EPA
United States
Environmental Protection
Agency

EPA/600/R-19/082 | August 2019
www.epa.gov/homeland-security-research

Sample Analysis of Native Air Filters for Characterization and Extent Mapping of Biological Incidents

Office of Research and Development
Homeland Security Research Program

EPA/600/R-19/082 August 2019

SAMPLE ANALYSIS OF NATIVE AIR FILTERS FOR CHARACTERIZATION AND EXTENT MAPPING OF BIOLOGICAL INCIDENTS

U.S. Environmental Protection Agency
Cincinnati, OH 45268

EPA/600/R-19/082
August 2019

Disclaimer

The United States Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center, funded and managed this evaluation. The document was prepared by Battelle Memorial Institute under EPA Contract Number EP-C-15-002; Task Order 0009. This document was reviewed in accordance with EPA policy prior to publication. Note that approval for publication does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

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Abbreviations and Acronyms

Acronym	Definition
AQ	Air Quality
Avg	Filters selected that had relatively moderate (average) particulate loadings for the site
B. a. Sterne	*Bacillus anthracis* Sterne
B. anthracis	*Bacillus anthracis*
BBCA	Brilliance *Bacillus cereus* Agar
BHIB	Brain Heart Infusion Broth
BSC	Biological Safety Cabinet
°C	Degree(s) Celsius
CFU	Colony Forming Unit(s)
Ct	Threshold Cycle
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
End	Filers removed at the end of their service life as defined by the scheduled maintenance schedule
EPA	U.S. Environmental Protection Agency
ERLN	Environmental Response Laboratory Network
FAM	Fluorescent reporter dye on 5' end of PCR probe (6-carboxyfluorescein); emits at ~517 nm
High	Filters selected that had relatively high particulate loadings for the site
HVAC	Heating, Ventilation, and Air Conditioning
L	Liter(s)
µL	Microliter(s)
Mid	Filters that were in use for approximately half of their scheduled service life
mL	Milliliter(s)
mM	Millimolar
ModG	Modified G
MYP	Mannitol Egg Yolk Polymyxin
NAF	Native Air Filter
New	Filters that were not yet used; no ambient particulate matter collected
NTC	No Template Control
NYCT	New York City Transit
PBS	Phosphate Buffered Saline
PBST	PBS plus 0.05% Tween
PC	Positive Control
PCR	Polymerase Chain Reaction
PE	Performance Evaluation
PES	Polyethersulfone
PMP	Paramagnetic Particles
PVDF	Polyvinyldiene Difluoride
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
QMP	Quality Management Plan
qPCR	quantitative PCR
rcf	Relative Centrifugal Force
rpm	Revolution(s) per Minute

Abbreviations and Acronyms (Cont.)

Acronym	Definition
RV-PCR	Rapid Viability PCR
SBA	Sheep Blood Agar
SOP	Standard Operating Procedure
T&E II	Testing and Evaluation II Program
TOCOR	Task Order Contracting Officer's Representative
TSA	Technical System Audit
TSA	Technical Systems Audit
UTR	Underground Transport Restoration
VIC	Fluorescent reporter dye on 5' end of PCR probe (emits at ~551 nm)

Acknowledgements

This document was developed by the Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) within EPA's Office of Research and Development. Dr. Worth Calfee was the project lead for this document. Contributions of the following individuals and organizations to the development of this document are acknowledged.

United States Environmental Protection Agency

Dr. Worth Calfee, National Homeland Security Research Center
Dr. Sanjiv Shah, National Homeland Security Research Center
Mr. Leroy Mickelsen, Office of Land and Emergency Management
Dr. Sang Don Lee, National Homeland Security Research Center
Mr. Francisco Cruz, Office of Compliance and Enforcement Assurance

New York City Department of Health and Mental Hygiene

Ms. Kobria Karim
Dr. Joel Ackelsberg

New York City Transit

Mr. Mike Gemelli

Battelle Memorial Institute

Mr. Scott Nelson
Mr. Anthony Smith
Ms. Hiba Shamma
Dr. Ryan James
Mr. Zachary Willenberg
Dr. Rachel Spurbeck
Dr. Aaron Wenzel
Ms. Delaney Pfister
Ms. Jennifer Beare
Mr. Nate Russart
Mr. Kent Hofacre

EXECUTIVE SUMMARY

The United States Environmental Protection Agency (EPA) is the federal lead supporting remediation of land and public infrastructure following the release of a hazardous substance to the environment that threatens public health. EPA's remediation responsibility includes responding to a bioterrorism incident, such as the release of *Bacillus anthracis* (*B. anthracis*) in an urban area. EPA, in coordination with other Government agencies, National Laboratories, and Stakeholders have conducted studies to support preparation for response and remediation following such a release. These studies have included releases of surrogates for *B. anthracis* spores in outdoor environments and subway stations to better understand the transport of aerosols and to assess models to predict their behavior. Those studies have also been used to establish and assess air and surface sampling methods.

In addition to sampling methods, EPA has also pioneered the development of new analytical methods supporting response and remediation efforts. EPA has developed culture, polymerase chain reaction (PCR), and Rapid Viability (RV) PCR quantification and identification protocols for *B. anthracis* species that are used by EPA's Office of Emergency Management Environmental Response Laboratory Network (ERLN) (EPA, 2012 and Shah, 2017). The analytical methods used in this current study were based on EPA's 2012 version of "Protocol for Detection of *B. anthracis* Spores from Environmental Samples During the Remediation Phase of an Anthrax Event," but with some updates based on EPA's 2017, Second Edition.

Following a biological contamination incident, the spatial extent of the contamination should be determined using established sampling and analytical methods such as those noted above. A previous study demonstrated that a sampling strategy involving native air samplers could be implemented in an urban area with the cooperation and collaboration of the public-private sector (Ackelsberg, et al., 2011). Particulate filters indigenous to the affected area, and although intended for other applications (e.g., ambient air quality particulate sampler or building heating, ventilation, and air conditioning [HVAC] filter), may be operating during and/or immediately after an incident. Thus, those ubiquitous native air filters (NAFs) offer the potential to better map an incident by having a potentially higher quantity of organisms collected and/or provide a higher fidelity of mapping. Inherently, those NAFs will have or will be collecting ambient particulate matter prior to and/or during the incident. The impact of the ambient particulate load on the NAFs may interfere with the current analytical methods to recover, identify, and quantify *B. anthracis*. In this study, EPA seeks to assess the feasibility of using NAFs for potential use in biological incident extent mapping. If feasible, it could facilitate subsequent sampling plans, increase the speed of a response, and potentially save cost.

The objective of this study was to identify and evaluate the compatibility of currently deployed NAF devices (e.g., PM10 samplers, building HVAC filters) with current *B. anthracis* analytical methods (culture and RV-PCR), for the ultimate goal of characterizing and mapping the extent of contamination following a biological contamination incident involving *B. anthracis* spores.

Literature containing pertinent information related to field air sampling equipment was surveyed to identify sources of filters and their filter types associated with native air samplers. Two classes of native air samplers were defined and from which filters were sought: 1) air quality (AQ) samplers such as those used at air quality monitoring sites around the United States and 2) non-air quality (non-AQ) filters such as those in a building HVAC system or various types of air filters associated with transportation vehicles that would be ubiquitous and likely operating in an

urban setting. These filter types were utilized in laboratory-based testing whereby a known quantity of *Bacillus anthracis* Sterne (*B. a.* Sterne) (non-pathogenic strain of *B. anthracis*, used as a surrogate for fully virulent strains) spores were spiked onto the filter media. Currently-recommended *B. anthracis* analytical methods, culture and RV-PCR, were attempted on the spiked media.

The key findings, conclusions, and recommendations from this research are:

- The foremost conclusion is that filters recovered from both AQ and non-AQ filters may be useful and beneficial to analyze for *B. anthracis* to help map the extent of biological incidents, recognizing there are limitations to their use. This conclusion is made based on the data showing that, even in the presence of other particulate matter having been collected on filters, *B. a.* Sterne spores that were spiked onto the filters could be recovered and successfully detected; however, the study results clearly indicate that the background flora and other particulate matter can adversely impact the method sensitivity and accuracy. Consequently, the NAF could be used to supplement results from other sampling plans but should not be relied upon solely as the definitive biological release incident mapping tool. The overall accuracy of the method properly detecting *B. a.* Sterne (combined true positives and true negatives) across all filter types was 82% for culture and 85% for RV-PCR.
- RV-PCR can be used to positively identify viable *B. a.* Sterne in the presence of complex, dirty sample matrices of NAFs. However, background flora and grime collected can impact the lower limit of detection and/or reduce the response to *B. a.* Sterne.
- Background flora and non-living material (dirt/grime) interferes with identification and quantifying *B. a.* Sterne using the traditional plate culture method, particularly for non-AQ filters. Presumptive *B. a.* Sterne colonies may not actually be the target organism because background flora can have an indistinguishable colony morphology, leading to false positives and an overestimate of the detection of the *B. a.* Sterne. Conversely, the apparent *B. a.* Sterne quantity recovered can be biased low due to suppression of *B. a.* Sterne growth with competing background flora. It is possible for so much background flora to be present on NAFs such that the presence of *B. a.* Sterne cannot be made, potentially leading to false negatives.
- The RV-PCR method requires great care and diligence to implement effectively. Most notably, glove changes were required between samples for each step, which is onerous and time consuming; however, it was found to be necessary to minimize cross-contamination.
- The primary recommendation is to assess the impact that spiking of *B. a.* Sterne spores onto the NAF substrates has on the recovery and subsequent analyses. The liquid suspension spiking method may bias the recovery efficiencies favorably (higher efficiency) or unfavorably. Specifically, it is recommended to expand the study by generating an aerosol of *B. a.* Sterne and then pulling the aerosol-laden air through the NAF rather than applying spores via a liquid suspension spike. The method would then be applied to recover and analyze for *B. a.* Sterne. This approach is expected to primarily affect spore recovery, which then may impact detection limits and or accuracy to identify.
- Priority should be placed on analyzing filters having the lowest loading of background particulate matter, to the extent that can be determined by the shortest duty cycle of non-AQ filters or by gravimetric analysis of AQ filters.

1.0 INTRODUCTION

1.1 Background

Under Emergency Support Function #10 of the National Response Framework, the United States Environmental Protection Agency (EPA) is responsible for the remediation of land and public infrastructure following a biological contamination incident such as an act of bioterrorism involving the release of *Bacillus anthracis* (*B. anthracis)* in an urban area (https://www.fema.gov/media-library/assets/documents/117791. EPA, in coordination with other Government agencies, National Laboratories, and Stakeholders have conducted studies to support preparation for that role. These studies have included releases of surrogates for *B. anthracis* in outdoor environments and subway stations to better understand the transport of aerosol releases and to assess models to predict the spread of the particles. Those studies have also been used to establish and assess air and surface sampling methods.

In addition to sampling methods, EPA has also pioneered the development of new analytical methods supporting response and remediation efforts. EPA has developed culture, polymerase chain reaction (PCR), and Rapid Viability (RV) PCR quantification, and identification protocols for *B. anthracis* species that are used by EPA's Office of Emergency Management Environmental Response Laboratory Network (ERLN) (EPA, 2012 and Shah, 2017). The analytical methods used in this study were based on EPA's 2012 version of "Protocol for Detection of *B. anthracis* Spores from Environmental Samples During the Remediation Phase on an Anthrax Event," but with some updates based on EPA's 2017, Second Edition.

In the case of a biological contamination incident, EPA must characterize the extent of the spread of the biological threat agent using established sampling and analytical methods such as those noted above. Although a biological contamination incident may occur where BioWatch samplers are operated, they may not be present in sufficient numbers and optimally spaced or located to adequately characterize the biological threat agent spread. A previous study demonstrated that a sampling strategy involving native air samplers could be implemented in an urban area with the cooperation and collaboration of the public-private sector (Ackelsberg, et al., 2011). Particulate filters indigenous to the affected area, and although intended for other applications (e.g., ambient air quality particulate sampler or building heating, ventilation, air conditioning [HVAC] filter) are likely to be more prevalent, widely dispersed, and operating during and/or immediately after

an incident. Thus, those ubiquitous native air filters (NAFs) offer the potential to better map an incident by having a potentially higher quantity of organisms collected and/or provide a higher fidelity of mapping through their higher abundance/density in comparison to purposefully deployed counterterrorism air samplers. Inherently, those NAFs will have or will be collecting ambient particulate matter prior to and/or during the incident. The impact of the ambient particulate load on the NAFs may interfere with the current analytical methods to recover, identify, and quantify *B. anthracis.* EPA seeks to assess the feasibility of using NAFs for potential use in biological incident extent mapping by assessing their compatibility with current *B. anthracis* analytical approaches. If feasible, it could facilitate subsequent sampling plans, increase the speed of a response, and potentially save cost.

1.2 Objective

The objective of this study was to identify and evaluate the compatibility of *B. anthracis* analytical methods with currently deployed NAF devices (e.g., PM10 samplers, building HVAC filters) for the ultimate purpose of assessing whether NAFs are feasible to use for characterizing and mapping the extent of contamination following a biological contamination incident involving *B. anthracis* spores.

1.3 Scope

The scope of the research reported here was to assess the EPA methods to recover and subsequently analyze for the positive identification of *B. anthracis* spores – specifically, *Bacillus anthracis* Sterne (*B. a.* Sterne) spores that were spiked by applying droplets of a stock spore suspension onto both air quality (AQ) filters (PM2.5 and PM10 filters from ambient air quality monitoring sites) and non-air quality (non-AQ) filters from bus engine air intake filters, building HVAC filters, subway platform filters, and subway rolling stock filters. A total of 377 filter samples were spiked with *B. a.* Sterne, recovered per EPA protocols and analyzed using both culture and molecular methods based on those previously developed by EPA (EPA, 2012). (Initially, EPA's 2012 version of "Protocol for Detection of *B. anthracis* Spores from Environmental Samples During the Remediation Phase of an Anthrax Event" was used because the 2017 version was not yet finalized.) Elements of the EPA 2017, Second Edition version

(Shah, 2017) were incorporated as noted in this report in order to ensure the results reflected the state-of-the-art of the methods and gave the best indication of method capability.

The performance of the culture method was assessed by determining percent recovery efficiency of presumptive *B. a.* Sterne spores spiked onto the filter, which was also used to define frequency of false positives and false negatives. The performance of the molecular method was assessed by whether a positive identification was made, which was then used to determine a frequency of accurate identification, false positives, and false negatives.

It is important to note that this study was not solely an assessment of the analytical method to identify and/or quantify *B. anthracis*, but rather an assessment of the method end-to-end, to include physical recovery from the filter media (and other grime or flora associated with the filter operation in its intended use) followed by the *B. a.* Sterne analytical method. It is that end-to-end analysis that was the key element to assess method performance. Consequently, the study provided information on the limitations and opportunity for improvement of the methods, as well as providing a baseline of processing and analyzing samples that may be encountered in an actual incident response.

2.0 MATERIALS AND METHODS

2.1 Filter Categories and Selection

Two categories of NAFs were assessed: those used in AQ samplers (for PM2.5 and PM10 collection for ambient air quality monitoring) and those used in non-AQ applications (e.g., bus filter, building HVAC filter, subway platform filter, and subway rolling stock filter).

2.1.1 AQ Filters

The selection of PM2.5 and PM10 samples for use during this NAF project was based on a query of EPA's Air Quality System, which revealed that there are 2,585 active PM2.5 monitors and 4,719 active PM10 monitors located across the United States. Therefore, if an incident involving a biological aerosol release occurred, one or more of those samplers may be able to be used to help determine the extent of the release (i.e., the area of contamination). The AQ filters used were 47-mm diameter Teflon for the PM2.5 and 8- x 10-inch (20 x 25 cm) glass fiber filter for the PM10. Representative images of each AQ type is shown in Figure 1. The 47-mm-diameter filters were cut into quarters to prepare test coupons for testing. Coupons, 4 x 4 cm, were cut from the flat sheet PM10 filters, avoiding the edges of the filter. The filters were recovered from four targeted geographic regions, nominally from the Northeast, Southeast, North, and West to the extent possible. The PM2.5 filters recovered for use were from Arizona (AZ), Florida (FL), Massachusetts (MA), and Wisconsin (WI). The PM10 filters recovered were from California (CA), New Hampshire (NH), South Carolina (SC), and Wisconsin (WI).

AQ filters with relative ambient particulate loading levels of average (Avg) and high (High), as determined by gravimetric analysis data accompanying the filters, were obtained from each geographic region for each filter type. The relative particulate load descriptors of Avg and High were assigned by comparing the mass loading for filters from that region. Filters with the highest mass loading were selected and denoted as High and those near the middle of the mass loadings were denoted as Avg. Filters that had not been used to sample ambient air and thus had no ambient particulate loading were denoted as New filters or New media. (Because the same filter media was used, the New media was obtained independent of the geographic region and represented all.) Sample availability and project scope did not allow for selecting filters for specific times of the year.

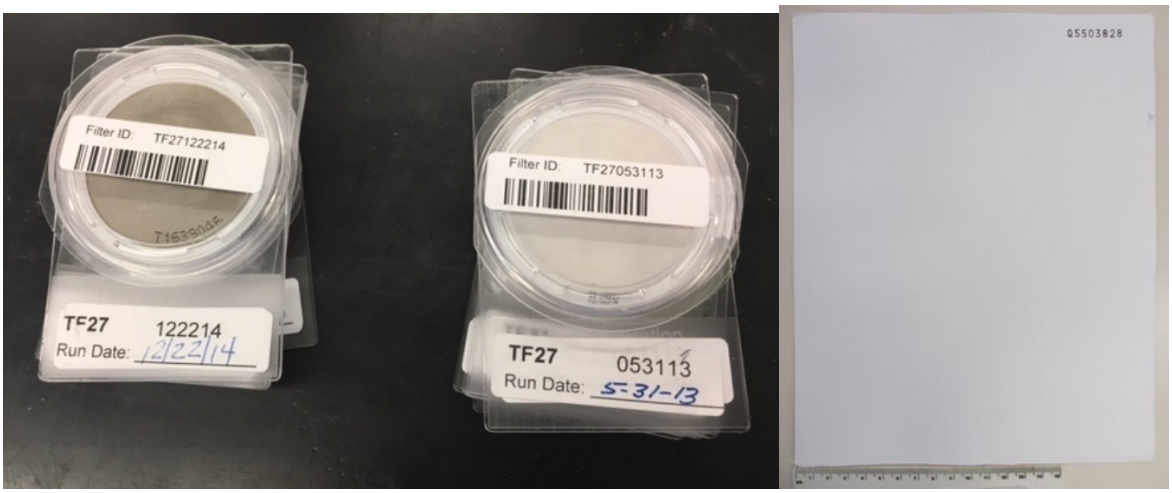

Figure 1. Air Quality Filter Types: PM2.5 (Left: High and Avg Particulate Loads) and PM10 (Right: New Media)

2.1.2 Non-AQ Filters

Representative images of each non-AQ filter type – building HVAC filter, bus filter, subway platform filter, and the subway rolling stock filter – are shown in Figure 2. All four filter types were pleated, but no additional information regarding the media type, fiber properties, or fiber density were provided. The swatches of filter media cut from the filters and used as coupons were generally obtained from the flat surface of the pleat, avoiding the pleat peaks and valleys. The bus filter, subway platform filter, and the subway rolling stock filter were all obtained from the New York City Transit (NYCT) system. The NYCT Authority has been involved in prior, related studies such as the Underground Transport Restoration (UTR) 2017 project, so there are additional data related to those filters that may complement these results (Serre and Oudejans, 2017), but are beyond the scope of this project. EPA requested that NYCT provide filters from each of the applications/locations that were unused (New), in the middle portion of their duty cycle (Mid), and at the end-of-duty cycle (End) as determined by their maintenance schedule. NYCT provided filters following these guidelines, so filters were obtained that contained varying quantities of ambient particulate matter. No specific duty cycle information (time, mileage) was provided from NYCT.

Figure 2. Non-AQ Filter Types: Bus Filter (Top Left); Building HVAC Filter (Top Right); Subway Platform Filter (Bottom Left); and Subway Rolling Stock Filter (Bottom Right)

2.2 Test Matrix

Each of the filter samples described in Section 2.1 was spiked with *B. a.* Sterne spores, extracted, and the extract analyzed to quantify and identify recovered *B. a.* Sterne to assess the EPA-provided methods using culture analysis and RV-PCR to determine the percent recovery for each of the three ambient particulate loading conditions.

The completed test matrix for the AQ filters and non-AQ filters are provided in Table 1 and Table 2, respectively. In total, 377 filter samples were analyzed, comprising 108 PM2.5 filters, 111 PM10 filters, and 158 non-AQ filters (approximately 40 for each of the four non-AQ filter types). The columns of Particle Loads in Table 1 and Duty Stage in Table 2 are as defined in Section 2.1 and represent new filters (New), average (Avg), or high (High) particle loading for AQ filters (Table 1) and new filters (New), middle-of-duty cycle (Mid), and end-of-duty cycle (End) for non-AQ filters (Table 2). The target spore load of 0, 30, 300, or 3,000 was the number of spores intended to be spiked onto the filters. Following physical extraction, the sample volume was split nominally in half to result in 0, 15, 150, 1,500 spore load challenges for each of two detection assays (culture and RV-PCR). These details are discussed further in Section 2.3.2. Generally, three replicates were completed for each target *B. a.* Sterne spore loading quantity,

but there were instances of higher number of replicates, especially early in the study as the analytical methods were being refined and implemented. The 0-spore load (no purposeful application of *B. a.* Sterne onto the test filter) served as a negative control. Use of New filter media served as a baseline to represent the expected best-case performance of the method because of the absence of potentially competing or interfering grime or flora.

The analytical methods of culture and RV-PCR were used to quantify or identify recovered *B. a.* Sterne spores spiked and subsequently recovered in the sample extracts. Sheep Blood Agar (SBA) was the primary medium used for all culture analyses; Mannitol Egg Yolk Polymyxin (MYP) and/or Brilliance *Bacillus cereus* agar (BBCA) were used for a subset of samples. MYP and BBCA are both chromogenic media that have been developed to aid in differentiating target pathogen microbial growth from background flora. They were assessed early in the study analyses to determine whether there was a benefit to unambiguously quantifying *B. a.* Sterne. As will be discussed in Section 3.1.1, it was decided to discontinue the culture assays with the chromogenic agar and continue using only the SBA media because no benefit was gained using the chromogenic media (Calfee, 2017). Details regarding the analytical methods are discussed in Section 2.3.

Table 1. Test Matrix for AQ Filters

Filter Type	Geographic Region	Particle Loads[a] (µg/cm²)	Target Spore Loads onto Filter[b]	Nominal Spores Available per Analytical Method[c]	Replicates	Analytical Method[d]	
						Culture	Molecular
PM2.5	N/A	New (0)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
	Wisconsin (WI)	Avg (14)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/BBCA	RV-PCR
		High (47)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/BBCA	RV-PCR
	Florida (FL)	Avg (13)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (39)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
	Arizona (AZ)	Avg (16)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (39)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
	Massachusetts (MA)	Avg (12)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (29)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
PM10	N/A	New (0)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	Wisconsin (WI)	Avg (41)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (104)	0/30/300/3000	0/15/150/1500	5/4/3/3	SBA/MYP	RV-PCR
	New Hampshire (NH)	Avg (69)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (199)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
	California (CA)	Avg (118)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/BBCA	RV-PCR
		High (277)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/BBCA	RV-PCR
	South Carolina (SC)	Avg (65)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR
		High (132)	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA	RV-PCR

(a) Ambient particle load reported from the air quality station from which the filters were received.
(b) Target number of spores spiked onto filter – See Section 2.3.2 for discussion.
(c) Nominally half of the target quantity of spores spiked onto the filter were available for each of the two analytical filter – See Section 2.3.3 for discussion.
(d) BBCA (selective); SBA; MYP agar (chromogenic); RV-PCR assay, chromosomal and pXO1 gene targets.

Table 2. Test Matrix for Non-AQ Filters

Filter Type	Duty Stages	Target Spore Loads onto Filter[a]	Nominal Spores Available per Analytical Method[b]	Replicates	Analytical Method[c]	
					Culture	Molecular
Bus	New	0/30/300/3000	0/15/150/1500	5/3/3/3	SBA/MYP	RV-PCR
	Mid	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	End	0/30/300/3000	0/15/150/1500	5/5/5/3	SBA/MYP	RV-PCR
Building HVAC	New	0/30/300/3000	0/15/150/1500	4/4/3/3	SBA/MYP	RV-PCR
	Mid	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	End	0/30/300/3000	0/15/150/1500	4/4/4/4	SBA/MYP	RV-PCR
Subway Rolling Stock	New	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	Mid	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	End	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
Subway Platform	New	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	Mid	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR
	End	0/30/300/3000	0/15/150/1500	3/3/3/3	SBA/MYP	RV-PCR

(a) Target number of spores spiked onto filter – See Section 2.3.2 for discussion.
(b) Nominally half of the target spore loaded onto the filter were available for each of the two analytical methods – See Section 2.3.3 for discussion.
(c) SBA; MYP agar (chromogenic); RV-PCR assay, chromosomal and pXO1 gene targets.

2.3 Microbiological Methods

All sample processing and analytical methods used were from those provided by the EPA Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident (EPA, 2012), with any differences or revisions noted. Both a culture and molecular analytical method were assessed as will be discussed in Sections 2.3.4 and 2.3.5.

Early application of the RV-PCR method to samples not purposely spiked with *B. a.* Sterne resulted in measurable levels of *B. a.* Sterne. There were instances of solution leakage of the capping tray and poor welds of the membrane in the filter vials that were potentially the source or contributor to the contamination. The capping tray was replaced with a new unit and filter vial leakage did not persist. Also, with subsequent refinement of glove change frequency and technique of equipment operation, cross-contamination was reduced and ultimately eliminated.

Following are sections that summarize specific procedures and steps applied to conduct the study.

2.3.1 Spore Bank

B. a. Sterne spores were used as the biological test agent for the entire study. This organism is a vaccine strain produced by Colorado Serum Company and is frequently used as surrogate to fully virulent *B. anthracis* strains such as Ames. The *B. a.* Sterne strain was handled as a Risk Group II agent following the Biosafety in Microbiological and Biomedical Laboratories and Battelle biosafety work practices for such agents. A spore bank was produced using sporulation broth as follows and used as needed for the duration of the study.

A cell bank of *B. a.* Sterne 34F2 prepared previously at Battelle from BEI Resources (BEI NR-1400) was used to grow an overnight culture on Tryptic Soy Agar. Isolated colonies were then used to inoculate 50 milliliter (mL) aliquots of nutrient broth and incubated overnight at 35 to 37 degrees Celsius (°C) with shaking at 200 revolutions per minute (rpm). Modified G (ModG) (500 mL) sporulation broth (see Appendix A, Table 1 for formulation details) was inoculated with 50 mL of the overnight *B. a.* Sterne culture, and then incubated in a 3-liter (L) Fernbach flask at 35 to 37°C with shaking at 200 rpm. The culture was observed via wet mount microscopy every 1 to 3 days for sporulation. Following 5 days of incubation, the ModG culture reached > 99% sporulation. (Note, a spore bank was also prepared using Leighton-Doi medium [see Appendix A, Table 2 for formulation details], but spores from the ModG medium were used because > 99% sporulation was not achieved with Leighton-Doi, and there was more cellular debris compared to spores prepared in the ModG medium.)

The sporulated culture was centrifuged at 10,000 relative centrifugal force (rcf) at 4°C for 12 minutes in multiple 250-mL bottles. After removing and discarding the supernatant, the resulting pellets were resuspended to a total volume of approximately 100 mL with sterile distilled water (dH_2O), transferred into a sterile glass vessel, and heat shocked at 60 to 65°C for 1 hour in a water bath with gentle agitation. (Note: A control flask with a thermometer was used to ensure the desired temperature was achieved and maintained during the heat-shock step.) The spores were then washed twice by repeated centrifugations at 10,000 rcf at 4°C for 12 minutes using 100 mL dH_2O per wash. After the final centrifugation, the spores were resuspended to a total volume of 100 mL in sterile dH_2O. The spore bank was assigned a unique lot number and stored refrigerated at 2 to 8°C.

2.3.2 Spore Loading (Spiking)

PM2.5 filters were received as circular 47-mm swatches and were quartered prior to spiking; PM10 and all non-AQ filter types were cut to 4-cm^2 swatches. Note that the EPA protocol (EPA, 2012) was originally developed for processing of 37-mm vacuum filter cassette samples, thus the area of filter sample analyzing the AQ and non-AQ filters was about 2.5 times less than that of the 37-mm filters. All filter manipulations were performed within a surface-decontaminated, certified biological safety cabinet (BSC) and handled using sterile forceps and scissors.

B. a. Sterne spiking stocks were vortex-mixed and diluted using sterile dH_2O to the three spiking stock target concentrations shown in Table 3. Each spiking stock was spread plated onto SBA on the day of testing to calculate the actual concentration of spores spiked in colony forming units (CFU)/mL. The loading levels in Table 3 represent loadings that yielded enough *B. a.* Sterne spores in the sample recovery extracts to make meaningful measurements with both the culture and RV-PCR methods and covered a range that was expected to span their lower limit of detection or quantification, which was an important consideration to assess whether grime or flora associated with the filter samples affected the sensitivity or lower limits of the analytical method.

Table 3. Target *B. a.* Sterne Spore Loading Levels onto Each Filter Substrate

Loading Level	Stock Concentration (CFU/mL)	Target Total CFU per Filter[(a)]	Extract Volume (mL)	Theoretical Concentration in Extract (CFU/mL)
High	3.0×10^4	3,000	25	120
Medium	3.0×10^3	300	25	12
Low	3.0×10^2	30	25	1.2

(a) 100 µL of stock suspension applied (20, 5-µL drops).

Each swatch to be spiked with *B. a.* Sterne spores was transferred to a Petri dish and 20 5-µL droplets were pipetted onto the surface of each filter swatch (see Figure 3) for a total of 100 µL of stock suspension applied. Negative control swatches that were included in each batch were transferred directly into sealed 50-mL conical tubes prior to spiking swatches with *B. a.* Sterne. The spiked swatches then dried overnight inside of a BSC.

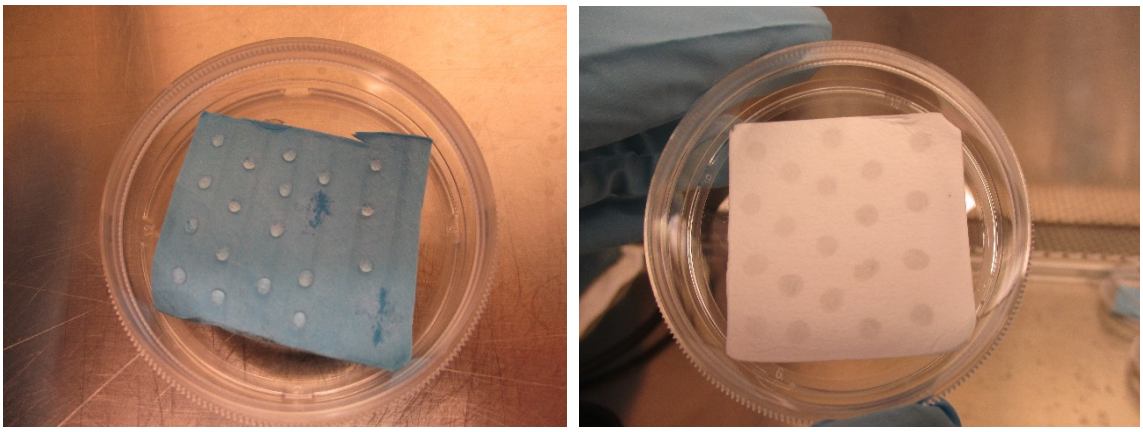

Figure 3. Photographs of Metro Bus Engine Filter (left) and PM10 Air Quality Filter (right) After Spiking with the *B. a.* Sterne Suspension

2.3.3 Spore Recovery

Throughout the recovery procedure, gloves were changed between handling samples to limit the likelihood of cross-contamination between samples.

Following spiking and drying, the filter swatches were placed into a 50-mL tube and a mesh support was placed over the filter swatch. Fifteen (15) mL of cold (4°C) extraction buffer with Tween® 20 (0.22 µm polyethersulfone (PES) filter sterilized 700 mL 1X phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.4, and 300 mL 200 proof ethanol) was added to each sample and the lids were sealed using Parafilm. Samples were vortexed for 20 minutes on a platform vortex set to speed 7 (VWR, Cat. 945057). After vortexing on a single-tube vortex for 3 to 5 seconds each, the samples sat for 2 minutes to enable large particles to settle prior to transferring ~12.5 mL of the suspension into a corresponding labeled 50-ml conical collection tube. A second spore extraction was then completed by addition of 10 mL of cold (4°C) extraction buffer without Tween 20 (0.22 µm PES filter sterilized 700 mL 1X PBS, pH 7.4 and 300 mL 200 proof ethanol) to each sample tube and the lids were sealed using Parafilm. Samples were vortexed for 10 minutes on a platform vortex set to speed 7. After vortexing on a single-tube vortex for 3 to 5 seconds, the samples sat for 2 minutes to enable large particles to settle prior to transferring the remaining ~12.5 mL of the suspension into the corresponding labeled 50-ml conical collection tube. After vortex mixing, 10.5 mL of the recovered suspension aliquot was transferred into a labeled 15-ml conical tube to be used for the culture-based microbial analysis described in Section 2.3.4, and the remaining volume (nominally ~12.5 ml) was transferred into a labeled filter vial for RV-PCR analysis as described in Section 2.3.5.

2.3.4 Culture Method

Culture-based microbiological analysis was performed on each sample by filtering the recovered extract through filter funnels and filter media (Pall, Cat. 4804) then placing the filters onto solid bacterial growth media and incubating. Serial dilution and spread-plating procedures, as prescribed by the full EPA *B. anthracis* method (EPA, 2012), were not performed since the spike levels were at/near the detection limit for the assay (i.e., spread-plating 0.1 mL of the undiluted extract from a sample spiked with 3,000 spores would have resulted in 12 CFU if 100% efficient). Accordingly, milliliter volumes of the recovered extract were captured onto MicroFunnel™ filters in the current study.

Initially, each MicroFunnel filter was pre-wetted with 5 mL of PBS with 0.05% Tween (PBST), then 10 mL of PBST was added to each MicroFunnel filter to suspend 1 mL or 4 mL of the sample extract followed by vacuum filtration. The walls of each filter funnel were rinsed with 10 mL of PBST and filtered through the MicroFunnel, then the filter membrane was removed and placed onto MYP, BBCA, and/or SBA media. (As shown in Table 1 and Table 2, MYP and BBCA were used in a subset of the sample conditions in the test matrix and SBA was the only culture medium carried throughout all test conditions.)

For the culture method, colonies with a typical *B. a.* Sterne morphology following overnight incubation at 35 to 37°C were counted to determine percent spore recovery. Typical *B. a.* Sterne morphology on SBA are 2 to 5 mm in diameter, flat or slightly convex with edges that are irregular, have a ground-glass appearance, and are not ß-hemolytic. When *B. a.* Sterne grows on MYP, the expected colony color is pink, and when grown on BBCA, the expected colony color is turquoise green. Figure 4 shows representative images of *B. a.* Sterne colonies on each culture medium used in this study.

Two different microbiologists enumerated colonies over the course of the project, all of whom were trained by the lead microbiologist on the project to most consistently identify presumptive *B. a.* Sterne based on colony morphology. The lead microbiologist periodically reviewed the enumeration results to help ensure consistency and integrity, which is an important consideration and factor in the application of the method because the culture analysis was subjective to the assessment of colony morphology. The samples were not blinded for analysis. The microbiologists were aware of which samples should and should not contain *B. a.* Sterne.

Nonetheless, as the results demonstrated, there were instances of the presence of presumptive *B. a.* Sterne on samples that should not have any (false positive) and no colonies where there should have been (false negatives).

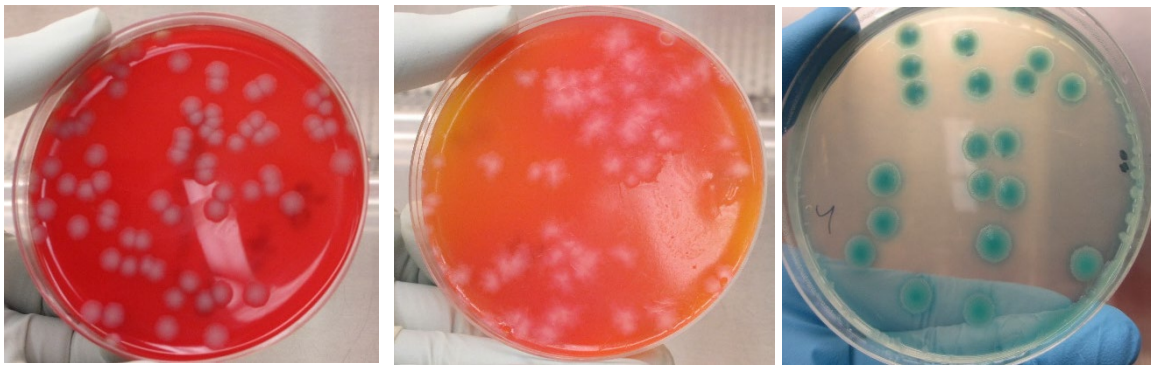

Figure 4. From left to right: *B. a.* Sterne on SBA, MYP, and BBCA

2.3.5 RV-PCR Method

Positive Control Preparation

Genomic deoxyribonucleic acid (DNA) of *B. a.* Sterne was extracted for use as a positive control for RV-PCR-based analysis. The *B. a.* Sterne vegetative cell culture that DNA was extracted from originated from the spore stock used for spike/recovery tests. The Wizard® Genomic DNA Kit (Promega, Madison, WI) was used following an internal Battelle method specific for extracting *B. anthracis* DNA. The resulting DNA was quantified by Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, Cat. P11496). The purified DNA was assigned a unique lot number, dispensed as multiple aliquots, stored frozen at ≤ -20°C, and used as needed as the positive control for PCR analysis.

Sample Processing (per EPA Method 2017, Second Edition (Shah, 2017))

Following filtration of ~12.5 mL of recovered extract through the Whatman™ Autovial™ filter vials (with polyvinyldiene difluoride [PVDF] membrane; Whatman Cat. AV125NPUAQU or Polyethersulfone [PES] membrane; Whatman Cat. AV125NPUPSU), two buffer washes were performed—the first wash was 12.5 mL of cold (4°C) high salt buffer (10X PBS) followed by 12.5 mL of cold (4 °C) low salt wash buffer (1X PBS). The top portion of the manifold was then removed and placed into a capping tray with pre-filled luer lock caps to seal the filter vials. Cold (4°C) Brain Heart Infusion Broth (BHIB) (5 mL) was then added to each filter vial, the vials

were capped, and then vortex-mixed for 10 minutes on a setting of 7. Images of the manifold and capping tray are depicted in Figure 5. Following the vortex step, the broth was mixed by pipetting up and down ~10 times and a 1-mL aliquot was transferred to a screw cap tube and stored at -20°C as the time zero (T_0) aliquot. The capped filter vials were then incubated overnight (~16 hours) in an incubator shaker set to 37 ± 1°C at 230 rpm.

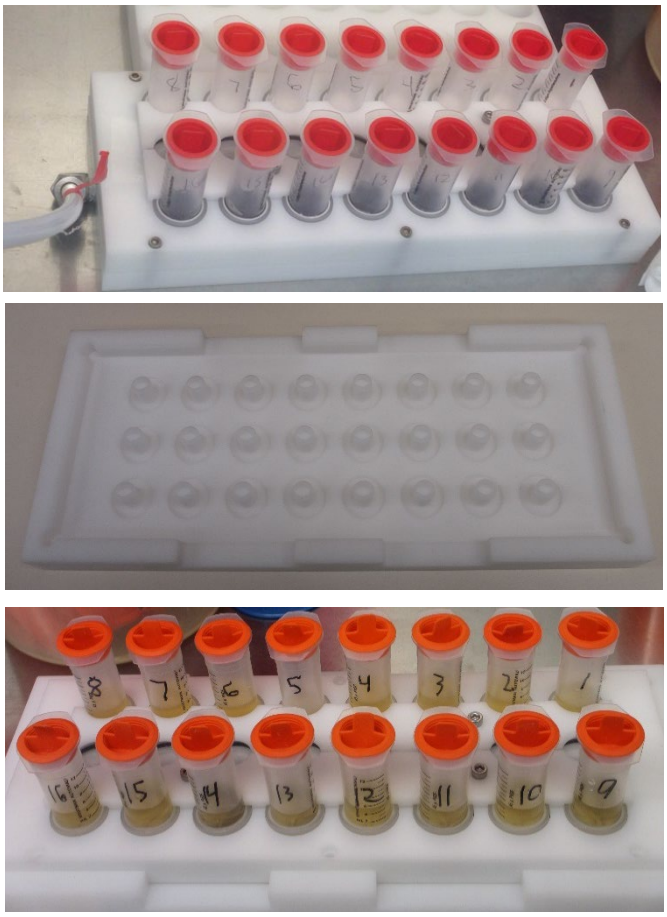

Figure 5. Top: Manifold Containing 16 Filter Vials; Middle: Capping Tray; Bottom: Capped Filter Vials Containing BHIB

Following overnight incubation of the filter vials with BHIB, the vials were mixed on the platform vortex for 10 minutes with speed set to 7. (The 16-hour incubation allowed for a standard work schedule to be maintained rather than require an overnight shift that would have been required by a 9-hour incubation.) The culture suspension was mixed by pipetting up and down ~10 times, and a 1-mL aliquot was transferred to screw cap tubes and labeled as the final time (T_{final}) aliquot.

DNA Extraction and Purification

Prior to extraction of DNA, the lysis buffer with anti-foam reagent, and the alcohol wash was added according to the manufacturer's instructions in the Magnesil Blood Genomic, Max Yield System, Kit (Promega, Cat. MD1360) and a heat block was pre-heated to 80°C. All screw capped 1-mL aliquots were thawed and centrifuged at 14,000 rpm for 10 minutes (4°C), and 800 µL of the supernatant from each tube was removed and discarded. To extract the DNA, 800 µL of lysis buffer was added to each tube and the pellets in remaining 200 µL were mixed by vortexing on high (~1,800 rpm) in 10-second pulses for a total of 60 seconds. Each tube was then vortex-mixed for 10 seconds at low speed directly before the lysate was transferred to a 2-mL labeled Eppendorf tube. The lysate tube was then incubated at room temperature for 5 minutes. Uniformly resuspended paramagnetic particles (PMPs) (600 µL) were added to each lysate tube and the samples were mixed by vortexing. After vortexing each T_0 and T_{final} tube for 10 seconds (high, ~1,800 rpm), the samples were incubated at room temperature for 5 minutes.

The samples were then placed on the magnetic stand with the hinged-side of the tube facing toward the magnet after briefly resuspending the particles by vortexing. The magnetic rack was then inverted to ensure all PMPs contacted the magnet. After 10 seconds, the tubes were opened, and the liquid removed without disturbing the PMPs. Lysis buffer (360 µL) was then added to each T_0 and T_{final} tube, capped, and vortexed for 10 seconds. The tubes were then placed on the magnetic stand and inverted again. The supernatant was then removed and 360 µL of salt wash solution was added to each tube. The tubes were capped and vortexed for 10 seconds, placed on the magnetic stand, and inverted. The supernatant was removed without disturbing the PMP pellet. The pelleted PMPs were washed a second time with 360 µL of salt wash solution.

After removal of the second salt wash supernatant, 500 µL of alcohol wash was added to each tube. The tubes were vortexed for 10 seconds, placed on the magnetic stand, and inverted. The supernatant was then removed, and two more alcohol washes were conducted for a total of three 500-µL alcohol washes. A fourth alcohol wash was then conducted using 500 µL of 70% ethanol. After the supernatant from the 70% ethanol wash was removed, all tubes were opened and allowed to air dry for 2 minutes. The open tubes were then heated at 80°C in a heat block inside a BSC until the PMPs were dry (~20 minutes). DNA was then eluted from the PMPs by the addition of 200 µL of elution buffer to each T_0 and T_{final} tube. The tubes were then closed, vortexed for 10 seconds, and incubated in the heat block for 80 seconds. The tubes were then

vortexed another 10 seconds and incubated in the heating block for 1 minute. The vortexing and heating for 1 minute was repeated four more times for a total of five times. The tubes were then removed from the heating block and incubated at room temperature for at least 5 minutes. Each tube was briefly vortexed and then centrifuged at 2,000 rpm at 4ºC for 1 minute. The tubes were then vortexed and placed on the magnetic stand for at least 30 seconds. The elute was collected (~80 to 90 µL) and transferred to clean, labeled, 1.5-mL tubes on a cold block. The tubes were centrifuged at 14,000 rpm at 4°C for 5 minutes to pellet any particles remaining with the eluted DNA. The supernatant was carefully removed and transferred to a new 1.5-mL tube using a new tip for each tube. The T_0 and T_{final} DNA extracts were stored at 4ºC until RV-PCR analysis or at -20ºC if RV-PCR could not be performed within 24 hours.

RV-PCR Assay

The EPA protocol originally provided (EPA, 2012) uses singleplex, real-time PCR assays for *B. anthracis* detection and quantification. Battelle assessed the feasibility to combine two singleplex assays targeting the chromosome and pXO1 assays described in the EPA protocol into a duplex assay to reduce analysis time and cost associated with filter extract analysis. It was demonstrated that the RV-PCR performance was unchanged when conducted using the duplex assay in a single analysis or using the singleplex assays in two independent analyses. Consequently, it was agreed to use the duplex assay method for this study. A summary report of assessment with details of the approach and supporting results is provided in Appendix B.

The duplex TaqMan® real-time PCR assay utilized FAM and VIC reporter dyes for detection of two *B. a.* Sterne DNA sequence targets simultaneously in a single reaction. (FAM and VIC are Applied Biosystems trademark fluorescent reporter dyes on 5' end of PCR probe that emit at ~517 nm and ~551 nm, respectively.) The two assays target sequences on the *B. anthracis* chromosome and pXO1 plasmid and were previously described as singleplex, real-time PCR assays (Letant et al., 2011). The duplex PCR assay Master Mix was prepared using the conditions provided in Appendix A. Each sample DNA extract was assayed in triplicate reactions. Controls consisted of four positive control wells containing 50 pg of DNA extracted from *B. a.* Sterne 34F2 (NR-1400, BEI Resources) and four no template controls were also included with each assay. Applied Biosystems 7500 Fast Real-Time PCR Instrument was used for PCR assay development and testing. Thermocycler conditions with a fast ramp rate were:

- Stage 1: 1 cycle at 95°C for 20 seconds
- Stage 2: 45 cycles at 95°C for 3 seconds followed by 60°C for 30 seconds

Note, the Stage 1 cycle conditions were slightly modified from the original EPA method, and concurrence to proceed with the revisions was provided by EPA (Shah, 2018).

2.4 Method Implementation

The primary microbial methods used to spike/recover/analyze the NAFs, shown as they occurred in chronological order, are depicted graphically in the process flow diagram of Figure 6.

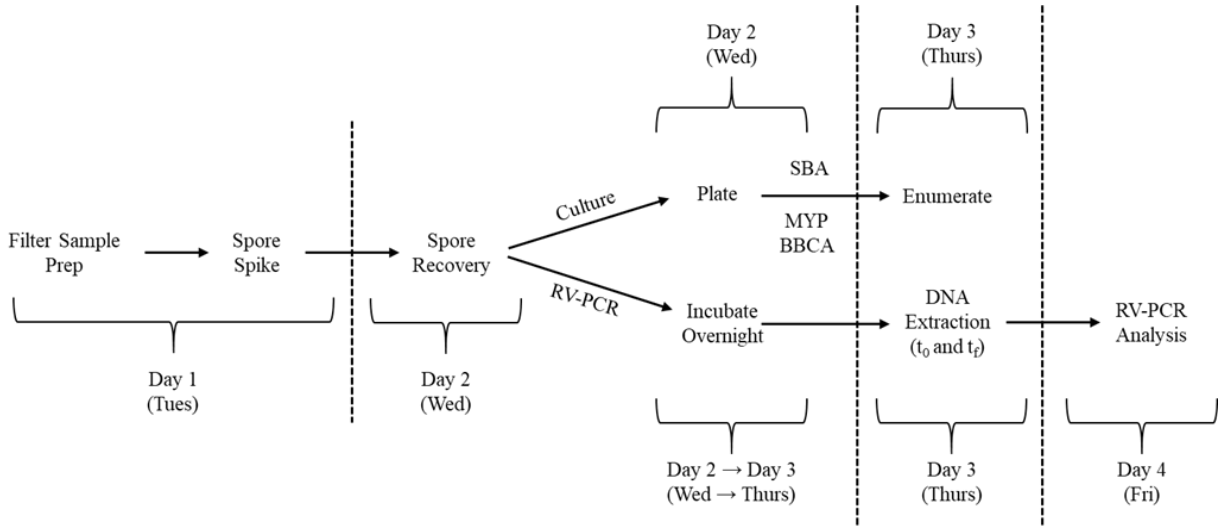

Figure 6. Process Flow Chart Depicting Key Method Process Steps in Chronological Order

The methods implemented, in the form of work instructions followed by the analytical staff, are provided in Appendices C through H. These work instructions also complement those microbiological methods described in Section 2.3, and emphasize glove-changing schedules that were implemented to minimize cross-contamination. The work instructions were reviewed in detail to refine and ensure proper implementation of the methods (Calfee, 2017).

The above method was used to analyze 16 filter samples per trial, with 1 trial conducted per week. For each weekly test, filters were cut into swatches and spiked using *B. a.* Sterne spores suspended in water. Each test consisted of swatches loaded with 0, 30, 300, or 3,000 spores per filter swatch per "Work Instruction for Dosing Filter Swatches with *Bacillus anthracis* Spores" in Appendix C. The spiked filters were dried overnight before spores being recovered following the "Work Instruction for *Bacillus anthracis* Spore Recovery," as detailed in Appendix D. The

recovered suspension volume was then split between the traditional culture method (10.5 mL) and RV-PCR method (12.5 mL) analyses. The aliquot for culture was divided into 1-mL or 4-mL volumes and filter-plated onto media and incubated overnight as outlined in the "Work Instruction for Culture of *Bacillus anthracis* Spores Recovered from Air Filters" in Appendix E. The T_0 RV-PCR aliquot was stored frozen while the recovered spores enriched overnight, then the T_{final} aliquot was removed and the DNA was extracted from both T_0 and T_{final} aliquots per "Work Instruction for Manual DNA Extraction and Purification from *Bacillus anthracis"* in Appendix F. The extracted DNA was then analyzed using a duplex, real-time PCR assay targeting the chromosome and pXO1 of *B. anthracis* per "Work Instruction for RV-PCR for *Bacillus anthracis* Spores" in Appendix G. PCR was also used to confirm or refute presumptive *B. a.* Sterne colonies selected from the culture analysis per "Work Instruction for Selecting Presumptive *B. a.* Sterne Colonies for quantitative PCR (qPCR) Confirmation" in Appendix H.

2.5 Data Reduction and Analysis

2.5.1 Culture – Percent Recovery

The percent recovery efficiency ($E_{recovery}$) of *B. a.* Sterne from each spiked filter sample was calculated by dividing the number of presumptive *B. a.* Sterne CFUs recovered ($N_{recover}$) from the filter by the actual number of *B. a.* Sterne spores spiked (N_{spike}) onto the filter (determined from the stock suspension titer for each test) and multiplying by 100. $N_{recover}$ is a product of the presumptive *B. a.* Sterne spore concentration ($C_{recover}$) (CFU/mL) and the total volume of extract used to recover the spores ($V_{extract}$) (mL). Mathematically, the percent recovery is expressed as follows:

$$E_{recovery}(\%) = \frac{C_{recover} * V_{extract}}{N_{spike}} * 100\%$$

The number of presumptive *B. a.* Sterne spores present in the volume of extract collected onto the MicroFunnel filter membrane was divided by the extract volume analyzed (either 1 mL or 4 mL) to yield a presumptive *B. a.* Sterne spore concentration ($C_{recover}$) (CFU/mL) that was then multiplied by the extract volume ($V_{extract}$) (25 mL) to determine the total presumptive *B. a.* Sterne CFUs recovered from the filter sample. The reported percent recovery was determined using the below rules:

1) Report the percent recovery from the aliquot (1-mL or 4-mL) that has between 20 to 80 CFU.

2) Report the 4-mL aliquot percent recovery if the CFU counted from both the 1-mL and 4-mL aliquots is less than 20.

3) Report the 4-mL aliquot percent recovery if the CFU counted from both the 1-mL and 4-mL aliquots is between 20 and 80.

4) Report the 1-mL aliquot percent recovery if the background flora on the 4-mL aliquot produces numerous colonies or a lawn of growth, thus complicating the identification of *B. a.* Sterne colonies.

The number of CFUs are reported as presumptive *B. a.* Sterne colonies. PCR analysis of presumptive colonies was required to positively confirm the presence of *B. a.* Sterne. A portion of the presumptive colony was collected into 100 µL of PCR-grade water in microcentrifuge tubes. The colony suspension was then heated for 5 minutes on a heat block at 95°C. The lysate was cooled and then centrifuged at 14,000 rpm for 2 minutes and the supernatant was analyzed using the real-time PCR assays targeting the *B. anthracis* chromosome and pXO1 gene targets.

2.5.2 RV-PCR

The cycle threshold (Ct) values for the T_0 and T_{final} timepoints as well as the delta Ct value (ΔCt) were reported. The ΔCt is generated by subtracting the average Ct (from triplicate reactions) generated by the T_{final} aliquot from the average Ct (from triplicate reactions) value generated by the T_0 aliquot. A positive ΔCt (≥ 9) value indicates that viable *B. a.* Sterne spores were recovered from the filter. For a sample to be considered positive, the below acceptance criterion had to be met:

- The ΔCt must be greater than or equal to 9 for both the chromosome and pXO1 targets ($\Delta Ct = Ct(T_0) - Ct(T_{final}) \geq 9$).

Additional criteria exist for the positive confirmation of a sample if analyzing samples obtained from an actual incident, but for this study the above criterion was used.

2.5.3 Presentation of Results

The method employed to recover *B. a.* Sterne spores spiked onto the NAFs was consistent with current EPA methods, as described in Section 2.3.4. The entire extract would be analyzed either using a culture method or a RV-PCR method, solely, in actual practice and application by EPA if analyzing samples collected after a biological release incident (Calfee, 2018). In the study

performed and reported here, however, the sample extract was split as described in Sections 2.3.4 and 2.3.5, so that approximately half of the extract sample was used for culture analysis and the other half for RV-PCR analysis. In this way, results from both methods could be compared in a pair-wise manner. Consequently, neither the culture nor the RV-PCR had the potential maximum quantity (assuming 100% recovery efficiency from the filter) of spores available in the extract for analysis. Rather, each split extract sample had a maximum of nominally half the actual spiked spore quantity available for their respective analyses. Therefore, in the presentation of results in tables and figures, unless explicitly noted otherwise, column headers or axes labels denote the nominal maximum number of spores available in the sample for its respective analysis, which was half of the target spore load.

For example, results were presented in plots of both spore recovery efficiency for the culture analyses and of ΔCt for RV-PCR analyses with an x-axis title of "Nominal Spores Available for Analysis (CFU)" with an x-axis label of 0, 15, 150, and 1,500. This convention of presenting the results was considered the most accurate and consistent representation and allowed for the most unambiguous discussion and interpretation of results across all the filter types and analytical methods, recognizing that the filters were originally spiked with target quantities of 0, 30, 300, and 3,000 *B. a.* Sterne spores.

As described in Section 2.3.2, the NAFs were spiked with a target quantity of spores by applying twenty (20) 5-µL drops of a *B. a.* Sterne spore stock suspension with a target titer of 30,000 spores/mL, diluted in log increments. The reported spore load for each filter analyzed was based on the *B. a.* Sterne spore suspension titer measured for each test trial in CFU. As expected, there was variability in the measured spore titer for each trial. Consequently, the summary tables of results also contain the average (± one standard deviation) of the measured or "determined" quantity of spores spiked onto the filter, which provides the reader with information other than the nominal spore load as defined in the test matrices to aide with interpretation of the results.

3.0 RESULTS AND DISCUSSION

A detailed discussion of the calculations and approach to presenting the results was provided in Section 2.5. In summary, all results presented in plots have an x-axis title and labels of 0, 15, 150, and 1,500, representing the nominal spores available for analysis (CFU). Similarly, the summary results in the tables contain the same nominal quantity of spores available, and also the determined quantity of spores applied to the filter. This convention of presenting the results was considered the most accurate and consistent representation and allowed for the most unambiguous discussion and interpretation of results across all the filter types and analytical methods, recognizing that the filters were originally spiked with target quantities of *B. a.* Sterne spores of 0, 30, 300, and 3,000, but extract samples were split in approximately equal volume for analysis by culture and RV-PCR methods.

Note that the spores available for analysis represent the maximum number of spores that could be available (assumes 100% recovery from the filter and no physical losses associated with processing of samples); it is not an absolute indication of the analytical method's limit of identification. Rather, it is a measure of the method's end-to-end performance to identify *B. a.* Sterne.

3.1 AQ Filter Analyses Results

3.1.1 Culture Method

A summary of the average and standard deviation of the measured recovery efficiencies of presumptive *B. a.* Sterne spores recovered from the AQ filter substrates spiked with *B. a.* Sterne and using the SBA medium are presented in Table 4 (PM2.5 filters) and Table 5 (PM10 filters). The determined number of spores available and the number of presumptive *B. a.* Sterne spores recovered are tabulated along with the nominal quantity of spores available for analysis (15, 150, and 1,500 CFU/filter sample). The quantity of presumptive *B. a.* Sterne colonies reported in the tables is half of the actual total recovered because in the context of the tables, only half of the extract samples was made available for analysis. The quantity of presumptive *B. a.* Sterne colonies for each filter sample, used in the percent recovery calculations, are reported in Appendix I for the culture method using the SBA medium. When either the MYP chromogenic agar or the BBCA growth medium was also used in the culture method, the recovery efficiencies are reported in Appendices J and K, respectively.

Table 4. Recovery Efficiencies for Presumptive *B. a.* Sterne Spores from PM2.5 Air Quality Filters Cultured in the SBA Medium

Location	Ambient Particle Load[a] (µg/cm²)	Sample Reps	Spores Available for Analysis (CFU)		Spores Recovered (CFU) (X ± σ)[d]	Spore Recovery Efficiency (%) (X ± σ)[e]
			Nominal[b]	Determined (X ± σ)[c]		
-- (New)	(0)	3	0	0	0	N/A
		3	15	18 ± 9	6.3 ± 0.0	46 ± 32
		3	150	180 ± 90	74 ± 46	44 ± 15
		3	1,500	1,800 ± 900	430 ± 40	33 ± 26
AZ	Avg (16)	3	0	0	1.0 ± 1.8	N/A
		3	15	18 ± 9	17 ± 4.8	120 ± 80
		3	150	180 ± 90)	91 ± 44	52 ± 3.4
		3	1,500	1,800 ± 900	720 ± 230	46 ± 16
	High (39)	3	0	0	2.1 ± 3.6	N/A
		3	15	7 ± 0	3.1 ± 3.1	42 ± 42
		3	150	70 ± 3	52 ± 15	72 ± 23
		3	1,500	700 ± 30	540 ± 150	74 ± 19
FL	Avg (13)	3	0	0	0	N/A
		3	15	15 ± 9	12 ± 9.5	71 ± 34
		3	150	150 ± 90	77 ± 21	59 ± 26
		3	1,500	1,500 ± 900	980 ± 160	75 ± 25
	High (39)	3	0	0	0	N/A
		3	15	15 ± 9	14 ± 7.9	110 ± 94
		3	150	150 ± 90	110 ± 24	81 ± 25
		3	1,500	1,500 ± 900	960 ± 82	75 ± 29
MA	Avg (12)	3	0	0	0	N/A
		3	15	14 ± 4	6.3 ± 5.4	51 ± 47
		3	150	140 ± 40	51 ± 34	36 ± 19
		3	1,500	1,400 ± 400	790 ± 210	57 ± 6.8
	High (29)	3	0	0	0	N/A
		3	15	14 ± 4	10 ± 1.8	76 ± 18
		3	150	140 ± 40	60 ± 40	39 ± 21
		3	1,500	1,400 ± 400	540 ± 400	34 ± 23
WI	Avg (14)	3	0	0	0	N/A
		3	15	11 ± 1	7.3 ± 4.8	67 ± 42
		3	150	110 ± 6	56 ± 27	54 ± 28
		3	1,500	1,100 ± 60	560 ± 220	53 ± 23
	High (47)	3	0	0	0	N/A
		3	15	11 ± 1	9.4 ± 0.0	88 ± 5
		3	150	110 ± 6	77 ± 10	73 ± 11
		3	1,500	1,100 ± 60	820 ± 36	77 ± 6.7

(a) Relative ambient particle load on the filter (with measured mass loading per area).

(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.

(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for culture analysis.

(d) Presumptive *B. a.* Sterne colonies based on morphology, and one-half of extract used for culture analysis.

(e) Calculated using the spore loading on each filter and presumptive *B. a.* Sterne spores recovered from each filter sample.

Table 5. Recovery Efficiencies for Presumptive *B. a.* Sterne Spores from PM10 Air Quality Filters Cultured in the SBA Medium

Location	Ambient Particle Load[(a)] (µg/cm²)	Sample Reps	Spores Available for Analysis (CFU)		Spores Recovered (CFU) (X ± σ)[(d)]	Spore Recovery Efficiency (%) (X ± σ)[(e)]
			Nominal[(b)]	Determined (X ± σ) [(c)]		
-- (New)	(0)	3	0	0	0	N/A
		3	15	6 ± 3	0.0 ± 0.0	0.0 ± 0.0
		3	150	60 ± 30	7.3 ± 4.8	15 ± 12
		3	1,500	700 ± 0	57 ± 15	8.8 ± 2.4
CA	Avg (118)	3	0	0	21 ± 3.6	N/A
		3	15	13 ± 5	18 ± 4.8	150 ± 29
		3	150	130 ± 50	14 ± 9.5	13 ± 10.2
		3	1,500	1,300 ± 500	68 ± 13	6.2 ± 3.6
	High (277)	3	0	0	9.4 ± 9.4	N/A
		3	15	13 ± 5	7.3 ± 4.8	55 ± 22
		3	150	130 ± 50	23 ± 6.5	22 ± 16
		3	1,500	1,300 ± 500	59 ± 8.3	5.1 ± 1.7
NH	Avg (69)	3	0	0	0	N/A
		3	15	14 ± 10	4.2 ± 7.2	17 ± 29
		3	150	140 ± 100	6.3 ± 6.3	6.0 ± 8.4
		3	1,500	1,400 ± 1000	83 ± 15	8.2 ± 4.8
	High (199)	3	0	0	0	N/A
		3	15	14 ± 10	0.0 ± 0.0	0.0 ± 0.0
		3	150	140 ± 100	7.3 ± 4.8	5.6 ± 2.0
		3	1,500	1,400 ± 1000	210 ± 130	24 ± 20
SC	Avg (65)	3	0	0	1.0 ± 1.8	N/A
		3	15	14 ± 1	4.2 ± 4.8	29 ± 33
		3	150	140 ± 6	14 ± 7.2	9.9 ± 5.5
		3	1,500	1,400 ± 60	84 ± 24	6.2 ± 2.0
	High (132)	3	0	0	3.1 ± 3.1	N/A
		3	15	14 ± 1	13 ± 3.1	91 ± 26
		3	150	140 ± 6	18 ± 7.2	13 ± 5.1
		3	1,500	1,400 ± 60	79 ± 49	5.8 ± 3.7
WI	Avg (41)	3	0	0	0	N/A
		3	15	9 ± 0	0.0 ± 0.0	0.0 ± 0.0
		3	150	90 ± 0	12 ± 6.5	13 ± 7.2
		3	1,500	900 ± 0	90 ± 71	10 ± 7.9
	High (104)	5	0	0	0	N/A
		4	15	6 ± 1	0.8 ± 1.6	12 ± 24
		3	150	60 ± 30	4.2 ± 7.2	9.3 ± 16
		3	1,500	900 ± 500	40 ± 9.5	5.2 ± 2.8

(a) Relative ambient particle load on the filter (with measured mass loading per area).

(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.

(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for culture analysis.

(d) Presumptive *B. a.* Sterne colonies based on morphology and one-half of extract used for culture analysis

(e) Calculated using the spore loading on each filter and presumptive *B. a.* Sterne spores recovered from each filter sample.

The presumptive *B. a.* Sterne recovery efficiencies on the SBA plates are plotted in Figures 7 through 14, one plot for each filter type. Note, a percent recovery is not tabulated or plotted for the 0-spore spike condition since, by definition, a meaningful recovery efficiency cannot be calculated, even though there could have been a finite number of presumptive *B. a.* Sterne colonies counted based on colony morphology. Nonetheless, there are instances when one or more colonies were counted as *B. a.* Sterne for the 0-spike condition, and the presumptive values are reported in Table 4 and Table 5.

Review of the percent *B. a.* Sterne spore recovery plots in Figures 7 through 10 for PM2.5 filters obtained from Arizona, Florida, Massachusetts, and Wisconsin, respectively, indicate that the percent recovery was lowest for the New filter material and generally higher for the Avg and High ambient particulate load filter load condition, which may be due to the applied spores adhering more strongly to the clean filter substrate than to the particulate matter present on the Avg or High ambient load filters and/or are physically removed with the particulate matter during recovery. The average percent recovery efficiencies were 35 to 45% for New media and the nominal *B. a.* Sterne spores available condition of 150 to 1,500; recovery efficiencies were typically 40 to 80% for the Avg and High ambient particles loads with the 150 and 1,500 available *B. a.* Sterne spores condition. The filters with a target of 15 *B. a.* Sterne spores generally had a higher standard deviation than the 150 and 1,500 nominal spores available condition, which was attributed to the relatively few (< 15) recovered presumptive *B. a.* Sterne colonies. In most filters analyzed (at least 90% of the filter samples spiked), colonies with a *B. a.* Sterne morphology were recovered from all samples.

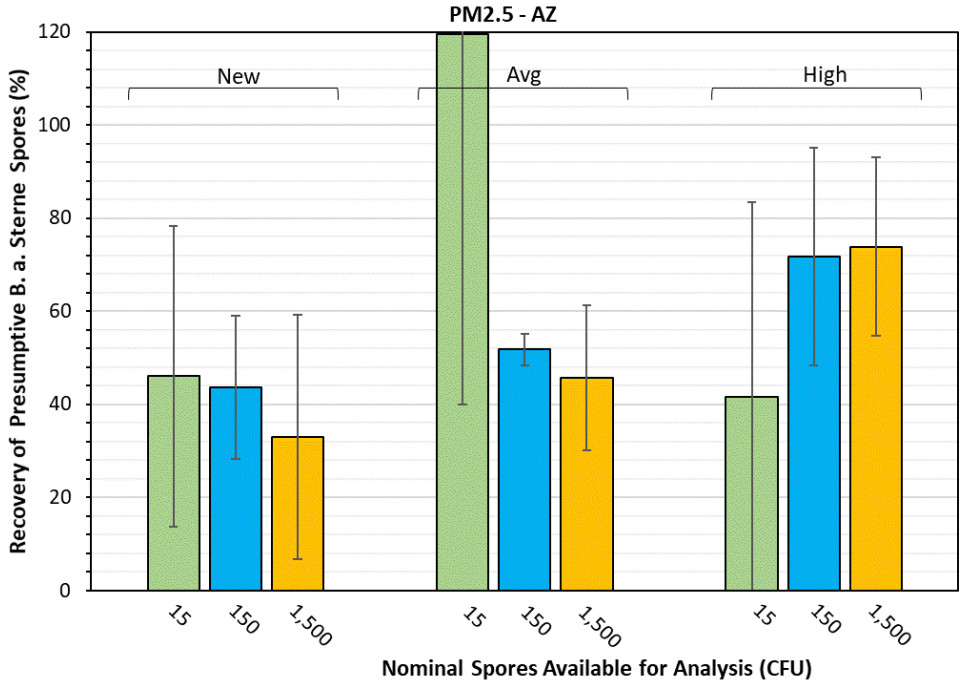

Figure 7. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM2.5 Filters from Arizona Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

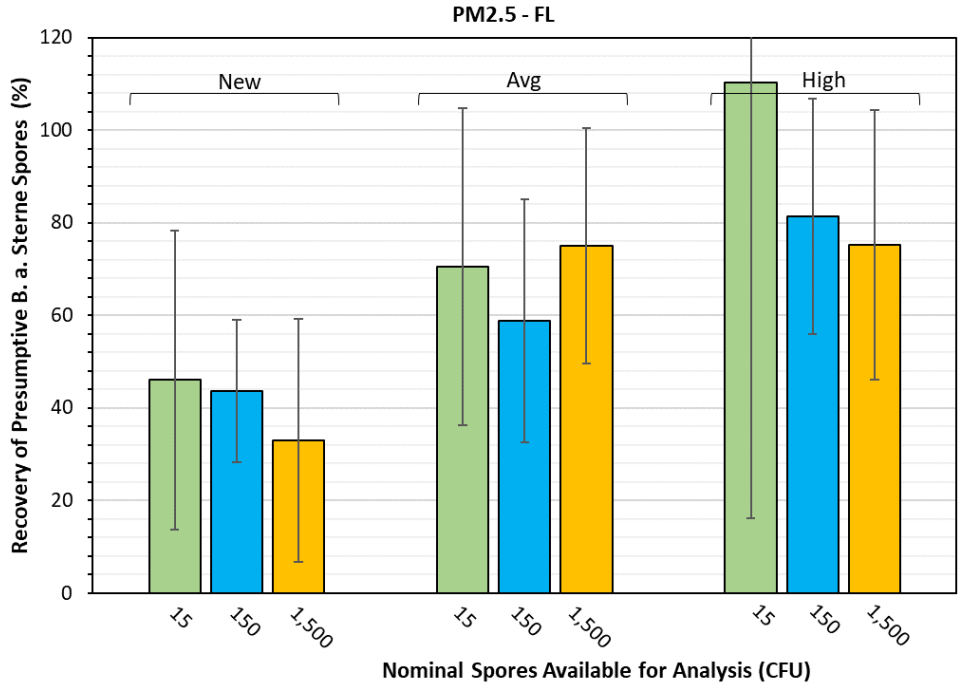

Figure 8. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM2.5 Filters from Florida Using the SBA Medium (New, Avg, and High refer to relative ambient particulate load)

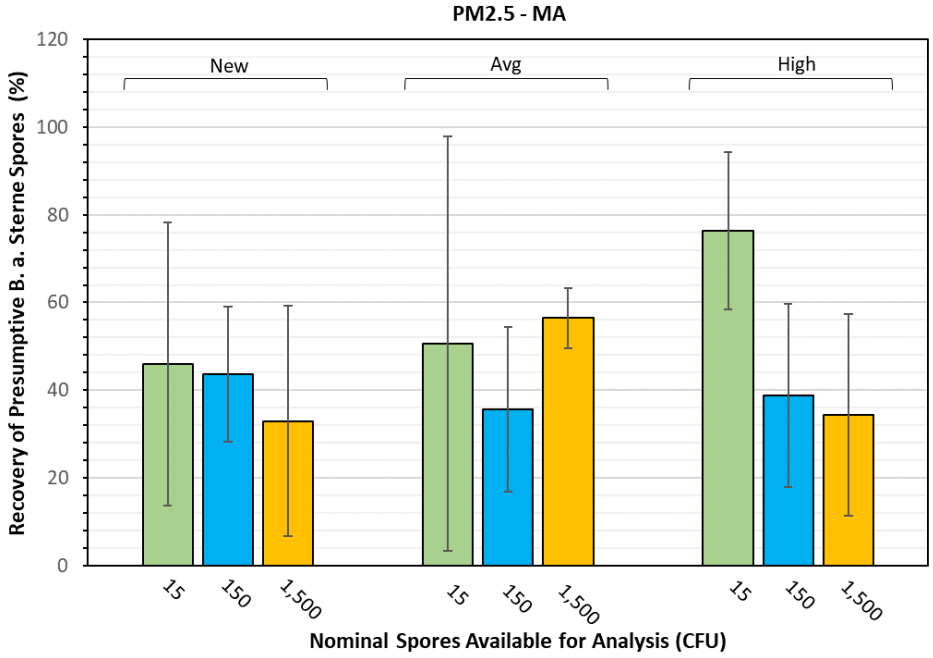

Figure 9. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM2.5 Filters from Massachusetts Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

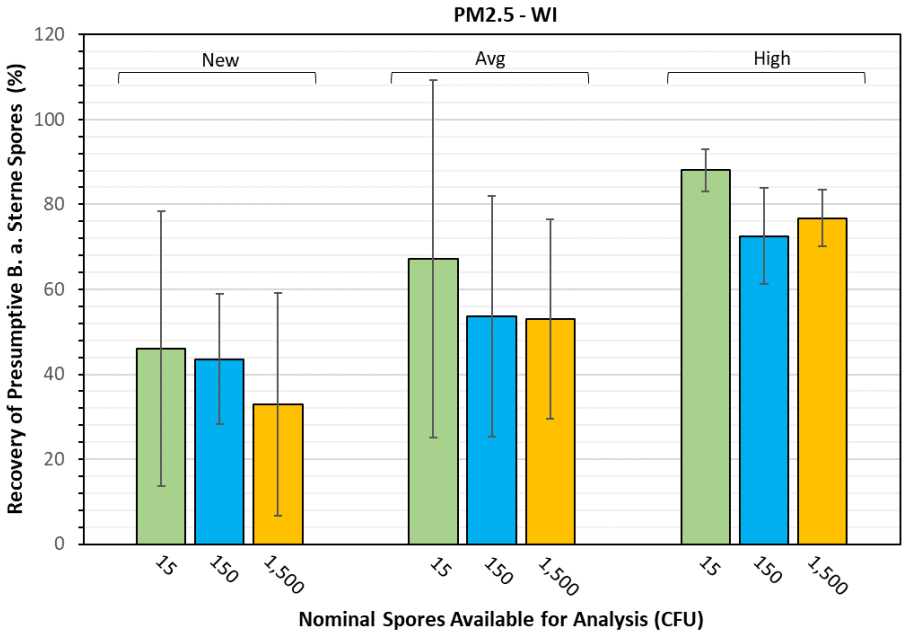

Figure 10. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM2.5 Filters from Wisconsin Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

Figures 11 through 14 include *B. a.* Sterne spore recovery efficiency plots for PM10 filters obtained from California, New Hampshire, South Carolina, and Wisconsin. They indicate generally low (5 to 20%) recovery efficiencies associated with the nominal 150 and 1,500 *B. a.* Sterne spores available. There were no presumptive *B. a.* Sterne spores recovered from the New PM10 filter substrate with a nominal 15 spores available for analysis. Additionally, the nominal 15-spores available condition for the Wisconsin, Avg filter and New Hampshire, High filter also yielded no quantifiable presumptive *B. a.* Sterne colonies. In those instances, the culture plates had other organism growth that was likely masking colonies of a *B. a.* Sterne morphology. Like the PM2.5 filters, the nominal 15 *B. a.* Sterne spore available filters had the greatest variability of measured percent recovery, attributed to so few (typically < 10 presumptive *B. a.* Sterne colonies) recovered from the spiked filter. When no presumptive *B. a.* Sterne colonies were recovered from spiked filters, it was noted as a false negative.

Filters obtained from California and South Carolina had instances of presumptive *B. a.* Sterne colonies counted for the 0-spores available condition, resulting in an overestimate of the number of true *B. a.* Sterne spores recovered. Figures 11 and 13 demonstrate that an over-estimation of recovered spores is most likely with the nominal 15-spore available condition. Specifically, the percent recovery of presumptive *B. a.* Sterne colonies from PM10 filters from California for Avg and High particle loads spiked with 30 spores (Figure 11) was 150 and 55%, respectively. The infeasibly high spore recovery efficiencies (> 100%) were attributed to presence of background flora on those filters with a colony morphology that was indistinguishable from *B. a.* Sterne, and thus counted as a presumptive *B. a.* Sterne colony.

Subsequent colony screening of presumptive *B. a.* Sterne colonies using real-time PCR assays targeting the chromosomal and pXO1 gene targets confirmed that there were instances where the presumed *B. a.* Sterne colony was shown not to be *B. a.* Sterne. Likewise, the South Carolina filters had background flora collected on the filters that had a colony morphology indistinguishable from *B. a.* Sterne but shown to not be *B. a.* Sterne by PCR analysis screening, and thus, artificially inflated the percent recovery values. These results demonstrate the importance of selected confirmation screening of presumptive *B. a.* Sterne when analyzing unknown samples from an actual incident. The impact of this background organisms/bacteria on percent recoveries is more apparent for the nominal 15-spore available condition because a few additional presumptive *B. a.* Sterne colonies greatly increases the calculated percent recovery.

For the 150 and 1,500 nominal spore level test conditions, the adverse impact of the background flora was diluted or suppressed because of greater competition from the *B. a.* Sterne spores.

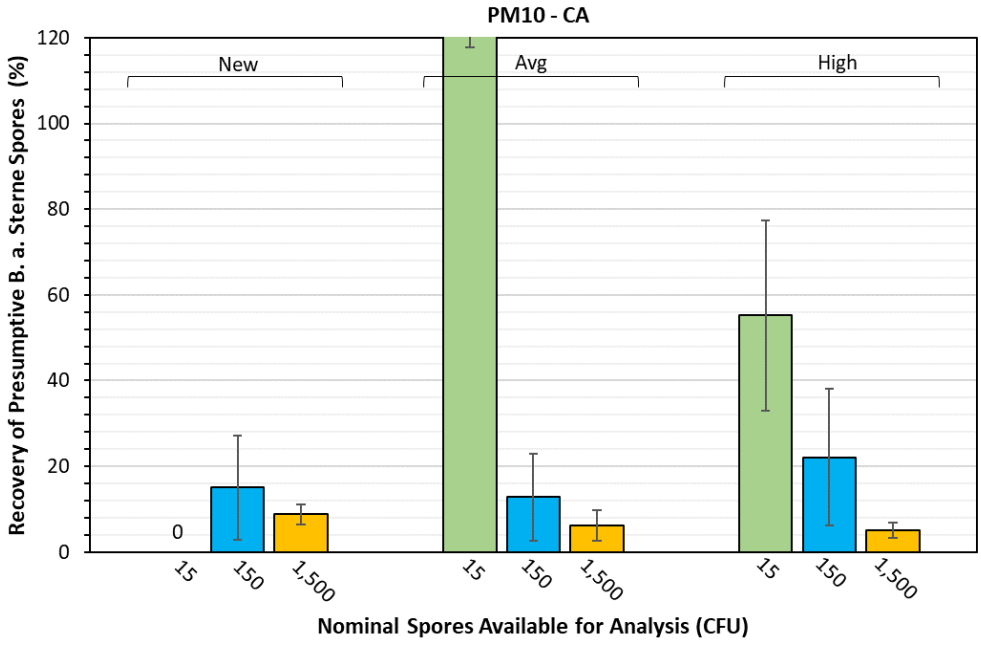

Figure 11. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM10 Filters from California Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

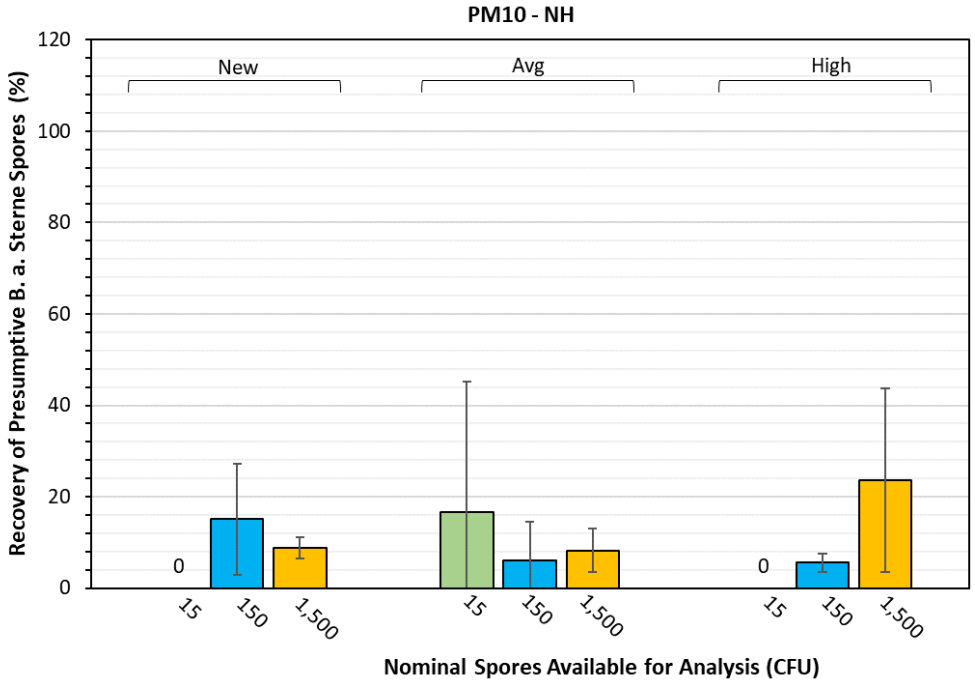

Figure 12. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM10 Filters from New Hampshire Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

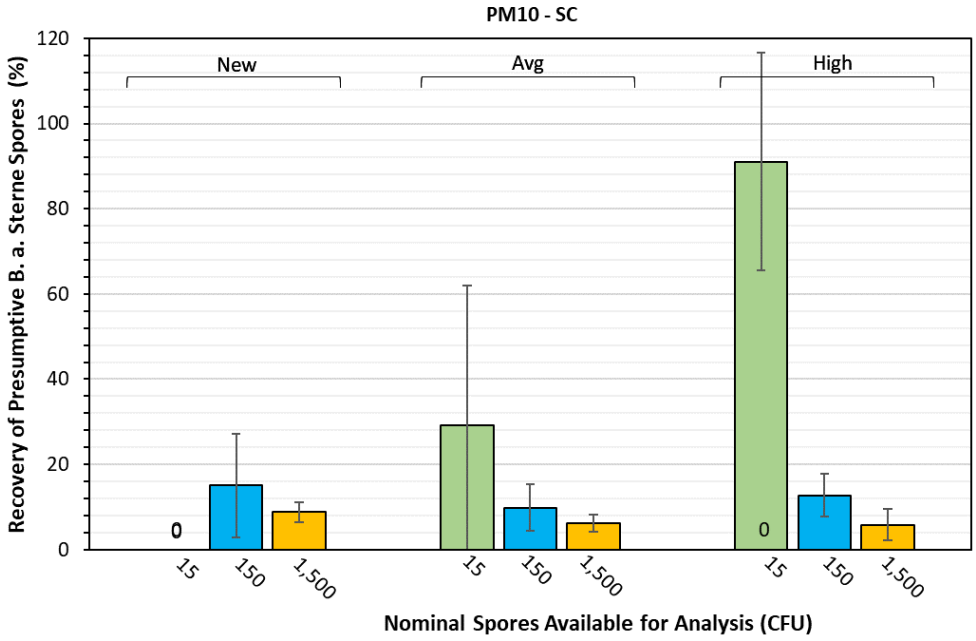

Figure 13 Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Three Replicates) of Presumptive *B. a.* Sterne Spores from PM10 Filters from South Carolina Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

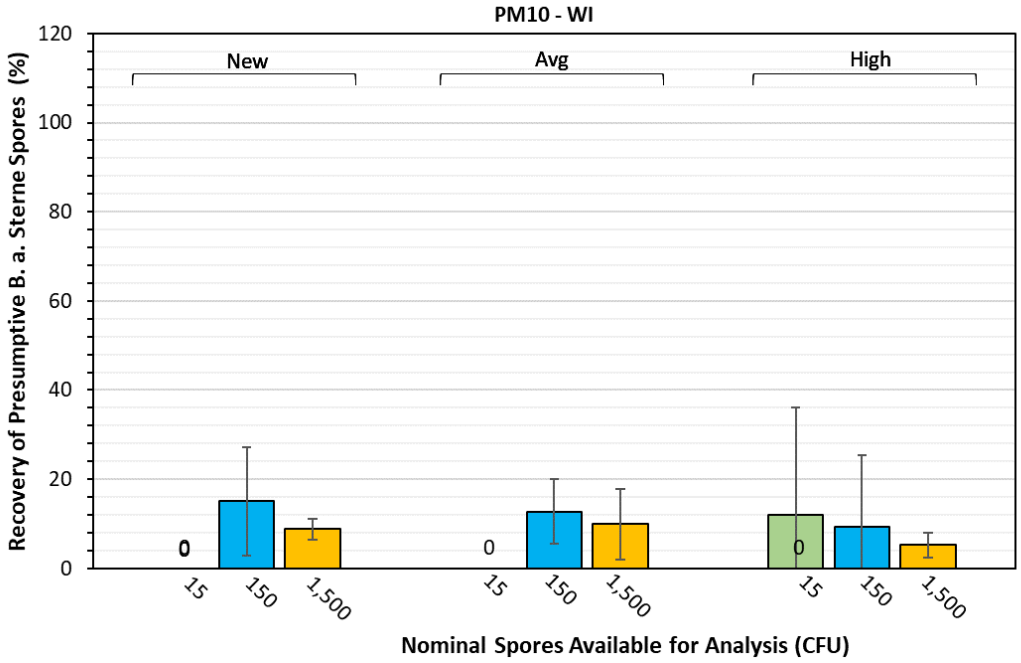

Figure 14 Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from PM10 Filters from Wisconsin Using the SBA Medium (New, Avg, and High refer to relative ambient particulate loads)

Average spore recovery efficiencies, for both PM2.5 and PM10 filter types, have the largest standard deviations associated with the spike condition resulting in nominally 15 spores available for analysis. This result is, in part, attributed to relatively few (< 10 colonies counted per plate) and/or the confounding effect of the presence of other colonies that have similar morphology that affect the estimated (presumptive) *B. a.* Sterne colonies. On average, with 100% recovery, the 4-mL aliquot plated would have five colonies to enumerate. Filters with the 150 and 1,500 nominal spore condition generally have a lower standard deviation as compared to the 15 nominal spore condition because more actual *B. a.* Sterne spores were recovered. As discussed in Section 2.3.4, the method for determining the number of *B. a.* Sterne spores recovered was determined based on colony morphology, and thus susceptible to biasing high due to non-*B. a.* Sterne organisms exhibiting an indistinguishable morphology to the microbiologist counting the colonies.

The fact that the recovery efficiency of the 15-spore available condition for the New media was the lowest compared to the filters with an ambient particulate load could also indicate that the applied *B. a.* Sterne spores were not as efficiently physically recovered from the filter substrate.

The existence of the ambient particulate matter of grime or flora on the filter substrate may reduce the adherence of spiked *B. a.* Sterne spores to the filter substrate.

The higher recovery efficiency of *B. a.* Sterne from PM2.5 filters compared to the PM10 filters is likely attributed to the spiked *B. a.* Sterne spores being adhered less strongly to the PM2.5 filter substrate than to that of the PM10 filter substrate. The PM2.5 filters were Teflon (MTL Corp Cat No. PT47-EP) and PM10 filters quartz fiber (Whatman Cat No. 1851-8531). One possible explanation is that the applied spores could distribute into the fiber matrix of the PM10 filter fibers and thus have an opportunity to contact individual fibers as a spore, whereas in the Teflon filter substrate, such distribution may not occur. The applied spores may remain present as agglomerates on the surface of the Teflon substrate and more readily removed. Assessing recovery mechanisms were beyond the scope of this study.

The lower recovery efficiencies of *B. a.* Sterne from PM10 filters with collected particulate matter may be attributed to more flora/grime compared to the PM2.5 filters, which may interfere with accurate quantification of *B. a.* Sterne. The ambient particulate loading on the Avg PM2.5 filter ranged from 12 to 16 $\mu g/cm^2$ compared to 41 to 118 $\mu g/cm^2$ for the PM10. Similarly, the High PM10 filters had an ambient particulate load 3 to 6 times that of the High PM2.5 filters, with PM2.5 ranging from 29 to 47 $\mu g/cm^2$ and PM10 ranging from 104 to 277 $\mu g/cm^2$.

The issue of whether the instances of recovery efficiency being less than 100% were due to less than complete physical recovery of the spores, other physical loss mechanisms such as retention on processing containers, or interference of growth due to the presence of grime or competing flora was beyond the scope of the study. It is also noted that spore/filter surface interactions may influence the percent recovery measured, which could be affected by the spore spiking method. The application of spores by pipetting droplets of a stock suspension may assist or hinder the ability to physically recover the spores. Spores collected as an aerosol, as would be expected during normal field operation, may adhere to the filter fiber or collection surface more strongly than when applied as a droplet of suspension. Conversely, they may be adhered less strongly because the spores are present with other inert particles that may benefit physical recovery. When spores are applied as a droplet spike as done in this study, they may more readily disperse into the extract solution either due to being a large agglomerate or weakly adhered to a surface. Conversely, the spores applied as a droplet may penetrate the filter substrate by capillary action

and be more difficult to physically recover. For those reasons and uncertainty, it is recommended to consider future research to assess whether the application method matters – whether "spiking" the filters with *B. a.* Sterne by aerosolizing and collecting onto the filter via air sampling yields different results than those obtained in this study.

The presence of other flora collected on the filters during their intended use can bias the average efficiency high due to counting actual non-*B. a.* Sterne colonies as *B. a.* Sterne or bias low because the background flora competed with the growth of *B. a.* Sterne and suppressed or masked *B. a.* Sterne growth. There were instances when no distinct *B. a.* Sterne colony morphology could be discerned, and in those occurrences, the recovery efficiency was reported as zero.

The uncertainty associated with or introduced by quantifying *B. a.* Sterne spore recovery efficiency based solely on colony morphology was revealed by PCR analysis of few representative colonies in each trial. A total of 76 colonies were screened using colony PCR. Fifteen (15) of these presumptive *B. a.* Sterne colonies were confirmed negative for the *B. anthracis* chromosome and pXO1 targets even though by morphology, these colonies were indistinguishable from *B. a.* Sterne, which highlights the importance for genetic confirmation of culture results. All 15 colonies that were identified incorrectly by morphology came from PM10 filters from two regions, California and South Carolina. Of the 29 colonies that were screened using colony PCR from these two regions, 52% were negative by real-time PCR analysis. The presence of a background flora with a morphology that is indistinguishable from *B. a.* Sterne artificially increased the percent spore recoveries for PM10 filters from these two regions. Eight (8) of these colonies were selected from zero spike samples, and by subtracting the number of colonies that appeared to have a *B. a.* Sterne morphology from zero spike samples, we could account for the number of colonies that contributed to the recovery values on spiked samples. Figure 15 depicts colonies and their morphology used to determine whether the colony was *B. a.* Sterne that was representative throughout the analysis of culture plates.

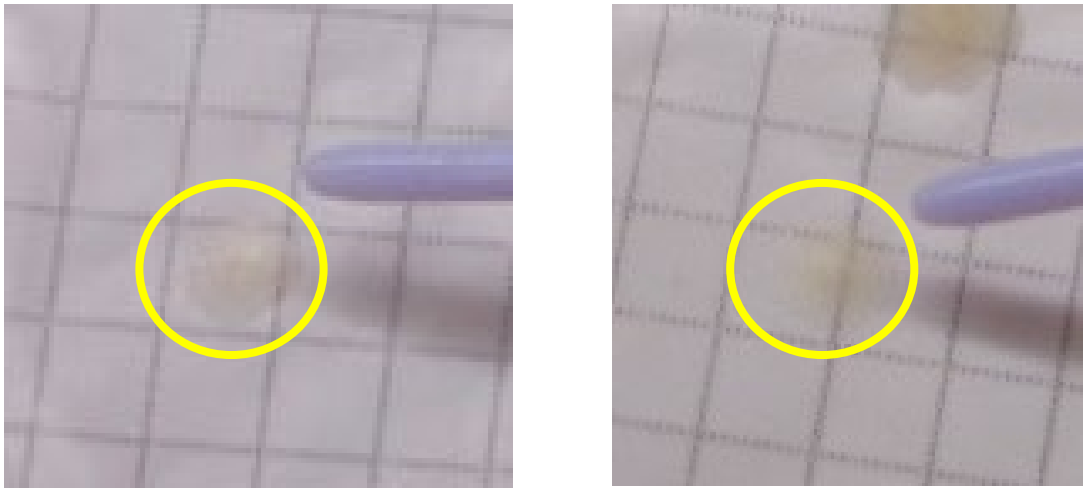

Figure 15. Colonies Recovered from PM10 California Filters Contained Background with *B. a.* Sterne Morphology (the colony on the left was confirmed negative and the colony on the right was confirmed positive for *B. a.* Sterne)

Chromogenic growth media (MYP and BBCA) were used to culture sample extracts from a subset of trials to assess whether it benefited differentiating *B. a.* Sterne morphology from background and to reduce the presence of background with the addition of antibiotics (Polymyxin B and Trimethoprim). The percent spore recoveries on the SBA and MYP growth media were equivalent when colonies were isolated on both media types. However, the growth of *B. a.* Sterne on MYP commonly spread into a lawn, interfering with the ability to accurately quantify and identify *B. a.* Sterne in many instances. Consequently, the MYP medium was no longer used. The BBCA percent recoveries were 5 to 7 times less than SBA, so use of the BBCA medium was also discontinued from the analytical methods at the direction of the EPA TOCOR (Calfee, 2017). A summary of those results and associated discussion are provided in Appendices J and K.

3.1.2 RV-PCR Method

A summary of the average and standard deviation of the RV-PCR ΔCt values for the detection of *B. a.* Sterne spores recovered from the AQ filter substrate are presented in Table 6 (PM2.5) and Table 7 (PM10). The ΔCt results are plotted in Figures 16 through 23 with each plot associated with one of the eight specific filter types. The summary tables and associated plots follow the same column header and x-axis labeling convention as used for the presentation of culture results. Most notably, the nominal number of spores available for analysis of 15, 150, and 1,500 CFU are used; it represents the maximum number of spores available assuming a 100% recovery

efficiency and half the sample extract is available for RV-PCR analysis. The average quantity of spores determined available are presented in the summary tables. The 0-spore available condition is included in the plots because meaningful RV-PCR results can be obtained, unlike that for a recovery efficiency. The plots all depict an area shaded in red that is the region of a negative confirmation result and an area of green that is a positive confirmation result, delineated by both the chromosomal and pXO1 gene target PCR ΔCt values having to be ≥ 9 to be a positive result. The RV-PCR results for each air quality filter sample analyzed are presented in Appendix L.

RV-PCR analyses of the PM2.5 filters generated average ΔCt values between 26.5 and 28.7 (chromosomal and pXO1 gene targets) and sample standard deviations typically < 5 for all locations and particle loads. Figures 16 through 19 indicate there is little difference in the ΔCt as a function of ambient particle loading for the filter and spike conditions assessed. (In Section 3.1.1, the culture results supported the recovery of *B. a.* Sterne spores from all the PM2.5 filters.) There were instances for the filters obtained from Florida that had a measurable ΔCt associated with the 0-spike condition. It is believed that the generated ΔCt may have been associated with low-level cross-contamination as those results were obtained early, within the first 10% of the matrix being completed and resolved within the first 25% of the matrix being completed, as the method was being implemented. The main issues were associated with leaking filter vials on the manifold and a warped manifold that were resolved with equipment change (new manifold implemented), refinement of technique, and rigorous care in method execution. The originally purchased filter manifold did not consistently seal well. Lawrence Livermore National Laboratory provided a filter manifold that was more effective at sealing and helped reduce occurrences of likely cross-contamination (non-zero ΔCt values for the 0-spike condition.) Also, there were instances where the PES filter vials exhibited leakage or by-pass flow that could have led to sample contamination. The impact was only apparent in the 0-spike condition of the filters analyzed because all other samples purposely (via spiking) contained the *B. a.* Sterne target organism.

As shown in Figures 20 through 23, RV-PCR analyses of the PM10 filters typically resulted in an average ΔCt > 20 at nominal spores available conditions of 150 or 1,500 and sample standard deviations < 5. Filters from three of the four geographic regions (South Carolina, California, and Wisconsin) all exhibited some suppression of ΔCt magnitude associated with a higher relative ambient particulate load. Attenuation of the ΔCt magnitude was more pronounced for the

nominal 15 *B. a.* Sterne spores available condition relative to that of the 150 or 1,500 nominal spores available, which suggests that the lower limit of detection of the RV-PCR method is near the nominal 15-spores available condition. Once the nominal *B. a.* Sterne spore load is at or above 150, there was little difference in measured ΔCt for the PM10 filters assessed. Results showing that sample ΔCt standard deviations are relatively large and the greatest with the nominal 15-spores-available condition relative to those measured at the 150 and 1,500 spores available condition also suggests that the method detection limit is being approached at the 15-spore load. Lastly, there were cases with the 15-spore load condition where one or two of the three replicates had a $\Delta Ct \geq 9$ and the remainder < 9, further indicating the limit of detection of the method is being approached.

Nearly half of the 0-spike conditions for all four regions of PM10 filters had at least one sample with a non-zero ΔCt. There were instances where T0 was a value other than 45, indicating potential initial contamination, which occurred early in the application of the method. Most all of these non-zero results were associated with analyses performed early in the study before some low-level cross-contamination had been minimized (e.g., sample handling technique and increased glove changes) as the method implementation progressed.

Comparing the ΔCt values of the PM2.5 filters versus the PM10 shows that the RV-PCR method was less adversely affected when applied to the PM2.5 filters to that of the PM10 filters. There was little difference between ΔCt values measured with the Avg or High ambient particulate loading condition compared to the New filter for PM2.5 filters, whereas a noticeable attenuation in ΔCt was observed for selected PM10 filters, which would suggest that the higher ambient particulate load on the PM10 filters compared to the PM2.5 filters was interfering with the method detection limit. Also, there appears to be some consistency in the culture results with that of the RV-PCR in that the recovery efficiencies for the PM10 were generally lower than that of the PM2.5. That low recovery efficiency could also partly explain why the RV-PCR detection limit appears to be approached around the nominal 15 spores available for the PM10 filters but lower with the PM2.5 filters.

Consistently, throughout all analyses, very good agreement (ΔCt differed by < 3 between the two gene targets) was obtained for the chromosomal and pXO1 gene targets for both the PM2.5 and PM10 filters and for all of the nominal spore loads.

Table 6. RV-PCR Analyses of PM2.5 Air Quality Filters for Detection of *B. a.* Sterne Spores Using Chromosomal and pXO1 Gene Targets (N ≥ 3 Replicates)

Location	Ambient Particle Load[a] (µg/cm²)	Spores Available for Analysis (CFU)		ΔCt (X ± σ)	
		Nominal[b]	Determined[c] (X ± σ)	Chromosomal Gene Target	pXO1 Gene Target
-- (New)	(0)	0	N/A	0.0 ± 0.0	-0.8 ± 1.3
		15	18 ± 9	27.9 ± 0.4	27.1 ± 1.6
		150	180 ± 90	27.5 ± 0.8	27.5 ± 0.6
		1,500	1,800 ± 900	27.1 ± 0.2	27.4 ± 0.2
AZ	Avg (16)	0	N/A	-0.1 ± 0.2	-0.4 ± 0.7
		15	18 ± 9	26.5 ± 1.0	25.3 ± 3.5
		150	180 ± 90	27.2 ± 1.1	24.8 ± 2.4
		1,500	1,800 ± 900	27.0 ± 1.3	26.1 ± 2.6
	High (39)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	7 ± 0	26.5 ± 3.0	26.5 ± 2.9
		150	70 ± 3	27.7 ± 0.9	27.8 ± 1.0
		1,500	700 ± 30	27.5 ± 0.7	27.9 ± 0.8
FL	Avg (13)	0	N/A	4.8 ± 6.5	4.3 ± 7.4
		15	15 ± 9	27.6 ± 1.0	27.9 ± 1.0
		150	150 ± 90	27.7 ± 0.9	27.9 ± 0.9
		1,500	1,500 ± 900	26.9 ± 1.4	27.8 ± 0.7
	High (39)	0	N/A	3.9 ± 6.7	4.1 ± 7.1
		15	15 ± 9	27.8 ± 1.4	28.0 ± 1.4
		150	150 ± 90	28.7 ± 0.6	29.0 ± 0.6
		1,500	1,500 ± 900	27.4 ± 0.7	27.8 ± 0.7
MA	Avg (12)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	14 ± 4	27.2 ± 0.3	27.4 ± 0.4
		150	140 ± 40	27.4 ± 0.5	27.5 ± 0.5
		1,500	1,400 ± 400	27.5 ± 1.0	27.7 ± 0.9
	High (29)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	14 ± 4	27.9 ± 0.7	28.0 ± 0.7
		150	140 ± 40	27.7 ± 0.6	27.9 ± 0.4
		1,500	1,400 ± 400	27.4 ± 0.6	27.7 ± 0.4
WI	Avg (14)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	11 ± 1	26.7 ± 3.1	26.8 ± 3.2
		150	110 ± 6	28.2 ± 0.8	28.4 ± 0.7
		1,500	1,100 ± 60	27.7 ± 0.2	28.0 ± 0.2
	High (47)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	11 ± 1	27.8 ± 0.9	27.9 ± 1.1
		150	110 ± 6	28.2 ± 0.4	28.4 ± 0.4
		1,500	1,100 ± 60	27.9± 0.5	28.2 ± 0.5

(a) Relative ambient particle load (with measured mass loading per area).

(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.

(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for RV-PCR analysis.

Table 7. RV-PCR Analyses of PM10 Air Quality Filters for Detection of *B. a.* Sterne Spores Using Chromosomal and pXO1 Gene Targets (N ≥ 3 Replicates)

Location	Ambient Particle Load[(a)] (µg/cm²)	Spores Available for Analysis (CFU)		ΔCt (X ± σ)	
		Target[(a)]	Determined[(c)] (X ± σ)	Chromosomal Gene Target	pXO1 Gene Target
-- (New)	(0)	0	N/A	2.7 ± 4.7	3.0 ± 5.3
		15	6 ± 3	14.3 ± 11.7	15.5 ± 10.7
		150	60 ± 30	26.8 ± 1.1	26.1 ± 2.8
		1,500	700 ± 0	26.6 ± 0.5	26.8 ± 0.2
CA	Avg (118)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	13 ± 5	9.8 ± 17.0	10.0 ± 17.4
		150	130 ± 50	23.1 ± 1.8	23.5 ± 1.9
		1,500	1,300 ± 500	26.3 ± 3.9	26.7 ± 4.1
	High (277)	0	N/A	0.0 ± 0.0	0.6 ± 1.0
		15	13 ± 5	11.5 ± 15.8	12.4 ± 15.6
		150	130 ± 50	26.5 ± 3.5	26.8 ± 3.2
		1,500	1,300 ± 500	28.0 ± 1.2	28.4 ± 0.9
NH	Avg (69)	0	N/A	1.7 ± 2.9	5.4 ± 4.2
		15	14 ± 10	21.8 ± 11.3	22.1 ± 10.7
		150	140 ± 100	26.7 ± 1.3	26.9 ± 1.3
		1,500	1,400 ± 1000	27.5 ± 0.2	27.8 ± 0.4
	High (199)	0	N/A	0.0 ± 0.0	3.3 ± 5.7
		15	14 ± 10	14.1 ± 11.7	15.7 ± 10.1
		150	140 ± 100	25.5 ± 3.0	25.6 ± 3.0
		1,500	1,400 ± 1000	25.8 ± 0.9	26.1 ± 0.8
SC	Avg (65)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	14 ± 1	13.8 ± 12.2	14.0 ± 12.4
		150	140 ± 6	28.1 ± 0.5	28.2 ± 0.4
		1,500	1,400 ± 60	25.0 ± 3.4	25.2 ± 3.3
	High (132)	0	N/A	0.0 ± 0.0	0.0 ± 0.0
		15	14 ± 1	10.8 ± 9.4	10.9 ± 9.5
		150	140 ± 6	22.5 ± 6.0	22.8 ± 6.0
		1,500	1,400 ± 60	23.8 ± 1.5	24.2 ± 1.4
WI	Avg (41)	0	N/A	0.0 ± 0.0	0.0 ± 0.1
		15	9 ± 0	14.5 ± 10.9	17.0 ± 7.5
		150	90 ± 0	24.0 ± 1.2	24.6 ± 1.4
		1,500	900 ± 0	27.5 ± 1.4	28.1 ± 1.5
	High (104)	0	N/A	12.1 ± 1.6	10.2 ± 2.9
		15	6 ± 1	16.5 ± 9.2	14.1 ± 9.1
		150	60 ± 30	23.3 ± 4.2	23.6 ± 3.9
		1,500	900 ± 500	26.3 ± 1.2	26.3 ± 0.8

(a) Relative ambient particle load (with measured mass loading per area).

(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.

(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for RV-PCR analysis.

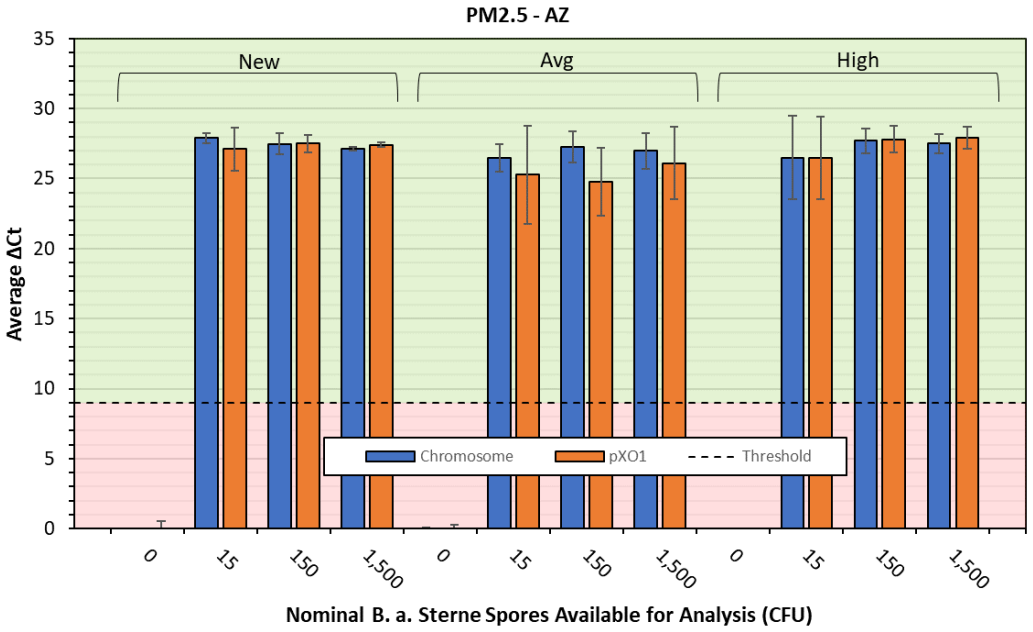

Figure 16. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM2.5 Filters from Arizona Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

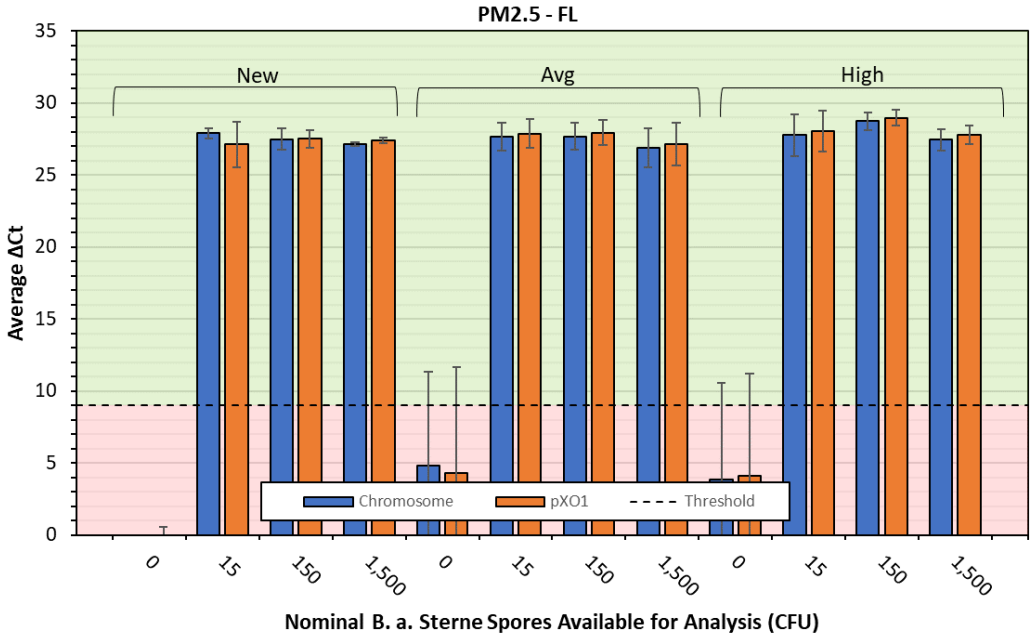

Figure 17. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM2.5 Filters from Florida Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

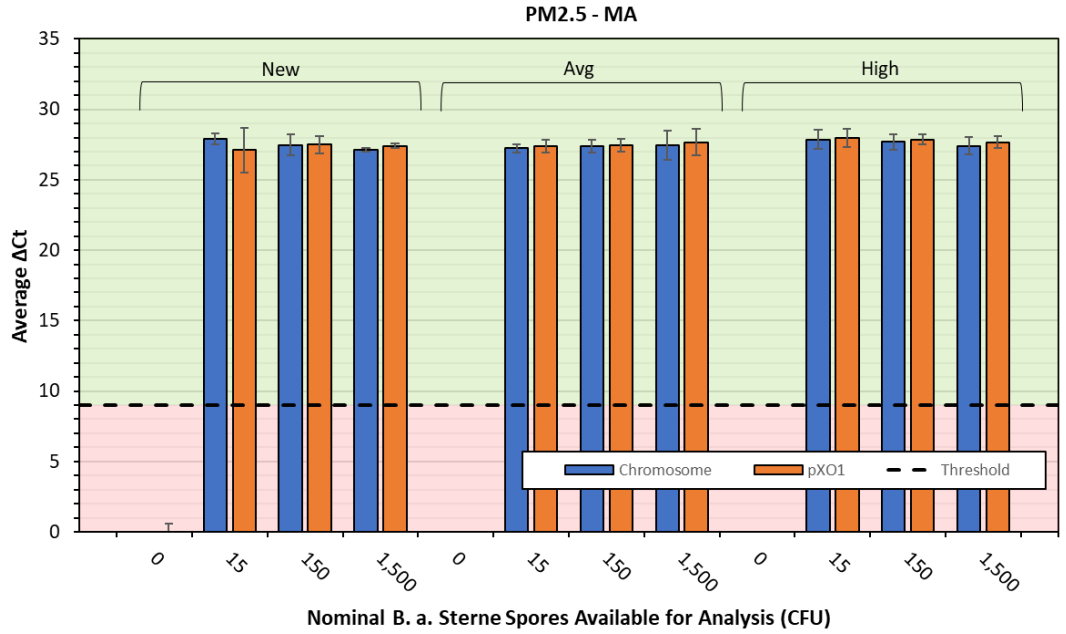

Figure 18. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM2.5 Filters from Massachusetts Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

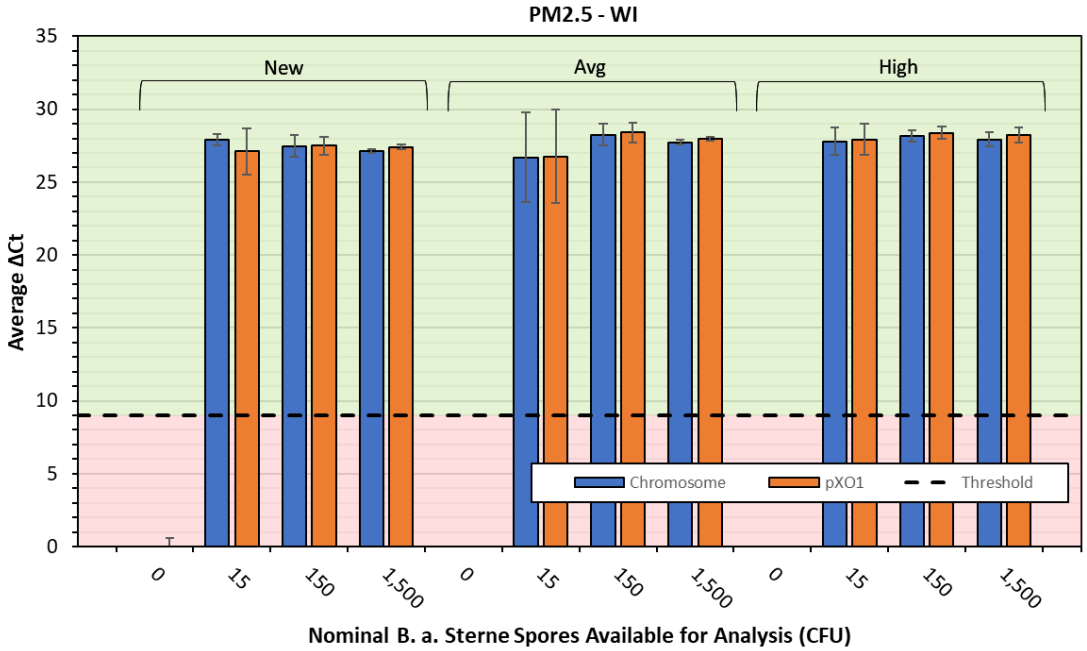

Figure 19. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM2.5 Filters from Wisconsin Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

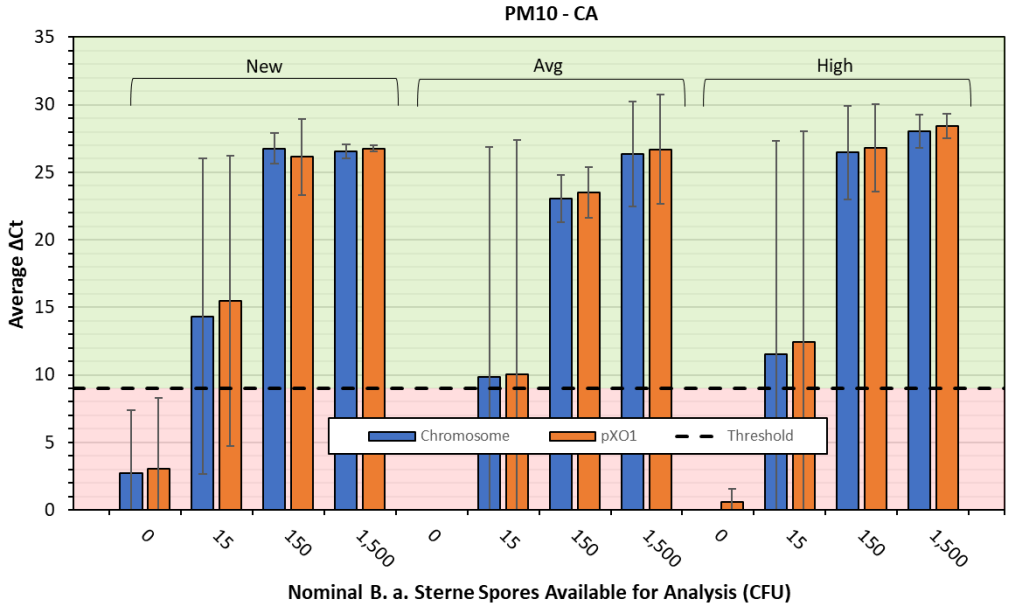

Figure 20. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM10 Filters from California Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

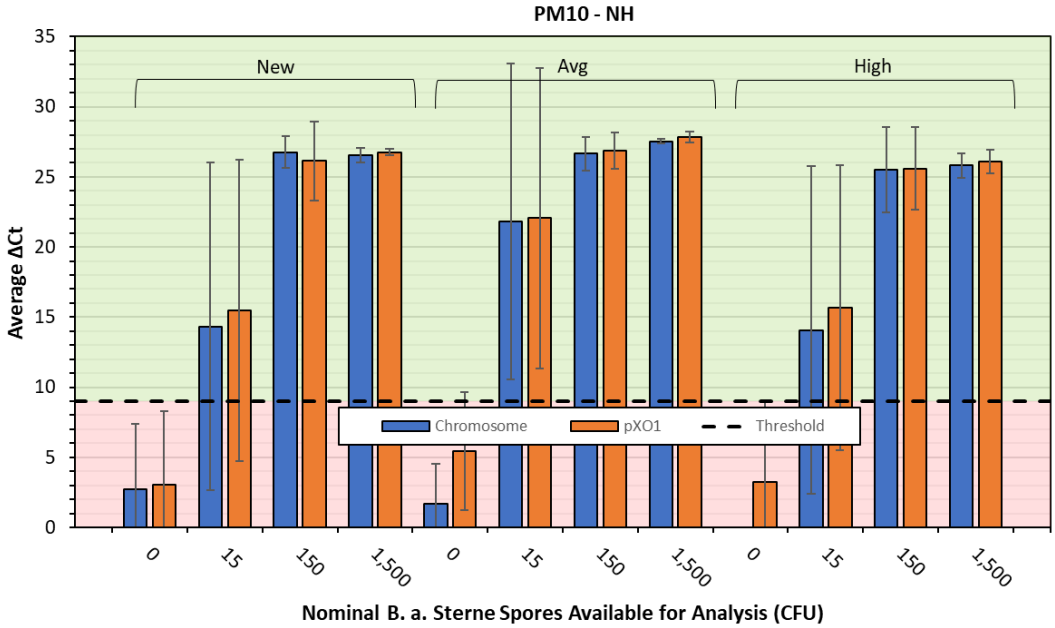

Figure 21. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM10 Filters from New Hampshire Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

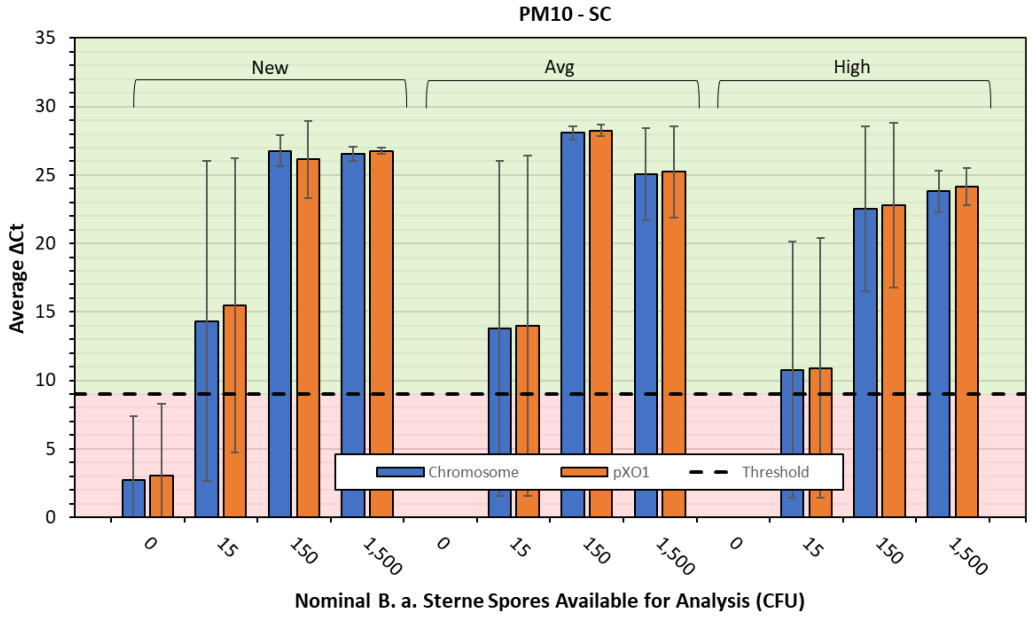

Figure 22. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM10 Filters from South Carolina Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

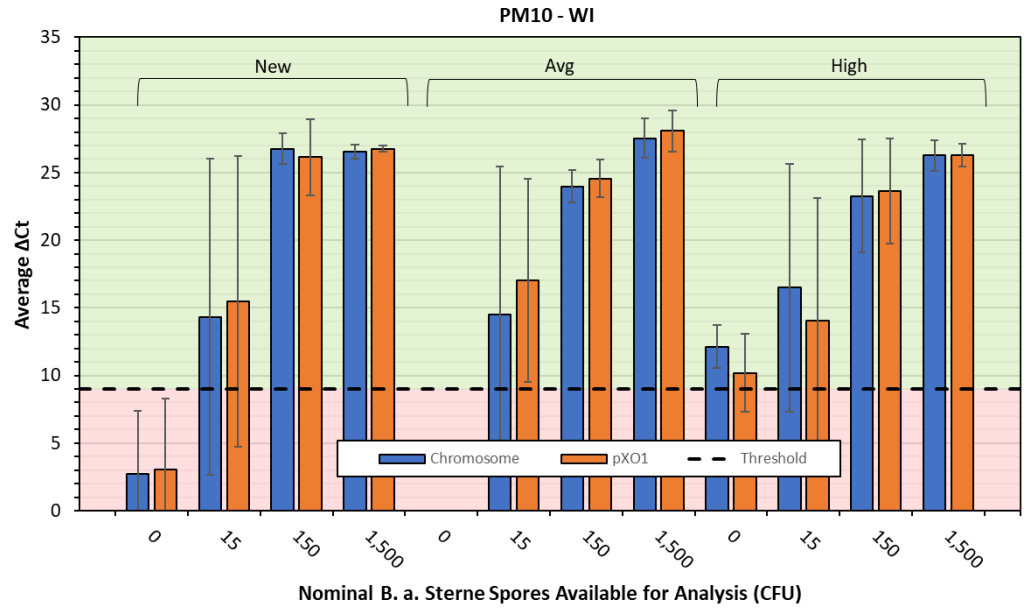

Figure 23. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from PM10 Filters from Wisconsin Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Avg, and High refer to relative ambient particulate loads)

3.2 Non-AQ Filter Analyses Results

3.2.1 Culture Method

A summary of the average and standard deviation of the measured recovery efficiencies of presumptive *B. a.* Sterne spores recovered from the non-AQ filter substrate spiked with *B. a.* Sterne spores and using the SBA medium to culture are presented in Table 8. The determined number of spores available and the number of presumptive *B. a.* Sterne spores recovered are tabulated along with the nominal quantity of spores available for analysis (15, 150, and 1,500 CFU/filter sample). The quantity of presumptive *B. a.* Sterne colonies reported in the tables is half of the actual total recovered because in the context of the tables, only half of the extract samples was made available for analysis. The quantity of presumptive *B. a.* Sterne colonies for each sample, used in the percent recovery calculations, are reported in Appendix M for the SBA culture medium. The recovery efficiencies are based on the culture method using the SBA medium. When the MYP chromogenic agar was also used in the culture method, the recovery efficiencies are reported in Appendix N.

The recovery efficiencies are plotted in Figures 25 through 28, one plot for each filter type. Note, a percent recovery is not tabulated or plotted for the 0-spore spike condition since, by definition, a meaningful recovery efficiency cannot be calculated, even though there could have been a finite number of presumptive *B. a.* Sterne colonies counted based on colony morphology.

Table 8. Recovery Efficiencies for Presumptive *B. a.* Sterne Spores from Non-Air Quality Filters Cultured in the SBA Medium (N ≥ 3 Replicates)

Filter Type	Duty Stage[a]	Sample Reps	Spores Available for Analysis (CFU)		Spores Recovered (CFU) (X ± σ)[d]	Spore Recovery Efficiency (%) (X ± σ)[e]
			Nominal[b]	Determined (X ± σ) [c]		
Bus Engine	New	5	0	0	0	N/A
		3	15	20 ± 4	5.2 ± 6.5	24 ± 30
		3	150	200 ± 40	13 ± 11	6.0 ± 4.8
		3	1,500	2,000 ± 400	100 ± 37	5.3 ± 1.7
	Mid	3	0	0	2.1 ± 1.8	N/A
		3	15	13 ± 7	6.3 ± 5.4	50 ± 52
		3	150	130 ± 70	21 ± 7.9	21 ± 13
		3	1,500	1,300 ± 700	170 ± 38	14 ± 6.0
	End	5	0	0	1.6 ± 1.8	N/A
		5	15	13 ± 8	19 ± 5.1	190 ± 110
		5	150	130 ± 80	27 ± 10	27 ± 16
		3	1,500	2,000 ± 400	340 ± 140	17 ± 4.4
Building HVAC	New	4	0	0	0	N/A
		4	15	18 ± 1	8.6 ± 3.0	48 ± 16
		3	150	160 ± 10	50 ± 23	32 ± 13
		3	1,500	1,600 ± 100	600 ± 81	38 ± 7.9
	Mid	3	0	0	0	N/A
		3	15	13 ± 7	2.1 ± 1.8	23 ± 20
		3	150	130 ± 70	47 ± 24	38 ± 16
		3	1,500	1,300 ± 700	600 ± 120	53 ± 22
	End	4	0	0	47 ± 56	N/A
		4	15	18 ± 1	31 ± 16	170 ± 79
		4	150	180 ± 10	69 ± 22	39 ± 15
		4	1,500	1,800 ± 100	740 ± 180	43 ± 13
Subway Platform	New	3	0	0	0	N/A
		3	15	10 ± 3	10 ± 3.6	110 ± 59
		3	150	100 ± 30	47 ± 29	43 ± 16
		3	1,500	1,000 ± 300	590 ± 64	61 ± 19
	Mid	3	0	0	13 ± 13	N/A
		3	15	17 ± 3	9.4 ± 3.1	59 ± 27
		3	150	170 ± 40	70 ± 12	42 ± 9.0
		3	1,500	1,700 ± 300	450 ± 51	28 ± 7.0
	End	3	0	0	21 ± 26	N/A
		3	15	10 ± 3	25 ± 22	290 ± 250
		3	150	100 ± 30	58 ± 29	57 ± 30
		3	1,500	1,000 ± 300	500 ± 280	56 ± 37

Table 8. Recovery Efficiencies for Presumptive *B. a.* Sterne Spores from Non-Air Quality Filters Cultured in the SBA Medium (N ≥ 3 Replicates) (Cont.)

Filter Type	Duty Stage[a]	Sample Reps	Spores Available for Analysis (CFU)		Spores Recovered (CFU) (X ± σ)[d]	Spore Recovery Efficiency (%) (X ± σ)[e]
			Nominal[b]	Determined (X ± σ) [c]		
Subway Rolling Stock	New	3	0	0	0	N/A
		3	15	28 ± 12	14 ± 1.8	57 ± 28.6
		3	150	280 ± 120	160 ± 46	63 ± 15.1
		3	1,500	2,800 ± 1200	1100 ± 530	38 ± 3.1
	Mid	3	0	0	6.3 ± 8.8	N/A
		3	15	17 ± 3	13 ± 22	60 ± 100
		3	150	170 ± 40	50 ± 33	31 ± 24
		3	1,500	1,700 ± 300	250 ± 330	16 ± 22
	End	3	0	0	8.3 ± 7.2	N/A
		3	15	28 ± 12	29 ± 26	130 ± 25
		3	150	280 ± 120	58 ± 71	25 ± 20
		3	1,500	2,800 ± 1200	UD[f]	UD

(a) Relative ambient particulate loading.
(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.
(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for culture analysis.
(d) Presumptive *B. a.* Sterne colonies based on morphology and one-half of extract used for culture analysis
(e) Calculated using the actual spore loading on each filter and presumptive *B. a.* Sterne spores recovered on each filter sample.
(f) Undetermined (UD) due to excess growth of collected ambient organisms on the filter and/or presumptive *B. a.* Sterne.

The basic trends, observations, general results, and discussion provided in Section 3.1.1 for the culture results (percent recovery efficiencies) for the air quality type filters applies here with the non-AQ filters. Most notable is the relatively large sample standard deviation and apparent recovery efficiencies exceeding 100% associated with the nominal 15-spores-available condition that are attributed to few *B. a.* Sterne spores recovered and/or impact of background flora that could bias the presumptive *B. a.* Sterne spore count high or low.

As examples, the highest overall spore recovery efficiencies approached 60% for the subway platform filters when loaded with nominal 150 to 1,500 spores available to a low of 0% (no colonies with *B. a.* Sterne morphology could be identified). The bus engine filter has the lowest percent recovery of the non-AQ filters when the filters were New. The subway rolling stock filters appeared (by visual observation) to be the dirtiest of the non-AQ filters and had an abundance of background flora that complicated the identification and quantification of

recovered *B. a.* Sterne. Figure 24 shows photographs of representative culture plates depicting the amount of background flora and grime that adversely affected *B. a.* Sterne quantification.

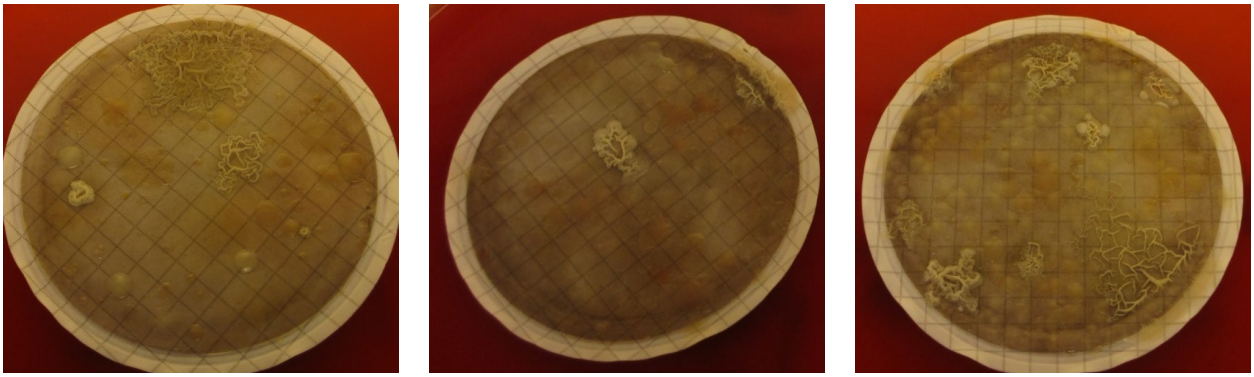

Figure 24. Subway Rolling Stock End-of-Service Filter Spiked with 30, 300, or 3,000 Spores (from left to right, respectively)

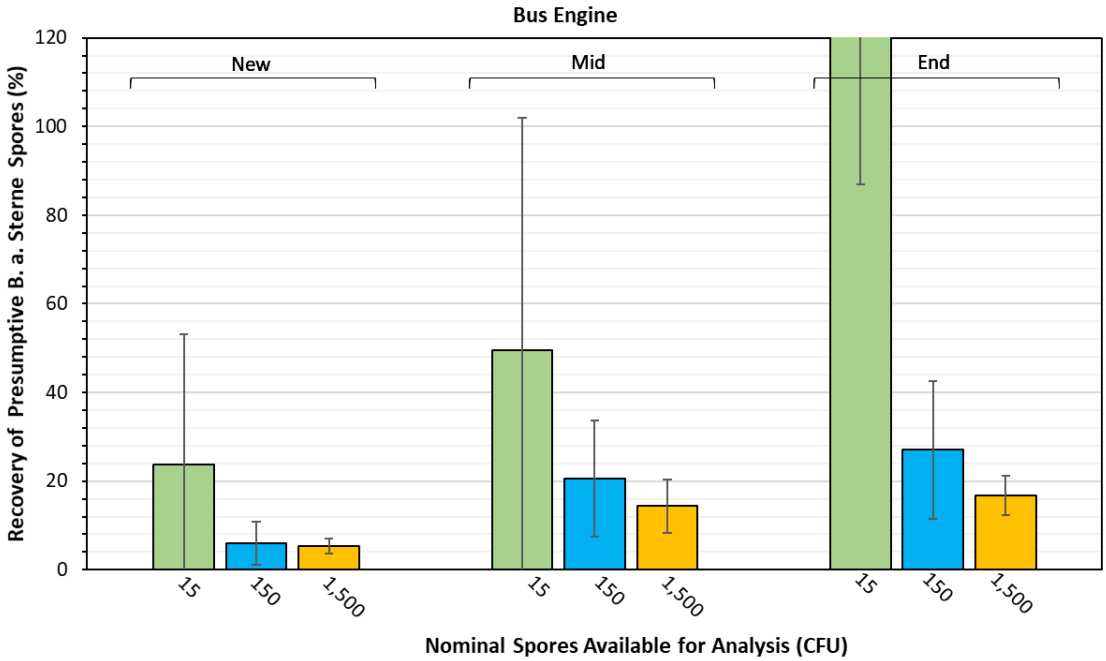

Figure 25. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from Bus Engine Filters Using the SBA Medium (New, Mid, and End refer to service life or duty cycle of the filter)

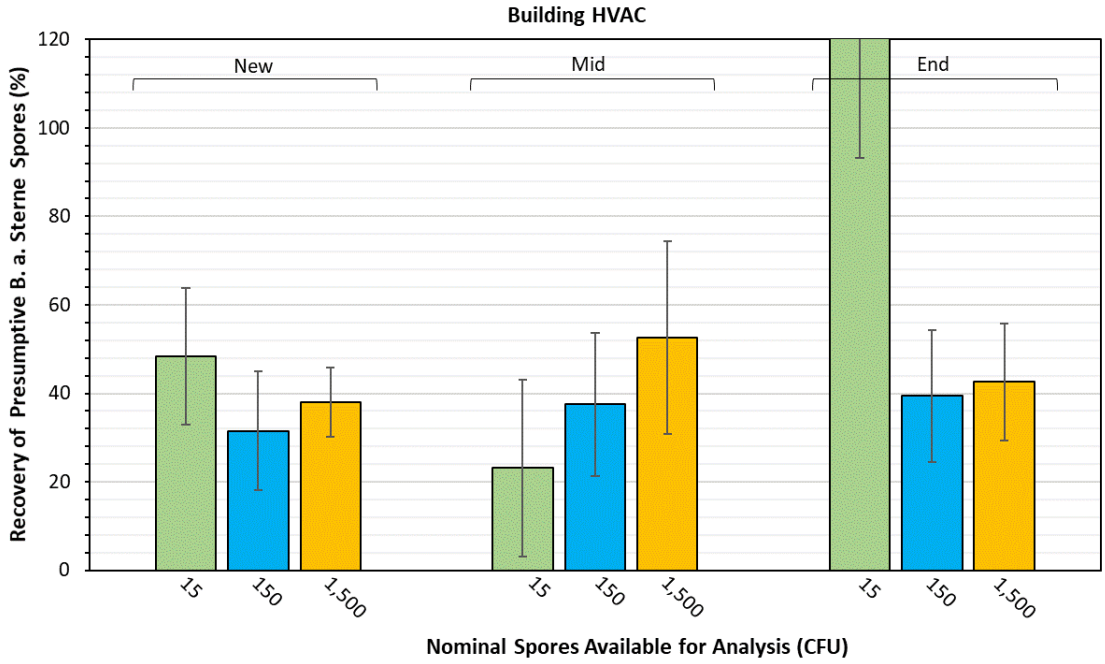

Figure 26. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from Building HVAC Filters Using the SBA Medium (New, Mid, and End refer to service life or duty cycle of the filter)

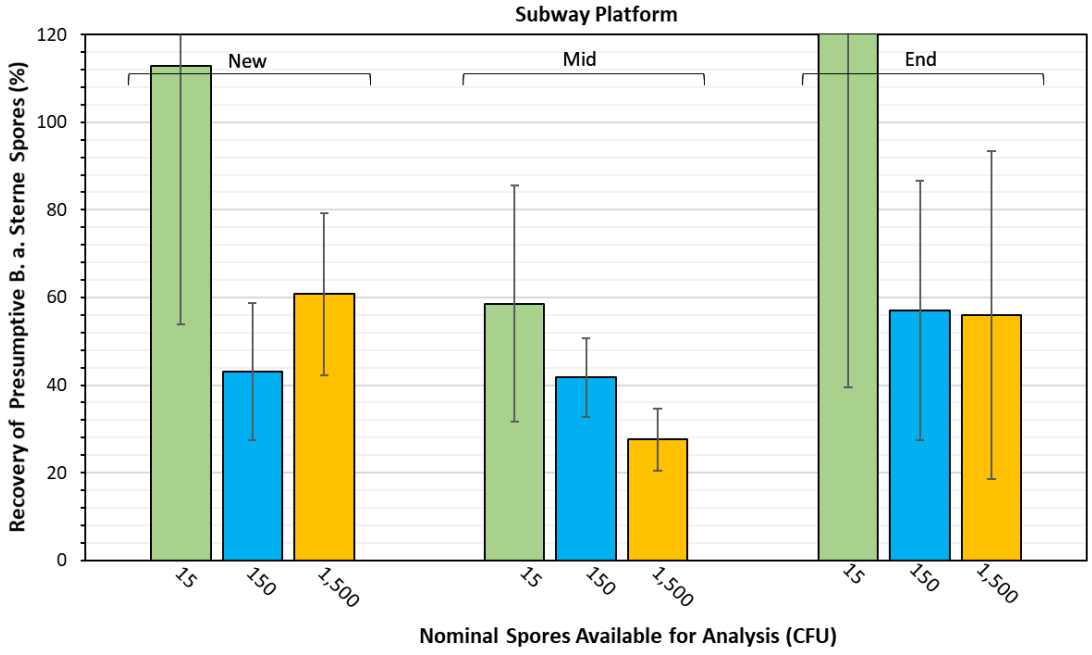

Figure 27. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from Subway Platform Filters Using the SBA Medium (New, Mid, and End refer to service life or duty cycle of the filter)

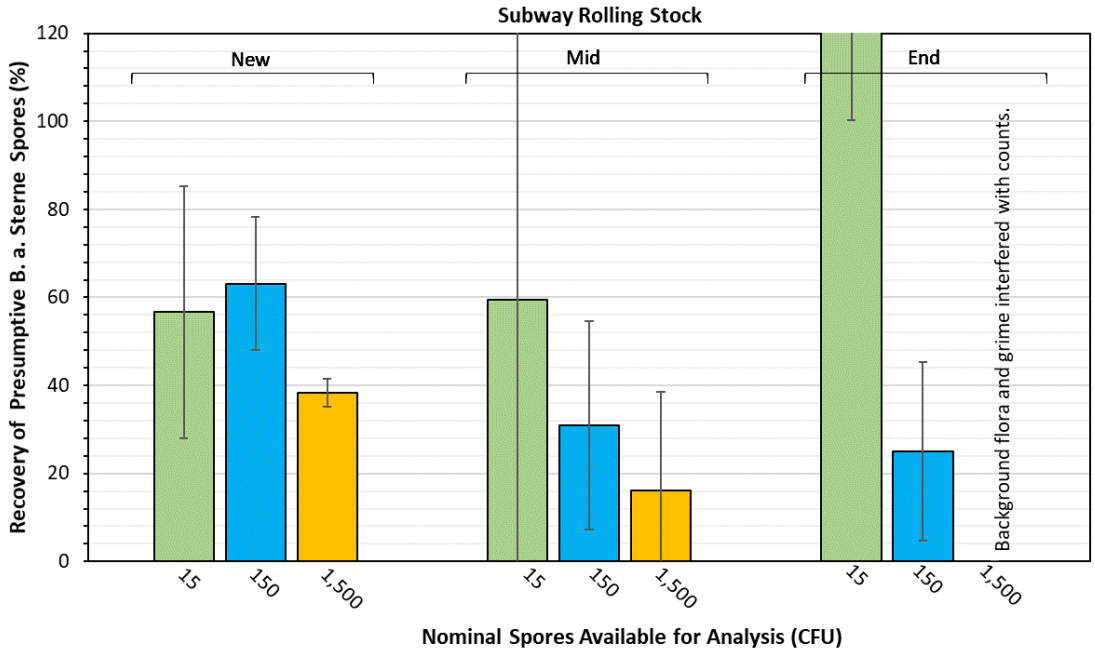

Figure 28. Percent Recovery Efficiencies (Average ± One Standard Deviation of N ≥ 3 Replicates) of Presumptive *B. a.* Sterne Spores from Subway Rolling Stock Filters Using the SBA Medium (New, Mid, and End refer to service life or duty cycle of the filter)

A representative, qualitative illustration of how dirty the filters were, and the associated suspension extract, is shown in Figure 29 for a bus engine filter. There was a range of observable discoloration and/or noticeable suspended particulate matter and the bus engine filter represented one of the dirtier filters. The images demonstrate that the analytical methods were applied to very challenging matrices and were far from being applied to pristine samples in a laboratory setting as that associated with method development.

Figure 29. Bus Engine End-of-Service Life Filter Deconstructed Prior to Spore Recovery (left) and Suspension After Recovered from Bus Engine Filters New (unused) in Top Row and End-of-Service Life in Bottom Row of RV-PCR Vacuum Manifold

3.2.2 RV-PCR Method

A summary of the average and sample standard deviation of the RV-PCR ΔCt values for the detection of *B. a.* Sterne spores recovered from the non-AQ filter substrates are presented in Table 9. The ΔCt results are plotted in Figures 30 through 33, with each plot associated with one of the four specific filter types. The summary tables and associated plots follow the same column header and x-axis labeling convention as used for the presentation of culture results. Most notably, the nominal number of spores available for analysis of 15, 150, and 1,500 CFU are used; it represents the maximum number of spores available assuming a 100% recovery efficiency and half the sample extract is available for RV-PCR analysis. The average quantity of spores determined available are presented in the summary tables. The 0-spore available condition is included in the plots because meaningful RV-PCR results can be obtained, unlike that for a recovery efficiency. The plots all depict an area shaded in red that is the region of a negative confirmation result and an area of green that is a positive confirmation result, delineated by both the chromosomal and pXO1 gene target ΔCt values having to be ≥ 9 to be a positive result. The RV-PCR results for each non-AQ filter sample analyzed are presented in Appendix O.

New non-AQ filters from bus engine, building HVAC, subway platform, and subway rolling stock generated a ΔCt value between 20.8 and 28.0 (chromosome and pXO1 target PCR assays). For used filters, both Mid and End, the ΔCt values increase with spore load, indicating inhibition from background, either due to growth inhibition during overnight incubation (enrichment step) or molecular inhibition of the real-time PCR assays, not due to physical recovery because the new filter material response for all spore loads are consistently above a ΔCt of 20. (The ΔCt values for both real-time PCR assay targets (chromosomal and pXO1) tracked similarly for each sample extract throughout the study.)

Table 9. RV-PCR Analyses of Non-Air Quality Filters for Detection of *B. a.* Sterne Spores Using Chromosomal and pXO1 Gene Targets (N ≥ 3 Replicates)

Filter Type	Duty Stage[a]	Spores Available for Analysis (CFU)		ΔCt (X ± σ)	
		Actual[b]	Determined[c] (X ± σ)	Chromosomal Gene Target	pXO1 Gene Target
Bus Engine	New	0	0	2.8 ± 4.2	3.0 ± 3.4
		15	20 ± 4	27.7 ± 1.1	27.6 ± 1.0
		150	200 ± 40	27.5 ± 0.9	27.7 ± 1.0
		1,500	2,000 ± 400	28.0 ± 0.1	27.6 ± 0.8
	Mid	0	0	0.0 ± 0.0	0.4 ± 0.7
		15	13 ± 7	14.1 ± 4.1	14.1 ± 3.3
		150	130 ± 70	20.1 ± 3.2	20.5 ± 2.8
		1,500	1,300 ± 700	22.0 ±1.7	22.8 ± 1.5
	End	0	0	4.5 ± 6.1	4.9 ± 6.2
		15	13 ± 8	14.9 ± 3.9	15.8 ± 3.4
		150	130 ± 80	19.2 ± 4.2	19.9 ± 3.8
		1,500	2,000 ± 400	24.1 ± 2.0	24.7 ± 1.9
Building HVAC	New	0	0	7.1 ± 3.6	9.3 ± 1.8
		15	18 ± 1	25.7 ± 1.5	26.4 ± 1.3
		150	160 ± 10	20.8 ± 5.1	21.2 ± 5.1
		1,500	1,600 ± 100	27.3 ± 1.2	27.6 ± 1.0
	Mid	0	0	0.0 ± 0.0	-0.3 ± 0.5
		15	13 ± 7	16.4 ± 1.1	16.9 ± 1.2
		150	130 ± 70	22.5 ± 1.0	22.9 ± 0.8
		1,500	1,300 ± 700	26.4 ± 1.2	26.9 ± 1.1
	End	0	0	6.3 ± 6.2	9.2 ± 3.6
		15	18 ± 1	16.9 ± 4.0	17.1 ± 3.8
		150	180 ± 10	19.2 ± 1.7	19.8 ± 1.8
		1,500	1,800 ± 100	23.8 ± 1.9	24.5 ± 1.9
Subway Platform	New	0	0	3.9 ± 3.4	3.7 ± 4.3
		15	10 ± 3	22.1 ± 10.9	19.3 ± 13.2
		150	100 ± 30	24.9 ± 5.8	24.9 ± 6.3
		1,500	1,000 ± 300	23.5 ± 3.5	22.1 ± 4.8
	Mid	0	0	0.0 ± 0.0	0.0 ± 0.0
		15	17 ± 3	11.0 ± 9.7	11.2 ± 9.8
		150	170 ± 40	19.6 ± 0.1	20.0 ± 0.2
		1,500	1,700 ± 300	20.0 ± 1.3	20.5 ± 1.2
	End	0	0	3.0 ± 5.2	1.7 ± 2.7
		15	10 ± 3	11.6 ± 4.8	11.2 ± 6.4
		150	100 ± 30	15.8 ± 1.8	13.1 ± 3.7
		1,500	1,000 ± 300	18.6 ± 5.2	18.5 ± 5.3

Table 9. RV-PCR Analyses of Non-Air Quality Filters for Detection of *B. a.* Sterne Spores Using Chromosomal and pXO1 Gene Targets (N ≥ 3 Replicates) (Cont.)

Filter Type	Duty Stage[a]	Spores Available for Analysis (CFU)		ΔCt (X ± σ)	
		Actual[b]	Determined[c] (X ± σ)	Chromosomal Gene Target	pXO1 Gene Target
Subway Rolling Stock	New	0	0	5.3 ± 5.2	6.7 ± 5.9
		15	28 ± 12	26.5 ± 3.5	26.5 ± 3.5
		150	280 ± 120	27.7 ± 0.7	27.9 ± 0.7
		1,500	2,800 ± 1200	27.6 ± 0.8	27.4 ± 0.4
	Mid	0	0	0.0 ± 0.0	0.0 ± 0.0
		15	17 ± 3	8.9 ± 7.8	9.2 ± 8.1
		150	170 ± 40	17.8 ± 2.7	18.3 ± 2.7
		1,500	1,700 ± 300	20.3 ± 1.7	20.9 ± 1.9
	End	0	0	9.0 ± 7.8	9.1 ± 7.9
		15	28 ± 12	12.0 ± 5.3	13.9 ± 3.1
		150	280 ± 120	17.2 ± 3.1	17.9 ± 3.0
		1,500	2,800 ± 1200	18.1 ± 4.1	18.8 ± 4.0

(a) Relative ambient particle loading.
(b) Nominally one-half of the target spore load onto the filter and assuming 100% recovery of spores.
(c) Based on the spiking suspension titer measured each test trial, 100% recovery efficiency, and one-half of extract used for RV-PCR analysis.

In all instances, the ΔCt value for both chromosomal and pXO1 gene targets were ≥ 9 when the filters had nominally 15 spores available for analysis. Consistently, very good agreement (average ΔCt differed by < 3 between the two gene targets) was obtained for the chromosomal and pXO1 gene targets for both all non-AQ filter types and for all of the nominal spore loads. There were instances (for example, with the subway rolling stock filter and its end-of-service life condition) where the ΔCt for the 0-spike condition was ≥ 9, indicating a positive presence of *B. a.* Sterne. Those non-zero ΔCt values were primarily associated with samples tested early in the study and were likely due to low-level cross-contamination that was subsequently eliminated through rigorous glove change-out, refinement of method execution technique, and extreme care in procedure execution. The originally purchased filter manifold did not consistently seal well. Lawrence Livermore National Laboratory provided a filter manifold that was more effective at sealing and helped reduce occurrences of likely cross-contamination (non-zero ΔCt values for the 0-spike condition.) Also, there were instances where the PES filter vials exhibited leakage or by-pass flow that could have led to sample contamination. The impact was only apparent in the 0-spike condition of the filters analyzed because all other samples purposely (via spiking) contained the *B. a.* Sterne target organism.

The RV-PCR method was adversely affected by the collection of ambient particulate matter collected on the non-AQ filters. The ΔCt values were highest for the New filters of each filter type compared to the ΔCt values for the Mid or End cycle filters for all quantities of *B. a.* Sterne spores available for analysis. Although the RV-PR response was attenuated, the RV-PCR method was able to detect the presence of *B. a.* Sterne. Only for the subway platform and the subway rolling stock were there instances where the nominal 15-spores-available condition did not yield a positive response. (On average, $\Delta Ct \geq 9$ was achieved for the subway platform filters, but there was one 15-spore spike condition for the New and one for the Mid service life that had one or both the chromosomal and pXO1 gene targets with $\Delta Ct < 9$, and there were two replicates for the End service life that had $\Delta Ct < 9$. Similarly, on average, $\Delta Ct \geq 9$ was achieved for the subway rolling stock filters, but there was one 15-spore-spike condition for the Mid and one for the End service life that had one or both the chromosomal and pXO1 gene targets with $\Delta Ct < 9$.
(See Appendix O for individual test replicate results.) The ΔCt values were the highest for the New filters for all four filter types for all three spore loading levels, which would suggest that the higher ambient particulate load on the filter had some interfering effect. The RV-PCR method detection across the four filters was around the 15-spores-available threshold, similar to that for the PM2.5 AQ filters.

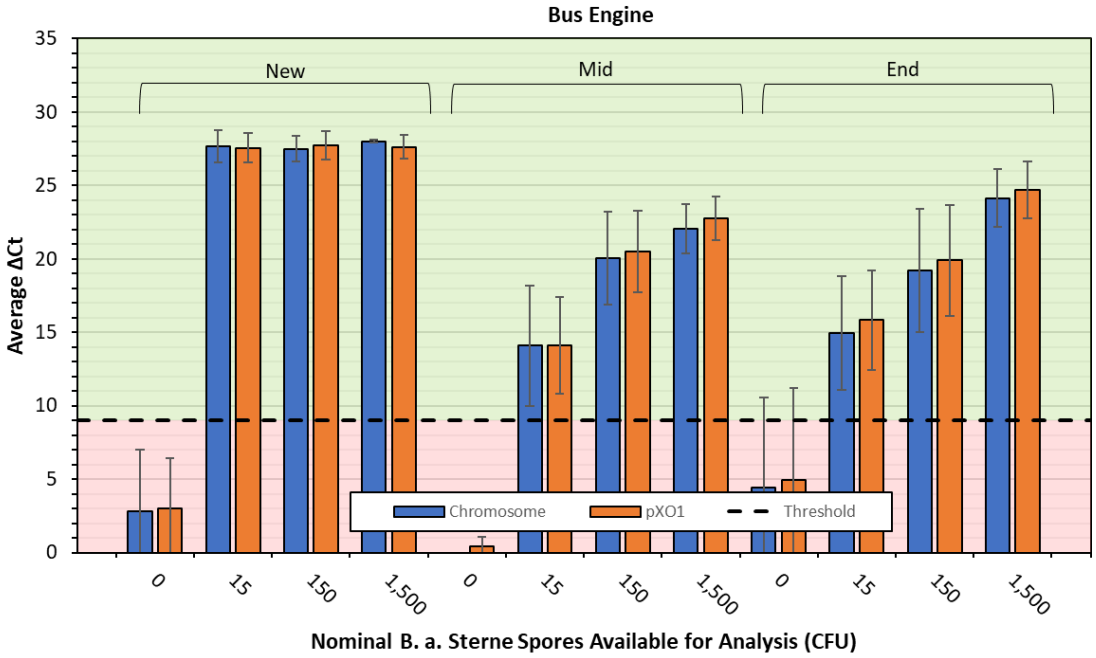

Figure 30. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from Bus Engine Filters Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Mid, and End refer to service life or duty cycle of the filter)

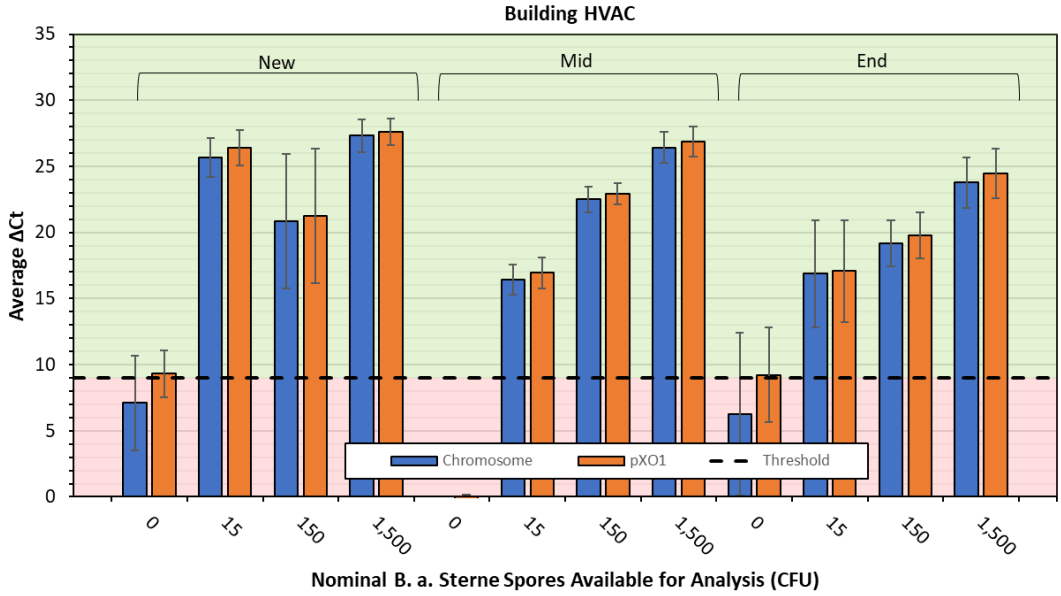

Figure 31. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from Building HVAC Filters Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Mid, and End refer to service life or duty cycle of the filter)

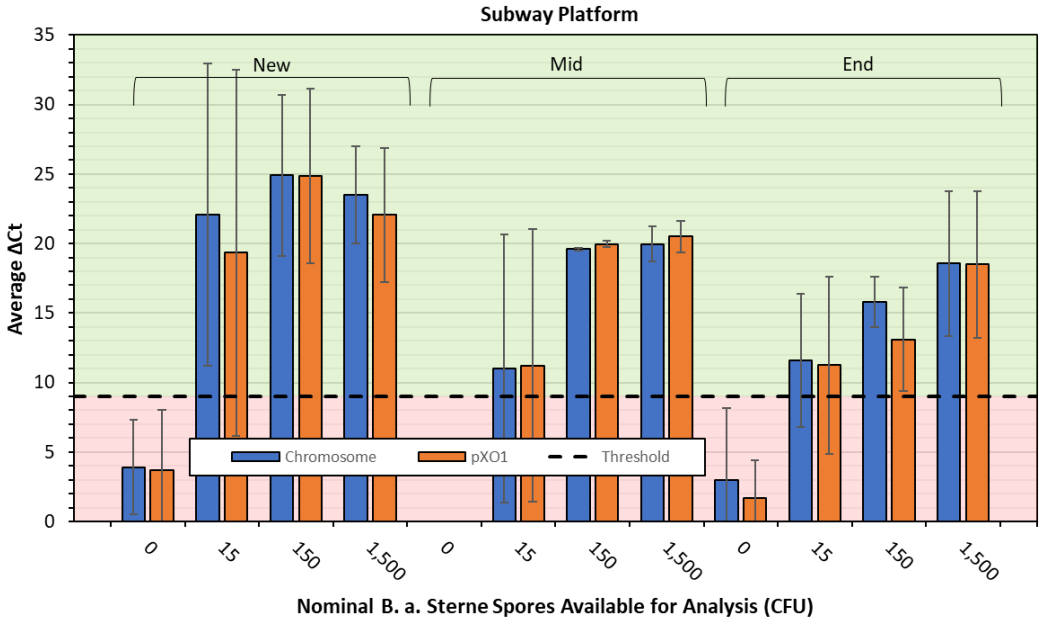

Figure 32. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from Subway Platform Filters Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Mid, and End refer to service life or duty cycle of the filter)

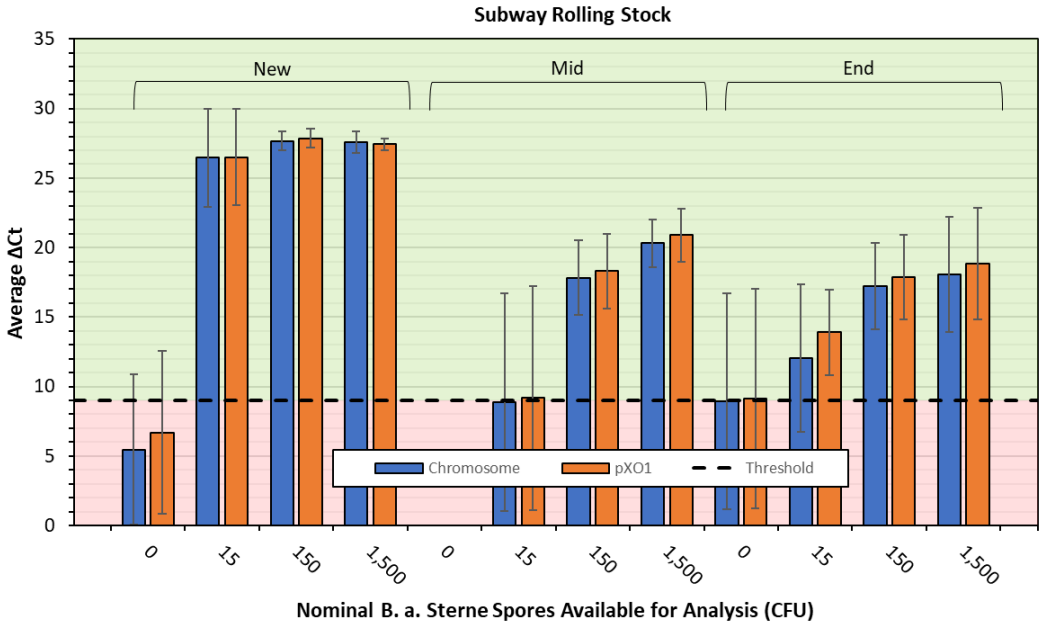

Figure 33. RV-PCR Analysis of *B. a.* Sterne Spores Recovered from Subway Rolling Stock Filters Using Chromosomal and pXO1 Gene Targets (Average ± One Standard Deviation for N ≥ 3 Replicates); Positive Response Equals ΔCt ≥ 9 (New, Mid, and End refer to service life or duty cycle of the filter)

3.3 Summary of Detection Accuracy

The results presented in Sections 3.1 and 3.2 can be further reduced to a high-level performance summary of detection accuracy of the two analytical methods and their associated false positive and negative frequencies. For culture method, false positive was defined as the identification (counting) of one or more presumptive *B. a.* Sterne spores when none were spiked onto the filter; false negative was defined as when no presumptive *B. a.* Sterne spores were counted, yet the filter was spiked, and an accurate detection when either no spores were identified in the 0-spike condition or identified for a filter spike condition. The positive identification for RV-PCR is as defined in Section 2.3.5 ($\Delta Ct \geq 9$ for both gene targets). A true positive was defined as correctly detecting *B. a.* Sterne in a spiked sample and a true negative as no detection of *B. a.* Sterne in a filter that was not spiked. A summary of those results is presented in Table 10 and expressed as percentage for each filter type assessed.

The false negative detections for culture were associated with either the 0-spike condition or when background flora of competing organisms did not permit identification of any colonies with *B. a.* Sterne morphology. The false positives for culture were attributed, and in some instances, confirmed with PCR analysis of selected colonies, to presumptive *B. a.* Sterne colonies not being correct, as discussed earlier.

The false negative for RV-PCR were believed to be due, in part, to poor physical recovery of spores from the filter as well as likely some loss in sensitivity due to the ambient particulate matter recovered along with the *B. a.* Sterne spores. The false positives for RV-PCR are attributed to likely cross-contamination that was suspected to have occurred early in the study as the method was being implemented.

Both methods performed poorest for the PM10 filters, which in part was believed due to having the lowest percent recovery of applied *B. a.* Sterne spores. The overall accuracy of the method properly detecting *B. a.* Sterne (combined true positives and true negatives) were 82% for culture and 85% for RV-PCR.

Table 11 gives similar summary of method response comparison as Table 10, but as a measure of the consistency of both methods yielding the same response of whether *B. a.* Sterne was detected or not.

Table 10. Summary of the Accuracy of the Method Response to Detect *B. a.* Sterne

Filter Type	Total Number of Samples			Culture / Molecular Response			
	Spiked	Not Spiked	Total	True Positive[a]	True Negative[b]	False Positive[c]	False Negative[d]
PM2.5	81	27	108	79 / 81	24 / 25	3 / 2	2 / 0
				98% / 100%	89% / 93%	11% / 7.4%	2.5% / 0%
PM10	82	29	111	64 / 68	22 / 25	7 / 4	18 / 14
				78% / 83%	76% / 86%	24% / 14%	22% / 17%
Bus	31	12	43	29 / 31	8 / 11	4 / 2	2 / 0
				94% / 100%	67% / 92%	33% / 17%	6.5% / 0%
HVAC	31	11	42	30 / 31	8 / 8	3 / 3	1 / 0
				97% / 100%	73% / 73%	27% / 27%	3.2% / 0%
Platform	27	9	36	25 / 23	5 / 9	4 / 0	2 / 4
				93% / 85%	56% / 100%	44% / 0%	7.4% / 15%
Rolling Stock	27	9	36	22 / 25	6 / 6	3 / 3	5 / 2
				81% / 93%	67% / 67%	33% / 33%	19% / 7.7%
Sum	279	97	376	249 / 259	73 / 74	24 / 14	30 / 20
All Filter Types				89% / 93%	75% / 76%	25% / 14%	10% / 7%

(a) Number of positive responses (for each analytical method) to filters that were spiked with *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of spiked samples.

(b) Number of negative responses (for each analytical method) to filters that were not spiked with *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of samples not spiked.

(c) Number of positive responses (for each analytical method) to filters that were not spiked with *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of samples not spiked.

(d) Number of negative responses (for each analytical method) to filters that were spiked with *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of spiked samples.

Table 11. Positive and Negative *B. a.* Sterne Detection Frequency for Culture and Molecular Analysis Methods

Filter Type	Total Number of Samples			Culture / Molecular Response			
	Spiked	Not Spiked	Total	Pos/Pos[(a)]	Neg/Neg[(b)]	Pos/Neg[(c)]	Neg/Pos[(d)]
PM2.5	81	27	108	79	22	3	4
				97.5%	81.5%	2.8%	3.7%
PM10	82	29	111	59	27	12	13
				72.0%	93.1%	10.8%	11.7%
Bus	31	12	43	29	8	2	4
				93.5%	66.7%	4.7%	9.3%
HVAC	31	11	42	30	7	2	3
				96.8%	63.6%	4.8%	7.1%
Platform	27	9	36	23	7	6	0
				85.2%	77.8%	16.7%	0.0%
Rolling Stock	27	9	36	24	6	1	5
				88.9%	66.7%	2.8%	13.9%
Sum	279	97	376	244	77	26	29
Percent				87.5%	79.4%	6.9%	7.7%

(a) Both the culture and molecular responses positively identified the presence of *B. a.* Sterne correctly in samples that were spiked; percentage calculated by dividing number of occurrences by number of spiked samples.

(b) Both the culture and molecular responses correctly gave a negative response to the presence of *B. a.* Sterne in samples that were not spiked; percentage calculated by dividing number of occurrences by number of samples not spiked.

(c) Culture yielded a positive response for *B. a.* Sterne and molecular response was negative for the presence of *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of samples analyzed.

(d) Culture yielded a negative response for *B. a.* Sterne and molecular response was positive for the presence of *B. a.* Sterne; percentage calculated by dividing number of occurrences by number of samples analyzed.

3.4 Ancillary Results

3.4.1 PES vs PVDF Membrane Filter Vials

One unexpected outcome of this project was the determination of the importance of consumable availability via supply chain. In the case of an actual event, time to results will be of utmost importance, and a method will need to be flexible enough to endure shortages of supplies. The method used here was tested in this way because of a back-order situation for the filter vials for the RV-PCR method that had to be resolved. During testing, an order for 10 boxes of filter vials with polyethersulfone (PES) membranes was placed in mid-October 2017 (GE Healthcare, Cat. No. AV125NPUPSU) and placed onto backorder and did not arrive until August 8, 2018. When the order was placed in mid-October, multiple vendors were showing the item number in stock. However, follow up with the vendors indicated they were not available, and the expected shipment date continued to be pushed to a later date. While in communication with the manufacturer (GE Healthcare), a filter vial with polyvinyldiene difluoride (PVDF) membrane was identified that was also available for similar applications and is manufactured as a stock item rather than a made-to-order item.

The filter vials with PES and PVDF membrane were compared side-by-side by spiking spores directly into the first extraction buffer (PBS with Tween 20 and Ethanol) followed by filtration then RV-PCR analysis. The test matrix in Table 12 shows the test matrix and number of replicates used to determine if the two product numbers were equivalent. Figures 34 and 35 show how the ΔCt and the final Ct values compare between the two different membranes. The binary result of "positive" or "negative" was unaffected and all ΔCt values were > 25 for all spike levels; the lone exception was a sample that leaked during enrichment (ΔCt was 23). All matched pair T_{final} Ct values were within ± 1.1 with the PVDF T_{final} Ct generally higher compared to its PES mate, indicating slightly less sensitivity. After this evaluation, testing continued using PES filter vials.

Table 12. Test Matrix for Comparing PES and PVDF Membranes

Target Spore Loading	PES Membrane (replicates)	PVDF Membrane (replicates)
0	2	2
1	4	4
150	5	5
1,500	5	5

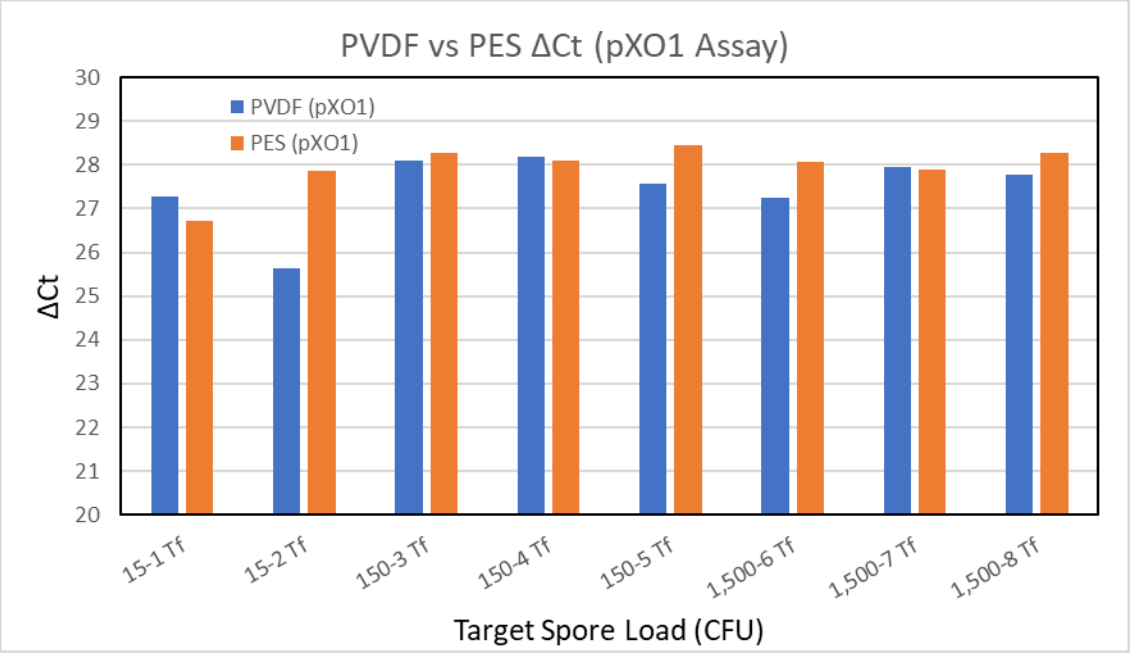

Figure 34. Side-by-Side Analysis of ΔCt Values Generated During the RV-PCR using PES and PVDF Filter Vials

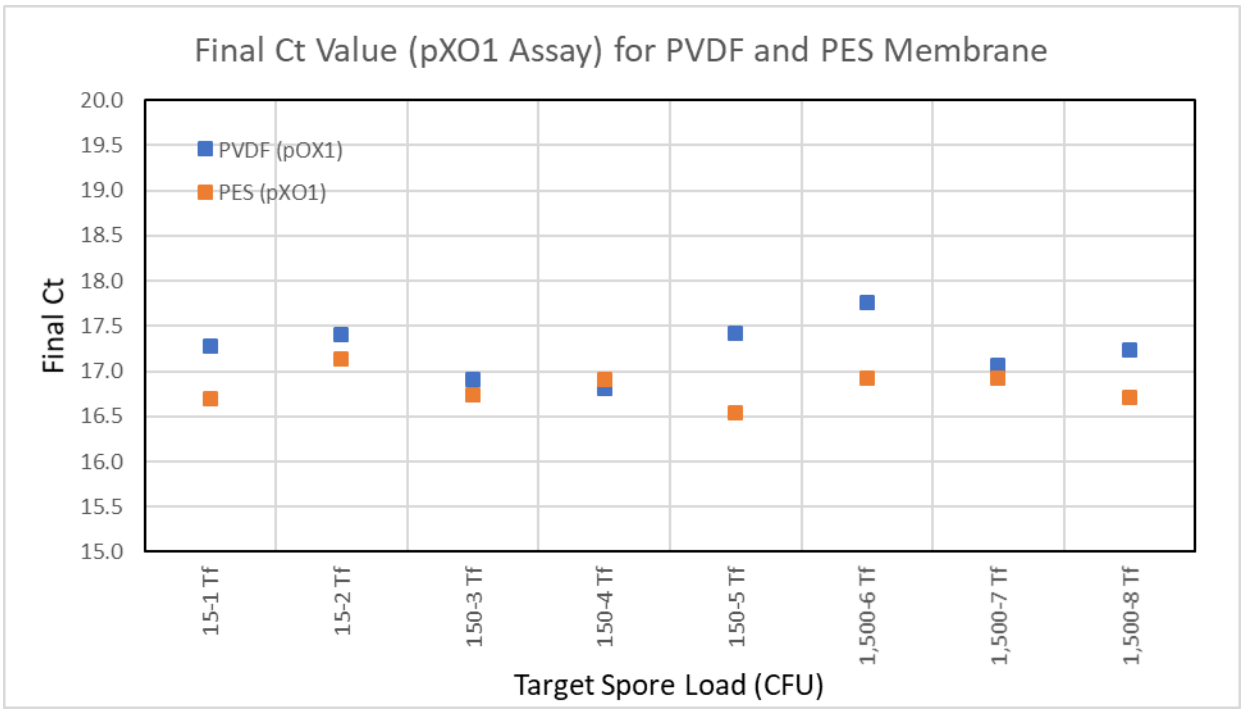

Figure 35. Side-by-Side Analysis of Final Ct Values Generated During the RV-PCR using PES and PVDF Filter Vials

4.0 QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance (QA)/quality control (QC) procedures were performed in accordance with the Testing and Evaluation (T&E II) Program Quality Management Plan (QMP), Version 1 and the TO 09 Quality Assurance Project Plan (QAPP) (Battelle, 2017). The QA/QC procedures and results are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, water baths, refrigerators/freezers) used at the time of the evaluation were verified as being certified, calibrated, or validated.

4.2 QC Results

QC efforts conducted during NAF testing included positive and negative controls for both spread plate samples and qPCR. In addition, both the spore bank and *B. a.* Sterne spike suspension concentrations (CFU/mL) were measured for each test so that known quantities of spores spiked onto the filter sample could be made.

Positive controls (PC) and no template controls (NTC) were included for each RV-PCR assay and in all cases performed as expected, with Ct values consistently in the mid-20s for the 50 pg PC and no Ct value generated for NTCs. 7500 Fast system performance was assessed according to internal standard operating procedure (SOPs) and maintained at regular intervals, monthly (optical and background calibration), every 6 months (dye calibration), and annual (RNase P calibration). For culture, the PC spore stock maintained a single morphological appearance consistent with *B. a.* Sterne throughout the study, as determined at the beginning of each trial. Media and reagents used for culture analysis were screened (negative controls) and had no growth, showing that reagents used were not the source of contamination.

4.3 Operational Parameters

Micropipettes, thermometers, and timers used were calibrated against a traceable standard at regular intervals (every 6 months or annual) and used only within acceptable calibration interval established by internal SOPs.

4.4 Audits

4.4.1 Performance Evaluation Audit

Performance evaluation (PE) audits were conducted to assess the quality of the results obtained during these experiments. Table 13 summarizes the PE audits that were performed. A 20-µL pipette (C20267) used for Master Mix addition and sample addition was found out of tolerance on 13 August 2018. Volumes pipetted were 5 µL (sample addition) and 20 µL (Master Mix addition) for RV-PCR analysis. The pipette was evaluated at three set volumes, 2 µL, 10 µL, and 20 µL and measurements ranged from 2.207 to 2.247; 10.183 to 10.334; and 20.247 to 20.407 for each of the set volumes, which is outside of specifications for internal SOPs. Controls on RV-PCR assay performed as expected and the pipette was adjusted before being returned to service.

Table 13. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10%	Passed calibration as found/as returned with one exception (C20267), as described above
Time	Compared to independent clock	± 2 seconds/hour	Passed calibration as found/as returned
Temperature	Compared to independent calibrated thermometer	± 2°C	Passed calibration as found/as returned

4.4.2 Technical Systems Audit

Observations and findings from the technical system audit (TSA) were documented and submitted to the laboratory technical lead for response. The TSA was conducted on June 15, 2018 to ensure that tests were being conducted in accordance with the appropriate QAPP and QMP. As part of the audit, test procedures were compared to those specified in the QAPP and work instructions, and data acquisition and handling procedures were reviewed. None of the findings of the TSA required corrective action.

4.4.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. Data were reviewed in December 2018 and January 2019. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported

results. All calculations performed on the data undergoing the audit were verified. Only minor issues were noted with the data, mostly data transcription errors that were corrected.

4.5 QA/QC Reporting

Each assessment and audit was documented in accordance with the QAPP and QMP. For these tests, findings were noted (none were significant) in the data quality audit, and no follow-up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. QA/QC procedures were performed in accordance with the QAPP.

4.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in this report.

5.0 SUMMARY OF METHOD OBSERVATIONS AND EXPERIENCES

While implementing the method, key observations and experiences were noted that will be useful to understand and/or take into consideration for future iterations or versions of the method. Key observations were:

- The PES membrane type of filter vial syringeless filter vials (GE Healthcare, Cat. AV125NPUPSU) was not always readily available from distributors and the lead time to manufacture was quoted to be 45 business days. The PES membrane type is a made-to-order product. The PVDF membrane type was a stock item and thus more readily available. Ten (10) boxes of PES filter vials were backordered from October 2017 to August 2018 (10 months). Both filter types were determined suitable for the RV-PCR assay.
- The RV-PCR method requires great care and diligence to implement effectively. Most notably, the RV-PCR method required changing gloves between samples for each step, which is onerous and time consuming. However, it was found necessary to minimize cross-contamination.
- Mixing the samples in the platform vortex resulted in loose lids. The method was revised from the original recommended 30-mL tubes to BD Falcon brand 50-mL conical tubes, and Parafilm was used to seal the lids during platform vortex steps.
- When applying vacuum to the filter vial manifold, the filtrate pooled in the manifold reservoir and contacted the bottom of the filter vials near the vacuum source. It is recommended to increase the depth of the bottom section of the manifold so that the filtrate does not pool and contact the bottom of filter vials.
- The filter analyses were performed in batches of 16 filter samples per trial using a single system based on initial trials to implement the methods. The 16 filter samples were the maximum that was deemed reasonable to process considering a normal 8:00 AM to 5:00 PM workday, without overtime and/or a night shift that may be used by the ERLN if actual samples were being processed. A single trial was completed over four consecutive days of operation, starting with filter sample spiking during the day and drying overnight. (Had these been actual filters collected post-biological release, this spiking activity would, obviously, not be performed by the ERLN.)
- A 16-hour incubation for RV-PCR was used in this study, but the EPA method typically uses a 9-hour incubation duration. It is reasonable to initially use the 9-hour incubation because the RV-PCR ΔCt was commonly over 15 for the AQ and non-AQ filters analyzed if the filters had 150 or more spores available. In practice, longer incubation times could be implemented for selected samples to confirm a negative response with a 9-hour incubation time.
- Estimated staff time to process 16 samples was approximately 64 hours and $1,500 of consumables. The 64 hours of staff time budget was approximately distributed by:
    - 8 hours for activities related specifically to the spiking of the filters being assessed, which was a requirement of the study, but not an activity that would be

performed had these been actual field samples. This task included time to prepare the stock suspensions, enumerate stock suspension, spike the filters, and complete associated documentation.

- 10 hours for spore recovery.
- 10 hours for culture analysis.
- 24 hours for RV-PCR analysis.
- Additionally, 4 hours was needed for PCR confirmation analysis of eight samples, when performed.

- Had the EPA 2012 method been followed without any changes (most notably the samples would not be split for analysis and either the culture only or the RV-PCR method only been used), a batch of 16 samples would take an estimated 34 labor hours and $1,000 in materials to perform culture analysis (with PCR confirmation of at least three colonies per sample). To process the same number of samples, an estimated 40 hours and $1,200 would be required using RV-PCR analysis. Each of the analytical methods would take 2 or 3 days.
    - The benefit to RV-PCR is that *B. a.* Sterne can be detected in sample matrices with high amounts of background flora and grime. For culture analysis, the growth of viable *B. a.* Sterne spores may be masked by background flora and grime in environmental samples, and therefore go undetected.

6.0 CONCLUSIONS AND RECOMMENDATIONS

The foremost conclusion is that filters recovered from both AQ and non-AQ filters may be useful to analyze for *B. anthracis* to help map the extent of contamination from biological incidents, recognizing there are limitations to their use. This conclusion is made based on the data showing that, even in the presence of other particulate matter having been collected on filters, *B. a.* Sterne spores that were spiked onto the filters could be recovered and successfully analyzed; however, the study results clearly indicate that the background flora and other particulate matter can adversely impact the method sensitivity and accuracy. Consequently, the NAF could be used to supplement results from other sampling plans but should not be relied upon solely as the definitive biological warfare incident mapping tool.

Both the culture and molecular methods can be adversely affected by the presence of ambient particulate matter on the filter being assessed. The methods' sensitivities were impacted by the presence of collected ambient particulate matter.

The filter substrate composition and/or structure may be important factor in the end-to-end performance of the methods because it could affect the physical recovery of organisms from the NAF being assessed. Furthermore, the results reported in this study are caveated by the fact that the NAFs were spiked by applying suspension droplets of *B. a.* Sterne, and that application method could impact physical recovery of *B. a.* Sterne spores.

The foremost recommendation is to assess the impact that spiking of *B. a.* Sterne spores onto the NAF substrates has on the recovery and subsequent analyses. The liquid suspension spiking method may bias the recovery efficiencies favorably (higher efficiency) or unfavorably. Specifically, it is recommended to expand the study by generating an aerosol of *B. a.* Sterne and then pulling the aerosol-laden air through the NAF rather than applying spores via a liquid suspension spike. The EPA method would then be implemented to recover and analyze for *B. a.* Sterne. This approach is expected to primarily affect spore recovery, which then may impact detection limits and or identification accuracy.

Other key conclusions and recommendations from the study include:

- RV-PCR can be used to positively identify viable *B. a.* Sterne in the presence of complex, dirty sample matrices of NAFs; however, background flora and grime also collected and present on the filter can impact the lower limit of detection and/or reduce the response to *B. a.* Sterne.
- Background flora and non-living material (dirt/grime) interferes with identification and quantifying *B. a.* Sterne using the traditional plate culture method, particularly for non-AQ filters. Presumptive *B. a.* Sterne colonies may not actually be *B. a.* Sterne because background flora can have an indistinguishable colony morphology, leading to false positives and an over-estimation of the number of actual *B. a.* Sterne spores. Conversely, the apparent *B. a.* Sterne quantity recovered can be biased low due to suppression of *B. a.* Sterne growth with competing background flora. It is possible for so much background flora to be present on NAFs such that the presence of *B. a.* Sterne cannot be made, potentially leading to false negatives.
- Priority should be placed on analyzing filters having the lowest loading of background particulate matter, to the extent that can be determined by the shortest duty cycle of non-AQ filters or by gravimetric analysis of AQ filters.
- Priority should be placed on use of PM2.5 filters over PM10 filters when both are available from the same area and would have been operating at an appropriate time relative to an incident.
- Recommend that the consumable supplies to execute the method be assessed for their availability in sufficient quantities to process the number of anticipated samples from an event. An alternative source of the consumable or qualifying an acceptable alternative material is recommended so that there is no single point failure in the supply chain.
- There was no apparent benefit of using a chromogenic agar for the filters tested. MYP media yielded results that were comparable to those obtained with SBA. BBCA media containing the selective supplement (Oxoid Cat. No. SR0230) yielded percent recovery of *B. a.* Sterne 5 to 7 times less than the counts from SBA.

The results from this study may be useful for sample plan development and interpretation of results following a large-scale biological incident where native air sample types provide utility or are available.

7.0 REFERENCES

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SAMPLE ANALYSIS OF NATIVE AIR FILTERS FOR CHARACTERIZATION AND EXTENT MAPPING OF BIOLOGICAL INCIDENTS

APPENDICES A-O

LIST OF APPENDICES

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APPENDIX A. FORMULATIONS OF RECIPES USED IN BIOLOGICAL TEST METHODS

Spore Production

Table 1. Components of Modified G Sporulation Medium

Ingredient	Amount/L
Yeast Extract	2.0 g
$(NH_4)_2SO_4$	2.0 g
$CaCl_2 \bullet 2H_2O$	0.03 g
$CuSO_4 \bullet 5H_2O$	0.005 g
$FeSO_4 \bullet 7H_2O$	0.0005 g
$MgSO_4 \bullet 7H_2O$	0.2 g
$MnSO_4 \bullet H_2O$*	0.06 g
$ZnSO_4 \bullet 7H_2O$	0.005 g
K_2HPO_4	0.5 g
dH_2O	1000 mL

*$MnSO_4 \bullet H_2O$ substituted for $MnSO_4 \bullet 4H_2O$. If $MnSO_4 \bullet 4H_2O$ is used, add 0.05 g.

Table 2. Components of Leighton-Doi Sporulation Medium

Component	Amount/L
KCl	1.88 g
$CaCl_2$	0.29 g
$FeSO_4$ x 7 H_2O	0.003 g
$MnSO_4$ x H_2O	0.0017 g
$MgSO_4$ x 7 H_2O	0.025 g
Dextrose	0.9 g
Nutrient Broth	16.0 g

Table 3. Duplex Assay Conditions

Component (Duplex Assay)	**Volume for one reaction (µL)**
2x FAST PCR Mix	12.5
PCR-grade water	1.5
pXO1 For Primer (25 µM)	1
pXO1 Rev Primer (25 µM)	1
pXO1 Probe (2 µM)	1
chromosome For Primer (25 µM)	1
chromosome Rev Primer (25 µM)	1
chromosome Probe (2 µM)	1
Template	5
Total volume	**25**

APPENDIX B. DUPLEX VERSUS SINGLEPLEX REAL-TIME PCR SPOT REPORT

SPOT REPORT

on

Task 4 Analysis Method Comparison: Duplex versus Singleplex PCR

Prepared under
Contract Number EP-C-15-002
Task Order Number 0009
EPA Task Order Contracting Officer Representative
Worth Calfee

Prepared by
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11/23/16

Purpose

The purpose of this experiment was to determine whether the *Bacillus anthracis* chromosome and pXO1 real-time PCR assays from Letant et al. (2011) and the EPA protocol EPA/600/R-12/577 (2012) can be performed as a duplex assay. This experiment will also determine the LOQ of the DNA (DNA) extracted from *B. anthracis Sterne* 34F2 (NR-1400, BEI Resources) and the limit of detection of the spore suspension that will be used to dose filters for this study. If successful, the duplex assay will produce the same quality data as the assays run in singleplex, and thus the duplex assay can be used instead of the singleplex assays, increasing the efficiency of analysis. The duplex method was the analytical method proposed, but does differ from the EPA's current singleplex approach. The duplex assay proposed by Battelle also differed from the EPA's RV-PCR method in that the DNA is not extracted prior to analysis, and instead the assay is run directly from spores. If the assay is successful in detecting and quantifying directly from spores, this will also increase the efficiency of the assay by removal of the DNA extraction step from the analytical process.

Materials and Methods

Materials

The materials used in the conduct of the assay are listed below:

- *Bacillus anthracis* Sterne 34F2 (NR-1400, BEI Resources) DNA.
- *Bacillus anthracis* Sterne 34F2 (NR-1400, BEI Resources) spore suspension.
- TaqMan Fast Advanced PCR Master Mix (4444557, Life Technologies).
- Unlabeled Sequence Detection Primers, (4304971, Life Technologies).
- Custom TaqMan Probe-MGBNFQ, (4316034, Life Technologies).
- MicroAmp Fast Optical 96-well Reaction Plate with Barcode (4346903, Life Technologies).
- MicroAmp Optical 8-Cap strips (4323032, Life Technologies).

Methods

The duplex master mix (Table 1) and singleplex master mixes (Table 2) were distributed into a single 96-well reaction plate. The master mix was prepared using the conditions described in the 2012 EPA protocol except that the non-fluorescent quencher of the DNA probes for this method were minor groove binder (MGB) groups rather than Black Hole Quencher.

Table 1. Duplex Assay Conditions

Component (Duplex Assay)	**Volume for One Reaction** (µL)
2x FAST PCR Mix	12.5
PCR-grade water	1.5
pXO1 For Primer (25 µM)	1
pXO1 Rev Primer (25 µM)	1
pXO1 Probe (2 µM)	1
chromosome For Primer (25 µM)	1
chromosome Rev Primer (25 µM)	1
chromosome Probe (2 µM)	1
DNA Template	5
Total volume	**25**

Table 2. Singleplex Assay Conditions

Component (Singleplex Assay)	**Volume for One Reaction** (µL)
2x FAST PCR Mix	12.5
PCR-grade water	4.5
pXO1 or Chromosome For Primer (25 µM)	1
pXO1 or Chromosome Rev Primer (25 µM)	1
pXO1 or Chromosome Probe (2 µM)	1
DNA Template	5
Total volume	**25**

A 10-fold dilution series of DNA extracted from *Bacillus anthracis* Sterne 34F2 (NR-1400, BEI Resources) ranging from 50 femtograms to 5 x 10^6 femtograms per 5 µL was used as a standard curve for the singleplex assays and the duplex assay. An additional four reactions of the DNA were included for each assay at 5 x 10^4 femtograms (50 pg) because the EPA method specified running positive controls at this concentration. A 10-fold dilution series of unextracted *Bacillus anthracis* Sterne 34F2 spores ranging from 5 spores to 5 x 10^5 spores per 5 µL was also included on each assay. No template controls (negative controls) were run in quadruplicate for each assay.

The reaction plate was run on a 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems) using the conditions outlined in Table 3 and data was analyzed using 7500 Fast System Software v1.4.0. The cycling conditions were set as described in the 2012 EPA method except that the extension time was increased from 20 seconds to 30 seconds in this method. This change was made because the thermocycler would not accept an extension time of less than 24 seconds and the Battelle real-time PCR assay for BaS is conducted with 30s extension times.

Table 3. Thermocycler Conditions on 7500 Fast

Stage	Temperature (°C)	Time (Min:Sec)	Repeat	Ramp Rate
1	50	2:00	1	Auto (Fast 7500 Mode)
	95	10:00		Auto (Fast 7500 Mode)
2	95	0:05	45	Auto (Fast 7500 Mode)
	60	0:30*		Auto (Fast 7500 Mode)

*7500 Fast does not allow an extension time of less than 24 seconds. The 2012 EPA method specifies an extension time of 20 seconds.

Limit of detection was defined as the lowest concentration of spores that resulted in a signal that crossed the threshold for both replicates. The limit of quantification was defined as the lowest quantification was the lowest concentration of the DNA standard curve that resulted in a signal that crossed the threshold for both replicates. A Student's paired t-test was performed on the Ct values from the singleplex compared to the duplex and duplex assay was considered successful when there was no significant difference found between the duplex and singleplex (*P*-value greater than 0.05 = no significant difference between the assays). Ct values of 36 or higher were excluded from analysis or when one of the two replicates was undetected.

Results and Discussion

The results of this experiment demonstrate that the *Bacillus anthracis* chromosome and pXO1 real-time PCR assays can be performed in a duplex format to reduce the reagent cost and labor associated with screening *B. anthracis Sterne* DNA (Table 4: chromosome, paired Student's t-test *P*-value = 0.747; Table 5: pXO1, paired Student's t-test *P*-value = 0.354) and unextracted spores (Table 6: chromosome, paired Student's t-test *P*-value = 0.554; Table 7: pXO1, paired Student's t-test *P*-value = 0.305).

Table 4. Chromosome Real-Time PCR Assay with DNA

Concentration (DNA in fg)	Singleplex Ct Values	Singleplex Average Ct	Singleplex Standard Deviation	Duplex Ct Values	Duplex Average Ct	Duplex Standard Deviation	%ΔCt ((singleplex-duplex)/singleplex)
5×10^6	15.3	15.3	0.0	15.1	15.2	0.1	0.6%
5×10^6	15.3			15.3			
5×10^5	18.7	18.6	0.1	18.5	18.5	0.0	0.4%
5×10^5	18.5			18.5			
5×10^4	21.8	22.1	0.2	22.1	22.0	0.2	0.2%
5×10^4	22.0			22.0			
$5 \times 10^{4*}$	22.1			22.2			
$5 \times 10^{4*}$	22.1			22.2			
$5 \times 10^{4*}$	22.2			22.1			
$5 \times 10^{4*}$	22.3			21.8			
5×10^3	25.6	25.6	0.1	25.5	25.5	0.1	0.1%
5×10^3	25.5			25.6			
5×10^2	29.0	29.0	0.0	29.4	29.4	0.1	-1.1%
5×10^2	29.0			29.3			
5×10^1	33.5	32.8	1.0	32.7	32.5	0.2	0.9%
5×10^1	32.1			32.3			

*These replicates were run as unknown samples, so were not included in the standard curve. The EPA method recommended running three positive controls at 50 pg (5×10^4 fg) per 96- well plate, so an additional four wells were included at this concentration per assay.

Table 5. pXO1 Real-Time PCR Assay with DNA

Concentration (DNA in fg)	Singleplex Ct Values	Singleplex Average Ct	Singleplex Standard Deviation	Duplex Ct Values	Duplex Average Ct	Duplex Standard Deviation	%ΔCt ((singleplex-duplex)/singleplex)
5×10^6	16.3	16.3	0.0	16.0	16.0	0.1	1.6%
5×10^6	16.2			16.0			
5×10^5	19.5	19.5	0.0	19.2	19.2	0.1	1.1%
5×10^5	19.5			19.3			
5×10^4	22.9	22.9	0.0	22.8	22.7	0.1	0.9%
5×10^4	22.9			22.8			
$5 \times 10^{4*}$	22.9			22.8			
$5 \times 10^{4*}$	22.9			22.6			
$5 \times 10^{4*}$	23.0			22.7			
$5 \times 10^{4*}$	22.9			22.5			
5×10^3	26.4	26.3	0.2	26.2	26.2	0.0	0.2%
5×10^3	26.1			26.2			
5×10^2	29.7	29.8	0.1	29.7	29.5	0.3	1.1%
5×10^2	29.9			29.2			
5×10^1	31.7	31.7	N/A	33.1	32.9	0.3	N/A
5×10^1	Undetected			32.7			

*These replicates were run as unknown samples, so were not included in the standard curve. The EPA method recommended running three positive controls at 50 pg (5×10^4 fg) per 96- well plate, so an additional four wells were included at this concentration per assay.

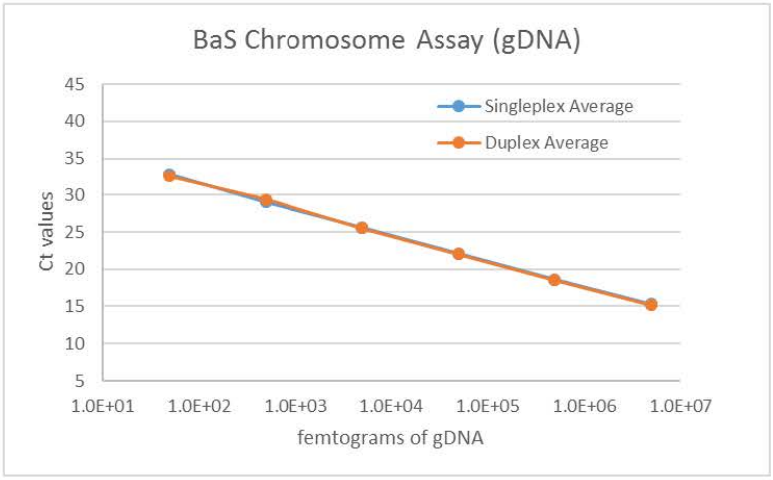
Table 6. Chromosome Real-Time PCR Assay Results with Spore Preparation

Concentration (spores)	Singleplex Ct Values	Singleplex Average Ct	Singleplex Standard Deviation	Duplex Ct Values	Duplex Average Ct	Duplex Standard Deviation	%ΔCt ((singleplex-duplex)/singleplex)
5×10^5	21.2	21.0	0.3	21.2	21.4	0.3	-2.0%
5×10^5	20.8			21.6			
5×10^4	24.8	24.7	0.0	25.0	24.9	0.2	-0.5%
5×10^4	24.7			24.7			
5×10^3	28.2	28.3	0.2	28.4	28.3	0.2	0.2%
5×10^3	28.5			28.2			
5×10^2	32.1	32.2	0.2	32.0	32.1	0.1	0.4%
5×10^2	32.3			32.1			
5×10^1	36.1	35.9	0.3	39.8	39.8	0.1	N/A
5×10^1	35.7			39.7			
5×10^0	Undetected	45.0	N/A	37.2	39.2	2.9	N/A
5×10^0	Undetected			41.2			

Table 7. pXO1 Real-Time PCR Assay Results with Spore Preparation

Concentration (spores)	Singleplex Ct Values	Singleplex Average Ct	Singleplex Standard Deviation	Duplex Ct Values	Duplex Average Ct	Duplex Standard Deviation	%ΔCt ((singleplex-duplex)/singleplex)
5×10^5	21.3	21.3	0.1	21.2	21.2	0.1	0.4%
5×10^5	21.2			21.1			
5×10^4	24.6	24.6	0.0	24.5	24.5	0.0	0.6%
5×10^4	24.7			24.5			
5×10^3	28.2	28.2	0.0	28.3	28.2	0.2	-0.1%
5×10^3	28.2			28.1			
5×10^2	31.5	31.4	0.1	31.6	31.5	0.1	-0.2%
5×10^2	31.3			31.4			
5×10^1	35.7	34.6	1.5	36.3	36.3	0.1	N/A
5×10^1	33.5			36.2			
5×10^0	Undetected	45.0	N/A	33.6	35.9	3.2	N/A
5×10^0	Undetected			38.1			

The assay performance (slope, intercept and R^2 values, Table 8) from the DNA standard curve did not show significant change nor did the cycle threshold (Ct) values when the assays were run individually or in the duplex format on either spores or extracted DNA (Figures 1 and 2). All negative controls were undetected when run in duplex or singleplex assay format. Furthermore, the percent delta Ct (defined as (singleplex Ct- duplex CT)/singleplex Ct)) for each input (spore or DNA) was calculated and since all %ΔCT were within 2% (Tables 4 through 8), this further supports that the performance of the duplex matches that of the singleplex assay.



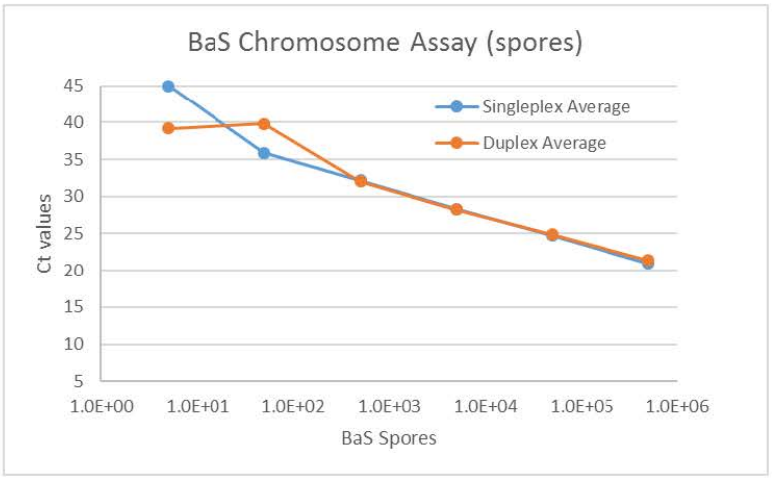


Figure 1. Chromosome Real-Time PCR Assay Results. Top: DNA titration. Bottom: spore titration

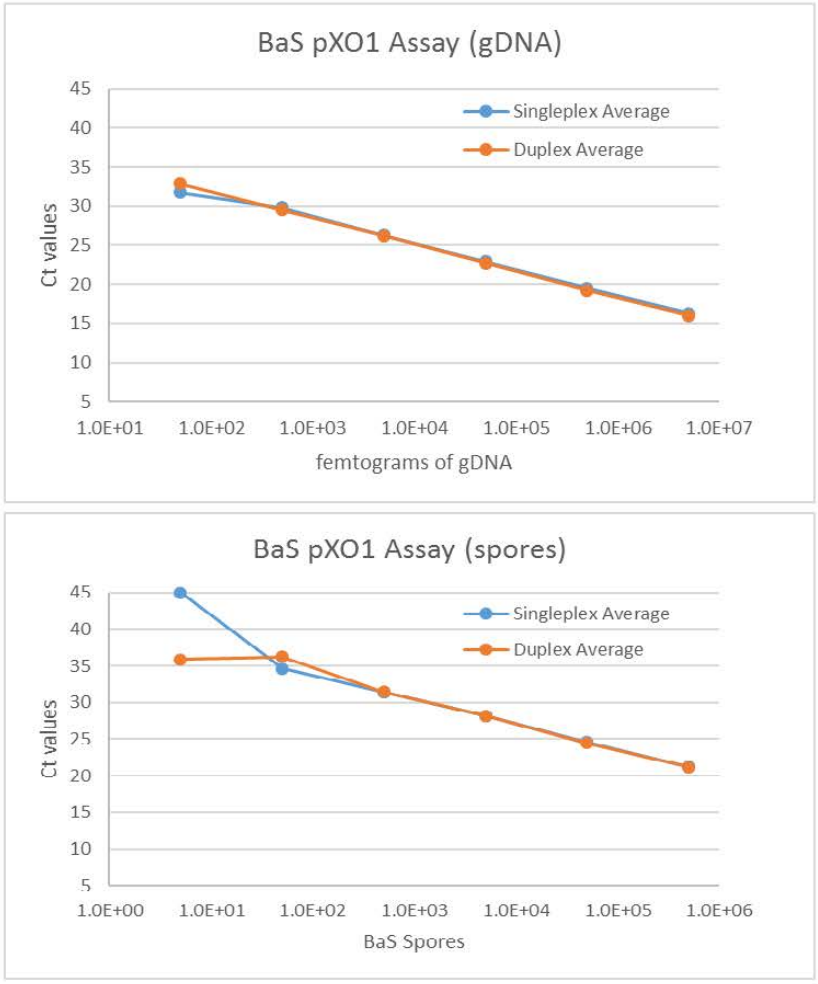


Figure 2. pXO1 Real-Time PCR Assay Results. Top: DNA and Bottom: spores

Table 8. Standard Curve (DNA) Performance per Assay

Assay	Slope	Intercept	R^2
Singleplex Chromosome assay	-3.50	38.58	0.997
Duplex Chromosome assay	-3.50	38.57	0.999
Singleplex pXO1 assay	-3.25	38.13	0.996
Duplex pXO1 assay	-3.38	38.64	0.999

For the *B. anthracis* Sterne chromosome target, the limit of quantification was 50 femtograms of DNA when run in a singleplex or duplex format; and the limit of detection (two replicates) was 500 spores for the singleplex and the duplex format (Table 9).

Table 9. Summary of Limit of Quantification and Limit of Detection for Both Assays When Run in Singleplex or Duplex Format

Assay	Limit of quantification (DNA)	DNA (*P*-value*)	Limit of detection (spores)	spores (*P*-value*)
Singleplex Chromosome	50 fg	0.747	50	0.554
Duplex Chromosome	50 fg		5	
Singleplex pXO1	500 fg	0.354	50	0.305
Duplex pXO1	50 fg		5	

*P-value from paired Student's t-test comparing Ct values between the singleplex and duplex assays on DNA or spores for each target.

For the *B. anthracis* Sterne pXO1 target, the limit of quantification was 500 femtograms of Total DNA when run in a singleplex format (one of the replicates at 50 fg being undetected) and 50 femtograms when run in a duplex format; and the limit of detection (two replicates) was 50 spores for both the singleplex format and the duplex format.

Conclusions and Recommendations

The duplex assay was able to perform as well as the singleplex assays targeting the *B. anthracis* chromosome and pXO1 targets, detecting as low as 50 femtograms of extracted DNA. Both the duplex and singleplex assays were able to amplify directly from spores, without the need for DNA extraction; with the ability to accurately detect the presence of 500 spores from the chromosome target or 50 spores from the pXO1 target. This difference in detection between the chromosome and pXO1 targets may be due to a difference in copy number present in the spores.

We recommended to proceed with testing of spiked filters using the duplex RV-PCR method proposed. The duplex assay produces the same data as the singleplex assay as demonstrated by Student's paired t-test and will save both time and reagent costs. Assaying directly from spores produces signal that can be used to reliably detect down to 500 spores. This method further reduces the time and cost of the assay by removing the DNA extraction step and reagents. To further determine the LOD for direct analysis from spores, a titration between 500 and 50 spores will need to be conducted, as at 50 and 5 spores some signal is produced, however, it was not consistent or linear. Therefore, based on the test conducted direct testing from spores recovered from the filters is recommended for filter swatches spiked with greater than 500 spores to reduce cost and ensure accurate detection and quantification. Taken together, the duplex RV-PCR method to directly assay spores recovered from the filter will streamline the RV-PCR method and is in line with the method originally proposed for this study. The results of the duplex RV-PCR method are equivalent to that of singleplex RV-PCR, and both can be used directly on spores for accurate detection of down to 500 spores.

APPENDIX C. WORK INSTRUCTION FOR DOSING FILTER SWATCHES WITH *BACILLUS ANTHRACIS* SPORES

WORK INSTRUCTION FOR DOSING FILTER SWATCHES WITH *BACILLUS ANTHRACIS* SPORES

I. PURPOSE/SCOPE

To dose filter swatches for the spore recovery testing.

II. Analyst/Reviewers

Role	Name	Initials	Date
Analyst			
Analyst			
Reviewer			

III. MATERIALS/EQUIPMENT

Materials

Item	Manufacturer	Lot Number	Exp. Date	Storage Temp.	Initials & Date
Bacillus anthracis 34F2 spores	Inhouse	34F2101716	TBD	2-8 °C	
Petri dishes				R.T.	
Sterile DI water	Teknova			R.T.	
Blood Agar	BBL				
15 mL tubes			N/A	R.T.	
1.5 or 2 mL tubes	Eppendorf		N/A	R.T.	
Sterile forceps			N/A	R.T.	
Polyester Mesh	McMaster Carr	8218T13	N/A	R.T.	

Equipment

Item	Manufacturer	Serial Number	Thermometer /Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)	The Baker Company	57553	N/A	9/2018	
Micropipette Type:L1000	Rainin		N/A		
Micropipette Type:L200	Rainin		N/A		
Micropipette Type:L20	Rainin		N/A		
vortex	VWR	N/A	N/A	N/A	
Refrigerator	Fisher	C3274822	115	4/2018	

N/A = Not Applicable

Other Supplies and Equipment

- Micropipette filter tips
- Biohazard bags
- Bench coat

- Filters

IV. PROCEDURE

A. Decontaminate the BSC with DNA Erase, bleach and isopropanol prior to use.
   1. Decontaminated by _____ Date_____
B. Cut mesh support and treat with UV.
   1. Remove sterile scissors from packaging a wipe with DNA erase, bleach and Isopropanol.
   2. Cut 4 cm^2 mesh support and expose to UV crosslinker for 5 minutes.
      UV start time _____ End time _____ Initials/date_____
C. Cut filter swatches
   1. Label petri dishes with the corresponding Filter ID.
   2. With sterile scissors, cut out the appropriate number of filter swatches from the filter with the dimensions 4 cm^2 (See diagram).
      a. For 47 mm filters, these will be cut into quarters, leading to slightly smaller than 4 cm^2 swatches.
   3. Zero-spike filter swatches will be added directly to 50 mL conical tubes and mesh support will be placed over the filter swatch prior to opening B. a. Sterne stock tubes in the Biosafety cabinet
   4. Using sterile forceps, transfer the filter swatches to the labeled petri dishes.
D. Name filters
   1. Use the T09Filter_Sample_Log_Nomenclature added.xlsx (in Native Filters box folder) to determine the filter ID for each filter.
      i. AAA-BBB-CCC-R#-Date
         1. AAA = Filter Type
         2. BBB = Geographical Region
         3. CCC = Particle Loading
         4. R# = Replicate number
         5. Date = MM/DD/YY
      ii. Electronically populate below table with sample names to be prepared on each day from the Sample Log.

Sample #	Filter type	Geographic Region	Particle Load	Filter Vial Type	Spore Spike level	Filter ID	Date Cut /initials	Date Dosed /initials
1	PM10	SC	AVG	PVDF	0	1-PM10-SC-AVG-0		
2	PM10	SC	AVG	PVDF	0	2-PM10-SC-AVG-0		
3	PM10	SC	AVG	PVDF	30	3-PM10-SC-AVG-30		
4	PM10	SC	AVG	PVDF	30	4-PM10-SC-AVG-30		
5	PM10	SC	AVG	PVDF	300	5-PM10-SC-AVG-300		
6	PM10	SC	AVG	PVDF	300	6-PM10-SC-AVG-300		
7	PM10	SC	AVG	PVDF	3,000	7-PM10-SC-AVG-3,000		
8	PM10	SC	AVG	PVDF	3,000	8-PM10-SC-AVG-3,000		
9	PM10	SC	High	PVDF	0	9-PM10-SC-High-0		
10	PM10	SC	High	PVDF	0	10-PM10-SC-High-0		
11	PM10	SC	High	PVDF	30	11-PM10-SC-High-30		
12	PM10	SC	High	PVDF	30	12-PM10-SC-High-30		
13	PM10	SC	High	PVDF	300	13-PM10-SC-High-300		
14	PM10	SC	High	PVDF	300	14-PM10-SC-High-300		
15	PM10	SC	High	PVDF	3,000	15-PM10-SC-High-3,000		
16	PM10	SC	High	PVDF	3,000	16-PM10-SC-High-3,000		

E. Spike Swatches

1. Prepare dosing stocks

i. Fill in information from stock tube.

Organism	Lot	Prep date	Concentration	Date of enumeration	Entered/verified by:
B. anthracis Sterne	34F2101716		3.2 X 10^8 cfu/mL	04/09/2018	

ii. Target stock concentration(s).

Stock #	Organism	Lot	Prep date	Concentration	Total spores per 100 µL	Entered/verified by:
1	*B. anthracis* Sterne	34F2101716		3.0 X 10^4 cfu/mL	3,000	
2	*B. anthracis* Sterne	34F2101716		3.0 X 10^3 cfu/mL	300	
3	*B. anthracis* Sterne	34F2101716		3.0 X 10^2 cfu/mL	30	

iii. Prepare dilutions of stock in sterile DI water. Vortex stock on high for 30 seconds prior to preparing dilutions.
Show calculations:

$(3.2 \times 10^8$ cfu/mL)*(X)=$(3.0 \times 10^7$ cfu/mL)(1mL) → 94µL of sample into 906µL H20

$(3.0 \times 10^7$ cfu/mL)*(X)=$(3.0 \times 10^6$ cfu/mL)(1mL) → 100µL of sample into 900µL H20

$(3.0 \times 10^6$ cfu/mL)*(X)=$(3.0 \times 10^5$ cfu/mL)(1mL) → 100µL of sample into 900µL H20

$(3.0 \times 10^5$ cfu/mL)*(X)=$(3.0 \times 10^4$ cfu/mL)(1mL) → 100µL of sample into 900µL H20

$(3.0 \times 10^4$ cfu/mL)*(X)=$(3.0 \times 10^3$ cfu/mL)(1mL) → 100µL of sample into 900µL H20

$(3.0 \times 10^3$ cfu/mL)*(X)=$(3.0 \times 10^2$ cfu/mL)(1mL) → 100µL of sample into 900µL H20

Dilutions Prepared By: _____ Date/Initials: _____

2. Dose swatches

Geographic Region	Filter Type	Particle Load	Spike Levels	Filter ID
South Carolina	PM10	Avg	0/30/300/3000	Q9563526 (04/27/2011) & Q0548769 (09/30/2011). Consume the Q9563526 filter first, if extra material is needed use Q0548769. Note Filter IDs used on WIs.
		High	0/30/300/3000	Q9563551 (07/02/2011) & Q9563559 (07/20/2011). Consume the Q9563551 filter first, if extra material is needed use Q9563559. Note Filter IDs used on WIs.

i. Prior to dosing filters, immediately vortex the stock for 30 seconds.
ii. Per swatch, transfer a 120 µL aliquot of the appropriate Stock tube (Low, Med., or High) into a 1.5 ml tube.
iii. Place twenty 5 µL droplets onto each filter swatch as shown in the below diagram. The same pipet tip can be used to place all twenty droplets, dispose of the 120 µL aliquot once each swatch has been dosed.
iv. Air dry in BSC overnight.
Start time: _____ Date/Initials: _____
End time: _____ Date/Initials: _____
v. Cover filter swatches with top to petri dish, use Parafilm to seal the petri dishes, and store at 4°C until ready for recovery.

3. Enumerate stock
i. Serially dilute the suspension in Sterile water (if necessary).
1. Fill 2mL dilution tubes for each sample with 900µL of Sterile water and label appropriately.
2. Vortex the stock on high for 30 seconds.
3. Transfer 100 µL of the stock into the first dilution tube containing 900µL of Sterile water. Recap the tube and vortex it on high for 30 seconds. This is the 10^{-1} suspension.

ii. Spread 100 µL aliquots of dilutions onto Blood Agar in triplicate.
iii. Incubate plates
    1. Invert the plates and incubate them at 37°C ± 2°C for 18 – 24 hours. *B. anthracis* produces flat or slightly convex, 2 – 5 mm colonies, with edges that are slightly irregular and have a "ground glass" appearance.

    Incubation start Date/Time: _____ Initials: _____
    Incubation end Date/Time: _____ Initials: _____
iv. Plate counts
    1. Record counts in the below table.

Stock	Media Type	Volume/ (Dilution on Plate)	Plate Counts			Average Counts	CFU/mL
			Plate 1	Plate 2	Plate 3		
1 (3.0×10^4 cfu/mL)	Blood Agar	100 µL/ (10^{-1})					
2 (3.0×10^3 cfu/mL)	Blood Agar	100 µL/ (10^{-1})					
3 (3.0×10^2 cfu/mL)	Blood Agar	100 µL/ (10^{-1})					

Recorded By: _____ Date/Initials: _____

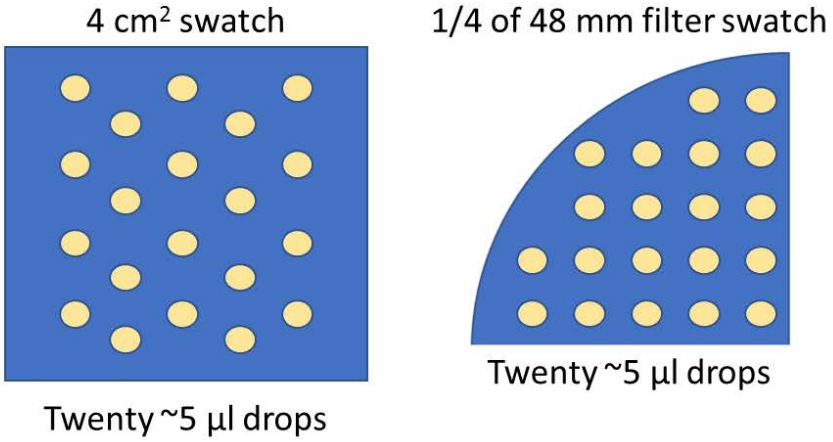


Figure 1. Dispersal layout for dosing filters.

APPENDIX D. WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

I. PURPOSE/SCOPE

To recover *B. anthracis* spores from air filters following the ***BACILLUS Analytical Methods 004*** published by the EPA December 2012.

II. MATERIALS/EQUIPMENT

Materials

Item	Manufacturer	Lot Number	Exp. Date	Storage Temp.	Initials & Date
extraction buffer with Tween® 20	Inhouse			2-8 °C	
extraction buffer without Tween® 20	Inhouse			2-8 °C	
10X PBS	Teknova			2-8	
1X PBS (pH 7.4)	Teknova			2-8	
BHI broth	Inhouse			2-8	
Conical tubes, 15 mL			N/A	R.T.	
Conical Tube 50mL			N/A	R.T.	
Screw top flask, 250 mL	Corning		N/A	R.T.	
0.45 µm filter vials	Whatmon		N/A	R.T.	
2mL screw cap tubes	VWR		N/A	R.T.	

N/A = Not Applicable

Equipment

Item	Manufacturer	Serial Number	Thermometer/ Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)	The Baker Company	57553	N/A	9/2018	
Micropipette Type:L1000	Rainin		N/A		
Incubator Shaker	New Brunswick	590644988	C25323	7/18/18	
Refrigerator	Fisher	35840	115	4/2018	
Platform Vortexer	VWR	5041	N/A	N/A	

N/A = Not Applicable

Filters – Electronically update this table with samples names from the Sample Log

Sample #	Filter type	Geographic Region	Particle Load	Filter Vial Type	Spore Spike level	Filter ID
1	PM10	SC	AVG	PVDF	0	1-PM10-SC-AVG-0
2	PM10	SC	AVG	PVDF	0	2-PM10-SC-AVG-0
3	PM10	SC	AVG	PVDF	30	3-PM10-SC-AVG-30
4	PM10	SC	AVG	PVDF	30	4-PM10-SC-AVG-30
5	PM10	SC	AVG	PVDF	300	5-PM10-SC-AVG-300
6	PM10	SC	AVG	PVDF	300	6-PM10-SC-AVG-300
7	PM10	SC	AVG	PVDF	3,000	7-PM10-SC-AVG-3,000
8	PM10	SC	AVG	PVDF	3,000	8-PM10-SC-AVG-3,000
9	PM10	SC	High	PVDF	0	9-PM10-SC-High-0
10	PM10	SC	High	PVDF	0	10-PM10-SC-High-0
11	PM10	SC	High	PVDF	30	11-PM10-SC-High-30
12	PM10	SC	High	PVDF	30	12-PM10-SC-High-30
13	PM10	SC	High	PVDF	300	13-PM10-SC-High-300
14	PM10	SC	High	PVDF	300	14-PM10-SC-High-300
15	PM10	SC	High	PVDF	3,000	15-PM10-SC-High-3,000
16	PM10	SC	High	PVDF	3,000	16-PM10-SC-High-3,000

Other Supplies and Equipment

- Forceps
- Biohazard bags
- Bleach
- 25mL Serological Pipettes
- 5mL Serological Pipettes
- Pipette aid
- Ziplock bags
- Bench paper

III. PROCEDURE

A. RV-PCR Sample Processing: Spore Recovery for Air Filter Samples

Note: Gloves should be used and changed between samples and as indicated below.

1. Prior to sample processing, prepare the following items:

□ Fill sample tube rack with 50 mL screw cap conical tubes and label as appropriate.

Performed By: _____ Date: _____

WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

- □ In a BSC, attach the vacuum manifold to the vacuum trap, waste container (with 250 ml of bleach), and vacuum source. Attach the filter vials to the manifold, using outer rows first. Verify that all filter vials are completely pushed down. Place a red pull tab tapered plug in each filter vial.
- □ 15 mL and 50 mL conical tube per sample
- □ For each sample prepare one 2 mL Eppendorf tube for enumeration.
- □ Document filter vial and sample tube labels.
- □ Prepare aliquots of the Extraction Buffer with Tween and Extraction Buffer without Tween in a 250mL screw capped bottle for washes.
  ~20mL/sample * _____ number of samples = _____ mL

2. Using gloved hands, place mesh support over filter swatches (dirty side facing mesh) in 50 mL tubes by holding the swatches to the side of the tube with sterile forceps and placing the coiled mesh support on top. Place swatch so that the spiked side is facing the inside of the tube. Ensure the sample and mesh are in the bottom half of the tube (avoiding the conical portion). Do not touch any other surface with gloved hand that was used to position mesh over spiked swatch in conical tube. Change gloves in between each sample.
   Note: The support keeps the swatch from interfering with pipetting activities and improves efficiency of spore extraction during vortexing.

3. **First Extraction:** Bleach wipe each tube. Add 15 mL cold (4°C) extraction buffer **with** Tween® 20 + Ethanol to samples. Uncap one tube at a time, add extraction buffer, close tube and Parafilm cap prior to moving to the next sample tube. Bleach wipe each tube.

4. Place tube rack in plastic bag, seal, double bag and bleach the bag prior to transferring to the platform vortexer located outside the BSC.

5. Vortex samples for 20 minutes on Platform vortexer with the speed set to 7. Make sure to clamp tube rack from the top and bottom of the vortexer.

   Start time: _____ End Time: _____ Speed: _____

6. After vortexing, transfer sample tube rack to the BSC. Remove tube rack from plastic bag and discard the bag.

7. Vortex up to 8 sample tubes on a single-tube vortexer in the BSC, for 3 – 5 seconds each. Let sit for at least 2 minutes to allow large particles to settle prior to aliquoting (for samples containing debris). If necessary, allow the tubes to settle for up to 5 minutes.

8. Remove Parafilm, bleach wipe the tube, uncap tubes one at a time. Using a 10 mL serological pipet carefully transfer 12.5 mL to clean clearly labeled 50 mL conical tube (extract pool). Recap 50 mL conical tube and move to the next sample. Change serological pipets and gloves between samples.

Performed By: _____ Date: _____

WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

9. **Second Extraction**: Add 10 mL of cold (4°C) extraction buffer + Ethanol **without** Tween® 20 to each sample tube, one at a time with a new 10 mL serological pipet for each sample and recapping each sample tube after buffer addition.

10. After adding extraction buffer to all the tubes, check that all caps are tight and Parafilm each cap. Place rack in double plastic bags, seal and bleach the outer bag. Transfer double bagged tube rack to platform vortexer located outside the BSC.

11. Vortex rack for 10 minutes, with speed set to 7.

    Start time:_____ End Time:_____ Speed:_____

12. Move the rack back to the BSC. Discard bags and vortex tubes for 3 - 5 seconds and allow large particles to settle for at least 2 minutes.

13. Remove Parafilm, bleach wipe the tube, uncap tubes one at a time. Using a 10 mL serological pipet carefully transfer ~12.5 mL to corresponding extract pool 50 mL conical tube from **Step 8**, but carefully avoid settled particles during aliquoting. Recap 50 mL conical tube and move to the next sample. Change serological pipets and gloves between each sample.

14. Vortex pooled extracts for 10 seconds, allow mixture to settle for approximately 2 minutes to allow particulates to settle.

15. Transfer 10.5 mL of the pooled extract to a 15 mL conical tube for microbiological analysis (**WI #4: Culture of Recovered Spores**). Store aliquot on ice or in refrigerator until processed on same day. Change serological pipets and gloves between each sample.

16. Transfer up to 12 mL of the pooled extract into corresponding filter vial. Recap the vial and move to the next sample. Change serological pipets and gloves between each sample. Turn on vacuum pump at 5 – 10 psi. Record measured volume in Table 1.

Performed By: _____ Date: _____

WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

Table 1. Volume of sample transferred to filter vial

Sample Number	Filter ID	Total volume transferred to filter vial for RV-PCR (mL)	Recorded by:
1	1-PM10-SC-AVG-0		
2	2-PM10-SC-AVG-0		
3	3-PM10-SC-AVG-30		
4	4-PM10-SC-AVG-30		
5	5-PM10-SC-AVG-300		
6	6-PM10-SC-AVG-300		
7	7-PM10-SC-AVG-3,000		
8	8-PM10-SC-AVG-3,000		
9	9-PM10-SC-High-0		
10	10-PM10-SC-High-0		
11	11-PM10-SC-High-30		
12	12-PM10-SC-High-30		
13	13-PM10-SC-High-300		
14	14-PM10-SC-High-300		
15	15-PM10-SC-High-3,000		
16	16-PM10-SC-High-3,000		

17. If necessary, transfer remaining volume of pooled extract into corresponding filter vial. Recap the vial and move to the next sample. Change serological pipets and gloves between samples. Turn on vacuum pump at 5 – 10 psi. Complete filtration of liquid through filter vials. Record volume in Table 1.

18. Proceed to RV-PCR processing section (section B) below, with filter vial manifold.

19. Discard tubes that contain filter swatches.

B. **RV-PCR Sample Processing: Buffer Washes and Broth Culture**

1. Place into BSC: a ziplock bag with orange caps (one per filter vial), 10 mL serological pipets and cold (4°C) 10X PBS in 250 mL screw cap bottle.

2. Transfer 12.5 mL of cold (4°C) **High salt wash buffer (10x PBS)** to each filter-vial using a 10 mL serological pipet. Change pipet and gloves between each sample.

3. Complete filtration of liquid through the filter vials.

Performed By: _____ Date: _____

WORK INSTRUCTION FOR *BACILLUS ANTHRACIS* SPORE RECOVERY

4. Place into the BSC: 10 mL serological pipets and cold (4°C) 1X low salt wash buffer in 250 mL screw cap bottle.

5. Transfer 12.5 mL cold (4°C) **low salt wash buffer (1x PBS)** to each filter-vial using a 10 mL serological pipet. Change pipet and gloves between each sample.

6. Complete filtration of liquid through filter vials. Turn off vacuum pump.

7. Using an Allen wrench, unscrew the top of the manifold and break the seal on manifold using a plate sealer to separate the top of the manifold.

8. Using a tray preloaded with caps, move the top of the manifold with the filters still in place and firmly press down, capping the bottoms of the filters. Repeat pressing down on each filter vial to ensure a good seal.

9. Place bleach soaked wipes onto the manifold to soak up the filtered waste and disinfect for 20 minutes.

10. Place into the BSC: 5 mL serological pipets, 1000 µL pipet, 1000 µL tips, cold (2-8°C) BHI broth aliquoted in 50 mL conical tubes, sharps container and orange caps.

11. Pipet 5 ml of **cold BHI broth** into each filter vial using a 5 mL serological pipet. Use a new pipet for each filter vial. Dispose of the red cap and place the orange cap firmly into the top of the filter. Change gloves between each sample.

12. Record the time of the BHI broth addition, this represents T_0. Bleach wipe the filter vial

    Time of BHI addition: _____

13. Place the rack of capped filter vials in a plastic bag, seal, double bag and bleach the bag.

14. Vortex the filter vials for 10 minutes on the platform vortexer, setting 7.

    Start time:_____ End Time:_____ Speed:_____

15. Place 2 mL screw cap tubes for T_0 aliquots onto ice in the BSC.

16. After vortexing, transfer filter vials to the BSC. Remove bag.

17. Uncap one filter vial at a time and open the corresponding 2 mL tube. Using a 1 mL pipette or serological pipet (if filter deteriorated), gently pipet up and down ~10 to mix. Transfer 1 mL from each vial to the corresponding pre-chilled (on ice) 2 mL screw cap tube for T_0. Cap the tube and place it back onto ice. Wipe the filter vial with a bleach soaked lab wipe. Change gloves between each sample

Performed By: _____ Date: _____

After transferring the T_0 aliquots for all samples, place the filter vial rack in a transfer container, seal, and bleach the container. Store the T_0 aliquot at -20 °C overnight.

T_0 -20 C storage start time: _____ End time: _____ Initial/Date:_____

18. Transfer the filter vial rack to the shaker incubator. Secure the rack. Incubate at 37°C at 230 rpm, overnight (i.e., 16 hours from the addition of BHI broth to the filter vials). These samples are referred to as the T_{final} samples. Following incubation record turbidity observation and volume remaining in the table below.

Start time:_____ End Time:_____ Speed:_____ Temperature:_____

Sample Number	Filter ID	Turbid (Yes/No)	Volume remaining (mL)	Recorded by:
1	1-PM10-SC-AVG-0			
2	2-PM10-SC-AVG-0			
3	3-PM10-SC-AVG-30			
4	4-PM10-SC-AVG-30			
5	5-PM10-SC-AVG-300			
6	6-PM10-SC-AVG-300			
7	7-PM10-SC-AVG-3,000			
8	8-PM10-SC-AVG-3,000			
9	9-PM10-SC-High-0			
10	10-PM10-SC-High-0			
11	11-PM10-SC-High-30			
12	12-PM10-SC-High-30			
13	13-PM10-SC-High-300			
14	14-PM10-SC-High-300			
15	15-PM10-SC-High-3,000			
16	16-PM10-SC-High-3,000			

19. Proceed to WI #3: DNA Purification to process T_0 and T_i samples

Performed By: _____ Date: _____

IV. Technical Review

Performed by: _____ Date:_____

APPENDIX E. WORK INSTRUCTION FOR CULTURE OF *BACILLUS ANTHRACIS* SPORES RECOVERED FROM AIR FILTERS

WORK INSTRUCTION FOR CULTURE OF *BACILLUS ANTHRACIS* SPORES RECOVERED FROM AIR FILTERS

I. PURPOSE/SCOPE

Culture of *B. anthracis* spores recovered from air filters following the ***BACILLUS Analytical Methods 004*** published by the EPA December 2012.

II. MATERIALS/EQUIPMENT

Materials

Item	Manufacturer	Lot Number	Exp. Date	Storage Temp.	Initials & Date
PBS with Tween (0.05%)	Teknova			2-8 °C	
Microfunnel filters	PALL			R.T.	
Blood Agar	BBL			2-8 °C	

N/A = Not Applicable

Equipment

Item	Manufacturer	Serial Number	Thermometer/ Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)	The Baker Company		N/A		
Stationary Incubator	Precision	9509-003	N/A	N/A	
Vacuum manifold	Gelman Sciences	N/A	N/A	N/A	
Platform Vortexer	VWR	N/A	N/A	N/A	

N/A = Not Applicable

Other Supplies and Equipment

- Forceps
- Bleach
- 5 mL, 10 mL, and 25mL Serological Pipettes
- Pipette aid
- Ziplock bags

WORK INSTRUCTION FOR CULTURE OF *BACILLUS ANTHRACIS* SPORES RECOVERED FROM AIR FILTERS

Sample #	Filter type	Geographic Region	Particle Load	Filter Vial Type	Spore Spike level	Filter ID
1	PM10	SC	AVG	PVDF	0	1-PM10-SC-AVG-0
2	PM10	SC	AVG	PVDF	0	2-PM10-SC-AVG-0
3	PM10	SC	AVG	PVDF	30	3-PM10-SC-AVG-30
4	PM10	SC	AVG	PVDF	30	4-PM10-SC-AVG-30
5	PM10	SC	AVG	PVDF	300	5-PM10-SC-AVG-300
6	PM10	SC	AVG	PVDF	300	6-PM10-SC-AVG-300
7	PM10	SC	AVG	PVDF	3,000	7-PM10-SC-AVG-3,000
8	PM10	SC	AVG	PVDF	3,000	8-PM10-SC-AVG-3,000
9	PM10	SC	High	PVDF	0	9-PM10-SC-High-0
10	PM10	SC	High	PVDF	0	10-PM10-SC-High-0
11	PM10	SC	High	PVDF	30	11-PM10-SC-High-30
12	PM10	SC	High	PVDF	30	12-PM10-SC-High-30
13	PM10	SC	High	PVDF	300	13-PM10-SC-High-300
14	PM10	SC	High	PVDF	300	14-PM10-SC-High-300
15	PM10	SC	High	PVDF	3,000	15-PM10-SC-High-3,000
16	PM10	SC	High	PVDF	3,000	16-PM10-SC-High-3,000

Filters – Electronically update this table with samples names from the Sample Log

III. PROCEDURE

Note: The following procedure is to be carried out with the 10 mL pooled extract taken from step 14 (refer to WI #2 for Bacillus anthracis spore recovery). Process 2-3 PBST only negative control filter funnels alongside samples.

A. Culture Method

1. Label two filter funnels per sample, one with 1 mL and one with 4 mL.
2. Place the filter funnels onto the vacuum manifold in a Class II BSC.
3. Add 5 mL of PBS with 0.05% Tween (PBST) to each filter funnel. Apply vacuum.
4. With the vacuum valve closed and the vacuum pressure released, place 10 mL of PBST into each filter cup. For each sample, add 1 mL of pooled extract to one filter funnel and 4 mL of pooled extract to one filter funnel. Apply vacuum.

Performed by: _____ Date: _____

WORK INSTRUCTION FOR CULTURE OF *BACILLUS ANTHRACIS* SPORES RECOVERED FROM AIR FILTERS

5. Close the vacuum valve and release the vacuum pressure. Rinse the walls of each filter funnel using 10 mL of PBST. Apply vacuum.

6. With the vacuum valve closed and the vacuum pressure released, remove the membrane from the filter funnel and place onto Blood Agar. Dispose of filter bases and then change glove.

7. Incubate plates inverted overnight at 37°C ± 2°C.
   Incubation start Date/Time: _____ Initials: _____
   Incubation end Date/Time: _____ Initials: _____

8. Enter results into the below table.

Filter ID	*B. anthracis* colonies		Total colonies (all morphologies)	
	CFU/ 1 mL	CFU/ 4 mL	CFU/ 1 mL	CFU/ 4 mL
PBST Negative #1				
PBST Negative #2				
1-PM10-SC-AVG-0				
2-PM10-SC-AVG-0				
3-PM10-SC-AVG-30				
4-PM10-SC-AVG-30				
5-PM10-SC-AVG-300				
6-PM10-SC-AVG-300				
7-PM10-SC-AVG-3,000				
8-PM10-SC-AVG-3,000				
9-PM10-SC-High-0				
10-PM10-SC-High-0				
11-PM10-SC-High-30				
12-PM10-SC-High-30				
13-PM10-SC-High-300				
14-PM10-SC-High-300				
15-PM10-SC-High-3,000				
16-PM10-SC-High-3,000				

Counts performed/recorded by: _____ Date: _____

Performed by: _____ Date: _____

IV. Technical Review

Reviewed by: _____ Date:_____

APPENDIX F. WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

I. PURPOSE/SCOPE

Manual DNA extraction and purification *B. anthracis* spores from recovered from air filters following the ***BACILLUS Analytical Methods 004*** published by the EPA December 2012.

II. MATERIALS/EQUIPMENT

Materials

Item	Manufacturer	Lot Number	Exp. Date	Storage Temp.	Initials & Date
Lysis Buffer	Promega			RT	
PMPs	Promega			RT	
Salt Wash solution	Promega			RT	
Alcohol Wash	Promega			RT	
70% Ethanol	Inhouse			RT	
Elution Buffer	Promega			RT	

N/A = Not Applicable

Equipment

Item	Manufacturer	Serial Number	Thermometer /Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)	The Baker Company	57553	N/A	9/2018	
Micropipette Type:L1000	Rainin		N/A		
Micropipette Type:L200	Rainin		N/A		
Micropipette Type:L1000	Rainin		N/A		
Micropipette Type:L200	Rainin		N/A		
Ultra-low Freezer	Woods	X34664	10	4/1/18	
Refrigerator	Thermo Fisher	35840	115		
Centrifuge	Eppendorf	X58983	N/A	N/A	
Heat block	VWR	949039	N/A	N/A	
Thermometer			N/A		

N/A = Not Applicable

Other Supplies and Equipment

- Micropipette tips
- Biohazard bags
- Bleach
- Prepare tubes

WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

III. PROCEDURE

A. Manual DNA Extraction and Purification

Prepare lysis buffer with anti-foam according to manufacturer's instructions in the Magnesil Blood Genomic, Max Yield System, Kit. Prepare the alcohol wash solution by adding ethanol and isopropyl alcohol according to manufacturer's instructions. Prepare 70% Ethanol by adding 6 mL sterile water to 14 mL EtOH. Transfer sufficient volume of buffer to sterile, 100 mL reservoir immediately before use. ***Pre-heat heat block to 80°C prior to Section 10.4.8.***

NOTE: Process samples from zero spike level to 3,000 spike level. Change gloves when moving from a spiked sample to a sample containing a lower spike level, or if contamination of gloves is suspected. Pre-aliquot reagents from the kit to prevent contamination of reagents between runs.

1. After the overnight (16 h) incubation, remove the filter vial manifold from the shaker incubator. Thaw T_0 aliquots if they were stored at -20°C.

2. Vortex filter vials for 10 minutes on platform vortexer with speed set to 7.
   Start: _____ End:_____ Speed:_____

3. Transfer the filter vial manifold to the BSC, remove and discard bags.

4. Set up 2 mL screw cap tubes for T_i aliquots in a tube. Do not use 1.5 mL tubes. Transfer T_i aliquot screw cap tubes to the BSC.

5. Transfer the filter vial rack to the BSC. Uncap one filter vial at a time and transfer 1 mL to corresponding 2 mL tube after gently pipetting up and down ~10 to mix. Change gloves in between each sample.

6. Centrifuge 2 mL screw cap tubes (both T_0 and T_i) at 14,000 rpm for 10 minutes (4°C).

   Start: _____ End:_____ Speed:_____

7. Remove 800 µL of the supernatant from each tube, using a 1000 µL pipet and dispose to waste. Do not disturb the pellet. Change gloves in between each sample.

8. Add 800 µL of lysis buffer using a 1000 µL pipet, using a new tip for each sample. Cap the tubes and mix by vortexing on high (~1800 rpm) in 10 second pulses for a total of 60 seconds. Change gloves in between each sample.

9. Vortex each screw-cap tube briefly (low speed, 5 – 10 seconds) and transfer the entire sample volume to a 2 mL Eppendorf tube (ensure the tubes are labeled correctly during transfer). Change gloves in between each sample. Incubate the T_0 and T_i lysate tubes at room temperature for 5 minutes.

Performed by: _____ Date: _____

WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

10. Vortex the PMPs on high (~1800 rpm) for 30 – 60 seconds, or until they are uniformly resuspended. Keep PMPs in suspension by briefly vortexing (3 – 5 seconds) before adding to each T_0 and T_i lysate tube.

11. Uncap one tube at a time and add 600 µL of PMPs to each T_0 and T_i tube (containing 1 mL sample). Change gloves in between each sample.

12. Vortex each T_0 and T_i tube for 5 – 10 seconds at high speed. Incubate at room temperature for 5 minutes, briefly vortex, and then place on the magnetic stand with hinged-side of the tube facing toward the magnet.

13. Invert tubes 180 degrees (upside-down) turning away from you, then right side-up, then upside down toward you, then right side-up (caps up) position, allowing all PMPs to contact the magnet.

14. Check to see if any beads are in the caps and if so, repeat the tube inversion cycle again. Let the tubes sit for 5 – 10 seconds before opening. Maintain the tube layout when transferring tubes between the magnetic stand and tube rack.

15. Uncapping one tube at a time, withdraw all liquid using a 1000 µL pipet, placing the pipet tip in the bottom of the 2 mL tube. Be sure to remove all liquid without disturbing PMPs. Use a new pipet tip to remove any residual liquid, if necessary. If liquid remains in the tube cap, remove by pipetting.

16. Uncap each tube one at a time and add 360 µL of lysis buffer using a 1000 µL pipet. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack.

17. Vortex each tube for 5 – 10 seconds (low) and place back on the magnetic stand. After all tubes are in the stand, follow tube inversion cycle, as described in Step A.13.

18. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube.

Wash Steps:

19. Uncap each tube one at a time and add 360 µL of **Salt Wash Solution**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **1st Salt Wash.**

20. Uncap each tube one at a time and add 360 µL of **Salt Wash Solution**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **2nd Salt Wash.**

Performed by: _____ Date: _____

WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

21. Uncap each tube one at a time and add 500 µL of **Alcohol Wash Solution**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **1st Alcohol Wash.**

22. Uncap each tube one at a time and add 500 µL of **Alcohol Wash Solution**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **2nd Alcohol Wash.**

23. Uncap each tube one at a time and add 500 µL of **Alcohol Wash Solution**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **3rd Alcohol Wash.**

24. Uncap each tube one at a time and add 500 µL of **70% Ethanol**. Remove tube rack off of magnetic stand. Vortex on low setting for 5 – 10 seconds, and transfer to tube rack. Place tube rack back on magnetic. Invert as described in step A.13. Remove all the liquid as described in Step A.17. Use a new tip for each T_0 and T_i tube. This is **4th Alcohol Wash.**

25. **If necessary, us**e a 200 uL pipet to remove remaining 70% ethanol, being careful to not disturb PMPs.

26. Open all T_0 and T_i tubes and air dry for 2 minutes.

27. Close tubes and transfer to heat block. Re open tubes once placed on the heat block at 80°C until the PMPs are dry (~20 minutes, or until dry). Allow all the alcohol solution to evaporate since alcohol may interfere with analysis. If residual condensation is present, do not remove, leave it in place.

Start: _____ End:_____ Temperature:_____

28. DNA elution: While they are in the heating block add 200 µL of elution buffer to each T_0 and T_i tube, and close tube. Vortex for 10 seconds and place back on heating block for 80 seconds.

29. Briefly vortex the tubes (5 – 10 seconds) taking care to prevent the liquid from entering the tube cap and let the tube sit in the heating block for 1 minute. Reduce vortex speed if liquid appears to enter the tube cap lid.

30. Repeat Step 29 four more times.

31. Remove the tubes from the heating block, place them in a tube rack in the BSC, and incubate at room temperature for at least 5 minutes.

Start: _____ End:_____

Performed by: _____ Date: _____

WORK INSTRUCTION FOR MANUAL DNA EXTRACTION AND PURIFICATION FROM *BACILLUS ANTHRACIS* SPORES

32. Briefly vortex each tube (5 – 10 seconds) on low speed and centrifuge at 2000 rpm, 4ºC for 1 minute..

33. Briefly vortex each tube and place on the magnetic stand for at least 30 seconds.

34. Collect liquid from each T_0 and T_i tube and transfer ~80-90 uL to a clean, labeled, 1.5 mL tube on ice (check tube labels to ensure the correct order). Use a new tip for each tube. Visually verify absence of PMP carryover during final transfer. If magnetic bead carryover occurred, place 1.5 mL tube on magnet, collect liquid, and transfer to a clean, labeled, 1.5 mL tube.

35. Centrifuge tubes at 14,000 rpm at 4°C for 5 minutes to pellet any particles remaining with the eluted DNA; carefully remove supernatant from all samples and transfer to a new 1.5 mL tube using a new tip for each tube.

Start: _____ End:_____

36. Store T_0 and T_i DNA extract tubes at 4°C until PCR analysis. Continue to ***WI-RV-PCR-Native Filters***.

Note: If PCR cannot be performed within 24 hours, freeze DNA extracts at -20ºC.

Labeled:_____

Date/Time:_____ Storage Temperature:_____

Storage Location:_____

Performed by: _____ Date: _____

IV. Technical Review

Performed by: _____ Date:_____

Comments:

_____

_____

_____

APPENDIX G. WORK INSTRUCTION DRV-PCR FOR *BACILLUS ANTHRACIS* SPORES

WORK INSTRUCTION DRV-PCR FOR *BACILLUS ANTHRACIS* SPORES

I. PURPOSE/SCOPE

Duplicate Rapid Viability (DRV)-PCR for *B. anthracis* spores from recovered from air filters modified from the single-plex RV-PCR described in ***BACILLUS Analytical Methods 004*** published by the EPA December 2012.

II. MATERIALS/EQUIPMENT

Materials

Enter materials used into Native Filters WI-RV-PCR-5v1 – FORM A

Equipment

Item	Manufacturer	Serial Number	Thermometer/ Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)			N/A		
Micropipette Type:			N/A		
Micropipette Type:			N/A		
Micropipette Type:			N/A		
Micropipette Type:			N/A		
Refrigerator					
Freezer					
Centrifuge	Eppendorf	X58983	N/A	N/A	
7500 Fast Dx	Applied Biosystems	275017115	N/A	6/2018	

N/A = Not Applicable

Other Supplies and Equipment

- Micropipette tips
- 96-well 0.1 mL FAST plates
- Optical caps
- Biohazard bags
- Bleach
- DNase Away
- 70% Isopropanol

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III. PROCEDURE

A. Prepare samples for qPCR

Note: This step must be performed in the BSC outside the PCR clean room set-up area. Prepare a fresh aliquot of PCR-grade water per sample batch to use for 1:10 dilutions and NTCs.

1. ***T_0 and T_i DNA extracts*:** Label 1.5 mL tubes with the sample identifier and "10-fold dilution". Add 90 µL of PCR-grade water to the tubes.

2. Mix T_0 and T_i DNA extracts by vortexing (3 – 5 seconds), spin at 14,000 rpm for 2 minutes, and transfer 10 µL of supernatant to 1.5-mL Eppendorf tubes with 90 µL of PCR-grade water, maintaining the plate layout.

   Note: No centrifugation is required if PCR analysis is conducted immediately after DNA elution.

B. Real-time PCR Analysis of DNA Extracts

1. Decontaminate the PCR workstation by treating all work surfaces with a DNA erase, bleach solution, followed by 70% Isopropanol. After decontamination, discard gloves and replace with a new clean pair.

   Note: If gloves become contaminated, they should be disposed of and fresh gloves donned. Only open one tube at a time throughout the process. At no point, should more than one tube be open. Do not allow hands (gloved or otherwise) to pass over an open tube, PCR plate, or any reagent container. All used pipet tips, gloves and tubes must be discarded in a biohazard autoclave bag.

2. Determine the number of reactions that are to be run. Prepare a sufficient volume of Master Mix to allow for one extra reaction for every ten reactions, so that there is enough Master Mix regardless of pipetting variations. *For each batch of samples, PCR Master Mix should be made for 4 PCs, 4 NTCs, 3 PNCs (Method blank) and 6 DNA extracts per sample (3 for T0 and 3 for Ti DNA extracts).* Record sample names and reaction numbers on ***WI-RV-PCR-5v1 – FORM A.***

3. In a clean PCR-preparation hood, pipet 20 µL of Master Mix into the wells of the PCR plate. Label four wells as NTC and four as PC.

4. Add 5 µL of PCR-grade water into the NTC wells.

5. Tightly seal the NTC wells with optical caps, cover all other wells of the plate using optical caps.

6. Vortex each sample briefly, then add 5 µL to each sample well. Tightly seal the sample wells with optical caps.

Performed by: _____ Date: _____

7. Vortex the PC (*B. anthracis* DNA [10 pg/µL or 50 pg/5 µL]) and add 5 µL to each of the PC wells. Tightly seal the PC wells with optical caps.

8. ***Note: This step must be performed in the BSC outside the PCR clean room set-up area (Dead Air Box).***

C. Within the Post-Amplification Lab (20-0-48) Load 96-well plates onto 7500 Fast Dx.

1. Set up 7500 Fast Dx
    a. Open the 7500 Fast Software and a new file.
        i. Configure the New Document dialog box:
            1. Assay – Select Standard Curve (Absolute Quantification)
            2. Container – Select 96 Wells Clear
            3. Template—Select Blank, or Browse for a previously saved file.
            4. Choose the Run mode—7500 FAST.
            5. Operator—Enter your name.
            6. Comments—Enter any comments pertaining to the run.
            7. Plate Name—Enter a plate name.
            8. Click Next.
        ii. Choose the Detector for this assay
            1. Choose 6-FAM-MGB from the list, or create a new one now by clicking the **New Detector** button. Multiple detectors can be selected by using the Ctrl key.
            2. Choose **Add** >> to add the detectors to the plate document.
            3. Choose ROX™ as the passive reference from the Passive Reference drop down box.
            4. Click Next.
        iii. The setup window is split into two panes. Use the layout grid to select indiviual wells, and the Setup tab of the Well Inspector pane to apply detectors and designate well assignments. As parameters are chosen in the Setup tab, they are recorded in the Table pane at the bottom of the window.
            1. Highlight the wells desired in the layout grid.
            2. Check the **Use** box next to each detector to be analyzed for each well.
            3. Click in the **Sample Name** box, and type in the name.
            4. Choose the **Task** (Standard or NTC) from the dropdown list. If the task is Standard, you must enter the concentration before proceeding to the next well. Proceed until all the wells have been assigned all of the appropriate detectors, names, task, and concentrations (standards only).
            5. Click Finish.
        iv. Set the method parameters by clicking on the Instrument tab of the Well Inspector pane. All parameters must be changed to match Table 1.
            1. To delete a stage, click on the bar separating the stages and drag to highlight the stage to be deleted. Click [Delete Step].

Performed by: _____ Date: _____

2. To add a parameter, click the step to the left of the location where you want to place the new parameter, and choose [Add Cycle], [Add Hold], or [Add Step].
3. To change cycle times and temperatures, click in the respective boxes in each stage and type in the appropriate settings.

Table 1. 7500 FAST Method Parameters.

Temperature (°C)	Time	Cycles
95.0	*0:20*	1
95.0	*0:03*	45
60.0	*0:30*	
25 µL Sample Volume		

v. Save the file. Only files with the (*.sds) extension can be run.
   1. From the top, choose File, Save As.
   2. If the document is a new plate, go to drive D, Applied Biosystems, SDS, Documents, and the appropriate folder. Name the file. From the drop down, "Files of type:" choose *ABI Prism SDS Single Plate (*.sds)*.
   3. If the document is a template, follow the same procedure. This file will already have a name and the *(*.sdt)* file extension. Change the name to identify this particular run, and change the file type to *ABI Prism SDS Single Plate (*.sds)*.
   4. Close the file.

vi. Centrifuge the plate at 300 x G for about 1 – 2 minutes at room temperature or in Labnet's MPS-1000 Mini Plate Spinner.

vii. Open the file created in step C. 1. a. v., load 96-well plate into 7500 and start run.

viii. When run is complete, burn the file to a CD.

ix. Remove 96-well plate from 7500 Fast Dx and dispose

D. Analysis

1. Open the assay with the most current version of 7500 Fast Dx software.
   a. Analysis can be performed using automatic settings. If required to manually set threshold and baseline, from the menu bar choose Analysis, and then choose Analysis Settings. Select Manual Ct, and Manual Baseline.
   b. From the menu bar choose Analysis, then choose Analyze from the drop down menu, or click on the large green triangle icon button in the toolbar.
   c. Highlight the unknowns, standards (only one set if there is more than one), and NTCs either in the Plate Grid pane or in the Table View pane.
   d. Click on the Results tab in the Well Inspector pane to view the Amplification Plot and Standard Curve Plot.

Performed by: _____ Date: _____

e. Ensure that the desired detector appears in the Detector box in both the Amplification Plot and Standard Curve Plot. If not, chose it from the drop down menus now.
f. If analyzing based on manual threshold and baseline settings,
    i. Look at the Ct values for the standards and unknowns in the Report View pane to determine the lowest Ct value. Change the value in the End (cycle) box on the right side of the plot to adjust the baseline setting to two Ct values below the lowest Ct value whole number, ignoring values to the right of the decimal. (For example, if the lowest Ct value is 22.610105, the right cursor should be placed at 20.)
    ii. Highlight the standards and NTCs only. Click on the line that represents the threshold in the amplification plot. Move the threshold so that it is above all background "noise" generated by non-amplification, and in the lowest part of the exponential phase of all the standards. Depending on the range of the standard curve, some standards at the lowest end of the curve may not amplify well; whether or not to include them will be decided by the Program Manager or Principal Investigator.
    iii. Check the values in the Report View pane. Moving the threshold occasionally causes the Ct values to change. If necessary, readjust the baseline and threshold by repeating steps 9a through b until the baseline remains at a level two Ct values below the lowest Ct value in the Report View pane and the threshold is properly set. If changes are made that would affect the software's response to the data, such as deleting a well or changing a detector, etc., the software will reset the analysis and the data will need to be reanalyzed.
    iv. Look in the Report View pane to ensure that the Ct value for all of the NTCs is Undetermined. This means that no amplification was detected in the number of cycles this analysis ran, and is considered to be a negative result. If any of the wells show amplification at this point, contact the Program Manager or Principal Investigator.
    v. From the menu bar, choose Tools and then Report Settings. Check the boxes for the data required to be printed by your project.
    vi. Click Print, Done.
g. Highlight each individual well with a Ct less than 45, and check the Multicomponent Plot to ensure there is actual amplification. Amplification is indicated by an upward curve in the line representing the reporter dye, and if a quencher is present, a downward curve in the line representing the quencher.
h. As specified by a project, print Multicomponent Plots.
i. Annotations to be made by the analyst on the printouts:
    - Initial and date (I/D) every printout.
    - Initial, date, and error or otherwise annotate all errors and comments.
    - Indicate which, if any, wells of the Standard Curve were omitted.
    - If required by the project, every Multicomponent Plot should indicate which sample number it represents, and the actual Ct value associated with the well

Performed by: _____ Date: _____

j. Repeat the preceding steps to this point for every detector OR each standard curve associated with this assay.
k. Attach all printouts to the worksheet. This constitutes one data package.

2. After the PCR run, discard sealed PCR plate.
3. Export the .csv file
   a. Go to File -> Export -> Results.
   b. Select folder location -> Native Filters
   c. Save results type as .csv.
   d. Select save.
      i. A dialog box will open.
      ii. Check the box, Apply Report Settings for Data Columns.
   e. Burn .sds and .csv files onto a CD.

IV. Data Calculations

Calculate an average CT from the replicate reactions for T0 and T9 DNA extracts of each sample. Subtract the average CT of the T9 DNA extract from the average CT of the T0 DNA extract. If there is no CT for the T0 DNA extract (i.e., the T0 is non-detect), use 45 (total number of PCR cycles used) as the CT. The change (decrease) in the average CT value from T0 to T9 (ΔCT) ≥ 9 indicates a positive result suggesting the presence of viable *B. anthracis* spores in the sample. If an incubation time longer than 9 hours was used for the RV-PCR, instead of T9, appropriate T× (incubation time) should be used. However, (ΔCT) ≥ 9 algorithm should still be used for a positive result. Depending upon the end user's requirement, sample complexity (dirtiness) and the phase of response during an event, a lower ΔCT criterion of ≥ 6 (a two log difference in DNA concentration) and a corresponding higher endpoint PCR CT of ≤ 39 could be set. A minimum of two out of three T0 PCR replicates must result in CT values ≤ 44 (in a 45-cycle PCR) to calculate the average CT. A minimum of two out of three T9 PCR replicates (or T× for other incubation time) must result in CT values ≤ 36 to calculate the average CT for a sample result to be considered positive. Negative controls (NTCs) should not yield any measurable CT values above the background level. If CT values are obtained as a result of a possible contamination or cross-contamination, prepare fresh PCR Master Mix and repeat analysis. In addition, field blank samples should not yield any measurable CT values. If CT values are observed as a result of a possible contamination or cross-contamination, a careful interpretation of the CT values for the sample DNA extracts and field blanks must be done to determine if the data is considered valid or if the PCR analyses must be repeated.
Print RV-PCR results and attach to this WI package, include relevant calculations and file name.

Performed by: _____ Date: _____

V. Technical Review

Performed by: _____ Date:_____

Comments:

_____
_____
_____

APPENDIX H. WORK INSTRUCTION FOR SELECTING PRESUMPTIVE *BACILLUS ANTHRACIS* STERNE COLONIES FOR QPCR CONFIRMATION

WORK INSTRUCTION FOR SELECTING PRESUMPTIVE *BACILLUS ANTHRACIS* STERNE COLONIES FOR QPCR CONFIRMATION

I. PURPOSE/SCOPE

Select and screen *B. anthracis* Sterne colonies recovered on culture plates using qPCR following the ***BACILLUS Analytical Methods 004*** published by the EPA December 2012.

II. MATERIALS/EQUIPMENT

Materials

Item	Manufacturer	Lot Number	Exp. Date	Storage Temp.	Initials & Date
PCR-Grade Water	Teknova			R.T.	
1 µL loop, 10 µL loop or inoculating needles				R.T.	
1.5 or 2 mL tubes				R.T.	

N/A = Not Applicable

Equipment

Item	Manufacturer	Serial Number	Thermometer/ Rees #	Calibration Due	Initials & Date
Biosafety Cabinet (BSC)	The Baker Company		N/A		
Heat Block	Precision	9509-003	N/A	N/A	
Thermometer		N/A	N/A	N/A	
Camera		N/A	N/A	N/A	

N/A = Not Applicable

Other Supplies and Equipment

- Bleach
- 5 mL, 10 mL, and 25mL Serological Pipettes

WORK INSTRUCTION FOR SELECTING PRESUMPTIVE *BACILLUS ANTHRACIS* STERNE COLONIES FOR QPCR CONFIRMATION

Tube #	Filter ID	Volume (mL)	Morphology (B. a. Sterne or Background)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			

Filters – Record Filter ID and Morphology for Selected Colonies

III. PROCEDURE

A. Selecting colonies

1. Pipette 100 µL of PCR-grade water into 1.5 or 2 mL tubes.
2. Select colonies. Take pictures of colonies that are selected.
3. Use 1 µL loop, 10 µL loop or inoculating needle to select the colony.
4. Immerse needle into PCR-grade water and rotate to dislodge cellular material.
5. Lyse the colony suspension for 5 minutes on a heat block at 95 ± 2 °C.

   Incubation start Date/Time: _____ Initials: _____
   Incubation end Date/Time: _____ Initials: _____

6. Store lysed suspension at – 20 °C for qPCR analysis.
7. Prior to qPCR analysis, thaw tubes, centrifuge @ 14,000 rpm for 2 minutes. Use supernatant for qPCR.

Performed by: _____ Date: _____

IV. Technical Review

Reviewed by: _____ Date: _____

APPENDIX I. CULTURE RESULTS FOR AIR QUALITY FILTERS USING SHEEP BLOOD AGAR MEDIUM

PM2.5 New							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PM2.5-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PM2.5-NA-NEW-PES-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-NA-NEW-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM2.5-NEW-30	1.5E+01	25	1	1	1.0	25.0	166.7
	1.5E+01	25	4	2	0.5	12.5	83.3
11-PM2.5-NA-NEW-PES-30	4.6E+01	25	1	0	0.0	0.0	0.0
	4.6E+01	25	4	2	0.5	12.5	27.4
12-PM2.5-NA-NEW-PVDF-30	4.6E+01	25	1	0	0.0	0.0	0.0
	4.6E+01	25	4	2	0.5	12.5	27.4
5-PM2.5-NEW-300	1.5E+02	25	1	2	2.0	50.0	33.3
	1.5E+02	25	4	12	3.0	75.0	50.0
13-PM2.5-NA-NEW-PES-300	4.6E+02	25	1	9	9.0	225.0	49.2
	4.6E+02	25	4	40	10.0	250.0	54.7
14-PM2.5-NA-NEW-PVDF-300	4.6E+02	25	1	1	1.0	25.0	5.5
	4.6E+02	25	4	19	4.8	118.8	26.0
7-PM2.5-NEW-3000	1.5E+03	25	1	38	38.0	950.0	63.3
	1.5E+03	25	4	172	43.0	1075.0	71.7
15-PM2.5-NA-NEW-PES-3,000	4.6E+03	25	1	33	33.0	825.0	18.1
	4.6E+03	25	4	156	39.0	975.0	21.3
16-PM2.5-NA-NEW-PVDF-3,000	4.6E+03	25	1	32	32.0	800.0	17.5
	4.6E+03	25	4	118	29.5	737.5	16.1
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Arizona Filter Average							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
2-PM2.5-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-PM2.5-AZ-AVG-PES-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	1	0.3	6.3	#DIV/0!
2-PM2.5-AZ-AVG-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
4-PM2.5-AVG-30	1.5E+01	25	1	1	1.0	25.0	166.7
	1.5E+01	25	4	5	1.3	31.3	208.3
3-PM2.5-AZ-AVG-PES-30	4.6E+01	25	1	1	1.0	25.0	54.7
	4.6E+01	25	4	4	1.0	25.0	54.7
4-PM2.5-AZ-AVG-PVDF-30	4.6E+01	25	1	1	1.0	25.0	54.7
	4.6E+01	25	4	7	1.8	43.8	95.7
6-PM2.5-AVG-300	1.5E+02	25	1	5	5.0	125.0	83.3
	1.5E+02	25	4	13	3.3	81.3	54.2
5-PM2.5-AZ-AVG-PES-300	4.6E+02	25	1	7	7.0	175.0	38.3
	4.6E+02	25	4	35	8.8	218.8	47.9
6-PM2.5-AZ-AVG-PVDF-300	4.6E+02	25	1	15	15.0	375.0	82.1
	4.6E+02	25	4	39	9.8	243.8	53.3
8-PM2.5-AVG-3,000	1.5E+03	25	1	38	38.0	950.0	63.3
	1.5E+03	25	4	99	24.8	618.8	41.3
7-PM2.5-AZ-AVG-PVDF-3000	4.6E+03	25	1	61	61.0	1525.0	33.4
	4.6E+03	25	4	188	47.0	1175.0	25.7
8-PM2.5-AZ-AVG-PVDF-3,000	4.6E+03	25	1	74	74.0	1850.0	40.5
	4.6E+03	25	4	222	55.5	1387.5	30.4
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Arizona Filter High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM2.5-HIGH-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	2	0.5	12.5	#DIV/0!
9-PM2.5-AZ-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM2.5-HIGH-30	1.5E+01	25	1	0	0.0	0.0	0.0
	1.5E+01	25	4	1	0.3	6.3	41.7
12-PM2.5-HIGH-30	1.5E+01	25	1	0	0.0	0.0	0.0
	1.5E+01	25	4	2	0.5	12.5	83.3
10-PM2.5-AZ-HIGH-30	1.4E+01	25	1	0	0.0	0.0	0.0
	1.4E+01	25	4	0	0.0	0.0	0.0
13-PM2.5-HIGH-300	1.5E+02	25	1	3	3.0	75.0	50.0
	1.5E+02	25	4	15	3.8	93.8	62.5
14-PM2.5-HIGH-300	1.5E+02	25	1	2	2.0	50.0	33.3
	1.5E+02	25	4	13	3.3	81.3	54.2
12-PM2.5-AZ-HIGH-300	1.4E+02	25	1	5	5.0	125.0	89.3
	1.4E+02	25	4	22	5.5	137.5	98.2
15-PM2.5-AZ-HIGHF-3,000	1.4E+03	25	1	41	41.0	1025.0	73.2
	1.4E+03	25	4	124	31.0	775.0	55.4
15-PM2.5-HIGH-3,000	1.5E+03	25	1	56	56.0	1400.0	93.3
	1.5E+03	25	4	120	30.0	750.0	50.0
16-PM2.5-HIGH-3,000	1.5E+03	25	1	33	33.0	825.0	55.0
	1.5E+03	25	4	125	31.3	781.3	52.1
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Florida Average							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PM2.5-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-PM2.5-FL-AVG-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM2.5-AVG-30	2.0E+01	25	1	1	1.0	25.0	124.4
	2.0E+01	25	4	3	0.8	18.8	93.3
4-PM2.5-AVG-30	2.0E+01	25	1	2	2.0	50.0	248.8
	2.0E+01	25	4	1	0.3	6.3	31.1
3-PM2.5-FL-AVG-PVDF-30	5.0E+01	25	1	2	2.0	50.0	99.4
	5.0E+01	25	4	7	1.8	43.8	87.0
5-PM2.5-AVG-300	2.0E+02	25	1	11	11.0	275.0	137.5
	2.0E+02	25	4	28	7.0	175.0	87.5
6-PM2.5-AVG-300	2.0E+02	25	1	8	8.0	200.0	100.0
	2.0E+02	25	4	17	4.3	106.3	53.1
5-PM2.5-FL-AVG-PVDF-300	5.0E+02	25	1	8	8.0	200.0	39.8
	5.0E+02	25	4	29	7.3	181.3	36.0
7-PM2.5-AVG-3000	2.0E+03	25	1	68	68.0	1700.0	85.0
	2.0E+03	25	4	200	50.0	1250.0	62.5
8-PM2.5-AVG-3,000	2.0E+03	25	1	75	75.0	1875.0	93.8
	2.0E+03	25	4	288	72.0	1800.0	90.0
7-PM2.5-FL-AVG-PVDF-3000	5.0E+03	25	1	93	93.0	2325.0	46.2
	5.0E+03	25	4	216	54.0	1350.0	26.8
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Florida High							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
9-PM2.5-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-FL-HIGH-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM2.5-HIGH-30	2.0E+01	25	1	2	2.0	50.0	250.0
	2.0E+01	25	4	7	1.8	43.8	218.8
12-PM2.5-HIGH-30	2.0E+01	25	1	0	0.0	0.0	0.0
	2.0E+01	25	4	2	0.5	12.5	62.5
4-PM2.5-FL-HIGH-PVDF-30	5.0E+01	25	1	2	2.0	50.0	99.4
	5.0E+01	25	4	4	1.0	25.0	49.7
13-PM2.5-HIGH-300	2.0E+02	25	1	7	7.0	175.0	87.5
	2.0E+02	25	4	33	8.3	206.3	103.1
14-PM2.5-HIGH-300	2.0E+02	25	1	3	3.0	75.0	37.5
	2.0E+02	25	4	28	7.0	175.0	87.5
6-PM2.5-FL-HIGH-PVDF-300	5.0E+02	25	1	21	21.0	525.0	104.4
	5.0E+02	25	4	43	10.8	268.8	53.4
15-PM2.5-HIGH-3,000	2.0E+03	25	1	76	76.0	1900.0	95.0
	2.0E+03	25	4	242	60.5	1512.5	75.6
16-PM2.5-HIGH-3,000	2.0E+03	25	1	71	71.0	1775.0	88.8
	2.0E+03	25	4	260	65.0	1625.0	81.3
8-PM2.5-FL-HIGH-PVDF-3,000	5.0E+03	25	1	84	84.0	2100.0	41.7
	5.0E+03	25	4	232	58.0	1450.0	28.8
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Massachusetts Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PM2.5-MA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-MA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-PM2.5-MA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM2.5-MA-AVG-30	3.2E+01	25	1	0	0.0	0.0	0.0
	3.2E+01	25	4	0	0.0	0.0	0.0
4-PM2.5-MA-AVG-30	3.2E+01	25	1	0	0.0	0.0	0.0
	3.2E+01	25	4	3	0.8	18.8	58.0
3-PM2.5-MA-AVG-30	2.0E+01	25	1	0	0.0	0.0	0.0
	2.0E+01	25	4	3	0.8	18.8	93.8
5-PM2.5-MA-AVG-300	3.2E+02	25	1	7	7.0	175.0	54.2
	3.2E+02	25	4	29	7.3	181.3	56.1
6-PM2.5-MA-AVG-300	3.2E+02	25	1	2	2.0	50.0	15.5
	3.2E+02	25	4	10	2.5	62.5	19.3
5-PM2.5-MA-AVG-300	2.0E+02	25	1	1	1.0	25.0	12.5
	2.0E+02	25	4	10	2.5	62.5	31.3
7-PM2.5-MA-AVG-3000	3.2E+03	25	1	63	63.0	1575.0	48.8
	3.2E+03	25	4	156	39.0	975.0	30.2
8-PM2.5-MA-AVG-PVDF-3,000	3.2E+03	25	1	80	80.0	2000.0	61.9
	3.2E+03	25	4	160	40.0	1000.0	31.0
7-PM2.5-MA-AVG-3000	2.0E+03	25	1	47	47.0	1175.0	58.8
	2.0E+03	25	4	103	25.8	643.8	32.2
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Massachusetts High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM2.5-MA-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-MA-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PM2.5-MA-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM2.5-MA-HIGH-30	3.2E+01	25	1	0	0.0	0.0	0.0
	3.2E+01	25	4	3	0.8	18.8	58.0
12-PM2.5-MA-HIGH-30	3.2E+01	25	1	0	0.0	0.0	0.0
	3.2E+01	25	4	4	1.0	25.0	77.4
11-PM2.5-MA-HIGH-30	2.0E+01	25	1	0	0.0	0.0	0.0
	2.0E+01	25	4	3	0.8	18.8	93.8
13-PM2.5-MA-HIGH-300	3.2E+02	25	1	5	5.0	125.0	38.7
	3.2E+02	25	4	29	7.3	181.3	56.1
14-PM2.5-MA-HIGH-300	3.2E+02	25	1	5	5.0	125.0	38.7
	3.2E+02	25	4	23	5.8	143.8	44.5
13-PM2.5-MA-HIGH-300	2.0E+02	25	1	3	3.0	75.0	37.5
	2.0E+02	25	4	5	1.3	31.3	15.6
15-PM2.5-MA-HIGHF-3,000	3.2E+03	25	1	62	62.0	1550.0	48.0
	3.2E+03	25	4	124	31.0	775.0	24.0
16-PM2.5-MA-HIGH-3,000	3.2E+03	25	1	61	61.0	1525.0	47.2
	3.2E+03	25	4	140	35.0	875.0	27.1
15-PM2.5-MA-HIGHF-3,000	2.0E+03	25	1	4	4.0	100.0	5.0
	2.0E+03	25	4	25	6.3	156.3	7.8
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Wisconsin Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PM2.5-WI-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-WI-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-WI-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM2.5-WI-AVG-30	2.2E+01	25	1	1	1.0	25.0	113.6
	2.2E+01	25	4	2	0.5	12.5	56.8
4-PM2.5-WI-AVG-30	2.2E+01	25	1	0	0.0	0.0	0.0
	2.2E+01	25	4	4	1.0	25.0	113.6
4-PM2.5-WI-AVG-30	2.0E+01	25	1	5	5.0	125.0	625.0
	2.0E+01	25	4	1	0.3	6.3	31.3
5-PM2.5-WI-AVG-300	2.2E+02	25	1	4	4.0	100.0	45.5
	2.2E+02	25	4	17	4.3	106.3	48.3
6-PM2.5-WI-AVG-300	2.2E+02	25	1	1	1.0	25.0	11.4
	2.2E+02	25	4	10	2.5	62.5	28.4
6-PM2.5-WI-AVG-300	2.0E+02	25	1	6	6.0	150.0	75.0
	2.0E+02	25	4	27	6.8	168.8	84.4
7-PM2.5-WI-AVG-3,000	2.2E+03	25	1	49	49.0	1225.0	55.7
	2.2E+03	25	4	146	36.5	912.5	41.5
8-PM2.5-WI-AVG-3,000	2.2E+03	25	1	25	25.0	625.0	28.4
	2.2E+03	25	4	92	23.0	575.0	26.1
8-PM2.5-WI-AVG-PVDF-3,000	2.0E+03	25	1	60	60.0	1500.0	75.0
	2.0E+03	25	4	108	27.0	675.0	33.8
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM2.5 Wisconsin High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM2.5-WI-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-WI-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-WI-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM2.5-WI-High-30	2.2E+01	25	1	3	3.0	75.0	340.9
	2.2E+01	25	4	3	0.8	18.8	85.2
12-PM2.5-WI-High-30	2.2E+01	25	1	1	1.0	25.0	113.6
	2.2E+01	25	4	3	0.8	18.8	85.2
12-PM2.5-WI-HIGH-30	2.0E+01	25	1	0	0.0	0.0	0.0
	2.0E+01	25	4	3	0.8	18.8	93.8
13-PM2.5-WI-High-300	2.2E+02	25	1	5	5.0	125.0	56.8
	2.2E+02	25	4	27	6.8	168.8	76.7
14-PM2.5-WI-High-300	2.2E+02	25	1	12	12.0	300.0	136.4
	2.2E+02	25	4	21	5.3	131.3	59.7
14-PM2.5-WI-HIGH-300	2.0E+02	25	1	13	13.0	325.0	162.5
	2.0E+02	25	4	26	6.5	162.5	81.3
15-PM2.5-WI-High-3,000	2.2E+03	25	1	62	62.0	1550.0	70.5
	2.2E+03	25	4	142	35.5	887.5	40.3
16-PM2.5-WI-High-3,000	2.2E+03	25	1	67	67.0	1675.0	76.1
	2.2E+03	25	4	144	36.0	900.0	40.9
16-PM2.5-WI-HIGH-3,000	2.0E+03	25	1	67	67.0	1675.0	83.8
	2.0E+03	25	4	144	36.0	900.0	45.0
Use values highlighted in green for reporting.							
Count based on half filter multiplied by 2							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 New							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
PM10HV-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
1-PM10-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
PM10HV-New-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
PM10HV-New-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
3-PM10-NEW-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	0	0.0	0.0	0.0
PM10HV-New-300	9.0E+01	25	1	1	1.0	25.0	27.8
		25	4	0	0.0	0.0	0.0
PM10HV-New-300	9.0E+01	25	1	1	1.0	25.0	27.8
		25	4	2	0.5	12.5	13.9
5-PM10-NEW-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	1	0.3	6.3	3.5
PM10HV-New-3,000	1.3E+03	25	1	4	4.0	100.0	7.7
		25	4	15	3.8	93.8	7.2
PM10HV-New-3,000	1.3E+03	25	1	5	5.0	125.0	9.6
		25	4	24	6.0	150.0	11.5
PM10HV-New-3,000	1.3E+03	25	1	3	3.0	75.0	5.8
		25	4	16	4.0	100.0	7.7
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 California Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PM10-CA-AVG-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	6	1.5	37.5	#DIV/0!
2-PM10-CA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	8	2.0	50.0	#DIV/0!
1-PM10-CA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	6	1.5	37.5	#DIV/0!
3-PM10-CA-AVG-30	3.1E+01	25	1	1	1.0	25.0	80.6
	3.1E+01	25	4	6	1.5	37.5	121.0
4-PM10-CA-AVG-30	3.1E+01	25	1	3	3.0	75.0	241.9
	3.1E+01	25	4	7	1.8	43.8	141.1
3-PM10-CA-AVG-30	1.4E+01	25	1	3	3.0	75.0	535.7
	1.4E+01	25	4	4	1.0	25.0	178.6
5-PM10-CA-AVG-300	3.1E+02	25	1	5	5.0	125.0	40.3
	3.1E+02	25	4	1	0.3	6.3	2.0
6-PM10-CA-AVG-300	3.1E+02	25	1	2	2.0	50.0	16.1
	3.1E+02	25	4	7	1.8	43.8	14.1
5-PM10-CA-AVG-300	1.4E+02	25	1	1	1.0	25.0	17.9
	1.4E+02	25	4	5	1.3	31.3	22.3
7-PM10-CA-AVG-3,000	3.1E+03	25	1	9	9.0	225.0	7.3
	3.1E+03	25	4	17	4.3	106.3	3.4
8-PM10-CA-AVG-3,000	3.1E+03	25	1	16	16.0	400.0	12.9
	3.1E+03	25	4	25	6.3	156.3	5.0
7-PM10-CA-AVG-3000	1.4E+03	25	1	6	6.0	150.0	10.7
	1.4E+03	25	4	23	5.8	143.8	10.3
Use values highlighted in green for reporting.							
B. a. Sterne morphology present on zero spike sample							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 California High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM10-CA-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	3	0.8	18.8	#DIV/0!
10-PM10-CA-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM10-CA-HIGH-0	0.0E+00	25	1	3	3.0	75.0	#DIV/0!
	0.0E+00	25	4	6	1.5	37.5	#DIV/0!
11-PM10-CA-High-30	3.1E+01	25	1	2	2.0	50.0	161.3
	3.1E+01	25	4	4	1.0	25.0	80.6
12-PM10-CA-High-30	3.1E+01	25	1	0	0.0	0.0	0.0
	3.1E+01	25	4	2	0.5	12.5	40.3
4-PM10-CA-HIGH-30	1.4E+01	25	1	0	0.0	0.0	0.0
	1.4E+01	25	4	1	0.3	6.3	44.6
13-PM10-CA-High-300	3.1E+02	25	1	2	2.0	50.0	16.1
	3.1E+02	25	4	5	1.3	31.3	10.1
14-PM10-CA-High-300	3.1E+02	25	1	2	2.0	50.0	16.1
	3.1E+02	25	4	0	0.0	0.0	0.0
6-PM10-CA-HIGH-300	1.4E+02	25	1	0	0.0	0.0	0.0
	1.4E+02	25	4	9	2.3	56.3	40.2
15-PM10-CA-High-3,000	3.1E+03	25	1	9	9.0	225.0	7.3
	3.1E+03	25	4	21	5.3	131.3	4.2
16-PM10-CA-High-3,000	3.1E+03	25	1	5	5.0	125.0	4.0
	3.1E+03	25	4	20	5.0	125.0	4.0
8-PM10-CA-HIGH-PVDF-3,000	1.4E+03	25	1	3	3.0	75.0	5.4
	1.4E+03	25	4	16	4.0	100.0	7.1
Use values highlighted in green for reporting.							
B. a. Sterne morphology present on zero spike sample							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 New Hampshire Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PM10-NH-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM10-NH-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PM10-NH-AVG-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM10-NH-AVG-30	1.6E+01	25	1	0	0.0	0.0	0.0
	1.6E+01	25	4	0	0.0	0.0	0.0
4-PM10-NH-AVG-30	1.6E+01	25	1	0	0.0	0.0	0.0
	1.6E+01	25	4	0	0.0	0.0	0.0
11-PM10-NH-AVG-PVDF-30	5.0E+01	25	1	1	1.0	25.0	49.7
	5.0E+01	25	4	0	0.0	0.0	0.0
5-PM10-NH-AVG-300	1.6E+02	25	1	0	0.0	0.0	0.0
	1.6E+02	25	4	0	0.0	0.0	0.0
6-PM10-NH-AVG-300	1.6E+02	25	1	1	1.0	25.0	15.6
	1.6E+02	25	4	0	0.0	0.0	0.0
13-PM10-NH-AVG-PVDF-300	5.0E+02	25	1	2	2.0	50.0	9.9
	5.0E+02	25	4	2	0.5	12.5	2.5
7-PM10-NH-AVG-3000	1.6E+03	25	1	9	9.0	225.0	14.1
	1.6E+03	25	4	23	5.8	143.8	9.0
8-PM10-NH-AVG-3,000	1.6E+03	25	1	6	6.0	150.0	9.4
	1.6E+03	25	4	32	8.0	200.0	12.5
15-PM10-NH-AVG-PVDF-3,000	5.0E+03	25	1	4	4.0	100.0	2.0
	5.0E+03	25	4	25	6.3	156.3	3.1
Use values highlighted in green for reporting.							
2 distinct large growths covering the majority of the filter							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 New Hampshire High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (cfu)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM10-NH-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM10-NH-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM10-NH-HIGH-PVDF-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM10-NH-HIGH-30	1.6E+01	25	1	0	0.0	0.0	0.0
	1.6E+01	25	4	0	0.0	0.0	0.0
12-PM10-NH-HIGH-30	1.6E+01	25	1	0	0.0	0.0	0.0
	1.6E+01	25	4	0	0.0	0.0	0.0
12-PM10-NH-HIGH-PVDF-30	5.0E+01	25	1	0	0.0	0.0	0.0
	5.0E+01	25	4	0	0.0	0.0	0.0
13-PM10-NH-HIGH-300	1.6E+02	25	1	0	0.0	0.0	0.0
	1.6E+02	25	4	1	0.3	6.3	3.9
14-PM10-NH-HIGH-300	1.6E+02	25	1	0	0.0	0.0	0.0
	1.6E+02	25	4	2	0.5	12.5	7.8
14-PM10-NH-HIGH-PVDF-300	5.0E+02	25	1	1	1.0	25.0	5.0
	5.0E+02	25	4	4	1.0	25.0	5.0
15-PM10-NH-HIGH-3,000	1.6E+03	25	1	28	28.0	700.0	43.8
	1.6E+03	25	4	88	22.0	550.0	34.4
16-PM10-NH-HIGH-3,000	1.6E+03	25	1	13	13.0	325.0	20.3
	1.6E+03	25	4	60	15.0	375.0	23.4
16-PM10-NH-HIGH-PVDF-3,000	5.0E+03	25	1	4	4.0	100.0	2.0
	5.0E+03	25	4	30	7.5	187.5	3.7
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 South Carolina Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PM10-SC-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM10-SC-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-PM10-SC-AVG-0	0.0E+00	25	1	7	7.0	175.0	#DIV/0!
	0.0E+00	25	4	1	0.3	6.3	#DIV/0!
3-PM10-SC-AVG-30	2.7E+01	25	1	0	0.0	0.0	0.0
	2.7E+01	25	4	0	0.0	0.0	0.0
4-PM10-SC-AVG-30	2.7E+01	25	1	0	0.0	0.0	0.0
	2.7E+01	25	4	1	0.3	6.3	23.1
3-PM10-SC-AVG-30	2.9E+01	25	1	1	1.0	25.0	86.2
	2.9E+01	25	4	3	0.8	18.8	64.7
5-PM10-SC-AVG-300	2.7E+02	25	1	1	1.0	25.0	9.3
	2.7E+02	25	4	7	1.8	43.8	16.2
6-PM10-SC-AVG-300	2.7E+02	25	1	0	0.0	0.0	0.0
	2.7E+02	25	4	3	0.8	18.8	6.9
5-PM10-SC-AVG-300	2.9E+02	25	1	1	1.0	25.0	8.6
	2.9E+02	25	4	3	0.8	18.8	6.5
7-PM10-SC-AVG-3,000	2.7E+03	25	1	13	13.0	325.0	12.0
	2.7E+03	25	4	32	8.0	200.0	7.4
8-PM10-SC-AVG-3,000	2.7E+03	25	1	11	11.0	275.0	10.2
	2.7E+03	25	4	31	7.8	193.8	7.2
7-PM10-SC-AVG-3,000	2.9E+03	25	1	5	5.0	125.0	4.3
	2.9E+03	25	4	18	4.5	112.5	3.9
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 South Carolina High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PM10-SC-HIGH-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM10-SC-HIGH-0	0.0E+00	25	1	2	2.0	50.0	#DIV/0!
	0.0E+00	25	4	1	0.3	6.3	#DIV/0!
2-PM10-SC-HIGH-0	0.0E+00	25	1	2	2.0	50.0	#DIV/0!
	0.0E+00	25	4	2	0.5	12.5	#DIV/0!
11-PM10-SC-HIGH-30	2.7E+01	25	1	0	0.0	0.0	0.0
	2.7E+01	25	4	4	1.0	25.0	92.6
12-PM10-SC-HIGH-30	2.7E+01	25	1	1	1.0	25.0	92.6
	2.7E+01	25	4	5	1.3	31.3	115.7
4-PM10-SC-HIGH-30	2.9E+01	25	1	0	0.0	0.0	0.0
	2.9E+01	25	4	3	0.8	18.8	64.7
13-PM10-SC-HIGH-300	2.7E+02	25	1	2	2.0	50.0	18.5
	2.7E+02	25	4	7	1.8	43.8	16.2
14-PM10-SC-HIGH-300	2.7E+02	25	1	0	0.0	0.0	0.0
	2.7E+02	25	4	3	0.8	18.8	6.9
6-PM10-SC-HIGH-300	2.9E+02	25	1	1	1.0	25.0	8.6
	2.9E+02	25	4	7	1.8	43.8	15.1
15-PM10-SC-HIGHF-3,000	2.7E+03	25	1	1	1.0	25.0	0.9
	2.7E+03	25	4	23	5.8	143.8	5.3
16-PM10-SC-HIGH-3,000	2.7E+03	25	1	11	11.0	275.0	10.2
	2.7E+03	25	4	42	10.5	262.5	9.7
8-PM10-SC-HIGH-3,000	2.9E+03	25	1	0	0.0	0.0	0.0
	2.9E+03	25	4	11	2.8	68.8	2.4
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 Wisconsin Average							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
2-PM10-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PM10-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM10-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
4-PM10-AVG-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	0	0.0	0.0	0.0
11-PM10-AVG-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	0	0.0	0.0	0.0
12-PM10-AVG-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	0	0.0	0.0	0.0
6-PM10-AVG-300	1.8E+02	25	1	2	2.0	50.0	27.8
	1.8E+02	25	4	2	0.5	12.5	6.9
13-PM10-AVG-300	1.8E+02	25	1	2	2.0	50.0	27.8
	1.8E+02	25	4	3	0.8	18.8	10.4
14-PM10-AVG-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	6	1.5	37.5	20.8
8-PM10-AVG-3,000	1.8E+03	25	1	5	5.0	125.0	6.9
	1.8E+03	25	4	14	3.5	87.5	4.9
15-PM10-AVG-3,000	1.8E+03	25	1	2	2.0	50.0	2.8
	1.8E+03	25	4	17	4.3	106.3	5.9
16-PM10-AVG-3,000	1.8E+03	25	1	15	15.0	375.0	20.8
	1.8E+03	25	4	55	13.8	343.8	19.1
Use values highlighted in green for reporting.							
80% of plate Lawn							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

PM10 Wisconsin High							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-High-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-High-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
PM10HV-High-30	1.3E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
PM10HV-High-30	1.3E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
PM10HV-High-30	1.3E+01	25	1	0	0.0	0.0	0.0
		25	4	1	0.3	6.3	48.1
PM10HV-High-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
PM10HV-High-300	9.0E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
PM10HV-High-300	9.0E+01	25	1	1	1.0	25.0	27.8
		25	4	4	1.0	25.0	27.8
7-PM10-High-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	0	0.0	0.0	0.0
PM10HV-High-3,000	1.3E+03	25	1	5	5.0	125.0	9.6
		25	4	12	3.0	75.0	5.8
PM10HV-High-3,000	1.3E+03	25	1	5	5.0	125.0	9.6
		25	4	16	4.0	100.0	7.7
14-PM10-WI-HIGH-3000	2.9E+03	25	1	4	4.0	100.0	3.4
	2.9E+03	25	4	10	2.5	62.5	2.2
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX J. CULTURE RESULTS FOR AIR QUALITY FILTERS USING MYP MEDIUM

July 10, 2017 Trial – PM10 Wisconsin Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	3	0	0.0	0.0	N/A
2-PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
3-PM10HV-High-N/A	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
4-PM10HV-High-30	1.3E+01	25	1	1	1.0	25.0	192.3
		25	4	0	0.0	0.0	0.0
5-PM10HV-High-30	1.3E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
6-PM10HV-High-30	1.3E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
7-PM10HV-High-3,000	1.3E+03	25	1	5	5.0	125.0	9.6
		25	4	10	2.5	62.5	4.8
8-PM10HV-High-3,000	1.3E+03	25	1	2	2.0	50.0	3.8
		25	4	5	1.3	31.3	2.4
9-PM10HV-New-3,000	1.3E+03	25	1	1	1.0	25.0	1.9
		25	4	20	5.4	135.1	10.4
10-PM10HV-New-3,000	1.3E+03	25	1	0	0.0	0.0	0.0
		25	4	20	5.3	131.6	10.1
11-PM10HV-New-3,000	1.3E+03	25	1	3	3.0	75.0	5.8
		25	4	20	5.0	125.0	9.6
Green highlighted cells are the values reported for percent recovery							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

July 17, 2017 Trial – PM10 Wisconsin Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PM10HV-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
2-PM10HV-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
3-PM10HV-New-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
4-PM10HV-New-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	2	0.5	12.5	138.9
5-PM10HV-New-300	9.0E+01	25	1	2	2.0	50.0	55.6
		25	4	4	1.0	25.0	27.8
6-PM10HV-New-300	9.0E+01	25	1	0	0.0	0.0	0.0
		25	4	1	0.3	6.3	6.9
7-PM10HV-High-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
8-PM10HV-High-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
9-PM10HV-High-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
10-PM10HV-High-30	9.0E+00	25	1	0	0.0	0.0	0.0
		25	4	1	0.3	6.6	73.1
11-PM10HV-High-300	9.0E+01	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
12-PM10HV-High-300	9.0E+01	25	1	2	2.0	50.0	55.6
		25	4	6	1.5	37.5	41.7
Green highlighted cells are the values reported for percent recovery							
Spilled during spore recovery							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX K. CULTURE RESULTS FOR AIR QUALITY FILTERS USING BBCA MEDIUM

April 9, 2018 Trial – PM10 California Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
PBST Neg.	0	25	4	**0**	0.0	0.0	#DIV/0!
		25	4	**0**	0.0	0.0	#DIV/0!
1-PM10-CA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM10-CA-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM10-CA-AVG-30	3.1E+01	25	1	0	0.0	0.0	0.0
	3.1E+01	25	4	0	0.0	0.0	0.0
4-PM10-CA-AVG-30	3.1E+01	25	1	0	0.0	0.0	0.0
	3.1E+01	25	4	0	0.0	0.0	0.0
5-PM10-CA-AVG-300	3.1E+02	25	1	1	1.0	25.0	8.1
	3.1E+02	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
6-PM10-CA-AVG-300	3.1E+02	25	1	0	0.0	0.0	0.0
	3.1E+02	25	4	0	0.0	0.0	0.0
7-PM10-CA-AVG-3,000	3.1E+03	25	1	0	0.0	0.0	0.0
	3.1E+03	25	4	1	0.3	6.3	0.2
8-PM10-CA-AVG-3,000	3.1E+03	25	1	1	1.0	25.0	0.8
	3.1E+03	25	4	3	0.8	18.8	0.6
9-PM10-CA-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM10-CA-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM10-CA-High-30	3.1E+01	25	1	0	0.0	0.0	0.0
	3.1E+01	25	4	0	0.0	0.0	0.0
12-PM10-CA-High-30	3.1E+01	25	1	0	0.0	0.0	0.0
	3.1E+01	25	4	0	0.0	0.0	0.0
13-PM10-CA-High-300	3.1E+02	25	1	0	0.0	0.0	0.0
	3.1E+02	25	4	0	0.0	0.0	0.0
14-PM10-CA-High-300	3.1E+02	25	1	1	1.0	25.0	8.1
	3.1E+02	25	4	2	0.5	12.5	4.0
15-PM10-CA-High-3,000	3.1E+03	25	1	0	0.0	0.0	0.0
	3.1E+03	25	4	4	1.0	25.0	0.8
16-PM10-CA-High-3,000	3.1E+03	25	1	0	0.0	0.0	0.0
	3.1E+03	25	4	3	0.8	18.8	0.6
Use values highlighted in green for reporting							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

April 16, 2018 Trial – PM2.5 Wisconsin Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
PBST Neg.	0	**25**	4	**0**	0.0	0.0	#DIV/0!
		25	4	**0**	0.0	0.0	#DIV/0!
1-PM2.5-WI-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PM2.5-WI-AVG-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PM2.5-WI-AVG-30	2.2E+01	25	1	0	0.0	0.0	0.0
	2.2E+01	25	4	0	0.0	0.0	0.0
4-PM2.5-WI-AVG-30	2.2E+01	25	1	0	0.0	0.0	0.0
	2.2E+01	25	4	0	0.0	0.0	0.0
5-PM2.5-WI-AVG-300	2.2E+02	25	1	0	0.0	0.0	0.0
	2.2E+02	25	4	3	0.8	18.8	8.5
6-PM2.5-WI-AVG-300	2.2E+02	25	1	0	0.0	0.0	0.0
	2.2E+02	25	4	2	0.5	12.5	5.7
7-PM2.5-WI-AVG-3,000	2.2E+03	25	1	8	8.0	200.0	9.1
	2.2E+03	25	4	26	6.5	162.5	7.4
8-PM2.5-WI-AVG-3,000	2.2E+03	25	1	1	1.0	25.0	1.1
	2.2E+03	25	4	20	5.0	125.0	5.7
9-PM2.5-WI-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PM2.5-WI-High-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PM2.5-WI-High-30	2.2E+01	25	1	0	0.0	0.0	0.0
	2.2E+01	25	4	0	0.0	0.0	0.0
12-PM2.5-WI-High-30	2.2E+01	25	1	0	0.0	0.0	0.0
	2.2E+01	25	4	0	0.0	0.0	0.0
13-PM2.5-WI-High-300	2.2E+02	25	1	1	1.0	25.0	11.4
	2.2E+02	25	4	3	0.8	18.8	8.5
14-PM2.5-WI-High-300	2.2E+02	25	1	2	2.0	50.0	22.7
	2.2E+02	25	4	5	1.3	31.3	14.2
15-PM2.5-WI-High-3,000	2.2E+03	25	1	12	12.0	300.0	13.6
	2.2E+03	25	4	37	9.3	231.3	10.5
16-PM2.5-WI-High-3,000	2.2E+03	25	1	1	1.0	25.0	1.1
	2.2E+03	25	4	7	1.8	43.8	2.0
Use values highlighted in green for reporting							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX L. RV-PCR RESULTS FOR AIR QUALITY FILTERS USING CHROMOSOMAL AND PXO1 GENE TARGETS

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
07/10/17 PM10 Wisconsin	1-PM10-WI-High-0 T0	0	45	0	10.7	Neg.*	39.3	1.4	5.8	Neg.
	1-PM10-WI-High-0 Tf		34.3	0.4			33.6	0.2		
	2-PM10-WI-High-0 T0	0	45	0	13.1	Pos.	43.3	2.9	11.9	Pos.
	2-PM10-WI-High-0 Tf		31.9	0.1			31.4	0.1		
	3-PM10-WI-High-0 T0	0	45	0	14.4	Pos.	43.5	2.7	13.3	Pos.
	3-PM10-WI-High-0 Tf		30.6	0.2			30.2	0		
	1-PM10-WI-High-30 T0	13	45	0	22.7	Pos.	43.4	2.8	21.5	Pos.
	1-PM10-WI-High-30 Tf		22.3	0.1			21.9	0		
	2-PM10-WI-High-30 T0	13	40.7	3.7	7	Neg.	39.3	2.6	6.4	Neg.
	2-PM10-WI-High-30 Tf		33.8	0.4			32.9	0		
	3-PM10-WI-High-30 T0	13	44.7	0.5	10.5	Neg.*	39.3	0.5	6	Neg.
	3-PM10-WI-High-30 Tf		34.2	0.3			33.2	0.1		
	1-PM10-WI-High-3000 T0	1,300	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	1-PM10-WI-High-3000 Tf		N/A	N/A	N/A		N/A	N/A	N/A	
	2-PM10-WI-High-3000 T0	1,300	45	0	27.4	Pos.	44.1	1.5	26.8	Pos.
	2-PM10-WI-High-3000 Tf		17.6	0			17.3	0		
	3-PM10-WI-High-3000 T0	1,300	45	0	25.1	Pos.	45	0	25.3	Pos.
	3-PM10-WI-High-3000 Tf		19.9	0			19.7	0		
	1-PM10-WI-NEW-3000 T0	1,300	45	0	26.7	Pos.	45	0	26.9	Pos.
	1-PM10-WI-NEW-3000 Tf		18.3	0			18.1	0		
	2-PM10-WI-NEW-3000 T0	1,300	45	0	26	Pos.	45	0	26.5	Pos.
	2-PM10-WI-NEW-3000 Tf		19	0.1			18.5	0.1		
	3-PM10-WI-NEW-3000 T0	1,300	45	0	27	Pos.	44.7	0.5	26.9	Pos.
	3-PM10-WI-NEW-3000 Tf		18	0			17.8	0.1		
07/17/17 PM10 Wisconsin	1-PM10-WI-NEW-0 (T0)	0	45	0	8.1	Neg.	45	0	9.1	Neg.*
	1-PM10-WI-NEW-0 (Tf)		36.9	0.7			35.9	0.2		
	2-PM10-WI-NEW-0 (T0)	0	45	0	N/A	N/A	44.3	1.1	N/A	N/A
	2-PM10-WI-NEW-0 (Tf)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	PM10-WI-NEW-30 1 (T0)	9	42.8	3.8	6.7	Neg.	45	0	9.3	Neg.*
	PM10-WI-NEW-30 1 (Tf)		36.1	0.2			35.7	0.2		
	PM10-WI-NEW-30 2 (T0)	9	45	0	8.5	Neg.	45	0	9.3	Neg.*
	PM10-WI-NEW-30 2 (Tf)		36.5	0.3			35.7	0.7		
	PM10-WI-NEW-300 1 (T0)	90	42.9	3.7	25.5	Pos.	40.1	1.2	22.9	Pos.
	PM10-WI-NEW-300 1 (Tf)		17.4	0			17.2	0		
	PM10-WI-NEW-300 2 (T0)	90	45	0	27.6	Pos.	45	0	28	Pos.
	PM10-WI-NEW-300 2 (Tf)		17.4	0			17	0		
	PM10-WI-HIGH-0 1 (T0)	0	42.4	4.4	10.8	Pos.	40.2	4.2	9.2	Pos.
	PM10-WI-HIGH-0 1 (Tf)		31.7	0.1			31	0.1		
	PM10-WI-HIGH-0 2 (T0)	0	45	0	11.7	Pos.	43.3	2.9	10.7	Pos.
	PM10-WI-HIGH-0 2 (Tf)		33.3	0.1			32.6	0.2		
	PM10-WI-HIGH-30 1 (T0)	9	45	0	25.8	Pos.	41.1	3.4	22.3	Pos.
	PM10-WI-HIGH-30 1 (Tf)		19.2	0			18.8	0		
	PM10-WI-HIGH-300 1 (T0)	90	45	0	21.1	Pos.	44.8	0.3	21.7	Pos.
	PM10-WI-HIGH-300 1 (Tf)		23.9	0			23.1	0		
	PM10-WI-HIGH-300 2 (T0)	90	45	0	28.1	Pos.	44.7	0.6	28.1	Pos.
	PM10-WI-HIGH-300 2 (Tf)		16.9	0			16.6	0		
10/09/17 PM10 Wisconsin	1-PM10-NEW-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM10-NEW-0 Tf		45	0			45	0		
	2-PM10-AVG-0 T0	0	45	0	0	Neg.	44.9	0.2	-0.1	Neg.
	2-PM10-AVG-0 Tf		45	0			45	0		
	3-PM10-NEW-30 T0	18	45	0	27.8	Pos.	45	0	27.9	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	3-PM10-NEW-30 Tf		17.2	0.1			17.1	0		
	4-PM10-AVG-30 T0	18	45	0	22.4	Pos.	45	0	22.9	Pos.
	4-PM10-AVG-30 Tf		22.6	0			22.1	0		
	5-PM10-NEW-300 T0	180	45	0	27.2	Pos.	45	0	27.5	Pos.
	5-PM10-NEW-300 Tf		17.8	0.1			17.5	0		
	6-PM10-AVG-300 T0	180	45	0	24.5	Pos.	45	0	25.1	Pos.
	6-PM10-AVG-300 Tf		20.5	0			19.9	0		
	7-PM10-High-300 T0	180	45	0	20.6	Pos.	45	0	21.1	Pos.
	7-PM10-High-300 Tf		24.4	0.1			23.9	0		
	8-PM10-AVG-3,000 T0	1,800	45	0	26.7	Pos.	45	0	27.1	Pos.
	8-PM10-AVG-3,000 Tf		18.3	0			17.9	0		
	9-PM10-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM10-AVG-0 Tf		45	0			45	0		
	10-PM10-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM10-AVG-0 Tf		45	0			45	0		
	11-PM10-AVG-30 T0	18	45	0	2.1	Neg.	45	0	8.6	Neg.
	11-PM10-AVG-30 Tf		42.9	3.6			36.4	0.4		
	12-PM10-AVG-30 T0	18	45	0	19.1	Pos.	45	0	19.6	Pos.
	12-PM10-AVG-30 Tf		25.9	0			25.4	0		
	13-PM10-AVG-300 T0	180	45	0	22.6	Pos.	45	0	23	Pos.
	13-PM10-AVG-300 Tf		22.4	0			22	0.1		
	14-PM10-AVG-300 T0	180	45	0	24.8	Pos.	45	0	25.6	Pos.
	14-PM10-AVG-300 Tf		20.2	0			19.4	0		
	15-PM10-AVG-3,000 T0	1,800	45	0	26.7	Pos.	45	0	27.3	Pos.
	15-PM10-AVG-3,000 Tf		18.3	0			17.7	0		
	16-PM10-AVG-3,000 T0	1,800	45	0	29.2	Pos.	45	0	29.8	Pos.
	16-PM10-AVG-3,000 Tf		15.8	0			15.2	0		
10/16/17 PM2.5 Florida	1-PM2.5-FL-AVG -0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM2.5-FL-AVG -0 Tf		45	0			45	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	2-PM2.5-FL-AVG -0 T0	0	45	0	2.2	Neg.	45	0	0.1	Neg.
	2-PM2.5-FL-AVG -0 Tf		42.8	3.9			44.9	0.2		
	3-PM2.5-FL-AVG -30 T0	20	45	0	27.8	Pos.	45	0	28	Pos.
	3-PM2.5-FL-AVG -30 Tf		17.2	0			17	0		
	4-PM2.5-FL-AVG -30 T0	20	45	0	28.5	Pos.	45	0	28.8	Pos.
	4-PM2.5-FL-AVG -30 Tf		16.5	0.1			16.2	0.1		
	5-PM2.5-FL-AVG -300 T0	200	45	0	28.2	Pos.	45	0	28.4	Pos.
	5-PM2.5-FL-AVG -300 Tf		16.8	0.1			16.6	0		
	6-PM2.5- FL-AVG -300 T0	200	45	0	28.2	Pos.	45	0	28.5	Pos.
	6-PM2.5- FL-AVG -300 Tf		16.8	0			16.5	0		
	7-PM2.5-FL-AVG -3,000 T0	2,000	45	0	27.5	Pos.	45	0	27.8	Pos.
	7-PM2.5-FL-AVG -3,000 Tf		17.5	0			17.2	0		
	8-PM2.5-FL-AVG -3,000 T0	2,000	45	0	27.8	Pos.	45	0	28.2	Pos.
	8-PM2.5-FL-AVG -3,000 Tf		17.2	0			16.8	0		
	9-PM2.5-FL-HIGH-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-FL-HIGH-0 Tf		45	0			45	0		
	10-PM2.5-FL-HIGH -0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-FL-HIGH -0 Tf		45	0			45	0		
	11-PM2.5-FL-HIGH -30 T0	20	45	0	28.8	Pos.	45	0	28.8	Pos.
	11-PM2.5-FL-HIGH -30 Tf		16.2	0.1			16.2	0		
	12-PM2.5-FL-HIGH -30 T0	20	45	0	0	Neg.	45	0	0	Neg.
	12-PM2.5-FL-HIGH -30 Tf		45	0			45	0		
	13-PM2.5-FL-HIGH -300 T0	200	45	0	29.4	Pos.	45	0	29.6	Pos.
	13-PM2.5-FL-HIGH -300 Tf		15.6	0			15.4	0		
	14-PM2.5-FL-HIGH -300 T0	200	45	0	28.2	Pos.	45	0	28.5	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	14-PM2.5-FL-HIGH - 300 Tf		16.8	0.1			16.5	0		
	15-PM2.5-FL-HIGH - 3,000 T0	2,000	45	0	27.8	Pos.	45	0	28.1	Pos.
	15-PM2.5-FL-HIGH - 3,000 Tf		17.2	0			16.9	0		
	16-PM2.5-FL-HIGH - 3,000 T0	2,000	45	0	27.9	Pos.	45	0	28.2	Pos.
	16-PM2.5-FL-HIGH - 3,000 Tf		17.1	0			16.8	0		
10/23/17 PM10 New Hampshire	1-PM10-NH-AVG-0 T0	0	45	0	0	Neg.	45	0	0.7	Neg.
	1-PM10-NH-AVG-0 Tf		45	0			44.3	1.1		
	2-PM10-NH-AVG-0 T0	0	45	0	5	Neg.	45	0	8.8	Neg.
	2-PM10-NH-AVG-0 Tf		40	4.4			36.2	0.6		
	3-PM10-NH-AVG-30 T0	16	45	0	27.9	Pos.	45	0	28	Pos.
	3-PM10-NH-AVG-30 Tf		17.1	0			17	0		
	4-PM10-NH-AVG-30 T0	16	45	0	8.8	Neg.	45	0	9.7	Neg.*
	4-PM10-NH-AVG-30 Tf		36.2	0.4			35.3	0.2		
	5-PM10-NH-AVG-300 T0	160	45	0	27.2	Pos.	45	0	27.5	Pos.
	5-PM10-NH-AVG-300 Tf		17.8	0.1			17.5	0		
	6-PM10-NH-AVG-300 T0	160	45	0	27.5	Pos.	45	0	27.7	Pos.
	6-PM10-NH-AVG-300 Tf		17.5	0			17.3	0		
	7-PM10-NH-AVG-3,000 T0	1,600	44.5	0.8	27.7	Pos.	45	0	28.3	Pos.
	7-PM10-NH-AVG-3,000 Tf		16.8	0.1			16.7	0		
	8-PM10-NH-AVG-3,000 T0	1,600	45	0	27.5	Pos.	45	0	27.6	Pos.
	8-PM10-NH-AVG-3,000 Tf		17.5	0.1			17.4	0		
	9-PM10-NH-HIGH-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM10-NH-HIGH-0 Tf		45	0			45	0		
	10-PM10-NH-HIGH-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM10-NH-HIGH-0 Tf		45	0			45	0		
	11-PM10-NH-HIGH-30 T0	16	45	0	10.9	Pos.	45	0	11.7	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	11-PM10-NH-HIGH-30 Tf		34.1	0.5			33.3	0		
	12-PM10-NH-HIGH-30 T0	16	45	0	4.3	Neg.	45	0	8.1	Neg.
	12-PM10-NH-HIGH-30 Tf		40.7	3.7			36.9	0.5		
	13-PM10-NH-HIGH-300 T0	160	45	0	27.5	Pos.	45	0	27.5	Pos.
	13-PM10-NH-HIGH-300 Tf		17.5	0.1			17.5	0		
	14-PM10-NH-HIGH-300 T0	160	45	0	27	Pos.	45	0	27.1	Pos.
	14-PM10-NH-HIGH-300 Tf		18	0			17.9	0		
	15-PM10-NH-HIGH-3,000 T0	1,600	45	0	25.1	Pos.	45	0	25.4	Pos.
	15-PM10-NH-HIGH-3,000 Tf		19.9	0			19.6	0		
	16-PM10-NH-HIGH-3,000 T0	1,600	45	0	26.8	Pos.	45	0	27	Pos.
	16-PM10-NH-HIGH-3,000 Tf		18.2	0.3			18	0		
10/30/17 PM2.5 Arizona	1-PM2.5-NA-New-0 T0	0	45	0	0	Neg.	42.7	3.9	-2.3	Neg.
	1-PM2.5-NA-New-0 Tf		45	0			45	0		
	2-PM2.5-AZ-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM2.5-AZ-AVG-0 Tf		45	0			45	0		
	3-PM2.5-NA-New-30 T0	15	45	0	28	Pos.	45	0	27.9	Pos.
	3-PM2.5-NA-New-30 Tf		17	0.1			17.1	0		
	4-PM2.5-AZ-AVG-30 T0	15	45	0	27.6	Pos.	45	0	28	Pos.
	4-PM2.5-AZ-AVG-30 Tf		17.4	0			17	0		
	5-PM2.5-NA-New-300 T0	150	45	0	27.9	Pos.	45	0	27.9	Pos.
	5-PM2.5-NA-New-300 Tf		17.1	0			17.1	0		
	6-PM2.5-AZ-AVG-300 T0	150	45	0	27.1	Pos.	45	0	27.5	Pos.
	6-PM2.5-AZ-AVG-300 Tf		17.9	0			17.5	0		
	7-PM2.5-NA-New-3,000 T0	1,500	45	0	27.1	Pos.	45	0	27.2	Pos.
	7-PM2.5-NA-New-3,000 Tf		17.9	0			17.8	0.1		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	8-PM2.5-AZ-AVG-3,000 T0	1,500	45	0	28.1	Pos.	45	0	28.6	Pos.
	8-PM2.5-AZ-AVG-3,000 Tf		16.9	0			16.4	0		
	9-PM2.5-AZ-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-AZ-High-0 Tf		45	0			45	0		
	10-PM2.5-AZ-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-AZ-High-0 Tf		45	0			45	0		
	11-PM2.5-AZ-High-30 T0	15	45	0	28.5	Pos.	45	0	28.3	Pos.
	11-PM2.5-AZ-High-30 Tf		16.5	0.1			16.7	0		
	12-PM2.5-AZ-High-30 T0	15	45	0	27.9	Pos.	45	0	28	Pos.
	12-PM2.5-AZ-High-30 Tf		17.1	0			17	0		
	13-PM2.5-AZ-High-300 T0	150	45	0	27.3	Pos.	45	0	27.3	Pos.
	13-PM2.5-AZ-High-300 Tf		17.7	0.1			17.7	0		
	14-PM2.5-AZ-High-300 T0	150	45	0	27.1	Pos.	45	0	27.2	Pos.
	14-PM2.5-AZ-High-300 Tf		17.9	0			17.8	0		
	15-PM2.5-AZ-High-3,000 T0	1,500	45	0	27.1	Pos.	45	0	27.3	Pos.
	15-PM2.5-AZ-High-3,000 Tf		17.9	0.1			17.7	0		
	16-PM2.5-AZ-High-3,000 T0	1,500	45	0	27.1	Pos.	45	0	27.6	Pos.
	16-PM2.5-AZ-High-3,000 Tf		17.9	0			17.4	0		
03/26/18 PM2.5 Arizona	1-PM2.5-AZ-AVG-PES-0 T0	0	44.6	0.7	-0.4	Neg.	43.8	2	-1.2	Neg.
	1-PM2.5-AZ-AVG-PES-0 Tf		45	0			45	0		
	2-PM2.5-AZ-AVG-PVDF-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM2.5-AZ-AVG-PVDF-0 Tf		45	0			45	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	3-PM2.5-AZ-AVG-PES-30 T0	23	43.7	2.2	25.8	Pos.	44.2	1.4	26.5	Pos.
	3-PM2.5-AZ-AVG-PES-30 Tf		17.9	0.1			17.7	0		
	4-PM2.5-AZ-AVG-PVDF-30 T0	23	43.3	2.9	26	Pos.	38.2	0.2	21.3	Pos.
	4-PM2.5-AZ-AVG-PVDF-30 Tf		17.3	0			16.9	0		
	5-PM2.5-AZ-AVG-PES-300 T0	230	43.4	2.7	26.2	Pos.	41.1	3.4	23.8	Pos.
	5-PM2.5-AZ-AVG-PES-300 Tf		17.2	0			17.3	0		
	6-PM2.5-AZ-AVG-PVDF-300 T0	230	45	0	28.4	Pos.	39.3	1.2	23	Pos.
	6-PM2.5-AZ-AVG-PVDF-300 Tf		16.6	0			16.3	0		
	7-PM2.5-AZ-AVG-PES-3,000 T0	2,300	45	0	27.2	Pos.	43.8	1.6	26.3	Pos.
	7-PM2.5-AZ-AVG-PES-3,000 Tf		17.8	0			17.5	0		
	8-PM2.5-AZ-AVG-PVDF-3,000 T0	2,300	44	1.8	25.6	Pos.	41.3	3.2	23.4	Pos.
	8-PM2.5-AZ-AVG-PVDF-3,000 Tf		18.3	0			17.9	0		
	9-PM2.5-NA-NEW-PES-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-NA-NEW-PES-0 Tf		45	0			45	0		
	10-PM2.5-NA-NEW-PVDF-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-NA-NEW-PVDF-0 Tf		45	0			45	0		
	11-PM2.5-NA-NEW-PES-30 T0	23	45	0	28.2	Pos.	45	0	28.1	Pos.
	11-PM2.5-NA-NEW-PES-30 Tf		16.8	0			16.9	0		
	12-PM2.5-NA-NEW-PVDF-30 T0	23	45	0	27.5	Pos.	42.5	3.2	25.3	Pos.
	12-PM2.5-NA-NEW-PVDF-30 Tf		17.5	0			17.2	0		
	13-PM2.5-NA-NEW-PES-300 T0	230	45	0	27.9	Pos.	45	0	27.8	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	13-PM2.5-NA-NEW-PES-300 Tf		17.1	0			17.2	0		
	14-PM2.5-NA-NEW-PVDF-300 T0	230	45	0	26.6	Pos.	45	0	26.8	Pos.
	14-PM2.5-NA-NEW-PVDF-300 Tf		18.4	0.1			18.2	0		
	15-PM2.5-NA-NEW-PES-3,000 T0	2,300	45	0	27.3	Pos.	45	0	27.5	Pos.
	15-PM2.5-NA-NEW-PES-3,000 Tf		17.7	0.1			17.5	0		
	16-PM2.5-NA-NEW-PVDF-3,000 T0	2,300	45	0	27	Pos.	45	0	27.5	Pos.
	16-PM2.5-NA-NEW-PVDF-3,000 Tf		18	0			17.5	0		
04/02/18 PM2.5 FL & PM10 New Hampshire	1-PM2.5-FL-AVG-0 T0	0	45	0	12.2	Pos.	45	0	12.8	Pos.
	1-PM2.5-FL-AVG-0 Tf		32.8	0.3			32.2	0.2		
	2-PM2.5-FL-High-0 T0	0	45	0	11.6	Pos.	45	0	12.3	Pos.
	2-PM2.5-FL-High-0 Tf		33.4	0.1			32.7	0.1		
	3-PM2.5-FL-AVG-30 T0	25	45	0	26.6	Pos.	45	0	26.8	Pos.
	3-PM2.5-FL-AVG-30 Tf		18.4	0.1			18.2	0.1		
	4-PM2.5-FL-High-30 T0	25	45	0	26.1	Pos.	45	0	26.4	Pos.
	4-PM2.5-FL-High-30 Tf		18.9	0.1			18.6	0.1		
	5-PM2.5-FL-AVG-300 T0	250	45	0	26.6	Pos.	45	0	26.9	Pos.
	5-PM2.5-FL-AVG-300 Tf		18.4	0			18.1	0		
	6-PM2.5-FL-HIGH-300 T0	250	45	0	No Data	No Data	45	0	No Data	No Data
	6-PM2.5-FL-HIGH-300 Tf		No Data	No Data			No Data	No Data		
	7-PM2.5-FL-AVG-3,000 T0	2,500	45	0	25.3	Pos.	45	0	25.4	Pos.
	7-PM2.5-FL-AVG-3,000 Tf		19.7	0			19.6	0.1		
	8-PM2.5-FL-HIGH-3,000 T0	2,500	45	0	26.6	Pos.	45	0	27	Pos.
	8-PM2.5-FL-HIGH-3,000 Tf		18.4	0			18	0		
	9-PM10-NH-AVG-0 T0	0	45	0	0	Neg.	45	0	6.8	Neg.
	9-PM10-NH-AVG-0 Tf		45	0			38.2	0.2		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	10-PM10-NH-HIGH-0 T0	0	45	0	0	Neg.	45	0	9.8	Neg. (chromosome assay ΔCt is negative)
	10-PM10-NH-HIGH-0 Tf		45	0			35.2	0.5		
	11-PM10-NH-AVG-30 T0	25	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
	11-PM10-NH-AVG-30 Tf		41.6	3.2			33.5	0.4		
	12-PM10-NH-HIGH-30 T0	25	45	0	27	Pos.	45	0	27.2	Pos.
	12-PM10-NH-HIGH-30 Tf		18	0.1			17.8	0.1		
	13-PM10-NH-AVG-300 T0	250	45	0	25.3	Pos.	45	0	25.4	Pos.
	13-PM10-NH-AVG-300 Tf		19.7	0.1			19.6	0.1		
	14-PM10-NH-HIGH-300 T0	250	45	0	22	Pos.	45	0	22.2	Pos.
	14-PM10-NH-HIGH-300 Tf		23	0			22.8	0		
	15-PM10-NH-AVG-3,000 T0	2,500	45	0	27.4	Pos.	45	0	27.6	Pos.
	15-PM10-NH-AVG-3,000 Tf		17.6	0			17.4	0		
	16-PM10-NH-HIGH-3,000 T0	2,500	45	0	25.5	Pos.	45	0	25.9	Pos.
	16-PM10-NH-HIGH-3,000 Tf		19.5	0			19.1	0		
04/09/18 PM10 California	1-PM10-CA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM10-CA-AVG-0 Tf		45	0			45	0		
	2-PM10-CA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM10-CA-AVG-0 Tf		45	0			45	0		
	3-PM10-CA-AVG-30 T0	16	44.9	0.2	29.5	Pos.	45	0	30.1	Pos.
	3-PM10-CA-AVG-30 Tf		15.4	0.1			14.9	0		
	4-PM10-CA-AVG-30 T0	16	45	0	0	Neg.	45	0	0	Neg.
	4-PM10-CA-AVG-30 Tf		45	0			45	0		
	5-PM10-CA-AVG-300 T0	160	45	0	23.3	Pos.	45	0	23.5	Pos.
	5-PM10-CA-AVG-300 Tf		21.7	0.1			21.5	0		
	6-PM10-CA-AVG-300 T0	160	45	0	24.7	Pos.	45	0	25.4	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	6-PM10-CA-AVG-300 Tf		20.3	0.1			19.6	0		
	7-PM10-CA-AVG-3,000 T0	1,600	45	0	22	Pos.	45	0	22.2	Pos.
	7-PM10-CA-AVG-3,000 Tf		23	0			22.8	0.1		
	8-PM10-CA-AVG-3,000 T0	1,600	45	0	29.5	Pos.	45	0	30.1	Pos.
	8-PM10-CA-AVG-3,000 Tf		15.5	0			14.9	0		
	9-PM10-CA-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM10-CA-High-0 Tf		45	0			45	0		
	10-PM10-CA-High-0 T0	0	45	0	0	Neg.	45	0	1.7	Neg.
	10-PM10-CA-High-0 Tf		45	0			43.3	2.8		
	11-PM10-CA-High-30 T0	16	45	0	5.1	Neg.	45	0	7.4	Neg.
	11-PM10-CA-High-30 Tf		39.9	4.5			37.6	1.4		
	12-PM10-CA-High-30 T0	16	45	0	29.5	Pos.	45	0	29.9	Pos.
	12-PM10-CA-High-30 Tf		15.5	0			15.1	0		
	13-PM10-CA-High-300 T0	160	45	0	28.9	Pos.	45	0	29.1	Pos.
	13-PM10-CA-High-300 Tf		16.1	0			15.9	0		
	14-PM10-CA-High-300 T0	160	45	0	28	Pos.	45	0	28.2	Pos.
	14-PM10-CA-High-300 Tf		17	0.1			16.8	0		
	15-PM10-CA-High-3,000 T0	1,600	45	0	28.7	Pos.	45	0.1	28.8	Pos.
	15-PM10-CA-High-3,000 Tf		16.3	0.1			16.2	0		
	16-PM10-CA-High-3,000 T0	1,600	45	0	26.6	Pos.	45	0	27.4	Pos.
	16-PM10-CA-High-3,000 Tf		18.4	0.1			17.6	0		
04/16/18 PM2.5 WI	1-PM2.5-WI-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM2.5-WI-AVG-0 Tf		45	0			45	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	2-PM2.5-WI-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM2.5-WI-AVG-0 Tf		45	0			45	0		
	3-PM2.5-WI-AVG-30 T0	22	45	0	#DIV/0!	No Data	45	0	#DIV/0!	No Data
	3-PM2.5-WI-AVG-30 Tf		#DIV/0!	#DIV/0!			#DIV/0!	#DIV/0!		
	4-PM2.5-WI-AVG-30 T0	22	45	0	27.96	Pos.	45	0	28.27	Pos.
	4-PM2.5-WI-AVG-30 Tf		17.04	0.05			16.73	0.01		
	5-PM2.5-WI-AVG-300 T0	220	45	0	27.84	Pos.	45	0	28.02	Pos.
	5-PM2.5-WI-AVG-300 Tf		17.16	0.06			16.98	0.02		
	6-PM2.5-WI-AVG-300 T0	220	45	0	27.81	Pos.	45	0	28.04	Pos.
	6-PM2.5-WI-AVG-300 Tf		17.19	0.09			16.96	0.05		
	7-PM2.5-WI-AVG-3,000 T0	2,200	45	0	27.6	Pos.	45	0	27.84	Pos.
	7-PM2.5-WI-AVG-3,000 Tf		17.4	0.06			17.16	0.02		
	8-PM2.5-WI-AVG-3,000 T0	2,200	45	0	27.69	Pos.	45	0	28.02	Pos.
	8-PM2.5-WI-AVG-3,000 Tf		17.31	0.04			16.98	0.03		
	9-PM2.5-WI-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-WI-High-0 Tf		45	0			45	0		
	10-PM2.5-WI-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-WI-High-0 Tf		45	0			45	0		
	11-PM2.5-WI-High-30 T0	22	45	0	28	Pos.	45	0	28.3	Pos.
	11-PM2.5-WI-High-30 Tf		17	0.03			16.7	0.05		
	12-PM2.5-WI-High-30 T0	22	45	0	28.55	Pos.	45	0	28.85	Pos.
	12-PM2.5-WI-High-30 Tf		16.45	0.01			16.15	0.03		
	13-PM2.5-WI-High-300 T0	220	45	0	#DIV/0!	No Data	45	0	#DIV/0!	No Data
	13-PM2.5-WI-High-300 Tf		#DIV/0!	#DIV/0!			#DIV/0!	#DIV/0!		
	14-PM2.5-WI-High-300 T0	220	45	0	28.07	Pos.	45	0	28.47	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	14-PM2.5-WI-High-300 Tf		16.93	0.06			16.53	0.06		
	15-PM2.5-WI-High-3,000 T0	2,200	45	0	27.61	Pos.	45	0	28	Pos.
	15-PM2.5-WI-High-3,000 Tf		17.39	0.04			17	0.03		
	16-PM2.5-WI-High-3,000 T0	2,200	45	0	#DIV/0!	No Data	45	0	#DIV/0!	No Data
	16-PM2.5-WI-High-3,000 Tf		#DIV/0!	#DIV/0!			#DIV/0!	#DIV/0!		
04/30/18 PM2.5 Massachusetts	1-PM2.5-MA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM2.5-MA-AVG-0 Tf		45	0			45	0		
	2-PM2.5-MA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM2.5-MA-AVG-0 Tf		45	0			45	0		
	3-PM2.5-MA-AVG-30 T0	32	45	0	27.3	Pos.	45	0	27.6	Pos.
	3-PM2.5-MA-AVG-30 Tf		17.7	0.1			17.4	0		
	4-PM2.5-MA-AVG-30 T0	32	45	0	27.5	Pos.	45	0	27.7	Pos.
	4-PM2.5-MA-AVG-30 Tf		17.5	0			17.3	0		
	5-PM2.5-MA-AVG-300 T0	320	45	0	27.1	Pos.	45	0	27.2	Pos.
	5-PM2.5-MA-AVG-300 Tf		17.9	0.1			17.8	0		
	6-PM2.5-MA-AVG-300 T0	320	45	0	27.1	Pos.	45	0	27.2	Pos.
	6-PM2.5-MA-AVG-300 Tf		17.9	0			17.8	0		
	7-PM2.5-MA-AVG-3,000 T0	3,200	45	0	27.2	Pos.	45	0	27.4	Pos.
	7-PM2.5-MA-AVG-3,000 Tf		17.8	0			17.6	0		
	8-PM2.5-MA-AVG-3,000 T0	3,200	45	0	26.6	Pos.	45	0	26.9	Pos.
	8-PM2.5-MA-AVG-3,000 Tf		18.4	0			18.1	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	9-PM2.5-MA-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-MA-High-0 Tf		45	0			45	0		
	10-PM2.5-MA-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-MA-High-0 Tf		45	0			45	0		
	11-PM2.5-MA-High-30 T0	32	45	0	27.1	Pos.	45	0	27.2	Pos.
	11-PM2.5-MA-High-30 Tf		17.9	0.1			17.8	0		
	12-PM2.5-MA-High-30 T0	32	45	0	28.2	Pos.	45	0	28.4	Pos.
	12-PM2.5-MA-High-30 Tf		16.8	0			16.6	0		
	13-PM2.5-MA-High-300 T0	320	45	0	27.1	Pos.	45	0	27.5	Pos.
	13-PM2.5-MA-High-300 Tf		17.9	0.1			17.5	0		
	14-PM2.5-MA-High-300 T0	320	45	0	27.8	Pos.	45	0	27.9	Pos.
	14-PM2.5-MA-High-300 Tf		17.2	0			17.1	0		
	15-PM2.5-MA-High-3,000 T0	3,200	45	0	27.1	Pos.	45	0	27.6	Pos.
	15-PM2.5-MA-High-3,000 Tf		17.9	0			17.4	0		
	16-PM2.5-MA-High-3,000 T0	3,200	45	0	27	Pos.	45	0	27.3	Pos.
	16-PM2.5-MA-High-3,000 Tf		18	0.1			17.7	0		
05/07/18 PM2.5 Massachusets and Wisconsin	1-PM2.5-MA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM2.5-MA-AVG-0 Tf		45	0			45	0		
	2-PM2.5-WI-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM2.5-WI-AVG-0 Tf		45	0			45	0		
	3-PM2.5-MA-AVG-30 T0	20	45	0	26.9	Pos.	45	0	26.9	Pos.
	3-PM2.5-MA-AVG-30 Tf		18.1	0.1			18.1	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	4-PM2.5-WI-AVG-30 T0	20	45	0	28.9	Pos.	45	0	28.9	Pos.
	4-PM2.5-WI-AVG-30 Tf		16.1	0.1			16.1	0		
	5-PM2.5-MA-AVG-300 T0	200	45	0	27.9	Pos.	45	0	28	Pos.
	5-PM2.5-MA-AVG-300 Tf		17.1	0.1			17	0		
	6-PM2.5-WI-AVG-300 T0	200	45	0	29.1	Pos.	45	0	29.2	Pos.
	6-PM2.5-WI-AVG-300 Tf		15.9	0			15.8	0		
	7-PM2.5-MA-AVG-3,000 T0	2,000	45	0	28.6	Pos.	45	0	28.7	Pos.
	7-PM2.5-MA-AVG-3,000 Tf		16.4	0.1			16.3	0		
	8-PM2.5-WI-AVG-3,000 T0	2,000	45	0	27.9	Pos.	45	0	28.1	Pos.
	8-PM2.5-WI-AVG-3,000 Tf		17.1	0			16.9	0		
	9-PM2.5-MA-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-MA-High-0 Tf		45	0			45	0		
	10-PM2.5-WI-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM2.5-WI-High-0 Tf		45	0			45	0		
	11-PM2.5-MA-High-30 T0	20	45	0	28.3	Pos.	45	0	28.3	Pos.
	11-PM2.5-MA-High-30 Tf		16.7	0.1			16.7	0		
	12-PM2.5-WI-High-30 T0	20	45	0	26.8	Pos.	45	0	26.7	Pos.
	12-PM2.5-WI-High-30 Tf		18.2	0			18.3	0		
	13-PM2.5-MA-High-300 T0	200	45	0	28.2	Pos.	45	0	28.2	Pos.
	13-PM2.5-MA-High-300 Tf		16.8	0.1			16.8	0		
	14-PM2.5-WI-High-300 T0	200	45	0	27.8	Pos.	45	0	27.9	Pos.
	14-PM2.5-WI-High-300 Tf		17.2	0			17.1	0		
	15-PM2.5-MA-High-3,000 T0	2,000	45	0	28.1	Pos.	45	0	28.1	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	15-PM2.5-MA-High-3,000 Tf		16.9	0			16.9	0		
	16-PM2.5-WI-High-3,000 T0	2,000	45	0	27.7	Pos.	45	0	27.9	Pos.
	16-PM2.5-WI-High-3,000 Tf		17.3	0			17.1	0		
05/14/18 PM2.5 Arizona, Florida, Wisconsin & PM10 California	1-PM10-CA-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM10-CA-AVG-0 Tf		45	0			45	0		
	2-PM10-CA-HIGH-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM10-CA-HIGH-0 Tf		45	0			45	0		
	3-PM10-CA-AVG-30 T0	14	45	0	0	Neg.	45	0	0	Neg.
	3-PM10-CA-AVG-30 Tf		45	0			45	0		
	4-PM10-CA-HIGH-30 T0	14	45	0	0	Neg.	45	0	0	Neg.
	4-PM10-CA-HIGH-30 Tf		45	0			45	0		
	5-PM10-CA-AVG-300 T0	140	45	0	21.2	Pos.	45	0	21.6	Pos.
	5-PM10-CA-AVG-300 Tf		23.8	0			23.4	0.1		
	6-PM10-CA-HIGH-300 T0	140	45	0	22.5	Pos.	45	0	23.1	Pos.
	6-PM10-CA-HIGH-300 Tf		22.5	0			21.9	0		
	7-PM10-CA-AVG-3,000 T0	1,400	45	0	27.5	Pos.	45	0	27.8	Pos.
	7-PM10-CA-AVG-3,000 Tf		17.5	0.1			17.2	0.1		
	8-PM10-CA-HIGH-3,000 T0	1,400	45	0	28.8	Pos.	45	0	29.1	Pos.
	8-PM10-CA-HIGH-3,000 Tf		16.2	0.1			15.9	0.1		
	9-PM2.5-AZ-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM2.5-AZ-High-0 Tf		45	0			45	0		
	10-PM2.5-AZ-High-30 T0	14	45	0	23.1	Pos.	45	0	23.1	Pos.
	10-PM2.5-AZ-High-30 Tf		21.9	0			21.9	0		
	11-PM2.5-WI-AVG-30 T0	14	45	0	23.2	Pos.	45	0	23.1	Pos.
	11-PM2.5-WI-AVG-30 Tf		21.8	0.1			21.9	0		
	12-PM2.5-AZ-HIGH-300 T0	140	45	0	28.7	Pos.	45	0	28.9	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	12-PM2.5-AZ-HIGH-300 Tf		16.3	0			16.1	0		
	13-PM2.5-FL-High-300 T0	140	45	0	28.6	Pos.	45	0	28.8	Pos.
	13-PM2.5-FL-High-300 Tf		16.4	0			16.2	0		
	14-PM2.5-WI-High-300 T0	140	45	0	28.6	Pos.	45	0	28.7	Pos.
	14-PM2.5-WI-High-300 Tf		16.4	0			16.3	0		
	15-PM2.5-AZ-High-3,000 T0	1,400	45	0	28.3	Pos.	45	0	28.8	Pos.
	15-PM2.5-AZ-High-3,000 Tf		16.7	0.1			16.2	0.1		
	16-PM2.5-WI-High-3,000 T0	1,400	45	0	28.5	Pos.	45	0	28.8	Pos.
	16-PM2.5-WI-High-3,000 Tf		16.5	0			16.2	0		
05/21/18 PM10 South Carolina	1-PM10-SC-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM10-SC-AVG-0 Tf		45	0			45	0		
	2-PM10-SC-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM10-SC-AVG-0 Tf		45	0			45	0		
	3-PM10-SC-AVG-30 T0	27	45	0	18.1	Pos.	45	0	18.2	Pos.
	3-PM10-SC-AVG-30 Tf		26.9	0.1			26.8	0		
	4-PM10-SC-AVG-30 T0	27	45	0	23.3	Pos.	45	0	23.8	Pos.
	4-PM10-SC-AVG-30 Tf		21.7	0			21.2	0		
	5-PM10-SC-AVG-300 T0	270	45	0	27.9	Pos.	45	0	28.1	Pos.
	5-PM10-SC-AVG-300 Tf		17.1	0.1			16.9	0		
	6-PM10-SC-AVG-300 T0	270	45	0	28.6	Pos.	45	0	28.7	Pos.
	6-PM10-SC-AVG-300 Tf		16.4	0			16.3	0		
	7-PM10-SC-AVG-3,000 T0	2,700	45	0	28.7	Pos.	45	0	28.8	Pos.
	7-PM10-SC-AVG-3,000 Tf		16.3	0.1			16.2	0.1		
	8-PM10-SC-AVG-3,000 T0	2,700	45	0	24.3	Pos.	45	0	24.7	Pos.
	8-PM10-SC-AVG-3,000 Tf		20.7	0			20.3	0		

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	9-PM10-SC-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM10-SC-High-0 Tf		45	0			45	0		
	10-PM10-SC-High-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	10-PM10-SC-High-0 Tf		45	0			45	0		
	11-PM10-SC-High-30 T0	27	45	0	16.9	Pos.	45	0	17.1	Pos.
	11-PM10-SC-High-30 Tf		28.1	0.1			27.9	0		
	12-PM10-SC-High-30 T0	27	45	0	15.4	Pos.	45	0	15.6	Pos.
	12-PM10-SC-High-30 Tf		29.6	0.1			29.4	0.1		
	13-PM10-SC-High-300 T0	270	45	0	25.4	Pos.	45	0	25.6	Pos.
	13-PM10-SC-High-300 Tf		19.6	0.1			19.4	0		
	14-PM10-SC-High-300 T0	270	45	0	15.6	Pos.	45	0	15.9	Pos.
	14-PM10-SC-High-300 Tf		29.4	0.1			29.1	0		
	15-PM10-SC-High-3,000 T0	2,700	45	0	22.4	Pos.	45	0	22.9	Pos.
	15-PM10-SC-High-3,000 Tf		22.6	0.3			22.1	0.1		
	16-PM10-SC-High-3,000 T0	2,700	45	0	23.6	Pos.	45	0	24	Pos.
	16-PM10-SC-High-3,000 Tf		21.4	0			21	0		
05/28/18 PM10 South Carolina and Various Filter types	1-PM10-SC-AVG-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	1-PM10-SC-AVG-0 Tf		45	0			45	0		
	2-PM10-SC-HIGH-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	2-PM10-SC-HIGH-0 Tf		45	0			45	0		
	3-PM10-SC-AVG-30 T0	29	45	0	0	Neg.	45	0	0	Neg.
	3-PM10-SC-AVG-30 Tf		45	0			45	0		
	4-PM10-SC-HIGH-30 T0	29	45	0	0	Neg.	45	0	0	Neg.
	4-PM10-SC-HIGH-30 Tf		45	0			45	0		
	5-PM10-SC-AVG-300 T0	290	45	0	27.7	Pos.	45	0	27.9	Pos.
	5-PM10-SC-AVG-300 Tf		17.3	0.1			17.1	0		
	6-PM10-SC-HIGH-300 T0	290	45	0	26.6	Pos.	45	0	26.9	Pos.
	6-PM10-SC-HIGH-300 Tf		18.4	0.1			18.1	0		
	7-PM10-SC-AVG-3,000 T0	2,900	45	0	22.1	Pos.	45	0	22.2	Pos.

Trial Date	Sample ID	Spore Load[1]	Chrom. Average Ct	Chrom. Stnd Dev	Chom. ΔCt	Chrom. Result	pXO1 Average Ct	pXO1 Stnd Dev	pXO1 Chom. ΔCt	pXO1 Chrom. Result
	7-PM10-SC-AVG-3,000 Tf		22.9	0.1			22.8	0		
	8-PM10-SC-HIGH-3,000 T0	2,900	45	0	25.4	Pos.	45	0	25.6	Pos.
	8-PM10-SC-HIGH-3,000 Tf		19.6	0			19.4	0		
	9-PM10-N/A-New-0 T0	0	45	0	0	Neg.	45	0	0	Neg.
	9-PM10-N/A-New-0 Tf		45	0			45	0		
	10-PM10-NH-AVG-30 T0	29	45	0	28.7	Pos.	45	0	28.5	Pos.
	10-PM10-NH-AVG-30 Tf		16.3	0.1			16.5	0.1		
	14-PM10-WI-High-3,000 T0	2,900	45	0	26.3	Pos.	45	0	26.7	Pos.
	14-PM10-WI-High-3,000 Tf		18.7	0.1			18.7	0.1		

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX M. CULTURE RESULTS FOR NON-AIR QUALITY FILTERS USING SHEEP BLOOD AGAR MEDIUM

Bus Filter New							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
1-BUS-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-BUS-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-BUS-NEW-30	4.4E+01	25	1	1	1.0	25.0	56.8
	4.4E+01	25	4	0	0.0	0.0	0.0
4-BUS-NEW-30	4.4E+01	25	1	0	0.0	0.0	0.0
	4.4E+01	25	4	1	0.3	6.3	14.2
11-BUS-NEW-30	2.9E+01	25	1	0	0.0	0.0	0.0
	2.9E+01	25	4	0	0.0	0.0	0.0
12-BUS-NEW-300	2.9E+02	25	1	0	0.0	0.0	0.0
	2.9E+02	25	4	1	0.3	6.3	2.2
5-BUS-NEW-300	4.4E+02	25	1	0	0.0	0.0	0.0
	4.4E+02	25	4	3	0.8	18.8	4.3
6-BUS-NEW-300	4.4E+02	25	1	1	1.0	25.0	5.7
	4.4E+02	25	4	8	2.0	50.0	11.4
15-BUS-NEW-3,000	2.9E+03	25	1	14	14.0	350.0	12.1
	2.9E+03	25	4	28	7.0	175.0	6.0
7-BUS-NEW-3,000	4.4E+03	25	1	13	13.0	325.0	7.4
	4.4E+03	25	4	24	6.0	150.0	3.4
8-BUS-NEW-3,000	4.4E+03	25	1	15	15.0	375.0	8.5
	4.4E+03	25	4	46	11.5	287.5	6.5

Use values highlighted in green for reporting.

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Bus Filters Mid							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-BUS-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-BUS-MID-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	1	0.3	6.3	#DIV/0!
1-BUS-Mid-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	1	0.3	6.3	#DIV/0!
3-BUS-MID-30	1.8E+01	25	1	1	1.0	25.0	138.9
	1.8E+01	25	4	3	0.8	18.8	104.2
4-BUS-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	0	0.0	0.0	0.0
3-BUS-Mid-30	4.2E+01	25	1	0	0.0	0.0	0.0
	4.2E+01	25	4	3	0.8	18.8	44.6
5-BUS-MID-300	1.8E+02	25	1	2	2.0	50.0	27.8
	1.8E+02	25	4	7	1.8	43.8	24.3
6-BUS-MID-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	9	2.3	56.3	31.3
5-BUS-Mid-300	4.2E+02	25	1	3	3.0	75.0	17.9
	4.2E+02	25	4	4	1.0	25.0	6.0
7-BUS-MID-3,000	1.8E+03	25	1	15	15.0	375.0	20.8
	1.8E+03	25	4	113	28.3	706.3	39.2
8-BUS-MID-3,000	1.8E+03	25	1	21	21.0	525.0	29.2
	1.8E+03	25	4	39	9.8	243.8	13.5
7-BUS-Mid-3,000	4.2E+03	25	1	13	13.0	325.0	7.7
	4.2E+03	25	4	60	15.0	375.0	8.9
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Bus Filters End							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
BUS-END-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	1	0.3	6.3	N/A
BUS-END-Blank		25	1	0	0.0	0.0	N/A
		25	4	1	0.3	6.3	N/A
BUS-END-Blank		25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
9-BUS-END-0	0.0E+00	25	1	Not plated	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	Not plated	#VALUE!	#VALUE!	#VALUE!
10-BUS-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
BUS-END-30	1.5E+01	25	1	2	2.0	50.0	333.3
		25	4	5	1.3	31.3	208.3
BUS-END-30	1.5E+01	25	1	2	2.0	50.0	333.3
		25	4	7	1.8	43.8	291.7
BUS-END-30	1.5E+01	25	1	3	3.0	75.0	500.0
		25	4	7	1.8	43.8	291.7
11-BUS-END-30	4.4E+01	25	1	1	1.0	25.0	56.8
	4.4E+01	25	4	0	0.0	0.0	0.0
12-BUS-END-30	4.4E+01	25	1	2	2.0	50.0	113.6
	4.4E+01	25	4	0	0.0	0.0	0.0
BUS-END-300	1.5E+02	25	1	3	3.0	75.0	50.0
		25	4	1	0.3	6.3	4.2
BUS-END-300	1.5E+02	25	1	2	2.0	50.0	33.3
		25	4	8	2.0	50.0	33.3
BUS-END-300	1.5E+02	25	1	2	2.0	50.0	33.3
		25	4	6	1.5	37.5	25.0
13-BUS-END-300	4.4E+02	25	1	6	6.0	150.0	34.1
	4.4E+02	25	4	12	3.0	75.0	17.0
14-BUS-END-300	4.4E+02	25	1	6	6.0	150.0	34.1
	4.4E+02	25	4	7	1.8	43.8	9.9
15-BUS-END-3,000	4.4E+03	25	1	28	28.0	700.0	15.9
	4.4E+03	25	4	69	17.3	431.3	9.8
16-BUS-END-3,000	4.4E+03	25	1	38	38.0	950.0	21.6
	4.4E+03	25	4	90	22.5	562.5	12.8
16-BUS-END-3,000	2.9E+03	25	1	24	24.0	600.0	20.7
	2.9E+03	25	4	60	15.0	375.0	12.9

Use values highlighted in green for reporting.

No discernable colonies due to grime/dirt on filter.

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

HVAC Filters New							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
9-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-HVAC-NEW-30	3.8E+01	25	1	1	1.0	25.0	65.8
	3.8E+01	25	4	2	0.5	12.5	32.9
12-HVAC-NEW-30	3.8E+01	25	1	0	0.0	0.0	0.0
	3.8E+01	25	4	4	1.0	25.0	65.8
3-HVAC-NEW-30	3.3E+01	25	1	0	0.0	0.0	0.0
	3.3E+01	25	4	3	0.8	18.8	56.8
4-HVAC-NEW-30	3.3E+01	25	1	1	1.0	25.0	75.8
	3.3E+01	25	4	2	0.5	12.5	37.9
5-HVAC-NEW-300	3.3E+02	25	1	7	7.0	175.0	53.0
	3.3E+02	25	4	24	6.0	150.0	45.5
6-HVAC-NEW-300	3.3E+02	25	1	4	4.0	100.0	30.3
	3.3E+02	25	4	10	2.5	62.5	18.9
13-HVAC-NEW-300	2.9E+02	25	1	2	2.0	50.0	17.2
	2.9E+02	25	4	14	3.5	87.5	30.2
17-hvac-new-3000	2.9E+03	25	1	54	54.0	1350.0	46.6
	2.9E+03	25	4	152	41.6	1039.1	35.8
7-HVAC-NEW-3,000	3.3E+03	25	1	48	48.0	1200.0	36.4
	3.3E+03	25	4	138	34.5	862.5	26.1
8-HVAC-NEW-3,000	3.3E+03	25	1	41	41.0	1025.0	31.1
	3.3E+03	25	4	124	31.0	775.0	23.5
Pieces of HVAC filter present on plated filter.							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

HVAC Filters Mid							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
9-HVAC-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	GRIME	GRIME	GRIME	GRIME
10-HVAC-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
2-HVAC-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
12-HVAC-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	1	0.3	6.3	34.7
4-HVAC-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	1	0.3	6.3	34.7
4-HVAC-MID-30	4.2E+01	25	1	0	0.0	0.0	0.0
	4.2E+01	25	4	0	0.0	0.0	0.0
13-HVAC-MID-300	1.8E+02	25	1	4	4.0	100.0	55.6
	1.8E+02	25	4	LAWN	LAWN	LAWN	LAWN
14-HVAC-MID-300	1.8E+02	25	1	4	4.0	100.0	55.6
	1.8E+02	25	4	7	1.8	43.8	24.3
6-HVAC-MID-300	4.2E+02	25	1	8	8.0	200.0	47.6
	4.2E+02	25	4	22	5.5	137.5	32.7
15-HVAC-MID-3000	1.8E+03	25	1	37	37.0	925.0	51.4
	1.8E+03	25	4	117	29.3	731.3	40.6
16-HVAC-MID-3000	1.8E+03	25	1	54	54.0	1350.0	75.0
	1.8E+03	25	4	114	28.5	712.5	39.6
8-HVAC-MID-3,000	4.2E+03	25	1	53	53.0	1325.0	31.5
	4.2E+03	25	4	102	25.5	637.5	15.2
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

HVAC Filters End							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-HVAC-END-0	0.0E+00	25	1	4	4.0	100.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-HVAC-END-0	0.0E+00	25	1	10	10.0	250.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-HVAC-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-HVAC-END-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-HVAC-END-30	3.8E+01	25	1	3	3.0	75.0	197.4
	3.8E+01	25	4	0	0.0	0.0	0.0
4-HVAC-END-30	3.8E+01	25	1	4	4.0	100.0	263.2
	3.8E+01	25	4	0	0.0	0.0	0.0
11-HVAC-End-30	3.3E+01	25	1	1	1.0	25.0	75.8
	3.3E+01	25	4	0	0.0	0.0	0.0
12-HVAC-END-30	3.3E+01	25	1	2	2.0	50.0	151.5
	3.3E+01	25	4	0	0.0	0.0	0.0
5-HVAC-END-300	3.8E+02	25	1	5	5.0	125.0	32.9
	3.8E+02	25	4	0	0.0	0.0	0.0
6-HVAC-END-300	3.8E+02	25	1	4	4.0	100.0	26.3
	3.8E+02	25	4	0	0.0	0.0	0.0
13-HVAC-END-300	3.3E+02	25	1	5	5.0	125.0	37.9
	3.3E+02	25	4	0	0.0	0.0	0.0
14-HVAC-END-300	3.3E+02	25	1	8	8.0	200.0	60.6
	3.3E+02	25	4	0	0.0	0.0	0.0
7-HVAC-END-3,000	3.8E+03	25	1	45	45.0	1125.0	29.6
	3.8E+03	25	4	0	0.0	0.0	0.0
8-HVAC-END-3,000	3.8E+03	25	1	56	56.0	1400.0	36.8
	3.8E+03	25	4	0	0.0	0.0	0.0
15-HVAC-END-3,000	3.3E+03	25	1	80	80.0	2000.0	60.6
	3.3E+03	25	4	0	0.0	0.0	0.0
16-HVAC-END-3,000	3.3E+03	25	1	57	57.0	1425.0	43.2
	3.3E+03	25	4	0	0.0	0.0	0.0
Grime prevents identification/counting of morphologies.							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Platform Filters New							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
9-PLAT-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PLAT-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
1-PLAT-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PLAT-NEW-30	1.7E+01	25	1	1	1.0	25.0	147.1
	1.7E+01	25	4	0	0.0	0.0	0.0
12-PLAT-NEW-30	1.7E+01	25	1	0	0.0	0.0	0.0
	1.7E+01	25	4	4	1.0	25.0	147.1
3-PLAT-NEW-30	2.8E+01	25	1	0	0.0	0.0	0.0
	2.8E+01	25	4	2	0.5	12.5	44.6
13-PLAT-NEW-300	1.7E+02	25	1	6	6.0	150.0	88.2
	1.7E+02	25	4	7	1.8	43.8	25.7
14-PLAT-NEW-300	1.7E+02	25	1	2	2.0	50.0	29.4
	1.7E+02	25	4	13	3.3	81.3	47.8
5-PLAT-NEW-300	2.8E+02	25	1	7	7.0	175.0	62.5
	2.8E+02	25	4	25	6.3	156.3	55.8
15-PLAT-NEW-3,000	1.7E+03	25	1	43	43.0	1075.0	63.2
	1.7E+03	25	4	208	52.0	1300.0	76.5
16-PLAT-NEW-3,000	1.7E+03	25	1	53	53.0	1325.0	77.9
	1.7E+03	25	4	220	55.0	1375.0	80.9
7-PLAT-NEW-3,000	2.8E+03	25	1	46	46.0	1150.0	41.1
	2.8E+03	25	4	146	36.5	912.5	32.6
Count is from half the plate filter multiplied by 2							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Platform Filters Mid							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PLAT-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	4	1.0	25.0	#DIV/0!
2-PLAT-MID-0	0.0E+00	25	1	2	2.0	50.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PLAT-Mid-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PLAT-MID-30	3.0E+01	25	1	1	1.0	25.0	83.3
	3.0E+01	25	4	0	0.0	0.0	0.0
4-PLAT-MID-30	3.0E+01	25	1	0	0.0	0.0	0.0
	3.0E+01	25	4	3	0.8	18.8	62.5
11-PLAT-Mid-30	4.2E+01	25	1	0	0.0	0.0	0.0
	4.2E+01	25	4	2	0.5	12.5	29.8
5-PLAT-MID-300	3.0E+02	25	1	4	4.0	100.0	33.3
	3.0E+02	25	4	25	6.3	156.3	52.1
6-PLAT-MID-300	3.0E+02	25	1	5	5.0	125.0	41.7
	3.0E+02	25	4	18	4.5	112.5	37.5
13-PLAT-Mid-300	4.2E+02	25	1	6	6.0	150.0	35.7
	4.2E+02	25	4	LAWN	LAWN	LAWN	#VALUE!
7-PLAT-MID-3,000	3.0E+03	25	1	41	41.0	1025.0	34.2
	3.0E+03	25	4	0	0.0	0.0	0.0
8-PLAT-MID-3,000	3.0E+03	25	1	34	34.0	850.0	28.3
	3.0E+03	25	4	0	0.0	0.0	0.0
15-PLAT-Mid-3,000	4.2E+03	25	1	34	34.0	850.0	20.2
	4.2E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
Grime makes counting impossible							
50% lawn							
TNTC/Grime							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Platform Filters End							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PLAT-END-0	0.0E+00	25	1	4	4.0	100.0	#DIV/0!
	0.0E+00	25	4	lawn	#VALUE!	#VALUE!	#VALUE!
2-PLAT-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
9-PLAT-END-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
3-PLAT-END-30	1.7E+01	25	1	3	3.0	75.0	441.2
	1.7E+01	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
4-PLAT-END-30	1.7E+01	25	1	3	3.0	75.0	441.2
	1.7E+01	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
11-PLAT-END-30	2.8E+01	25	1	0	0.0	0.0	0.0
	2.8E+01	25	4	0	0.0	0.0	0.0
5-PLAT-END-300	1.7E+02	25	1	6	6.0	150.0	88.2
	1.7E+02	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
6-PLAT-END-300	1.7E+02	25	1	2	2.0	50.0	29.4
	1.7E+02	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
13-PLAT-END-300	2.8E+02	25	1	6	6.0	150.0	53.6
	2.8E+02	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
7-PLAT-END-3,000	1.7E+03	25	1	57	57.0	1425.0	83.8
	1.7E+03	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
8-PLAT-END-3,000	1.7E+03	25	1	48	48.0	1200.0	70.6
	1.7E+03	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
15-PLAT-END-3,000	2.8E+03	25	1	15	15.0	375.0	13.4
	2.8E+03	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
50% lawn							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Rolling Stock New							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-ROLL-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-ROLL-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-ROLL-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-ROLL-NEW-30	7.0E+01	25	1	3	3.0	75.0	107.6
	7.0E+01	25	4	4	1.0	25.0	35.9
4-ROLL-NEW-30	7.0E+01	25	1	0	0.0	0.0	0.0
	7.0E+01	25	4	5	1.3	31.3	44.8
4-ROLL-NEW-30	2.8E+01	25	1	1	1.0	25.0	89.3
	2.8E+01	25	4	4	1.0	25.0	89.3
5-ROLL-NEW-300	7.0E+02	25	1	15	15.0	375.0	53.6
	7.0E+02	25	4	63	15.8	393.8	56.3
6-ROLL-NEW-300	7.0E+02	25	1	11	11.0	275.0	39.3
	7.0E+02	25	4	59	14.8	368.8	52.7
6-ROLL-NEW-300	2.8E+02	25	1	5	5.0	125.0	44.6
	2.8E+02	25	4	36	9.0	225.0	80.4
7-ROLL-NEW-3,000	7.0E+03	25	1	114	114.0	2850.0	40.7
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
8-ROLL-NEW-3,000	7.0E+03	25	1	111	111.0	2775.0	39.6
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
8-ROLL-NEW-3,000	2.8E+03	25	1	39	39.0	975.0	34.8
	2.8E+03	25	4	196	49.0	1225.0	43.8
Count is from half the plate filter multiplied by 2							
Use values highlighted in green for reporting.							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Rolling Stock Mid							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
9-ROLL-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-ROLL-MID-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-ROLL-Mid-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	GRIME	GRIME	GRIME	#VALUE!
11-ROLL-MID-30	3.0E+01	25	1	0	0.0	0.0	0.0
	3.0E+01	25	4	0	0.0	0.0	0.0
12-ROLL-MID-30	3.0E+01	25	1	0	0.0	0.0	0.0
	3.0E+01	25	4	0	0.0	0.0	0.0
12-ROLL-Mid-30	4.2E+01	25	1	3	3.0	75.0	178.6
	4.2E+01	25	4	GRIME	GRIME	GRIME	#VALUE!
13-ROLL-MID-300	3.0E+02	25	1	7	7.0	175.0	58.3
	3.0E+02	25	4	0	0.0	0.0	0.0
14-ROLL-MID-300	3.0E+02	25	1	2	2.0	50.0	16.7
	3.0E+02	25	4	0	0.0	0.0	0.0
14-ROLL-Mid-300	4.2E+02	25	1	3	3.0	75.0	17.9
	4.2E+02	25	4	GRIME	GRIME	GRIME	#VALUE!
15-ROLL-MID-3,000	3.0E+03	25	1	50	50.0	1250.0	41.7
	3.0E+03	25	4	0	0.0	0.0	0.0
16-ROLL-MID-3,000	3.0E+03	25	1	0	0.0	0.0	0.0
	3.0E+03	25	4	0	0.0	0.0	0.0
16-ROLL-Mid-3,000	4.2E+03	25	1	11	11.0	275.0	6.5
	4.2E+03	25	4	GRIME	GRIME	GRIME	#VALUE!
50% lawn							
Grime makes counting impossible							
75% lawn							
TNTC/Grime							
Use values highlighted in green for reporting							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

Rolling Stock End							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
9-ROLL-END-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
10-ROLL-END-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
10-ROLL-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
11-ROLL-END-30	7.0E+01	25	1	4	4.0	100.0	143.5
	7.0E+01	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
12-ROLL-END-30	7.0E+01	25	1	3	3.0	75.0	107.6
	7.0E+01	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
12-ROLL-END-30	2.8E+01	25	1	GRIME	#VALUE!	0.0	0.0
	2.8E+01	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
13-ROLL-END-300	7.0E+02	25	1	11	11.0	275.0	39.3
	7.0E+02	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
14-ROLL-END-300	7.0E+02	25	1	3	3.0	75.0	10.7
	7.0E+02	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
14-ROLL-END-300	2.8E+02	25	1	GRIME	#VALUE!	0.0	0.0
	2.8E+02	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
15-ROLL-END-3,000	7.0E+03	25	1	TNTC	#VALUE!	#VALUE!	#VALUE!
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
16-ROLL-END-3,000	7.0E+03	25	1	TNTC	#VALUE!	#VALUE!	#VALUE!
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
16-ROLL-END-3,000	2.8E+03	25	1	GRIME	#VALUE!	#VALUE!	#VALUE!
	2.8E+03	25	4	GRIME	#VALUE!	#VALUE!	#VALUE!
Use values highlighted in green for reporting							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX N. CULTURE RESULTS FOR NON-AIR QUALITY FILTERS USING MYP MEDIUM

July 24, 2017 Trial - Bus Filters (New and EOL)							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-Bus-High-Blank	0.0E+00	25	1	3	3.0	75.0	N/A
		25	3	5	1.6	40.3	N/A
2-Bus-High-Blank		25	1	0	0.0	0.0	N/A
		25	4	4	1.0	25.0	N/A
3-Bus-High-Blank		25	1	1	1.0	25.0	N/A
		25	4	1	0.3	6.3	N/A
4-Bus-High-30	1.5E+01	25	1	6	6.0	150.0	1000.0
		25	4	0	0.0	0.0	0.0
5-Bus-High-30	1.5E+01	25	1	2	2.0	50.0	333.3
		25	4	0	0.0	0.0	0.0
6-Bus-High-30	1.5E+01	25	1	1	1.0	25.0	166.7
		25	4	0	0.0	0.0	0.0
7-Bus-High-300	1.5E+02	25	1	0	0.0	0.0	0.0
		25	4	0	0.0	0.0	0.0
8-Bus-High-300	1.5E+02	25	1	4	4.0	100.0	66.7
		25	4	0	0.0	0.0	0.0
9-Bus-High-300	1.5E+02	25	1	3	3.0	75.0	50.0
		25	4	0	0.0	0.0	0.0
10-Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
11-Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
12-Bus-New-Blank	0.0E+00	25	1	0	0.0	0.0	N/A
		25	4	0	0.0	0.0	N/A
Formation of large lawn							
Lawn may be B.A.							
Used values highlighted green for percent recovery							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

August 25, 2017 Trial - Bus Filters (New and EOL)							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-BUS-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-BUS-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-BUS-NEW-30	4.4E+01	25	1	0	0.0	0.0	0.0
	4.4E+01	25	4	1	0.3	6.3	14.2
4-BUS-NEW-30	4.4E+01	25	1	0	0.0	0.0	0.0
	4.4E+01	25	4	0	0.0	0.0	0.0
5-BUS-NEW-300	4.4E+02	25	1	3	3.0	75.0	17.0
	4.4E+02	25	4	7	1.8	43.8	9.9
6-BUS-NEW-300	4.4E+02	25	1	2	2.0	50.0	11.4
	4.4E+02	25	4	9	2.3	56.3	12.8
7-BUS-NEW-3,000	4.4E+03	25	1	11	11.0	275.0	6.3
	4.4E+03	25	4	54	13.5	337.5	7.7
8-BUS-NEW-3,000	4.4E+03	25	1	12	12.0	300.0	6.8
	4.4E+03	25	4	41	10.3	256.3	5.8
9-BUS-END-0	0.0E+00	25	1	Not plated	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	Not plated	#VALUE!	#VALUE!	#VALUE!
10-BUS-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-BUS-NEW-30	4.4E+01	25	1	0	0.0	0.0	0.0
	4.4E+01	25	4	0	0.0	0.0	0.0
12-BUS-END-30	4.4E+01	25	1	3	3.0	75.0	170.5
	4.4E+01	25	4	0	0.0	0.0	0.0
13-BUS-END-300	4.4E+02	25	1	8	8.0	200.0	45.5
	4.4E+02	25	4	24	6.0	150.0	34.1
14-BUS-END-300	4.4E+02	25	1	3	3.0	75.0	17.0
	4.4E+02	25	4	0	0.0	0.0	0.0
15-BUS-END-3,000	4.4E+03	25	1	27	27.0	675.0	15.3
	4.4E+03	25	4	0	0.0	0.0	0.0
16-BUS-END-3,000	4.4E+03	25	1	28	28.0	700.0	15.9
	4.4E+03	25	4	0	0.0	0.0	0.0

Colonies counted through grime

No discernable colonies due to grime/dirt on filter

Half of filter appears to be unidentified lawn

Used values highlighted green for percent recovery

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

September 15, 2017 Trial - Bus & HVAC Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-BUS-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-BUS-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
3-BUS-MID-30	1.8E+01	25	1	1	1.0	25.0	138.9
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
4-BUS-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
5-BUS-MID-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	LAWN	LAWN	LAWN	LAWN
6-BUS-MID-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	LAWN	LAWN	LAWN	LAWN
7-BUS-MID-3,000	1.8E+03	25	1	15	15.0	375.0	20.8
	1.8E+03	25	4	0	0.0	0.0	0.0
8-BUS-MID-3,000	1.8E+03	25	1	6	6.0	150.0	8.3
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
9-HVAC-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
10-HVAC-MID-0	0.0E+00	25	1	LAWN	LAWN	LAWN	LAWN
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
11-HVAC-MID-30	1.8E+01	25	1	LAWN	LAWN	LAWN	LAWN
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
12-HVAC-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
13-HVAC-MID-300	1.8E+02	25	1	2	2.0	50.0	27.8
	1.8E+02	25	4	17	4.3	106.3	59.0
14-HVAC-MID-300	1.8E+02	25	1	1	1.0	25.0	13.9
	1.8E+02	25	4	13	3.3	81.3	45.1
15-HVAC-MID-3,000	1.8E+03	25	1	36	36.0	900.0	50.0
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
16-HVAC-MID-3,000	1.8E+03	25	1	22	22.0	550.0	30.6
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
Used values highlighted green for percent recovery							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

August 1, 2017 Trial – HVAC Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-HVAC-EOL-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	3	0	0.0	0.0	#DIV/0!
2-HVAC-EOL-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-HVAC-EOL-30	3.8E+01	25	1	0	0.0	Lawn	#VALUE!
	3.8E+01	25	4	0	0.0	0.0	0.0
4-HVAC-EOL-30	3.8E+01	25	1	0	0.0	0.0	0.0
	3.8E+01	25	4	0	0.0	Lawn	#VALUE!
5-HVAC-EOL-300	3.8E+02	25	1	7	7.0	175.0	46.1
	3.8E+02	25	4	0	0.0	0.0	0.0
6-HVAC-EOL-300	3.8E+02	25	1	0	0.0	0.0	0.0
	3.8E+02	25	4	0	0.0	Lawn	#VALUE!
7-HVAC-EOL-3,000	3.8E+03	25	1	0	0.0	Lawn	#VALUE!
	3.8E+03	25	4	0	0.0	0.0	0.0
8-HVAC-EOL-3,000	3.8E+03	25	1	0	0.0	Lawn	#VALUE!
	3.8E+03	25	4	0	0.0	0.0	0.0
9-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-HVAC-NEW-30	3.8E+01	25	1	2	2.0	50.0	131.6
	3.8E+01	25	4	3	0.8	18.8	49.3
12-HVAC-NEW-30	3.8E+01	25	1	0	0.0	0.0	0.0
	3.8E+01	25	4	2	0.5	12.5	32.9
Used values highlighted green for percent recovery							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

September 8, 2017 Trial – HVAC Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-HVAC-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-HVAC-NEW-30	3.3E+01	25	1	0	0.0	0.0	0.0
	3.3E+01	25	4	2	0.5	12.5	37.9
4-HVAC-NEW-30	3.3E+01	25	1	2	2.0	50.0	151.5
	3.3E+01	25	4	3	0.8	18.8	56.8
5-HVAC-NEW-300	3.3E+02	25	1	10	10.0	250.0	75.8
	3.3E+02	25	4	14	3.5	87.5	26.5
6-HVAC-NEW-300	3.3E+02	25	1	4	4.0	100.0	30.3
	3.3E+02	25	4	22	5.5	137.5	41.7
7-HVAC-NEW-3,000	3.3E+03	25	1	38	38.0	950.0	28.8
	3.3E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
8-HVAC-NEW-3,000	3.3E+03	25	1	38	38.0	950.0	28.8
	3.3E+03	25	4	121	30.3	756.3	22.9
9-HVAC-END-0	0.0E+00	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-HVAC-END-0	0.0E+00	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-HVAC-End-30	3.3E+01	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	3.3E+01	25	4	0	0.0	0.0	0.0
12-HVAC-END-30	3.3E+01	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	3.3E+01	25	4	0	0.0	0.0	0.0
13-HVAC-END-300	3.3E+02	25	1	4	4.0	100.0	30.3
	3.3E+02	25	4	0	0.0	0.0	0.0
14-HVAC-END-300	3.3E+02	25	1	5	5.0	125.0	37.9
	3.3E+02	25	4	0	0.0	0.0	0.0
15-HVAC-END-3,000	3.3E+03	25	1	LAWN	LAWN	#VALUE!	#VALUE!
	3.3E+03	25	4	0	0.0	0.0	0.0
16-HVAC-END-3,000	3.3E+03	25	1	LAWN	LAWN	#VALUE!	#VALUE!
	3.3E+03	25	4	0	0.0	0.0	0.0
Used this value in calculation							
Pieces of HVAC filter present on plated filter							
Grime prevents identification/counting of morphologies							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

September 15, 2017 Trial - HVAC & Bus Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-BUS-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-BUS-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
3-BUS-MID-30	1.8E+01	25	1	1	1.0	25.0	138.9
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
4-BUS-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
5-BUS-MID-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	LAWN	LAWN	LAWN	LAWN
6-BUS-MID-300	1.8E+02	25	1	0	0.0	0.0	0.0
	1.8E+02	25	4	LAWN	LAWN	LAWN	LAWN
7-BUS-MID-3,000	1.8E+03	25	1	15	15.0	375.0	20.8
	1.8E+03	25	4	0	0.0	0.0	0.0
8-BUS-MID-3,000	1.8E+03	25	1	6	6.0	150.0	8.3
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
9-HVAC-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
10-HVAC-MID-0	0.0E+00	25	1	LAWN	LAWN	LAWN	LAWN
	0.0E+00	25	4	LAWN	LAWN	LAWN	LAWN
11-HVAC-MID-30	1.8E+01	25	1	LAWN	LAWN	LAWN	LAWN
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
12-HVAC-MID-30	1.8E+01	25	1	0	0.0	0.0	0.0
	1.8E+01	25	4	LAWN	LAWN	LAWN	LAWN
13-HVAC-MID-300	1.8E+02	25	1	2	2.0	50.0	27.8
	1.8E+02	25	4	17	4.3	106.3	59.0
14-HVAC-MID-300	1.8E+02	25	1	1	1.0	25.0	13.9
	1.8E+02	25	4	13	3.3	81.3	45.1
15-HVAC-MID-3,000	1.8E+03	25	1	36	36.0	900.0	50.0
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
16-HVAC-MID-3,000	1.8E+03	25	1	22	22.0	550.0	30.6
	1.8E+03	25	4	LAWN	LAWN	LAWN	LAWN
Used this value in calculation							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

August 11, 2017 Trial – PLAT Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-PLAT-END-0	0.0E+00	25	1	Lawn	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
2-PLAT-END-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PLAT-END-30	1.7E+01	25	1	6	6.0	150.0	882.4
	1.7E+01	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
4-PLAT-END-30	1.7E+01	25	1	2	2.0	50.0	294.1
	1.7E+01	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
5-PLAT-END-300	1.7E+02	25	1	10	10.0	250.0	147.1
	1.7E+02	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
6-PLAT-END-300	1.7E+02	25	1	3	3.0	75.0	44.1
	1.7E+02	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
7-PLAT-END-3,000	1.7E+03	25	1	49	49.0	1225.0	72.1
	1.7E+03	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
8-PLAT-END-3,000	1.7E+03	25	1	37	37.0	925.0	54.4
	1.7E+03	25	4	Lawn	#VALUE!	#VALUE!	#VALUE!
9-PLAT-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-PLAT-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-PLAT-NEW-30	1.7E+01	25	1	0	0.0	0.0	0.0
	1.7E+01	25	4	2	0.5	12.5	73.5
12-PLAT-NEW-30	1.7E+01	25	1	1	1.0	25.0	147.1
	1.7E+01	25	4	2	0.5	12.5	73.5
13-PLAT-NEW-300	1.7E+02	25	1	2	2.0	50.0	29.4
	1.7E+02	25	4	11	2.8	68.8	40.4
14-PLAT-NEW-300	1.7E+02	25	1	2	2.0	50.0	29.4
	1.7E+02	25	4	12	3.0	75.0	44.1
15-PLAT-NEW-3,000	1.7E+03	25	1	31	31.0	775.0	45.6
	1.7E+03	25	4	115	28.8	718.8	42.3
16-PLAT-NEW-3,000	1.7E+03	25	1	30	30.0	750.0	44.1
	1.7E+03	25	4	130	32.5	812.5	47.8
Used this value in calculation							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

September 18, 2017 Trial - PLAT & ROLL Filters							
Sample ID	Spore Load[1]	Extraction Volume (mL)	Volume in Filter Cup (mL)	Plate Counts (CFU)	Average Sample Concentration		Percent Recovery
					CFU/mL	Total CFU	
1-PLAT-MID-0	0.0E+00	25	1	1	1.0	25.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-PLAT-MID-0	0.0E+00	25	1	2	2.0	50.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-PLAT-MID-30	3.0E+01	25	1	6	6.0	150.0	500.0
	3.0E+01	25	4	0	0.0	0.0	0.0
4-PLAT-MID-30	3.0E+01	25	1	3	3.0	75.0	250.0
	3.0E+01	25	4	0	0.0	0.0	0.0
5-PLAT-MID-300	3.0E+02	25	1	3	3.0	75.0	25.0
	3.0E+02	25	4	0	0.0	0.0	0.0
6-PLAT-MID-300	3.0E+02	25	1	5	5.0	125.0	41.7
	3.0E+02	25	4	0	0.0	0.0	0.0
7-PLAT-MID-3,000	3.0E+03	25	1	54	54.0	1350.0	45.0
	3.0E+03	25	4	0	0.0	0.0	0.0
8-PLAT-MID-3,000	3.0E+03	25	1	35	35.0	875.0	29.2
	3.0E+03	25	4	0	0.0	0.0	0.0
9-ROLL-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
10-ROLL-MID-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
11-ROLL-MID-30	3.0E+01	25	1	0	0.0	0.0	0.0
	3.0E+01	25	4	0	0.0	0.0	0.0
12-ROLL-MID-30	3.0E+01	25	1	0	0.0	0.0	0.0
	3.0E+01	25	4	0	0.0	0.0	0.0
13-ROLL-MID-300	3.0E+02	25	1	0	0.0	0.0	0.0
	3.0E+02	25	4	0	0.0	0.0	0.0
14-ROLL-MID-300	3.0E+02	25	1	0	0.0	0.0	0.0
	3.0E+02	25	4	0	0.0	0.0	0.0
15-ROLL-MID-3,000	3.0E+03	25	1	0	0.0	0.0	0.0
	3.0E+03	25	4	0	0.0	0.0	0.0
16-ROLL-MID-3,000	3.0E+03	25	1	0	0.0	0.0	0.0
	3.0E+03	25	4	0	0.0	0.0	0.0
Used for calculations							
100% lawn							
50% lawn							
75% lawn							
Grime makes counting impossible							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

August 14, 2017 Trial – ROLL Filters							
Sample ID	**Spore Load[1]**	**Extraction Volume (mL)**	**Volume in Filter Cup (mL)**	**Plate Counts (CFU)**	**Average Sample Concentration**		**Percent Recovery**
					CFU/mL	**Total CFU**	
1-ROLL-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
2-ROLL-NEW-0	0.0E+00	25	1	0	0.0	0.0	#DIV/0!
	0.0E+00	25	4	0	0.0	0.0	#DIV/0!
3-ROLL-NEW-30	7.0E+01	25	1	4	4.0	100.0	143.5
	7.0E+01	25	4	12	3.0	75.0	107.6
4-ROLL-NEW-30	7.0E+01	25	1	1	1.0	25.0	35.9
	7.0E+01	25	4	4	1.0	25.0	35.9
5-ROLL-NEW-300	7.0E+02	25	1	22	22.0	550.0	78.6
	7.0E+02	25	4	58	14.5	362.5	51.8
6-ROLL-NEW-300	7.0E+02	25	1	16	16.0	400.0	57.1
	7.0E+02	25	4	63	15.8	393.8	56.3
7-ROLL-NEW-3,000	7.0E+03	25	1	120	120.0	3000.0	42.9
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
8-ROLL-NEW-3,000	7.0E+03	25	1	98	98.0	2450.0	35.0
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
9-ROLL-END-0	0.0E+00	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
10-ROLL-END-0	0.0E+00	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	0.0E+00	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
11-ROLL-END-30	7.0E+01	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	7.0E+01	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
12-ROLL-END-30	7.0E+01	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	7.0E+01	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
13-ROLL-END-300	7.0E+02	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	7.0E+02	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
14-ROLL-END-300	7.0E+02	25	1	LAWN	#VALUE!	#VALUE!	#VALUE!
	7.0E+02	25	4	LAWN	#VALUE!	#VALUE!	#VALUE!
15-ROLL-END-3,000	7.0E+03	25	1	TNTC	#VALUE!	#VALUE!	#VALUE!
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
16-ROLL-END-3,000	7.0E+03	25	1	TNTC	#VALUE!	#VALUE!	#VALUE!
	7.0E+03	25	4	TNTC	#VALUE!	#VALUE!	#VALUE!
Used for calculations							

[1] Actual number of B. a. Sterne spores spiked onto filter based on spiking suspension titer and volume of suspension applied.

APPENDIX O. RV-PCR RESULTS FOR NON-AIR QUALITY FILTERS USING CHROMOSOMAL AND PXO1 GENE TARGETS

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
07/24/2017 BUS	BUS-High-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-High-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-High-0	0	45	45	0	Neg.	45	43.37*	1.21	Neg.
	BUS-High-30	15	45	34.06	10.94	Pos.	45	31.93	13.07	Pos.
	BUS-High-30	15	45	31.4	13.6	Pos.	45	30.27	14.73	Pos.
	BUS-High-30	15	45	31.54	13.46	Pos.	45	31.07	13.93	Pos.
	BUS-High-300	150	45	32.83	12.17	Pos.	45	31.45	13.55	Pos.
	BUS-High-300	150	45	25.93	19.07	Pos.	45	25.17	19.83	Pos.
	BUS-High-300	150	45	24.19	20.81	Pos.	45	23.57	21.43	Pos.
	BUS-New-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-New-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-New-0	0	45	45	0	Neg.	45	43.13*	1.87	Neg.
07/31/17 HVAC	HVAC-New-0	0	45	36.9 ± 1.9	8.1	Neg.	45	34.0 ± 0.3	11	Neg.[1]
	HVAC-New-0	0	45	42.9 ± 1.9	2.1	Neg.	45	37.9 ± 2.7	7.1	Neg.
	HVAC-New-30	38	45	19.3 ± 0.1	25.7	Pos.	45	18.8 ± 0.0	26.2	Pos.
	HVAC-New-30	38	45	17.2 ± 0.1	27.8	Pos.	45	16.7 ± 0.0	28.3	Pos.
	HVAC-END-0	0	45	44.8 ± 0.3	0.2	Neg.	45	40.7 ± 3.7	4.3	Neg.
	HVAC-END-0	0	45	43.2 ± 3.2	1.8	Neg.	45	36.1 ± 0.2	8.9	Neg.
	HVAC-END-30	38	45	28.2 ± 0.1	16.8	Pos.	45	27.6 ± 0.1	17.4	Pos.
	HVAC-END-30	38	45	25.2 ± 0.1	19.8	Pos.	45	24.3 ± 0.0	20.7	Pos.
	HVAC-END-300	380	45	25.8 ± 0.0	19.2	Pos.	45	25.2 ± 0.0	19.8	Pos.
	HVAC-END-300	380	45	23.4 ± 0.1	21.2	Pos.	45	22.7 ± 0.1	22.3	Pos.
	HVAC-END-3,000	3,800	45	20.2 ± 0.1	24.8	Pos.	45	19.5 ± 0.0	25.5	Pos.
	HVAC-END-3,000	3,800	45	19.6 ± 0.1	25.4	Pos.	45	18.9 ± 0.0	26.1	Pos.
9/5/2017 HVAC	HVAC-New-0	0	45	34.5 ± 0.6	10.5	Pos.	45	34.5 ± 0.4	10.5	Pos.
	HVAC-New-0	0	45	37.3 ± 1.8	7.7	Neg.	45	36.2 ± 0.0	8.8	Neg.
	HVAC-New-30	33	45	20.2 ± 0.1	24.8	Pos.	45	19.9 ± 0.0	25.1	Pos.
	HVAC-New-30	33	42.6 ± 4.2	18.1 ± 0.0	24.5	Pos.	43.9 ± 2.0	17.8 ± 0.0	26.1	Pos.
	HVAC-New-300	330	45	26.8 ± 0.2	18.2	Pos.	45	26.4 ± 0.0	18.6	Pos.
	HVAC-New-300	330	45	27.4 ± 0.1	17.6	Pos.	45	27.0 ± 0.0	18	Pos.
	HVAC-New-3,000	3,300	45	19.1 ± 0.1	25.9	Pos.	45	18.5 ± 0.0	26.5	Pos.

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
	HVAC-New-3,000	3,300	45	16.9 ± 0.0	28.1	Pos.	45	16.6 ± 0.0	28.4	Pos.
	HVAC-END-0	0	45	32.6 ± 0.2	12.4	Pos.	45	32.5 ± 0.2	12.5	Pos.
	HVAC-END-0	0	45	34.3 ± 0.5	10.7	Pos.	45	33.8 ± 0.0	11.2	Pos.
	HVAC-END-30	33	45	25.3 ± 0.1	19.7	Pos.	43.2 ± 3.2	24.7 ± 0.0	18.5	Pos.
	HVAC-END-30	33	45	33.8 ± 0.3	11.2	Pos.	45	33.3 ± 0.0	11.7	Pos.
	HVAC-END-300	330	45	27.0 ± 0.0	18	Pos.	45	26.6 ± 0.0	18.4	Pos.
	HVAC-END-300	330	45	27.1 ± 0.1	17.9	Pos.	45	26.3 ± 0.0	18.7	Pos.
	HVAC-END-3,000	3,300	45	21.2 ± 0.0	23.8	Pos.	45	20.5 ± 0.0	24.5	Pos.
	HVAC-END-3,000	3,300	45	23.9 ± 0.1	21.1	Pos.	45	23.2 ± 0.0	21.8	Pos.
08/7/2017 PLAT	PLAT-New-0	0	40.6 ± 3.0	34.5 ± 0.5	6.1	Neg.	42.3 ± 3.1	33.9 ± 0.3	8.5	Neg.
	PLAT-New-0	0	41.3 ± 3.5	35.6 ± 0.3	5.7	Neg.	36.9 ± 1.1	34.3 ± 0.1	2.6	Neg.
	PLAT-New-30	17	45	35.5 ± 0.6	9.5	Neg.[1]	38.8 ± 2.1	34.5 ± 0.3	4.2	Neg.
	PLAT-New-30	17	45	16.9 ± 0.0	28.1	Pos.	41.8 ± 2.8	16.5 ± 0.0	25.2	Pos.
	PLAT-New-300	170	36.2 ± 1.1	18.0 ± 0.1	18.2	Pos.	35.3 ± 1.1	17.7 ± 0.0	17.6	Pos.
	PLAT-New-300	170	45	16.5 ± 0.0	28.5	Pos.	45	16.1 ± 0.0	28.9	Pos.
	PLAT-New-3,000	1,700	38.8 ± 1.1	16.8 ± 0.1	21.9	Pos.	36.1 ± 1.2	16.3 ± 0.0	19.8	Pos.
	PLAT-New-3,000	1,700	38.1 ± 1.4	17.0 ± 0.0	21.1	Pos.	35.3 ± 0.1	16.5 ± 0.0	18.8	Pos.
	PLAT-END-0	0	34.3 ± 1.23	34.3 ± 0.8	0	Neg.	33.7 ± 0.4	33.4 ± 0.4	0.3	Neg.
	PLAT-END-0	0	42.3 ± 4.5	33.4 ± 0.2	9	Neg.[1]	37.9 ± 1.6	33.1 ± 0.1	4.8	Neg.
	PLAT-END-30	17	34.9 ± 0.2	28.3 ± 0.0	6.7	Neg.	34.3 ± 0.1	27.9 ± 0.0	6.4	Neg.
	PLAT-END-30	17	43.3 ± 3.0	27.0 ± 0.0	16.3	Pos.	45	26.5 ± 0.1	18.5	Pos.
	PLAT-END-300	170	38.2 ± 1.9	24.5 ± 0.0	13.7	Pos.	35.7 ± 0.3	23.9 ± 0.1	11.9	Pos.
	PLAT-END-300	170	45	28.1 ± 0.2	16.9	Pos.	37.7 ± 0.2	27.6 ± 0.2	10.2	Pos.
	PLAT-END-3,000	1,700	43.3 ± 1.9	21.7 ± 0.1	21.6	Pos.	42.1 ± 2.6	21.1 ± 0.0	21	Pos.
	PLAT-END-3,000	1,700	34.8 ± 0.7	22.3 ± 0.5	12.5	Pos.	33.9 ± 0.3	21.5 ± 0.4	12.4	Pos.
08/14/2017 ROLL	ROLL-New-0	0	45	34.2 ± 0.4	10.8	Pos.	45	34.2 ± 0.1	10.8	Pos.
	ROLL-New-0	0	45	39.4 ± 1.5	5.6	Neg.	45	35.7 ± 0.1	9.3	Neg.[1]
	ROLL-New-30	70	45	16.4 ± 0.1	28.6	Pos.	45	16.4 ± 0.0	28.6	Pos.

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
	ROLL-New-30	70	45	16.6 ± 0.0	28.4	Pos.	45	16.4 ± 0.0	28.6	Pos.
	ROLL-New-300	700	45	16.8 ± 0.0	28.2	Pos.	45	16.6 ± 0.0	28.4	Pos.
	ROLL-New-300	700	45	17.1 ± 0.0	27.9	Pos.	45	16.9 ± 0.0	28.1	Pos.
	ROLL-New-3,000	7,000	45	16.6 ± 0.1	28.4	Pos.	43.6 ± 2.3	16.3 ± 0.0	27.3	Pos.
	ROLL-New-3,000	7,000	44.1 ± 1.6	16.7 ± 0.1	27.4	Pos.	44.2 ± 1.3	16.3 ± 0.0	27.9	Pos.
	ROLL-END-0	0	45	31.7 ± 0.1	13.3	Pos.	45	31.6 ± 0.1	13.4	Pos.
	ROLL-END-0	0	45	31.4 ± 0.1	13.6	Pos.	45	31.0 ± 0.1	14	Pos.
	ROLL-END-30	70	45	31.7 ± 0.1	13.3	Pos.	45	31.4 ± 0.2	13.6	Pos.
	ROLL-END-30	70	45	28.4 ± 0.1	16.6	Pos.	45	27.9 ± 0.0	17.1	Pos.
	ROLL-END-300	700	45	27.3 ± 0.1	17.7	Pos.	45	26.8 ± 0.0	18.2	Pos.
	ROLL-END-300	700	45	24.9 ± 0.0	20.1	Pos.	45	24.3 ± 0.0	20.7	Pos.
	ROLL-END-3,000	7,000	45	27.4 ± 0.1	17.6	Pos.	45	26.7 ± 0.0	18.3	Pos.
	ROLL-END-3,000	7,000	45	22.6 ± 0.1	22.4	Pos.	45	21.9 ± 0.0	23.1	Pos.
08/21/17 BUS	BUS-New-0	0	45	35.7 ± 0.6	9.3	Neg.[1]	41.1 ± 1.0	35.3 ± 0.6	5.8	Neg.
	BUS-New-0	0	45	40.1 ± 4.6	4.9	Neg.	43.4 ± 1.5	36.0 ± 0.3	7.4	Neg.
	BUS-New-30	44	45	16.9 ± 0.1	28.1	Pos.	45	16.7 ± 0.0	28.3	Pos.
	BUS-New-30	44	45	16.5 ± 0.1	28.5	Pos.	44.2 ± 1.4	16.2 ± 0.0	28	Pos.
	BUS-New-300	440	45	17.0 ± 0.0	28	Pos.	45	16.7 ± 0.0	28.3	Pos.
	BUS-New-300	440	45	17.0 ± 0.0	28	Pos.	45	16.7 ± 0.0	28.3	Pos.
	BUS-New-3,000	4,400	45	17.1 ± 0.1	27.9	Pos.	45	16.8 ± 0.0	28.2	Pos.
	BUS-New-3,000	4,400	45	16.9 ± 0.1	28.1	Pos.	45	16.6 ± 0.0	26.7	Pos.
	BUS-END-0	0	45	33.8 ± 0.6	11.2	Pos.	45	33.4 ± 0.2	11.6	Pos.
	BUS-END-0	0	45	33.9 ± 0.0	11.1	Pos.	45	33.1 ± 0.1	11.9	Pos.
	BUS-END-30	44	45	29.5 ± 0.1	15.5	Pos.	45	29.2 ± 0.1	15.8	Pos.
	BUS-END-30	44	45	23.8 ± 0.0	21.2	Pos.	45	23.4 ± 0.0	21.6	Pos.
	BUS-END-300	440	45	21.9 ± 0.1	23.1	Pos.	45	21.7 ± 0.0	23.3	Pos.
	BUS-END-300	440	45	24.0 ± 0.1	21	Pos.	45	23.6 ± 0.0	21.4	Pos.
	BUS-END-3,000	4,400	45	22.2 ± 0.1	22.8	Pos.	45	21.5 ± 0.0	23.5	Pos.

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
	BUS-END-3,000	4,400	45	21.8 ± 0.1	23.2	Pos.	45	21.3 ± 0.0	23.7	Pos.
09/11/17 BUS and HVAC	BUS-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-Mid-30	18	45	26.9 ± 0.5	18.1	Pos.	43.2 ± 3.1	26.3 ± 0.0	16.9	Pos.
	BUS-Mid-30	18	45	30.7 ± 0.2	14.3	Pos.	45	30.1 ± 0.1	14.9	Pos.
	BUS-Mid-300	180	45	24.9 ± 0.1	20.1	Pos.	45	24.6 ± 0.1	20.4	Pos.
	BUS-Mid-300	180	45	28.1 ± 0.1	16.9	Pos.	45	27.2 ± 0.0	17.8	Pos.
	BUS-Mid-3,000	1,800	45	24.8 ± 0.0	20.2	Pos.	45	23.8 ± 0.0	21.2	Pos.
	BUS-Mid-3,000	1,800	45	22.6 ± 0.1	22.4	Pos.	45	21.7 ± 0.0	23	Pos.
	HVAC-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	HVAC-Mid-0	0	45	45	0	Neg.	44.2 ± 1.4	45	-0.8	Neg.
	HVAC-Mid-30	18	45	28.9 ± 0.1	16.1	Pos.	45	28.1 ± 0.1	16.9	Pos.
	HVAC-Mid-30	18	45	27.3 ± 0.0	17.7	Pos.	45	26.9 ± 0.0	18.1	Pos.
	HVAC-Mid-300	180	45	21.9 ± 0.0	23.1	Pos.	45	21.5 ± 0.1	23.5	Pos.
	HVAC-Mid-300	180	45	23.6 ± 0.1	21.4	Pos.	45	23.0 ± 0.0	22	Pos.
	HVAC-Mid-3,000	1,800	45	19.8 ± 0.1	25.2	Pos.	45	19.4 ± 0.0	25.6	Pos.
	HVAC-Mid-3,000	1,800	45	18.4 ± 0.1	26.6	Pos.	45	17.8 ± 0.0	27.2	Pos.
09/18/17 PLAT and ROLL	PLAT-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	PLAT-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	PLAT-Mid-30	30	45	26.9 ± 0.1	18.1	Pos.	45	26.7 ± 0.0	18.3	Pos.
	PLAT-Mid-30	30	45	30.1 ± 0.0	14.9	Pos.	45	29.6 ± 0.0	15.4	Pos.
	PLAT-Mid-300	300	45	25.5 ± 0.1	19.5	Pos.	45	25.1 ± 0.0	19.9	Pos.
	PLAT-Mid-300	300	45	25.3 ± 0.1	19.7	Pos.	45	24.8 ± 0.0	20.2	Pos.
	PLAT-Mid-3,000	3,000	45	23.9 ± 0.0	21.1	Pos.	45	23.4 ± 0.1	21.6	Pos.
	PLAT-Mid-3,000	3,000	45	24.8 ± 0.1	20.2	Pos.	45	24.4 ± 0.0	20.6	Pos.
	ROLL-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	ROLL-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	ROLL-Mid-30	30	45	30.1 ± 0.1	14.9	Pos.	45	29.8 ± 0.0	15.2	Pos.
	ROLL-Mid-30	30	45	33.3 ± 0.1	11.7	Pos.	45	32.7 ± 0.2	12.3	Pos.
	ROLL-Mid-300	300	45	30.0 ± 0.1	15	Pos.	45	29.6 ± 0.1	15.4	Pos.

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
	ROLL-Mid-300[1]	300	45	26.8 ± 0.1	18.2	Pos.	45	26.2 ± 0.0	18.8	Pos.
	ROLL-Mid-3,000	3,000	45	23.7 ± 0.0	21.3	Pos.	45	23.0 ± 0.0	22	Pos.
	ROLL-Mid-3,000	3,000	45	23.7 ± 0.1	21.3	Pos.	45	23.0 ± 0.0	22	Pos.
09/25/17 non-AQ mid-duty	BUS-Mid-0	0	45	45	0	Neg.	45	43.8 ± 2.0	1.2	Neg.
	HVAC-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	BUS-Mid-30	42	45	35.1 ± 0.6	9.9	Pos.	45	34.5 ± 0.2	10.5	Pos.
	HVAC-Mid-30	42	45	29.5 ± 0.1	15.5	Pos.	45	29.2 ± 0.1	15.8	Pos.
	BUS-Mid-300	420	45	21.8 ± 0.1	23.2	Pos.	45	21.7 ± 0.0	23.3	Pos.
	HVAC-Mid-300	420	45	22.0 ± 0.1	23	Pos.	45	21.8 ± 0.0	23.2	Pos.
	BUS-Mid-3,000	4,200	45	21.5 ± 0.0	23.5	Pos.	45	20.9 ± 0.0	24.1	Pos.
	HVAC-Mid-3,000	4,200	45	17.5 ± 0.0	27.5	Pos.	45	17.2 ± 0.0	27.8	Pos.
	PLAT-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	ROLL-Mid-0	0	45	45	0	Neg.	45	45	0	Neg.
	PLAT-Mid-30[1]	42	45	45	0	Neg.	45	45	0	Neg.
	ROLL-Mid-30[1]	42	45	45	0	Neg.	45	45	0	Neg.
	PLAT-Mid-300	420	45	25.4 ± 0.1	19.6	Pos.	45	25.2 ± 0.0	19.8	Pos.
	ROLL-Mid-300	420	45	24.7 ± 0.0	20.3	Pos.	45	24.3 ± 0.0	20.7	Pos.
	PLAT-Mid-3,000	4,200	45	26.4 ± 0.0	18.6	Pos.	45	25.7 ± 0.0	19.3	Pos.
	ROLL-Mid-3,000	4,200	45	26.7 ± 0.1	18.3	Pos.	45	26.3 ± 0.0	18.7	Pos.
10/02/17 PLAT & ROLL	PLAT-New-0	0	45	45	0	Neg.	45	45	0	Neg.
	ROLL-New-0	0	45	45	0	Neg.	45	45	0	Neg.
	PLAT-New-30	28	45	16.4 ± 0.0	28.6	Pos.	45	16.5 ± 0.0	28.5	Pos.
	ROLL-New-30	28	45	22.6 ± 0.0	22.4	Pos.	45	22.5 ± 0.0	22.5	Pos.
	PLAT-New-300	280	45	17.0 ± 0.0	28	Pos.	45	16.9 ± 0.0	28.1	Pos.
	ROLL-New-300	280	45	18.1 ± 0.0	26.9	Pos.	45	17.9 ± 0.0	27.1	Pos.
	PLAT-New-3,000	2,800	45	17.5 ± 0.1	27.5	Pos.	45	17.4 ± 0.1	27.6	Pos.
	ROLL-New-3,000	2,800	45	18.1 ± 0.0	26.9	Pos.	45	17.9 ± 0.0	27.1	Pos.
	PLAT-END-0	0	45	45	0	Neg.	45	45	0	Neg.
	ROLL-END-0	0	45	45	0	Neg.	45	45	0	Neg.

Trial Date	Sample ID	Spore Load	B. a. chromosome Ct (t0)	B. a. chromosome Ct (tf)	B. a. chromosome ΔCt	Result	B. a. pXO1 Ct (t0)	B. a. pXO1 Ct (tf)	B. a. pXO1 ΔCt	Result
	PLAT-END-30	28	41.0 ± 3.5	29.1 ± 0.1	11.9	Neg.[1]	38.0 ± 1.1	29.1 ± 0.1	8.9	Neg.
	ROLL-END-30	28	45	38.8 ± 5.6	6.2	Neg.	45	34.0 ± 1.3	11	Neg.[1]
	PLAT-END-300	280	45	28.2 ± 0.1	16.8	Pos.	45	27.7 ± 0.1	17.3	Pos.
	ROLL-END-300	280	45	31.1 ± 0.1	13.9	Pos.	45	30.3 ± 0.1	14.7	Pos.
	PLAT-END-3,000	2,800	45	23.5 ± 0.0	21.5	Pos.	45	22.9 ± 0.0	22.1	Pos.
	ROLL-END-3,000	2,800	45	30.8 ± 0.1	14.2	Pos.	45	29.9 ± 0.0	15.1	Pos.
05/28/18 PM10 South Carolina and Various Filter types	11-BUS-NEW-30	29	45	18.6 ± 0.1	26.4	Pos.	45	18.6 ± 0.1	26.4	Pos.
	12-BUS-NEW-300	290	45	18.5 ± 0.1	26.5	Pos.	45	18.5 ± 0.1	26.6	Pos.
	13-HVAC-NEW-300	290	45	18.3 ± 0.1	26.7	Pos.	45	18.3 ± 0.1	27.1	Pos.
	15-BUS-NEW-3,000	2,900	45	17 ± 0.1	28	Pos.	45	17 ± 0.1	28	Pos.
	16-BUS-END-3,000	2,900	45	18.6 ± 0.1	26.4	Pos.	45	18.6 ± 0.1	26.9	Pos.
	17-HVAC-NEW-3,000	2,900	45	17 ± 0.0	28	Pos.	45	17 ± 0.0	28	Pos.

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