experts from EPA and the CDC.

1.2 Project Objectives

The objective of this project was to evaluate four currently-available vacuum-based devices for their ability to recover *Bacillus* spores from environmental surfaces. Performance (relative recovery) of the devices was compared to recoveries from pre-moistened gauze wipes, used to sample reference stainless steel coupons that were inoculated at the same time as the test coupons. Evaluation of operational parameters included time required for sample collection, the physical impact on the sampling team during collection, time required for sample analysis, and the cost of media and analysis equipment and supplies. Another objective of this study was to evaluate vacuum-based sampling of contaminated HVAC filters to that of extraction-based methods.

1.3 Experimental Approach

The experimental approaches that were used to meet the objectives of this project are:

- Use of controlled chambers, standardized sections and spore inoculums.
- Inoculation of material coupons via aerosol deposition of bacterial spores.
- Quantitative assessment of spore recoveries with each device, by sampling representative sections of materials and then extraction of sampling media to recover collected spores (enumerated and reported as colony forming units (CFU)).
- Use of reference stainless steel coupons to quantify and standardize results for cross-comparison of tests.
- Documentation of operational considerations (e.g., cross-contamination, procedural time, impacts on materials and personnel).

All testing was conducted at EPA's Research Triangle Park, NC, campus.

1.3.1 Vacuum-based Sampling Device Evaluation Tests – Phase 1

The Vacuum-based Sampling Device Evaluation Tests were conducted with four different device types and one sampling variation (sampling speed) for one device type (Table 2-1). Samples were collected from three surface types (carpet, upholstery, and concrete) for each device with ten replicate samples. Pre-moistened polyester rayon blend (PRB) wipe samples were also collected from reference stainless steel coupons as a method to standardize results collected on different days and with different inoculation devices. The foundation for this test matrix was described in the Quality Assurance Project Plan (QAPP) entitled, "Evaluation of Vacuum-based Sampling Devices for Collection of Biological Agent (Decontamination and Consequence Management Division (DCMD) 3.60)" (available upon request).

1.3.2 HVAC Inoculation Tests – Phase 2

HVAC Inoculation (HI) Tests were conducted to determine the best inoculation method for HVAC filters. Contamination of HVAC filters during a biological incident, unlike most surfaces, is expected to occur under flow conditions, with HVAC blowers pulling spores to the interior of the filter. Also, the porous nature of the substrate presents challenges to even the settling-based aerosol inoculation. For this reason, a modified version of the method developed by Calfee et al., 2013, for depositing aerosolized spores on material surfaces, was utilized for this substrate. These tests were conducted to verify that the inoculation target (quantity of spores) could be achieved without excessive variability.

1.3.3 HVAC Sampling Tests – Phase 2

HVAC Sampling (HS) Tests were conducted to determine spore recovery from HVAC filters. Two types of HVAC filters (electrostatic and mechanical) were inoculated under flow conditions. The contaminated surfaces were then evaluated using three methods: direct extraction, vacuum sock sampling, and 37 mm MCE filter vacuum sampling. In addition, the variability of the inoculating method was evaluated with two

sampling methods: sponge-wipe sampling of stainless steel coupons and direct extraction of stainless steel coupons.

1.3.4 Inoculum Variability and Level Tests – Phase 3

Inoculum Variability and Level (INOC) Tests were conducted to determine the repeatability and magnitude of inoculation from metered dose inhaler (MDI) dosing method. Three inoculum levels (10^3, 10^6, 10^7 spores per coupon) were deposited onto as many as ten replicate stainless steel coupons. Coupons were sampled by sponge-wipe following the required 18-h deposition time.

1.3.5 Linearity Recovery Tests and Laboratory Variability Tests – Phase 3

Linearity Recovery (LR) Tests and Laboratory Variability (LV) Tests were conducted to determine: (1) if vacuum-based recoveries were linear over the three inoculation levels demonstrated in the INOC tests; and (2) if recoveries varied significantly between two different technicians processing (extracting and plating) the samples. During these tests, carpet coupons were sampled using two chosen vacuum sampling methods: vacuum sock and 37 mm MCE filters. Following collection, all samples were extracted and plated by two laboratory technicians. All samples for both devices and all three inoculum levels were collected on a single day so that comparisons could be made across devices, inoculum levels, and laboratory technicians. Recoveries were compared between the two laboratory technicians to determine if technician-induced variability was significant. The linearity of recoveries for each device was determined graphically by plotting recovery versus the targeted inoculum.

1.4 Definition of Sampling Efficiency

The recovery from vacuum sampling methods was compared to recovery from wipe-based methods (PRB wipes or sponge-wipes, or both, dependent upon the test) used to sample stainless steel surfaces with identical inoculums. Surface samples from stainless steel were considered the best estimate of the number of spores inoculated by the aerosol method due to the high repeatability and high recovery efficiencies when wipe sampling from this surface type. When side-by-side comparisons could not be made (from separate test days due to the large number of vacuum samples prescribed by the test matrix), results were normalized to the stainless steel surface samples.

2 Materials and Methods

2.1 Test Materials and Deposition

2.1.1 Coupon Preparation

Carpet coupons (Figure 2-1) were 30.5 cm x 30.5 cm (12” x 12”) carpet tiles (Shaw Living Berber sand loop, Home Depot, Model # 3W05300100) attached to a 35.6 cm x 35.6 cm (14” x 14”) square of 1.9 cm (¾”)-thick plywood. The tiles were attached to the plywood using their self-adhesive backs plus three staples on each side.

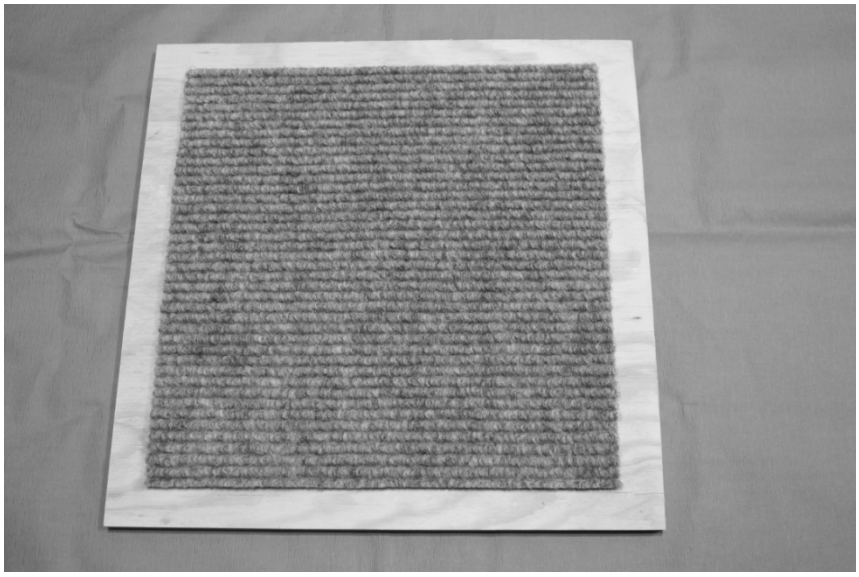

Figure 2-1. Carpet Coupon

Upholstery coupons (Figure 2-2) were constructed from a 61.0 cm x 61.0 cm (24” x 24”) piece of fabric (Indoor/Outdoor Modern Houndstooth Red Fabric, www.fabric.com, Part# UJ-849) covering a 30.5 cm x 30.5 cm (12” x 12”) piece of upholstery padding (432 Poly Foam, 2.5 cm by 61.0 cm (1” x 24”), OnlineFabricStore, Item# 1243310), placed in the center of a 35.6 cm x 35.6 cm (14” by 14”) square of 1.9 cm (¾”)-thick plywood. The fabric square was stretched over the foam and wood, and excess fabric was folded underneath and stapled to the back side of the plywood backing.

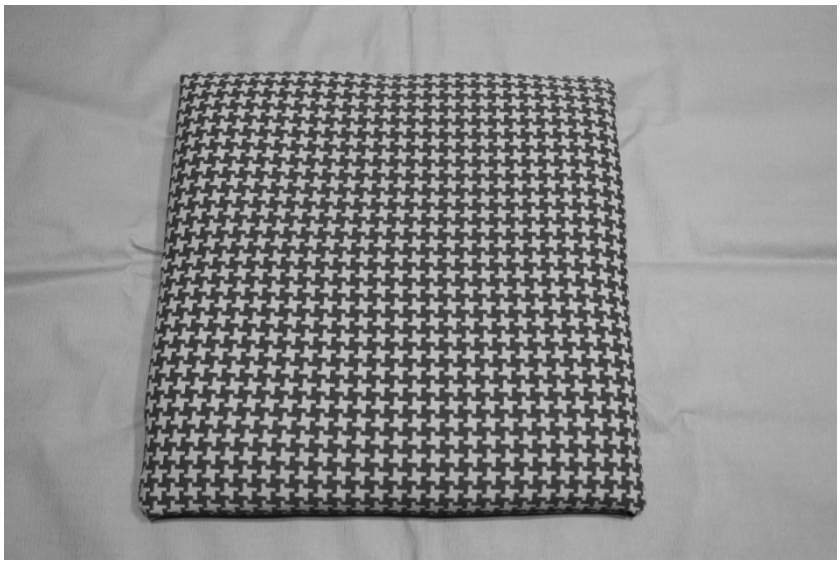

Figure 2-2. Upholstery Coupon

Concrete coupons (Figure 2-3) were formed from Sakrete Sand Mix poured into custom 35.6 cm x 35.6 cm (14” by 14”) forms. The sand mix was prepared according to indications on the package using a trough and garden hose for the water supply. The coupon was smoothed with a trowel and allowed to set and dry overnight. The coupons were allowed to cure under humid conditions for at least five days.

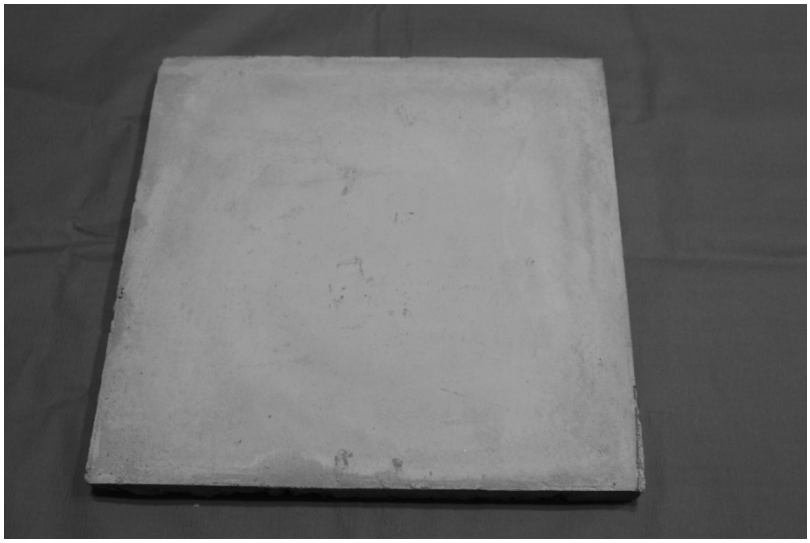

Figure 2-3. Concrete Coupon

Mechanical MERV 8 HVAC filters were 35.6 cm x 35.6 cm x 2.5 cm (14” x 14” x 1”) Purafilter 2000 Blue series (Purafilter 2000, Las Vegas, NV; http://purafilter2000.com/products.php). Electrostatic MERV 8 filters were 35.6 cm x 35.6 cm x 2.5 cm (14” x 14” x 1”) Eco-Aire MERV 8 High Cap (Con-Air Industried, Inc., Orlando, FL). Stainless steel coupons were Grade 316, cut to 35.6 cm x 35.6 cm (14” by 14”) or 10.2 cm x 15.2 cm (4” x 6”). Only the centermost 30.5 cm x 30.5 cm (12” x 12”) of each 35.6 cm x 35.6 cm (14” by 14”) coupon was sampled.

All coupons were placed in sterilization bags and sterilized prior to use. Carpet and upholstery coupons were sterilized by a minimum 250 ppmv Vaporous Hydrogen Peroxide (VHP®) cycle for four hours using a STERIS ED1000. Concrete and stainless steel coupons were sterilized prior to use by steam autoclave utilizing a gravity cycle program consistent with the EPA NHSRC Microbiology Laboratory Miscellaneous Operating Procedure (MOP) 6570 (see Appendix A for all associated MOPs). HVAC filters were sterilized with ethylene oxide. Sterility was evaluated by swab sampling one coupon from each sterilization batch according to MOP 3135. Prior to use, the coupons treated with VHP® were incubated at 30-35 °C for a minimum of two days to force off-gassing of residual hydrogen peroxide (H_2O_2) from material coupons as suggested by Baron et al. [4], so that biocidal activity was prevented.

2.1.2 Bacillus Spore Preparation

The test organism for this work was a powdered spore preparation of *B. atrophaeus* (American Type Culture Collection (ATCC) 9372) and silicon dioxide particles. The preparation was obtained from the U.S. Army Dugway Proving Ground (DPG) Life Science Division, in Dugway Utah. The preparation procedure is reported in Brown et al. [5]. Briefly, after 80 – 90 percent sporulation, the suspension was centrifuged to generate a preparation of approximately 20 percent solids. A preparation resulting in a powdered matrix containing approximately 1 x 10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Deguss, Frankfurt am Main, Germany). The powdered preparation was loaded into MDIs by the U.S. Army Edgewood Chemical Biological Center (ECBC) or by ARCADIS according to a proprietary protocol. Control checks for each MDI were included in the batches of coupons contaminated with a single MDI.

2.1.3 Coupon Inoculation

Coupons were inoculated with spores of *B. atrophaeus* from an MDI using the procedure described by Calfee et al. [6] and detailed in MOPs 3161-LD and 3161-HD for low dose ($\leq$2 x 10^4) and high dose (>2 x 10^4) concentrations. Briefly, each coupon was inoculated independently by being placed into a separate aerosol dosing apparatus (ADA) designed to fit one 35.6 cm x 35.6 cm (14” by 14”) coupon of any thickness. In accordance with MOP 3161-LD or -HD, the MDI was discharged into the ADA a single time for most concentrations, but three discharges were administered when the 2 x 10^2 colony forming units (CFU) per dose MDI was used, for a total dose of ~6 x 10^2 CFU per coupon for these inoculations. The spores were allowed to settle onto the coupon surfaces for a minimum period of 18 h. When porous coupons were used (HVAC filters), coupons were placed on double-coated carpet tape (Polyken Model 105C) to prevent re-entrainment of spores during handling and sampling. After the minimum 18-h period, the coupons were then removed from the ADA and sampled. The ADAs were removed from only those coupons required for a single sample at a time (some samples were comprised of three coupons, others a single coupon). After use, the coupons were placed in a bin of soapy water before disposal. The handling of the contaminated coupons was done in a way to minimize or control spore dispersal. One person was tasked with removing the clamps holding the ADA to the coupon and the removal of the ADA and gasket from the coupon. A second person, wearing new gloves for each coupon, was then tasked with placing the sampling template atop the coupon. A third person executed the actual sample collection procedure (e.g., vacuum-device usage, wipe-based sample collection, etc). The first person then removed the coupon for neutralization. All personnel conducted the same job throughout the entirety of a test operation.

The MDIs are claimed to provide 200 discharges per MDI. The number of discharges per MDI was tracked so that use did not exceed this value. For any inoculation event for all three materials, a new MDI had to be used to avoid exceeding 200 discharges per MDI. Additionally, in accordance with MOP 3161-LD and -HD, the weight of each MDI was determined after completion of the inoculation of each coupon. For quality control of the MDIs, on each day of testing at least three reference stainless steel inoculation control coupons were inoculated simultaneously and interspersed with test coupons. The reference coupons were inoculated as the first, middle, and last coupons within a single group of coupons, all inoculated by the same MDI within a single test. These inoculation control (reference) coupons were a stainless steel coupon (35.6 cm by 35.6 cm) inoculated in accordance with MOP 3161-LD or -HD, sampled and analyzed in accordance with Section 2.3.2 or Section 2.3.3. If the results from the inoculation controls were outside the acceptance criteria (Table 4-2), the results were discussed immediately to determine the corrective action.

A log was maintained for each set of coupons that was dosed via the method of MOP 3161-LD or -HD. Each record contained the unique coupon identifier, the MDI unique identifier, the date, the operator, the weight of the MDI before dissemination into the coupon dosing device, the weight of the MDI after dissemination, and the difference between these two weights. The coupon codes were pre-printed on the log sheet prior to the start of coupon inoculation (dosing).

A second method was used to inoculate HVAC filters under air flow (~1000 cubic feet per minute, CFM). To provide the flow, the back of the filter was connected to a blower from a high-volume air sampler (Thermo Scientific High Volume Air Sampler VFC-PM10.for FRM RFPS-1287-063 (https://www.thermo.com/eThermo/CMA/PDFs/Various/File_52267.pdf)). The outlet of the blower was directed to the intake of the H130 enclosure exhaust system to control contamination from breakthrough. On top of the filter was an ADA assembled exactly as described in MOP 3161-LD or -HD. Positioned between the HVAC filter and the blower, a sterile quartz filter was used to collect any spores that escaped capture by the HVAC filter (see Figure 2-4). The quartz filter was installed as outlined in MOP 3168. The blower was operated for 15 sec, then the MDI was activated, and the blower operated for another 15 sec before deactivation. Once the blower ceased, the filter and ADA were lifted from the blower assembly and placed on carpet tape as the filters inoculated using the first method. The quartz filter was collected as outlined in MOP 3168. The blower adapter was cleaned and decontaminated between coupons with Dispatch™ disinfectant wipes (Clorox Corp, CA), followed by 3% sodium thiosulfate (STS) and ethanol wipes. This method is described in more detail in MOP 3161-F.

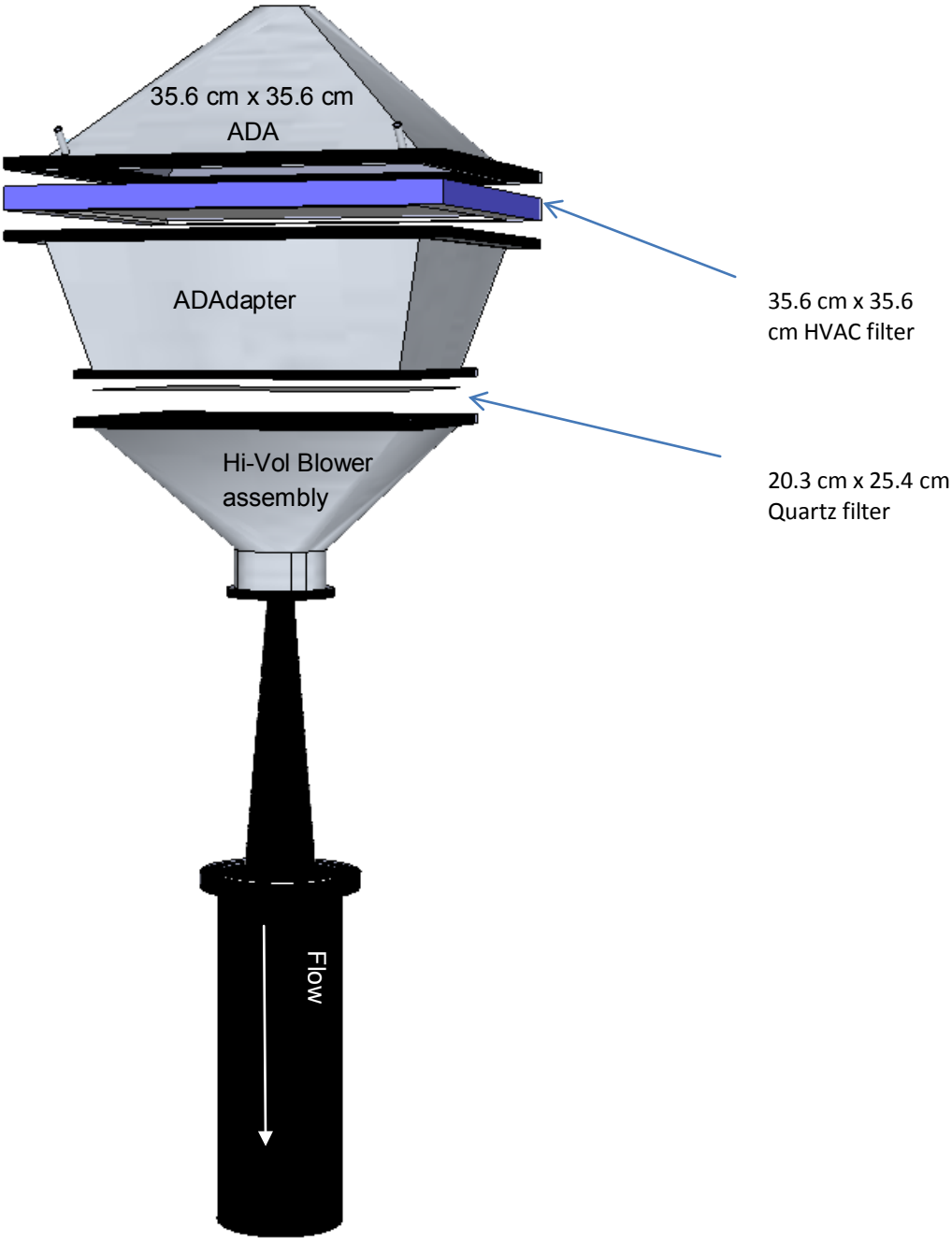

Figure 2-4. Schematic of Deposition Apparatus for Inoculation under Flow Conditions

Additionally, after a coupon was dosed via the above procedure, the coupon was labeled with the unique identifier described in Section 2.5.5. The identification (ID) was written on the ADA with a Sharpie® or equivalent permanent marker.

2.2 Test Matrix

Testing was conducted in three phases. Data from the first phase of testing were used to compare recoveries from the various vacuum devices. The second phase was designed to perform side-by-side comparisons of the two devices with the highest recoveries (from Phase 1) when used to collect spores from HVAC filters. The third phase was designed to better understand the variability between different laboratories in performing the inoculation, recovery, and extraction activities. The Phase 1 test matrix is shown in Table 2-1.

Table 2-1. Phase 1Test Matrix

Vacuum Device	Material	Target Inoculum (CFU/coupon)	Replicate Test Samples (Total number of coupons)	Negative Controls Samples	Total Coupons
Method 1 (Vacuum sock – fast sampling)	Carpet	10^7	10 (30 coupons)	1 (3 coupons)	33
	Upholstery	10^7	10 (30 coupons)	1 (3 coupons)	33
	Concrete	10^7	10 (30 coupons)	1 (3 coupons)	33
Method 1 (Vacuum sock – slow sampling)	Carpet	10^7	10 (30 coupons)	1 (3 coupons)	33
	Upholstery	10^7	10 (30 coupons)	1 (3 coupons)	33
	Concrete	10^7	10 (30 coupons)	1 (3 coupons)	33
Method 2 (37 mm MCE)	Carpet	10^7	10 coupons	1 coupon	11
	Upholstery	10^7	10 coupons	1 coupon	11
	Concrete	10^7	10 coupons	1 coupon	11
Method 3 (Trace Evidence Filter)	Carpet	10^7	10 (30 coupons)	1 (3 coupons)	33
	Upholstery	10^7	10 (30 coupons)	1 (3 coupons)	33
	Concrete	10^7	10 (30 coupons)	1 (3 coupons)	33
Method 4 (37 mm PTFE)	Carpet	10^7	10 coupons	1 coupon	11
	Upholstery	10^7	10 coupons	1 coupon	11
	Concrete	10^7	10 coupons	1 coupon	11

MCE = Mixed Cellulose Ester, PTFE = Polytetrafluoroethylene

Vacuum methods applicable to large areas were challenged with three coupons per sample (Methods 1 and 3), while methods requiring a longer sample time per area were challenged with only one coupon per sample (Methods 2 and 4).

The Phase 2 test matrix is shown in Table 2-2.

Table 2-2. Phase 2 Test Matrix

Test Series	Surface Type	Sampling Methods	Deposition Method(s)	Purpose
HI1	Clean electrostatic HVAC filters	Extraction	Settling vs. under flow, 10^8 spores/actuation MDI used for inoculation	Measure the recovery based on extraction and test the validity of the inoculation procedure
HI2	Clean electrostatic HVAC filters	Vacuum sock, 37 mm MCE, and 37 mm PTFE vs. extraction	Under flow, 10^8 spores/actuation MDI used for inoculation	Compare recovery of vacuum methods to extraction method
HS	Clean electrostatic or mechanical HVAC filters	Vacuum sock, 37 mm MCE, and 37 mm PTFE vs. extraction	Under flow, 10^8 spores/actuation MDI used for inoculation	Compare recovery of vacuum methods to extraction method

One test from the HS series was performed twice (Test HS1 and Test HS1b). Test HS1b included PRB wipe sampling of stainless steel coupons in addition to sponge-wipe sampling.

The Phase 3 test matrix is shown in Table 2-3.

Table 2-3. Phase 3 Test Matrix

Test Series (Test Name)	Target Inoculum Level (CFU/coupon)	Material Type	Sample Method	Sample Type	Reps	Purpose
Inoculation (INOC)	10^3	Stainless steel	Sponge-wipe	Experimental	10	To determine the repeatability and magnitude of inoculation from the MDI/ADA dosing method
	10^6	Stainless steel	Sponge-wipe	Experimental	10	
	10^7	Stainless steel	Sponge-wipe	Experimental	10	
Linearity Recovery (LR)	10^3	Carpet	Vacuum sock and 37 mm	Experimental	5	To determine linearity of recovery as a function of inoculation dose
	10^6	Carpet	Vacuum sock and 37 mm	Experimental	5	
	10^7	Carpet	Vacuum sock and 37 mm	Experimental	5	
Laboratory Variability (LV)	10^7	Carpet	Vacuum sock and 37 mm	Experimental to Laboratory	10 each	To determine variability between laboratory technicians (laboratories)
	10^7	Carpet	Vacuum sock and 37 mm MCE	Experimental to Laboratory B	10 each	

Each sample analysis day required laboratory blank samples analyzed in parallel with test samples.

2.3 Sampling and Analytical Procedures

Within a single test, surface sampling was first completed for all negative control coupons before sampling of any inoculated coupon was performed. Surface sampling was conducted either by PRB wipe sampling, sponge-wipe sampling, or vacuum sampling in accordance with the protocols documented below. These methods encompass those currently used by the EPA and CDC during biological contamination sampling events.

Laboratory surfaces were covered with new bench liner each day immediately prior to testing.

Paper sampling templates were sterilized with ethylene oxide (EtO) before each use.

Prior to the sampling event, all materials needed for sampling were prepared using aseptic techniques. The materials specific to each protocol are indicated in the relevant sections below. In addition, general sampling supplies were also needed. A sampling material bin was stocked for each sampling event. The bin contained enough wipe sampling and vacuum sampling kits to accommodate all required samples for the specific test. Additional kits of each type were also included for backup. A sample collection bin was used to transport samples to the NHSRC Microbiology Laboratory after collection. The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a 5000 ppm hypochlorite solution prior to transport from the sampling location to the NHSRC Microbiology Laboratory.

2.3.1 Swab Sampling

Swab sampling was used to verify sterility of coupons prior to inoculation. One coupon per sterilization batch, or one coupon per 10 coupons in large sterilization batches, was swab-sampled (approx. 25 cm^2) according to MOP 3135 and analyzed according to MOP 6563.

2.3.2 Polyester-Rayon Blend (PRB) Wipe Sampling

The centermost 30.5 cm x 30.5 cm area of a coupon was delineated by a sterile paper template and sampled with a pre-moistened PRB wipe. Wipe sampling is typically used for small sample areas (i.e., 1 ft^2) and is effective on nonporous, smooth surfaces such as ceramics, vinyl, metals, painted surfaces, and plastics [7]. Wipe sampling was used for stainless steel control samples only and conducted according to MOP 3144. The general approach is that a moistened sterile nonwoven PRB pad is used to wipe a specified area to recover bacteria, viruses, and biological toxins. The protocol that was used in this project is described in MOP 3144 and has been adapted from that provided by Busher et al. [7], Brown et al. [5], and documented in the Idaho National Laboratory (INL) 2008 Evaluation Protocols . Wipe samples were extracted in 20 mL Phosphate Buffered Saline (PBS) with 0.05% TWEEN®20 (PBST) according to MOP 6567 and subjected to serial 10-fold dilution and spread-plating onto Tryptic Soy Agar (TSA, BD, Franklin Lakes, NJ) according to MOP 6535a.

2.3.3 Sponge-wipe Sampling

The centermost 30.5 cm x 30.5 cm area of the coupon was delineated by a sterile paper template and sampled with a pre-moistened sponge. Sponge-wipe samples, described in MOP 3165, were collected using the following five patterns: (1) using the flat side of the sponge-wipe, the surface was sampled using horizontal S-strokes, covering the entire template area; (2) the sponge-wipe was then flipped over to the opposite side to sample the surface in a vertical pattern, covering the entire template area; (3) using the narrow edges of the sponge-wipe, the surface was sampled using the same S-strokes but applied diagonally across the template, (4) rotating the sponge to use the opposite side starting at the midway point of the coupon; and (5) the tip of the sponge-wipe was then used to sample the perimeter of the sampling area. The sampling method is described in detail in the study Rose et al. [8] and are consistent with the CDC-developed field collection procedures (www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html). Sponge-wipe samples were extracted in 90 mL PBST as described in MOP 6580 and subjected to 10-fold serial dilution and spread-plating according to MOP 6535a.

2.3.4 Vacuum Sock Sampling and Analysis

Vacuum sock sampling was conducted in accordance with MOP 3145 with the following modification: three coupons were vacuumed per sample for some Phase 1 tests. A single coupon was vacuumed for Phase 2 and 3 tests. The centermost 30.5 cm x 30.5 cm (12” x 12”) square area was vacuumed on each coupon. For Phase 1, vacuum sock nozzles were moved across the coupon at two speeds, approximately 1 sec per 30.5 cm pass (fast) and approximately 3 sec per 30.5 cm pass (slow). For example, on one square foot of surface area (30.5 cm x 30.5 cm), sampling with the vacuum sock (slow) or vacuum sock (fast) required approximately 60 passes each (30 passes in one direction, then 30 additional passes in a direction oriented 90 degrees to the first), and approximately 240 or 90 sec, respectively. For the three coupon sample, the collection time required was 720 or 270 sec for the slow or fast method, respectively. Each speed was considered a different vacuum method. All vacuum samples were collected using an OmegaVac (Atrix, Int.; Burnsville, MN), which supplied airflow of approximately 2000 L min^{-1}. This equipment was powered by alternating current (120 V) supplied by a wall receptacle. Phases 2 and 3 used only the slow speed technique. Vacuum socks were extracted in 20 mL PBST according to MOP 6572 and subjected to 10-fold serial dilution and spread-plating according to MOP 6535a. Figure 2-5 shows an assembled vacuum sock kit with cardboard nozzle and connection tubes, and the vacuum sock removed from the tubes.

Figure 2-5. Vacuum Sock Kit and Individual Sock.

2.3.5 37 mm MCE and 37 mm PTFE Sampling

Filter samples (37 mm MCE and PTFE) were collected according to Section A of MOP 3164, based on methods developed jointly by EPA and CDC. In short, a vacuum pump (Vac-U-Go, SKC, Inc., Eighty Four, PA) at the back end of the filter pulled 20 L min^{-1} of air through the filter. This pump was powered by 120 V alternating current supplied by a wall receptacle. A section of Tygon® (~3 cm) tubing was cut to an angle of 45° on one end, the non-angled terminus was attached to the cassette via a polyvinyl chloride (PVC) adapter (SKC, Inc., P/N 225-132A), and the angled end was used as a nozzle. The nozzle and filter were moved along the coupon at roughly ten cm sec^{-1} (three seconds per 30.5 cm pass) in both directions (i.e., horizontally and vertically). For example, on one square foot of surface area (30.5 cm x 30.5 cm), sampling with the 37 mm cassette (MCE or PTFE) required approximately 100 total passes and approximately 400 sec. A single coupon was vacuumed when sampling with either of these two devices. The nozzle was extracted separately (described in MOP 6579), the nozzle extract was then combined with the filter extraction vessel, and filter extraction commenced. The combined resulting extract was subjected to 10-fold serial dilution and spread-plating according to MOP 6535a.

Figure 2-6 shows the 37 mm cassette with nozzle and tubing.

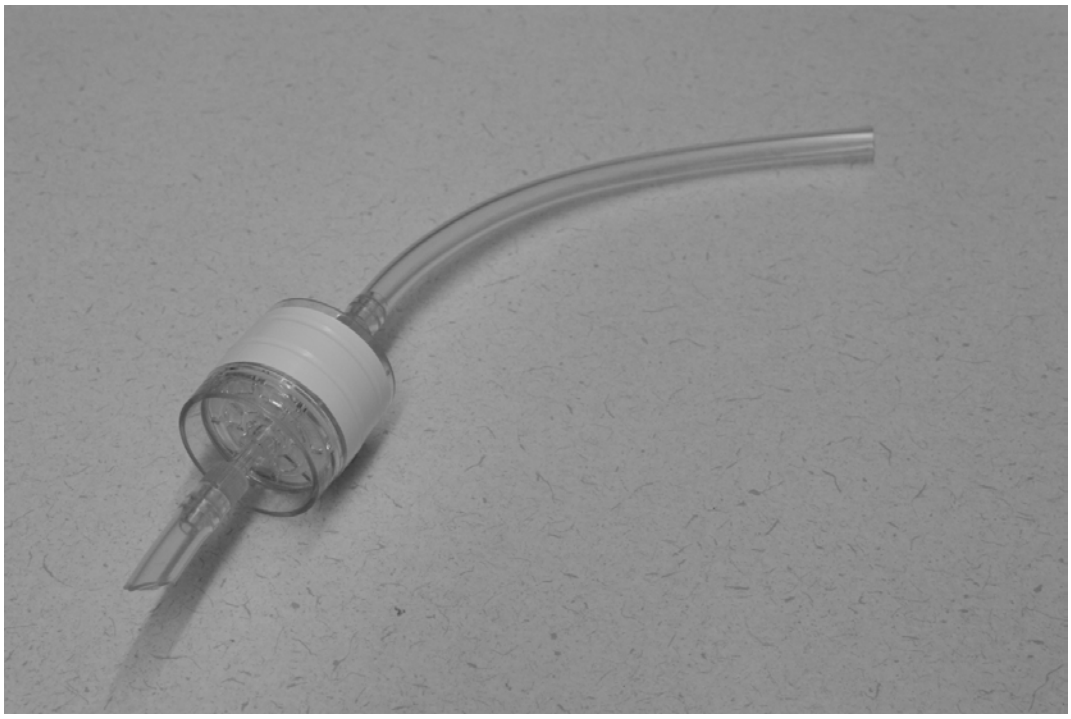

Figure 2-6. Filter (37 mm) Cassette with Nozzle and Tubing

2.3.6 Trace Evidence Filter Sampling

Trace Evidence Filter (TEF) samples were collected according to Section B of MOP 3164, based on methods developed jointly by EPA and CDC. In short, a vacuum (Atrix OmegaVac, operating at 50% voltage to prevent overcoming filter housing) pulled 790 L min^{-1} (28 standard cubic feet per minute (SCFM)) of air through the filter. The nozzle and filter were moved along the coupon at roughly ten cm sec^{-1} (three seconds per 30.5 cm pass) in both directions (i.e., horizontally and vertically). On one square foot of surface area (30.5 cm x 30.5 cm), sampling with the vacuum TEF required approximately 30 total passes and approximately 120 sec for sample collection. Three coupons per sample were vacuumed when sampling with this device. TEF filters were extracted in 90 mL PBST as described in MOP 6582 and subjected to 10-fold serial dilution and spread-plating according to MOP 6535a. Figure 2-7 shows the capped TEF cassette.

2.3.7 Operational Assessment

Operational data, such as time required for sample collection, ease of sample collection, ease of device use, malfunction of device, and ease of laboratory analytical procedures, were collected for each sampling method. These data were used to qualitatively compare methods based on their ease of use.

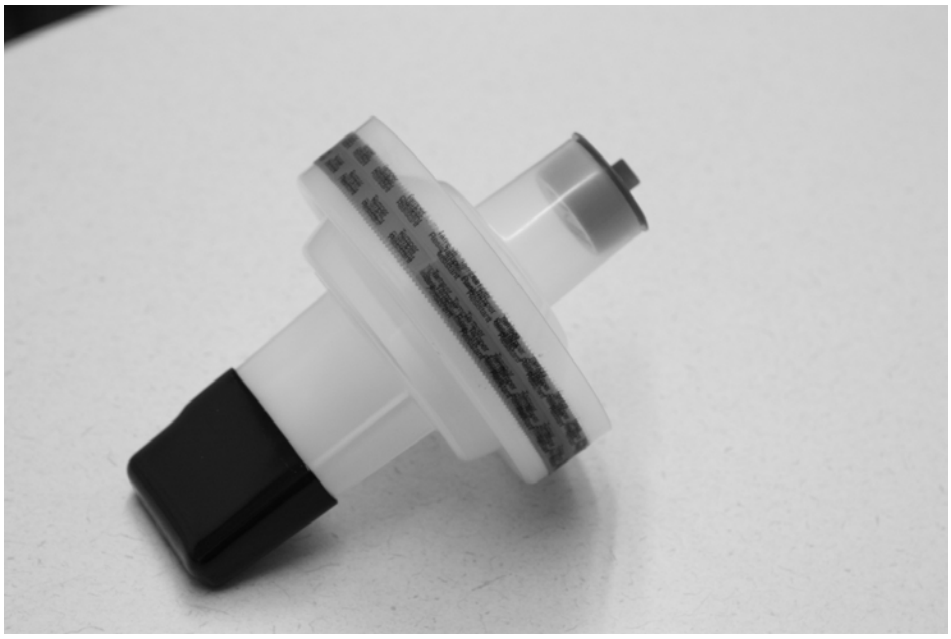

Figure 2-7. 3M® Trace Evidence Filter (TEF) Vacuum-based Sampling Device

2.3.8 HVAC filter extraction

Sections (930 cm^2, 1 ft^2) of HVAC filters were excised and further cut into half or quarter sections (15.3 cm x 30.5 cm (6" x 12") or 15.3 cm x 15.3 cm (6" x 6")) and using sterile scissors, folded, and placed in a sterile 1 L container (one section per container). The size chosen for filter sub-sections was based upon optimization experiments conducted with the same 1 L containers at CDC (data not shown). Excision and sectioning of the filter typically required 90 sec (each 30.5 cm x 30.5 cm (12" x 12") section) once cutting was initiated. HVAC filters were then extracted in PBST for 30 min using an orbital shaker (300 rpm). Quarter-sections of the filters were extracted in 500 mL PBST, while half-sections of the filters were extracted in 700 mL PBST. Details on the method are provided in MOP 6593.

2.3.9 Quartz extraction

Quartz filters were extracted in 100 mL of PBST as described in MOP 6586.

2.3.10 Direct Extraction of Stainless Steel

Six 15.3 cm x 30.5 cm (4" x 6") stainless steel coupon parts were aseptically transferred straight from the ADA to a sterile 10L beaker. Two sterile glass rods, bent symmetrically at a 90 degree angle, were placed in the bottom of the beaker, followed by one sextant of the inoculated coupon, followed by another two sterile glass rods. The process of stacking the glass rods and coupons continued until there were no more inoculated coupon pieces. Each coupon was placed in the beaker so the inoculated side was upright. The glass beaker was sealed (sterile aluminum foil; the same foil with which the beaker was autoclaved) and transported to the NHSRC Microbiology Laboratory for extraction.

Sterile PBST (1.5 liters) was aseptically added to the 10L beaker containing the sample and then re-sealed with the aluminum foil. The entire beaker was placed into an ultrasonic cleaner (Branson model 8510, Danbury, CT) and the sample was sonicated (40 kHz) for 15 min. Immediately following sonication, 1L of extraction liquid was removed and transferred to a 1L specimen container. The sample was then homogenized by manual agitation/swirling before being 10-fold serially diluted and plated according to MOP 6535a.

2.4 Sampling Strategy

2.4.1 Sampling/Monitoring Points

The experimental samples are listed in Section 2.2. For each inoculation event, additional samples collected from stainless steel surfaces were used as control samples. These control samples included wipe samples (used as inoculation controls or reference coupons) and vacuum samples (to compare collection efficacy among vacuum sampling methods). Wipe and vacuum samples were collected by sampling within a 30.5 cm x 30.5cm (12" x 12") sampling template (SKC, Inc., P/N 225-2416) centered on the coupons. Direct extraction techniques were also used to quantify inoculation levels on stainless steel and HVAC filters. Each coupon was sampled only once.

The time required to collect and analyze vacuum samples, both singly and in the aggregate, was logged in the laboratory notebook.

Table 2-4 lists the samples collected for each test.

Table 2-4. Frequency of Sampling Monitoring Events

Sample Type	Sample Number	Purpose
Vacuum sample	3 to 10 per test condition per coupon material	To determine the number of viable spores recovered via the vacuum method
Negative coupon sample (vacuum sample of sterile coupon)	1 per vacuum method per test	To determine extent of cross-contamination from coupon handling and sampling
Field blank sample (1 minute sample of laboratory air)	1 per vacuum method per test	To determine extent of cross-contamination from laboratory air
Positive control coupons sampled either by extraction, wipe, or sponge	1 set of 3 stainless steel coupons per sampling method inoculated at the beginning, middle, and end of test coupon inoculations	To provide the best estimate of the number of viable spores deposited onto the material test coupons
Swab samples	1 per material or equipment per sterilization batch	To demonstrate sterility of coupons and inoculation materials
Microbiology Laboratory material blanks	3 per material	To demonstrate sterility of extraction and plating materials

Table 2-5 lists the critical and noncritical measurements for each sample.

Table 2-5. Critical and Non-Critical Measurements

Sample Type	Critical Measurements	Non-critical Measurement
Vacuum sample	Plated volume, incubation temperature, extracted volume, CFU, collection area	Storage time, storage temperature, collection time, extraction time
Negative coupon sample (vacuum sample of sterile coupon)	Plated volume, incubation temperature, extracted volume, CFU, collection area	Storage time, storage temperature, collection time, extraction time
Field blank sample (1 minute sample of laboratory air)	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature, collection time, extraction time
Positive control coupons sampled either by extraction, wipe, or sponge	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature, collection time, extraction time
Swab samples	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Microbiology Laboratory material blanks	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature

2.5 Sampling Handling and Custody

2.5.1 Preventing Cross-contamination during Coupon Preparation

Coupon preparation included the activities performed on each pre-fabricated material coupon and procedural blank coupon prior to the inoculation procedure. Sterilization methods depended on the sample type: concrete coupons were sterilized using the gravity cycle of the autoclave; carpet and upholstery coupons were sterilized using VHP®. Swab sampling of coupons from sterilization batches were used to confirm sterility of the materials after sterilization. The sterilization procedure was repeated if results were positive for the target organism. Swabs showing foreign contamination could be cause for repeating the sterilization procedure or taking other corrective action. The blank coupon sampling occurred before sampling of any inoculated coupons.

2.5.2 Preventing Cross-contamination during Sampling

Sampling poses a significant opportunity for cross-contamination of samples. In an effort to minimize the potential for cross-contamination, several management controls were followed.

- In accordance with aseptic technique, a sampling team made up of a "sampler," a "support person," and a "sample handler" was utilized.
- The sample handler was the only person to handle deposition pyramids (ADAs) or material coupons during the sampling event. The support person had the responsibility of handing sterile templates to the sampler.
- The sampler handled only the sampling media and the support person handled all other supplies. The sampler sampled the surface according to the appropriate procedure as described in Section 2.3.
- The collection medium was then placed into a sample container that was opened, held and closed by the support person.
- The sealed sample was handled only by the support person.
- All of the following actions were performed only by the support person, using aseptic technique:
    - The sealed bag with the sample was placed into another sterile plastic bag that was then sealed; that bag was then decontaminated using a bleach wipe.
    - The double-bagged sample was then placed into a third sterile bag that was sealed and then placed into a sterile sample container for transport.
    - The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a solution of 5000 ppm hypochlorite prior to transport from the sampling location to the NHSRC Microbiology Laboratory.
- After the sample was placed into the container for transport, the sample handling team placed the sampled coupon in soapy water for eventual disposal.

The sampling crew then changed their gloves in preparation for working with the next sample.

Additionally, and equally important, the order of sampling was as follows: (1) first field blank; (2) all blank coupons; (3) second field blank (when required by test plan); (4) inoculated coupons; and (5) last field

blank (when required by test plan). This order ensured that test coupons were handled in an order from least level of contamination to the most, and field blank control samples provided evidence that samples were handled properly, without cross-contamination.

2.5.3 Preventing Cross-contamination during Analysis

General aseptic laboratory technique was followed and was embedded in the standard operating procedures (SOPs) and MOPs used by the NHSRC Microbiology Laboratory to recover and plate samples. The SOPs and MOPs document the aseptic technique employed to prevent cross-contamination. Additionally, the order of analysis was (1) all blank coupons, then (2) all inoculated coupons.

2.5.4 Sample Containers

For each PRB wipe sample, the primary containment was an individual sterile 50 mL conical tube. Conical tubes (15mL) were primary containment for swab samples. Secondary containment for swab and PRB wipe samples was sterile sampling bags. The sponge-wipe primary containment was the stomacher bag used for extraction, and secondary containment was sterile sampling bags. The primary containment of the vacuum sock was a sterile sampling bag. A four inch cable tie was also used to close the open end of the newly-collected vacuum sock sample. The secondary containment of each vacuum method sample was separate sterile sampling bags. All biological samples from a single test were then placed in a sterilized container. After samples were placed in the container for storage and transport to the NHSRC Microbiology Laboratory, the container was wiped with a towelette saturated with a ≥5000 ppm hypochlorite solution. A single container was used for storage of materials during sampling and for transport of samples to the NHSRC Microbiology Laboratory.

2.5.5 Sample Identification

Each coupon or sample was identified by a unique sample ID. The sampling team maintained an explicit laboratory log which included records of each unique sample ID and its associated test number, inoculum level, sampling method, and the date sampled. Each coupon was marked with only the material descriptor and unique code number. Sample IDs included descriptors, where necessary, for project number (WA 10), test ID, coupon material type, vacuum or other sample type, inoculation type, sample purpose (test, control, field blank, etc.) and replicate number. The sample codes eased written identification. A typical sample ID was 10-HS1-F-V-4, which identified the sample as from WA **10**, Test **HS1**, inoculated under **F**low, sampled by **V**acuum sock, replicate **4**. Once samples were transferred to the NHSRC Microbiology Laboratory for microbiological analysis, each sample (plate) was additionally identified by replicate number and dilution. The NHSRC Microbiology Laboratory also included on each plate the date it was placed in the incubator.

The samples from blank coupons had a two-letter material code, with the first letter being “X”.

The sequence number was added to the test number to distinguish control samples in the case where all materials were not inoculated at the same time for a single vacuum method. For instance, a stainless steel PRB wipe sample control coupon for Test 1 for carpet and upholstery may be labeled 10-1.1-S-W-1, while for the concrete, inoculated on a different day, the sample would be labeled 10-1.2-S-W-1.

2.5.6 Information Recorded by Field Personnel

The sampling team members' names, date, run number, and all sample codes with corresponding coupon codes were recorded in the laboratory notebook, along with sample times and durations. Any deviations from sampling protocols were documented in the laboratory notebook, along with any observations.

Digital video was collected during sampling of each material using each vacuum method in order to document the process.

2.5.7 Sample Preservation

Following transfer to the NHSRC Microbiology Laboratory, all samples were stored at 4 ± 2 °C until analyzed. All samples were allowed to equilibrate at room temperature for one hour prior to analysis.

2.5.8 Sample Holding Times

After sample collection for a single test was complete, all biological samples were transported to the NHSRC Microbiology Laboratory immediately, with appropriate chain of custody (COC) form(s). Liquid samples were stored no longer than 24 h prior to analysis. Samples of other matrices were stored no longer than five days before the primary analysis. Typical hold times, prior to analyses, for most biological samples was ≤ two days.

2.5.9 Sample Custody

Careful coordination with the NHSRC Microbiology Laboratory was required to achieve successful transfer of uncompromised samples in a timely manner for analysis. Test schedules were confirmed with the Microbiology Laboratory prior to the start of each test. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody or possession is mandatory. Accurate records were maintained whenever samples were created, transferred, stored, analyzed, or destroyed. The primary objective of these procedures was to create an accurate written record that could be used to trace the possession of the sample from the moment of its creation through the reporting of the results. A sample was in custody in any one of the following states:

- In actual physical possession
- In view, after being in physical possession
- In physical possession and locked up so that no one could tamper with it
- In a secured area, restricted except to authorized personnel
- In transit.

Laboratory test team members received copies of the test plans prior to each test. Pre-study briefings were held to apprise all participants of the objectives, test protocols, and COC procedures to be followed. These protocols were required to be consistent with any protocols established by EPA. In the transfer of custody, each custodian signed, recorded, and dated the transfer on the COC. Sample transfer could be on a sample-by-sample basis or on a bulk basis. The following protocol was followed for all samples as they were collected and prepared for distribution:

- A COC record accompanied the samples. When turning over possession of samples, the transferor and recipient signed, dated, and noted the time on the record sheet. This record sheet allowed

transfer of custody of a group of samples from the sample collection laboratory to the NHSRC Microbiology Laboratory.

- If the custodian had not been assigned, the laboratory operator had the responsibility of packaging the samples for transport. Samples were carefully packed and hand-carried between on-site laboratories. The COC record showing the identity of the contents accompanied all packages.

2.5.10 Sample Archiving

All samples and diluted samples were archived for a minimum of two weeks following completion of analysis. This time allowed for review of the data to determine if any re-plating of selected samples was required. Samples were archived by maintaining the primary extract at 4 ± 2 °C in a sealed extraction vessel.

2.6 Statistical Analysis Methods

Relative recovery data were analyzed using a one-way analysis of variance (ANOVA). A three-way ANOVA model with full interactions between Device, Material, and Experiment/Control was used to analyze raw recovery data. For the three-way ANOVA, mean $\log_{10}$ reduction was then computed as a linear contrast of model coefficients to assess recovery rate. Mean differences in $\log_{10}$ reduction between devices were also computed as linear contrasts. Statistical significance ($p \leq 0.05$) of differences between devices was then assessed via *t*-tests on the contrasts, and two sets of *p*-values were computed. First, p-values were computed without adjusting for multiple comparisons. Subsequently, p-values which accounted for multiple comparisons were computed, based on the multivariate *t*-distribution of the test statistics.

Both statistical methods were deemed valid by a statistical contractor (Neptune, Inc.). The advantage of the one-way ANOVA is that it compared relative recovery data that were normalized across experiments (normalized by PRB wipe recoveries). The disadvantage is that one-way ANOVA is not robust with regards to multiple sources of variation (i.e., variation in treatment groups and variation in control recoveries). The three-way ANOVA compared recoveries of the sampling methods using raw recovery values, and included recovery data from PRB wipe samples as a treatment group. While this method accounted for multiple sources of variation and interaction, the method, as used, was less robust for direct comparisons of sampling methods across tests.

The results of both statistical approaches are presented and discussed.

3 Results and Discussion

3.1 Phase 1

The results of the vacuum method scoping tests comparing recovery of five techniques from three materials are summarized in Table 3-1 and Figure 3-1.

Table 3-1. Recovery from Materials for each Vacuum Method

	Mean CFU/cm^2 (n = 10) (Standard Deviation in parentheses)				
Material	**Vacuum Sock – Fast Speed**	**Vacuum Sock – Slow Speed**	**37 mm MCE**	**TEF**	**37 mm PTFE**
Carpet	2.01×10^4 (9.33×10^3)	2.66×10^4 (8.49×10^3)	2.83×10^4 (1.19×10^4)	1.28×10^4 (2.21×10^3)	2.18×10^4 (1.05×10^4)
Concrete	1.44×10^4 (3.55×10^3)	3.18×10^4 (5.55×10^3)	7.41×10^4 (3.88×10^4)	3.47×10^4 (1.06×10^4)	2.57×10^4 (1.49×10^4)
Upholstery	1.86×10^4 (4.16×10^3)	7.29×10^3 (2.73×10^3)	2.08×10^4 (5.64×10^3)	3.76×10^3 (3.78×10^3)	1.39×10^4 (8.60×10^3)

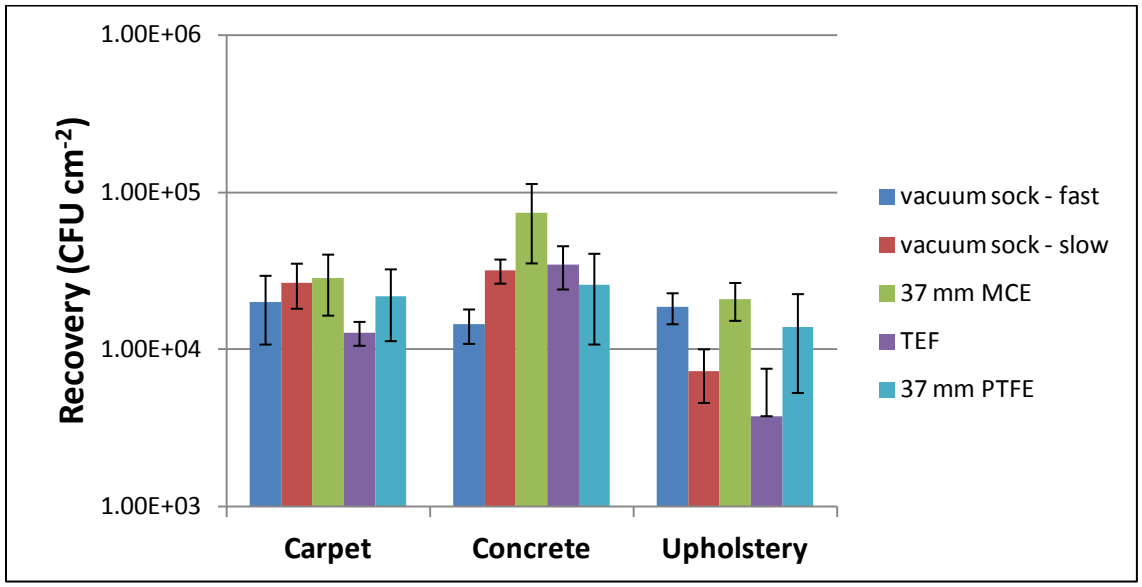

Figure 3-1. Recovery from Materials for each Vacuum Method. Data are plotted on a log-scale, as mean ± standard deviation.

These data are standardized for number of coupons sampled, with three coupons per sample for all methods except the 37 mm MCE and PTFE methods, which used only one coupon. Even without this standardization, the 37 mm MCE vacuum method shows higher recoveries from concrete (i.e., more

spores recovered by this method even though surface area sampled was one-third that of the vacuum sock and TEF methods).

To account for differing inoculation levels achieved across numerous test days and MDIs, recovery was further standardized by normalizing vacuum recoveries to PRB wipe samples collected from stainless steel coupons inoculated by the same MDI and collected on the same test day as the test samples. Figure 3-2 and Table 3-2 summarize these results. Interestingly, the 37 mm MCE method demonstrated higher recovery on concrete than the wipe-based method on stainless steel, resulting in a relative recovery ≥100%. One explanation is that the 37 mm MCE device efficiently collected fine dust or debris particles from the concrete surface. Spores bound to these debris particles may have been collected more efficiently as a consequence, because the ease of particle resuspension from surfaces increases proportionately with cross-sectional area (i.e., larger particles are more easily resuspended than smaller particles).

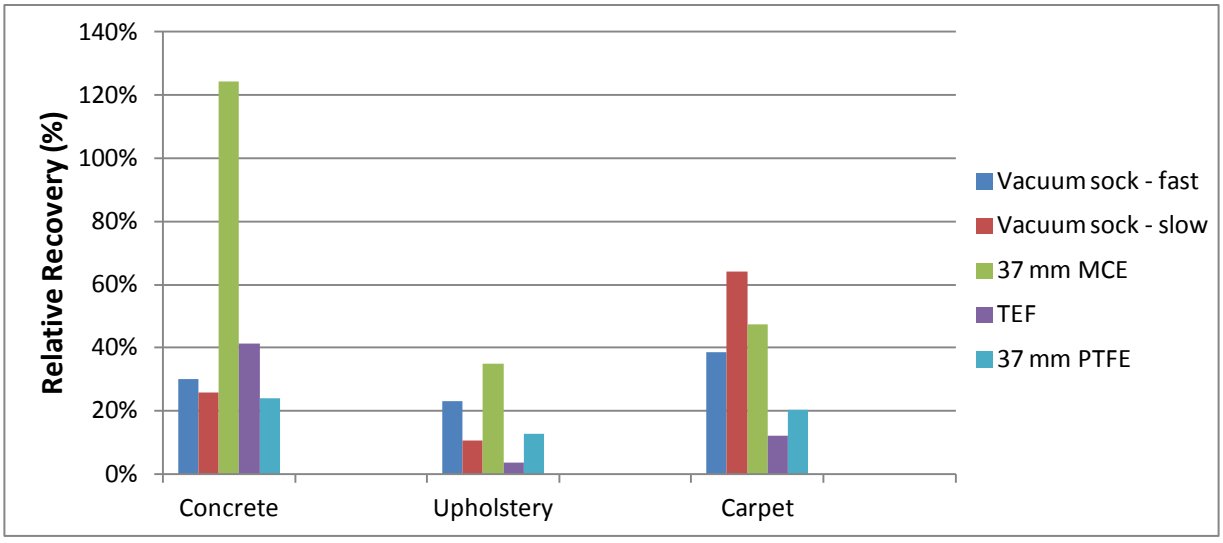

Figure 3-2. Relative Recovery – Data presented as mean relative recovery (RR). RR calculated as percent of wipe recovery from stainless steel surface.

Table 3-2. Relative Recoveries from all Devices and Material Surface Types

Average CFU/cm^2 vacuum method / Average CFU/cm^2 stainless steel wipe					
	Vacuum Sock – Fast Speed	**Vacuum Sock – Slow Speed**	**37 mm MCE**	**TEF**	**37 mm PTFE**
Concrete	30%	26%	124%	41%	24%
Upholstery	23%	11%	35%	3%	13%
Carpet	39%	64%	47%	12%	20%

The null hypothesis that recoveries obtained during slow operation of the vacuum sock nozzle were not significantly different ($p > 0.05$) from the recoveries obtained during fast operation was not disproven by these tests. Recovery using the slow vacuum sock method was higher from carpet coupons but lower (than the fast vacuum sock method) from upholstery and concrete coupons, possibly due to the rapid motion of the nozzle agitating the coupon surface. Operation of the vacuum sock nozzle is expected to be highly variable between individual operators. Based on these data, operational variability may not greatly affect recoveries.

Preliminary research (based upon direct spiking, conducted at the CDC) suggested that recovery from PTFE was higher than recovery from MCE for the 37 mm filter media. The current data suggest that overall recovery (collection from the surface, retention on the filter, then extraction from the filter) with MCE filters was higher. The TEF vacuum method performed poorly for upholstery and carpet coupons, compared to the other methods. The sock (slow) method performed poorly on upholstery.

3.1.1 Results of Statistical Analyses

For carpet, the slow vacuum sock method has the highest recovery rate (one-way ANOVA, $p \leq 0.001$). When analyzed by three-way ANOVA without correcting for multiple comparisons, the vacuum sock had significantly higher recoveries than the 37mm PTFE ($p = 0.004$) and TEF ($p = 0.003$) methods. On carpet, none of the method comparisons were statistically significant after correcting for multiple comparisons (three-way ANOVA, $p > 0.05$).

For concrete, the 37mm MCE method demonstrated the highest recovery (one-way ANOVA, $p \leq 0.001$). Contrasts of the methods were also significant when analyzed by three-way ANOVA, before adjusting for multiple comparisons (all $p \leq 0.02$). After adjusting for multiple comparisons, the 37mm MCE recoveries were statistically significant only when contrasted with recoveries of the 37 mm PTFE method ($p = 0.03$).

For upholstery, the 37 mm MCE method demonstrated the highest recovery (one-way ANOVA, $p \leq 0.001$). When analyzed by three-way ANOVA, the recoveries using the 37 mm MCE method were significantly higher than those of the 37 mm PTFE, TEF, and slow vacuum sock methods. After adjusting for multiple comparisons, was the 37 mm MCE method recoveries were only statistically higher than those using the TEF method. The TEF method demonstrated the lowest recovery for upholstery compared to all other devices. This result was statistically significant even after adjusting for multiple comparisons (three-way ANOVA, all $p \leq 0.001$).

3.1.2 Operational Parameters

3.1.2.1 Sampling Time and Ease

As discussed above, two sampling durations were used for vacuum sock sampling. Based on those data, there was conflicting effects of sampling time on vacuum sock samples.

For the 37 mm devices, sampling is very time consuming due to the small surface area of the vacuum nozzle. A sample time of five min ft^{-2} limits the number of samples that can be collected per person per day, especially within the confines of Hazardous Materials (HAZMAT) operations. The TEF method sampling time was comparable to that of the vacuum sock.

The vacuum sock and 37 mm vacuums are both commercially-available items. The recommended vacuum for the TEF filters caused the filters to warp within the housing, which caused loss of sample. For these tests, the recommended vacuum had to be operated at 50% power to prevent filter collapse. Defining the operation of the TEF vacuum was thus more complicated and may be more difficult to implement in the case of a large response.

3.1.2.2 Analysis Time and Ease

Vacuum Socks
Sample kit preparation, as well as sampling and extraction procedures, is straightforward. In the current study, the extraction was completed in 20 mL of PBST. Increasing the volume of PBST may be beneficial. With the 20 mL, the entire white (sample) portion of the sock cannot be wetted during the filter segmentation steps without allowing the nonsterile blue portion of the sock to touch the inside of the extraction cup. Durable, ergonomic scissors that are resilient to repeated sterilizations are strongly recommended. Custom-made racks for the orbital shaker were needed to accommodate the extraction vessels. Vacuum sock samples required many plating iterations (each iteration was conducted in triplicate) to meet QA goals for variability. This variability may be due to the presence of debris in the vacuum sock extraction fluid. Average processing time (all unpackaging and extraction procedures, not plating and analysis) for a typical sample batch (12 sock samples) was 90 min, or 7.5 min per sample.

Filter (37 mm)
Assembly of the 37 mm sampling kits and extracting the samples was time-consuming. Transferring liquid from the filter cartridge can be difficult, especially when large amounts of debris are present. The nozzle was extracted separately and usually contained large fragments of carpet or other debris. The debris from the concrete, when mixed with the PBST, created a paste-like substance that was difficult to transfer via pipet. The carpet debris also was difficult to pipet, as it frequently obstructed pipet tips. Average processing time (all unpackaging and extraction procedures, not plating and analysis) for a typical sample batch (12 sock samples) was 120 min, or 10 min per sample.

TEF
Aseptically removing the TEF filter from the filter housing proved difficult as it was impossible to predict which half of the two-part housing the filter would align itself with upon opening. Rinsing the filter and cartridge was also time consuming and difficult, especially with samples collected from concrete. The dust and debris from the concrete samples generated a paste during sample extraction and was difficult to manipulate. Occasionally the filters punctured the stomacher bag, resulting in a compromised sample. Sample throughput was limited by the stomacher. Average processing time (all unpackaging and extraction procedures, not plating and analysis) for a typical sample batch (10 sock samples) was 180 min, or 18 min per sample.

3.1.2.3 Cost

While the cost of all three methods was tracked, it is difficult to determine if any method was significantly more expensive than any another. The capital costs of each method would vary as well from laboratory to laboratory, depending on the availability of equipment. The following extraction equipment is recommended for each laboratory handling 50 samples daily:

Vacuum Sock method: Orbital shaker with appropriate rack for extraction vessels;
37 mm filter samples: Ultrasonic water bath (sonicator);

TEF filter samples: Stomacher (preferably two), bench-top centrifuge.

3.2 Phase 2

The results from Test HI1 are summarized in Table 3-3 and suggest that MOP 3161-F, the proposed method for inoculating HVAC filters under flow, worked as expected and yielded repeatable results (the data quality objective was to achieve 1.0 x 10^6 recovered spores (CFU) and less than 100% RSD). During inoculation, an average 1.1 x 10^8 spores (CFU) were captured on the quartz filter having passed completely through the HVAC MERV 8 filter. However, the amounts recovered from HVAC filters inoculated under flow were similar to the HVAC filters inoculated with settling conditions only. Because similar recoveries were obtained from both methods and the flow-based method is likely to be more representative of HVAC filter contamination in the field, MOP 3161-F (flow-based method) was used on all subsequent HVAC filter inoculations.

Table 3-3. Average Recovery from HVAC Filter Extraction – Test HI1

Inoculation Method	Mean Recovery (CFU) from HVAC Filter (n=3)	RSD* (%)
Flow	3.96 x 10^6	54%
Settling	9.47 x 10^6	39%

*RSD – Relative Standard Deviation, i.e., Coefficient of Variation as a percentage

Test HI2 was a scoping test to evaluate the feasibility of sampling flow-inoculated filters using vacuum methods. Table 3-4 and Figure 3-3 show the average recovery (CFU) from HVAC filters using four vacuum methods, as well as direct extraction. The method for direct extraction can be used on the small, thin filters chosen for this project but is not expected to be feasible for larger or thicker HVAC filters.

Table 3-4. Recovery from HVAC filters – Test HI2

Vacuum Method	Mean Recovery (CFU) (n=3)	RSD* (%)
37 mm PTFE	1.83 x 10^8	15%
TEF	1.17 x 10^8	98%
37 mm MCE	5.41 x 10^7	33%
Vacuum sock (slow)	1.37 x 10^8	51%
Extraction	1.63 x 10^8	21%

*RSD – Relative Standard Deviation, i.e., Coefficient of Variation as a percentage

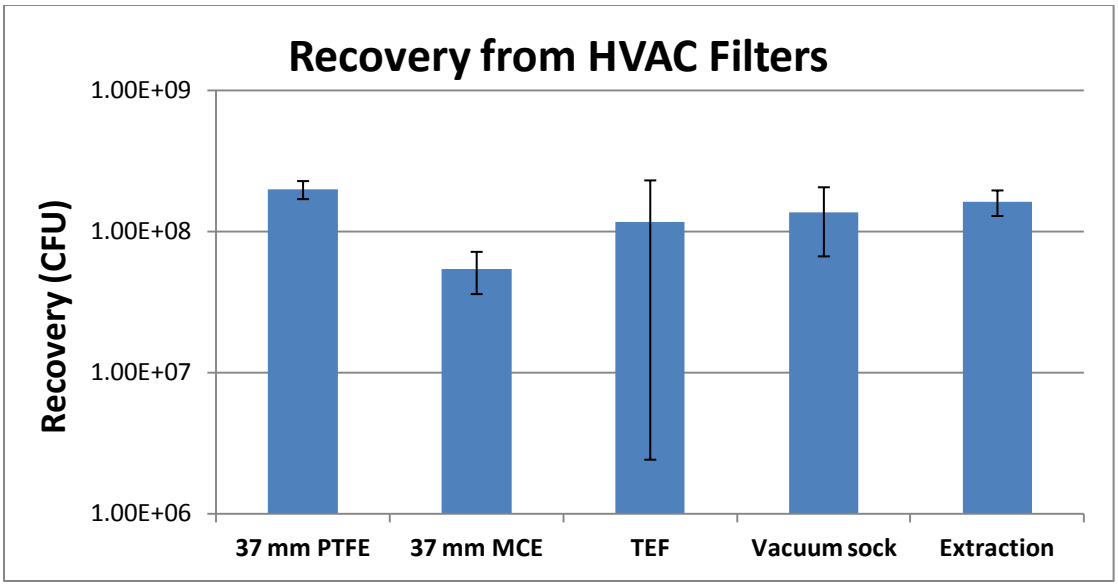

Figure 3-3. Recovery from HVAC Filters Using 5 Sampling Methods – Test HI2. Data are plotted on a log-scale, as mean ± standard deviation.

Results from Tests HS1 and HS2 are shown in Table 3-5. All tested methods performed well, with some higher variability in the TEF recovery. Though in Phase 2 the 37 mm PTFE filters provided better recovery than the 37 mm MCE filters (Figure 3-3), the MCE filters were chosen for side-by-side evaluation with vacuum socks based on the results from Phase 1 (Table 3-2).

Table 3-5. HVAC Vacuuming Recovery – Test HS

Sample Type	Mechanical MERV 8 Filter			Electrostatic MERV 8 filter		
	Mean CFU/Sample (n=5)	RSD (%)	Comparison to Extraction (T-test p-value)	Mean CFU/Sample (n=5)	RSD (%)	Comparison to Extraction (T-test p-value)
Extraction	4.90×10^6	50%		4.60×10^6	84%	
Vacuum sock [a]	1.34×10^6	43%	0.029	1.39×10^6	43%	0.26
37 mm MCE	1.58×10^6	40%	0.036	3.36×10^5	40%	0.16

[a] One electrostatic filter sample was lost during extraction. n = 4.

The single factor ANOVA *p*-value of 4.4×10^{-3} suggests a difference between all three recovery methods from Minimum Efficiency Reporting Value (MERV) 8 mechanical filters and the heteroscedastic Student's t-test values between the vacuum methods and extraction recovery indicate neither vacuum method provided recoveries as high as extraction from the mechanical MERV 8 filter. Figure 3-4 shows recovery data from the mechanical MERV 8 filter with the three collection methods. Neither vacuum method provided a statistically significant difference in recovery (heteroscedastic Student's t-test, p = 0.56).

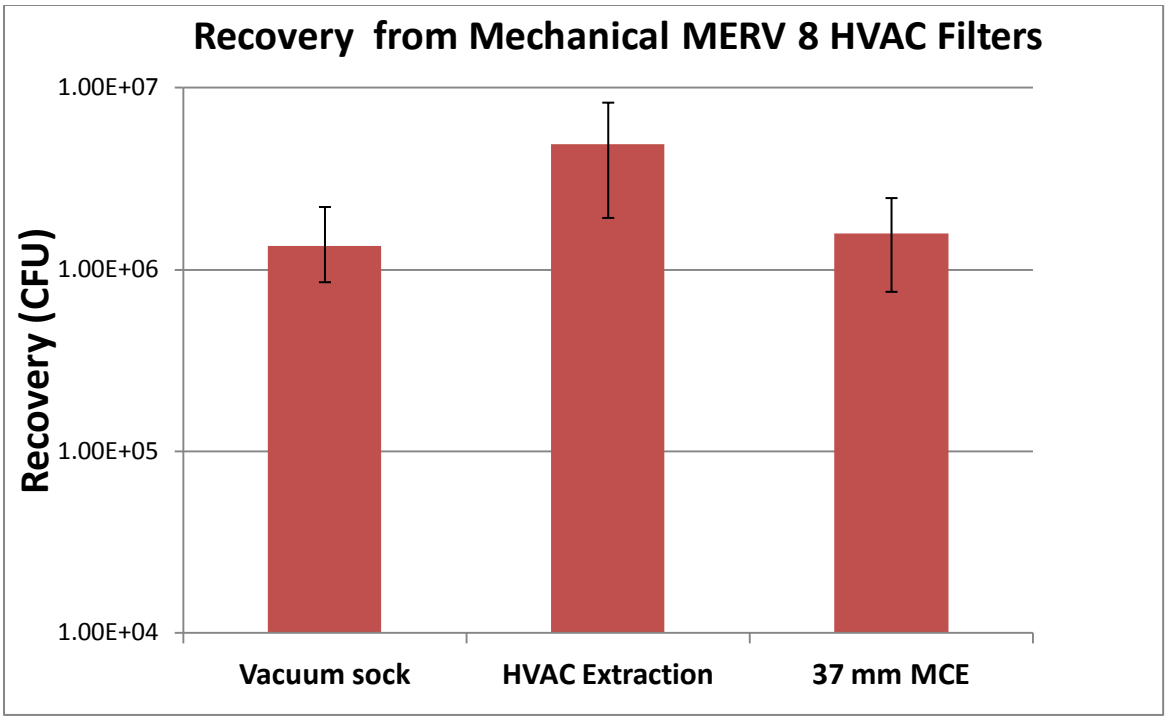

Figure 3-4. Recovery from Mechanical MERV 8 Filter – Test HS. Data are plotted on a log-scale, as mean ± standard deviation.

Unlike the case for mechanical type filters, the single factor ANOVA suggests no statistical difference between all three recovery methods from electrostatic MERV 8 filters (p = 0.16) (Figure 3-5). However, reanalysis using $\log_{10}$-transformed recovery, thus stabilizing variability, indicates there is a statistically significant difference between the three methods (ANOVA, p = 0.002). This difference is indicative in part to a high uncertainty in the number of spores deposited. Table 3-6 shows the heteroscedastic Student's t-test between method pairs for both raw recovery and log-transformed recovery.

Table 3-6. Student's t-test values from Electrostatic filters

Method Pairs	Heteroscedastic Student's T-test p-values	
	Recovery	Log_{10} Recovery
vacuum sock vs. 37 mm	0.036147	0.005965
vacuum sock vs. extraction	0.262986	0.187705
37 mm vs. extraction	0.157613	0.007319

Table 3-6 suggests a statistically significant difference between vacuum sock and 37 mm MCE vacuum methods, indicating higher recovery from vacuum sock. Table 3-6 also suggests a potential difference between 37 mm and extraction methods.

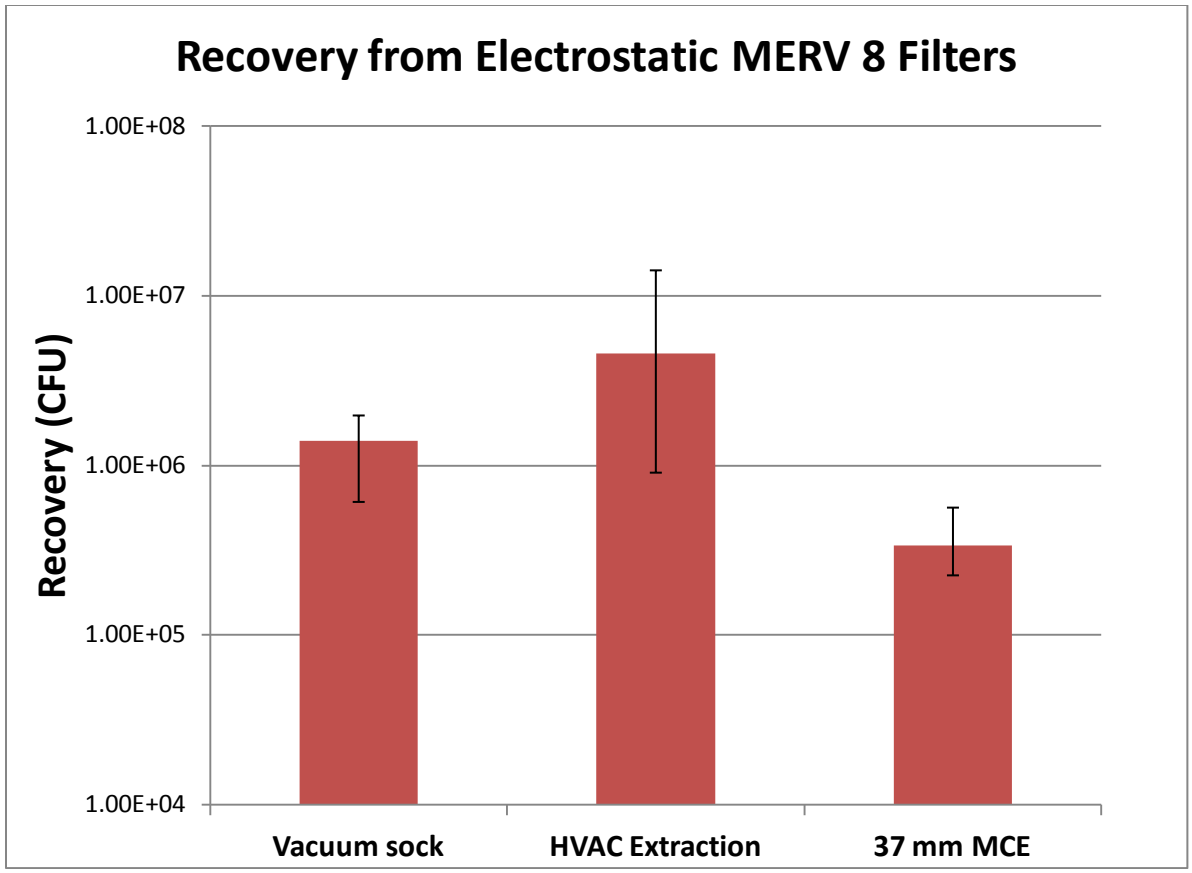

Figure 3-5. Recovery from Electrostatic HVAC Filter – Test HS. Data are plotted on a log scale, as mean ± standard deviation.

The vacuum sock method was much easier to perform on HVAC filters than the operation of the 37 mm filters. However, the ease of performance may be true only for certain types of HVAC filters. Filter types with very deep crevices may require a sampling method such as the 37 mm filter method, which uses a more narrow nozzle.

The Phase 2 test matrix also included comparisons of recovery methods from stainless steel deposition. While these depositions were in part included for quality control − as a measure of the stability of the inoculation MDI − they also provide information on spore recovery from nonporous surfaces. These data are not directly comparable to the HVAC data, due to the spores that passed completely through the filter as discussed above (Test HI1). Tables 3-7 and 3-8 summarize the recovery results from stainless steel surface samples, collected and analyzed during Phase 2 tests. The data are segregated by test because different MDIs were used for each. But taken collectively, the three recovery methods were equivalent, with no method producing statistically significant higher recovery.

Table 3-7. Mean Recovery from Stainless Steel Coupons (n = 3), Phase 2 Tests

Sampling Method	Log_{10} CFU (± Standard Deviation)		
	HS2	HS1b	HS1
extraction	2.2×10^7 (6.61×10^6)	1.75×10^7 (5.23×10^6)	1.11×10^8 (2.68×10^7)
sponge-wipe	2.63×10^7 (8.8×10^6)	1.90×10^7 (6.61×10^6)	7.52×10^7 (2.10×10^7)
PRB wipes	1.93×10^7 (3.02×10^6)	2.05×10^7 (5.11×10^6)	NA

Note: Standard deviation is in parentheses.

Table 3-8. ANOVA – Comparison of the Three Stainless Steel Recovery Methods within each Phase 2 Test Run (n = 3)

Test	*Source of Variation*	*df*	*F*	*P-value*	*F crit*
HS2	Between Groups[a]	2	0.87	0.46	5.14
HS1b	Between Groups	2	0.20	0.82	5.14
HS1	Between Groups	2	3.21	0.15	7.71

[a] Groups: Direct extraction, sponge-wipe, and PRB wipe of stainless steel.

3.3 Phase 3

The results from the INOC test series are summarized in Table 3-9 and graphically displayed in Figure 3-6.

Table 3-9. Mean Recoveries from Stainless Steel using the Sponge-wipe Method - Test INOC (n = 10)

	MDI (Target Dose)		
	10^3	10^4	10^7
Mean Recovery (CFU)	1.27×10^3	1.48×10^4	1.39×10^7
RSD	37%	31%	52%

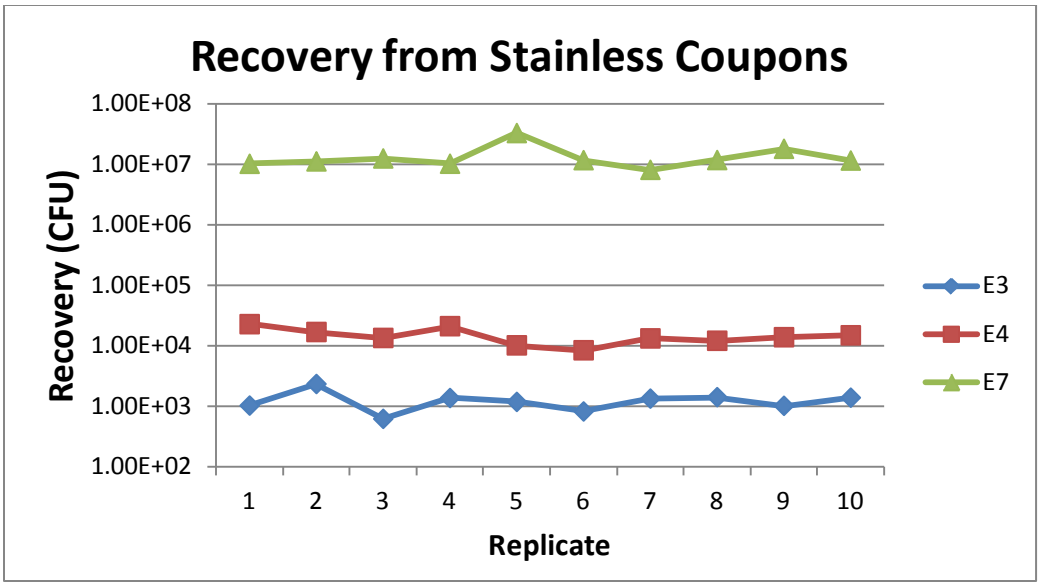

Figure 3-6. Recovery from Stainless Coupons during INOC Series Tests.

These results demonstrate the ability of both the deposition method and the sponge-wipe recovery method over a range of spore concentrations. This approach was then applied to carpet coupons so that vacuum-based methods could be evaluated over a range of spore concentrations. The mean recoveries (CFU) for each vacuum-based method are shown in Table 3-10 and Figure 3-7. These data were gathered to evaluate the linearity of recoveries by each method when challenged with inocula spanning several orders of magnitude.

Table 3-10. Mean Recoveries (CFU/sample) from Test LR (n=5)

	Mean Recoveries (CFU/sample)			
Inoculation Level	**Vacuum Sock (Carpet)**	**37 mm MCE (Carpet)**	**Extraction (Stainless Steel)**	**Sponge-wipe (Stainless Steel)**
10^4	1.06×10^3	2.48×10^3	1.60×10^4	1.27×10^4
10^6	2.02×10^5	5.07×10^5	1.93×10^6	1.81×10^6
10^7	2.45×10^6	8.65×10^6	2.27×10^7	1.69×10^7

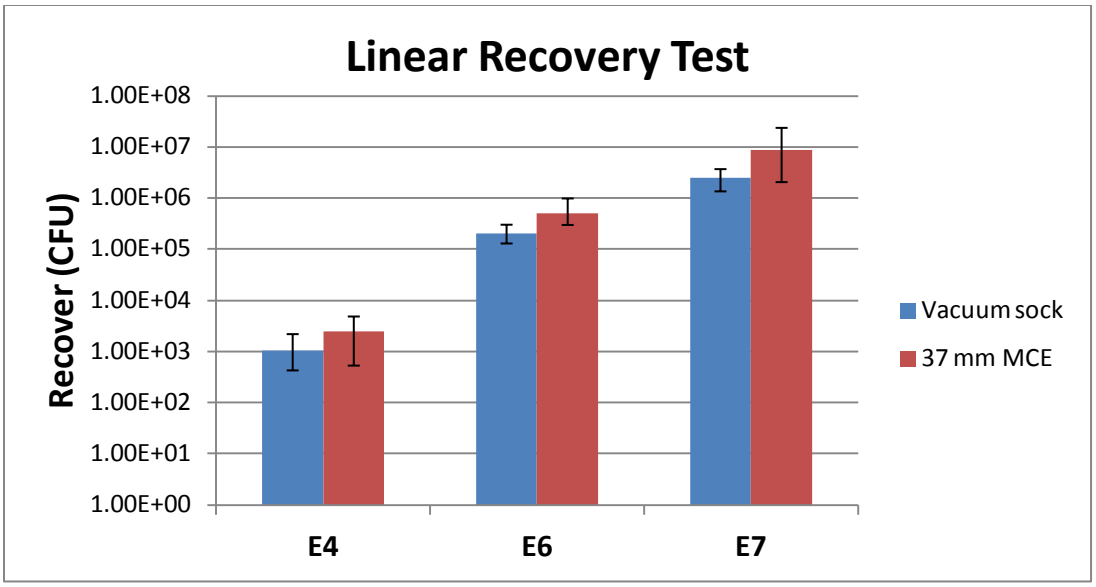

Figure 3-7. Mean CFU/Sample from Linear Recovery (LR) Test. Data are plotted on a log-scale as mean ± standard deviation.

Table 3-11 shows the relative recovery (percentage) based on stainless steel extraction method.

Table 3-11. Percent Relative Recovery of Vacuum Devices (Test LR) (n = 5)

Target Inoculum	Relative Recovery (percent of stainless steel extraction recovery)		Mean Relative Recovery (percent of stainless steel extraction recovery)	
	Vacuum Sock	37 mm MCE	Vacuum Sock	37 mm MCE
10^4	4%	30%	7%	15%
	14%	12%		
	3%	3%		
	7%	11%		
	6%	21%		
10^6	11%	21%	10%	26%
	7%	15%		
	16%	19%		
	9%	51%		
	10%	25%		
10^7	12%	35%	11%	38%
	11%	104%		
	16%	20%		
	9%	9%		
	6%	22%		

The relative recovery (RR%) consists of the mean recovery (CFU) from each vacuum sample divided by the mean recovery (CFU) from sponge-wipe sampling stainless steel (Table 3-12). These data suggest a slight advantage of the 37 mm MCE vacuum method over the vacuum sock method, though the Student t-test returned p-values ≥ 0.05 for each comparison.

Table 3-12. Relative Recovery of Vacuum Methods to Sponge-Wipe – Test LR (n=5).

Target Inoculum	Relative Recovery (percent of sponge-wipe recovery)		Mean Relative Recovery (percent of sponge-wipe recovery)	
	Vacuum Sock	37 mm MCE	Vacuum Sock	37 mm MCE
10^4	5%	38%	8.3%	19.6%
	17%	15%		
	3%	4%		
	8%	14%		
	8%	27%		
10^6	12%	22%	11.1%	28.0%
	7%	16%		
	17%	21%		
	9%	54%		
	11%	27%		
10^7	17%	47%	14.5%	51.2%
	14%	140%[1]		
	22%	27%		
	12%	12%		
	8%	30%		

[1] – Data point is within two standard deviations about the mean and the value was therefore not considered an outlier.

ANOVA analysis of CR% over the three inoculums suggested that there was no difference in the recovery over the entire range for either vacuum method. (i.e., no statistical difference in CR% between devices at each inoculum)

Results from Test LV showing variation in laboratory and personnel for two vacuum methods are shown in Figure 3-8 and Table 3-13.

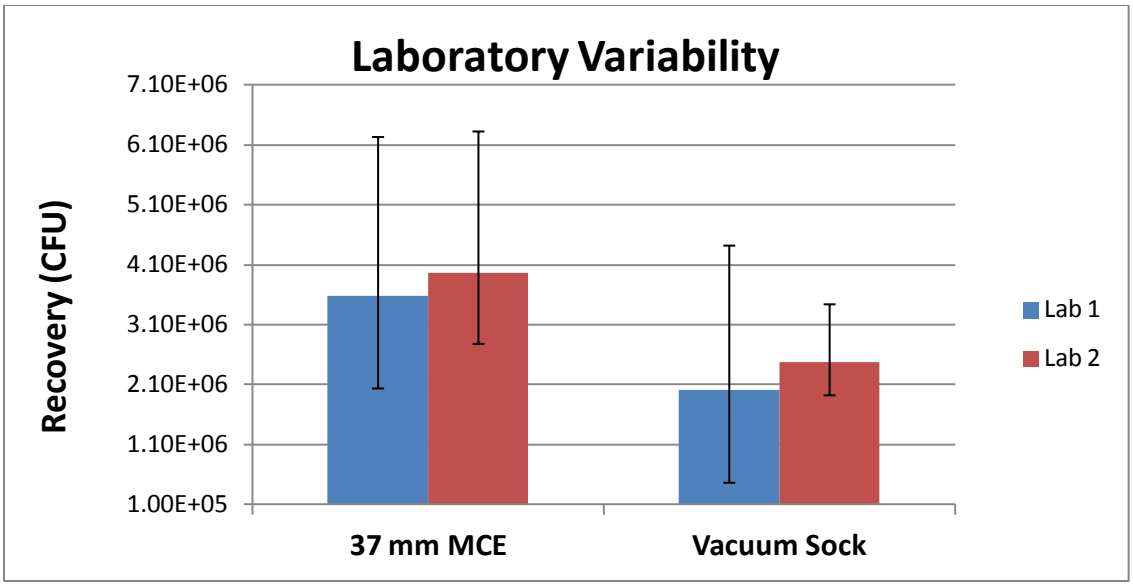

Figure 3-8. Laboratory Variability for Two Vacuum Methods - Test LV

Table 3-13. Laboratory Variability for Two Vacuum Methods (Test LV)

		Average CFU/Sample		% Recovery of Extraction Controls	
Sample Type	**n**	**Lab 1**	**Lab 2**	**Lab 1**	**Lab 2**
37 mm MCE	10	3.58×10^6	4.12×10^6	12.4 %	13.7 %
Vacuum Sock	10	2.01×10^6	2.47×10^6	6.8%	8.4%
		Standard Deviation		**Standard Deviation**	
Sample Type	**n**	**Lab 1**	**Lab 2**	**Lab 1**	**Lab 2**
37 mm MCE	10	1.34×10^6	1.25×10^6	4.6 %	4.6%
Vacuum Sock	10	1.06×10^6	4.86×10^5	3.7 %	1.7%
		CV%		**CV%**	
Sample Type	**n**	**Lab 1**	**Lab 2**	**Lab 1**	**Lab 2**
37 mm MCE	10	37%	34%	37%	34%
Vacuum Sock	10	53%	20%	53%	20%
		T-Test Between Laboratories		**T-Test Between Laboratories**	
Sample Type	**n**				
37 mm MCE	10	0.522		0.522	
Vacuum Sock	10	0.234		0.234	

There was no statistically significant difference between the recoveries of the two laboratories. Pooling the results of the two laboratories, however, does show a significant difference between the results of the two vacuum methods, with a Student's t-test value of 4.9×10^{-5}. With this large number of replicates, the 37 mm MCE vacuum method did provide better recovery than the vacuum sock method.

4 Quality Assurance

This project was performed under an approved Category III QAPP titled *Evaluation of Vacuum-based Sampling Devices for Collection of Biological Agent (DCMD 3.60) (October 2011)*.

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

There were SOPs for the maintenance and calibration of all laboratory and NHSRC Microbiology Laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA's APPCD on-site (RTP, NC) Metrology Laboratory at the time of use. Standard laboratory equipment such as balances, pH meters, biological safety cabinets and incubators were routinely monitored for proper performance. Calibration of instruments was done at the frequency shown in Table 4-1. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 h. If tolerances were not met after recalibration, additional corrective action was taken, possibly including recalibration or/and replacement of the equipment.

Table 4-1. Sampling and Monitoring Equipment Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Thermometer	Compare to independent NIST thermometer (this is a thermometer that is recertified annually by either NIST or an International Organization for Standardization (ISO)-17025 facility) value once per quarter	±1°C
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days.	±1 min/30 days
Clock	Compare to office U.S. Time @ time.gov every 30 days.	±1 min/30 days
Micropipets	All micropipets will be certified as calibrated at time of use. Pipettes are recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every year.	±5%
Scale	Compare reading to Class S weights	±1%

4.2 Data Quality

The primary objective of this project was to evaluate up to four currently-available vacuum-based devices for biological sampling efficiency. Performance (recovery) of devices was compared to the currently-preferred method, the "Vacuum Sock" (Midwest Filtration; Cincinnati, OH). Evaluation of operational parameters included time required for sample collection, the physical impact on the sampling team during collection, time required for sample analysis, and the cost of media and analysis equipment and supplies. This section discusses the Quality Assurance/Quality Control (QA/QC) checks (Section 4.3) and Acceptance Criteria for Critical Measurements (Section 4.4) considered critical to accomplishing the project objectives.

4.3 QA/QC Checks

Uniformity of the test materials was a critical attribute to ensuring reliable test results. Uniformity was maintained by obtaining a large enough quantity of material so that multiple material sections and coupons could be constructed with presumably uniform characteristics. Samples and test chemicals were maintained to ensure their integrity. Samples were stored away from standards or other samples which could cross-contaminate them.

Supplies and consumables were acquired from reputable sources and were National Institute of Standards and Technology (NIST)-traceable when possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were not used. All examinations were documented and supplies were appropriately labeled. Project personnel checked supplies and consumables prior to use to verify that they met specified task quality objectives and did not exceed expiration dates.

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, the CFU were enumerated manually and recorded. QC checks for critical measurements/parameters are shown in Table 4-2. These checks also served as DQI goals. The acceptance criteria were set at the most stringent level that could be routinely achieved and are consistent with the DQOs described in Section 4.4. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Verification of the sterility of samples prior to inoculation and other background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions. MOPs using qualified, trained and experienced personnel were used to ensure data collection consistency. The confirmation procedure, controls, blanks, and method validation efforts were the basis of support for biological investigation results. If necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research.

Table 4-2. QA/QC Sample Acceptance Criteria

Sample Type	Purpose	Frequency	Acceptance Criteria	Corrective Actions
Negative control (coupon without biological agent)	Controls for sterility of materials and methods used in the sampling procedure.	1 per test	No observed CFU	Identify and remove source of contamination. Consult WAM.
Wipe Control (wipe sample from stainless steel coupon inoculation with biological agent)	Verify inoculation level on the coupons and to Demonstrate plate's ability to support growth.	3 replicates per MDI use	For high inoculation, target loading of 10^7 CFU per sample with a standard deviation of $< 0.5 \log_{10}$. ($5 \times 10^6 - 5 \times 10^7$ CFU/sample); Grubbs outlier test (or equivalent).	Outside target range: discuss potential impact on results with EPA WAM; correct loading procedure for next test and repeat, depending on decided impact. Outlier: evaluate stability of MDIs.
Blank plating of microbiological supplies	Controls for sterility of supplies used in dilution plating, includes beads, PBST, dilution tubes, and could include other supplies if filter plates are needed.	3 of each supply per plating event	No observed growth following incubation	Sterilize or dispose of source of contamination. Re-plate samples.
Blank Tryptic Soy Agar Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates.	Each plate is incubated at least 18 but fewer than 24 h	No observed growth following incubation.	All plates are incubated prior to use; all contaminated plates will be discarded.
Field Blank Samples (Sample matrices handled in sampling area without contact with surfaces)	The level of contamination present during sampling	3 per sampling event	No observed growth following incubation	Clean up environment. Sterilize sampling materials before use.

Tests with conditions falling outside these criteria were rejected and repeated. Decisions to accept or reject tests were based upon engineering judgment used to assess the likely impact of the parameter on the conclusions drawn from the data. For the current study, no tests required repeating.

Potential confounding organisms were excluded or controlled by sterilization of the materials and use of aseptic technique, procedural blank controls, and a pure initial culture. Aseptic technique was used to ensure that the culture remained pure. Blank controls were set up and sampled in parallel with the

contaminated materials. Infrequently, colonies were observed from negative control and procedural blank samples. The magnitude of the recoveries from these samples was always at least 2 orders of magnitude lower than test samples, and therefore did not significantly affect results.

4.4 Acceptance Criteria for Critical Measurements

The DQOs are used to determine the CMs needed to address the stated objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- Enumeration of spores recovered from the surface of the coupons.
- Total number of coupons vacuumed per sample.

The DQIs listed in Table 4-3 are specific criteria used to quantify how well the collected data met the DQOs. Failure to provide a measurement method or device that meets these goals results in the rejection of results derived from the CM. For instance, if the plated volume of a sample is not known (i.e., is not 100% complete), then that sample is invalid.

Table 4-3. Critical Measurement Acceptance Criteria

Critical Measurement	Measurement Device	Accuracy	Precision	Detection Limit	Completeness
Plated Volume	Pipet	±2%	±1%	NA	100%
CFU/Plate	Enumeration by visual inspection	±10% (between 2 counters)	±5	1 CFU	100%
Number of coupons per plate	Enumeration by visual inspection	±1%	±0.5	1	100%

Plated volume critical measurement goals were met. All pipets are calibrated yearly by an outside contractor (Calibrate, Inc., Carborro, NC) and verified gravimetrically at the conclusion of testing.

Plates were analyzed quantitatively (CFU/plate) using a visual inspection-based counting method. For each set of results (per test), a second enumeration was performed on 25 percent of the plates within the desirable range (30-300 CFU per plate). All second counts were found to be within 10 percent of the original count.

There are many QA/QC checks used to validate microbiological measurements. These checks include samples that demonstrate the ability of the NHSRC Microbiology Laboratory to culture the test organism, as well as to demonstrate that materials used in this effort do not themselves contain spores. The checks include:

- Field blank coupons: sterile coupons sampled at the same time as inoculated coupons.
- Field blank sample: vacuum-based sampling media attached to vacuum device, and device was activated for one minute. Unfiltered laboratory air was collected, no surfaces were sampled.
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Microbiology Laboratory in sample analysis.
- Inoculation control coupons: stainless steel coupons inoculated at beginning, middle, and end of each inoculation campaign. After 18 – 24 h, surfaces were sampled with PRB wipes and analyzed to assess the precision and accuracy of the MDI during the inoculation operation.

4.5 Data Quality Audits

This project was assigned QA Category III and did not require technical systems or performance evaluation audits.

4.6 QA/QC Reporting

QA/QC procedures were performed in accordance with the QAPP for this investigation.

5 Summary

5.1 Phase 1

Four vacuum methods (vacuum sock, 37 mm MCE filter, 37 mm PTFE filter, and TEF), were evaluated for sampling porous materials (concrete, carpet, and upholstery). The vacuum sock method was tested additionally at two sampling speeds. The vacuum sock and TEF method afforded more ease of use to those collecting the samples and may therefore be more desirable for larger sample areas. However, the 37 MCE filter method demonstrated higher recoveries per unit area than did the vacuum sock device. The TEF and vacuum sock (slow) method performed poorly on upholstery. The TEF also performed poorly on carpet. There are advantages and disadvantages for each device.

Some conclusions can be reached based on Phase 1 recovery:

- the speed of vacuum sock sampling (rate of speed the vacuum device traversed the coupon surface) does not necessarily impact collection; and
- overall recovery (including collection from the surface, retention on the filter, then extraction from the filter) of spores using the 37 mm MCE vacuum method was often higher than the recovery achieved from the other devices.

5.2 Phase 2

A method was developed which successfully deposited spores on (and through) HVAC filters under flow conditions. Three methods of sampling HVAC filters (two vacuum methods and one extractive) performed reliably on mechanical and electrostatic filters. The data suggest extractive methods may be more efficient than vacuum-based recovery methods, depending on filter type. Vacuum sock sampling provided higher recovery from mechanical filters, but both sock and 37 mm cassettes performed similarly when sampling from electrostatic filters. Vacuum-based methods may be more applicable to HVAC filter media that are not easily sectioned.

Phase 2 data also suggested there was no statistical difference between extraction, sponge-wipes, or PRB wipes for recovery from stainless steel coupons.

5.3 Phase 3

The MDI inoculation method and the sponge-wipe recovery method were demonstrated to be effective over a broad range of concentrations, from 1 to 1×10^4 CFU/cm^2 (1×10^3 to 1×10^7 CFU/sample). Two vacuum methods were also demonstrated effective over the same range. Compared to sponge-wipes (from stainless steel), the vacuum sock method demonstrated recoveries between 3% and 22%, while the 37 mm MCE filter method demonstrated recoveries between 4% and 140% from carpet samples. The extraction procedures for both vacuum methods were evaluated by two independent laboratory technicians with no statistical difference in the number of spores recovered.

5.4 Lessons Learned and Application of Vacuum-based Methods to Field Use

Based on the data generated during this study, the 37 mm MCE and the vacuum sock sampling methods were most efficient among the combination of materials and vacuum-based methods evaluated. When

looking at these methods from an operational perspective, the vacuum sock and trace evidence filter methods offer advantages in that they allow a greater amount of surface area to be sampled in a given amount of time. Increasing the amount of area sampled increases the representativeness of the sample. Collecting samples more rapidly decreases the sampler's time down range (in the contaminated area), resulting in decreasing health and safety-related risks. However, each method has a unique set of advantages and disadvantages. Table 5-1 below summarizes these advantages and disadvantages.

Table 5-1. Summary of Advantages and Disadvantages of Each Vacuum-Based Sampling Method.

37 mm (MCE or PTFE)		Vacuum Sock		Trace Evidence Filter	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**	**Advantages**	**Disadvantages**
High collection efficiency for some surfaces (i.e., concrete and upholstery)	AC power required for the Vac-U-Go SKC sampling pump (SKC, Inc.)	Larger surface area collected	AC power required; however, newer Omega vacuum (Global Industrial, Port Washington, NY) comes with battery option	Larger surface area collected	AC power required; however, newer Omega vacuum comes with battery option
Sample is easily contained and packaged following collection	Long sample collection time, >5 min per 1 ft^2	Short sample collection time: 1.5 to 4 min per 1 ft^2	Heavy equipment, cumbersome in the field	Short sample collection time: 2 min per 1 ft^2	Volumetric flow above 790 L min^{-1} will cause filter to unseat from base, allowing air to bypass filter
Relatively simple extraction procedure	Increases health and safety concerns due to longer sampling duration required for a given number of samples	Vacuum housing can be decontaminated by wiping	Poor quality control in device construction (some have visible holes in the seams)	Vacuum housing can be decontaminate d by wiping	Expensive (~$20 per sample device)
		Shortest time requirement of the methods evaluated for laboratory extraction		Sample is easily contained and packaged following sample collection	Longest time requirement of methods evaluated for laboratory extraction

In summary, all three methods are available and could be utilized during a large-scale event. The results of the current study suggest the 37 mm and vacuum sock methods perform well and with less variability than the TEF. When selecting a sampling method for nonporous surfaces, the method should be based on sampling efficiency, portability, sampling time, surface area sampled, and health and safety of sampling personnel.

5.5 Future Research

Future research efforts may focus on improving the 37 mm filter cassette sampling method. These improvements could involve increasing the sampling speed of the 37 mm MCE collection to determine the effect of sampling speed. Alternatively, the width of the 37 mm nozzle could be increased while maintaining the cross-sectional area of the nozzle. Further testing would need to be conducted to determine the effect of this nozzle alteration.

More testing should be conducted on a variety of HVAC filters to vet the methods more thoroughly. In addition, further study is needed to investigate the effect of surface grime or conditions on recovery.

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Appendix A: Miscellaneous Operating Procedures

MOP 3135	Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems
MOP 3144	Procedure for Wipe Sampling of Coupons
MOP 3145	Procedure for Vacuum Sock Sampling of Large and Small Coupons
MOP 3161-F	Aerosol Deposition of Spores on HVAC Filter Under Flow
MOP 3161-HD	Aerosol Deposition of Spores onto Material Coupon Surfaces Using The Aerosol Deposition Apparatus (ADA) – High Dosing
MOP 3161-LD	Aerosol Deposition of Spores onto Material Coupon Surfaces Using The Aerosol Deposition Apparatus (ADA) – Low Dosing
MOP 3164	Procedure for 37MM Cassette and Trace Evidence Filter Vacuum Sampling of Large and Small Coupons
MOP 3165	Sponge Sample Collection Protocol
MOP 3168	Aggressive Air Sampling (AAS) for WA 3-28: Phase 1 Sampling Approach
MOP 6535a	Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spore
MOP 6555:	Petri Dish Media Inoculation Using Beads
MOP 6562	Preparing Pre-Measured Tubes with Aliquotted Amounts of Phosphate Buffered Saline with Tween 20 (PBST)
MOP 6563	Swab Streak Sampling and Analysis
MOP 6565	Filtration and Plating of Bacteria from Liquid Extracts
MOP 6567	Recovery of *Bacillus* Spores from Wipe Samples
MOP 6570	Use of STERIS Amsco Century SV 120 Scientific Pre-vacuum Sterilizer
MOP 6572	Recovery of Spores from Vacuum Sock Samples
MOP 6579	Recovery of *Bacillus* Spores from 37MM Filter Cassettes
MOP 6580	Recovery of *Bacillus* Spores from 3M Sponge-Stick™ Samples
MOP 6582	Recovery of *Bacillus* Spores from Trace Evidence Filters
MOP 6586	Recovery of *Bacillus* Spores from Quartz Filters
MOP 6593	Recovery of Spores from HVAC Filters

Appendix B: Miscellaneous Operating Procedures

MOP 3135	Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems
MOP 3144	Procedure for Wipe Sampling of Coupons
MOP 3145	Procedure for Vacuum Sock Sampling of Large and Small Coupons
MOP 3161-F	Aerosol Deposition of Spores on HVAC Filter Under Flow
MOP 3161-HD	Aerosol Deposition of Spores onto Material Coupon Surfaces Using The Aerosol Deposition Apparatus (ADA) – High Dosing
MOP 3161-LD	Aerosol Deposition of Spores onto Material Coupon Surfaces Using The Aerosol Deposition Apparatus (ADA) – Low Dosing
MOP 3164	Procedure for 37MM Cassette and Trace Evidence Filter Vacuum Sampling of Large and Small Coupons
MOP 3165	Sponge Sample Collection Protocol
MOP 3168	Aggressive Air Sampling (AAS) for WA 3-28: Phase I Sampling Approach
MOP 6535a	Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spore
MOP 6555:	Petri Dish Media Inoculation Using Beads
MOP 6562	Preparing Pre-Measured Tubes with Aliquoted Amounts of Phosphate Buffered Saline with Tween 20 (PBST)
MOP 6563	Swab Streak Sampling and Analysis
MOP 6565	Filtration and Plating of Bacteria from Liquid Extracts
MOP 6567	Recovery of *Bacillus* Spores from Wipe Samples
MOP 6570	Use of STERIS Amsco Century SV 120 Scientific Prevacuum Sterilizer
MOP 6572	Recovery of Spores from Vacuum Sock Samples
MOP 6579	Recovery of *Bacillus* Spores from 37MM Filter Casettes
MOP 6580	Recovery of *Bacillus* Spores from 3M Sponge-Stick™ Samples
MOP 6582	Recovery of *Bacillus* Spores from Trace Evidence Filters
MOP 6586	Recovery of *Bacillus* Spores from Quartz Filters
MOP 6593	Recovery of Spores from HVAC Filters

Miscellaneous Operating Procedure (MOP) 3135: Procedure for Sample Collection Using BactiSwab™ Collection and Transport Systems

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MOP 3135

TITLE: Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems

SCOPE: This MOP describes the procedure for collecting swab samples for Low Tech Decontamination Technique Testing

PURPOSE: The purpose if this MOP is to ensure all swab sampling is performed in a consistent manner.

Equipment/Reagents

- Disposable lab coat
- Nitrile examination gloves
- P95 Respirator
- Shoe covers
- Bouffant cap
- Safety glasses
- BactiSwab™ Collection and Transport System

1.0 PROCEDURE

1. Before starting the swabbing procedure, make sure you are wearing the appropriate, project-specific PPE (at a minimum gloves, lab coat, and safety glasses).

2. Through the sleeve, crush the BactiSwab™ ampule at midpoint.

3. Hold BactiSwab™ tip end up for at least five seconds to allow the medium to wet the swab.

4. Open the package and remove the BactiSwab™.

5. Label the plastic tube appropriately using the following scheme:

   X-Y-N where,

   X is the test number,
   Y is the material abbreviation, and
   N is the material number

6. Remove the cap-swab from the plastic tube.

7. Swab the surface while spinning the cap-swab between the thumb and index fingers. Swabbing should be conducted by following the recommend guidelines for each material as detailed in the project documentation (usually the QAPP).

8. Return cap-swab to tube.

9. Date and initial each sample tube. Enter this information into the lab notebook.

10. Complete the chain of custody form and relinquish the samples to the BioLab.

Miscellaneous Operating Procedure (MOP) 3144: Procedures for Wipe Sampling of Coupons

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MOP-3144

TITLE: PROCEDURE FOR WIPE SAMPLING OF COUPONS

SCOPE: This MOP describes the procedure for wipe sampling both small and large coupons.

PURPOSE: The purpose of this MOP is to ensure consistent and representative sampling of such coupons.

EQUIPMENT (quantities are per sampling kit)

- Sterile sampling bag (10" x 14") – outer bag
- Sterile sampling bag (5.5" x 9 ") – inner "sample collection sterile sampling bag"
- Two sterile 50 mL Falcon Blue-Max™ Polypropylene Conical Tubes
- Sterile Kendall (ref. # 8402) 4-ply all-purpose sponge
- Sterile phosphate buffered saline with 0.005% TWEEN®-20, prepared according to MOP -6562
- Pipette or other method for aseptic dispensing of 5 mL liquid
- Sterile Posi-grip© forceps
- P-95 Particulate Respirators – to prevent contamination and for respiratory protection. (Specific projects may require additional respiratory protection and will be addressed in the project Quality Assurance Project Plan (QAPP), e.g, SAR)
- Powder-free Nitrile gloves (support person) and Kimtech Pure G3 Sterile Nitrile gloves (sampler)
- Dispatch® bleach wipes

1.0 PREPARATION

1. All materials needed for collection of each sample will be prepared in advance using aseptic technique. A sample kit for a single wipe sample will be prepared as follows:

    a. Two sterile sampling bags (10" x 14", 5.5" x 9 ") and a 50 mL conical tube, capped, will be uniquely labeled as specified in the project QAPP. These bags and conical tube will have the same label. The 5.5" x 9" labeled sterile sampling bag will be referred to as the sample collection sampling bag.

    b. A sterile all-purpose sponge will be placed in an unlabeled sterile 50 mL conical tube using sterile forceps and aseptic technique. The all-purpose sponge will be moistened

by adding 2.5 mL of sterile phosphate buffered saline with 0.005% TWEEN®-20. The tube will then be capped.

c. The labeled 50 mL conical tube (capped), the unlabeled conical tube containing the pre-moistened all-purpose sponge, and the 5.5" x 9" labeled sampling bag will be placed into the 10" x 14" labeled sampling bag. Hence, each labeled sampling bag will contain a labeled 50 mL conical tube (capped), an unlabeled capped conical tube containing a pre-moistened all-purpose sponge, and an empty labeled sampling bag.

d. Each prepared bag is one sampling kit.

2.0 SAMPLING PROCEDURE FOR SMALL 14"x14" COUPONS

1. A three person team will be used, employing aseptic technique throughout. The team will consist of a sampler, sample handler, and support person.
2. Throughout the procedure, the support person will log anything they deem to be significant into the laboratory notebook.
3. In general, the team works from the least contaminated sample set (i.e., control blanks) towards the most contaminated sample set (i.e., positive controls).
4. The sampling team will each don a pair of sampling gloves (a new pair per sample, non-sterile, as they will only be handling non-sterile items); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. All members shall wear dust masks to further minimize potential contamination of the samples. Depending on the situation, respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP). New disposable lab coats are required for the sample handler when changing between different types of materials or when direct contact between the coupon and lab coat occurs.
5. The sample handler will remove the coupon from the appropriate cabinet and place it on the sampling area, being careful to handle the coupon only around the edges.
6. The support person will record the coupon code on the sampling log sheet.
7. The support person will remove a template from the bag and aseptically unwrap it such that the sampler may grab it wearing sterile gloves.
8. The sampler will place the template onto the coupon surface and align it such that the edges of the coupon are visible through the holes on the template.
9. The support person will remove a sample kit from the sampling bin and record the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.
10. The sampler and support person will verify the sample code and ensure that the correct coupon and location are being sampled.
11. The support person will:
    a. Open the outer sampling bag touching the outside of the bag.

b. Touching only the outside of the (10" x 14") bag, remove and open the unlabeled conical tube and pour the pre-moistened all-purpose sponge onto the sample or into the sampler's hands.

c. Discard the unlabeled conical tube.

d. Remove the sample collection sample bag (5.5" x 9"), being careful to not touch the inside of the outer sampling bag, and open it touching only the outside.

e. Maneuver the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosen the cap.

f. Remove the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.

12. The sampler will:

    a. Wipe the surface of the sample horizontally using S-strokes to cover the entire sample area of the coupon using a consistent amount of pressure.

    b. Fold the all-purpose sponge concealing the exposed side and then wipe the same surface vertically using the same technique.

    c. Fold the all-purpose sponge over again and roll up the folded sponge to fit into the conical tube.

    d. Carefully place the all-purpose sponge into the 50 mL conical tube that the support person is holding, being careful not to touch the surface of the 50 mL conical tube or plastic sterile sampling bag.

13. The support person will then immediately close and tighten the cap to the 50 mL conical tube and slide the tube back into the sample collection sampling bag and seal it.

14. The support person will then wipe the sample collection sampling bag with a Dispatch® bleach wipe and place it into the outer sampling bag.

15. The support person will then seal the outer sample collection bag now containing the capped 50 mL conical tube (containing the all-purpose sponge) inside a sealed 5.5" x 9" sample collection bag.

16. The support person will then decontaminate the outer sample bag by wiping it with a Dispatch® bleach wipe.

17. The support person will then place the triply contained sample into the sample collection bin.

18. All members of the sampling team will remove and discard their gloves.

19. Steps 2 – 18 will be repeated for each sample to be collected.

3.0 SAMPLING METHOD FOR LARGE (4'x4' or larger) COUPONS

3.1 Sample Layout

The sampling of large coupons is carried out using a sample grid to divide the large coupons into representative sections. These sections are then numbered and selected to be sampled at different times during the course of the experiment as a blank, a control, or an experimental group sample. This selection grid is pre-determined and the Project Quality Assurance Project Plan (QAPP) may overrule the template shown in Figure 1 if otherwise specified.

As in the example below, the first cell is sampled as a Blank before contamination. Starting in cell 3, every third cell is sampled as a positive Control. This sample is to be taken post-contamination and before decontamination. Every cell directly following a Control cell is sampled as Experimental and is taken following decontamination. The sample kit labeling will be based on this grid and the sampling team must ensure to correctly sample the coupons based on this template.

1 Blank	2	3 Control	4 Experimental
5	6 Control	7 Experimental	8
9 Control	10 Experimental	11	12 Control
13 Experimental	14	15 Control	16 Experimental

Figure 1. 4' x 4' Material Section Template and Sample Grid

3.2 Sampling Procedure

1. A two-person team will be used, employing aseptic technique throughout. The team will consist of a sampler and a support person.
2. Throughout the procedure, the support person will log anything they deem to be significant into the laboratory notebook.
3. The sampling team will each don a pair of sampling gloves (a new pair per sample, non-sterile, as they will only be handling non-sterile items); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. All members shall wear dust masks to further minimize potential contamination of the samples. Depending on the situation, respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP).
4. The support person will record the coupon code on the sampling log sheet.
5. The sampler will place the template onto the coupon surface (using clamps as necessary).
6. The support person will remove a sample kit from the sampling bin and record the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.
7. The sampler and support person will verify the sample code and ensure that the correct coupon and location (cell) is being sampled.
8. The support person will:
    a. Open the outer sampling bag touching the outside of the bag.
    b. Touching only the outside of the (10" x 14") bag, remove and open the unlabeled conical tube and pour the pre-moistened all-purpose sponge onto the sample or into the sampler's hands.
    c. The unlabeled conical tube is retained for Step 9.
    d. Remove the sample collection sample bag (5.5" x 9") being careful to not touch the inside of the outer sampling bag and open it touching only the outside.
    e. Maneuver the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosen the cap.
    f. Remove the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.
9. The sampler will:
    a. For a vertical coupon, the sampler will squeeze excess moisture from the sampling sponge to prevent dripping down the sampling surface. The excess moisture is caught in the unlabeled conical tube from Step 8c, and is then discarded.
    b. Wipe the surface of the sample using S-strokes to cover the entire sample area of the coupon (inside the grid) using a consistent amount of pressure.
    c. Fold the all-purpose sponge concealing the exposed side and then wipe the same

surface vertically using the same technique.

d. Fold the all-purpose sponge over again and roll up the folded sponge to fit into the conical tube.

e. Carefully place the all-purpose sponge into the 50 mL conical tube that the support person is holding being careful not to touch the surface of the 50 mL conical tube or plastic sterile sampling bag.

10. The support person will then immediately close and tighten the cap to the 50 mL conical tube and slide the tube into the sample collection sampling bag and seal it.
11. The support person will then wipe the sample collection sampling bag with a Dispatch® bleach wipe and place it into the outer sampling bag.
12. The support person will then seal the outer sample collection bag now containing the capped 50 mL conical tube (containing the all-purpose sponge) inside a sealed 5.5" x 9" sample collection bag.
13. The support person will then decontaminate the outer sample bag by wiping it with a Dispatch® bleach wipe.
14. The support person will then place the triply contained sample into the sample collection bin.
15. All members of the sampling team will remove and discard their gloves.
16. Steps 2 – 15 will be repeated for each sample to be collected.

Miscellaneous Operating Procedure (MOP) 3145:
Procedure for Vacuum Sock Sampling of Large and Small Coupons

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MOP-3145

TITLE: PROCEDURE FOR VACUUM SOCK SAMPLING OF LARGE AND SMALL COUPONS

SCOPE: This MOP describes the procedure for vacuum sampling of porous areas.

PURPOSE: The purpose of this MOP is to ensure consistent and representative sampling of such areas.

EQUIPMENT (quantities are per sampling kit)

- 1 – Gamma irradiated vacuum sock filtration kit
- 2 - Fisherbrand bags with round wire enclosure, 5.5” x 15” (Fisher Scientific, p/n 14-955-181)
- 1 - Fisherbrand bag with round wire enclosure, 10” x 14” (Fisher Scientific, p/n 01-002-53)
- 1 – 7.5” “cable-tie” (McMaster Car, Item# 7130K59)
- Dispatch wipes
- Fisher Scientific CLEAN_WIPES (Catalog No. 06-664-14) prepared with 400 mL of 3% Sodium Thiosulfate solution (90mL of 1N STS in 310mL DI water)
- Fisher Scientific CLEAN_WIPES (Catalog No. 06-664-24) premoistened with 70% isopropyl alcohol
- Permanent marker
- Nitrile gloves
- OMEGA HEPA vacuum

1.0 PREPARATION

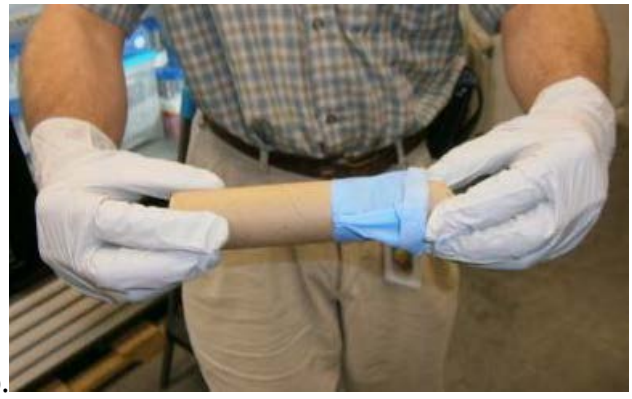
All materials needed for each sample to be collected will be prepared in advance. A sample kit for a single vacuum sock sample will be prepared using the procedure in MOP-3141A, Procedure for Assembling Irradiated Vacuum Sock Sampling Kits.

2.0 VACUUM SAMPLING OF SMALL (14” by 14”) COUPONS

The following procedure will be used in this study for vacuum sock sampling of each coupon surface:

1. A two person team will be used, employing aseptic technique. The team will consist of a sampler and a support person.
2. Both members of the sampling team will each don a pair of sampling gloves (a new pair per sample); the sampler's gloves shall be sterile sampling gloves if he/she is placing a template onto the sample. Both members shall wear dust masks to further minimize potential contamination of the samples. Further respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP).
3. The sampler will plug in the vacuum power cord and then don his/her sterile gloves.
4. The vacuum will be powered through a foot switch to allow the sampler to turn the vacuum on and off. In general, care should be taken to direct the vacuum exhaust away from samples.
5. The support person will aseptically unwrap a template (if used) from the bag and present it to the sampler, taking care to not touch the template.
6. The sampler will place the template onto the coupon surface.
7. The sampler will wipe the hose connection end (that receives the vacuum sock) first with a fresh dispatch wipe, followed with a fresh CLEAN-WIPE containing 3% STS, followed by a fresh CLEAN-WIPE containing the 70% isopropyl alcohol.
8. The sampler will hold the vacuum nozzle for the support person to place the vacuum sock assembly onto the nozzle.
9. The support person will open the sampling supply bin and remove the vacuum sock sample kit from the bin.
10. The support person will record the sample collection bag ID number on the sampling log sheet or in the laboratory notebook.
11. The sampler and support person will ensure that the correct sample coupon has been selected, referencing the coupon code on the sampling bag.
12. The support person will record the coupon code on the sampling log sheet next to the corresponding vacuum sock collection bag number that was just recorded.
13. The support person will:
    a. Open the vacuum sock sample kit outer bag and remove the unlabelled vacuum sock assembly bag.
    b. Tear open the bag containing the vacuum sock assembly and, working from the outside of the bag, maneuver the assembly from the bottom to expose the cardboard applicator tube opening.
    c. Firmly place the vacuum sock assembly onto the nozzle of the vacuum tube, using the bag to handle the vacuum sock assembly, while the sampler holds the vacuum nozzle.

14. The sampler will:
    a. Ensure that the sock is correctly placed on the nozzle and adjust, if necessary. Care must be taken to not puncture or tear the sock.
    b. Turn on the vacuum.
    c. Vacuum “horizontally” using S-strokes to cover the entire area of the material surface not covered by the template, while keeping the vacuum nozzle angled so that the tapered opening of the vacuum sock is flush with the sample surface.
    d. Vacuum the same area “vertically” using the same technique.
    e. Turn off the vacuum when sampling is completed.
15. The sampler will remove the vacuum sock assembly from the nozzle, taking care to only touch the cardboard and blue sections of the sock.
    a. Remove the sock assembly from the vacuum hose. Take care not to touch the sock inside the tube (Figure 1a shows the sock assembly).
    b. Loosen the nozzle by pulling it free of the longer tube, while holding onto the blue sock filter, and lightly replacing it in the longer tube.
    c. Unfold the blue portion of the sock filter so that it is folded over the nozzle tubing (Figure 1b).
    d. While holding both section of cardboard tubing (one in each hand), partially remove the angled section of tubing.
    e. Present the sock to the support person, who will cinch the sock closed using a plastic “cable tie” at the blue portion between the cardboard tubes (Figures 1c and 1d).
    f. Reconnect the two cardboard tubes (Figure 1e).
    g. Place nozzle end up in a labeled 5.5” x 15” bag held by the support person.

a.  b. 

c.  d. 

e. 

Figure 1. (a) Vacuum sock assembly, (b) blue portion of sock over nozzle tubing, (c,d) cinching the sock with the "cable tie", and (e) the two cardboard tubes reconnected.

16. The support person will then seal the outer sterile sampling bag and wipe it with a Dispatch® wipe.

17. The support person will then place this into the labeled 10" x 14" sample bag now containing the outer and inner bags, the inner containing the vacuum sock assembly. The outermost bag will then be wiped with a Dispatch® wipe.

18. The sampler will wipe down the nozzle (inside and out) and end of the tubing first with a Dispatch® wipe, next with a wipe pre-moistened with 3% STS, next with a wipe pre-moistened with 70 % ethanol.

19. The support person will then place the triply contained sample into the sample collection bin.

20. All members of the sampling team will remove and discard their gloves.

21. Steps 2 – 20 will be repeated for each sample to be collected.

Miscellaneous Operating Procedure (MOP) 3161-F: Aerosol Deposition of Spores on HVAC Filter Under Flow

Prepared by: _____ Date: 4/29/2013

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Prepared for

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MOP 3161-F

TITLE: **AEROSOL DEPOSITION OF SPORES ON HVAC FILTER UNDER FLOW**

SCOPE: This MOP outlines the procedure for the deposition of a spore loaded aerosol onto an HVAC filter under high flow conditions.

PURPOSE: To provide a procedure for the consistent deposition of spores onto HVAC filters under flow.

MATERIALS

- One 14" x 14" Aerosol Deposition Apparatus (ADA) (per inoculation)
- One 14" x 14" gasket (per inoculation)
- ADAdapter (see Figure 2)
- Dispatch® wipes
- STS Wipes
- Isopropyl Alcohol Wipes
- High Volume Sampler (Thermo Andersen 100042-00)
- MOP 3161 (and associated materials)
- MOP 3168
- Sterile forceps – Posi-Grip® Ref # 7190
- Quartz filter sampling kits (prepared by BioLab)
- Sterile filter sampling bag (VHP bag sterilized with EtO)
- Enclosure aeration duct
- Fisherbrand ® labeling tape – Catalog # - 15-951
- Foot power switch – McMaster Item #96618
- Sterilized Quartz filter (1 per inoculation) - WHATMAN® G653 Cat#-18209932
- Sterilized 14" x 14" HVAC filter (1 per inoculation) - Purafilter 2000®
- Medium binder clips – Staples ® Model#-10668-CC
- Large binder clips - Staples ® Model#-10669
- Blue spring clamps – Workforce 573-682
- 12" Double-sided sticky tape – Polyken 105C Carpet Tape

1.0 PREPARATION AND ASSEMBLY

1. Sterilize one 14" x 14" ADA, one 14" x 14" gasket, and an ADAdapter **at least 3 days prior** to allow for off-gassing.

2. Sterilize one VHP bag per coupon using ethylene oxide (EtO) **at least 3 days prior** to testing.

3. Set up the High Volume Sampler in the enclosure vented to the enclosure aeration system.

   NOTE: Refer to Figure 1 to ensure assembly is correct during Steps 3-8. Figure 2 shows the completed blower and ADA assembly.

4. The filter base on the blower assembly must be lifted above the top face of the sampler using angle iron (See Figure 1a) to allow the seal between the base and ADAdapter to be clamped.

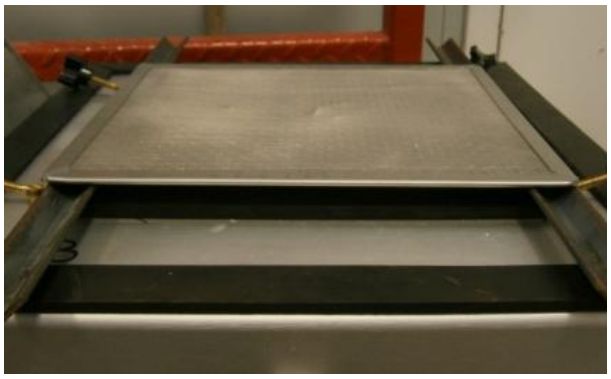
5. A two-person team is employed to aseptically install the quartz filter on top of the blower assembly base as in MOP 3168, except there is no need for a third person to hold the bracket as it is not required with this assembly (see Figure 1b).

6. Install the ADAdapter on top of the filter ensuring contact with the gasket. It is important that the filter is installed such that all edges are covered by the gasket on the bottom of the ADAdapter to ensure all airflow passes through it. Clamp the ADAdapter to the assembly using small binder clips (see Figure 1c).

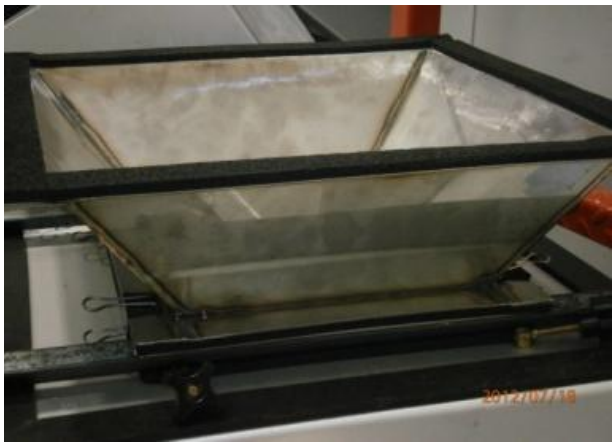
7. Once the ADAdapter is securely in place, aseptically install the 14" x14" HVAC filter. Remove the filter from the bag using sterile gloves and place on top of the ADAdapter. Ensure the filter is installed in the orientation pictured in Figure 1d.

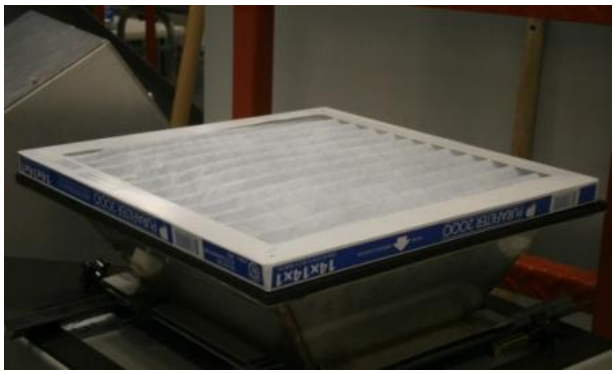
8. Finally, Install the 14" x 14" gasket and ADA on top of the filter and secure the seal with the spring clamps. Ensure the ADA is equipped with 4 filters and the top port is sealed with the included mechanism (see Figure 1e).

9. Before proceeding to Section 2.0, prepare adequate space for the number of filters to be puffed on cleaned tables. Use double-sided tape to create 16" x 16" settling areas, one for each puffed filter. It is necessary to clean the table surfaces with Dispatch® wipes, water and alcohol. **Do not** use paper coverings due to using double-sided tape.

a. 

b. 

c. 

d. 

e. 

Figure 1. (a) Blower assembly base, (b) Quartz filter installed, (c) ADAdapter installed and clamped, (d) HVAC filter installed, and (e) Completed assembly.

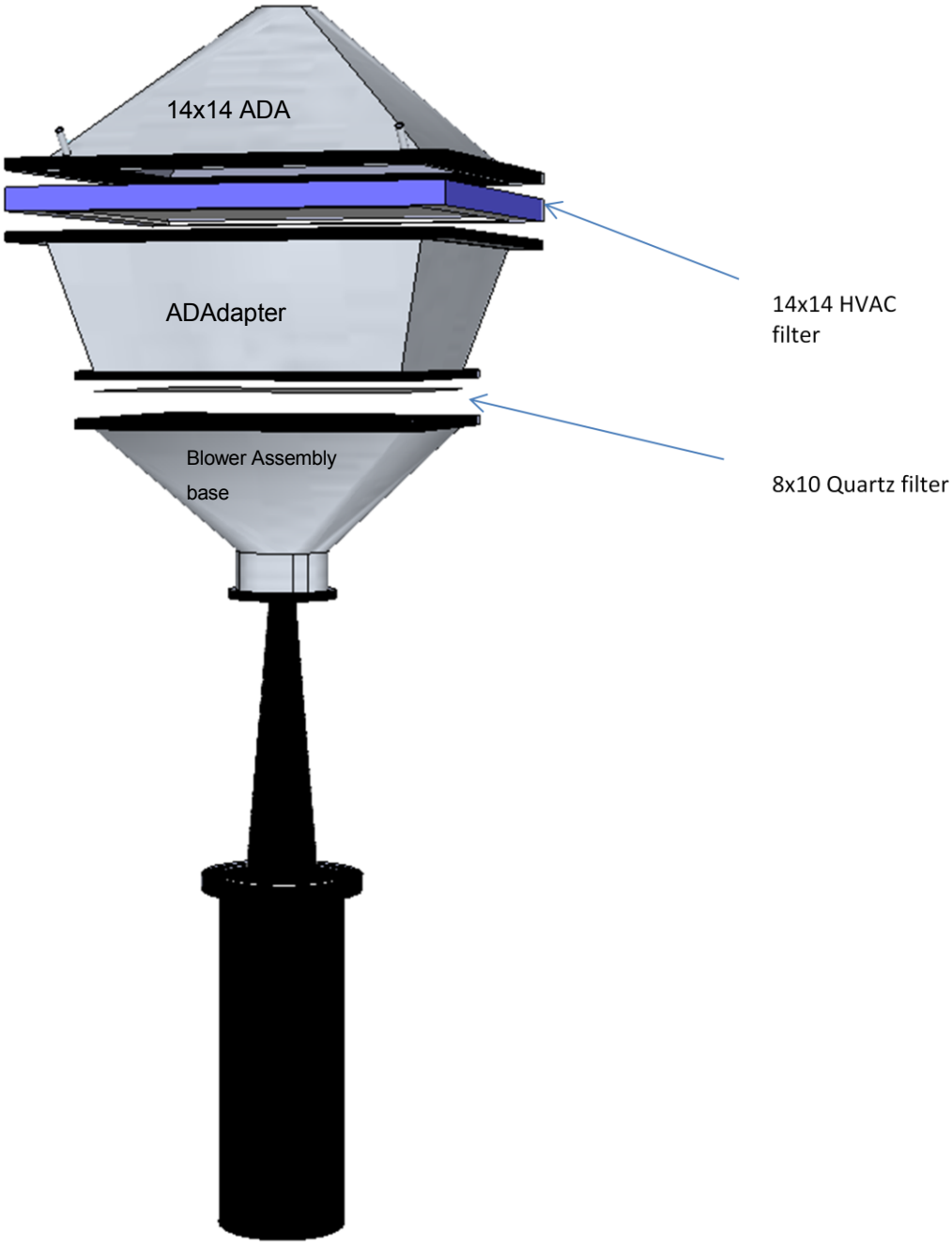


Figure 2. Blower and ADA assembly

2.0 PUFFING PROCEDURE

1. Determine the weight of the MDI canister using a balance. Record the MDI ID number and the weight (to the nearest 0.01g) in lab notebook. In addition, keep a record of the total number of 'puffs' dispensed for each MDI canister.

   NOTE: The MDI canister full is approximately 15 grams, an empty canister is approx 9.5 grams. To ensure the canister contains adequate spore suspension for dosing, canisters should be retired from use when their weight falls below 10.5 grams.

2. Next, assemble the MDI and actuator by inserting the MDI into the actuator, taking care not to activate the MDI.

3. Vortex the MDI/actuator assembly for 30 seconds (the MDI canister should be in direct contact with the vortex mixer).

4. Holding the MDI/actuator assembly upright, in a swift, firm motion, dispense three test 'puffs' into the aerosol trap to prime the MDI. It is important to vortex the assembly 10 seconds before every puff (except 30 seconds prior to the initial puff of the experiment).

5. Vortex the assembly 10 seconds, and attach it to the ADA lid by mating the ADA adaptor to the hole in the ADA lid. Loosen the lid screws enough to allow the lid to be slid into the 'open' position. Secure the lid in the open position by tightening the lid screws.

   NOTE: The 'open' position is achieved when the hole in the lid aligns with the hole in the top of the ADA.

6. Using the foot switch, start the blower and allow the unit to flow for 15 seconds.

7. At 15 seconds, dispense the spores by activating the MDI with a swift, firm motion. Hold the MDI in the activated position for 3 seconds before releasing. Activation is best achieved by grasping the MDI/actuator with two hands, and using a thumb to press the bottom of the MDI canister.

8. After 30 seconds total, follow the reverse order of the lid opening procedure to close the ADA lid, and turn off the blower using the foot switch.

9. Determine the weight of the actuator-MDI using a balance, and record the weight in lab notebook.

   NOTE: If the dosing puff is faulty, return to Step 5 and attempt a second puff on the current filter. Do not proceed to the next filter until a 'successful' puff has been delivered. A 'successful' puff is achieved when the weight of the actuator- MDI assembly has a

0.04gram-0.07gram loss. Familiarity and professional judgment will be needed to determine the success of a puff.

10. Once the filter is successfully puffed, remove the ADA , gasket, and filter assembly. Clamp the filter to the assembly using large binder clips, and place on the double sided tape.

11. Remove the ADAdapter and place on a clean surface.

12. Aseptically recover the quartz filter as in MOP 3168.

13. Clean the ADAdapter with Dispatch® wipes, STS wipes, and isopropyl alcohol wipes before proceeding to Step 14.

14. For each additional filter, proceed to apparatus assembly, using a new sterile ADA and gasket for each inoculation (Step 5, Section 1).

15. Repeat Steps 1.5 to 2.14 until all filters have been puffed (see test plan for number of samples).

16. Once all coupons have been puffed, remove the MDI from the actuator and weigh. Record the final weight and total number of puffs.

17. Allow spores to settle onto the coupon surface for at least 18 hours. Settling time should not exceed 26 hours.

3.0 RECOVERY

1. After 18 hours settling time, carefully remove binder clips and remove ADA and gasket from coupon surface, taking care not to disturb the surface of the coupon.

2. Don sterile gloves and transfer the filter to sterilized VHP bag. Carefully tape the bag closed, ensuring an air tight seal.

3. The VHP bag should be placed in tertiary containment before transport to the APPCD Biocontaminant Laboratory for analysis.

Miscellaneous Operating Procedure (MOP) 3161-HD:

Aerosol Deposition of Spores onto Material Coupon Surfaces using the Aerosol Deposition Apparatus (ADA) – High Dosing

Prepared by: _____ Date: 11/15/2012
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Reviewed by: _____ Date: 11/15/2012
Dahman Touati, ARCADIS Project Manager

Approved by: _____ Date: 11/15/2012
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MOP 3161-HD

TITLE: AEROSOL DEPOSITION OF SPORES ONTO MATERIAL COUPON SURFACES USING THE AEROSOL DEPOSITION APPARATUS (ADA) – HIGH DOSING

SCOPE: This MOP outlines the procedure for assembly and usage of the Aerosol Deposition Apparatus (ADA).

PURPOSE: Precise and highly repeatable aerosol deposition of bacterial spores onto material surfaces for detection, sampling, and/or decontamination studies.

Materials:

- Aerosol Deposition Apparatus (ADA) (shown in Figure 1)
- Metered Dose Inhaler (MDI) preloaded with a bacterial spore suspension of known concentration (i.e., 1 x 10^9 spores per puff)
- Vertical MDI Actuator (shown in Figure 2)
- Material coupon (with dimensions at least that of the ADA)
- ADA-coupon gasket (1 per ADA) (see Figure 1)
- Clamping devices (i.e., medium-size steel binder clips, C-clamps (8 per ADA))
- Vortex mixer (shown in Figure 4)
- Aerosol trap (described in Appendix A and shown in Figure 4)
- Personal Protective Equipment (PPE) (gloves, lab coat, safety goggles)
- pH-adjusted bleach (pAB) (MOP 3128-A)
- 0.22μm pore-size syringe filters (shown in Figure 1)
- PVC tubing (3/8" OD, 1/4" ID)
- Mass balance (with 0.01 gram accuracy)
- Bench liner

1.0 STERILIZATION OF MATERIALS

Prior to the start of any experiment, all components must be sterilized and stored in a sterile environment until usage. Sterilization **is not necessary** for binder clips, MDI, vortex, or the aerosol trap.

ADAs can be sterilized by autoclave, VHP, or by wiping with pH-adjusted bleach (pAB) with subsequent deionized (DI) water and ethanol rinse/wipes. The ADA lid should be attached and in the closed position during the sterilization.

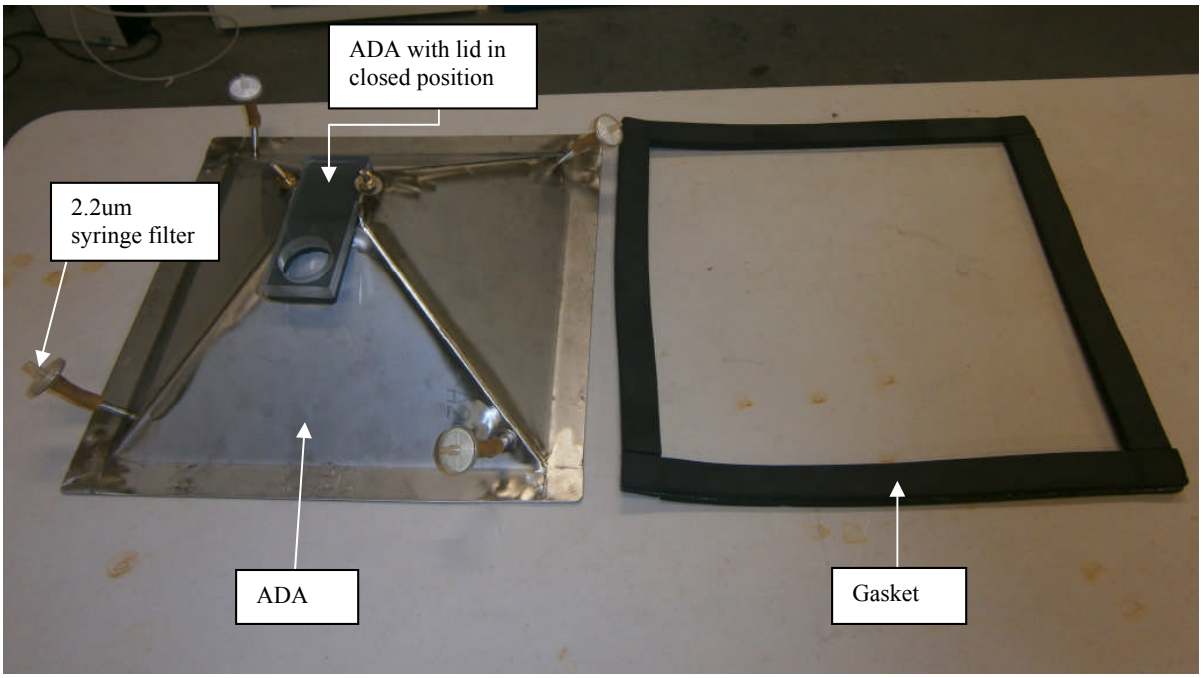

Figure 1. ADA apparatus

The MDI actuator, with attached MDI adaptor, can be wiped with pAB then rinsed with DI water.

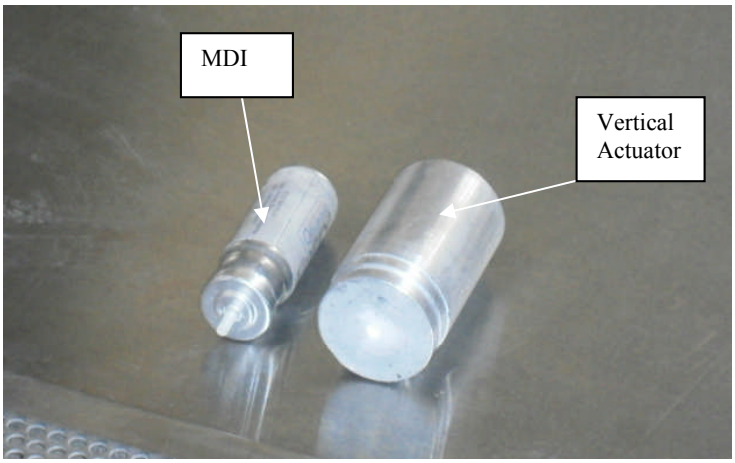

Figure 2. MDI and vertical actuator

Sterilization requirements for coupons vary by material. Regardless of the sterilization method, quality control (QC) checks (typically by collecting a swab sample per MOP 3135) should be administered to ensure the effectiveness of the sterilization method.

Gasket sterilization may also vary by material. Care should be taken to thoroughly degas gaskets if sterilized via fumigation.

2.0 PROCEDURE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).
2. Clean the workspace by wiping with pAB, next with DI water , and lastly with a 70-90% solution of denatured ethanol. Alternatively, new, clean bench liner may be placed on the work surfaces. Make sure the workspace is clean and free of debris.
3. Discard gloves and replace with fresh pair.
4. Using aseptic techniques (when possible) assemble the coupon/ADA by first placing the sterilized material coupon onto the clean lab bench or workspace, next place the sterilized gasket on top of the coupon, and lastly seat the ADA on the coupon + gasket. Orient each component so that it fits squarely with the previously placed item. Take care not to touch the inside of the ADA or the coupon surface. Secure these components by attaching medium-size binder clips, one at each corner, and one at the midpoint of each of the four sides of the ADA. The binders should firmly secure the coupon to the ADA, and apply sufficient pressure to the gasket to seal the union. If material coupons are too large to use binder clips other methods may be used to secure the coupon and gasket to the ADA (i.e., larger clamps, weight added to the ADA, etc.). Lastly, attach 0.2 um syringe filters to each vent tube on all ADAs (4 per ADA). Syringe filters can be attached using PVC tubing (3/8" OD, 1/4" ID).
5. Determine the weight of the MDI canister using a balance. Record the MDI ID number and the weight (to the nearest 0.01g) in lab notebook. In addition, keep a record of the total number of 'puffs' dispensed for each MDI canister.

   NOTE: The MDI canister full is approximately 15 g, an empty canister is approx 9.5 g. To ensure the canister contains adequate spore suspension for dosing, canisters should be retired from use when their weight falls below 10.5 g.

6. Next, assemble the MDI and actuator by inserting the MDI into the actuator, taking care not to activate the MDI.
7. Vortex the MDI/actuator assembly for 30 seconds (the MDI canister should be in direct contact with the vortex mixer).
8. Holding the MDI/actuator assembly upright (Figure 3), with a swift, firm motion, dispense three test 'puffs' into the aerosol trap to prime the MDI. It is important to vortex the

assembly 10 seconds before every puff (the exception being 30 seconds prior to the initial puff of the experiment, as prescribed in Step 7).

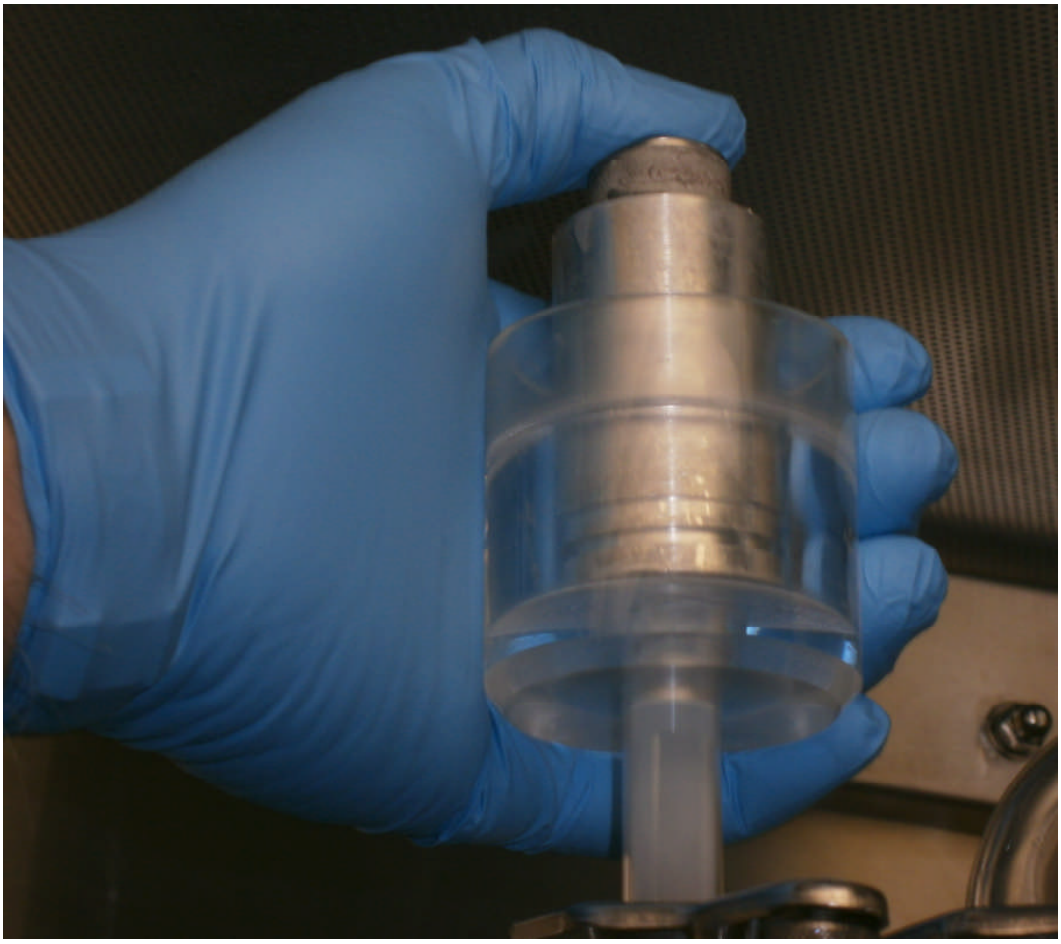

Figure 3. MDI orientation while dispensing test puffs into the aerosol trap.

9. Vortex the assembly for 10 seconds and then attach to the ADA lid by mating the ADA adaptor to the hole in the ADA lid. Loosen the lid screws enough to allow the lid to be slid into the 'open' position. Secure the lid in the open position by tightening the lid screws.

   NOTE: The 'open' position is achieved when the hole in the lid aligns with the hole in the top of the ADA.

10. With a swift, firm motion, dispense the spores by activating the MDI. Hold the MDI in the activated position for 3 seconds before releasing. Activation is best achieved by grasping the MDI/actuator with two hands, and using a thumb to press the bottom of the MDI canister.

11. Follow the reverse order of the lid opening procedure to close the ADA lid.

12. Determine the weight of the actuator-MDI using a balance, and record the weight in lab notebook.

    NOTE: If the dosing puff is faulty, return to Step 9 and attempt a second puff on the current coupon. Do not proceed to the next coupon until a 'successful' puff has been delivered. A 'successful' puff is achieved when the weight of the actuator-MDI assembly has a 0.04 g to 0.07 g loss. Familiarity and professional judgment will be needed to determine the success of a puff.

13. Vortex the assembly for 10 seconds, then proceed to dosing the next coupon (Step 9).
14. Repeat Steps 9 through 13 until all coupons have been dosed.
15. Once all coupons have been puffed, remove the MDI from the actuator and weigh. Record the final weight and total number of puffs.
16. Allow spores to settle onto the coupon surface for at least 18 hours. Settling time should not exceed 26 hours.
17. Carefully remove binder clips (or other attachment device), and remove ADA and gasket from coupon surface, taking care not to disturb the surface of the coupon.
18. Test coupon is now ready for use.
19. Decontaminate the ADA and associated components with the same procedures utilized during the initial sterilization.

APPENDIX A - Aerosol Trap

Purpose: This device allows test puffs of the MDI to be deployed without contamination of the surrounding area. Spores are pulled into the trap, contained, and inactivated.

This device consists of a suction source, a trap (containing pAB), and an inlet funnel. Aerosolized spores are pulled into the funnel, and forced into the trap. The spores are collected and inactivated as the aerosol flows through the pAB solution. The effluent air traveling toward the suction device is spore-free downstream of the trap. See Figure 4.

The aerosol trap should be assembled inside a biological safety cabinet (BSC) or chemical fume hood.

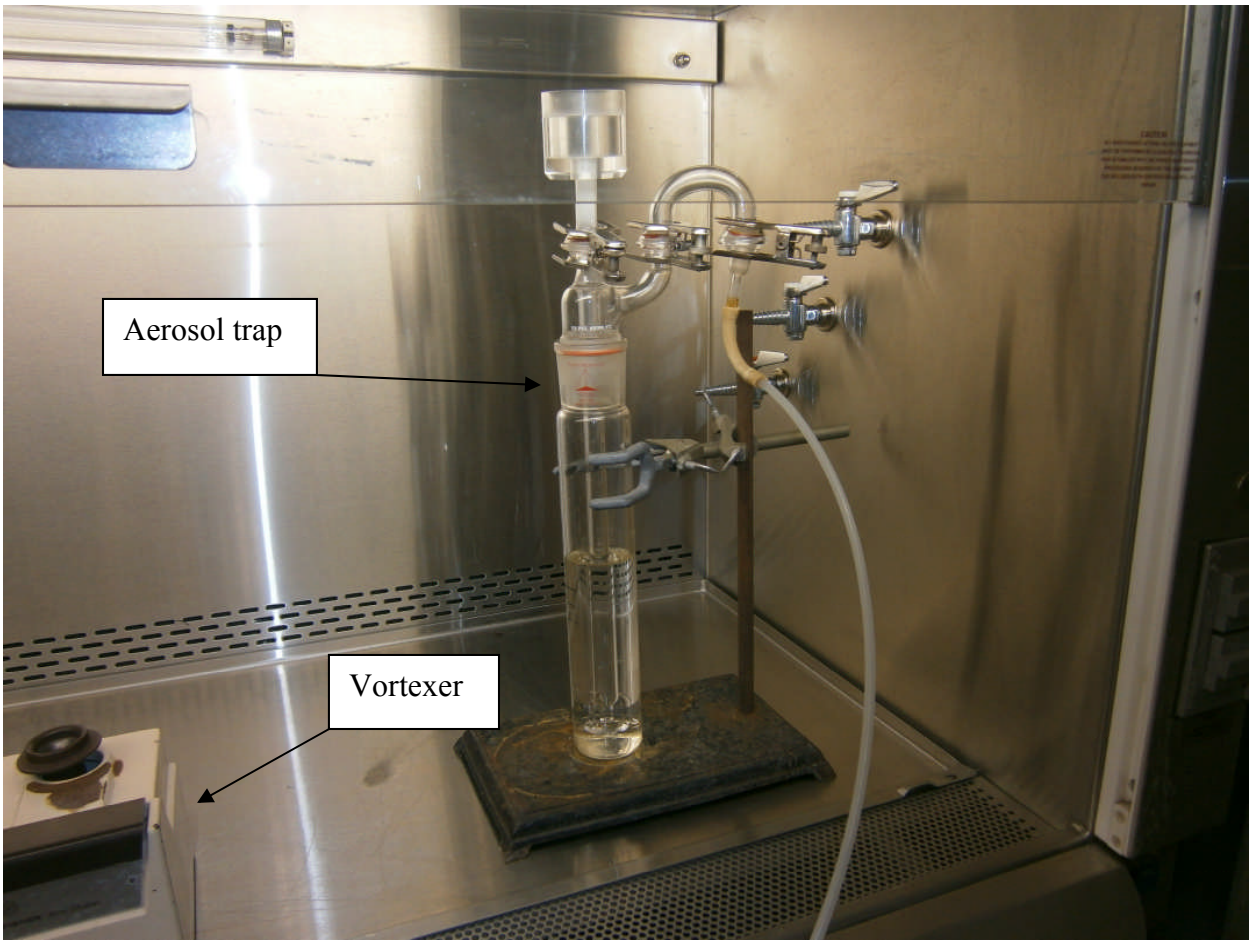

Figure 4. Aerosol trap

Miscellaneous Operating Procedure (MOP) 3161-LD:

Aerosol Deposition of Spores onto Material Coupon Surfaces using the Aerosol Deposition Apparatus (ADA) – Low Dosing

Prepared by: _____ Date: 4/29/2013

Matt Clayton, ARCADIS Work Assignment Leader

Reviewed by: _____ Date: 4/29/2013

Dahman Touati, ARCADIS Project Manager

Approved by: _____ Date: 4/29/2013

Worth Calfee, EPA Work Assignment Manager

Prepared for

National Homeland Security Research Center
Office of Research and Development
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MOP 3161-LD

TITLE: AEROSOL DEPOSITION OF SPORES ONTO MATERIAL COUPON SURFACES USING THE AEROSOL DEPOSITION APPARATUS (ADA) – LOW DOSING

SCOPE: This MOP outlines the procedure for assembly and usage of the Aerosol Deposition Apparatus (ADA) for low dosing.

PURPOSE: To provide a procedure for repeatable aerosol deposition of bacterial spores onto material surfaces for detection, sampling, and/or decontamination studies.

MATERIALS

- Aerosol Deposition Apparatus (ADA)
- Metered Dose Inhaler (MDI) preloaded with a bacterial spore suspension of known concentration (i.e., 5 x 10^6 spores per puff or less, also designated as 5E6)
- MDI Actuator with ADA adaptor (adaptor required for proper attachment of actuator to ADA lid). The MDI actuator label must match the concentration and organism of the MDI.
- Material coupon (with dimensions at least that of the ADA)
- ADA-coupon gasket (1 per ADA)
- Clamping devices (i.e., medium-size steel binder clips, C-clamps (8 per ADA))
- Vortex mixer
- Aerosol trap (described in Appendix A)
- PPE (gloves, lab coat, P95 dust mask, cap or hair bonnet, safety goggles)
- pH-adjusted bleach (pAB; prepared per MOP 3128)
- 0.22µm pore-size syringe filters
- PVC tubing (3/8” OD, 1/4” ID)
- mass balance (with 1 mg accuracy)
- canned air duster

1.0 DISINFECTION OF MATERIALS

Prior to the start of any experiments, all components must be disinfected, and stored in as close to a sterile environment as possible until usage. (Disinfection is not necessary for binder clips, MDI, vortex, and the aerosol trap).

ADAs can be disinfected by autoclave, VHP, or pAB wiping with subsequent deionized water (diH_2O) and ethanol rinse/wipes. The ADA lid should be attached and in the closed position during the disinfection.

MDI actuator with attached MDI adaptor can be wiped with pAB then rinsed with diH_2O.

> **NOTE**: **DO NOT** expose to ethanol, as ethanol will shatter the ADA adaptor.

Disinfection requirements for coupons vary by material. Regardless of disinfection method, QC checks should be administered to ensure the effectiveness of the disinfection method.

Gasket disinfection may also vary by material.

2.0 PROCEDURE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).
2. Cover the tables with fresh bench liner.
3. Discard gloves and replace with fresh pair.
4. Using aseptic techniques (when possible) assemble the coupon/ADA by first placing the disinfected material coupon onto the clean lab bench or workspace. Next place the disinfected gasket on top of the coupon, and then seat the ADA on the coupon + gasket.

   Orient each component so that it fits squarely with the previously placed item. Take care not to touch the inside of the ADA or the coupon surface. Secure these components by attaching medium-size binder clips, one at each corner, and one at the midpoint of each of the four sides of the ADA. The binders should firmly secure the coupon to the ADA and apply sufficient pressure to the gasket to seal the union. If material coupons are too large to use binder clips, other methods may be used to secure the coupon and gasket to the ADA (i.e., larger clamps, weight added to the ADA, etc.).

   Lastly, attach 0.2 um syringe filters to each vent tube on all ADAs (4 per ADA). Syringe filters can be attached using PVC tubing (3/8” OD, 1/4” ID).
5. Determine the weight of the MDI canister using a balance. Record the weight (to the nearest mg) in lab notebook. In addition, keep a record of the total number of ‘puffs’ dispensed for each MDI canister.

NOTE: A full MDI canister is usually approximately 20 grams (although they are sometimes 15 grams); an empty canister is approximately 9.5 grams. To ensure the canister contains adequate spore suspension for dosing, canisters should be retired from use when their weight falls below 10.5 grams.

6. Next, assemble the MDI and actuator (with ADA adaptor) by inserting the MDI into the actuator, taking care not to activate the MDI. Weigh the MDI and actuator assembly and record the weight (to the nearest mg) in lab notebook.

7. Vortex the MDI/actuator assembly for 30 seconds (the MDI canister should be in direct contact with the vortex mixer).

8. Holding the MDI/actuator assembly upright (MDI canister on top, MDI nozzle pointing down, and actuator + adaptor outlet pointing horizontal, in contact with the aerosol trap inlet adapter − see Figure 1), dispense three test 'puffs' into the aerosol trap to prime the MDI. It is important to vortex the assembly 10 seconds before every puff (except 30 seconds prior to the initial puff of the experiment, as prescribed in Step 7). Weigh the MDI/actuator assembly after each puff and record in the Deposition Log. A weight reduction of 0.04-0.07g will indicate a successful puff.

Figure 1. MDI orientation while dispensing test puffs into the aerosol trap.

9. Vortex the assembly 10 seconds, and attach it to the ADA lid by mating the ADA adaptor to the hole in the ADA lid. Loosen the lid screws enough to allow the lid to be slid into the 'open' position. (The 'open' position is achieved when the hole in the lid aligns with the hole in the top of the ADA.) Secure the lid in the open position by tightening the lid screws.

10. With a swift firm motion, dispense the spores by activating the MDI. Hold the MDI in the activated position for 3 seconds before releasing. Activation is best achieved by grasping the MDI/actuator with two hands, and using a thumb to press the bottom of the MDI canister. Weigh the MDI and record in the Deposition Log assembly.

    NOTE: If the dosing puff is faulty, return to Step 8 and attempt a second puff on the current coupon. Do not proceed to the next coupon until a 'successful' puff has been delivered. A 'successful' puff is achieved when the plume can be seen through the clear ADA adaptor immediately following the puff. The puff results in audible characteristics typical of a successful puff, and the proper weight reduction is achieved. Familiarity and professional judgment will be needed to determine the success of a puff.

11. Follow the reverse order of the lid opening procedure to close the ADA lid. Detach the MDI/actuator assembly from the lid.

12. Remove the MDI from the actuator and determine the weight of the MDI using a balance, record the weight in lab notebook.

13. Place the empty actuator into the aerosol trap and remove dosing residue from the inner surface of the actuator using the canned air duster. Take care to blow the compressed air into the bottom of the actuator so that the residue is blown directly into the aerosol trap.

14. Vortex the assembly for 10 seconds, holding the assembly upright (MDI canister on top, MDI nozzle pointing down, and actuator + adaptor outlet pointing horizontal, towards the aerosol trap inlet − see Figure 1), dispense a test puff into the aerosol trap (expect a faulty puff, as the MDI requires recharging after a puff in the non-upright orientation). Repeat once more (vortex 10 seconds, puff). Expect the subsequent puff to be characteristic (visually, audibly, and with expected weight loss) of a successful puff. If the second test puff is successful, proceed to dosing the next coupon (Step 9). If the second puff is faulty, repeat Step 13.

15. Repeat Steps 9 through 13 until all coupons have been dosed.

16. Allow spores to settle onto the coupon surface for at least 18 hours. Settling time should not exceed 26 hours.

17. Wearing the proper PPE, carefully remove binder clips (or other attachment device), and remove ADA and gasket from coupon surface, taking care not to disturb the surface of the coupon.

18. Test coupon is now ready for use.

19. Decontaminate the ADA and associated components with the same procedures utilized during the initial disinfection.

APPENDIX A – AEROSOL TRAP

Purpose: This device allows test puffs of the MDI to be deployed without contamination of the surrounding area. Spores are pulled into the trap, contained, and inactivated.

This device consists of a suction source, a trap (containing pAB), and an inlet funnel. Aerosolized spores are pulled into the funnel and forced into the trap. The spores are collected and inactivated as the aerosol flows through the pAB solution. The effluent air traveling toward the suction device is spore-free downstream of the trap. See Figure 2.

The aerosol trap should be assembled inside a biological safety cabinet (BSC) or chemical fume hood.

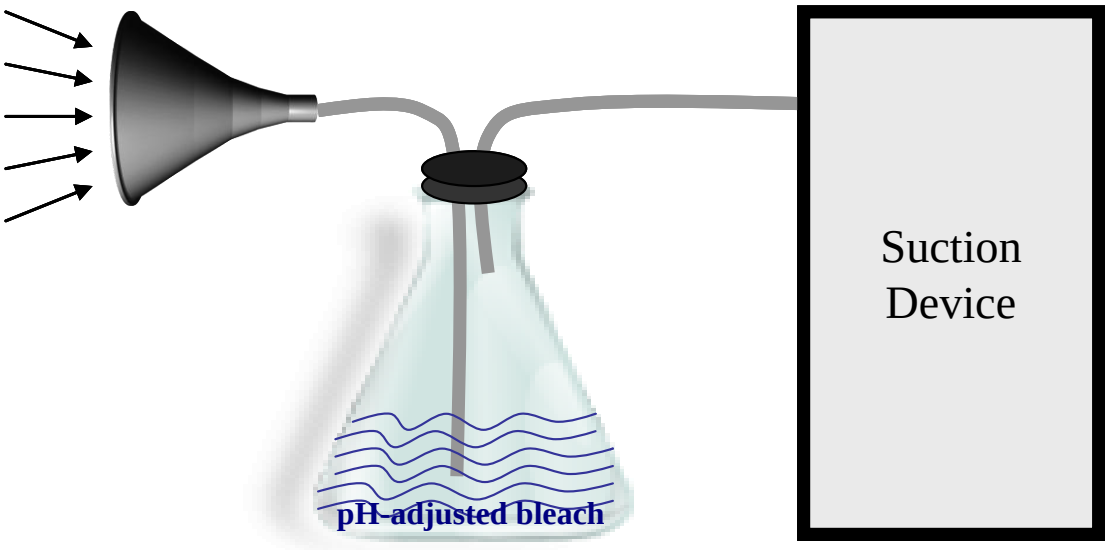

Figure 2. Schematic drawing of Aerosol Trap.

Miscellaneous Operating Procedure (MOP) 3164:

Procedure for 37MM Cassette and Trace Evidence Filter Vacuum Sampling of Large and Small Coupons

Prepared by: _____ Date: 4/29/2013

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Reviewed by: _____ Date: 4/29/2013

Dahman Touati, ARCADIS Project Manager

Approved by: _____ Date: 4/29/2013

Worth Calfee, EPA Work Assignment Manager

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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Prepared by

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TITLE: PROCEDURE FOR 37MM CASSETTE AND TRACE EVIDENCE FILTER VACUUM SAMPLING OF LARGE AND SMALL COUPONS

SCOPE: This MOP describes the procedure for vacuum sampling of porous areas.

PURPOSE: The purpose of this MOP is to ensure consistent and representative sampling of such areas.

A: 37 mm filter

EQUIPMENT (starred* quantities are per sampling kit)

- *1 – 37 mm Filter cassette loaded with desired filter
    - 0.3 µm pore size PTFE membrane (SKC 225-1723)
    - 0.8 µm pore size MCE membrane (SKC 225-3-01)
- Vac-U-Go pump (SKC 228-9605)
- Rotameter (SKC 320-100)
- Tygon tubing, 1/4 in ID, 7/16 in OD, 50ft (SKC 225-1345)
- *Two PVC adapters, 250/pkg (SKC 225-132A)
- *One BD Falcon Sterile 15 mL Polyprolene Conical Tubes (fishersci.com, P/N 225-352196)
- *Two 5.5” x 9” 3.5 mil sterile sampling bags with flat-wire closures (fishersci.com, Item# 14-955-187)
- *10” x 14” overpack sample bag with round wire enclosure (fishersci.com, Item# 01-002-53)
- 12” x 12” Template
- Permanent marker
- Nitrile gloves
- Timer

1.0 PREPARATION

All materials needed for each sample to be collected will be prepared in advance. A sample kit for a single cassette vacuum sample will be prepared using the following procedure.

1) Don nitrile gloves.
2) In laboratory, assemble nozzles:

a) Cut the sampling nozzle from tygon tubing as follows: Using scissors, cut a section of tygon tubing 1-inch long, then cut one end of the tubing at approximately a 45-degree angle.

b) Place the nozzle onto a PVC adapter.

c) Prepare 25% more nozzles than required by the testing protocol.

d) Sterilize the nozzles with a 15 minute gravity cycle of the autoclave.

3) In laboratory, assemble cassette kit:

   a) Label the 37 mm cassette with a unique sample ID.

   b) Label the 15 mL conical tube with the same unique sample ID.

   c) Aseptically remove the cassette plugs and place a PVC adaptor onto each end of the cassette. Save the removed plugs for step f) below.

   d) Cut a 20 cm long piece of tubing with the scissors.

   e) Place the 20 cm tubing onto the downstream end of the cassette..

   f) Place the sampling nozzle (1-inch section of tubing) onto the upstream end of the cassette with the angled side furthest from the cassette.

   g) Place the cassette with PVC adaptors, 20 cm tubing, and nozzle into a 5.5" x 9" 3.5 mil bag, making sure the end of the cassette that attaches to the vacuum tubing is closest to the bag opening. (see Figure 1)

   h) Place the red plugs removed from the cassette in step a) above into the same 5.5" x 9" bag.

   i) Place the 15 mL conical tube and the 5.5" x 9" bag with 37mm cassette assembly and plugs into the 10" x 14" overpack bag.

   j) Place an additional sterile small bag into the overpack bag.

4) Label the 10" x 14" bag with the sample ID.

5) Store sample kits in a clean dry location.

Figure 1. 37mm Cassette Vacuum Inner Bag

2.0 VACUUM SAMPLING OF SMALL (12" by 12") COUPONS

The following procedure will be used in this study for 37mm vacuum sampling of each coupon surface:

1. A two-person team will be used, employing aseptic technique. The team will consist of a sampler and a support person. In times when a third person (sample handler) is present, he or she will act as an assistant to the support person for data recording.
2. The sampler will plug in the Vac-U-Go pump power cord, and attach the calibrated rotameter to the pump. Turn on the pump and adjust the pump valve until the flow rate is 20 ± 2 LPM. Record the flow rate in the project notebook. The sampler will then don his/her sterile gloves.
3. Both members of the sampling team will each don a pair of sampling gloves (a new pair per sample); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. Both members shall wear dust masks to further minimize potential contamination of the samples. Further respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP).
4. The Vac-U-Go pump will be maintained on a rolling cart for easy movement into place.
5. The support person will aseptically unwrap a template from the bag and present it to the sampler, taking care to not touch the template.
6. The sampler will place the template onto the coupon surface.
7. The support person will open the sampling supply bin and remove the 37mm cassette sample kit from the bin.

8. The support person or assistant will record the sample collection bag ID number on the sampling log sheet or in the laboratory notebook.
9. The sampler and support person will ensure that the correct sample coupon has been selected, referencing the coupon code on the sampling bag.
10. The support person or assistant will record the coupon code on the sampling log sheet next to the corresponding 37mm cassette collection bag number that was just recorded.
11. The support person will:
    a. Open the 37mm cassette sample kit outer bag and remove the unlabeled 37 mm cassette assembly bag.
    b. Open the small unlabeled bag containing the 37 mm cassette assembly.
    c. Hold the bag so that the sampler can remove the kit.
    d. The support person will hold the tubing for the sampler to place the 37mm cassette assembly onto the tubing.
12. The sample handler will remove the 37 mm cassette assembly from the bag and attach to the tygon vacuum tube held by the support person.
13. The support person or assistant will be prepared to record the duration of sampling. Prompts should be given to the sampler so that the sample duration is close to the values in the Table 1.

    Table 1: Suggested Sample Duration and Speed for 12" x 12" surface area

Material	**Total Sampling duration**	**Single Pass duration**	**Number of passes per direction**
Concrete	300 seconds	3 seconds	50
Upholstery	300 seconds	3 seconds	50
Carpet	300 seconds	3 seconds	50

14. The sampler will:
    a. Ensure that the filter is correctly placed on the tygon vacuum tube and adjust, if necessary.
    b. Turn on the vacuum.
    c. Vacuum "horizontally" using S-strokes to cover the entire area of the material surface not covered by the template, while keeping the tygon nozzle angled so that the tapered opening of the tygon nozzle is flush with the sample surface.

       Note: a target duration of time to vacuum each coupon should be determined prior to testing for each material type. The sampler should pace the speed of the nozzle such that the target sampling time is achieved for each coupon.

d. Vacuum the same area "vertically" using the same technique.

e. Turn off the vacuum when sampling is completed.

f. Hold the nozzle and remove it from the cassette.

15. The support person will remove the 17 mm tube from the sample kit and open the tube.
16. The sampler will place the nozzle into the tube with the adapter end down while holding the cassette in the opposite hand.
17. The support person will seal the tube and place in the small unlabeled bag.
18. The sampler will use the "nozzle" hand to remove the tubing from the outlet side of the cassette and hold the cassette out to the support person.
19. The support person will
    a. Don a fresh pair of gloves.
    b. Seal the cassette with the two red plugs found in the small unlabeled sample collection bag.
20. The support person will open the small unlabeled sample collection bag, and the sampler will place the secured 37 mm cassette inside with the 15 mL conical tube.
21. The support person will then seal the small unlabeled sample collection bag and wipe it with a Dispatch® wipe.
22. The support person will open the labeled 10" x 14" overpack bag and place the smaller unlabeled collection bag containing the cassette inside.
23. The support person will then seal the labeled 10" x 14"overpack bag and wipe it with a Dispatch® wipe.
24. The sampler will remove the used 20 cm length of tubing and discard.
25. The support person will then place the double contained sample into the sample collection bin.
26. All members of the sampling team will remove and discard their gloves.
27. Steps 3 – 22 will be repeated for each sample to be collected.
28. At the completion of testing, determine the final flow rate of the vacuum using the rotameter and record in the project notebook.

B: 3M Trace Evidence Filter

Materials:

- PPE (gloves, lab coat, safety goggles)

- Sterile, sealed 10”x15” Twirl-em bags
- Alcohol wipes
- Dry wipes moistened with 3% sodium thiosulfate
- Sharpie and/or pre-printed labels
- Secondary containment such as a large Tupperware bin
- Lab notebook
- QAPP for project that is utilizing the Trace Evidence Filter samples
- 3M Forensic Vacuum Filter (Trace Evidence Filter) (Precision Data Products, Grand Rapids, MI catalog #FF-1), referred to as TEF

1.0 PROCEDURE

1.1 Assembly of Trace Evidence Filter Kits

TEF kits are assembled in the following manner:

1. TEF kits can be assembled outside of the biological safety cabinet, in a dry, clean area. Make certain to use proper PPE, including gloves, while handling all TEF kit materials. Gather all materials to assemble the kits before assembly. These materials include:

    - 3M Forensic Vacuum Filters
    - 10”x15” Twirl-em bags
    - Sharpie or pre-printed labels

2. Obtain a copy of the labeling scheme for the samples. This may be detailed in the QAPP. For each TEF kit, use a Sharpie and label a large 10” x 15” Twirl-em bag with the correct sample ID. Using 2 pre-printed labels, label the bag containing the TEF with a permanent label and a label that can be easily removed. The removable label will be placed on the TEF after sampling.

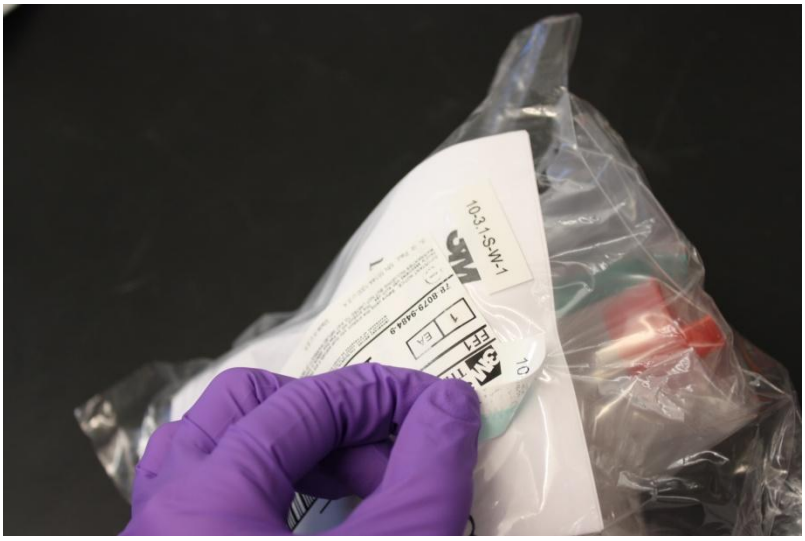

Figure 1: Example of a permanent and removable label on a TEF

3. Open the labeled, 10” x 15” Twirl-em bags one at a time. Place the labeled, TEF in the 10” x 15” Twirl-em bags that have the corresponding label. Add a non-labeled, 10” x 15” Twirl-em bag in the labeled 10” x 15” Twirl-em bag containing the TEF. This completes the TEF kit assembly. In the lab notebook, record the date and project for which the TEF kits were assembled.

4. Place the assembled TEF kits into a secondary containment, such as a large Tupperware bin. When moving the kits to a sampling location, always have them in secondary containment.

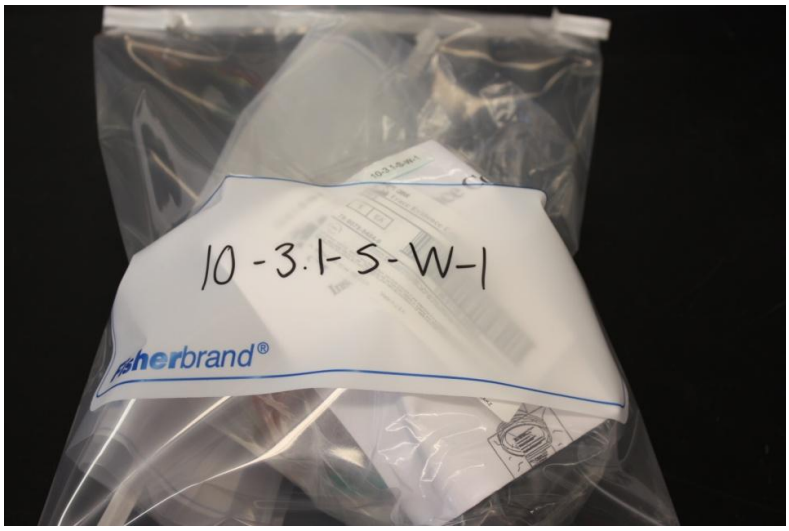

Figure 2: Example of an assembled TEF kit

2.0 VACUUM SAMPLING OF SMALL (12" by 12") COUPONS

The following procedure will be used in this study for TEF vacuum sampling of each coupon surface:

A two-person team will be used, employing aseptic technique. The team will consist of a sampler and a support person. In times when a third person (sample handler) is present, he or she will act as an assistant to the support person for data recording.

1) The Omega vacuum will be maintained on a rolling cart for easy movement into place.
2) The sampler will plug in the Omega Vacuum into a variable transformer (Staco Energy Products Co., Model 3PN1010B) set on 50%. The sampler will wipe down the nozzle (inside and out) and end of the tubing first with a Dispatch® wipe, next with a wipe pre-moistened with 3% Sodium thiosulphate, next with a wipe pre-moistened with 70 % ethanol, then don his/her sterile gloves.
3) Both members of the sampling team will each don a pair of sampling gloves (a new pair per sample); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. Both members shall wear dust masks to further minimize potential contamination of the samples. Further respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP).
4) The support person will aseptically unwrap a template from the bag and present it to the sampler, taking care to not touch the template.
5) The sampler will place the template onto the coupon surface.
6) The support person will open the sampling supply bin and remove the TEF sample kit from the bin.
7) The support person or assistant will record the sample collection bag ID number on the sampling log sheet or in the laboratory notebook.
8) The sampler and support person will ensure that the correct sample coupon has been selected, referencing the coupon code on the sampling bag.
9) The support person or assistant will record the coupon code on the sampling log sheet next to the corresponding TEF collection bag number that was just recorded.
10) The support person will:
    a. Open the TEF sample kit overpack bag and remove the labeled TEF assembly bag.
    b. Open the labeled assembly bag containing the TEF cassette assembly.
    c. Hold the bag so that the sampler can remove the TEF cassette without touching the outside of the bag.
    d. The sampler will remove the red round plug from the TEF cassette, and return it to the inside of the bag.

e. The sampler will attach the TEF to the vacuum hose.

f. The sampler will remove the black end cap from the TEF cassette, and return it to the inside of the bag.

11) The support person or assistant will be prepared to record the duration of sampling. Prompts should be given to the sampler so that the sample duration is close to the values in the Table 2.

Table 2: Suggested TEF Sample Duration and Speed for 12" x 12" surface area

Material	**Total Sampling duration per coupon**	**Single Pass duration**	**Number of passes per direction**
Concrete	90 seconds	3 seconds	15
Upholstery	90 seconds	3 seconds	15
Carpet	90 seconds	3 seconds	15

12) The sampler will:

a. Ensure that the filter is correctly placed on the vacuum hose and adjust, if necessary.

b. Turn on the vacuum.

c. Vacuum "horizontally" using S-strokes to cover the entire area of the material surface not covered by the template, while keeping the nozzle flush with the sample surface.

d. Vacuum the same area "vertically" using the same technique.

e. Turn off the vacuum when sampling is completed.

f. Reseal the inlet with the black end cap. This will be provided to him by the support person working the cap to the lip of the bag, touching only the outside of the bag, until the sampler can grab it.

g. Remove the filter from the vacuum hose.

h. Reseal the outlet with the red plug. This will be provided to him by the support person working the cap to the lip of the bag, touching only the outside of the bag, until the sampler can grab it.

13) The support person will remove the second removable label from the assembly bag and place on the filter housing.

14) The support person will hold open the evidence bag while the sampler places the filter inside.

15) The support person will seal the evidence bag with the self-adhering strip and wipe the outside with a Dispatch wipe.

16) The support person will open the labeled 10" x 14" overpack bag and place the evidence

collection bag containing the cassette inside.

17) The support person will then seal the labeled 10” x 14”overpack bag and wipe it with a Dispatch® wipe.

18) The support person will then place the double contained sample into the sample collection bin.

19) All members of the sampling team will remove and discard their gloves.

20) The sampler will wipe down the nozzle (inside and out) and end of the tubing first with a Dispatch® wipe, next with a wipe pre-moistened with 3% STS, next with a wipe pre-moistened with 70 % ethanol.

21) Steps 4 – 24 will be repeated for each sample to be collected.

Miscellaneous Operating Procedure (MOP) 3165:
Sponge Sample Collection Protocol

Prepared by: _____ Date: 11/15/2012
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MOP 3165

Title: **SPONGE SAMPLE COLLECTION PROTOCOL**

Scope: This MOP outlines the procedure for collecting spores using a 3M Sponge-Stick™.

Purpose: To provide a procedure for the collection of spore samples using a Sponge-Stick™ in a consistent and repeatable manner.

MATERIALS

- 3M Sponge-Sticks™ (P/N SSL10NB), hereafter referred to as 'sponge'
- One Seward stomacher bag (P/N BL6041/CLR) per kit
- Disposable gloves
- Sterilized sampling templates
- One Fisher Sterile sampling bag with flat wire enclosure (7" x 12", P/N 14-955-194) per kit
- One Fisher Sterile sampling bag with flat wire enclosure (10" x 14", P/N 01-002-53) per kit for overpack
- Dispatch wipes

1.0 PREPARATION

All materials needed for collection of each sample will be prepared in advance using aseptic technique. A sample kit for a single sponge sample will be prepared as follows:

1.1 One stomacher bag will be uniquely labeled as specified in the project QAPP.

1.2 A 10" x 14" bag will be labeled with the same ID as the stomacher bag.

1.3 One stomacher bag, and one 9.5" x 12" unlabeled bag will be placed in the overpack bag.

1.4 A sterile Sponge-Stick will be added to the overpack bag.

1.5 Each prepared bag is one sampling kit.

2.0 PROCEDURE

A two person team will be used, employing aseptic technique throughout. The team will consist of a sampler and a sample handler. In some cases, a third person may be needed to move samples.

Throughout the procedure, the support person will log anything they deem to be significant into the laboratory notebook.

In general, the team works from the least contaminated sample set (i.e., control blanks) towards the most contaminated sample set (i.e.,positive controls).

All members shall wear dust masks to minimize potential contamination of the samples. Depending on the situation, respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP). New disposable lab coats are required for the sample handler when changing between different types of materials or when direct contact between the coupon and lab coat occurs.

2.1 Wearing a clean pair of gloves over existing gloves, the sampler will place the disposable template over the area to be sampled.

2.2 The support person will remove a sample kit from the sampling bin and record the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.

2.3 The sampler and support person will verify the sample code and ensure that the correct coupon and location are being sampled.

2.4 The support person will:

a) Open the outer sampling bag touching the outside of the bag.

b) Touching only the outside of the (10” x 14”) bag, remove the sponge, and hand it to the sampler.

c) Remove the stomacher bag, being careful to not touch the inside of the outer sampling bag, and open it touching only the outside.

2.5 The sampler will remove the sterile sponge from its package. Grasp the sponge near the top of the handle. Do not handle below the thumb stop.

2.6 The sampler will wipe the surface to be sampled using the moistened sterile sponge by laying the widest part of the sponge on the surface, leaving the leading edge slightly lifted. Apply gentle but firm pressure and use an overlapping ‘S’ pattern to cover the entire surface with horizontal strokes (Figure 1). Use the other hand to hold the template during sampling, being careful not to touch the surface.

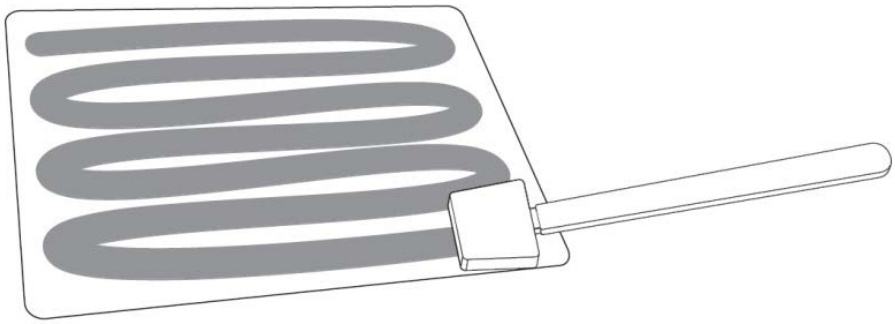

Figure 1. First pass with sponge – horizontal strokes using one side of the sponge

2.7 The sampler will turn the sponge over and wipe the same area again using vertical 'S'-strokes (Figure 2).

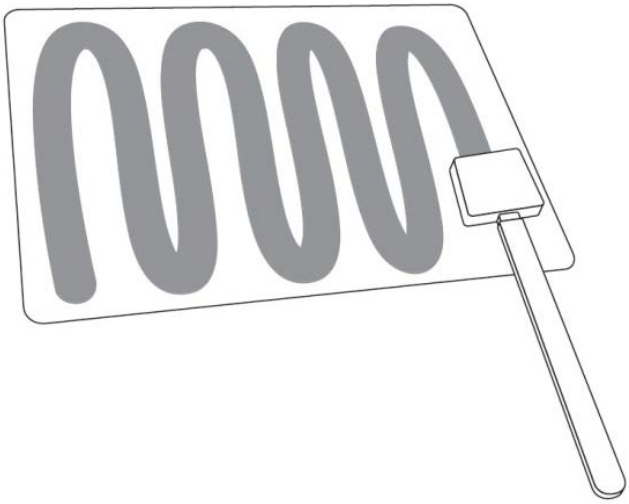

Figure 2. Second pass with sponge – vertical strokes using the other side of the sponge

2.8 The sampler will the use the edges of the sponge (narrow sides) to wipe the same area using diagonal 'S'-strokes (Figure 3). The sponge will be flipped to use the opposite side immediately after the longest stroke at opposite corners.

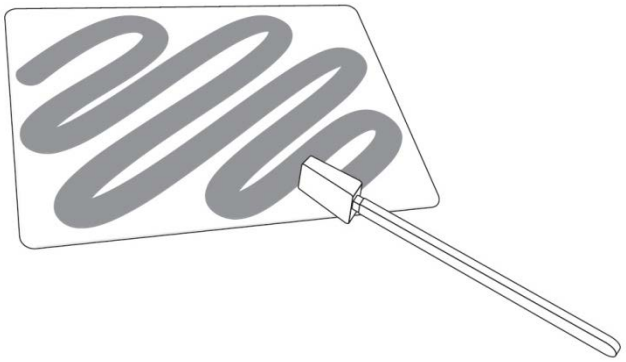

Figure 3. Third pass with sponge – diagonal strokes using the edges of the sponge

2.9 The sampler will use the tip of the sponge to wipe the perimeter of the sampling area (Figure 4).

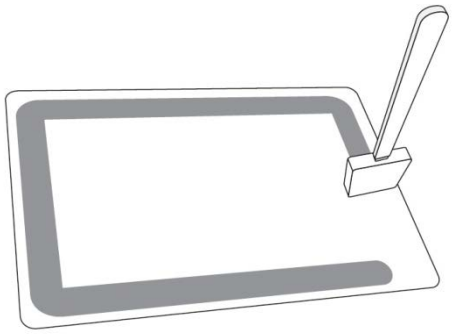

Figure 4. Final (fourth) pass with sponge – perimeter wipe using the tip of the sponge

2.10 The sample handler will open the stomacher bag, careful not to touch the inside of the bag.

2.11 The sampler will place the end of the sponge in the bag, holding the handle outside the opening of the bag.

2.12 The sample handler will grasp the sponge from outside of the bag, and help the sample break off the handle of the sponge. The handle below the thumbstop should not touch the inside of the stomacher bag.

2.13 The sample handler will securely seal the stomacher bag and wipe the outside with a disinfecting wipe.

2.14 The sample handler will then place the stomacher bag inside the unlabeled sterile bag.

2.15 The sample handler will place this in the overpack bag and wipe the overpack bag with disinfecting wipes.

2.16 The sample handler will place the overpack bag in the sample bin.

NOTE: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

2.17 The sampler will dispose of the template.

2.18 Both members will remove outer gloves and discard. Clean gloves should be worn for each new sample.

3.0 REFERENCES

Sponge sample collection protocol adapted from:

National Validation Study of a Cellulose Sponge Wipe-Processing Method for Use after Sampling Bacillus anthracis Spores from Surfaces. Rose, Laura J.; Hodges, Lisa; O'Connell, Heather; Noble-Wang, Judith. *Appl. Environ. Microbiol.* 2011, 77(23):8355.

Miscellaneous Operating Procedure (MOP) 3168:
Aggressive Air Sampling (AAS) for WA 3-28: Phase I Sampling Approach

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MOP 3168

TITLE: AGGRESSIVE AIR SAMPLING (AAS) FOR WA 3-28: PHASE I SAMPLING APPROACH

SCOPE: This MOP outlines the setup, operation, and timeline schedule for conducting AAS testing in COMMANDER.

PURPOSE: To provide a standardized and repeatable procedure for all AAS tests to be conducted under WA 3-28's Phase I sampling matrix using high volume (HiVol) samplers.

1.0 INTRODUCTION

Preparations for each test will be conducted according to the schedule listed in this procedure. Any deviations will be noted in the laboratory notebook, along with the reason for the deviation.

Section 2.0 lists the preparation steps that need to be taken the Thursday before testing is to occur (Day 1). Sections 3.0 and 4.0 detail the pre- and post-decon procedures to be followed for AAS testing.

2.0 PREPARATION

NOTE: Materials needed **before** Day 1 include material coupons and gaskets, placed in VHP bags, and exposed to a VHP sterilization cycle. These should be left to degas for a **minimum of 3 days** before use.

Day 1 – Thursday

1. Ensure the following materials for inoculation are in airlock:

- (2) 3 x 3 Grids
- (6) ADAs and clamps for single coupons
- (18) ADAs and clamps for 3 x 3 coupons
- Oscillating fan
- Tables for coupon staging come Monday

2. Ensure all equipment needed for testing is in COMMANDER and COMMANDER is setup (see Figure 1):

    - SAR inlet filter box, hoses and masks
    - (2) Blowers and needed power cords
    - (3) HiVol Samplers in open orientation (no air-tight seals)
    - HiVol samplers should have been calibrated the day prior
    - Hydrogen peroxide (H_2O_2) air monitors are calibrated and functional

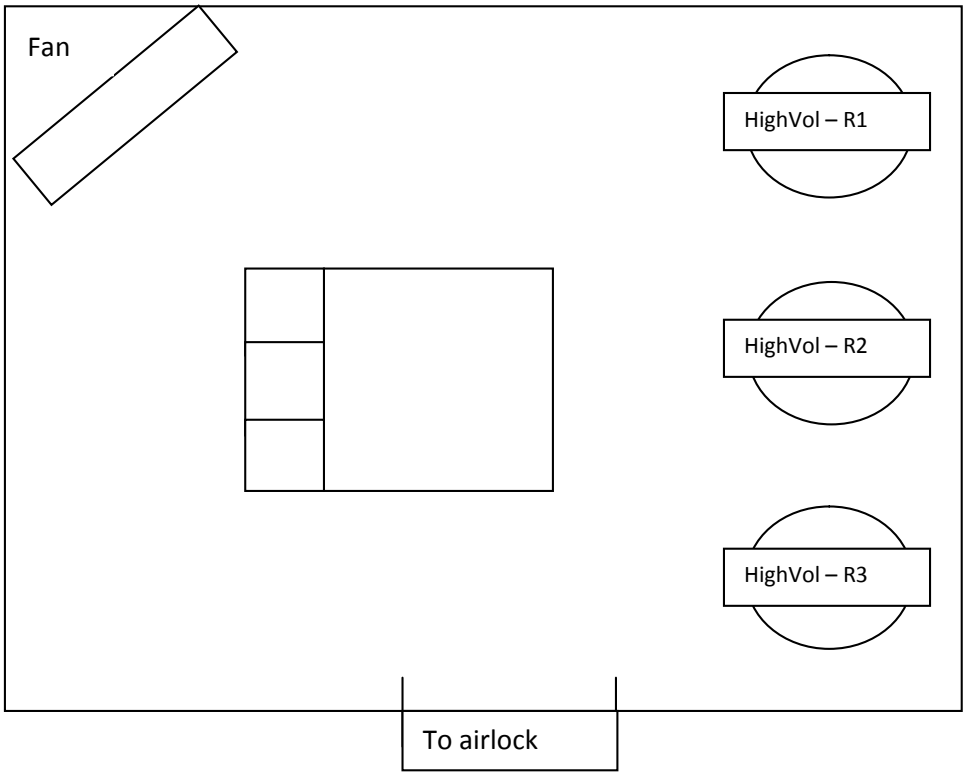

Figure 1. COMMANDER layout showing HiVol sample numbers

3. Close and seal COMMANDER door.

4. Close airlock door.

5. VHP airlock per MOP-3120 (*VHP Operation)*.

Day 2 – Friday

1. Aerate the airlock upon arrival in the morning.

2. VHP COMMANDER per MOP-3120 **in the morning**, so that external aeration can begin in mid afternoon.

3. Clear the airlock for entry as early as possible using a Draeger tube.

4. Set up coupons in H130, outside of the enclosure, using equipment from the airlock and previously sterilized coupons and gaskets allowed to degas in VHP bags.

5. Inoculate coupons according to MOP 6561 (*Aerosol Deposition of Spores onto Material Coupon Surfaces using the Aerosol Deposition Apparatus (ADAs)*. Fill out Attachment B, WA 3-28 Coupon Deposition Log. The list of coupon IDs follows, where M is the material ID; L(Laminate), D(Drywall), or C(Carpet):

42" x 42" not inoculated	28-[test ID]-XM,
42" x 42"	28-[test ID]-M
14" x 14" material	28-[test ID]-M1-R[1,2,3]
14" x 14" stainless steel	28-[test ID]-S-R[1,2,3]
14" x 14" material not inoculated	28-[test ID]-XM1-F1

6. Calibrate the COMMANDER ATI H_2O_2 sensor outside of COMMANDER using MOP 3136 (*General Procedure for Calibration of ATI Hydrogen Peroxide Gas Transmitters using Solution Wells).*

7. Place the ATI sensor back into COMMANDER.

8. Mop the enclosure with Clorox Clean-up.

9. Close enclosure doors.

3.0 PRE-DECON AAS PROCEDURES

Day 3 - Monday

△ Conduct a pre-job safety briefing prior to initiating work.

△ A "buddy" equipped with appropriate respiratory protection is required to be present outside of COMMANDER when personnel are working inside.

1. Set up the ELPI system from outside of COMMANDER and zero according to MOP 3133 (*Basic Operation and Maintenance of the Dekati Electrical Low Pressure Impactor (ELPI)*).

2. Wipe down the outside of the 42" x 42" coupons, the 14" x 14" test coupons, and ADA's with dispatch wipes.

3. Transfer the coupons from H130 to the airlock. This operation will be done with a clean team inside the airlock, and a team in H130. The H130 team (in clean garb) will complete the wipe down, and then change garb. The airlock team will enter the airlock in clean garb and booties. During the moving operation, the H130 team will pass the coupon to the airlock team with no direct contact between alternate teams. Once both coupons have been passed into the airlock, all personnel will leave the airlock, and it will be purged for 30 minutes. An oscillating fan will be used to prevent settling.

4. Positive control 14" x 14" coupons should be moved at this same time, but put back in their original location. This is to simulate the movement undergone by the test coupons.

5. Verify the camera is recording, and that the DAS is operational and recording data.

6. Assemble the supplies:
   a. Sterilized PM_{10} filters (8)
   b. Sterilized collection shims (8)
   c. Sterile gloves (2 packs) and laboratory gloves
   d. Sterile garb x 8(coat, p95, hair net, 12 boot covers)
   e. EtO'd notebook, AAS event log and pen
   f. Digital timer (2)
   g. Duct tape
   h. Blank sample kits (Filters Sample IDs : 28-05a-XAF-1-R(1-3), and 28-05a-XAF-1-F1),(Shims Sample IDs : 28-05a-XAS-1-R(1-3) and 28-05a-XAS-1-F1)

(Sponge sticks: 28-05a-M-1-R(1-3), 28-05a-XM-1-F1 28-05a-S-1-R(1-3) and 28-05a-S-1-F1)

i. Cooling vests

j. Dispatch wipes

k. Molykote® Grease Spray

l. Anemometer

7. Three personnel (referred to as "Clean Man", "Dirty Man 1", and Dirty Man 2", or CM, DM1 and DM2) will enter the enclosure and wipe in bins of supplies, then close the enclosure doors.
   - Plug in and record position of power cords for blowers and fans.
   - Install the relative humidity (RH) and vaprous hydrogen peroxide (VHP) sensors to the COMMANDER chamber.
   - Verify supply quantities against the WA 28 packing list (found on DTRL/WA 3-28).
   - ELPI should be monitoring enclosure PM, and should be changed to COMMANDER immediately before entry.
   - Personnel should spend 10 minutes minimum in enclosure before opening airlock door.

8. Three personnel (CM, DM1, and DM2) will enter the airlock wearing cooling vests, C-suits and carrying the supply bins:

9. Once the airlock door is closed, open the door to COMMANDER. Put on clean garb over the C-suit. Install the sterile filters on top of the vents of the three HiVol samplers (vent seen in Figure 2a). To do this, the support person (DM1) will grab the filter frame with sterile gloves and hold it out of the way. The sample handler (DM2) will open the bag containing the filter and the sampler (CM) will use sterile thumb forceps to put the filter in place. The DM1 will then return the frame and secure it in place. Unlatch the hinge of the head unit and pull down. Secure latches around the base of the unit (Figure 2b). CM can help with this last step before changing into new sterile gloves.

10. The DM1 will unwrap the aluminum foil covering the collection shim, leaving the shim inside and exposed. The DM2 will spray the shim with Molykote® Grease Spray, ensuring full coverage of the shim. Wearing sterile gloves, the CM will aseptically pull the shim out of the foil and place in each of the three HiVol inlet heads, minimizing contact with the greased surface. Align the shim and secure in place with the Teflon tabs (Figure 2c).

11. CM will unlatch the top covering of the head unit and pull down. Secure in place with the latches (Figure 2d).

12. DM1 and DM2 will return to the airlock.

13. Wearing sterile gloves, the DM1 will remove the apparatus brace, ADAs, and gaskets from the sterile coupon and put aside in the airlock. DM1 and DM2 will pick up the coupon, being careful not to touch the surface. Move the coupon into COMMANDER and place in the designated sampling position. To do this, the coupon will need to be rotated vertically so that it can fit through the door.

14. DM1 and DM2 remove their gowns and don SAR. The CM returns to the airlock and closes the airlock door.

△ Inspect SAR masks and hoses and the filter box prior to donning.

a. 

b. 

c. 

d. 

Figure 2. a) HiVol sampler with head unit and top open, and filter vent secured; b) head unit closed and latched; c) placement of shim; and d) top closed and latched.

15. Turn on the fan to the 70% speed setting. Measure the wind speed at the HiVol inlets. These should be less than 30 mph.

16. Test the force of the leaf blower, pointed away from the coupon, using the anemometer.

17. The DM1 will agitate the surface of the coupon using a leaf blower according to MOP 3166 (*Aerosolization of Contaminated Coupons Using the Toro Power Sweep Electric Blower for Aggressive Air Sampling (AAS)*). At the very moment the blower is turned on, DM2 will turn on all three HiVol samplers. DM2 will monitor the blowing time as per MOP 3166 and document the intervals by filling out the Aggressive Air Sampling (AAS) Event Log (Attachment A).

18. At the completion of the agitation, the DM1 and DM2 remain in COMMANDER. After 20 minutes total (10 minutes after finishing aggressive agitation), DM2 turns off the HiVol samplers.

19. Turn off (unplug) the fan.

20. CM will re-enter COMMANDER.

21. Collect blank samples:

    Filters Sample IDs : XAF-1-R(1-3) + XAF-1-F1 (Field blank)

    Shims Sample IDs : XAS-1-R(1-3) + XAS-1-F1 (Field blank)

    a. DM1 and DM2 don outer layer of clean garb.

    b. The DM1 will open the first HiVol sampler and latch in it place.

    c. The DM1 will don a pair of sterile gloves and remove the collection shim, being careful not to touch the sides of the flow ports.

    d. The DM2 and CM will then use a sponge kit to sample the top surface of the shim according to MOP 3169 (*Sponge Sample Collection Protocol for AAS Shims*).

    e. The DM1 will then discard the shim to the airlock for sterilization. The DM1 will unlatch the base of the HiVol head unit and tilt the unit up to reveal the vent (see Figure 2a).

    f. The DM1 will don a new pair of sterile gloves and remove the vent bracket and top grate, being careful not to touch the filter.

    g. Donning new gloves, the CM will remove the filter using sterile thumb forceps. Fold the filter in half and then in half again, keeping the top side of the filter inside of the fold. Using the forceps, transfer the filter directly to a stomacher bag. The DM2 will hold the stomacher bag open for this transfer.

    h. Sample the shim plates and remove the filters for the two remaining HiVol samplers using the same protocol.

22. Remove the blank coupon and place against the COMMANDER wall.

23. All personnel change into new sterile garb.

24. CM will install sterile filters on top of the vents of the three HiVol samplers and secure in place. Unlatch the hinge of the head unit and pull down. Secure latches around the base of the unit.

25. The DM1 will unwrap the aluminum foil covering the collection shim, leaving the shim inside and exposed. The DM2 will spray the shim with Molykote® Grease Spray, ensuring full coverage of the shim. Wearing sterile gloves, the DM2 will aseptically pull the shim out

of the foil and place in each of the three HiVol inlet heads, minimizing contact with the greased surface. Align the shim and secure in place with the Teflon tabs (see Figure 2c).

26. DM2 will unlatch the top covering of the head unit and pull down. Secure in place with the latches.

27. Personnel will return to the airlock and DM1 and DM2 will don 3 pairs of boot covers.

28. Wearing gloves, the DM1 will remove the apparatus brace, ADAs, and gaskets from the inoculated coupon and put aside in the airlock. DM1 and DM2 will pick up the coupon, being careful not to touch the inoculated surface, and move the coupon into COMMANDER and place in the designated sampling position. DM1 and DM2 will remove one pair of boot covers while crossing the threshold into COMMANDER with the help of CM.

29. In the same aseptic manner, remove ADAs from material coupons and place inside on COMMANDER floor just above the test coupon, removing a second pair of boot covers while entering COMMANDER. This process will make one large 42" x 56" coupon.

30. The DM1 and DM2 remove their gowns and don SAR. CM closes the airlock door.

31. CM communicates to the buddy outside to unplug blowers 2 and 3. Confirm this is done to DM2. Alternatively, the CM can unplug as he leaves the airlock.

32. DM2 then plugs in blowers 2 and 3 (now, or after the 12 minute AAS operation).

33. DM2 turns on the fan to the 70% speed setting.

34. Agitate the surface of the inoculated coupon according to MOP 3166. At the very moment the blower is turned on, a DM2 will turn on the first HiVol sampler as specified in the QAPP. The DM2 will monitor the blowing time as per MOP 3166 and document the intervals by filling out the AAS Event Log.

35. When agitation is complete, remove SAR and leave in COMMANDER.

36. CM to exit airlock, leaving clean garb in the airlock upon exit.

37. DM 1 and DM2 will open the airlock door, move to the airlock and close the airlock door.

38. DM1 and DM2 will shower out and exit the airlock.

39. After 20 minutes of air sampling, turn off the first HiVol sampler. Immediately turn on the second sampler.

40. After 20 minutes of sampling with the second HiVol sampler, turn off the second sampler and immediately turn on the third. Again, sample for 20 minutes and then turn off.

41. Wearing clean garb, collect samples from the stainless steel and laminate control coupons according to MOP 3165 (*Sponge Sample Collection Protocol*). A blank laminate coupon (L1-F1) and a field blank for sponge sticks (S1-F1) should also be sampled at this time.

    Reference Positives IDs : S-1-R(1-3), L1-R(1,2,3), L1-F1, and S1-F1

42. VHP airlock and start aeration as soon as possible (MOP-3120). Allow 18 hours for the agitated spores to settle in COMMANDER.

Day 4 – Tuesday

1. Verify the camera is recording, and that the DAS is operational and recording data.

2. After waiting the specified spore-settling time, a three-person sampling team (CM, DM1, and DM2) will enter COMMANDER in C-suits carrying :

    a. Sterile gloves and laboratory gloves
    b. Sterile garb (coat, p95, hair net, 8 boot covers)
    c. EtO'd notebook, AAS event log and pen
    d. Digital timer
    e. Duct tape
    f. Sample kits: Kits: 28-05a-MAF-1-R{1-3) and 28-05a-MAF-1-F1 28-05a-MAS-1-R{1-3) and 28-05a-MAS-1-F1
    g. Dispatch wipes

3. Each will don sterile garb and boot covers.

4. Enter COMMANDER, removing one pair of boot covers while crossing the threshold.

5. Remove the air filters from the HiVol samplers. The team will consist of a CM, DM2 and a DM1.

Filter Sample IDs: MAF-1-R(1-3), where M is material ID – C-carpet, L-laminate, and W-drywall

Shim Sample IDs: MAS-1-R(1-3), where M is material ID – C-carpet, L-laminate, and W-drywall

6. The DM1 will open the first HiVol sampler and latch in it place.

7. The DM1 will don a pair of sterile gloves and remove the collection shim, being careful not to touch the sides of the flow ports.

8. The DM2 and CM will then use a sponge kit to sample the top surface of the shim according to MOP 3169 (*Sponge Sample Collection Protocol for AAS Shims*). CM will manipulate the sponge stick.

9. The DM1 will then discard the shim into the airlock for disinfection. The DM1 will unlatch the base of the head unit and tilt the unit up to reveal the vent.

10. The DM1 will don a new pair of gloves and remove the vent bracket and top grate, being careful not to touch the filter.

11. Donning new gloves, the CM will remove the filter using sterile thumb forceps. Fold the filter in half and then in half again, keeping the top side of the filter inside of the fold. Using the forceps, transfer the filter directly to a stomacher bag. The DM2 will hold the stomacher bag open for this transfer.

12. Sample the shim plates and remove the filters for the two remaining HiVol samplers using the same protocol.

13. All HiVol samplers should be in the fully open position.

14. All personnel shower out of the airlock.

15. VHP COMMANDER as per MOP 3120. Conditions will be determined by the WAM.

16. Start aeration of COMMANDER.

17. VHP the airlock as per MOP 3120.

4.0 POST-DECON AAS PROCEDURES

Day 5 - Wednesday

1. Start airlock aeration as soon as possible and ensure conditions are safe for entry.

2. Verify the camera is recording, and that the DAS is operational and recording data.

3. Three personnel will enter the airlock wearing C-suits and carrying:

    a. Sterilized PM_{10} filters (3)
    b. Sterilized collection shims (3)
    c. Sterile gloves and laboratory gloves
    d. Sterile garb (coat, p95, hair net, boot covers)
    e. EtO'd laboratory notebook, AAS event log and pen
    f. Digital timer
    g. Duct tape
    h. Dispatch wipe
    i. Sample kits Sponge stick kits 28-05a-L-2-R(1-3) and 28-05a-L-2-F1

4. Once the airlock door is closed, open the door to COMMANDER. Don boot covers (2 pairs), being careful not to contaminate the first pair with the airlock floor.

5. Move into COMMANDER, removing one pair of boot covers when crossing the threshold.

6. Collect post-decon sponge stick surface samples (or vacuum sock samples of carpet coupons) of the three positive coupons per MOP 3165. CM will serve as the sampler, DM1 will serve as the support person.

    Sample IDs: M-2-R(1-3), where M is material type as listed above

7. DM2 will place coupons in soapy water in airlock after sampling.

8. All personnel to change into new clean garb.

9. CM will install the sterile filters on top of the vents of the HiVol samplers and secure in place. DM2 will unlatch the hinge of the head unit and pull down. Secure latches around the base of the unit.

10. The DM1 will unwrap the aluminum foil covering the collection shim, leaving the shim inside and exposed. The DM2 will spray the shim with Molykote® Grease Spray, ensuring full coverage of the shim. Wearing sterile gloves, the DM2 will aseptically pull the shim out of the foil and place in each of the three HiVol inlet heads, minimizing contact with the greased surface. Align the shim and secure in place with the Teflon tabs (see Figure 2c).

11. The DM1 and DM2 will remove their gowns and don SAR, while CM retires to airlock.

12. CM to communicate to buddy outside to verify Blower 1 power cord is plugged in, and Blowers 2 and 3 are off.

△ Inspect the SAR masks and hoses and the filter box prior to donning.

13. Turn on the fan to the 70% speed setting. Measure the wind speed at the HiVol inlets. These should be less than 30 mph.

14. Test the force of the leaf blower, pointed away from the coupon, using the anemometer.

15. Agitate the surface of the coupon according to MOP 3166. At the very moment the blower is turned on, DM2 will plug in the first HiVol sampler. The DM2 will monitor the blowing time as per MOP 3166 and document the intervals by filling out the AAS event log.

16. When agitation is complete, DM1 and DM2 move to airlock, remove SAR and drop their hose and mask in COMMANDER, close the airlock door and shower out of the COMMANDER airlock.

17. After 20 minutes of air sampling, turn off the first HiVol sampler. Immediately turn on the second sampler.

18. After 20 minutes of sampling with the second HiVol sampler, turn off the second sampler and immediately turn on the third. Again, sample for 20 minutes and then turn off.

19. Allow chamber to settle for 2 hours.

20. After the settling time, a three-person sampling team will enter COMMANDER carrying post-decon sample kits: filter kits 28-05a-LAF-2-R{1-3) and 28-05a-LAF-2-F1 and shim kits 28-05a-LAS-2-R{1-3) and 28-05a-LAS-2-F1

21. Sample the collection shims and collect the filters.

Filter Sample IDs: MAF-2-R(1-3), where M is material code , listed above

Shim Sample IDs: MAS-2-R(1-3), where M is material code , listed above

a. The DM1 will open the first HiVol sampler and latch in it place.

b. The DM1 will don a pair of sterile gloves and remove the collection shim, being careful not to touch the sides of the flow ports.

c. The DM2 and CM will then use a sponge kit to sample the top surface of the shim according to MOP 3169.

d. The DM1 will then discard the shim to the airlock. The DM1 will unlatch the base of the head unit and tilt the unit up to reveal the vent.

e. The DM1 will don a new pair of gloves and remove the vent bracket and top grate, being careful not to touch the filter.

f. Donning new gloves, the CM will remove the filter using sterile thumb forceps. Fold the filter in half and then in half again, keeping the top side of the filter inside of the fold. Using the forceps, transfer the filter directly to a stomacher bag. The DM2 will hold the stomacher bag open for this transfer.

g. Sample the shim plates and remove the filters for the two remaining HiVol samplers using the same protocol.

22. Conduct post-test flow calibration as outlined in MOP 3170 (*HiVol Calibration Check*).

23. Verify that two blowers are placed in COMMANDER and that the samplers are opened. Ensure all SAR related equipment is located in COMMANDER.

24. Exit COMMANDER and shower out of the airlock.

25. VHP COMMANDER per MOP-3120.

Attachment A

Aggressive Air (AA) Sampling Event Log

Test ID _____

Agitator _____
DM2 _____
Buddy 2 _____

Reference:

Dimensions (in^2)	Agitation Time (s)	Horizontal Sweeps	Vertical Sweeps	Sweep rate (s/sweep)
14 x 14	60	10	10	3
28 x 28	240	20	20	6
42 x 42	540	30	30	9

Test Day/ date	Coupon ID	High Vol 1 start	High Vol 2 start	High Vol 3 start
2/	Sterile Blank			
2/	Test			
4/	Test			

Test Day/ date	Coupon ID	Vertical start	Vertical end	# sweeps	Horizontal start	Horizontal end	# sweeps
2/	Sterile Blank			30			30
2/	Test			30			30
3/	Positive 1			10			10
3/	Positive 2			10			10
3/	Positive 3			10			10
4/	Test			30			30

Attachment B

WA 3-28 Coupon Deposition Log Page ____ of ___

Test ID		MDI ID:	
Date		Initial Weight (g):	
Personnel / Title		Final Weight (g):	

Time	Coupon ID	Vortex Interval (s)	Puff Number	MDI Weight (g)	Comments

Miscellaneous Operating Procedure (MOP) 6535a:

Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spores

Prepared by: _____ Date: 2/11/2013
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Reviewed by: _____ Date: 2/11/2013
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Approved by: _____ Date: 2/11/2013
Worth Calfee, EPA Work Assignment Manager

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MOP 6535a

TITLE: SERIAL DILUTION: SPREAD PLATE PROCEDURE TO QUANTIFY VIABLE BACTERIAL SPORES

SCOPE: Determine the abundance of bacterial spores in a liquid extract

PURPOSE: Determine quantitatively the number of viable bacterial spores in a liquid suspension using the spread plate procedure to count colony-forming units (CFU)

Materials:

- Liquid suspension of bacterial spores
- Sterile centrifuge tubes
- Diluent as specified in QAPP or Test Plan (e.g., sterile water, Phosphate Buffered Saline with Tween 20 (PBST))
- Media plates as specified in QAPP or Test Plan (e.g., Trypticase Soy Agar (TSA) plates)
- Microliter pipettes with sterile tips
- Sterile beads placed inside a test tube (used for spreading samples on the media surface according to MOP 6555 (*Petri Dish Media Inoculation Using Beads)* or cell spreaders
- Vortex mixer

1.0 PROCEDURE (This protocol is designed for 10-fold dilutions.)

1. For each bacterial spore suspension to be tested label microcentrifuge tubes as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}... (The number of dilution tubes will vary depending on the concentration of spores in the suspension). Aseptically, add 900 uL of sterile diluent to each of the tubes.

2. Label three media plates for each dilution that will be plated. These dilutions will be plated in triplicate.

3. Mix original spore suspension by vortexing thoroughly for 30 seconds. Immediately after the cessation of vortexing, transfer 100 uL of the stock suspension to the 10^{-1} tube. Mix the 10^{-1} tube by vortexing for 10 seconds, and immediately pipette 100 uL to the 10^{-2} tube. Repeat this process until the final dilution is made. It is imperative that used pipette tips be exchanged for a sterile tip each time a new dilution is started.

4. To plate the dilutions, vortex the dilution to be plated 10 seconds, immediately pipette 100 uL of the dilution onto the surface of a media plate, taking care to dispense all of the liquid

from the pipette tip. If less than 10 seconds elapses between inoculation of all replicate plates, then the initial vortex mixing before the first replicate is sufficient for all replicates of the sample. Use a new pipette tip for each set of replicate dilutions.

5. Carefully and aseptically spread the aliquotted dilution on the surface of the media either by use of glass beads (MOP 6555) or cell spreader (the method used may be directed in the QAPP or Test Plan) until the entire sample is distributed on the surface of the agar plate. Repeat for all plates.

6. Incubate the plates for the optimum time period at the optimum growth temperature for the target organism (incubation conditions will vary depending on the organism's optimum growth temperature and generation time. This information can be found in Bergey's Manual of Determinative Bacteriology or it will be provided with the ATCC certification.

7. Manually enumerate the colony forming units (CFU) on the media plates by manually counting with the aid of a plate counting lamp and a marker (place a mark on the surface of the Petri dish over each CFU when counting, so that no CFU is counted twice). A hand held tally counter or an electronic counting pen may be used to assist the person counting, but may not be used as the primary source for the count.

   Quality control (QC) requirements for bacterial enumeration will be addressed per QAPP or test plan. However, in general, the following QC practices should always be adhered to:

   a. The arrangement of plates and tubes, and the procedure for preparing dilutions and enumerating CFU should be done the exact same way each time. This helps prevent systematic errors and often helps determine the cause of problems when a discrepancy is found.

   b. A visual check of the graduated pipette tip should be made during each use to ensure the pipette is pulling properly.

   c. Samples should acclimate to room temperature for 1 hour prior to plating.

   d. Samples should be processed (extracted and plated) from the least contaminated to the most contaminated.

   e. When a target range of CFU is known, three dilution factors are plated to bracket the expected results (0, -1, and -2, if the -1 dilution factor was the target).

   f. Enumerated colonies and results should be verified that the results are the target organism, and that second counts have been performed. Second counts must be completed on 25% of significant data, and must be within 10% of the first count. If CFUs are found to have more than a 10% difference between first and second counts, then a third count is to be completed.

g. Pictures should be taken of any plates that are contaminated or have results out of the normal

8. Record all quantitative data in the "Serial Dilution/Plating Results Sheet". Target range for statistically significant counts is 30-300 CFU. Data that fall out of the 30-300 CFU range are addressed in MOP 6584 (*Procedure for Replating Bacteria Spore Extract Samples*) and MOP 6565 (*Filtration and Plating of Bacteria from Liquid Extracts*).

2.0 CALCULATIONS

Total abundance of spores (CFU) within extract:

(Avg CFU / volume (mL) plated) X (1 / tube dilution factor) X extract volume

For example:

Tube Dilution	Volume plated	Replicate	CFU
10^{-3}	100 µL (0.1 mL)	1	150
10^{-3}	100 µL (0.1 mL)	2	250
10^{-3}	100 µL (0.1 mL)	3	200

Extract total volume = 20 mL

(200 CFU / 0.1 mL) X $(1/10^{-3})$ X 20 mL =

(2000) X (1000) X 20 = 4.0×10^7 CFU

Note: The volume plated (mL) and tube dilution can be multiplied to yield a 'decimal factor' (DF). DF can be used in the following manner to simplify the abundance calculation.

Spore Abundance per mL = (Avg CFU) X (1 / DF) X extract volume

Serial Dilution/Plating Results Sheet **Page 1 of _____**

TEST INFORMATION			
EPA Project No.		PI	
Technician Name		Test Date	
Technician Signature		Test No.	

RESULTS								
Date:	**Volume Plated:**							
		Tube Dilution						
Sample ID	**Plate Repl.**	**10^{0}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							

NOTES: _____

_____

_____

Page 2 of ______

Sample ID	Plate Repl.	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							

NOTES: ____

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Page _____ of ______

Sample ID	Plate Repl.	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
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	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							

NOTES: ____

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Page _____ of ______

Sample ID	Plate Repl.	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
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	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							

NOTES: ____

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Miscellaneous Operating Procedure (MOP) 6555:
Petri Dish Media Inoculation Using Beads

Prepared by: _____ Date: 11/15/2012
Nicole Griffin Gatchalian, ARCADIS Work Assignment Leader

Reviewed by: _____ Date: 11/15/2012
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Approved by: _____ Date: 11/15/2012
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MOP 6555

TITLE: PETRI DISH MEDIA INOCULATION USING BEADS

SCOPE: This MOP outlines the procedure for cleaning, assembling, and using beads to inoculate agar plates.

PURPOSE: To provide an easily repeatable method for spreading liquid inoculation onto agar plates.

Equipment:

- #13 test tubes
- 6 x 12 test tube racks (which hold 72 tubes)
- Beads of various sizes (glass)
- Glass autoclavable trays (stainless steel is eventually corroded by the bleach and autoclaving processes)
- Bleach
- DI water
- Hot gloves
- Amber bottle for collecting hazardous waste (with hazardous waste label)
- Funnel
- Aluminum foil
- Label tape
- Chemical hood
- Autoclave
- Oven
- Labeled bead container or cup (All mold and bacteria beads must be kept separately)

1.0 CLEANING BEADS

1. When a sufficient number of beads have been collected, or at least once a day when beads are being used to spread colonies, place the used beads into a tray with a solution containing a 1:5 ratio of bleach to deionized water.

   Add the bleach to the beads first, under the protection of a chemical safety cabinet. Then

add the deionized water. Cover the pan with aluminum foil and label it with the contents (for example: "bacteria beads in 1:5 bleach to DI water solution"). Soak the beads 12-24 hours (usually overnight) in a chemical fume hood.

2. After soaking, take a bottle brush and thoroughly scrub the beads.

3. Decant the bleach solution (collect the bleach for proper disposal) and rinse with deionized water 6 to 8 times, collecting the rinsate after the first rinse for disposal (subsequent rinses can be discarded in the lab sink). Rinse until the decanted liquid is clear. Use a funnel to add the bleach waste to a labeled amber waste bottle. These liquids must be labeled "hazardous waste" and can then be stored, collected or disposed of properly.

4. Cover the beads with deionized water and autoclave for 1 hour on the liquid cycle.

5. Decant the deionized water and place the tray of beads in the Thelco lab oven at 121 °C until dry (a minimum of 3 hours).

6. Remove the beads from the oven using proper safety equipment (heat gloves) and cover with clean aluminum foil to prevent contamination. Label each tray with the following information:
   "Clean bacteria (or mold) beads," the date beads were cleaned, initials of the person who cleaned them.

7. These beads are then ready for use as described in "PLACING BEADS IN TUBES".

3.0 PLACING BEADS IN TUBES

1. Fill a 6 x 12 rack with tubes

2. Place clean beads into a shallow pan, and then manually fill each tube with 7-15 beads/tube.
   Note: Beads vary in size and will therefore fill the tubes to different heights.

3. Tightly attach a cap to each tube

4. Autoclave for 1 hour using a gravity sterilization cycle (see MOP 6570). Autoclave tape must be placed on the top of each rack to provide evidence that the beads have been sterilized

4.0 SPREADING BEADS

1. To spread inoculum on the agar surface, one tube of beads should be used for each individual plate.

2. After the beads have been added, the plates can be stacked up to six plates high. The plates are then shaken 10 times from side-to-side. Turn the stack of plates ¼ turn, and again shake 10 times from side-to-side. Repeat this procedure (¼ turn and 10 shakes) two more times, so that the beads are shaken a total of forty times.

3. Turn the plates over (upside down), and tap the beads into the lid.

4. Aseptically dump the beads into a labeled bead container (mold and bacteria beads must be labeled and collected separately), which should be considered contaminated one plate at a time, replacing the lid as quickly as possible to prevent contamination.

References

1. http://serc.carleton.edu/microbelife/k12/LIMW/dilution.html

2. http://www.qbiogene.com/businessdivisions/platformnews/news0005-b.shtml

3. http://www.genlantis.com/objects/catalog/Product/extras/C400050.pdf

4. EPA - SHEM Chemical Hygiene Plan

Miscellaneous Operating Procedure (MOP) 6562:

Preparing Pre-Measured Tubes with Aliquoted Amounts of Phosphate Buffered Saline with Tween 20 (PBST)

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MOP 6562

TITLE: PREPARING PRE-MEASURED TUBES WITH ALIQUOTED AMOUNTS OF PHOSPHATE BUFFERED SALINE WITH TWEEN 20 (PBST)

SCOPE: This MOP provides the procedure for preparing PBST.

PURPOSE: This procedure will ensure that that the PBST is prepared correctly and that all measured tubes are filled aseptically.

1.0 PREPARING STERILE PHOSPHATE BUFFERED SALINE WITH TWEEN 20 (PBST)

Phosphate Buffered Saline with Tween 20 (PBST) is prepared 1 L at a time in a 1 L flask.

1. Add 1 packet of SIGMA Phosphate Buffered Saline with Tween 20 (P-3563) to 1 L of deionized (DI) water.

2. Shake vigorously to mix until dissolved.

3. Label bottle as "non-sterile PBST" and include date and initials of person who made PBST.

4. Filter sterilize into two 500 mL reagent bottles using 150 ml bottle top filter (w/ 33mm neck and .22 μm cellulose acetate filter) for sterilization. Complete this by pouring the liquid into the non-sterile PBST into the top portion of the filtration unit 150 ml at a time, while using the vacuum to suck the liquid through the filter. Continue to do this until 500 ml have been sterilized into a 500 ml bottle. Change bottle top filter units between each and every 500 ml bottle.

5. Change label to reflect that the PBST is now sterile. Include initials and date of sterilization. The label should now include information on when the PBST was initially made and when it was sterilized and by whom.

6. Each batch of PBST should be used within 90 days.

2.0 PREPARING 20 ML/5 ML PBST TUBES FOR USE DURING EXPERIMENTATION

Twenty (20) ml or five (5) ml of the prepared PBST will be added to each sterile 50-ml conical tube as detailed below. Each flat of conical tubes contains 25 tubes, so one 500 ml sterile bottle of PBST should fill approximately one flat when 20 ml tubes are needed and four flats when 5 ml tubes are needed.

1. Prepare the hood by wiping down with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or Techwipe. Then stock the hood with the following items if they are not already there:

    - The flats of sterile conical tubes you need to fill with PBST.
    - Sufficient bottles of sterile PBST to fill these tubes.
    - Ample 25 ml serological pipettes (at least 3 per flat) for 20 ml transfers and 10 ml serological pipettes for the 5 ml transfers.
    - Serological pipetter (automatic, hand-held pipette).
    - Burner and striker.

2. Light the burner and adjust the flame for a width adequate to flame the lips of the PBST bottles.

3. Take one flat of sterile conical tubes and loosen each cap on the outside edges (about ½ turn).

4. Open a serological pipette and insert into the serological pipetter, taking care to not touch the tip to any surface.

5. Hold the pipetter with the first three fingers of your right (or dominant) hand. With your left hand (or non-dominant hand), pick up a bottle of the PBST and use the bottom of your right hand to unscrew the lid. Place the lid upside down on the benchtop and quickly flame the lip of the bottle. Turn the bottle and repeat, taking care to thoroughly flame the lip without getting the glass so hot that it shatters.

6. Inset the tip of the pipette into the bottle and fill to the 20 ml line. Flame the bottle lip and place the bottle on the benchtop.

    NOTE: If the tip of the pipette touches the outside of the bottle or any other surface in the hood, consider it contaminated. Discard the pipette and reload a new one.

7. Quickly pick up one of the tubes that you have loosened the cap on, and use the bottom of your right hand to remove the cap. Completely discharge the entire pipette into the tube, taking care to not touch anything with the tip of the pipette. Recap the tube and place back into the flat (the lid does not have to be tight – you will tighten the lids after you have completed filling the 10 outside tubes).

    NOTE: If the tip touches the outside or rim of the tube (or any other surface in the hood), consider the tube and pipette contaminated. Discard both the tube and the pipette.

8. Pick up the PBST bottle and flame the lip. Repeat Steps 6 and 7 until all 10 of the tubes on the outside of the flat have been filled. Flame the lip of the PBST bottle and replace the cap. Slide the used pipette back into the plastic sleeve and put to the side of the hood for disposal. Then tighten the lid of each tube you just filled. But rather than placing it back into its original spot in the flat, switch it for the empty tube from the next row. When this has been completed, go around the outside of the flat again and loosen the lids of these 10 tubes. Repeat steps 4 through 7 to fill and cap these tubes.

9. This same procedure is used to fill the middle row of tubes from the flat, and if more than one flat of tubes is being filled, can be done at the same time as the outside rows of a second flat.

10. When all tubes have been filled, label each flat as follows, and place on the shelf in room E390B:

    "PBST Tubes (20 ml or 5 ml)"
    Date prepared
    Your initials

11. These tubes should be made at least 14 days before they need to be used so that they can be verified as sterile. Any tubes that are cloudy or that have any floating matter/turbidity should be discarded. The tubes are stable for and should be used within 90 days.

3.0 CLEANUP FOR 20 ML/5 ML PBST TUBES

1. Dispose of the used pipettes in the nonregulated waste.

2. Plug in the serological pipetter so that it can recharge.

3. Replace any unused PBST in the liquid containment on the shelf. Make sure that the bottle is labeled as having been opened (date opened and initials of whomever used it).

4. Turn off the burner.

5. Wipe down the hood benchtop with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or TechWipe.

4.0 PREPARING 900µL PBST TUBES FOR USE DURING EXPERIMENTATION

1. Prepare the hood by wiping down with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or Techwipe. Then stock the hood with the following items if they are not already there:

- A sterile beaker of microcentrifuge tubes.
- Sufficient tubes of sterile PBST to fill these tubes (PBST may be aseptically transferred to 50 ml conical tubes for an easier aseptic transfer to the microcentrifuge tubes- it is easier than working from a 500 ml reagent bottle. Make certain that these 50 ml conical tubes are labeled to when the PBST was made, sterilized, etc.).
- 1000 µL micropipette.
- 1000 µL sterile pipette tips
- Microcentrifuge tube racks.
- Labeled beaker or waste container used to hold non-regulated waste, such as tips, under the hood.

2. Carefully remove the microcentrifuge tubes one at a time from the beaker and close the top on each one before placing it in the tube rack. Place the tubes in the rack skipping every other row. Fill up two racks doing this.

3. Add 900 µL of PBST to the microcentrifuge tubes by aseptically transferring the PBST from the sterile 50 ml conical tube containing the PBST. Do this by using the 1000 µL micropitte and tips. Change tips whenever after two rows of tubes are completed or whenever a contamination event (such as touching the outside of the 50 ml tube or the microcentrifuge tube) occurs. Put the dirty tips in the beaker or container used to contain waste (tips, tubes) in the hood. If any 900 µL tubes are contaminated during the transfer, dispose of them in the waste container used to hold tips under the hood. If a new box of tips has to be opened, make certain the date it was opened and initials of the person who opened it are clearly labeled on the box.

4. After both racks are full, carefully move all the tubes from one rack to fill in the empty rows on the other rack. In this manner, one rack should be completely filled with tubes at this point.

5. Label the rack of tubes as “Sterile 900 µL PBST Tubes”, along with the name of the person who completed the transfer, along with the date. Also, include the date that the original stock of PBST was made and the date it was sterilized, along with the initials of the person who completed those steps.

5.0 CLEANUP FOR 900µL PBST TUBES

1. Dispose of the waste that was put in the labeled beaker or waste container (micropipette tips and tubes) in the nonregulated waste. Then, place this beaker in the “To be decontaminated via sterilization- contaminated glassware” bin or if it is a disposable container, then it can be put in the non-regulated waste container.

2. Put the unused sterile tips and the micropipetter back in its original location.

3. Replace any unused 50 ml conicals of PBST in the liquid containment on the shelf. Make sure that the tube is labeled as having been opened (date opened and initials of whomever used it). If the tube could possibly be contaminated in any way, dispose of it in non-regulated waste.

4. Wipe down the hood benchtop with ethanol, followed by bleach, followed by DI water and a clean Kimwipe or TechWipe.

Miscellaneous Operating Procedure (MOP) 6563:
Swab Streak Sampling and Analysis

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MOP 6563

TITLE: SWAB STREAK SAMPLING AND ANALYSIS

SCOPE: This MOP provides the procedure for the process of completing a swab streak plate and subsequent qualitative analysis.

PURPOSE: This procedure will ensure that the swab streak plate sampling and analysis methods are standardized and that the collection and plating of samples are free from contamination.

1.0 PREPARING THE MATERIALS

There are two types of prepared swabs that can be used in this procedure:

Environmental Transport Swabs – purchased swabs that are individually packaged and pre-sterilized.

In-house Sterilized Swabs – swabs placed into autoclave pouches and sterilized using a 1-hour gravity cycle.

This procedure requires the following materials and equipment:

- Tryptic soy agar (TSA) media plates
- 32 °C incubator
- Nitrile (non-sterile) gloves
- Sharpie for writing on plates

2.0 COLLECTING AND PLATING SAMPLES

The procedure for collecting and plating samples is dependent on the type of swab being used. Appropriate PPE should be worn in both cases and includes a lab coat, nitrile gloves, and safety glasses.

2.1 Environmental Transport Swabs

2.1.1 Collection of Environmental Transport Swab Sample

1. Break the seal on the individually packaged and sterile swab. Collect the specimen with the swab applicator as detailed in the specific test protocol, then replace the swab in the tube.

2. Label the tube with what is being swabbed (sample ID), the date, time, and initials of the person performing the procedure.

3. Place the swab into a secondary container, such as a sterile bag, and label the bag with the same information placed on the tube label.

4. Transport the sample(s) to the Microbiology Laboratory for processing.

2.1.2 Plating of Environmental Transport Swab Sample

1. When the sample is received in the Microbiology Laboratory, label one TSA plate using a Sharpie with the information from the swab packaging. Verify that the sample ID and date match.

2. Place labeled plates and swab samples under the biological safety cabinet. Remove the sample swab from the secondary container and the tube. Press onto the plate in an S-stroke motion, turning the swab as it is plated to ensure that all of the surface area of the swab touches the plate. Press firmly, but not so hard that the surface of the media is broken.

3. Replace the swab into its tube and discard in the non-regulated waste container.

4. Repeat steps #1 through #4 for each sample.

5. Label three TSA plates as **Swab Blank A**, **Swab Blank B,** and **Swab Blank C.** These plates will serve as negative controls for the swabs.

6. Open a new/unused Environmental Transport Swab and use it to plate the three blank plates as detailed in Step #2.

7. Stack the triplicate plates media side up and place in a 35 °C ± 2 °C incubator for at least 18 hours. Note the time the plates were placed in the incubator.

2.2 In-house Sterilized Swabs

When In-house Sterilized Swabs are being used to collect samples, they need to be plated immediately (unlike the Environmental Transport Swabs which are transported back to the Microbiology Laboratory for plating). Therefore, prior to travelling to the sample site, collect the following materials and supplies which will be needed:

- One TSA media plate (in a media bag) per sample to be collected plus three additional plates to be used as negative controls for swab blanks.
- One In-house Sterilized Swab (in their autoclave pouches) per sample to be collected, one swab for the control plates, plus a few extras.
- Sharpie for labeling plates.

Use the following procedure to collect and plate samples.

1. Once at the sample collection site, take the TSA plates out of the media bag and label one plate for each sample with what is being swabbed (sample ID), date, time, and initials of the person performing the procedure.
2. As carefully and as aseptically as possible, remove the swab from the autoclave pouch by the stick end. Be sure and not touch the swab end to anything but the sample. If the swab's sterility is compromised, dispose of the swab and use one of the extras.
3. Collect the specimen with the swab applicator as detailed in the specific test protocol.
4. Press onto the plate in an S-stroke motion, turning the swab as it is plated to ensure that all of the surface area of the swab touches the plate. Press firmly, but not so hard that the surface of the media is broken. Because these samples are being plated in the open air and not in a biological safety cabinet, be certain to limit the time that the lid is removed from the TSA plate.
5. Replace the swab into the autoclave pouch it came in and discard in the non-regulated waste container.
6. Repeat steps #1 through #6 for each sample.
7. Label three TSA plates as **Swab Blank A**, **Swab Blank B,** and **Swab Blank C.** These plates will serve as controls for both the swabs and the TSA.
8. Open another in-house sterilized swab from the autoclave pouch and use it to plate the three blank plates as detailed in Step #2.

9. Put the TSA plates back into the media bag and transport to the Microbiology Laboratory.

10. When received by the laboratory, the plates should be stacked media side up and placed in a 35 °C ± 2 °C incubator for at least 18 hours. Note the time the plates were placed in the incubator.

3.0 ANALYZING THE SAMPLES

The Swab Results Template, which follows this section, is used to record the results of the sampling. Some quantities of samples may require more than one form. Make certain that the data is filled in completely on each page. The analyst will use the information on the TSA plates to fill in the following blanks at the top of the form:

- Swab samples taken on: *(date)*
- Swabbed by: *(person)*
- Plating completed on: *(date)*
- Plated by: *(person)*

The following procedure is used to analyze the samples and complete the remainder of the Swab Results Template form.

1. Fill in the final two sections at the top of the form: **Plate results read on** and **Results read by**.

2. Observe the agar surface on the plates and note the sample IDs on the first three lines in the **Sample** column.

3. For each plate, check whether there was growth **(G)** or no growth **(NG).** Growth is indicative of an organism(s) being present, and should be described on the form. Be as detailed as possible, noting colony morphology (size, shape, color and any other distinctive things that can be seen concerning the growth).

4. The Swab Results Template form serves as the sample report and should be provided to the Project Manager.

Swab Results Template

Swab samples taken		**Swabbed by:**	
Plating completed on:		**Plated by:**	
Plate results read on:		**Results read**	

Sample Name	**Result**	**If growth, describe**
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
	G ❒ NG ❒	
Controls	**Result**	**If growth, describe**
Swab blank A	G ❒ NG ❒	
Swab blank B	G ❒ NG ❒	
Swab blank C	G ❒ NG ❒	

Key

G = Growth.

NG = No Growth.

All plates are plated in triplicate resulting in sample identification of “A”, “B”, and “C”.

Miscellaneous Operating Procedure (MOP) 6565: Filtration and Plating of Bacteria from Liquid Extracts

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MOP 6565

TITLE: FILTRATION AND PLATING OF BACTERIA FROM LIQUID EXTRACTS

SCOPE: This MOP outlines the procedure for filtration and subsequent cultivation of bacterial spores from a liquid extract.

PRUPOSE: This method is deployed when results from spread-plate methods yield less than 30 colony-forming units (CFU) per plate. This method allows a lower limit of detection for bacterial recovery/survivorship assays. This method can also be used to analyze liquid samples such as decon rinsates.

Materials:

- Petri dishes with appropriate agar
- 0.2 μm pore-size disposable analytical filter units (2-3 per sample)
- P1000 pipette and sterile tips
- Sterile forceps
- Pipettman and sterile serological pipettes

1.0 PROCEDURE

1. For each liquid sample to be analyzed, gather the required number of disposable analytical filter units and Petri dishes containing the desired sterilized/QC'd media.

    NOTE #1: For analysis of 5 to 30 ml extracts, 1 ml and remainder should be filtered; for 31 to 200 ml samples, 1 ml, 10 ml, and remainder should be filtered; for samples over 200 ml, more filter samples may be needed.

    NOTE #2: For previously plated samples where 10 – 19 CFU were observed, replating using a 400 μl inoculum, and plates where 20 – 29 CFU were observed, replating using a 200 μl inoculum can be executed rather than filter plating. For inoculua greater than 200 μl, a sterile spreader should be used rather than the bead method).

2. Label plates.

3. Vortex liquid extract vigorously for 2 minutes, using 10 second bursts. (for larger volume samples, a vigorous mixing by shaking of the sample container can be substituted for vortex mixing)

4. Using a P1000, sterile tip, and aseptic techniques, immediately following vortexing, pipette 1 ml of the extract into one of the filter units.

5. Apply vacuum to the filter unit, to pull the liquid through the filter and collect the spores on the surface of the filter.

6. Using a sterile serological pipette, rinse the filter unit by pipetting 10 ml of sterile deionized water along the inner sides of the unit while it is under vacuum.

7. Aseptically remove the filter from the filter apparatus using sterile forceps, and lay the filter onto the agar surface within the Petri dish (spore side up).

8. Vortex the liquid extract vigorously for 10 seconds.

9. Use the appropriate volume serological pipette to transfer the remaining aliquots into their respective filtration units (one at a time).

10. Repeat steps 5 through 7 taking time to vortex or mix the sample 10 seconds immediately before removing an aliquot.

    Important: Be sure to note and record the volume of the "remainder" sample.

11. Incubate all plates at the optimal growth temperature for the organism used for 16 – 28 hours.

12. Enumerate and record the number of CFU on each plate.

2.0 DATA CALCULATIONS

Utilize the following equation to determine the total abundance of recovered spores:

$$N = CFU \times \frac{V_{Extract}}{V_{filtered}}$$

Where, N is the total number of spores recovered in the extract, CFU is the abundance of colonies on the agar plate, $V_{Extract}$ is the total volume of the extract (before any aliquots were removed), $V_{Filtered}$ is the volume of the extract filtered.

Miscellaneous Operating Procedure (MOP) 6567: Recovery of *Bacillus* Spores from Wipe Samples

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MOP 6567

TITLE: RECOVERY OF *BACILLUS* SPORES FROM WIPE SAMPLES

SCOPE: This MOP outlines the procedure for recovering *Bacillus* spores from wipe samples.

PURPOSE: To aseptically extract and quantify *Bacillus* spores from wipe samples in order to determine viability and obtain quantifiable data.

Materials:

- PPE (gloves, lab coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-amended bleach
- Deionized water
- 70% solution of denatured ethanol
- Kimwipes
- Disbatch® bleach wipes
- Non-regulated waste container
- 50 mL sterile conical tubes containing 20 mL of sterile phosphate buffered saline with Tween 20 solution (PBST) (MOP 6562)
- Vortex mixer
- Cart
- Wire or foam rack for 50mL conical tubes
- Tryptic soy agar plates
- 900 µL tubes of sterile PBST
- Pipettor and pipette tips for dilutions
- Incubator set to appropriate growth temperature for target organism (35°C or 55°C)
- Light box for counting colonies
- Lab notebook
- QAPP for project that is utilizing the wipe samples

1.0 PROCEDURE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).

2. Obtain wipe samples that may contain *Bacillus* spores. Wipe samples should be received as one wipe/sponge in a sterile 50 mL conical tube delivered in secondary containment. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If variation has occurred, make a note of it in the notebook.

3. Clean the workspace (biological safety cabinet) by wiping surfaces with pH-amended bleach, next with deionized water, and lastly with a 70-90 % solution of denatured ethanol. Wipe with a Kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that, once the procedure has begun, the task may be performed without interruptions.

4. Discard gloves and replace with fresh pair.

5. One at a time, under the biological safety cabinet, remove the sample tube containing the wipe sample from the secondary containment bag in which it arrived. Using the Dispatch® bleach wipes, wipe each sample tube with one wipe, and then wipe it with a clean Kimwipe. Discard the used bleach wipe and the used Kimwipe in the secondary containment bag and place them in the non-regulated waste container. Remove gloves and don a fresh pair of gloves. Repeat this procedure for every sample. After each sample has been cleaned, place the tubes containing the wipe samples in an appropriate sized wire or foam rack to hold the tubes in an upright, vertical position.

6. Leaving the tubes in the rack underneath the biological safety cabinet, aseptically add 20 mL of PBST solution (this should be in a pre-measured, sterile conical tube, per MOP 6562) to each sample tube containing a wipe, one a time. Remove the rack containing wipe samples from hood when all samples have had the PBST added. Place the rack with the samples on the cart.

7. Using the procedure to clean the biological safety cabinet, as found in Step 3, clean the biological safety cabinet again. Afterwards don a fresh pair of gloves.

8. Using a vortex mixer, agitate the wipe samples, four at a time, in a biological safety cabinet, for ten second bursts for two minutes total. Make certain to clean the biological safety cabinet after each set of four samples and change gloves between each set of samples.

   NOTE: The reason that four samples are done at one time is to limit the time between agitation and plating. The samples need to be processed immediately after

agitation, and agitation of more than four samples at a time leaves too much time between agitation and spread plating.

9. Using tryptic soy agar media plates that are appropriately labeled with the sample number, dilution set and date, complete dilution plating for the wipe samples immediately after the two minute agitation step is completed. The samples should also be agitated again for ten seconds directly prior to removing an aliquot from the sample tube. Each dilution tube should also be agitated for ten seconds prior to removal of aliquots. Dilutions should be completed using the techniques and methodology as described in MOP 6535a, and the 900 µL tubes should be made with sterile PBST to stay consistent with materials/solutions. Plating in this manner should be repeated for all samples, with any changes in protocol noted in the lab notebook.

10. Once the dilution plating has been completed, the plates are to be placed in an incubator. For non-thermophilic *Bacillus* species, the plates should be placed at 35°C +/- 2° C for 12-24 hours. For thermophilc *Bacillus* species, such as *Geobacillus stearothermophilis*, the plates should be incubated at 55°C ±2 °C for 12-24 hours. The target *Bacillus* organism that will be used for the wipe samples will be specific to the project and noted in the QAPP.

11. After the plates have incubated for a sufficient amount of time (12-24 hours) and the growth from any *Bacillus* colonies are quantifiable, the colonies should be manually counted using the light box and the data should be properly recorded as dictated per project by the QAPP. All results will be checked for quality assurance and all data will be reported to the proper personnel as listed in the QAPP.

Miscellaneous Operating Procedure (MOP) 6570:
Use of Steris Amsco Century SV 120 Scientific Prevacuum Sterilizer

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MOP 6570

TITLE: USE OF STERIS AMSCO CENTURY SV 120 SCIENTIFIC PREVACUUM STERILIZER

SCOPE: Basic instructions for use of the large Steris autoclave.

PURPOSE: To outline proper procedural use of the autoclave, using preprogrammed cycles, to effectively sterilize items, while complying with quality control standards.

Materials:

- Amsco Century SV 120 Scientific Prevacuum Sterilizer
- Items to be sterilized (liquids, solids, waste, etc)
- Pouches to contain materials during sterilization and maintain sterility until use
- Aluminum foil
- Autoclave indicator tape
- Sterilization verification ampoules (such as Raven ProSpore Ampoules)
- Thermally resistant gloves
- De-Ionized (DI) water

1.0 PROCEDURE

1.1 Start Up

1. Turn on the autoclave. The power switch is located behind the door in the top right corner. The digital touch screen on the front of the unit will power up and indicate that a memory test is in progress.
2. After the memory test is complete, the device will request that it be flushed. This should be conducted daily to minimize scaling inside the boiler. The flush valve is located behind the door on the bottom, left of the device (yellow handle). Move the valve to the open position and then press the “Start Timer” button on the touch screen. The flush will run for 5 minutes and will alert at completion with a single chime.
3. Once the flush is complete, close the flush valve and press the “Continue” button on the touch screen. The screen should then return to its default menu which has 2 choices “Cycle Menu” and “Options”

1.2 Basic Operation

1. Prepare any items that need to be sterilized. The items must be carefully wrapped or sealed in sterilization pouches in order to maintain sterility when removed from the autoclave. Examples of this include: wrapping any orifices with aluminum foil, placing whole items in autoclave pouches, loosely applying a cap on a bottle (to allow for the pressure changes inside).
2. Once prepared, each item should be outfitted with a sterility indicator such as a small piece of autoclave indicator tape; or by utilizing an autoclave pouch with a built-in sterility indicator strip. These indicators provide a visual verification that the sterilizing temperature (121°C) was reached.
3. To add items to the autoclave, open the autoclave door by pressing down on the foot pedal on the bottom right corner on the front of the device.
4. Place items that need to be sterilized into the autoclave, adding or moving racks to accommodate the load. If liquids are being autoclaved, then they must have secondary containment (usually a large plastic autoclave-safe tray) to contain any fluids in the event of a leak, spill or boil-over. Add an indicator ampoule to the first autoclave cycle of the day, regardless of the type of cycle.
5. Once the autoclave is loaded, press the foot pedal to close the autoclave door.
6. Once the door is sealed, a menu of the cycles can be seen by pressing the button on the touch screen labeled "Cycle Menu". Then choose the appropriate cycle by touching the corresponding button. If the cycle chosen is the one desired for the sterilization process, press the "Start Cycle" button. Otherwise, press "Back" to return to the prior menu screen.
7. After the cycle has started, the type of cycle, the number of the cycle, the items placed in the autoclave during the cycle, the time, whether or not an indicator ampuole was included in the load, and the initials of the person starting the cycle must be recorded in the autoclave log book, located in the drawer across form the unit labeled "Autoclave Supplies."
8. Quality control (QC) indicator ampoules, usually Raven ProSpore Ampoules with *Geobacillus stearothermophilus* (at a concentration 10E6), are added to one cycle each day to ensure that the autoclave is functioning properly. These ampoules are used according to manufacturer's instructions. These ampoules must be properly labeled with the date in which they were autoclaved and the initials of the individual that completed the cycle. At the beginning of each week, a positive control ampoule must be processed, where the ampoule is placed directly into the 55°C water bath, without being autoclaved. The positive control indicator ampoule should change from purple to yellow in color, indicating growth. All test ampoules should be placed in a water bath following the end of the cycle in which they are run. These ampoules should not change color (from purple to yellow, but instead should remain a purple to

purple-brown color). Ampoules should be checked at both 24 and 48 hour intervals for growth and then finally recorded and disposed of after 48 hours. All QC information concerning ampoules should be recorded in the autoclave notebook.

9. Upon completion of any cycle, the autoclave will alarm with a repeating beep for approximately one minute. Any time after this alarm starts, it is safe to open the main door (take caution because the steam escaping the chamber will be very hot when the door is opened). The contents from the autoclave will be very hot; use protection to remove items from the autoclave (thermally resistant gloves).
10. Place the contents of the autoclave in an appropriate place to cool, and close the autoclave door using the foot pedal.

1.3 Cycles

1.3.1 Gravity Cycles

Gravity cycles are used to sterilize glassware and other utensils, which are not submerged in nor contain any volume of liquid. These cycles are typically used for "dry" materials. Currently there are two different gravity cycles programmed for daily operations: a 1-hour cycle and a 30-minute cycle. The time that the chamber is held at the sterilization temperature (121 °C) is the only difference between these two cycles. The different sterilization times allow for the compensation of the various sizes of materials and more resilient organisms. The 30-minute cycle is primarily used for a small quantity of material. The 1 hour cycle is used for large loads or items containing a large amount of contamination. The 1 hour cycle is recommended for inactivation of gram positive spore-forming bacteria.

1.3.2 Liquid Cycles

Liquid cycles are used to sterilize a variety of liquids and solutions. The solutions are typically mixed prior to sterilization. It is important to have secondary containment to contain any fluids in the event of a leak, spill or boil-over. The 30-minute liquid cycle is used to sterilize small volumes of liquid (usually less than 2L total). When attempting to sterilize any volume larger than 2L, the 1-hour liquid cycle should be used to ensure complete sterilization. The 1-hour liquid cycle is the preferential cycle used as the destruction cycle for waste. In the event of materials (liquid or otherwise) being contaminated/exposed microorganisms, the 1-hour liquid cycle will be used as the initial means of decontamination. When completing a decontamination cycle, if there is no liquid inside of a container, then deionized water must be added to the container or the item must be submerge prior to the start of the cycle. Only items that are being decontaminated can go in destruction cycles. Decontamination cycles cannot be mixed with sterilization cycles.

Miscellaneous Operating Procedure (MOP) 6572: Recovery of Spores from Vacuum Sock Samples

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MOP 6572

TITLE: RECOVERY OF SPORES FROM VACUUM SOCK SAMPLES

SCOPE: This MOP outlines the procedure for recovering spores from vacuum sock samples

PURPOSE: To aseptically extract and quantify spores from vacuum sock samples in order to determine viability and obtain quantifiable data

MATERIALS

- PPE (gloves, lab coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-amended bleach
- Deionized water
- 70% solution of denatured ethanol
- Kimwipes
- Disbatch® bleach wipes
- Non-regulated waste container
- 3 oz. sterile specimen cup containing 20 mL of sterile phosphate buffered saline with Tween 20 solution (PBST) (MOP 6562)
- Sterile scissors
- Vortex mixer
- Cart
- Tryptic soy agar plates
- 900 µL tubes of sterile PBST
- Pipettor and pipette tips for dilutions
- Incubator set to appropriate growth temperature for target organism (35 °C or 55 °C)
- Light box for counting colonies
- Lab notebook
- QAPP for project that is utilizing the vacuum sock samples

1.0 PROCEDURE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).

2. Obtain vacuum sock samples that may contain *Bacillus* spores. Vacuum sock samples should be received as one vacuum sock in a sterile 5.5' x 9 bag secondarily contained in a 10' x 15' bag. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If variation has occurred, make a note of it in the notebook.

3. Clean the workspace (biological safety cabinet) by wiping surfaces with pH-amended bleach, next with deionized water, and lastly with a 70-90 % solution of denatured ethanol. Wipe with a Kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that once the procedure has begun the task may be performed without interruptions.

4. Discard gloves and replace with fresh pair.

5. Label a 3 oz. specimen cup to match the vacuum sock sample ID. The specimen cup contains 20 mL of sterile PBST.

6. When extracting samples, handle one sample at a time from start to finish. Begin by removing the inner bag from the outer bag. Discard the outer bag in the non-regulated waste container. Place the inner bag containing the vacuum sock under the hood. Loosen the cap on the 3 oz. specimen cup and open a pack of sterile scissors. Open the bag and remove the sock, careful not to touch the white part. Roll the non-sterile blue portion of the vacuum sock onto the smaller cardboard ring. Dispose of the larger cardboard ring. Wet the vacuum sock by holding the upper blue portion of the vacuum sock (around the smaller cardboard ring) and dipping the lower 1-inch of the vacuum sock into the PBST. The vacuum sock will be allowed to absorb the PBST for a few seconds. After wetting, the vacuum sock will be lifted up just above the opening of the specimen bottle, and a 1-inch vertical slit will be cut up the center from the bottom of the sock using sterile scissors (a new pair of scissors should be used for each sample). The vacuum sock is then cut horizontally from side to side, about 1 inch from the bottom allowing the two pieces to fall into the specimen bottle. The vacuum sock should be cut only where the sock has been wetted. Repeat the dip/cutting procedure until the entire collection portion of the sock has been excised. The upper top blue portion of the vacuum sock will then be discarded. Place used scissors in a discard pan. After samples are all extracted, scissors will be immediately autoclaved using a one hour gravity destruction cycle in preparation for use with the next sample batch. Remove gloves and don a fresh pair of gloves. Repeat the extraction procedure for every sample, while maintaining aseptic technique.

7. After cutting all vacuum sock samples, all specimen cups (up to sixty samples at a time) should be loaded into the sample cup holder of the orbital shaker-incubator. The samples are then

agitated in the shaker incubator at 300 rpm for 30 minutes at room temperature. The samples are then removed from the shaker incubator and brought to the Biological Safety Cabinet for dilution plating.

8. Using the procedure to clean the biological safety cabinet, as found in Step 3, clean the biological safety cabinet again. Afterwards don a fresh pair of gloves.

9. Using tryptic soy agar media plates that are appropriately labeled with the sample number, dilution set and date, complete dilution plating for the vacuum sock samples immediately after the thirty minute agitation step is completed. The samples should also be agitated again for ten seconds directly prior to removing an aliquot from the specimen cup. Each specimen cup should also be agitated for ten seconds prior to removal of aliquots. Dilution-plating should be carried out according to MOP 6535a. Dilution tubes used in MOP 6535a should contain PBST to stay consistent with materials/solutions. Repeat procedure for all samples.

10. Once the dilution plating has been completed, the plates should be incubated. For non-thermophilic *Bacillus* species, the plates should be placed at 35°C ±2 °C for 18-24 hours. For thermophilc *Bacillus* species such as *Geobacillus stearothermophilis*, the plates should be incubated at 55°C ±2 °C for 18-24 hours. The target *Bacillus* organism that will be used for the vacuum sock samples will be specific to the project and noted in the QAPP.

11. After the plates have incubated for a sufficient amount of time (18-24 hours) and the growth is quantifiable, the colonies should be manually counted with the assistance of a light box. The data should be properly recorded as dictated per project by the QAPP. All results will be checked for quality assurance and all data will be reported to the proper personnel as listed in the QAPP.

Miscellaneous Operating Procedure (MOP) 6579: Recovery of *Bacillus* Spores from 37MM Filter Cassettes

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MOP: **6579**

Title: **RECOVERY OF *BACILLUS* SPORES FROM 37MM FILTER CASSETTES**

Objective: To aseptically extract and quantify recovery of *Bacillus* spores from 37mm filters used to sample porous surfaces.

MATERIALS AND EQUIPMENT

Equipment:

- Biological Safety Cabinet (BSC, Class II)
- Sonicating cleaner (such as Branson 8510, 40KHz)
- Incubator set to appropriate growth temperature for target organism (35 °C)
- Light box for counting colonies
- Vortex mixer
- Cassette opening tool (SKC cat# 225-8372, www.SKCshopping.com)
- Specimen cup rack (cat # 5534M1, http://www.medicus-health.com)

Materials:

- Personal Protective Equipment (PPE) (gloves, lab coat, safety goggles)
- 1:10 Bleach, pH-amended to 7.0 with acetic acid
- Dispatch bleach wipes
- Disposable 500 ml beaker (such as Fisher Scientific cat #14-955-111D) (for discarding used tips and beads/spreaders)
- 70% Solution of denatured ethanol
- Kimwipes
- Waste container
- Sterile Phosphate Buffered Saline with Tween 20 solution (PBST) (MOP 6562)
- Tryptic Soy Agar (TSA) plates
- Sterile scissors or scalpels, preferably one for each sample
- Sterile 1500 µL tubes filled with 900µL of sterile PBST
- 15 ml centrifuge tube containing 6 ml of sterile PBST (one for each sample)
- Autoclaved 2 oz polypropylene jars with lids (cat # J037, cat # L208 www.containerandpackaging.com)

- Pipettor and pipette tips for dilutions (100 µl, 1000 µl, and 5000 µl.)
- Pall Filter units 0.45 um MCE (VWR; catalog #28143-544)
- Vacuum Pressure Gauge (McMaster; catalogue # 38545K16)
- Disposable cell spreaders or glass beads for cell spreading
- Disposable sterile thumb forceps

1.0 REMOVING FILTER AND DUST FROM CASSETTE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).
2. Each cassette should be received separately in a small sealable bag (primary containment), then placed within secondary containment. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If discrepancies are found, make a note of each one in the notebook.
3. Clean the workspace (BSC) by wiping surfaces with pH-amended bleach, next with deionized water, and lastly with a 70-90 % solution of denatured ethanol. Wipe with a kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task.
4. Discard gloves and replace with fresh pair.
5. For each cassette sample, label one 2 oz polypropylene jar with the sample identifier.
6. One at a time, under the BSC, remove the conical tube and cassette from the primary containment bag in which it arrived.
7. First wipe down the outside of the conical tube containing the vacuum tubing and adapter with a disinfecting wipe, and place it into a rack. Then, aseptically add 5ml of premeasured PBST. Set the rack to the side.
8. Remove the band from around the cassette using a sterile scalpel or sterile pair of scissors. Using the Dispatch bleach wipes, wipe each cassette with a wipe, and then a clean kimwipe. Discard the used bleach wipe and the used kimwipe in the secondary containment bag and place them in the non-regulated waste container. Remove gloves and don a fresh pair of gloves before proceeding.
9. With the plug in place on the back side of the filter, remove the red plug from the front filter side of the cassette, use a pipette and aseptically dispense about 1mL µl of PBST (taken from the tube containing 6ml PBST) into the cassette. Roll the cassette around to allow the liquid to touch all surfaces of the inside of the cassette. If there is a large quantity of dust inside, more PBST may be needed to dampen the dust before opening the cassette.
10. Once a visual check has been completed to assure that the dust is dampened enough to prevent its aerosolization, use the cassette tool to pry open the top section of the cassette by carefully rotating the cassette while using the tool to pry the edges up. Be careful to hold the cassette right side up while prying open, so as not to spill the liquid inside.

11. When the cassette is open, use a pipette to aseptically rinse the inside walls of the cassette with 1 to 2 ml of PBST (additional aliquots from the same 5ml tube used previously), then remove the rinse eluent (using the same transfer pipette) and place it in the 2 oz sterile cup labeled with the sample identifier.
12. Use the cassette opening tool to then remove the middle section of the cassette in the same manner as the top section, thereby allowing the filter to be removed. Using sterile forceps, aseptically remove the filter, being careful not to pick up the support filter underneath, and place it in the 2 oz polypropylene jar with the rinse eluent.
13. Use the remainder of the 6 ml PBST to rinse the walls of the middle and top sections of the cassette while holding them over the open 2 oz cup. The entire 6 ml PBST, the filter and as much dust as can be rinsed from the cassette should now be inside the 2 oz polypropylene jar.
14. Discard the cassette sections, support filter, cassette plugs, disposable pipettes and disposable thumb forceps into the proper waste. Close the 2 oz jar tightly. Wipe outside of the jar with disinfecting wipe and place in specimen cup rack or sample retention bin.
15. Wipe down the cassette opening tool thouroughly with a disinfecting wipe, and wipe down the surface of the BSC as described in Step 3 above.
16. Change gloves, and repeat from Step 5 for each remaining sample set.

2.0 PROCESSING 37 MM FILTERS

1. Place the rack of conical tubes containing the 5mL of PBST with the vacuum tubing and adapters into the sonicating bath with a weighted, waterproof, rectangular, flat surface on top of the tubes to keep them from floating when submerged. Sonicate the conical tubes for 1 minute.
2. Remove the tubes from the sonicator and dry them with a kimwipe and wipe them with a disinfecting wipe.
3. Vortex the conical tubes for 2 continuous minutes.
4. Aseptically transfer the 5mL of PBST from the conical tube samples to the 2oz specimen cup containing the filter sample that corresponds with the conical tube sample. The labels on the conical tube should match the label on the specimen cup.
5. After the aseptic transfer of extraction liquid from the conical tube to the specimen cup, using a 10 ml serological pipette, measure the amount of PBST extraction liquid that has been collected in the 2oz specimen cup. Record this number.
6. Place all 2 oz polypropylene cups containing the 37mm filters and 5 ml PBST in the specimen cup rack, place a weighted waterproof, rectangular, flat surface on top of the jars to keep them from floating when submerged in the sonicating bath.
7. Place the rack with the cups and the weighted surface into the bath so that they are submerged about 1 – 2 inches, leaving about 1 inch between the level of the bath water and the jar lid.

NOTE: This may require one or two wire racks (test tube racks or additional cup holders) in the bottom of the sonicating bath to achieve the correct level for the cups. Make sure the sonicating bath is filled with water to the correct level as designated by the line on the inside of the bath. A series of open racks is preferable to a basket for this purpose, as the baskets provided by the bath manufacturers tend to have more solid walls with small holes, which can restrict the sonic waves more than a wire rack.

8. When the correct level of submersion is achieved, turn on the sonicating bath (no heat) and sonicate for 3 minutes, rotating the cup rack within the bath at the 1 min and 2 min mark (pick up, turn 90 degrees, re-submerge).
9. Remove the rack of cups from the sonicating bath and place on an absorbent pad. Remove each 2 oz cup from the rack, wipe with a kimwipe to dry and place back inside the BSC.

3.0 FILTRATION AND PLATING OF BACTERIA FROM LIQUID EXTRACTS

1. Follow MOP 6535a for spread-plating samples in triplicate from the 2 oz cups.
2. Filtration – detailed in the steps below – is required only if extremely low recovery is expected.
    a. Label three TSA plates with information specific to the sample and amount to be filtered.
    b. Set up filter manifold with three filter unit cups.
    c. Set the vacuum, with proper gauge, to no more than 10 inches Hg (4.9 psi).
    d. Add 10 ml PBST to filter cup.
    e. Add 1 ml sample eluent from sample cup to each filter cup on the manifold.
    f. Open the vacuum valve and vacuum liquid through filter.
    g. Rinse sides of filter cup with 10 ml of sterile PBST, vacuum through again.
    h. Pop off sides of the filter cup by squeezing and lifting.
    i. Use sterile forceps to lift filter and place it on the TSA, making sure good contact with the agar surface is made and air pockets are minimized.
3. Repeat for subsequent sample jars, completing samples in batches of no more than 4. This will help to avoid too much settling time between vortexing and plating.
4. Place all TSA plates in incubator set at 35 ºC and incubate 18 – 24 hrs before counting colonies.

4.0 CALCULATIONS

For spread plates: total CFU recovered = (mean CFU on plate / 0.1ml volume plated) x (1/dilution factor) x (5 ml total in jar)

For vacuum filter plates: total CFU recovered = (mean CFU on vacuum filter / volume filtered) x 5 ml total in jar

Miscellaneous Operating Procedure (MOP) 6580: Recovery of *Bacillus* Spores from 3M Sponge-Stick™ Samples

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MOP 6580

TITLE: RECOVERY OF BACILLUS SPORES FROM 3M SPONGE-STICK™ SAMPLES

SCOPE: This MOP provides the procedure for recovering spores from 3M Sponge-Stick™ samples.

PURPOSE: To extract and quantify bacterial spores from 3M Sponge-Stick™ samples using a highly repeatable procedure.

MATERIALS

- pH-amended bleach
- 70-90 % Solution of denatured ethanol
- Deionized (DI) water
- Kimwipes
- 3M Sponge-Stick™ samples (P/N SSL10NB), hereafter referred to as 'sponge'
- Seward Stomacher® bags (P/N BA6041/CLR)
- Phosphate buffered saline with 0.05% TWEEN®20 (PBST) (SIGMA-ALDRICH, Co, P/N P3563-10PAK)
- MicroFunnel Disposable Filter Funnels, Pall Life Sciences (VWR P/N 55095-060) or Nalgene Sterile Analytical Filter Unit (Fisher P/N 130-4020)
- Disposable polystyrene serological pipettes (5mL and 10mL)
- Tryptic Soy Agar (TSA) plates
- Vortex mixer
- Disposable sterile 10µl loops
- Disposable sterile forceps
- Disposable gloves
- Cell spreaders or glass beads for spreading
- Racks for 15 mL and 50 mL centrifuge tubes
- Sterile, plastic, screw-cap 50 mL centrifuge tubes (e.g. Fisher Cat# 14-959-49A)

- Sterile, plastic, screw-cap 15 mL centrifuge tubes (e.g. Fisher Cat# 14-959-49D)
- Pipette tips with aerosol filter for 1 mL and 200 µL

1.0 PREPARATION

Personnel must be familiar with all procedures prior to start.

1.1 Equipment Preparation

a) Begin by donning personal protective equipment (PPE) such as gloves, lab coat, and protective eyewear.

b) Clean the workspace (Biological Safety Cabinet; BSC) by wiping surfaces with pH-amended bleach, next with DI water, and lastly with a 70-90% solution of denatured ethanol. Allow any excess liquid to dry prior to beginning procedure. Make sure the workspace is clean and free of debris.

c) Assemble equipment in the BSC as needed: vortex mixer, filtration manifold, automatic pipettors, tips, racks, etc.

d) Assemble extra supplies, such as stomacher and reagents, near BSC.

1.2 Supply Preparation

a) Unpack shipping containers directly into a BSC.

b) If sponges are not in Stomacher® bags, label one Stomacher® bag for each sponge and place in a bag rack.

c) Label two sterile 50 mL centrifuge tubes for each sponge sample and place in tube rack.

d) For each sample, label TSA plates on the agar side of the plate with the sample number and the appropriate dilution factors, as per MOP 6535a (*Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spores*).

e) Label two additional plates for filter-plate analysis.

2.0 PERFORM SPORE EXTRACTION, ELUTION, AND CULTURE PROCEDURE

2.1 Dislodge Spores from the Sample Sponges

a) Begin by donning a new pair of gloves. All subsequent procedures involving manipulation of sponges or spore suspensions must be carried out in a BSC. (Stomaching may occur outside the BSC when samples are double-contained inside the indicated bags.)

b) If the sponges are not in Stomacher® bags, aseptically transfer each sponge to a Stomacher® bag (labeled during step 1.2b) using sterile disposable forceps. Change forceps between samples.

c) Aseptically add 90 mL of PBST to each bag that contains a sponge.

d) Stomach sponges in the PBST by completing the following:

- Make certain the Stomacher® is set to MANUAL. Program the Stomacher® speed to 260 RPM and the timer to 1 minute.

- Open the Stomacher® door by raising the lid fully upward and back. The DOOR OPEN icon will be displayed.

- Place the stomacher bag containing the sponge sample into a second stomacher bag to contain any leakage in the event the primary containment is compromised. Place the combined bags such that 50 to 60 mm of the top portions protrude above the bag clamp, while making certain that the sponge sample rests evenly between the homogenizer paddles.

- Close the door to the Stomacher®. The DOOR OPEN icon will no longer be illuminated.

- Stomach each sponge for 1 min by pressing the START button.

- When the cycle ends, the Stomacher® will stop. If there is an emergent reason to stop the stomacher during the 1 minute stomaching period, press the red button or the power button to do so prior to opening the Stomacher®. Stopping the Stomacher® by opening the door can damage the equipment.

- Open the door of the Stomacher® and remove the bags containing the sponge. Grab the sponge from the outside of the bag with your hands. Move the sponge

to the top of the bag while using your hands to squeeze excess liquid from the sponge.

- Remove and discard the sponge using sterile forceps.

e) Repeat steps (b) through (d) for all samples.

f) Allow bags to sit for 10 min to allow elution suspension foam to settle before beginning the concentration step.

2.2 Remove Sponge Elution Suspension

a) Gently mix elution suspension up and down with a 50 mL pipette three times.

b) Split elution suspension volume equally.

- Remove half of the suspension volume (~45 mL) with a sterile 50 mL pipette and place it in a 50 mL screw capped centrifuge tube.

- Place remaining suspension (~45 mL) into a second 50 mL tube.

c) Record suspension volumes on tubes and data sheet.

d) Repeat steps (a) through (c) for all samples.

2.3 Concentrate Sponge Elution Suspension (Optional)

a) Centrifuge 50 mL centrifuge tubes

- Prior to daily use and before placing tubes into centrifuge, follow MOP 6558 (*Centrifuge Cleaning Procedure*) for cleaning this equipment.

- Add centrifuge tubes to rotor, evenly distributing weight.

- Centrifuge tubes at 3500 x g for 15 min. Do not use the brake option on the centrifuge to slow the rotor, as re-suspension of pellet may occur.

b) Carefully remove about 42mL of supernatant with a 50 mL pipette and discard to leave approximately 3 mL in each tube. The pellet may be easily disturbed and not visible, so place pipette tip away from the tube bottom or side.

c) Vortex and sonicate tubes as follows:

- Set vortex mixer to level 10 and touch activation.
- Turn on sonicator water bath.
- Vortex tubes for 30 sec.
- Transfer tubes to sonicator bath and sonicate for 30 sec.
- Repeat vortex and sonication cycles two additional times.

d) Remove suspension from one tube with a sterile 5 mL pipette and place it in the other tube of the same sample. The combined result is the final sponge elution suspension.

e) Measure final volume of the final sponge elution suspension with 5 mL pipette and record on tube and data sheet.

f) Repeat steps (e) through (i) for all samples.

2.4 Serially Dilute and Plate the Final Spore Elution Suspension

a) Use MOP 6535a to serially dilute and plate samples.

NOTE: If the samples are turbid, wide-orifice pipette tips may be used to prevent clogging of pipette tips.

b) Place all plates in an incubator set at 35 ± 2 ºC for a maximum of 3 days. Plates should be examined within 18-24 hours after start of incubation. Manually enumerate CFU of target organism and record data.

- If the CFU is <300/plate, record actual number.
- If the CFU is >300/plate, record as "too numerous to count" (TNTC)

2.5 Capture Spores on Filter Membranes and Culture on TSA

Choose one of the following to methods to filter the final spore elution suspension:

a) Complete filter plating using MOP 6565 (*Filtration and Plating of Bacteria from Liquid Extracts*).

b) Complete filter plating using the following method:

1) Place two 0.45 μm (pore-size) Microfunnels on a Pall vacuum manifold (Pall Cat# 15403).

2) Moisten Microfunnel membranes with 5 ml PBST, open vacuum, and vacuum through the filter. All filtering should be done with a vacuum pressure <20 cm Hg.

3) Make certain that the manifold vacuum valve is closed. Turn on the vacuum.

4) With the vacuum valve closed, place 10 mL of PBST into each filter cup.

5) Add 1.0 mL of the final sponge elution suspension to each filter cup.

6) Open valves and allow the suspension to flow through the filter, close the valve.

7) Rinse the walls of each Microfunnel cup with 10 mL of PBST. Reopen the valve to allow the suspension to flow through the filter.

8) Close the valve, turn off the vacuum pump. Slowly reopen the valve to equalize the pressures.

9) Squeeze the walls of the Microfunnel cup gently and separate the walls from the base holding the filter. Remove each filter membrane with sterile disposable forceps and place grid-side up on a TSA plate. Make sure that the filter is in good contact with the surface of the agar. If an air pocket occurs under the filter, use the sterile forceps to lift the edge of the filter to release the air pocket for better contact with the agar.

10) Record exact volume of the sponge elution suspension filtered on each plate. It should be 1 mL. (Greater sample volumes may be used to lower detection limits)

11) Repeat steps (1) through (8) for all each sample.

12) Incubate TSA plates with filter membranes at 35 ± 2 ºC for a maximum of 3 days. Plates should be examined within 18-24 hours after start of incubation. Manually enumerate CFU of target organism and record data.

- If the CFU is <300/plate, record actual number.
- If the CFU is >300/plate, record as “too numerous to count” (TNTC)

Miscellaneous Operating Procedure (MOP) 6582:
Recovery of *Bacillus* Spores from Trace Evidence Filters

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Prepared for

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Prepared by

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MOP: **6582**

Title: **RECOVERY OF *BACILLUS* SPORES FROM TRACE EVIDENCE FILTERS**

Objective: To aseptically extract and quantify recovery of *Bacillus* spores from 3M Trace Evidence filters (TEF) used to sample porous surfaces.

MATERIALS AND EQUIPMENT

Equipment:

- Biological Safety Cabinet (BSC, Class II)
- Incubator set to appropriate growth temperature for target organism (35 °C)
- Light box for counting colonies
- Vortex mixer
- Stomacher (Seward Stomacher® 400 Circulator (Seward; catalog #0400/001/AJ)
- Closure bags (catalog # BA6141/CLR)
- Stomacher bag rack (Seward catalog # BA6090)
- Specimen cup rack
- 50 mL Tube rack

Materials:

- Personal Protective Equipment (PPE) (gloves, lab coat, safety goggles)
- 1:10 Bleach, pH-adjusted (pAB) to 7.0 with acetic acid
- Dispatch bleach wipes
- Disposable 500 mL beaker (such as Fisher Scientific cat #14-955-111D) (for discarding used tips and beads/spreaders)
- 70% Solution of denatured ethanol
- Kimwipes
- Waste container
- Sterile Phosphate Buffered Saline with Tween 20 solution (PBST) (MOP 6562)
- Tryptic Soy Agar (TSA) plates
- 3M Forensic Vacuum Filter (Trace Evidence Filter) (Precision Data Products, Grand Rapids, MI catalog #FF-1)
- Stomacher bags, closure type (Seward catalog # BA6141/CLR)

- Sterile scalpels, preferably one for each sample
- Sterile 50 mL centrifuge tubes filled with 45 mL of sterile PBST, two for each sample
- Pipettor and sterile 50 mL pipettes
- Pipettor and pipette tips for dilutions (100 µl, 1000 µl, and 5000 µl.)
- Disposable cell spreaders or glass beads for cell spreading
- Disposable sterile thumb forceps
- Disposable sterile 2 mL bulb pipettors (such as Fisher Sci cat #13-711-7M)

1.0 REMOVING FILTER AND DUST FROM TRACE EVIDENCE FILTER

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).
2. Each TEF should be received separately in a small sealable bag (primary containment), and within secondary containment. Make certain that all of the samples are labeled. Review any chain of custody forms that may accompany the samples to ensure that all of the labels are consistent and that there is no notable variation in the samples. If discrepancies are found, make a note of each one in the notebook.
3. Clean the workspace (BSC) by wiping surfaces with pAB, next with deionized water, and lastly with a 70-90% solution of denatured ethanol. Wipe with a kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task.
4. Discard gloves and replace with fresh pair.
5. For each TEF sample, label one closure-type stomacher bag and two 50 ml centrifuge tubes (containing 45 ml PBST each) with the sample identifier.
6. One at a time, under the BSC, remove the TEF from the primary containment bag in which it arrived. Using the Dispatch bleach wipes, wipe each cassette with a wipe, and then a clean kimwipe. Discard the used bleach wipe and the used kimwipe in the secondary containment bag and place them in the non-regulated waste container. Remove gloves and don a fresh pair of gloves before proceeding.
7. With the plug in place on the back side of the filter, remove the black nozzle cover from the front filter side of the cassette, use a pipette and aseptically dispense 5 mL of PBST (taken from the centrifuge tubes containing 45 ml PBST) into the cassette (Figure 1).

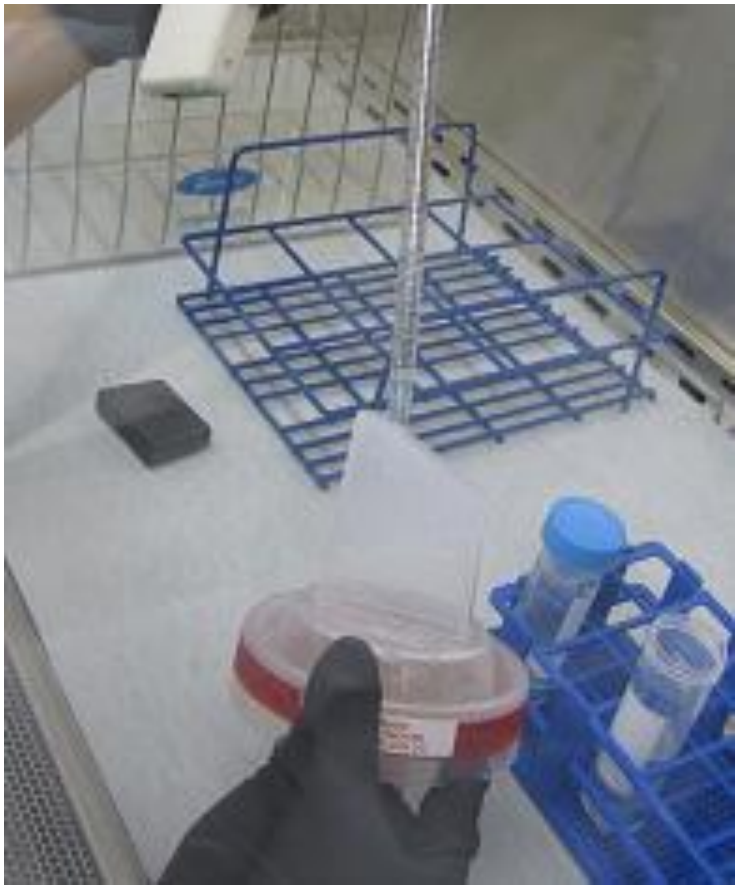

Figure 1. Dispensing 5 mL PBST into TEF nozzle

8. **Replace the black nozzle cover** and roll the TEF around to allow the liquid to touch all surfaces of the inside of the cassette. If there is a large quantity of dust inside, more PBST may be needed to dampen the dust before opening the TEF. Be careful not to hold or squeeze the black nozzle cover, as this may cause the seal to be disrupted, resulting in loss of PBST.
9. Once a visual check has been completed to assure that the dust is dampened enough to prevent its aerosolization, use the sterile scalpel to cut through the red tape seal around the circumference of the TEF.
10. Holding the TEF nozzle side up, open the plastic case of the filter. Turn the top of the filter case upside down (nozzle side down with the black nozzle cover in place) and set aside for a moment, inside the BSC on a rack (specimen cup rack will work) so that it will not be touching anything. (Figure 2)

Figure 2. Place the top half of the filter case onto a rack so that the filter can be removed from the bottom half of the filter case.

11. Position the labeled stomacher bag in the bag rack so that the closure wires can be bent in a manner so that the bag remains open while standing up in the rack (Figure 3).

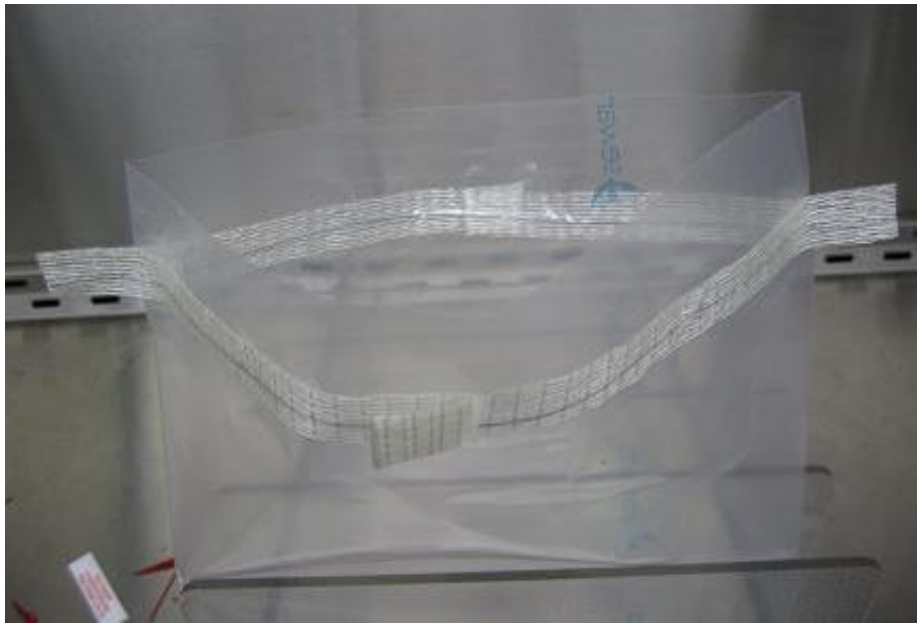

Figure 3. Stomacher bag in bag holder with wires in open position, ready to accept filter and liquid.

12. Remove the moistened filter with sterile forceps and place in the stomacher bag (Figure 4).

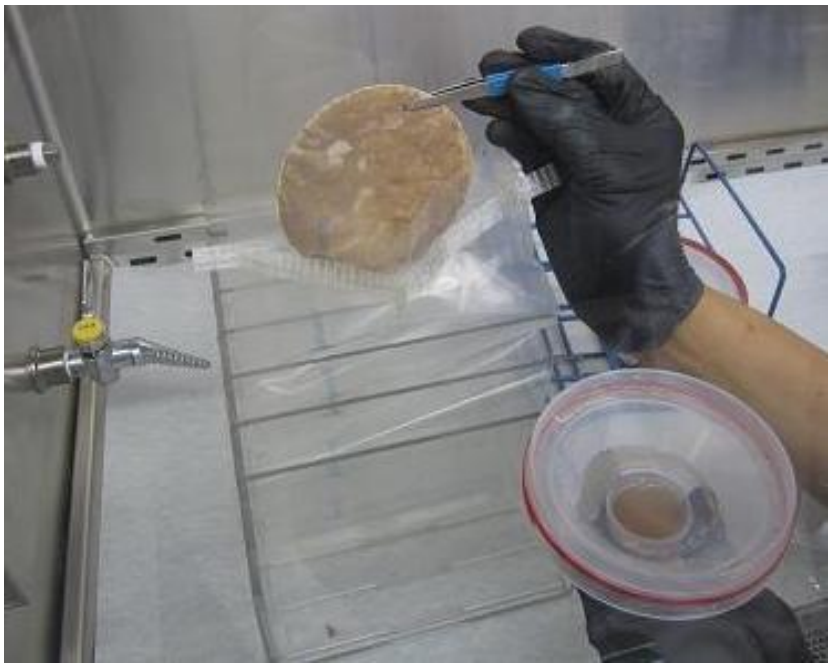

Figure 4. Placing moistened filter into open stomacher bag.

13. Use a pipette to aseptically remove liquid that has collected under the filter during the moistening step (Step 7 above). Place the liquid into the stomacher bag with the filter (Figure 5).

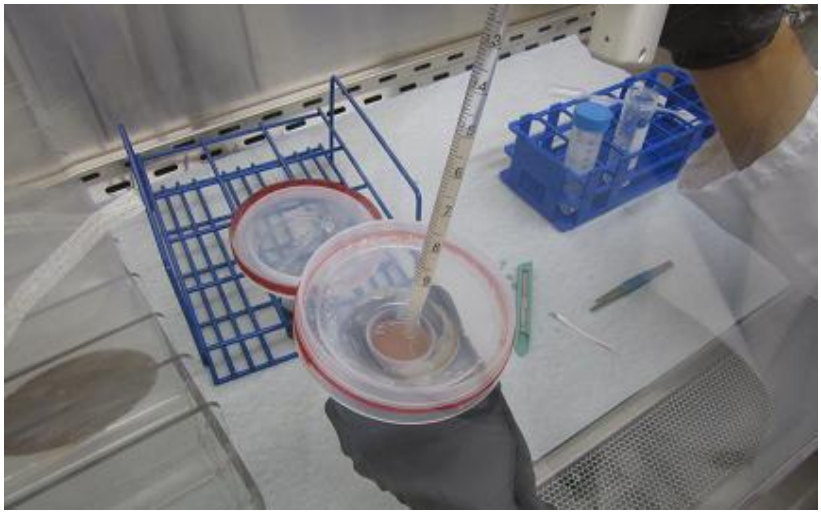

Figure 5. Transferring moistening liquid to stomacher bag.

14. Use a disposable bulb pipette to aseptically rinse the inside walls of the bottom portion of the filter case with 1 to 2 mL of PBST (additional aliquots from the same 45 mL tubes used previously), then remove the rinse eluent (using the same transfer pipette) and place it into the stomacher bag along with the filter (Figure 6).

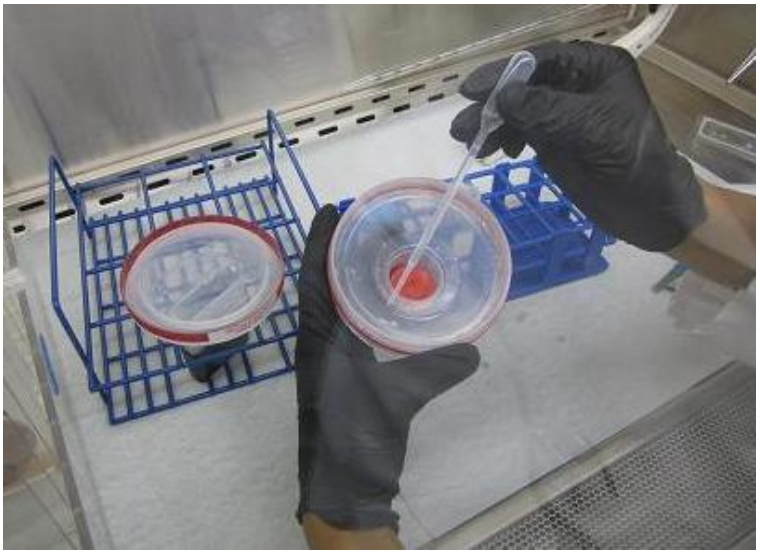

Figure 6. Rinsing inside walls of filter case.

15. Repeat the rinsing step for the top portion of the filter case that was set aside (Step 10).
16. Pipette or pour the remainder of the 90 mL PBST (two tubes of 45 mL each) into the stomacher bag that contains the filter and the rinsate of the filter case (Figure 7).

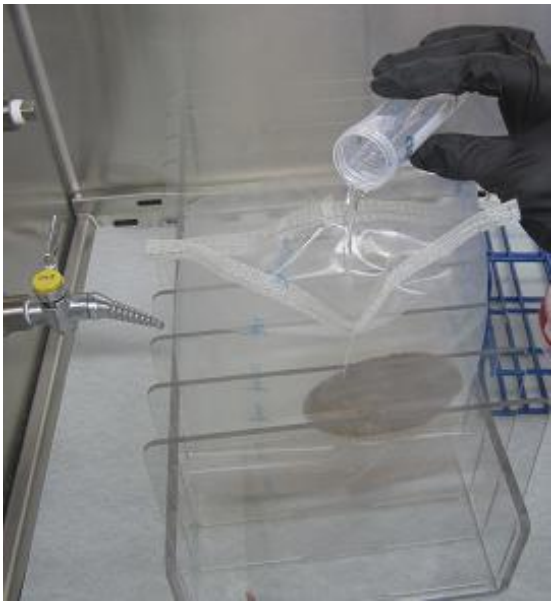

Figure 7. Pouring remainder of 90 mL PBST into stomacher bag.

17. Discard the filter case, red plug nozzle cover, disposable pipettes and disposable thumb forceps into the proper waste containers. **DO NOT** discard the 50 mL centrifuge tubes, as you will use these again for centrifuging the eluent after processing in the stomacher.
18. Close stomacher bags by folding multiple times and folding the wire closures into the center of the bag. Wipe the outside of the bag with a disinfecting wipe and place in a secondary stomacher bag rack or sample retention bin.
19. Wipe down the surface of the BSC as described in Step 3 above.
20. Change gloves, and repeat from Step 5 for each remaining TEF sample.

2.0 PROCESSING TRACE EVIDENCE FILTERS

1. Set the stomacher to 1 min, 260 rpm (setting P3). Place one stomacher bag from Section 1.0, Step 18 (above) into the stomacher and run the cycle. Remove the stomacher bag and place back in the bag rack.
2. Repeat for each sample.
3. Hold the samples at room temp for 10 min to allow the foam to settle in the stomacher bag (Figures 8 and 9).

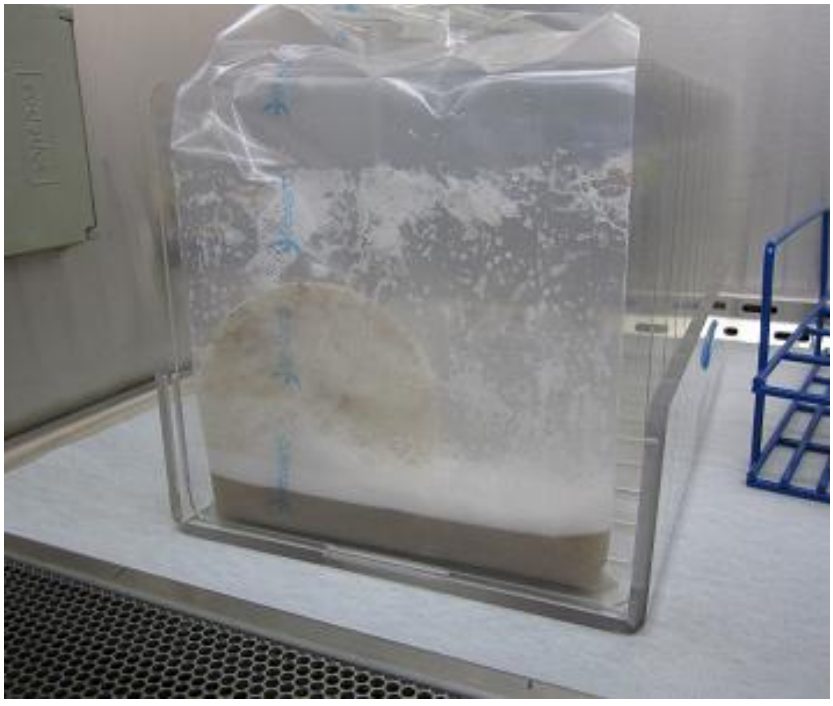

Figure 8. Stomacher bag with sample; foam in stomacher bag immediately after 1 min stomacher cycle.

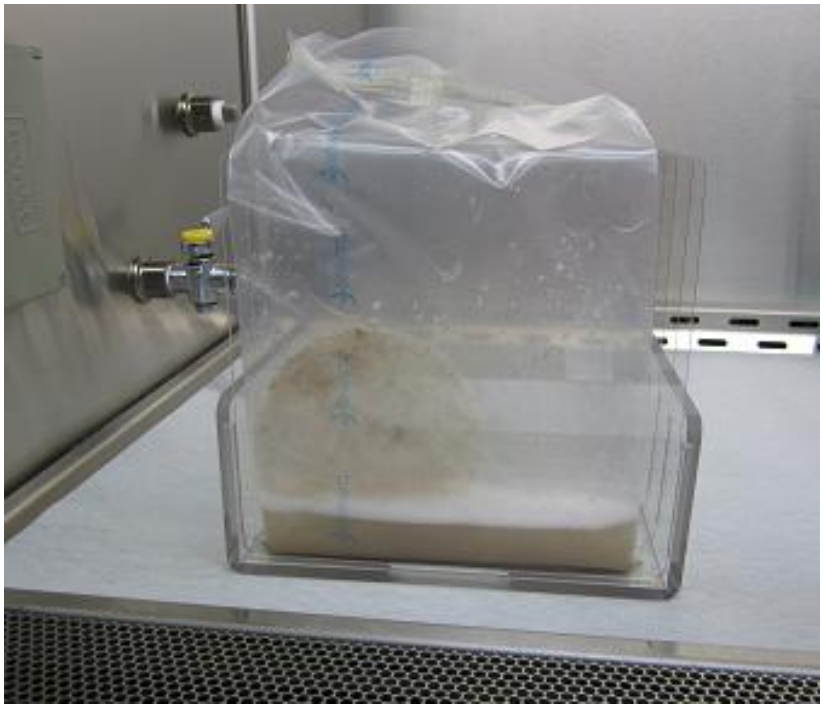

Figure 9. Stomacher bag with sample; settled foam after 10 min holding time.

4. Using a 50 mL pipette, transfer the eluent evenly (so the tubes will be balanced in the centrifuge) into the same two labeled 50 mL centrifuge tubes saved from Section 1.0, Step 17 above (~43 mL in each tube, since ~ 4 mLs is absorbed by the filter).
5. Weigh the tubes to guarantee that they will be balanced (+/- 0.25g).
6. Centrifuge the eluent at 3500g for 15 min.
7. Carefully remove all but approximately 3 mL (use the 5mL gradient on the side of the tube to guide the amount) from each tube using a 50 mL pipette, being careful not to disturb the pellet. Vortex vigorously to disperse the pellet.
8. Vortex the remaining liquid in the tubes and then aseptically combine the remaining eluent from the two tubes so that approximately 6 mL of eluent is now in one tube. Vortex and sonicate to break up aggregates before plating.
9. **Measure and record the exact volume of the remaining liquid within each sample**.

3.0 PLATING AND FILTRATION OF BACTERIA FROM LIQUID EXTRACTS

1. Follow MOP 6535a for serial dilutions and spread-plating samples in triplicate from the ~6mL solution containing the concentrated sample.

2. Filtration – detailed in the steps below – is required only if extremely low recovery is expected.

a. Label three TSA plates with information specific to the sample and amount to be filtered.
b. Set up filter manifold with three filter unit cups.
c. Set the vacuum, with proper gauge, to no more than 10 inches Hg (4.9 psi).
d. Add 10 mL PBST to filter cup.
e. Add 1 mL sample eluent from sample cup to each filter cup on the manifold.
f. Open the vacuum valve and vacuum liquid through filter.
g. Rinse sides of filter cup with 10 mL of sterile PBST, vacuum through again.
h. Pop off sides of the filter cup by squeezing and lifting.
i. Use sterile forceps to lift filter and place it on the TSA, making sure good contact with the agar surface is made and air pockets are minimized.

3. Repeat for subsequent sample tubes, completing samples in batches of no more than 4. This will help to avoid too much settling time between vortexing and plating.
4. Place all TSA plates in incubator set at 35 ºC and incubate 18 – 24 hrs before counting colonies.

4.0 CALCULATIONS

For spread plates:

total CFU recovered = (mean CFU on plate / 0.1 mL volume plated) x (1/dilution factor) x (total volume in centrifuge tube after decanting)

For vacuum filter plates:

total CFU recovered = (mean CFU on vacuum filter /volume filtered) x total volume in centrifuge tube after decanting

Miscellaneous Operating Procedure (MOP) 6586: Recovery of *Bacillus* Spores from Quartz Filters

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Approved by: _____ Date: 2/12/2013
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Prepared for

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MOP: **6586**

Title: **RECOVERY OF *BACILLUS* SPORES FROM QUARTZ FILTERS**

Objective: To aseptically extract and quantify recovery of *Bacillus* spores from quartz filters used to sample air.

MATERIALS AND EQUIPMENT

Equipment:

- Biological Safety Cabinet (BSC, Class II)
- Seward 400 Circulator Stomacher®
- Incubator set to appropriate growth temperature for target organism (35 °C ± 2 ºC)
- Light box for counting colonies
- Vortex mixer
- Stomacher bag rack
- Serological pipette

Materials:

- Personal Protective Equipment (PPE) (gloves, lab coat, safety goggles)
- 1:10 Bleach, pH-amended to 7.0 with acetic acid (pAB)
- Dispatch bleach wipes
- Disposable 500 ml beaker (such as Fisher Scientific, cat #14-955-111D) (for discarding used tips and beads/spreaders)
- 70% Solution of denatured ethanol
- Kimwipes
- Waste container
- Sterile Phosphate Buffered Saline with Tween 20 solution (PBST) (MOP 6562)
- Tryptic Soy Agar (TSA) plates
- Sterile 1500 µL tubes filled with 900µL of sterile PBST
- 120 mL specimen cup containing 100 mL of sterile PBST

- Pipettor and pipette tips for dilutions (100 µl, 1000 µl, and 5000 µl.)
- Disposable cell spreaders or glass beads for cell spreading
- 100 mL serological pipette tips
- Disposable sterile thumb forceps
- 10" x 15" Twirl'em bag (Fisher, cat # 01-002-53)
- Quartz filter (Whatman, cat # 18209932)
- Seward stomacher 400 bags (BA6141/CLR closure bags x 10)

1.0 PREPARATION

Personnel must be familiar with all procedures prior to start.

1.1 Equipment Preparation

a) Begin by donning personal protective equipment (PPE) such as gloves, lab coat, and protective eyewear.

b) Clean the workspace (Biological Safety Cabinet; BSC) by wiping surfaces with pH-amended bleach (pAB), next with deionized (DI) water, and lastly with a 70-90% solution of denatured ethanol. Allow any excess liquid to dry prior to beginning procedure. Make sure the workspace is clean and free of debris.

c) Assemble equipment in the BSC as needed: vortex mixer, filtration manifold, automatic pipettors, tips, racks, etc.

d) Assemble extra supplies, such as stomacher and reagents, near BSC.

e) Wipe down workspace once again with 70-90% solution of denatured ethanol.

1.2 Supply Preparation

a) Unpack shipping containers onto a cart near a BSC.

b) For each sample, label TSA plates on the agar side of the plate with the sample number and the appropriate dilution factors, as per MOP 6535a (*Serial Dilution: Spread Plate Procedure to Quantify Viable Bacterial Spores*).

c) Pre-label sterile, empty 120 mL specimen cups to collect the extraction liquid from each sample.

2.0 PERFORM SPORE EXTRACTION AND CULTURE PROCEDURE

2.1 Dislodge Spores from the Sample Filters

a) Begin by donning a new pair of gloves. All subsequent procedures involving manipulation of the filters or spore suspensions must be carried out in a BSC. (Stomaching may occur outside the BSC when samples are double-contained inside the indicated bags.)

b) Outside of the BSC, open the secondary (10" x 15") containment bag containing the labeled stomacher bag containing the sample filter. Check to make sure the secondary bag and the stomacher bag have matching labels. Wipe down the stomacher bag with a dispatch wipe and place in the stomacher rack located in the BSC. Discard the outer 10" x15" bag in the non-regulated waste container.

c) Aseptically add 100 mL of PBST to each labeled stomacher bag containing a filter. The 100 mL of PBST should be pre-measured in a 120 mL specimen cup. Discard this specimen cup when empty.

d) Place the stomacher bag containing the quartz filter sample and PBST into a second stomacher bag to contain any leakage in the event the primary containment is compromised. Place the combined bags such that 50 to 60 mm of the top portions protrude above the bag clamp, while making certain that the quartz filter rests evenly between the homogenizer paddles.

e) Stomach filters in the PBST by completing the following:

1) Make certain the Stomacher® is set to MANUAL. Program the Stomacher® speed to 230 RPM and the timer to 2 minutes.

2) Open the Stomacher® door by raising the lid fully upward and back. The DOOR OPEN icon will be displayed.

3) Close the door to the Stomacher®. The DOOR OPEN icon will no longer be illuminated.

4) Stomach each filter for 2 min by pressing the START button.

5) When the cycle ends, the Stomacher® will stop.

> Note: If there is an emergency, stop the stomacher by pressing the red button or the power button prior to opening the Stomacher®. Stopping the Stomacher® by opening the door can damage the equipment.

6) Open the door of the Stomacher® and remove the bags containing the filter. Remove the labeled stomacher bag containing the filter sample and place it into the stomacher bag rack. The same secondary stomacher bag may be used for each sample provided there was no leak. If a leak was detected, leave the labeled stomacher bag in the secondary bag and place both into the stomacher bag rack.

f) Repeat steps (b) through (d) for all samples.

g) Allow bags to sit for 10 min to allow the suspension foam to settle before beginning the extraction step.

2.2 Recovery of Suspension

a) Obtain a new, empty, pre-labeled 120 mL specimen cup with the same sample ID as that of the stomacher bag.

b) The suspension will be removed using a 100 mL pipette. Open the stomacher bag and remove the liquid by first tilting the bag to one side, moving the liquid away from the filter. Remove as much liquid as possible using the pipette.

c) When liquid can no longer be collected this way, simultaneously squeeze the filter and collect liberated liquid via pipette. Make attempts to locate pipette tip in a location within the bag that reduces collection of filter debris.

NOTE: The filter particles can become lodged in the pipette tip. If this happens it may be necessary to eject some of the liquid to dislodge the particle.

d) After all the liquid has been removed from the bag, place it into the pre-labeled specimen cup. Make sure to note the volume collected on the specimen cup as well.

e) Discard the labeled stomacher bag with the filter into a non-regulated waste container.

f) Repeat steps (a) through (e) for each sample.

2.3 Serially Dilute and Plate the Final Spore Elution Suspension

a) Use MOP 6535a to serially dilute and plate samples.

NOTE: If the samples are turbid, wide-orifice pipette tips may be used to prevent clogging of the pipette tips.

Miscellaneous Operating Procedure (MOP) 6593: Recovery of Spores from HVAC Filters

Prepared by: _____ Date: 4/30/2013

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Reviewed by: _____ Date: 4/30/2013

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Approved by: _____ Date: 4/30/2013

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MOP: 6593

Title: **RECOVERY OF SPORES FROM HVAC FILTERS**

Scope: This MOP outlines the procedure for recovering spores from HVAC filter sections

Purpose: To aseptically extract and quantify spores from sections of HVAC filters in order to determine viability and obtain quantifiable data

1.0 MATERIALS

- PPE (gloves, lab coat, safety goggles)
- Class II Biological Safety Cabinet (BSC)
- pH-amended bleach
- Deionized (DI) water
- 70% solution of denatured ethanol
- Kimwipes
- Dispatch bleach wipes
- Non-regulated waste container
- Bottles of sterile Phosphate Buffered Saline with Tween 20 solution (PBST) (MOP 6562)
- Vortex mixer
- Shaker table with clamps or brackets to hold Nalgene bottles
- Cart
- Trypticase soy agar (TSA) plates
- 900uL dilution tubes of sterile PBST
- Pipettor and pipette tips for dilutions
- Incubator set to appropriate growth temperature for target organism (35°C+/-2°C)
- Light box for counting colonies
- Lab notebook
- Sterile graduated cylinder
- Parafilm

2.0 PROCEDURE

1. Begin by donning PPE (gloves, lab coat, and protective eyewear).

2. Samples will be received as follows: Half portions of HVAC filters will be in 32oz white capped Nalgene jars (Fisher cat no. 2118-0032) and quarter portions of HVAC filters will be in 1L clear bottles (Fisher cat no. 02-893D). The samples should be secondarily contained by large sterile bags (10” x 15” sterile bags Fisher cat no. 01-002-53). Chain of custody forms that accompany the samples will need to be

reviewed to ensure that all of the samples IDs are consistent and that there is no notable variation in the samples. If variation has occurred, make a note of it in the notebook.

3. Clean BSC workspace by wiping surfaces with pH-amended bleach, followed by DI water, and lastly with a 70% solution of denatured ethanol. Wipe with a kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the BSC, within arm's reach so that once the procedure has begun the task may be performed without interruptions.

4. Discard gloves and replace with a fresh pair.

5. Place the samples one at a time, under the BSC. Samples should be handled in order of least contaminated to most contaminated, with any negative controls or blank samples being handled first, test samples being handled next, and lastly the positive controls. Carefully unwrap the secondary containment bag from around the jar or bottle, discard in non-regulated waste container and then wipe down the jars or bottles with a dispatch wipe, followed by a Kimwipe. Change gloves after handling each sample.

6. Once all of the samples are under the BSC and disinfected, change gloves and place the sterile graduated cylinder under the BSC, along with several bottles of sterile PBST. Again, in order from least contaminated to most contaminated, handle the samples individually, and carefully open the jars or bottles to aseptically add 700 mL of PBST to the sample. The PBST is first aseptically poured into the graduated cylinder and measured at 700 mL, and then it is aseptically poured from the graduated cylinder into the sample. Carefully transfer all liquid as to not spill any or disturb/touch the filter or its container in any way. Once the 700 mL of PBST has been added, carefully place the jar lid or bottle top back on the sample, and make certain it is tightly closed.

7. Again, wipe the outside of the samples with Dispatch wipes, followed by a Kimwipe to remove any moisture. After all samples have 700 mL of sterile PBST added, wrap the sample lids/bottle tops with Parafilm to ensure a tight seal and to prevent sample leakage.

8. Place all sample jars or bottles into an orbital shaker table, using the specially designed platform holders. Make certain all samples are secure, and shake at room temperature for 30 min at 300 rpm.

9. Immediately after the thirty minute agitation step is completed, remove the samples from the shaker and place them, again from least contaminated to most contaminated, into the BSC for dilution plating. Don a fresh pair of gloves, remove and discard the Parafilm, and follow MOP 6535a to complete serial dilutions using TSA media plates that are appropriately labeled with the sample ID, dilution set, and date. Each sample should also be manually agitated for ten seconds prior to removal of aliquots. Dilution tubes used in MOP 6535a should contain PBST to stay consistent with materials/solutions.

10. Once the dilution plating has been completed, incubate the plates at 35°C +/- 2°C for 18-24 hours, and archive the samples in at refrigerator at 4°C+/-2°C.

11. Count the colonies with the assistance of a light box. Record data.

12. Colony forming units for each 6" x 12" half section or for each 6" x 6" quarter section will be determined and the sum of the CFUs for the two or four sections making up one 12" x 12" sample will be determined. Data will be reported as total CFU for the 12" x 12" sample.

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