EPA/600/R-20/113 | May 2020
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United States
Environmental Protection
Agency

Comparison of DNATrax and *Bacillus anthracis* Surrogate Resuspension from Subway Surfaces

Office of Research and Development
Homeland Security Research Program

EPA/600/R-20/113

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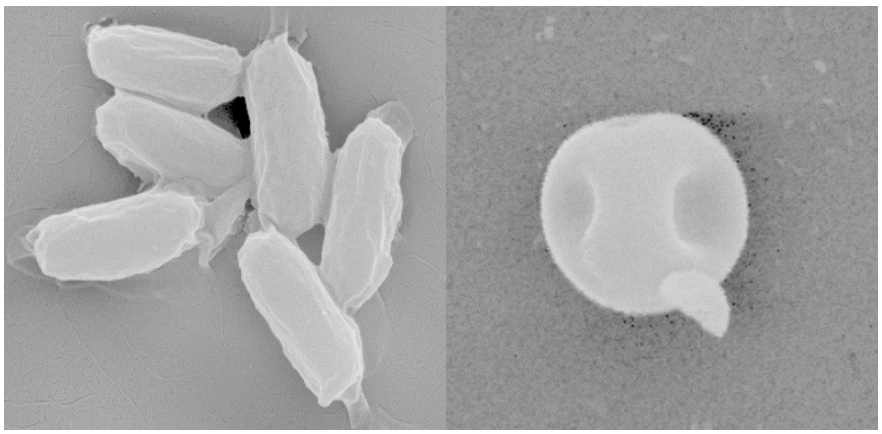
Assessment and Evaluation Report

Homeland Security and Materials Management Division
Center for Environmental Solutions and Emergency Response
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711



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The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's Center for Environmental Solutions and Emergency Response (Homeland Security and Materials Management Division), directed and managed this research through Contract No. EP-C-15-008 with Jacobs Technology, Inc. The research described in this study has been funded wholly or in part by the U.S. Department of Homeland Security (DHS) Science and Technology Directorate (S&T) under an interagency agreement (EPA No. RW-070-95935001 and DHS S&T IA# 70RSAT18KPM000176).

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Foreword

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Acknowledgments

The principal investigator from the Office of Research and Development's Center for Environmental Solutions and Emergency Response (CESER), Homeland Security and Materials Management Division (HSMMD) directed this effort with support of EPA and interagency project teams. Special thanks to the EPA Microbiology Laboratory (BioLab) staff for all of their support with microbiological sample analysis. Contributions of the following individuals were a valued asset throughout this effort:

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Acronyms and Abbreviations

µm	Micron or micrometer
ANL	Argonne National Laboratory
APS	Aerodynamic Particle Sizer
ATF	Aerosol Test Facility
AWT	Aerosol Wind Tunnel
Ba	*Bacillus anthracis*
$B_{\mu m}$	Barcodes/μm^3
BioLab	EPA Microbiology Laboratory
Btk	*Bacillus thuringiensis* var. *kurstaki*
°C	Degree(s) Celsius
CBRN	Chemical, Biological, Radiological, and Nuclear
CCSEM	Computer-Controlled Scanning Electron Microscope
CCDC CBC	Combat Capability Development Command Chemical Biological Center
CESER	Center for Environmental Solutions and Emergency Response
CFU	Colony Forming Unit(s)
CMAD	Consequence Management Advisory Division
CV	Coefficient of Variation
DCB	Disaster Characterization Branch
DE	Dextrose equivalent
DHS	US Department of Homeland Security
DNA	Deoxyribonucleic Acid
DNATrax	DNA Tagged Reagents for Aerosol eXperiments
DQI	Data Quality Indicator
DQO	Data Quality Objective
EPA	US Environmental Protection Agency
°F	Degree(s) Fahrenheit
HEPA	High-efficiency particulate air
HSMMD	Homeland Security and Materials Management Division
LL	Lincoln Laboratory
LLNL	Lawrence Livermore National Laboratory
Lpm	Liter(s) per minute
MIT	Massachusetts Institute of Technology
MMAD	Mass Median Aerodynamic Diameter
OEM	Office of Emergency Management
PBST	Phosphate-buffered saline with 0.05% Tween® 20

P_c	Particles collected
P_d	Particles deposited
psi	Pound(s) per square inch
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
qPCR	Quantitative Polymerase Chain Reaction
ReBoUndS	Resuspension of *Bacillus anthracis* surrogates on Underground Subway Surfaces
RF	Resuspension fraction
RH	Relative Humidity
RPM	Revolution(s) Per Minute
RTP	Research Triangle Park
RWT	Resuspension Wind Tunnel
S&T	Science and Technology Directorate
SEM	Scanning electron microscope
SPORE	Scientific Program On Reaerosolization and Exposure
SD	Standard Deviation
SS	Stainless Steel
T	Temperature
T_{DNA}	Total DNA Barcodes
T_p	Total Particles
T_v	Total DNATrax volume
$V_{i\%}$	Particle Volume Percent
V_i	Particle Volume
WAIDB	Wide Area Infrastructure and Decontamination Branch

Executive Summary

This project supports the interests of the US Environmental Protection Agency (EPA) and the Department of Homeland Security Science and Technology Directorate (DHS S&T). Specifically, this research supports their missions to understand the spread of biological contaminants due to a bioterrorism incident after the contaminants settle onto surfaces in an urban environment.

The main objective of this study was to compare the resuspension of an inert sugar-based surrogate, known as DNA Tagged Reagents for Aerosol eXperiments (DNATrax) developed by Lawrence Livermore National Laboratory (LLNL), under variable humidity conditions for an underground subway system to the resuspension of an established biological surrogate of *Bacillus anthracis* (*Bacillus thuringiensis* var. *kurstaki* [*Btk*]). The two surrogates were deposited on representative porous and nonporous subway surfaces, and we determined under what conditions, if any, DNATrax is an appropriate surrogate for *Bacillus anthracis (Ba)* when resuspension is considered. A dry powder micro-eductor deposition system was developed to accurately and repeatedly deposit the materials at the desired coupon load, spatial distribution, and with the majority of particles in their singlet and doublet forms with reduced agglomeration. Resuspensions were conducted in a custom wind tunnel using a wind shear velocity of approximately 45 mph and particles immediately captured onto filters.

Results from the comparative resuspension testing are shown below in Table ES-1. Quantitative polymerase chain reaction (qPCR) was used to quantify DNATrax particles and culture counting methods were used for *Btk*. *Btk* resuspension data should be treated as a maximum because culture counting of colony forming units could potentially lead to overestimation due to agglomerate disassociation. There was no statistically significant difference in resuspension fractions (RFs) from deposited *Btk* and DNATrax for the 30% and 80% relative humidity (RH) environmental test conditions. In contrast, the variable diurnal cycle (30% → 85% → 30% for stainless steel (SS); 30% → 80% → 30% for concrete) RH conditions resulted in statistically significant differences in RFs between the two surrogates.

For the surrogate materials on stainless steel following a diurnal cycle, the RF of DNATrax was reduced significantly below the RF of *Btk.* However, for concrete, the RF for *Btk* was reduced to significantly less than the RF of DNATrax. The lower RF for DNATrax on stainless steel is hypothesized to be due to softening of the DNATrax material due to the RH extending beyond its glass transition condition and potentially leading to greater adhesion to stainless steel. Glass transition is defined as the transition in amorphous materials from a hard and relatively brittle “glassy” state into a viscous or rubber state. This transition can be abrupt or gradual. For concrete, the conditions remained below the glass transition, which may explain the higher RF. The mechanism for the lower *Btk* RF on concrete is undetermined, though this lower *Btk* RF on concrete could potentially be due to a combination of the surface roughness of the concrete combined with the exosporium (hairy nap) on the surface of the *Btk*, which is not present on DNATrax. The variability in results makes it difficult to draw strong conclusions and recommendations. However, we hypothesize from the results that combinations of temperatures and relative humidities greater than the glass transition of DNATrax or the presence of standing water could lead to significant differences in the resuspension of DNATrax and *Btk*/*Ba*. For conditions that remain well below the glass transition (~80% RH), DNATrax (specifically formulated to be similar in size to *Ba*) is statistically indistinguishable from *Btk* (representative

Ba surrogate). Therefore, based on current data there is no evidence to suggest that the resuspension of the DNATrax formulation examined here would be significantly different than *Ba* under conditions well below the glass transition. Future work is recommended to examine the high variation in RFs of DNATrax under all conditions, especially following RH cycles, saturated porous substrates, and rain events.

Table ES-1. Summary of Resuspension Fraction Findings [Mean (SD)]

Surface, %RH Condition	DNATrax	*Btk*
SS, 30%	6.32 (±7.42) %	2.26 (±0.91) %
SS, 80%	1.79 (±1.88) %	0.73 (±0.47) %
SS, 30% → 85% → 30%	0.31 (±0.21) %	2.77 (±0.84) %
Concrete, 30%	8.36 (±7.92) %	3.75 (±1.00) %
Concrete, 80%	9.49 (±7.10) %	3.25 (±1.10) %
Concrete, 30% → 80% → 30%	12.76 (±2.5) %	0.35 (±0.36) %

SD = standard deviation
SS = Stainless Steel
Highlight across row indicates statistically significant difference at 95% Confidence Level

1.0 Introduction

1.1 Background

Release of a biological agent aerosol in an urban area has the potential for widespread contamination and risk to civilian life. Human activity and complex wind dynamics make determining levels and locations of contamination difficult. To coordinate evacuation and remediation efforts to protect human health, the US Environmental Protection Agency (EPA) and Department of Homeland Security Science and Technology Directorate (DHS S&T) are tasked with determining where these agents may be transported and settle. To that end, these agencies have employed computational contaminant transport models to examine multitudes of release scenarios. One such scenario is a biological agent release in an urban subway system. High wind speeds associated with train traffic in confined spaces and human activities such as walking may allow biological agents such as *Bacillus anthracis* (*Ba*) to travel considerable distances after an initial release, resuspend after depositing on surfaces, and be transported on human clothing (fomite transport) far away from the initial release point. To validate these contaminant transport models, it is necessary to conduct surrogate releases inside real-life subway environments. However, it is preferable if not mandatory to utilize surrogates for *Ba* that are inert and non-pathogenic due to safety (and perceived safety) concerns. One such inert nonbiological surrogate was developed by Lawrence Livermore National Laboratory (LLNL) to assist in validating these aerosol transport models, and this surrogate is known as DNA Tagged Reagents for Aerosol eXperiments (DNATrax). However, the degree to which DNATrax interacts with and resuspends from surfaces similarly to *Ba* is currently unknown. This information will aid in understanding how DNATrax, an inert nonbiological surrogate, compares to biological agents and biological spore surrogates in terms of resuspension from surfaces and will provide a basis for interpretation of future DNATrax test data.

1.2 Objectives

The purpose of the research conducted under the Resuspension of *Bacillus anthracis* Surrogates on Underground Subway Surfaces (ReBoUndS) project was to fill some of the key knowledge gaps whether DNATrax is a representative surrogate for actual biological spores, e.g., *Ba*, specifically, for resuspension/reaerosolization. The primary objective of this work was to evaluate and statistically compare reaerosolization of surrogate spores, *Bacillus thuringiensis* var*. kurstaki* (*Btk*), and DNATrax from surfaces and materials found in subways under typical environmental conditions. To meet the primary objective, this study includes a secondary objective of developing a repeatable small-scale dry deposition method for inoculation of subway materials with DNATrax and the *Ba* surrogate *Btk*.

2.0 Experimental Approach

This project involved determining the degree to which the resuspension of dry-deposited DNATrax under various environmental conditions is similar to a dry-deposited *Ba* surrogate, *Btk*. Previous experiments conducted at the EPA under the Scientific Program on Reaerosolization and Exposure (SPORE) demonstrated that *Btk* is a suitable *Ba* surrogate for reaerosolization studies (EPA 2014). Thus, the conditions under which DNATrax resuspends similarly to *Btk* are the same conditions where DNATrax is an acceptable surrogate for *Ba* in field studies. In order to make such a comparison, the parameter Resuspension Fraction is determined experimentally. Resuspension Fractions (**RFs**) are calculated by the following equation:

$$RF = \frac{P_c}{P_d}$$

where P_c is the number/mass of particles collected after a resuspension experiment, and P_d is the number/mass of particles deposited onto the surface used for resuspension testing. Therefore, two things must be measured: the amount of material deposited onto the test coupon surface and the amount of material resuspended from the test coupon surface. Due to the size and complexity of the surfaces examined, non-destructive methods of enumerating deposited materials such as optical microscopy or fluorescence could not be used. Therefore, prior to the resuspension studies, characterization studies of the depositions were carried out. These studies were used to show that the materials were deposited similarly and that small reference discs could be used as an estimate for the coupon surface coverage. The test surfaces chosen for this study were concrete and stainless steel, to represent a porous and nonporous surface, respectively. Both surfaces are found throughout a subway system and are representative of the surfaces most likely to be exposed to surface stresses sufficient to resuspend particulate matter. Relative humidity (RH) can be a major factor in the ability of a particle to resuspend. Differences in surface characteristics of the particles and adhering surfaces can potentially cause different resuspension characteristics with a change in humidity. Under normal conditions, particles can have a thin liquid layer on the surface, creating a capillary adhesive force between the particle and the surface. The curvature and roughness of the particle and adhering surface can affect the magnitude of that force (Hinds 1999). In addition, reduction in RH from a state in which capillary formation has occurred can cause the particle to be pulled closer to the surface as the capillary recedes, resulting in an increase in the adhesion force. Therefore, a comparative study must also include varying the relative humidity both statically (before deposition) and dynamically (after deposition).

The general experimental approach used to meet the project objectives is described below:

1. **Evaluation of eductor-based laboratory-scale dry deposition system.** To evaluate the eductor-based deposition system repeatability, multiple tests were conducted:
    a. The repeatability of the size distribution exiting the eductor was measured by repeated particle size measurements using an Aerodynamic Particle Sizer (APS) and both DNATrax and *Btk.*
    b. The repeatability and uniformity of the dispersion of test material over the surface of the test coupons was measured by sampling an array of discs at the bottom of the deposition chamber for multiple depositions.

c. The repeatability of the total surface load was examined by sampling stainless-steel surfaces after deposition. A nominal load of $1x10^7$ particles ($1.5x10^5$ particles/in^2) for both surrogates was desired and the amount of each surrogate material required to achieve this goal was established. Variation was desired to be within an order of magnitude and each coupon's load for resuspension was measured.

d. Scanning electron microscopy (SEM) imaging was conducted to show that particles on surfaces were dominated by singlets and doublets, and agglomeration of particles was minimized so that particle-to-particle comparisons could be made.

2. **Comparative resuspension of particles on subway materials (coupons) and conditions.** To compare resuspension of test particles, the general testing procedure is shown below:

   a. Subway material coupons were equilibrated to test conditions for a minimum of 24 hours prior to deposition of DNATrax or *Btk.*

   b. The subway material coupons for resuspension were inoculated or seeded with the surrogate particles under controlled conditions at a load of approximately 1 $x10^7$ particles ($1.5x10^5$ particles/in^2).

   c. Subway material coupons were subjected to a wind shear stress in a customized resuspension wind tunnel normalized to test environment conditions, and resuspended material was captured onto filters.

   d. Filters were removed from the system and test particles extracted into fluid and enumerated either via quantitative polymerase chain reaction (qPCR) (for DNATrax) or culture techniques (for *Btk*).

   e. Additional resuspension tests were conducted without filter capture to estimate the size distribution of resuspended test particles by using an aerodynamic particle sizer (APS) to aid in particle enumeration and characterization.

   f. Resuspension fractions of surrogate materials were calculated by dividing the number of resuspended particles by the number of deposited particles.

   g. Statistical analysis was conducted to compare resuspension fractions of test surrogates directly.

3.0 Experimental Materials and Methods

This section describes the test materials, environmental chambers, test surrogates, deposition system, resuspension wind tunnel, and particle counting methods used to achieve the project objectives.

3.1 Test Materials

The representative subway materials chosen for this test were stainless steel and concrete. Coupons of 7.75” x 7.75” x 0.75” of these materials were created in bulk at the EPA Research Triangle Park (RTP) facility to ensure uniformity of material surfaces. Stainless steel coupons were created by attaching 7.75” x 7.75” sheets of 22-gauge #4 polished stainless steel to 7.75” x 7.75” x 0.75” thick plywood with spray adhesive (Super 77™, 3M™, Maplewood, MN). Concrete coupons were created by mixing dry concrete mix (Quikrete® Concrete Mix PN 1101, The QUIKRETE Companies, Atlanta, GA) to manufacturers’ specifications at an approximate 10:1 by weight mix-to-water ratio, pouring into 7.75” x 7.75” x 0.75” molds and curing for five days at 70 degrees Fahrenheit (°F) and 30% RH. The concrete coupons were removed from the molds and allowed to continue curing in an environmental chamber at 20 degrees Celsius (°C) and 30% RH for a minimum of two weeks before use. Figure 3-1 shows completed stainless steel and concrete test coupons. Loose particles were removed from the concrete surface by spraying with an air nozzle at 30 pounds per square inch (psi) compressed house air prior to particle seeding/inoculation. Stainless steel coupons were wiped clean with a lint free cloth and methanol prior to particle seeding/inoculation.

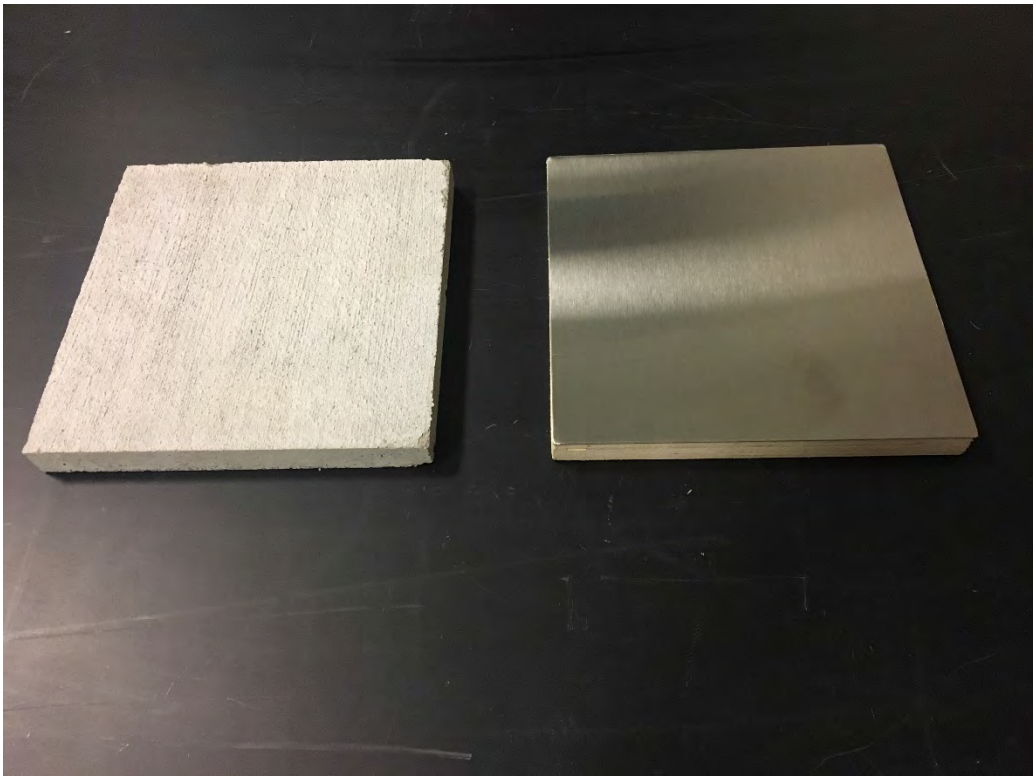


Figure 3-1. Concrete (left) and Stainless Steel (right) Coupons for Resuspension Testing

3.2 Environmental Systems

To achieve specific environmental conditions for material coupon storage/conditioning and resuspension testing, the EPA RTP facility has multiple environmental chambers to ensure controlled conditions and containment of particles. These chambers include an environmental test/conditioning chamber (shown in Figure 3-2) used for storage and conditioning of test material coupons and the EPA's recirculating aerosol wind tunnel (AWT, shown in Figure 3-3) used to house a small resuspension wind tunnel (Section 3.5) for reaerosolization of materials from test coupons and subsequent sampling for resuspended material and test coupon surfaces. Both containment systems are high-efficiency particulate air (HEPA)-filtered and are at negative pressure relative to the surrounding laboratory spaces. Both systems are temperature (T)- and RH-controlled. All set conditions were monitored throughout the experiments via a calibrated probe (VWR 35519-041, VWR International, Radnor, PA), HOBO Micro Station data logger (Onset Computer Corp., Bourne, MA), and Humicap HMT330 (Vaisala Inc., Louisville, CO, USA).

Figure 3-2. T- and RH-Controlled Environmental Storage Chamber

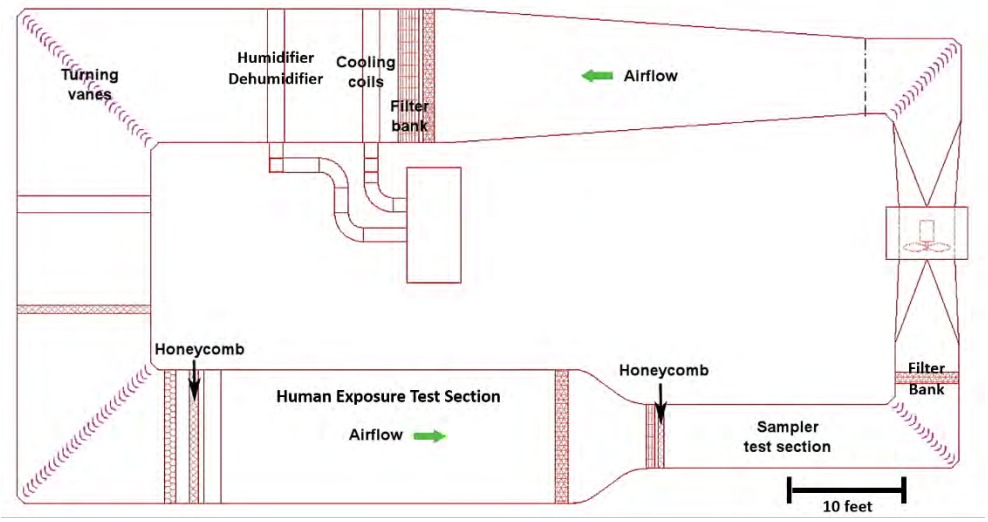
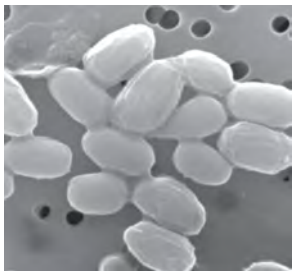
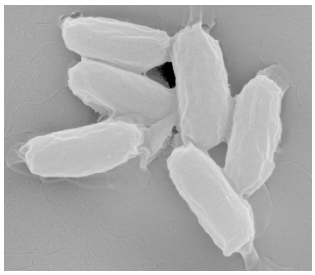
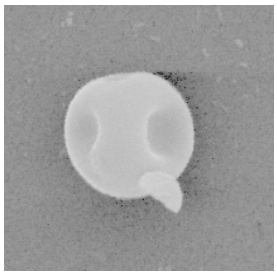


Figure 3-3. Plan view of the EPA Aerosol Wind Tunnel

3.3 Test Surrogates and Preparation

For the resuspension comparison tests, two different *Ba* surrogates were used: DNA-barcoded maltodextrin particles known as DNATrax (Section 3.3.1) and barcoded *Btk* (Section 3.3.2). The nominal properties of each surrogate from literature are discussed in the following sections and summarized in Table 3-1 along with the properties of the Ames strain of *Ba*. Images of DNATrax and *Btk* in Table 3-1 are of the materials used and properties measured for the specific batches of DNATrax and *Btk* used in this study and are discussed in the results.

Table 3-1. *Ba, Btk,* and DNATrax Properties

	***Ba* Ames** [†]	***Btk***	**DNATrax**
Physical Shape	 **Capped Cylinder**[††]	 **Capped Cylinder**	 **Spheroid**
Median Dimension	~1.53 µm x 0.81 µm	~1.61 µm x 0.80 µm	~1.83 µm*
Cross Sectional Area	1.10 μm^2 max 0.52 μm^2 min	1.15 μm^2 max 0.5 μm^2 min	2.63 μm^2
Volume	0.58 μm^3	0.608 μm^3	3.2 μm^3
Aspect Ratio	~1.89	~2.01	~1 – 1.2
Surface Roughness	Hairy Nap	Hairy Nap	Smooth
Density	Dry – 1.42 g/cm^3	Dry – ~1.4 g/cm^{-3} (assumed)	1.54 g/cm^3

*Mass Median Aerodynamic Diameter
[†] *Ba* Ames Data from Carrera et al. 2007
[††] *Ba* Ames Image from EPA 2012
References for DNATrax and *Btk* properties are in Section 3.3.1 and 3.3.2, respectively.

3.3.1 DNATrax

DNATrax was developed by Lawrence Livermore National Laboratory (LLNL) as a non-toxic test particle for aerosol fate and transport experiments in populated areas where the use of other *Ba* surrogates is restricted. The DNATrax particles are created by spray drying an aqueous solution of maltodextrin containing copies of a short DNA barcode (~100 base pairs) to achieve nominally spherical particles. The size of these particles can be varied depending on specific application, with small particles (~2 µm) being made of pure maltodextrin and particles on the order of 5-10 micrometers (µm) being achieved by seeding with silica particles. In the current experiments, the 2-µm nominal particle size was chosen as particles of this size are more similar to the size of *Btk* and have less of a potential for material shedding. The DNATrax samples were received from LLNL with a projected mass mean aerodynamic diameter of 1.83 µm. This mass mean aerodynamic diameter gives a cross sectional area of approximately 2.63 μm^2 and a volume of approximately 3.2 μm^3, assuming a perfect sphere. Specifications from LLNL were that the average 2-µm particle contains 469 DNA fragments for qPCR counting. The maltodextrin chosen for the DNATrax has a dextrose equivalent (DE) of 10 and a nominal density of 1.54 (g/cm^3) (Kaeser 2017). The density of *Ba*, however, is approximately 1.43 g/cm^3, making DNATrax slightly heavier on average than *Ba* (EPA 2012). A DE of 10 makes DNATrax moderately resistant to absorption of water vapor onto the surface and into the bulk of the particle. This resistance to absorption also increases its resistance to softening due to a glass transition. Glass transition is defined as the transition in amorphous materials from a hard and relatively brittle “glassy” state into a viscous or rubber state. This glass transition is abrupt as the water absorption isotherm exhibits a cascade absorption at the critical RH and temperature (Abramovič 2002). Materials with a DE of approximately 10 have a glass transition at 20 °C at approximately 80% RH, as the RH is lowered, the glass transition temperature increases to 65% RH/34 °C, 53% RH/41 °C, etc. (Nurhadi 2016). Once sufficient water is present on the surface, DNATrax becomes fully soluble, making deoxyribonucleic acid (DNA) extraction for qPCR highly efficient, though the presence of water on a deposition surface could destroy particle integrity (Nurhadi 2016). The surface of DNATrax particles is nominally smooth under SEM analysis (Harding 2016). DNATrax material was stored in an environmental chamber set to 20 °C and 30% RH when not in use. In addition, the tubes containing the material were kept in a Ziploc® bag filled with desiccant to further lower the RH.

3.3.2 Btk

The barcoded *Btk* used for this project is a genetically modified strain that was developed by the U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC, Aberdeen Proving Ground, MD) to allow the spores to be distinguished from naturally occurring *Btk* via qPCR analysis. The barcoded *Btk* was obtained from Dugway Proving Ground (Dugway, UT) as a dry powder of lyophilized spores. *Btk* is a gram-positive, spore-forming, rod-shaped (hemisphere capped cylinder) bacterium found in soil and has been used extensively in the past as a surrogate for *Ba* as many of the physical properties are identical (Carrera 2007; EPA 2012). *Btk* spores have a length of approximately 1.61 µm and a width of approximately 0.8 µm (EPA 2012). Assuming a hemispherical capped cylinder, these measurements give an average cross-sectional area of 1.15 μm^2 at maximum, 0.5 μm^2 at minimum, and a volume of 0.608 μm^3. The dried density of *Btk* has not been measured directly. However, the wet density

is identical to *Ba* at 1.17 g/cm^3. Other bacterial spores of similar size and shape matching the wet density of *Ba* match the dry density. Therefore, we assumed that the dry density of *Btk* is very close to the dry density of *Ba* (EPA 2012). *Btk* and *Ba* spores are covered in a hairy nap, making the surface rough on the nanometer scale (Plomp 2005; Tufts 2014). *Btk* and *Ba* have been shown to be relatively hydrophobic compared to other *Bacillus* species. However, *Btk* and *Ba* have been shown to change sizes by 4-10% over both semi-major and semi-minor axes as relative humidity changes from dry (~3% RH) to >95%. This expansion corresponds with a decrease in particle density from dry to wet spores of approximately 1.42 g/cm^3 to 1.17 g/cm^3. The spore core/cortex has been shown to be the main driver of this expansion as the spore-coat surface area remains relatively constant compared to the volume, i.e., under dry conditions, the spore coat has nano-sized wrinkles underneath the hairy nap and smooths when the spore is expanded (Plomp 2005; Carrera 2007; Westphal 2003). The onset RH of this expansion has not currently been explored. It is possible that this increased smoothness could cause a larger contact area and decrease resuspension for hydrated spores compared to dry spores.

3.4 Surrogate Particle Deposition System

The surrogate dry particle deposition system developed for these experiments is shown in Figures 3-4, 3-5, and 3-6. The eductor injection system was designed based on the large-scale system used for DNATrax dispersion experiments previously conducted, but the system was reduced in size to laboratory scale with a lower flow rate to deposit smaller masses of particles (Kaeser 2017). The settling chamber is similar to the chamber used for spray dry depositions in previous *Btk* resuspension experiments (EPA 2014). The method of operation uses a compressed air flow into the eductor, which causes a vacuum at the particle inlet. This vacuum causes air to rush into the bottom of the eductor, carrying particles through the eductor system into the settling chamber. Figure 3-4 shows the base microflow eductor system, which consists of a microflow venturi eductor (Micro-Flo Eductor, Jacobs Process Analytics, Inc., Williamsburg, VA), a dry compressed air connection, an inlet for particle introduction, and a 90-degree curved exit tube (elbow) for particle introduction into the settling chamber. The vacuum flow from the inlet is approximately 1.3 liters per minute (Lpm), and the total flow into the settling chamber is approximately 2 Lpm. Figure 3-5 shows the system with a more complex particle inlet into the eductor for elimination of large particles/agglomerates. This system contains two additional 90-degree bends and a 2” diameter x 1” tall cylindrical settling chamber prior to the eductor particle inlet. The flows of this system are identical to the flows in the base system. Figure 3-6 shows the settling chamber with internal mixing fans. The settling chamber is 18” x 8” x 8” to contain the 7.75” square material coupons and allow thorough mixing of particles prior to settling over three hours. Scouting experiments were used to determine the proper setup for deposition prior to validation for each test surrogate. It was shown that DNATrax could not be delivered at high enough surface concentrations using the complex setup in Figure 3-5. Therefore, the base system was used (Figure 3-4). Proper material preparation and the violent nature of the eductor was demonstrated to be sufficient to remove major agglomerates. The system in Figure 3-5 was used for *Btk* deposition/inoculation.

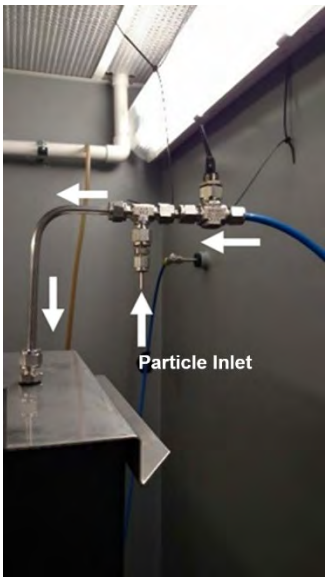


Figure 3-4. Micro-Venturi Eductor with Flow Field

Figure 3-5. Micro-Venturi Eductor Fitted with Large Particle Separator

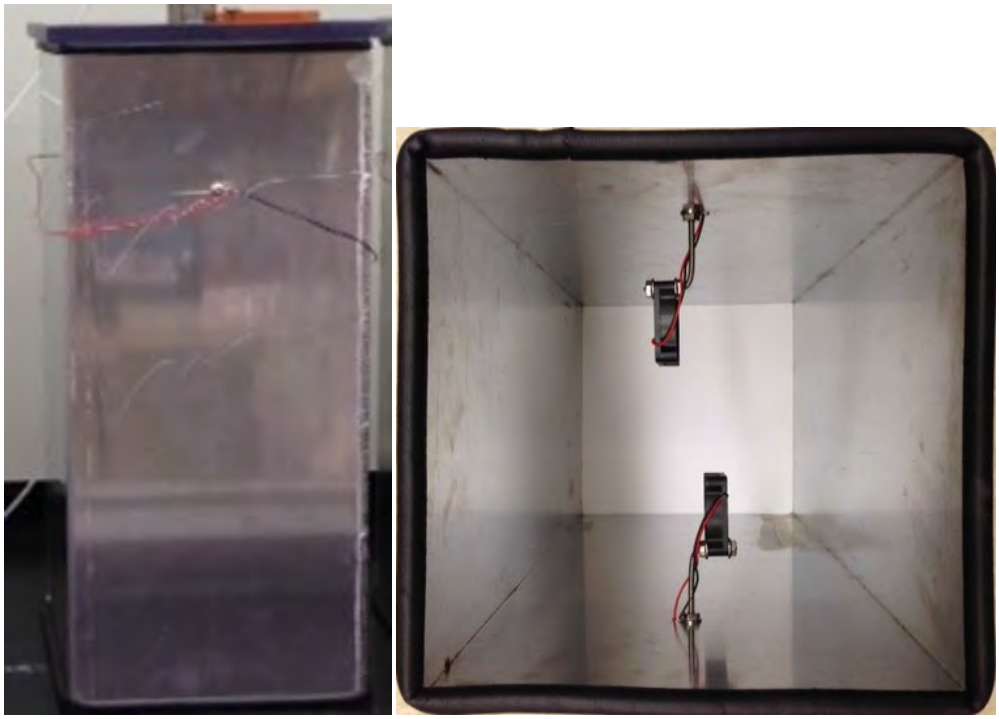


Figure 3-6. Dry Particle Settling Chamber and Internal Mixing Fans

3.5 Resuspension Wind Tunnel

The resuspension wind tunnel (RWT) shown in Figure 3-7 is 6 feet long with an approximately 9 in x 9 in cross section. The RWT is open-ended by design and thus can take advantage of the T and RH environmental settings available inside the environmental chamber. When operating under total collection of reaerosolized material from the test surface, the tunnel air is continually pulled through four polyester felt filters (EQXSCIEN-001, Superior Felt and Filtration, Ingleside, IL, USA) mounted in custom filter holders shown in Figure 3-8 by a blower at a nominal velocity of 2.5 meters per second (5.5 miles per hour [mph]). With the felt filters removed, the RWT can be fitted with an aerodynamic particle sizer (APS) (TSI 3321, TSI Inc., Shoreview, MN) to measure the size distribution of resuspended particles. The RWT is fitted with a HEPA filter on the inlet to provide clean sweeping air into the RWT and another HEPA filter just in front of the outlet to provide clean air and prevent contamination of the outer experimental chamber and the blower. The RWT is constructed of aluminum and stainless steel to allow for easy decontamination. The top of the RWT has a door that provides access to load a coupon. The outlet of the tunnel is hinged to provide access to the filter holders. In addition, the hinged door can be removed so that the RWT can be fitted with an extended chamber and APS sampling nozzle for particle sizing experiments as shown in Figure 3-9. Resuspension shear forces are achieved by a custom air knife mounted to an actuator as shown in Figure 3-10. The air knife is connected to a clean high-pressure dry air line to prevent particle impingement onto the surface through the air knife. The actuator traverses the air knife over the coupon at a steady speed. The height of the jet, angle of the air impinging on the coupon, and the speed of the actuator can all be adjusted as desired. In these experiments, the shear air speed across the coupon

was set to 45 mph to simulate subway conditions when trains are actively moving, and the air knife traversed the coupon in 60 seconds or 0.13 inches/second.

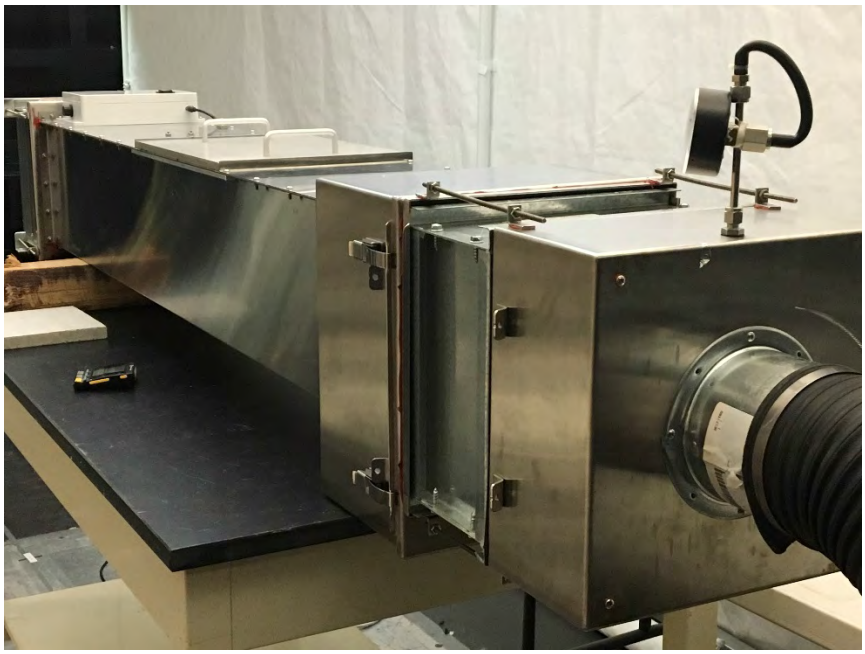


Figure 3-7. RWT Positioned in T- and RH-Controlled AWT

Figure 3-8. Four Filter Holders Mounted in RWT

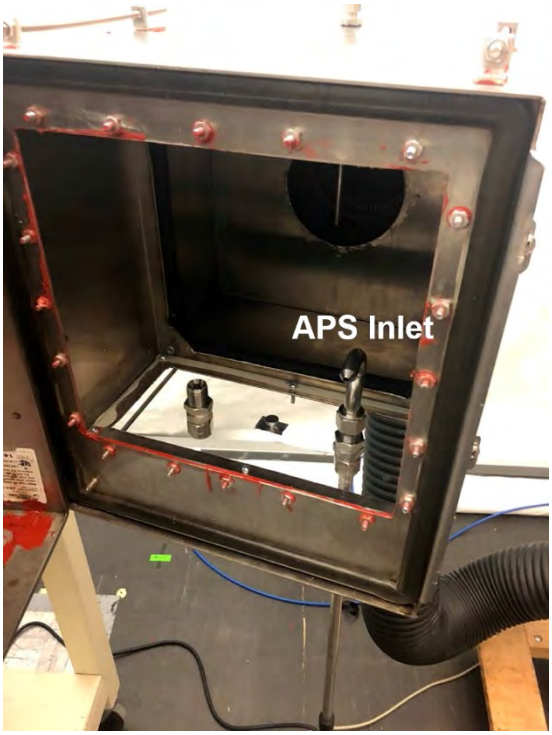


Figure 3-9. RWT Particle Sizing Extension with APS Nozzle

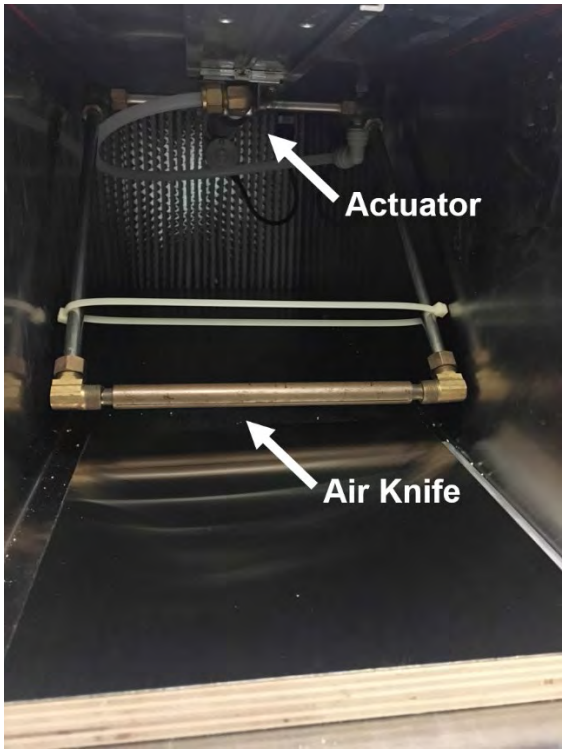


Figure 3-10. Air Knife and Actuator

3.6 Test Surrogate Enumeration

3.6.1 DNATrax

As stated previously, the DNATrax particles were tagged with a DNA barcode for enumeration via quantitative polymerase chain reaction (qPCR). Collected material, either on filters or aluminum deposition reference discs, was dissolved in phosphate buffered saline containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO) and processed using a qPCR machine (ABS 7500 Fast, Applied Biosystems, Bedford, MA) using standard practices (Kaeser 2017). A standard curve was generated daily using DNA sequences and concentrations provided by LLNL. The resulting output from processed samples was compared with the standard to provide the total number of DNA barcodes in each sample.

The process for converting the total number of recovered DNA barcodes into a number of DNATrax particles requires some assumptions and additional measurements. The basis for the enumeration rests on knowledge of the number of DNA barcodes present in each particle based on the spray-dry procedure. Information provided by LLNL stated that a 2-µm diameter particle contains approximately 496 DNA barcodes. If the particles were monodispersed (single size), then the process would have been to simply divide the total number of DNA barcodes by the number of barcodes per particle. However, analysis of the DNATrax material received from LLNL showed a somewhat broad polydispersed size distribution (via APS and SEM measurements) ranging from <500 nm to 10 µm with a mass peak between 1-2 µm and 90% of mass below 4 µm. Therefore, some assumptions, additional measurements, and calculations were made to avoid overestimating or underestimating particles depending on the measured size distribution.

First, all particles were assumed to be spheres of uniform maltodextrin density (i.e., solid) and second, the DNA barcodes were assumed to be evenly dispersed throughout each particle (469 barcodes/2-µm sphere = 112 barcodes/μm^3). Next, the size distribution of material that was to be enumerated needed to be determined. For depositions, measurement was done during the deposition process via an APS. For resuspended particles, however, as stated in the previous section, APS measurements were conducted separately from tests that collected material for qPCR, as the tunnel wind speeds were too great and material was removed from the chamber too quickly for appropriate APS measurements to be conducted. In addition, the porous concrete material shed small particles under wind shear, and those background particles would likely have dominated the APS signal. Therefore, the size distribution from stainless steel resuspension was used for all resuspension calculations.

Once the size distributions of the particles were established by APS, the count or number distributions were converted to percent volume distributions as the amount of DNA was dependent on the volume of each particle. The total number of DNA barcodes (T_{DNA}) was divided by the assumed number of DNA barcodes per μm^3 ($B_{\mu m}$ = 112 barcodes/μm^3) to obtain the total volume of DNATrax present in solution (T_V), Equation 3.1. This value was then multiplied by the sum of each particle volume percent ($V_{i\%}$) divided by the volume of the particle (V_i) to obtain the total number of particles in solution (T_P), Equation 3.2. The APS displays measured particle sizes sorted into 51 size bins, so the sum is over 51 to reflect all 51 particle

volume bins. If the particles are solid, the actual number of barcodes per µm^3 ($B_{\mu m}$) is inconsequential when calculating resuspension fractions, as it is found multiplicatively in the numerator and denominator of the RF equation after particle enumeration. However, if the particles are not solid, the volume of DNA per particle would be dependent on the wall thickness and may not be linearly related to the apparent volume of the particle. In that case, a counting error would occur.

$$T_V = \frac{T_{DNA}}{B_{\mu m}} \qquad \text{Equation 3.1}$$

$$T_P = T_V \times \sum_{i=1}^{51} \frac{V_{i\%}}{V_i} \qquad \text{Equation 3.2}$$

3.6.2 Btk

Initially, we desired to enumerate *Btk* spores via qPCR, in a fashion similar to DNATrax. However, we determined that agar plating and colony forming unit (CFU) counting was a more robust and repeatable measurement of the number of spores than qPCR measurement for a number of reasons. First, the small number of qPCR targets contained in each spore raises the minimum countable range of spores to a level where the amount of material collected during resuspension studies would likely be below the range of qPCR analysis. Second, the efficiency of extracting DNA from a spore is not known and is likely very low due to the hard spore coat. Experiments to estimate this efficiency would be prohibitively expensive and outside the realm of this current work. Third, significant internal work has been conducted at EPA microbiological laboratories to show that culture spore enumeration is a highly repeatable process with low variability using triplicate plating. Therefore, the process for enumerating *Btk* particles in the current experiments is much more simplified than DNATrax analysis. CFU enumeration does not account for particles that are non-viable and how they contribute to resuspension. However, non-viable particles are not counted when determining the number of spores deposited on the surface. Thus, they do not contribute to either the numerator or denominator of the resuspension fraction equation and would not bias the calculation. Spores that are viable but do not germinate would be the same small fraction of deposited particles versus resuspended particles and cancel out in the RF equation.

Btk spores were extracted from the filter collection media using a Stomacher® system (Seward Ltd., Worthing, West Sussex, UK), spread onto agar plates, incubated, and the number of colonies counted either automatically (QCount, Advanced Instruments, Norwood, MA) or visually by a laboratory technician, depending on the concentration. These processes are more fully described in Section 5.2.2.

The major assumption with the *Btk* analysis is that agglomerates of particles do not break apart when being extracted from the filters or aluminum deposition discs and form only a single

colony. For deposition, nearly 90% of particles are below 1.6 µm (singlets and doublets). Using APS data from deposition in Section 6.1.1, treating particles as spheres, and knowing that particle diameters increase as a cube function of the number of particles in the agglomerate, the overestimation of particles deposited due to deagglomeration can be calculated as a factor of ~2 (Hinds 1999). For resuspension, however, as large particles are more easily resuspended, deagglomeration would lead to a much larger overestimation of the number of resuspended particles compared to deposited particles (EPA 2015; Hinds 1999). Using the same method for resuspension as for deposition and the size distribution measured as shown in Section 6.2.1, the maximum overestimation of resuspended particles would be a factor of ~13. This method gives an overall overestimation of the RF as a factor of ~6.5, and any RF derived from CFU enumeration should be treated as a maximum.

4.0 Dry Deposition Evaluation

This section discusses the resuspension test matrix and approach for evaluation of the dry deposition method. The dry deposition system was developed and evaluated for reliable and repeatable deposition of dry particles to surfaces of interest.

4.1 Test Matrix

There were four main components to evaluation of the dry deposition method as listed in Section 2. The first is confirmation that the eductor system produces a consistent size distribution of particles, the second is evaluating the distribution of particles over the surface of the coupons, and the third is determining the repeatability of surface loading and confirmation that 1 x 10^7 particles surface loading can be achieved. Lastly, SEM confirmation of particle size distribution was conducted. Each of the first three components consisted of five replicates to obtain an estimate of the variability. The SEM analysis of deposited particles was only conducted once due to time constraints. Since the APS data showed low variability and the SEM size distribution was similar for the single analysis, it was determined that the SEM particle size analysis was representative. Additionally, the SEM particle sizing was conducted to corroborate the APS data and was not deemed a critical measurement. A summary of the deposition test matrix can be found in Table 4-1.

Table 4-1. Deposition Test Matrix

Surrogate	Test	Replicates
DNATrax	APS Size Distribution	5
	Particle Dispersion	5
	Particle Load	5
	SEM Analysis	1
Btk	APS Size Distribution	5
	Particle Dispersion	5
	Particle Load	5
	SEM Analysis	1

4.2 Dry Deposition Testing Approach and Procedure

All of the above experiments occurred on the same day to minimize inter-day variability. The particle dispersion and particle seeding/inoculation experiments were conducted concurrently, and the APS measurements were conducted between each of the deposition replicate tests. All experiments were conducted inside an environmental chamber set to 20 °C and 30% RH. For each deposition dispersion/load test, a new array of nine 1.125” diameter aluminum foil discs

was placed on top of a stainless steel pan at locations marked by a grid and labeled A-I as shown in Figure 4-1. The settling chamber was placed over the top of the grid array, and one of the two eductor arrangements was connected to the lid, as shown in Figure 3-4 for DNATrax and Figure 3-5 for *Btk*. Immediately prior to deposition, the *Btk* and DNATrax were vigorously agitated to break up the clumps and provide a more consistent powder of singlets and doublets. For *Btk,* 500 mg of the lyophilized powder was placed into a plastic vortex tube with five 3-mm glass beads and vortexed for two minutes in 30-second bursts. DNATrax was processed similarly with only 3 beads and 200 mg of powder to reduce stress on the material. After processing, approximately 2 mg of the processed powder to be tested was weighed, the mixing fans inside the deposition chamber were turned on, and the material was injected into the settling chamber. The mixing fan and eductor system were allowed to operate for five minutes before the compressed air valve was closed, and the mixing fans were turned off. Material inside the settling chamber was allowed to settle for three hours prior to collection of the aluminum discs for analysis. Immediately following completion of material injection into the settling chamber, the eductor system was removed, and the microflow portion was cleaned with water and ethanol and allowed to dry. After cleaning, the eductor was reconnected to the compressed air and inlet system. Once the components were reattached, the system was placed directly over an APS inlet tube, and 2 mg of material was injected into the APS for five minutes to allow time to obtain a particle size distribution, and the eductor was cleaned again. This cycle was repeated five times for a total of 10 runs for surrogate material, five depositions onto aluminum discs and five injections into the APS. For the final DNATrax deposition experiment, an SEM stub (PN 16111, Ted Pella Inc., Redding, CA) was placed next to the center aluminum disc (Labeled E) to collect settled material for analysis. For SEM imaging of *Btk,* a 25-mm polycarbonate filter with a 0.5 µm pore size was used to capture *Btk* material exiting the eductor, and the filter was mounted to a carbon tape-coated SEM stub.

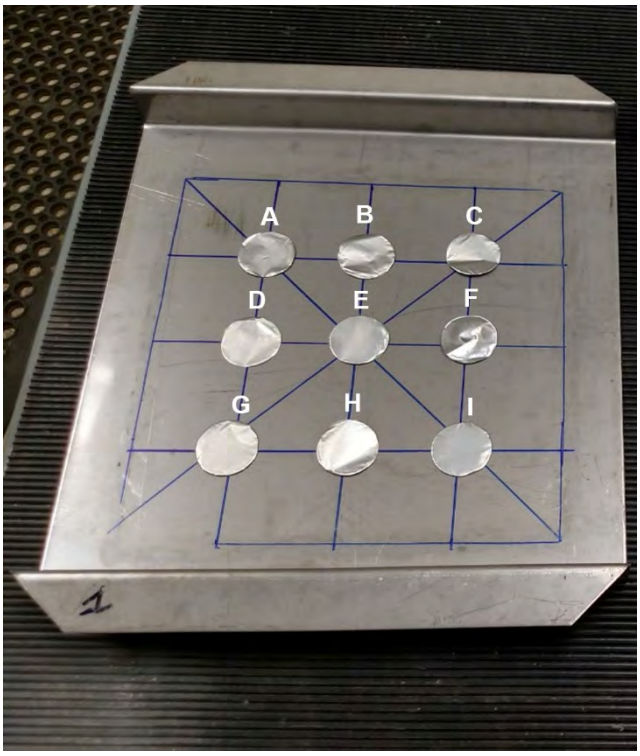


Figure 4-1. Distribution Disc Sampling Array

After the three-hour settling time was complete, each deposition stack was removed, and each aluminum disc collected into an individually labeled 50-mL vortex tube (Falcon 50 mL, Corning Inc., Corning, NY) so that disc position and deposition experiment number could be maintained. Material was extracted from the discs by adding 5 mL of phosphate buffered saline containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO) to the tube and vortexing for two minutes. For DNATrax, qPCR analysis was then conducted to determine the number of DNA barcodes on each disc and for *Btk,* culture plating was conducted to determine the number of CFU deposited onto each disc.

The peak concentration of the APS signal during the size distribution measurements was used as the benchmark for the size distribution for each run. The results were averaged, and standard deviations were calculated. Once the average size distribution was determined for DNATrax, it was used along with the qPCR results in the enumeration process for DNATrax using the method found in Section 3.6.1.

Following determination of the number of particles on each disc, deposition heat maps were created to show if any deposition contained highly skewed concentrations of surrogate material. The total number of particles deposited for each run was determined by averaging the surface concentration of the nine deposition discs and multiplying it by the total area of the stainless steel or concrete coupon (60 in^2).

Both *Btk* and DNATrax particles were sputter-coated in ~30 nanometers of gold prior to SEM analysis to prevent charging (Harding 2016). The imaging was conducted using a TESCAN

Mira3 field emission SEM (Tescan USA, Inc., Warrendale, PA), and the computer controlled particle analysis was done using IntelliSEM software (RJ Lee Group, Monroeville, PA).

5.0 Resuspension Comparison Tests

This section discusses the test matrix and procedures for comparing the resuspension fraction of DNATrax and *Btk* from select subway-type surfaces and conditions. All procedures discussed in this section follow standards for quality required by EPA.

5.1 Resuspension Text Matrix

The resuspension tests consisted of two phases. The first phase was resuspended particle collection on felt filters for enumeration, and the second phase was APS measurements of resuspended particle size distributions.

5.1.1 Filter Sampling of Resuspended Surrogate Materials

The surfaces and conditions for resuspension comparison were chosen to reflect common subway surface materials and the environmental conditions the settled particles may experience. Three main variables were represented in this study: particles, surface types, and environmental conditions. The traverse speed of the air knife over the coupon (0.13 in/second) and the air shear speed (45 mph angled at 15° below horizontal) were kept constant. The surface types used for resuspension tests with DNATrax and *Btk* were concrete and stainless steel. The temperature for each environmental test condition was kept constant at 20 °C, and only the RH was varied. RH values tested were 30%, 80%, and a cycle from 30% to 80% and back to 30% - referred to hereafter as a diurnal cycle - prior to resuspension tests. Each day of testing consisted of a single environmental condition and a single surface with the only variable being the deposited surrogate for each day. Finally, five replicates in a day were conducted on each surrogate for a total of 10 resuspension tests per day. A summary of the test matrix can be found in Table 5-1 with alternating row colors indicating a separate test day.

Table 5-1. Filter Collection Resuspension Test Matrix

Material	Surface	RH/Temp	Wind Speed	Replicates
DNATrax	Steel	30%/20 °C	20 m/s (45 mph*)	5
Btk	Steel	30%/20 °C	20 m/s (45 mph)	5
DNATrax	Concrete	30%/20 °C	20 m/s (45 mph)	5
Btk	Concrete	30%/20°C	20 m/s (45 mph)	5
DNATrax	Steel	80%/20 °C	20 m/s (45 mph)	5
Btk	Steel	80%/20 °C	20 m/s (45 mph)	5
DNATrax	Concrete	80%/20 °C	20 m/s (45 mph)	5
Btk	Concrete	80%/20 °C	20 m/s (45 mph)	5
DNATrax	Steel	30%-80%-30%/20 °C	20 m/s (45 mph)	5
Btk	Steel	30%-80%-30%/20 °C	20 m/s (45 mph)	5
DNATrax	Concrete	30%-80%-30%/20 °C	20 m/s (45 mph)	5
Btk	Concrete	30%-80%-30%/20 °C	20 m/s (45 mph)	5

*miles per hour (mph)

5.1.2 Particle Size Measurements of Resuspended Material

In general, particle size measurements of resuspended material can be difficult because low target particle counts and background particles from porous material coupons make isolation of the test material particles challenging. Therefore, to maximize the probability of detection, only ideal conditions (30% RH and 20 °C) and surfaces (clean surface/low background particles) were considered for these measurements, and assumptions were made that changes in conditions and surfaces did not affect the size distribution of resuspended material dramatically. The surface chosen for these tests was stainless steel, and the temperature and humidity were set to 20 °C and 30%, respectively. Five replicates of each surrogate were tested on the same day for a total of 10 resuspension tests. Shear wind speed and traverse speed were kept identical to the filter collection tests. SEM analysis of resuspended material was not conducted as not enough material could be collected during a test run for analysis.

5.2 Resuspension Testing Approach and Procedures

5.2.1 Coupon Preparation and Particle Seeding

Prior to deposition, all coupons were equilibrated according to experimental conditions to ensure that coupon surface conditions were static during deposition. There were two seeding/inoculation/post-inoculation conditions: 20 °C/30% RH for the 30% and diurnal cycle resuspension tests and 20 °C/80% RH for the 80% RH resuspension tests. After equilibration, all stainless steel coupons were wiped clean with methanol and allowed to dry fully prior to inoculation. All concrete coupons were sprayed with compressed air at 30 psi to remove loose dust prior to seeding/inoculation. All coupon depositions occurred the day before resuspension tests with a total of five DNATrax coupons and five *Btk* coupons inoculated per day. After equilibration, the deposition procedure for each surrogate was identical for all environmental conditions. However, for the 80% RH deposition condition, care was taken to limit the exposure of the surrogate materials to the atmosphere to avoid agglomeration. The process for deposition after coupon preparation was as follows.

1. Five coupons for single surrogate seeding/inoculation were placed on individual stainless steel bases inside an environmental chamber under resuspension environmental conditions.

2. A 1.125” aluminum foil disc was placed on the center of each coupon as a measure of deposition level.

3. All coupons were then covered by a settling chamber and lid.

4. One stack was taken to the deposition table.

5. The fans in the settling chamber were turned on, and an eductor system corresponding to the surrogate being deposited was connected to the lid.

6. Approximately 2 mg of surrogate material was weighed and delivered through the eductor system, and air flow was maintained for 5 minutes.

7. Airflow through the eductor and the mixing fans was stopped.
8. The eductor system was removed from the settling chamber, was cleaned with water and ethanol, and allowed to dry.
9. The eductor system was then connected to the APS, and a size distribution was measured.
10. Steps 3-9 were repeated until all five depositions were completed and five reference APS measurements taken.
11. Steps 1-10 were then repeated for the next surrogate seeding/inoculation.
12. After three hours of settling, all coupons were removed from their settling chambers, the aluminum disc was collected and placed into a labeled vortex tube, and the coupons were placed into labeled individual closed aluminum trays for storage until resuspension testing.
13. All aluminum foil deposition reference discs were then transported to the EPA BioLab for analysis.

For the 30% and 80% RH resuspension tests, the environment was maintained at a constant temperature and humidity. For the diurnal cycle tests, after all coupons were transferred to the aluminum trays, the humidity set point for the environmental chamber was set from 30% to 80% RH, and the chamber was maintained at 80% for ~14 hours. Then, the tunnel RH was set back to 30%. Total transition time from 30%→80% and from 80%→30% was approximately one hour each.

5.2.2 Filter Sampling Resuspension Procedure

Resuspension testing was conducted using the RWT described in Section 3.5. The testing follows procedures similar to the procedures used in previous studies (EPA 2014). All five coupons for a single surrogate inoculation were tested in succession, followed by five coupons of the other surrogate. Scouting runs showed that no significant background material was present inside the RWT after each run, thus no decontamination of the tunnel was conducted between the five replicate test runs. The tunnel was decontaminated before the second set of five coupons was tested using DNA Away™ (ThermoFisher Scientific, Waltham, MA) for DNATrax or pH-adjusted bleach (acetic acid plus bleach) for *Btk,* depending on which surrogate had been used in the last set of tests. The procedure for filter collection resuspension tests was as follows.

1. The back of the RWT was opened, four felt folder holders were inserted into the wind tunnel, and the section was closed.
2. A coupon in its closed aluminum tray was carefully moved from the deposition zone to directly next to the RWT.

3. The top of the tunnel was opened, the coupon for resuspension testing was slowly removed from its tray and was gently inserted to minimize airflow over the surface. The technician changed gloves and sealed the top of the tunnel.

4. The pressure regulator on the air knife was set to emit air at 45 mph.

5. The blower controlling the tunnel sampling flow was started and run for 30 seconds prior to resuspension testing.

6. The air knife valve was opened, and the traverse electronics were engaged.

7. The air knife and traverse ran for 60 seconds. Then, the valve was closed and the traverse electronics reversed to return the air knife to its initial condition (the traverse position was monitored to ensure full extension at 60 seconds.)

8. The blower was allowed to run for 30 seconds to ensure all remaining resuspended particles were pulled into the felt filters.

9. The blower was disengaged. The filter holders were removed from the tunnel and placed into a sterile labeled Ziploc® bag.

10. The back of the tunnel was closed. The coupon was removed and placed into a storage container to be discarded.

11. After removal of the coupon, the technician changed gloves, the tunnel was resealed, the blower was turned on, the air knife valve was opened, and the traverse was swept back and forth for 3 cycles to remove any additional particles from the tunnel prior to the next resuspension test.

12. Steps 1-10 were repeated for the additional four single-surrogate coupons.

13. After all five coupons were tested, the tunnel was decontaminated and prepared for the next set of five coupons to be tested that day.

After all resuspension testing for that day was completed, the felt filters were removed from their holders using aseptic techniques, and all four filters from each test were placed into a labeled Stomacher® bag (Seward Ltd., Worthing, West Sussex, UK). Each test day generated five DNATrax and five *Btk* Stomacher® bags containing four felt filters from the resuspension studies as well as two bags containing field blanks. All samples were delivered to the BioLab for particle extraction and processing. Material from filters was extracted by adding 120 mL of phosphate buffered saline containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO) to the filter bags and stomaching the bags for two minutes at 230 revolutions per minute (RPM). The eluent was removed without squeezing the filters. Filters were not squeezed due to a slight potential of dilution during *Btk* extraction. *Btk* spores loosened from the filter material but still entrapped with the filter body may not move through the filter as easily as the extraction liquid and become entrapped. Internal extraction efficiency measurements have shown ~99% particle removal, so it is assumed that the Btk material is evenly distributed throughout the extraction

fluid and that determination of the number of CFU/ml in an aliquot is representative of the total fluid including the portion remaining in the filter. DNATrax filter extract solutions were analyzed by qPCR to determine the number of DNA barcodes on each filter. *Btk* filter extracts were spiral plated, incubated, and counted by computer to determine the number of CFU in the filter extraction solution. If the *Btk* material was not countable by computer methods, the extract solution was spread plated or filter plated, incubated, and counted manually. The aluminum foil deposition reference discs were extracted and analyzed as described previously in Section 4.2.

5.2.3 APS Measurement of Resuspended Material Procedure

To measure the size distribution of the resuspended material, the RWT, prior to blower connection, was fitted with an extended sampling chamber and exit HEPA filter as show in Figure 3-9. The sampling chamber contained sampling ports for installation of isokinetic nozzles internally and connection ports for the APS externally. A ¼" sampling nozzle was installed parallel to the tunnel flow, and an APS was connected to the external connection port. The APS samples at 5 liters per minute generated a nozzle sampling velocity of ~5.9 mph. The front of the nozzle was approximately 3 feet (ft) from the leeward edge of the coupon and approximately 4 inches below the top of the coupon. Though the 5.9 mph sampling velocity was significantly below the 45-mph wind shear speed directly out of the air knife, the significant distance from the edge of the coupon and the larger space to capture particles over time increased the likelihood of particle capture.

As stated above, particle sizing of resuspended materials requires ideal conditions and surfaces. The particle sampling was conducted with stainless steel coupons at 30% RH. All coupons were cleaned, equilibrated to 30% RH, and tested for background particles by APS in the tunnel prior to particle seeding/inoculation for resuspension. The tunnel was thoroughly cleaned and measured for a particle background prior to each resuspension test to show that only particles coming from the seeded/inoculated surface were measured by the APS. Resuspension tests were conducted without the tunnel blower on to maximize the time resuspended particles were inside the chamber. The air knife was set to 45 mph and the traverse for 60 seconds (same settings as the resuspension tests). Ten coupons were tested for a particle background. Then, five were inoculated with *Btk* and five seeded with DNATrax. Coupon deposition, settling time, and storage prior to resuspension followed the procedure for the 30% RH resuspension studies described in Section 5.3.2. Identically to Section 5.3.2, all five of a single surrogate type were tested before the next surrogate type. The process for a single sampling event was as follows.

1. The seeded/inoculated coupon was placed inside the RWT, and the RWT was closed.

2. The APS sampling was initiated for 10-second sampling intervals, and six 10-second samples were taken for a 60-second total background sample.

3. The air knife and traverse were initiated, and the APS sampled during resuspension for 60 seconds.

4. The air knife valve was then closed, and the traverse was returned to its initial starting point. The APS continued to sample for five more minutes for a post-resuspension sample.

5. The blower was turned on for 30 seconds to clear the tunnel of residual aerosolized particles and then turned off.

6. The coupon was then removed from the tunnel.

7. The tunnel was resealed, the blower was turned on, the air knife valve was opened, and the traverse was swept back and forth for three cycles to remove any additional particles from the tunnel prior to the next resuspension test.

8. Steps 1-7 were repeated for the additional four single-surrogate coupons.

The five minutes of post-resuspension sampling were used to measure the particle size distribution. The APS sampled in 10-second intervals. Therefore, 30 sample intervals were summed together to determine the distribution. All five coupon runs for each surrogate were then averaged to show the final distribution and variance. The DNATrax distribution was then used for the calculation of the number of resuspended DNATrax particles from the filter extraction qPCR results.

6.0 Results and Discussion

This study sought to examine the resuspension properties and behavior of a suitable *Ba* surrogate (*Btk*) compared to an inert maltodextrin-based surrogate, DNATrax. This comparison was accomplished by carefully depositing known amounts of each material, using a specially designed dry deposition eductor system for controlled deposition of DNATrax and *Btk* onto two types of subway surfaces: stainless steel (nonporous) and concrete (porous). After deposition, particles were resuspended from the surfaces under controlled conditions (temperature, relative humidity, and wind speed), resuspended material was captured onto filters, and resuspension fractions of both materials were calculated and compared against one another. The results of the deposition and resuspension experiments are presented in this section.

6.1 Dry Deposition System Performance

In this section, the performance of the dry deposition eductor system is discussed. Since deposited DNATrax particle counting using qPCR requires knowledge of the size distribution, the APS results are presented first.

6.1.1 APS Size Distribution and SEM Analysis

Both DNATrax and *Btk* were tested for repeatable deposition onto coupon surfaces using the dry deposition eductor system. Initial runs of DNATrax through the eductor system led to significant clogging of the system and highly variable size distributions from deposition to deposition. After initiating a cleaning procedure of water and ethanol, the variation of the distributions was significantly reduced. Figure 6-1 shows the average of five depositions of DNATrax through the eductor system after the implementation of the cleaning procedure. Figure 6-1A shows the relative number and volume size distributions plotted with standard deviations as error bars. Figure 6-1B, on the right, shows the cumulative size distributions for DNATrax. The volume distributions correspond to the distribution of mass in the system. The mass median aerodynamic diameter (MMAD) through the deposition system was measured to be 2.28 µm, which is very similar to the LLNL specifications provided with the DNATrax shipment (1.83 µm MMAD), and the median aerodynamic particle size from the number distribution was 0.9 µm. These data show that the DNATrax is polydispersed with a wide range of particle sizes with 50% of the particles less than 1 µm in aerodynamic diameter and 90% of the particles having a diameter less than 2 µm.

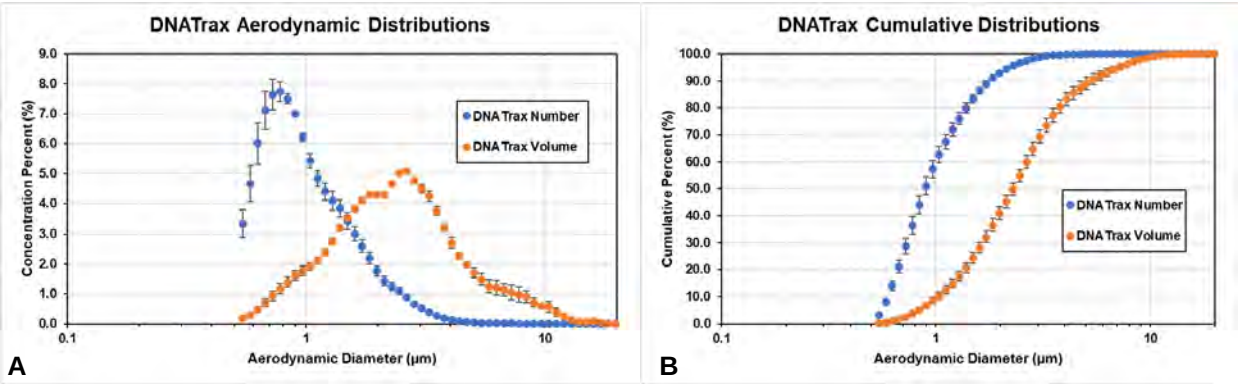


Figure 6-1: DNATrax Number/Volume Relative and Cumulative Distributions

Following the APS distribution analysis, the deposited DNATrax captured onto a silicon SEM stub was analyzed by a computer-controlled scanning electron microscope (CCSEM) capable of automated particle imaging, sizing, and shape analysis. Figure 6-2 shows an example SEM image of the deposited DNATrax (A) and a close-up image of a particle with a circle area equivalent diameter of 1.8 µm (B). The surface morphology of all observed DNATrax particles showed dimples or indentations on the surfaces of the particles, possibly indicating that the DNATrax initially formed hollow spheres during the spray dry process, and these hollow spheres collapsed as the internal moisture was removed (Vehring 2008). The results from the CCSEM analysis plotted with the average APS measurements can be seen in Figure 6-3. The 9000 particles were measured over an approximately 8-mm^2 area. The results of the automatic scan and visual inspection of the resultant images showed that the DNATrax deposited in mostly singlet and doublet form with a large spectrum of singlet sizes. The shape of the distributions from CCSEM match closely to the number size distribution measured through the APS. The APS measures the aerodynamic diameter whereas the CCSEM gives the circle area equivalent diameter or the diameter of a circle with the same cross sectional area as the measured particle. For spherical and near-spherical particles, this value is very close to the actual geometric diameter. Direct comparison between the aerodynamic diameter and the circle equivalent diameter requires knowledge of the dynamic shape factor and the particle density (Hinds 1999). In the case of DNATrax in Figure 6-3, the two graphs align very closely without correction, suggesting that the density of the DNATrax particles is close to the density of water (1 g/cm^3) instead of the 1.54 g/cm^3 reported for the bulk material. This observation suggests that the particles are partially hollow shells, or that the apparent dimples on the particle surface change the dynamic shape factor from the dynamic shape factor of a perfect sphere, or a combination of the two. More experimentation in particle settling and density measurements must be conducted to determine the source of this discrepancy. In addition, the images show no sign of loss of particle integrity (shearing/fracturing) as all particles remain spheroidal below 1 micron. The CCSEM serves to show that the APS measurement is not missing larger particles and that the dry eductor deposition system with prior material vortexing delivers the DNATrax to the coupon surface in its natural state with few agglomerates.

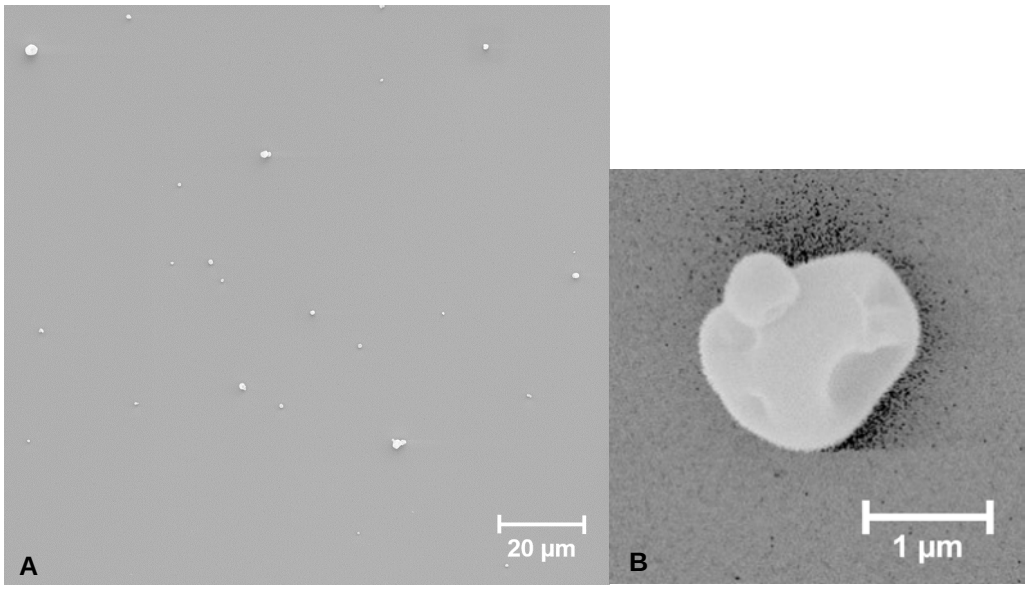


Figure 6-2. Overview Scan of Deposited DNATrax (A) and 1.8-µm Particle (B)

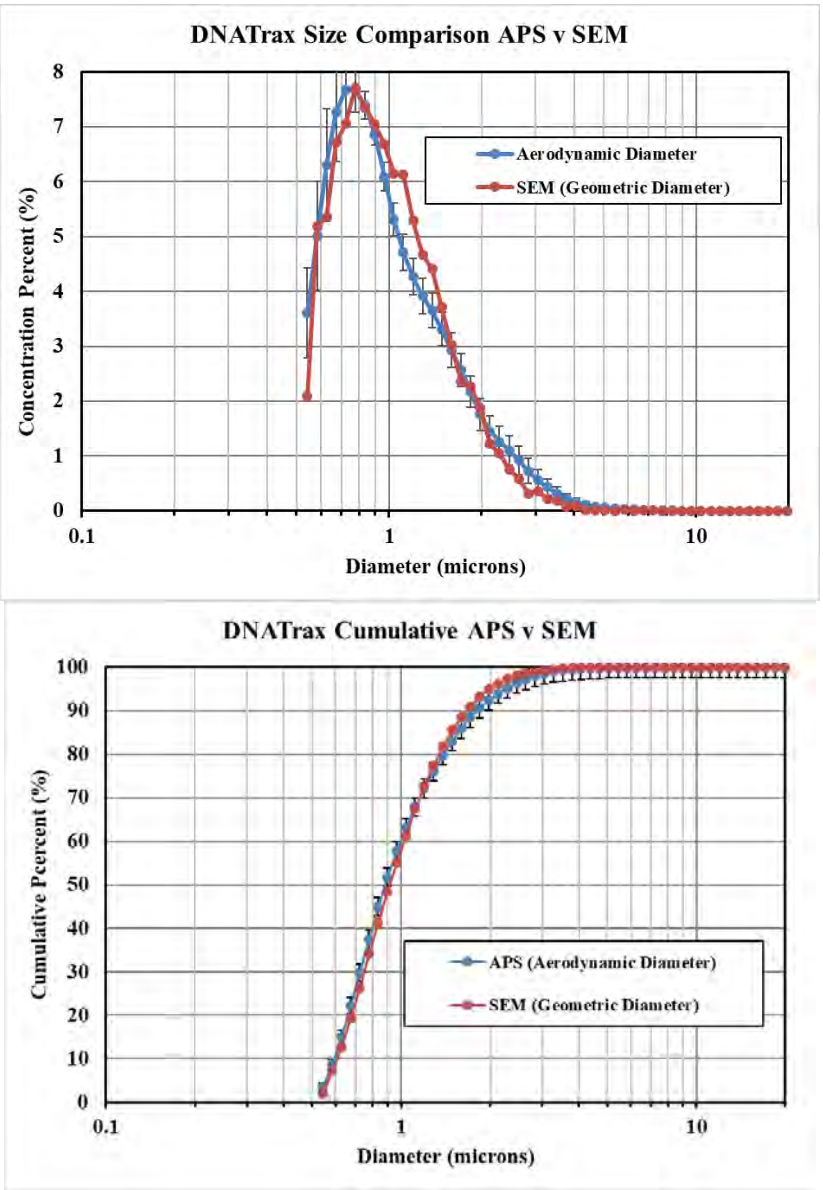


Figure 6-3. SEM Size Measurement and APS Comparison for DNATrax

Figure 6-4 shows the size distribution results for the *Btk* deposition system. This figure shows a strong sharp peak in the distribution for *Btk*. The mass mean particle size for the *Btk* was observed to be 1.7 µm and the mean aerodynamic size derived from the number distribution was 1.2 µm, with 90% of particles being below 1.6 µm. Particles below 0.8 µm are not viable *Btk* spores and are non-colony forming, thus the particles below 0.8 µm are not counted during plating. The mass distribution does demonstrate that a significant portion of the mass is associated with agglomerates—either doublets or larger—as single spores have a very tight size distribution. However, this is not a problem if the spore agglomerates do not break apart significantly when extracted from the filters or aluminum discs, which is the main assumption as described in Section 3.6.2.

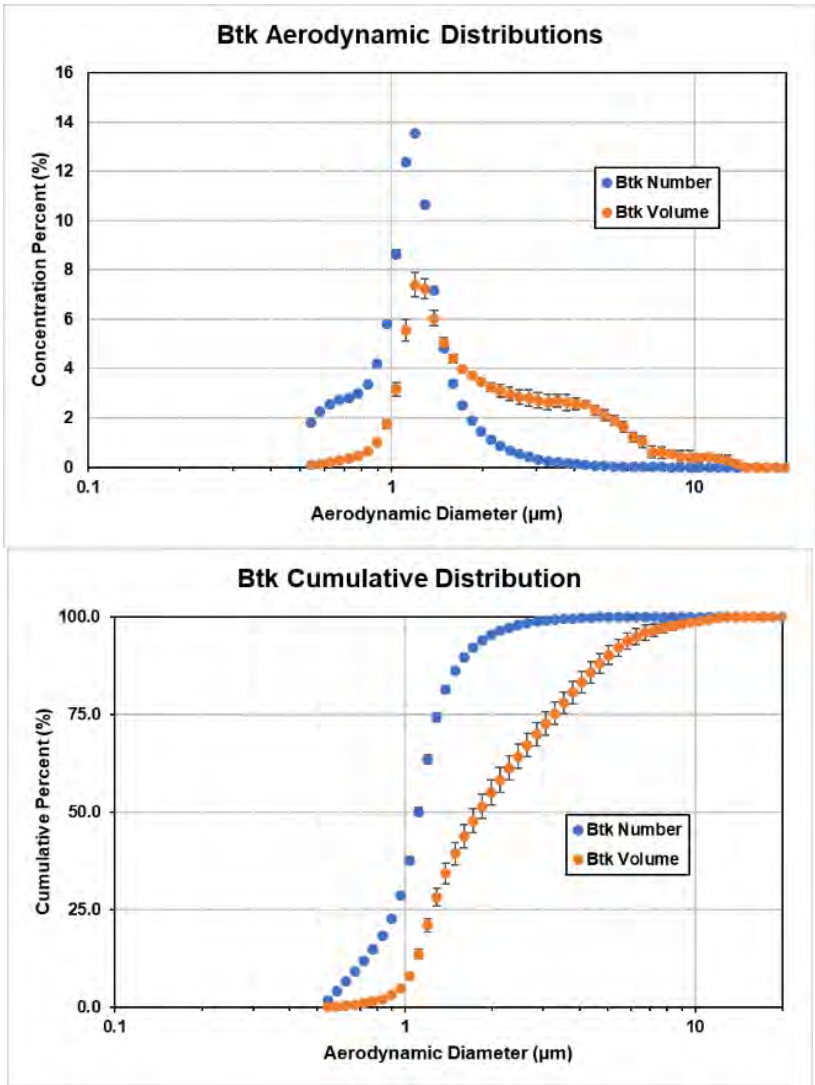


Figure 6-4: *Btk* Number/Volume Relative and Cumulative Distributions

Qualitative SEM analysis was conducted for the *Btk* sample collected on a polycarbonate filter. However, the presence of pores on the filter surface and additional non-spore material (crystalline proteins, etc.) that was on the filter interfered with the computer-controlled sizing. Therefore, quantitative CCSEM was not conducted. This situation was not an issue for the DNATrax as it was captured on a smooth silicon SEM stub, and the material is pure. Figure 6-5 shows representative images of the distribution of *Btk* particles delivered through the dry deposition eductor system. As this figure shows, agglomerates do exist, but the bulk of the deposited particles is dominated by singlets and doublets. Manual analysis from ImageJ (National Institute of Health, Bethesda, MD) shows that 85% of the particles are identifiable as *Btk* singlets/doublets with 15% being agglomerates of higher order, but with no agglomerates present above 10 µm in size. The APS measurements and SEM analysis show that the sizes of the singlets and doublets of DNATrax are more broadly dispersed than the sizes of the singlets and doublets of *Btk.*

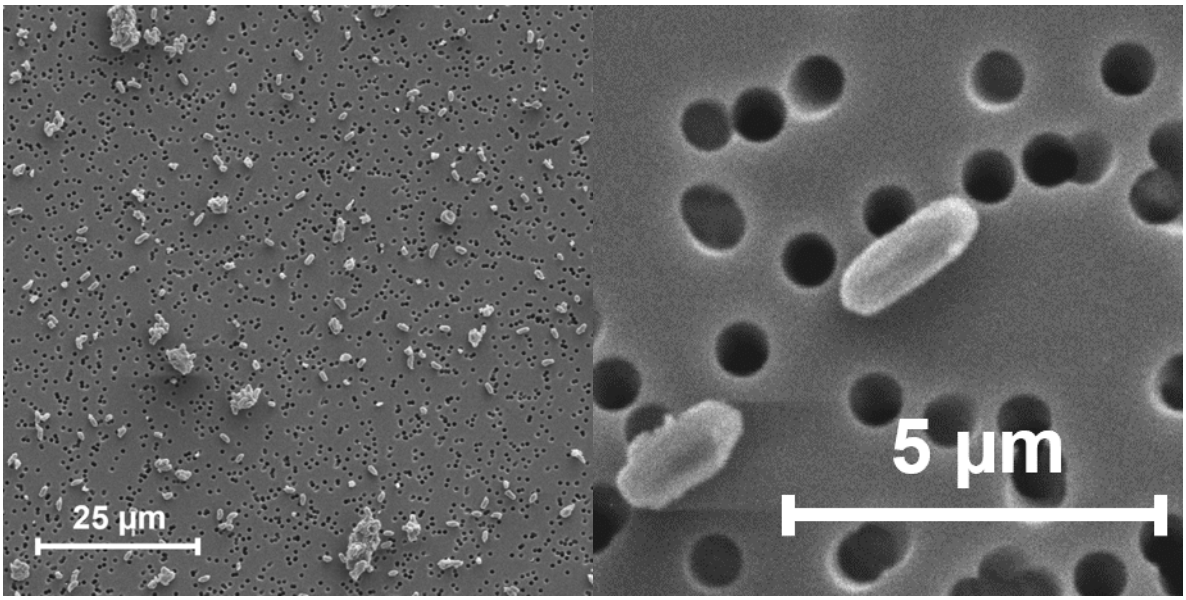


Figure 6-5. Overview Scan of *Btk* deposition and Single Spore

6.1.2 Deposition Spatial Distribution and Load Variation

The results from the foil-based deposition spatial distribution and repeatability tests are presented below. Figure 6-6 shows representative heat maps of the distribution of material on coupon surfaces for: A) DNATrax and B) *Btk.* The material is delivered relatively evenly across the surface measured by the nine-foil array. However, a slight diagonal skew was occasionally detected due to the orientation of the mixing fans in the deposition stack. In general, this slight diagonal skew is not an issue for resuspension tests as the air knife in the RWT sweeps the entire coupon surface. Table 6-1 shows the variation in material sampled on the nine-foil array for each deposition as well as the variation in number of particles per milligram delivered by the dry powder system. The variation in the nine foils represented by the coefficient of variation (CV) was 20% for both DNATrax and *Btk* with the center foil (E) being a good representation of the average foil/surface coverage. The number of particles delivered per mg for DNATrax was, on average, 2×10^7 with 58% variation from run to run. The 58%variation is attributed to the DNATrax having a tendancy to agglomerate in storage and clog the eductor system. However, as discussed in Section 6.1.1, the material that exited the eductor was generally free of large agglomerates. The *Btk* system delivered particles to the surface at a rate of 1.35×10^6 CFU/ mg with a variation of 35%. Thus, it requires roughly twice as much *Btk* to attain the same surface coverage as DNATrax. However, the deposition of *Btk* is highly repeatable due to the lack of large particles clogging the eductor system. These results paired with the size distribution data show that the dry powder delivery system delivers relatively repeatable size and spatial distributions of material to the coupon surface and that a single foil in the center of the coupon during deposition is a good reference for the total amount of material deposited. The number of particles measured from a foil placed in the center of test coupons during inoculation was

therefore used to derive the total number of particles for the denominator (i.e., amount deposited) of resuspension fraction calculations.

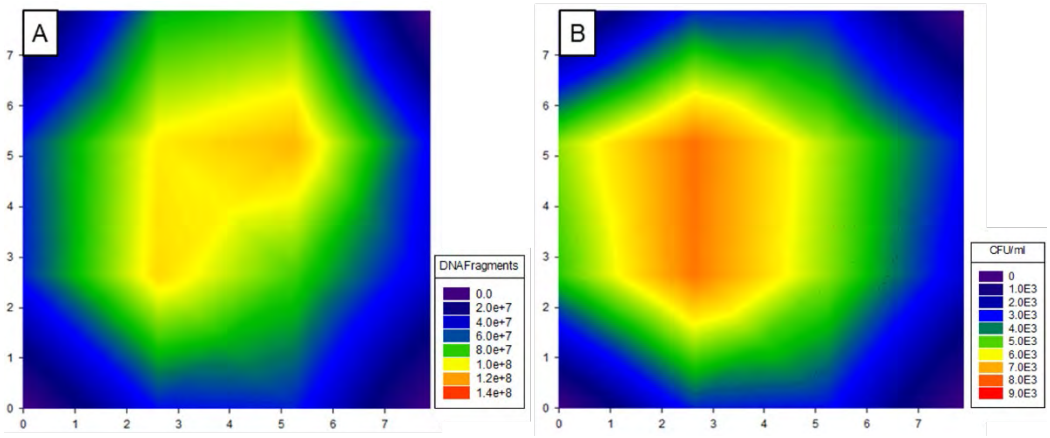


Figure 6-6. Coupon Spatial Distribution Heat Maps: A) DNATrax, B) *Btk*

Table 6-1. Deposition Variation and Particle Counts per mg of Particles Deposited

	DNATrax		*Btk*	
	Particles per mg	**9 Foil Coefficient of Variation (CV)**	**CFU per mg**	**9 Foil Coefficient of Variation (CV)**
Depo 1	1.02×10^7	19.2%	2.19×10^6	22.7%
Depo 2	1.02×10^7	20.2%	1.21×10^6	24.4%
Depo 3	1.31×10^7	17.8%	1.10×10^6	20.6%
Depo 4	3.47×10^7	10.4%	1.08×10^6	27.4%
Depo 5	3.33×10^7	32.9%	1.18×10^6	15.2%
Average	2.09×10^7	20.1%	1.35×10^6	22.1%
Standard Deviation	1.20×10^7	8.1%	4.70×10^5	4.6%
Overall CV	58%		35%	

6.2 Resuspension Testing

The results for the DNATrax and *Btk* resuspended particle size distributions and calculated resuspension fractions for the surfaces under varying environmental conditions are presented in this section.

6.2.1 Resuspended Particle Size Distribution

The particle size distributions from the resuspension of surrogate materials from stainless steel coupons at 30% RH are shown in Figures 6-7 and 6-8. Time constraints did not permit resuspended particle size analysis at 80% humidity. Each set of deposition and resuspension

data is the average of five depositions and resuspensions. Figure 6-7 shows the relative counts of the resuspended particles compared to the deposited size distribution. Both surrogates show a distinct peak shift towards larger particles because the deposited *Btk* size distribution is dominated by singlets of narrow size distribution whereas DNATrax has a broader size distribution for singlet particles. The shift towards a larger size distribution is to be expected since a larger diameter particle experiences a larger shear stress from wind due to its larger cross section compared to a smaller particle (Hinds 1999). Figure 6-7 also shows that the relative size distributions of resuspended DNATrax and *Btk* are similar, likely because although their deposited mean particle diameter is different, the cumulative percentage of particles deposited above approximately 1.2 µm is similar, and that similarity appears to contribute considerably to the resuspension distribution, as shown by the cumulative deposition distributions presented in Figure 6-8 where the >80% cumulative contributions are similar. In addition, Figure 6-8 reinforces the conclusion that the resuspended distributions are similar, as the mean diameters are separated by only 300 nanometers, 1.5 µm for DNATrax and 1.8 µm for *Btk*, respectively. The large error bars on the resuspension size distributions are due to relatively low particle counts sampled by the APS. And these large error bars are the main complication with resuspension size distribution sampling of sub-monolayer (i.e., single layer of particles with no particle-particle interactions) deposited microparticles.

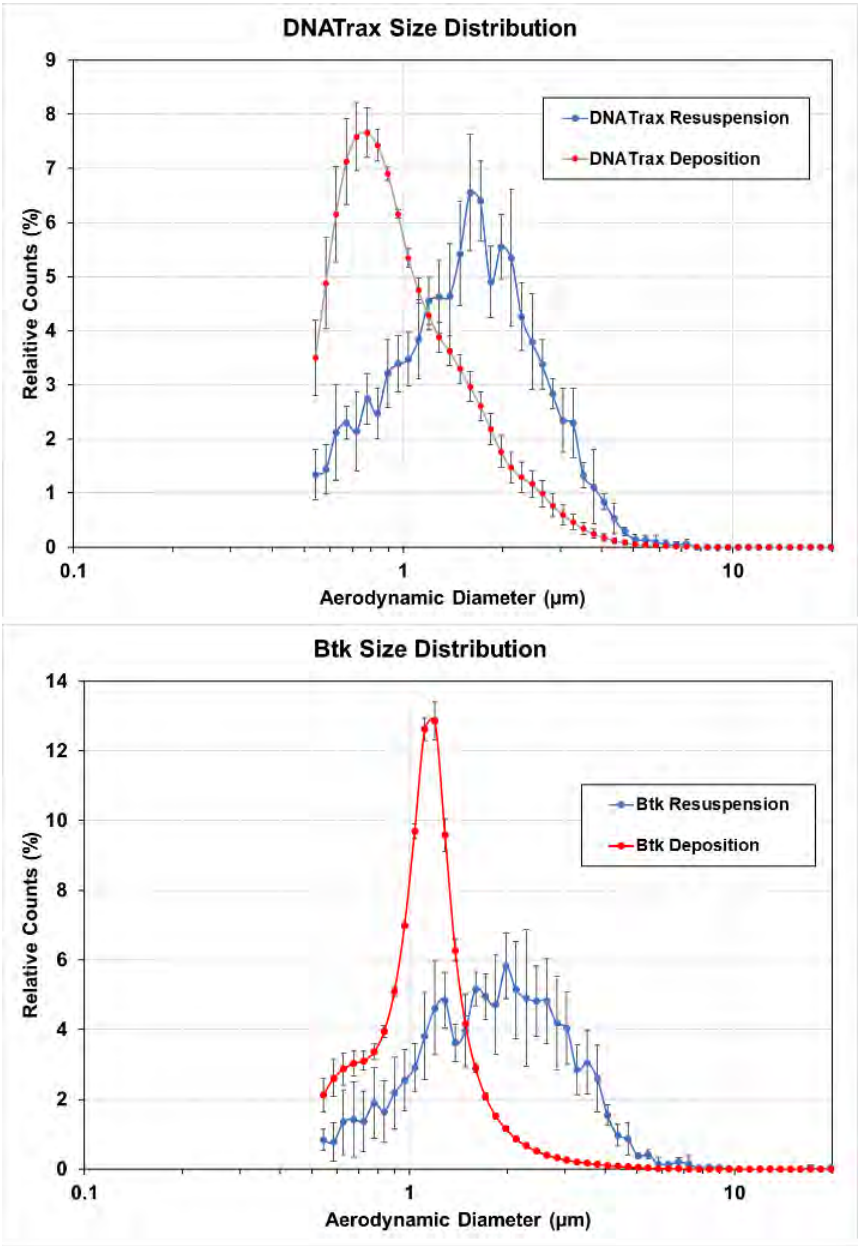


Figure 6-7. Size Distribution - Resuspension versus Deposition: (Top) DNATrax (Bottom) *Btk*

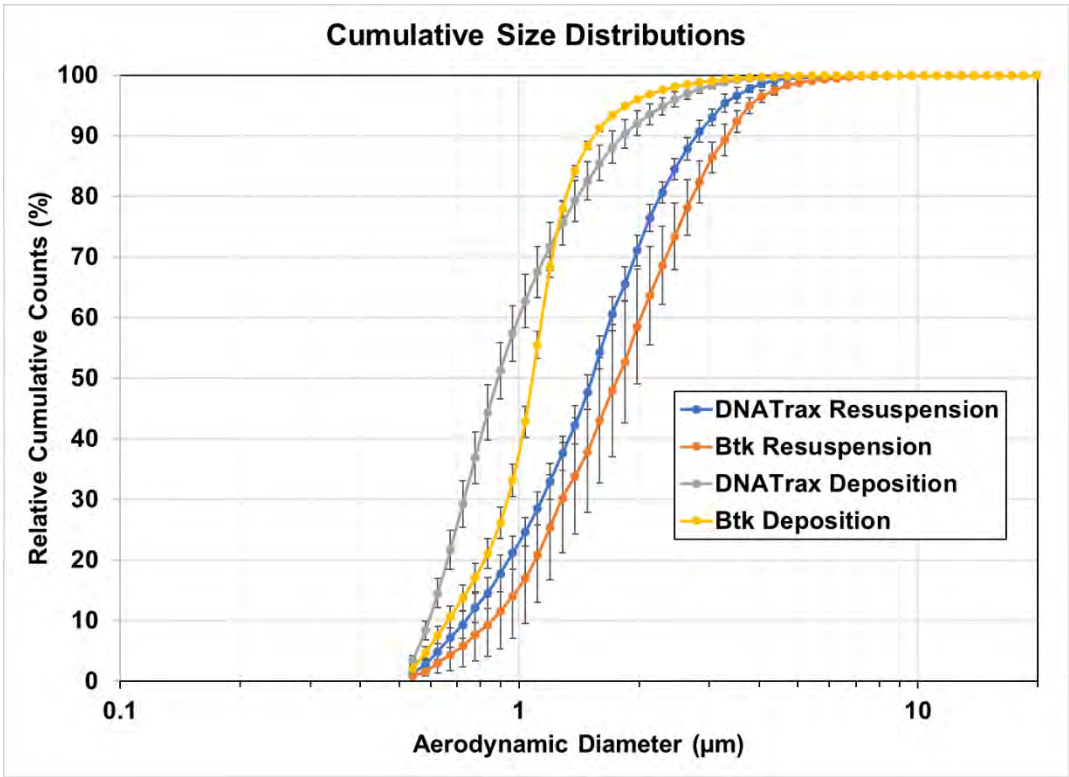


Figure 6-8. Cumulative Size Distributions for Resuspension versus Deposition

6.2.2 Resuspension Fraction Comparison between Surrogates

After size distributions of the DNATrax deposition and resuspension were measured, the results of qPCR analysis from the resuspension filters and deposition foils could be properly interpreted and particle counts derived. The calculations for resuspension fraction (RF) then followed from the equation shown in Section 2.0. *Btk* culture counting results required no such correction due to changes in size distribution as the assumption was made that one CFU counted was equivalent to one spore, and the *Btk* spores are all essentially the same size.

The test average results from the five tests used to calculate resuspension fractions from stainless steel nonporous surfaces are presented for both DNATrax and *Btk* in Figure 6-9. DNATrax had higher variability than *Btk* with CVs between 69 and 117% compared to 30-65% for *Btk*. Qualitatively, it is evident that the two surrogates had similar resuspension fractions for the 30% and 80% RH resuspension tests. However, the similarity diverged for the diurnal cycle RH condition. Statistical analysis of the data can be done on a per day basis to compare the resuspension fractions of paired surrogate resuspension (i.e., *Btk* versus DNATrax at 30%). However, an overall specific condition-to-condition comparison is difficult as the variation from day to day within the same environmental conditions could not be fully assessed. Therefore, no statistical analysis was conducted to compare the absolute change in resuspension fractions due to changes in environmental conditions (i.e., DNATrax at 30% versus DNATrax at 80%). All data sets were tested for normality prior to comparative statistical analysis, and all data sets met

the normality standard of the Shapiro-Wilk test (p-value > 0.05). For the 30% and 80% resuspension fractions from stainless steel, no statistically significant difference ($p < 0.05$) in resuspension could be detected via a two-sample Welch's T-test at the 95th percentile (p value = 0.29 for 30% RH and 0.28 for 80% RH). However, for the diurnal cycle, a statistically significant difference was observed (p-value = 0.026) with the *Btk* RF on stainless steel being an order of magnitude higher than the RF of DNATrax.

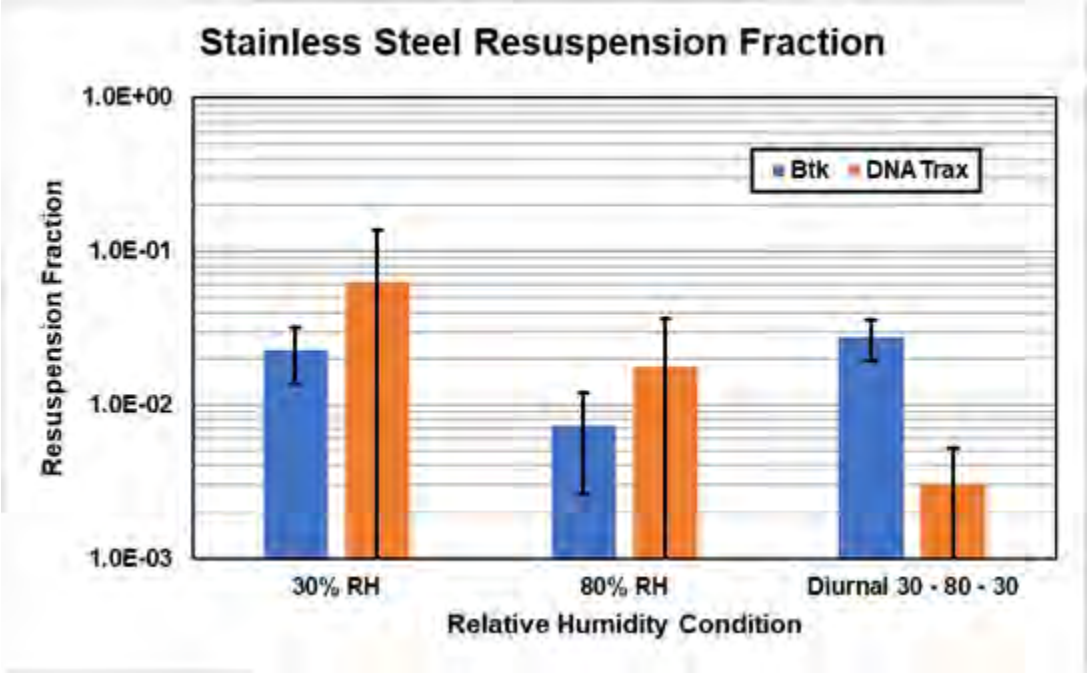


Figure 6-9. Stainless Steel Resuspension Fraction

Figure 6-10 shows the five-coupon average resuspension fractions of the two surrogate materials on the porous concrete surfaces under varying conditions. As with the stainless steel experiments, an absolute overall comparison between RFs of the same surrogate under different conditions is dubious. However, a surrogate-to-surrogate comparison under the same environmental conditions is entirely possible. The Welch's T-test once again showed no statistically significant difference between the two surrogates at 30% and 80% RH at the 95th percentile (p = 0.26 at 30% and 0.12 at 80%). The diurnal cycle, however, showed a statistically significant difference (p-value of 0.0031) between RFs of DNATrax and *Btk.* However, in this instance (concrete), the *Btk* RF was an order of magnitude lower than the RF of DNATrax.

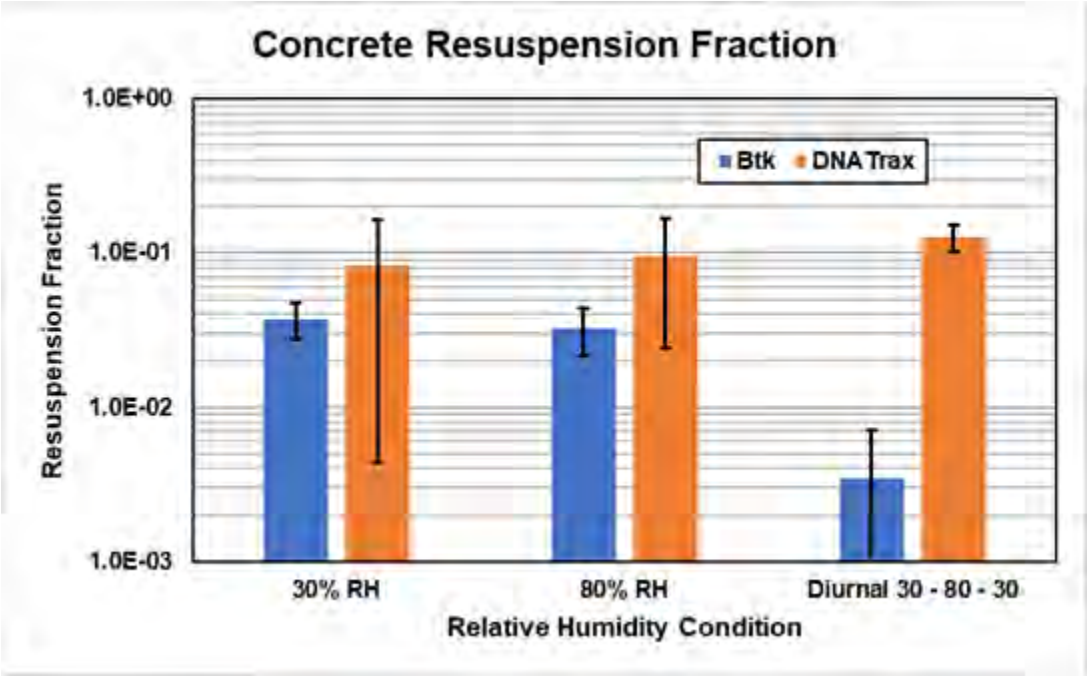


Figure 6-10. Concrete Resuspension Fraction

Table 6-2 shows the average resuspension fractions and the variation of all environmental conditions, surrogates, and surfaces. Tables of the RF for each coupon measured can be found in Appendix A. The magnitude of the difference in each surrogate resuspension fraction per condition should be viewed as large for all tests. However, those differences for the 30% and 80% RH conditions could be due to outliers and potential variation in resuspended particle sizes. The differences in the diurnal cycle are considered to be outside the variation due to shifts in the particle size distribution.

Table 6-2. Average Surrogate Resuspension Fractions under Varied RH Values [Mean (SD)]

Condition	DNATrax	Btk
SS 30% RH	6.32 (±7.42) %	2.26 (±0.91) %
SS 80% RH	1.79 (±1.88) %	0.73 (±0.47) %
SS 30 → 80 → 30 (diurnal cycle)	0.31 (±0.21) %	2.77 (±0.84) %
Concrete 30% RH	8.36 (±7.92) %	3.75 (±1.00) %
Concrete 80% RH	9.49 (±7.10) %	3.25 (±1.10) %
Concrete 30 → 80 → 30 (diurnal cycle)	12.76 (±2.5) %	0.35 (±0.36) %

To understand the separation in RFs for the SS diurnal cycle, Figure 6-11 shows the temperature and RH conditions over the course of the cycle. As the figure shows, the relative humidity for the SS experiments extended past 80% for approximately one hour before returning to the 80% set point. This extension may have resulted in softening and an increase in the adhesive properties of the DNATrax due to a glass transition compared to the *Btk,* which would not soften considerably (Nurhadi 2016). Nurhadi et al. established glass transition temperatures and humidities for a variety of dextrose equivalent maltodextrin particles. They showed that particles with DEs similar to DNATrax have a glass transition at approximately 20 °C and 80% RH. This process would be irreversible, and the DNATrax would thus resuspend less than *Btk*. This is the only condition under which *Btk* resuspended at a higher fraction than DNATrax, which would point to a change in the surface properties of the DNATrax or a capillary condensation not experienced by *Btk*.

The diurnal humidity cycle for concrete, however, did not extend above 80% and remained just at or below the glass transition for DNATrax (Nurhadi 2016). However, the *Btk* RF (0.35%) dropped significantly below the RF of DNATrax (12.8%) due to the diurnal cycle in this case. It is possible that the increased surface roughness of the *Btk* with the concrete causes a more complex interaction than that of DNATrax and that surface dynamics of the 30% RH-equilibrated concrete with the 80% external humidity during the cycle affect the *Btk* differently than the DNATrax.

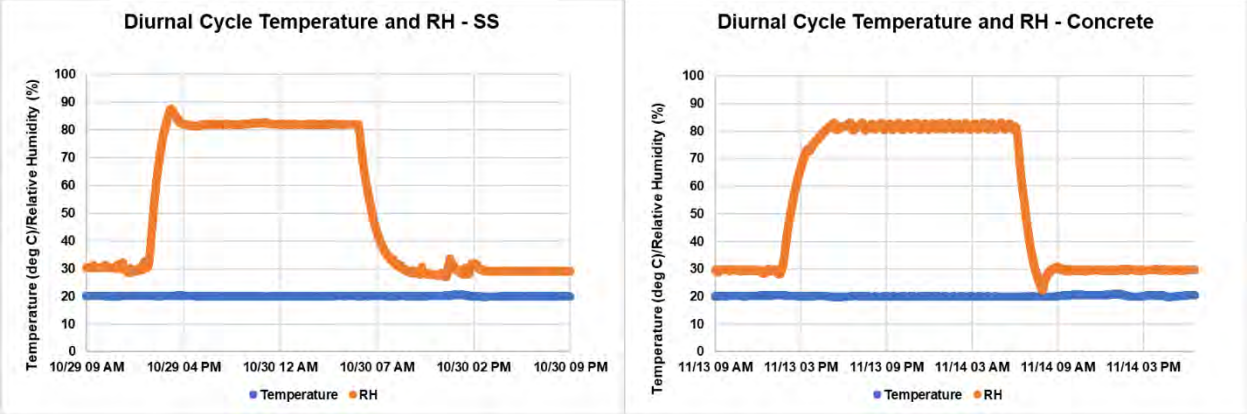


Figure 6-11. Diurnal Cycle Temperature and RH for (Left) SS and (Right) Concrete

Finally, using the total resuspension fraction for DNATrax and Btk at 30% RH on stainless steel, the deposition distribution, and the resuspension distribution, we can examine the resuspension fraction for each APS size bin, shown in Figure 6-12. Though this graph contains significant variation in particles above two microns, the DNATrax particles in the size range of the singlet *Btk* particles (1.19 µm) appear to resuspend more readily than the particles of *Btk*. The variation in the larger-sized particles occurs because there were very few particles in that size range counted by the APS. The error bars presented here do not reflect the total RF standard deviation presented in Table 6-2, but only the RF standard deviation of the APS measurements. Time constraints did not allow for additional size bin characterization for other environmental conditions. Additional research needs to be conducted to evaluate particle size differences in RF under variable RH conditions.

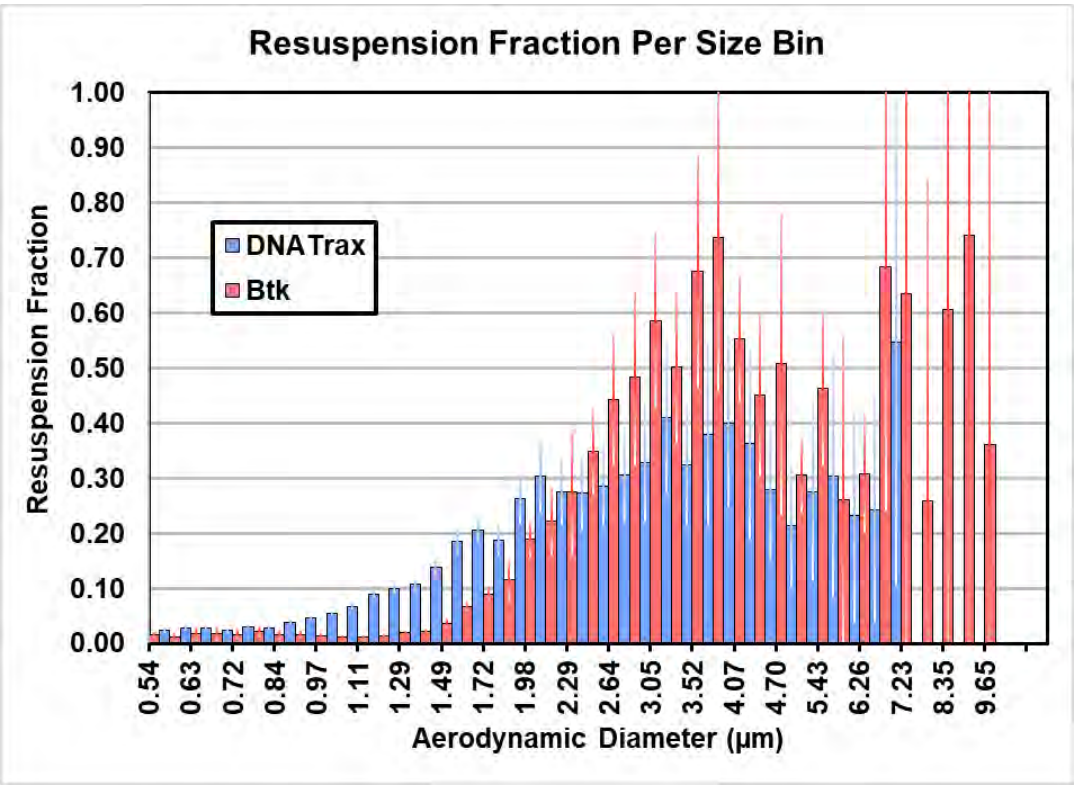


Figure 6-12. Resuspension Fraction Per Size Bin SS 30% RH

6.3 Summary and Conclusions

The results of these resuspension fraction comparison experiments provided information under what conditions an inert sugar-based particle is a suitable surrogate for a biological agent when comparing resuspension from two common subway surfaces. The dry deposition eductor system developed by EPA to mimic the system used in previous subway disseminations was shown to deliver consistent size distributions of the chosen surrogate particles with depositions consisting of mostly singlets and doublets. The surface loading and spatial distribution were considered sufficient for an accurate comparison of the resuspension fractions of each surrogate material from various surfaces and conditions. The surface morphology of the DNATrax was determined by SEM to be “dimpled” as opposed to perfectly spherical, suggesting partially collapsed hollow spheres that resulted from loss of water under different humidity conditions. The size distributions of the two resuspended surrogate materials at 30% RH from stainless steel were similar.

The size distributions of the resuspended materials were measured for 30% RH on stainless steel and were shown to be similar. In addition, no statistically significant difference was evident in resuspension fractions of DNATrax versus *Btk* for the 30% and 80% RH conditions for either surface. Analysis of the size bins from the 30% RH stainless steel experiments does show some differences between *Btk* and DNAtrax resuspension in the *Btk* singlet size region. However,

even with this variation, the overall RF values between the two surrogates were statistically similar. When subjected to a diurnal RH cycle, DNATrax and *Btk* resuspension fractions differed significantly with DNATrax being lower than *Btk* on stainless steel but higher than *Btk* on concrete. There is evidence that the RH cycle for stainless steel rose above 80% to 85% RH at 20 °C, which is hypothesized to have irreversibly changed the surface structure of the DNATrax. This irreversible change did not occur for the concrete surface. However, the resulting *Btk* resuspension fraction could potentially be attributed to the changing surface dynamics and the porosity of the concrete.

It is therefore evident that DNATrax under environmental conditions below 80% relative humidity can be viewed as not statistically different from *Btk,* and subsequently *Ba,* when considering resuspension from representative conditions and these two surfaces present in subway systems. However, there was considerable variation in resuspension of DNATrax among replicate experiments even when depositions of the material were of low variability, possibly indicative of the difference in surface properties, size, or shape of DNATrax as compared to *Btk*, which is more uniform. Additional experiments with higher numbers of replicates and analysis of inter-day variation are indicated.

We also recommend further evaluation of DNATrax and *Btk* resuspension under rain conditions, with wet porous surfaces, and RH cycles be explored to give a better understanding of the extrema of resuspension conditions and the role a rain event would play in the use of DNATrax as a *Ba* surrogate. Finally, “dirty” surface coupons should be considered to more completely assess the role of surface conditions on DNATrax resuspension that may exist in the environment.

7.0 Quality Assurance (QA) and Quality Control (QC)

To maintain quality assurance/quality control (QA/QC), this project was conducted under the approved category B/Applied Research Quality Assurance Project Plan (QAPP), Comparison of DNATrax and *Bacillus anthracis* Surrogate Resuspension from Subway Surfaces QAPP-3J18-001.0.

7.1 Equipment Calibration

Before beginning experiments, all of the following monitoring equipment was checked against either a primary or a secondary standard to ensure that the monitoring equipment was operating within acceptance criteria:

- A Rotronic (Hauppauge, NY, USA) model HT205 probe was used to monitor and control temperature and RH inside the B155A test chamber.
- A Model DA 410 vane anemometer (Pacer Instruments, Keene, NH, USA) was used to measure the reaerosolization wind tunnel velocity. The anemometer was returned to the factory for calibration.
- A National Institute of Standards and Technology-traceable temperature probe was used to record temperatures in the incubators.
- A Humicap HMT330 (Vaisala Inc., Louisville, CO, USA) provided temperature and humidity measurements inside the AWT.
- Class A volumetric glassware was used where possible.
- Ohaus GA200D (Ohaus Corporation, Parsippany, NJ) or Sartorius ME 5-F (Sartorius AG, Goettingen, Germany) microbalances were used to measure weight deposition material.

All equipment used for critical measurements in the project was maintained and verified as being certified, calibrated, or having calibration validated by the EPA Metrology Laboratory once per year, or immediately following an event that could cause damage (e.g., power surge) or an equipment modification according to operation manual specifications and/or previous investigations.

7.2 QA/QC Checks

The QA/QC checks were performed following the guidelines set forth in EPA 815-B-04-001 and EPA 841-B-96-003. (EPA 2004; EPA 1996) A list of QA/QC checks can be found in Table 7-1. For each deposition and sampling day, one positive control and one negative control were performed. The sample for the positive control was pulled directly from the stock material. A negative control (blank) of the phosphate buffered saline containing 0.1% Triton X-100 (PBST) that was used was analyzed via qPCR. In addition, a wind tunnel blank or background sample was collected. The background test was run and collected onto filters just as the test runs were

but with a sanitized test surface. QA/QC checks and calibrations of all equipment served to establish quality data and fulfill the necessary data quality indicators (DQIs).

Table 7-1. QA/QC Checks and DQIs

QC Sample	Information Provided	Frequency	Acceptance Criteria	Corrective Action
Chamber Temperature/RH	Experiments were performed under the desired conditions	Continuous	Temperature ± 2 °C RH ± 5 %	Stop sampling and correct as necessary.
Procedural blank (sample matrix without aerosol)	Controls for sterility of materials and methods used in the procedure	1 per sample matrix	DNA/CFU below limit of detection	Reject results of samples of the same order of magnitude
Blank analysis of microbiological supplies	Controls for sterility of supplies used in dilution procedure	3 of each supply per event	DNA/CFU below limit of detection	Sterilize or dispose of contamination source Rerun samples
Procedural blank samples	Contamination level present during sampling	1 per sample matrix	DNA/CFU below limit of detection	Clean up environment. Sterilize sampling materials before use.
Aerosol material positive control	Sample contains the DNA copy numbers by weight as expected, no material degradation	1 per deposition	DNA/CFU results fall within 10% of original qPCR calibration curve	Outside qPCR calibration curve, a new curve must be developed and reasons for degradation must be explored.
Field blank (unexposed sample matrix transported with samples)	Contamination due to handling	1 per sample matrix	DNA/CFU below limit of detection	Clean up environment. Identify contamination route.
Check weighing of Laboratory Balance with Check Weights	Ensures that the balance is measuring accurately and precisely	1 check per day of measuring	Check each scale for acceptable balance range for each check weight.	Contact Metrology Laboratory for new calibration and certification of balance.
Air Knife Velocity	Shows pressure gauge and wind delivery system functioning normally	1 per day	Air knife velocity at edge of coupon should be within 5% of expected value.	Vary pressure gauge to achieve proper velocity.

7.3 Data Quality Objectives

The precision and accuracy goals have been established for each measurement parameter based on: (1) scientific requirements needed to achieve the primary objectives, (2) knowledge of the measurement system, (3) in-house experience with the sampling and measurement methods, and (4) other similar research studies. Data quality objectives (DQOs) for each major measurement parameter are listed in Table 7-2.

Table 7-2. DQOs for Critical Measurements

Critical Measurement	Measurement Device	Accuracy/Precision
DNA Copy Numbers	PCR	Replicates within 30%
CFU Triplicate	QCount	CFU triplicate within 30%
Powder Delivery Vessel Weight Change	Laboratory Scale	95% of material delivered into eductor
Chamber Temperature/RH	Laboratory Probe	RH should be within 5% of expected value
Air Knife Velocity	Anemometer	Velocity should be within 5% of expected average value

Substantial effort was expended to ensure that samples and measured parameters were representative of the media and conditions being measured. All data were calculated and reported in units that were consistent with similar measurements from other organizations to allow for comparability of data among organizations. DQOs for precision and accuracy were based on prior knowledge of the measurement system employed and method verification studies, which include the use of replicate samples and duplicate analyses. During data analysis for this project, the DQOs were observed and met in every instance. Definitions of DQOs are given below.

Accuracy: the degree of agreement of measurements (or an average of measurements) with an accepted reference or true value. Accuracy is a measure of the bias or systematic error in a system. Accuracies of each measurement technique were established by measurement of laboratory standards. PCR equipment was calibrated daily to reference standards. Automated colony counting software was checked to laboratory standards for each counting run. All environmental measurement instruments were calibrated prior to experimentation. Variation in colony counting and PCR measurements checked against standards fell well within the precision variation, thus the measurements were deemed accurate and representative.

Precision: a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. Precision is best expressed in terms of the standard deviation. Various measurements of precision exist depending on the prescribed similar condition. The precision goals for this project were replicate PCR measurements within 30% of each other and CFU triplicate counts within 30%. The actual average variation measured for each test was below 30%.

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Appendices

Appendix A: RF Data Summary

This appendix presents the data summary tables for resuspension fraction (RF) of each of the five individual tests per surrogate, environmental condition, and surface. Colored bars represent relative scale for each deposited surface, i.e., full bar equals largest RF.

Table A-1. Stainless Steel RF Compendium

	Stainless Steel Resuspension Fraction 45 mph					
	DNATrax 30 %	DNATrax 80%	DNATrax Diurnal Cycle	BTK 30%	BTK 80%	BTK Diurnal Cycle
Run 1	0.0588	0.0022	0.0017	0.0212	0.0112	0.0197
Run 2	0.0063	0.0039	0.0014	0.0370	0.0098	0.0254
Run 3	0.0536	0.0417	0.0015	0.0245	0.0021	0.0402
Run 4	0.0088	0.0068	0.0057	0.0168	0.0111	0.0215
Run 5	0.1885	0.0348	0.0050	0.0135	0.0024	0.0315
Average	**0.063**	**0.018**	**0.003**	**0.023**	**0.007**	**0.028**
Stdev	**0.074**	**0.019**	**0.002**	**0.009**	**0.005**	**0.008**
CV(%)	**117.4%**	**105.2%**	**68.7%**	**40.0%**	**64.1%**	**30.2%**

Table A-2. Concrete RF Compendium

	Concrete Resuspension Fraction 45 mph					
	DNATrax 30 %	DNATrax 80%	DNATrax Diurnal Cycle	BTK 30%	BTK 80%	BTK Diurnal Cycle
Run 1	0.2128	0.0531	0.1130	0.0405	0.0441	0.0023
Run 2	0.0465	0.0247	0.1061	0.0392	0.0224	0.0068
Run 3	0.0841	0.0727	0.1423	0.0335	0.0431	0.0004
Run 4	0.0740	0.1178	0.1118	0.0236	0.0321	0.0078
Run 5	0.0004	0.2065	0.1645	0.0509	0.0207	0.0002
Average	**0.084**	**0.095**	**0.128**	**0.038**	**0.032**	**0.003**
Stdev	**0.079**	**0.071**	**0.025**	**0.010**	**0.011**	**0.004**
CV(%)	**94.7%**	**74.8%**	**19.6%**	**26.7%**	**34.0%**	**102.7%**

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