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Evaluation of a Future BSL-3 Capability: Aerosol Generation and Collection on Filters

Jana Kesavan
RESEARCH AND TECHNOLOGY DIRECTORATE

Daniel Mcgrady
MAG AEROSPACE
Fairfax, VA 22033-4901

Jerold Bottiger
EXCET, INC.
Springfield, VA 22150-2519

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Director, DEVCOM CBC, ATTN: FCDD-CBR-IT, APG, MD 21010-5424
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This study evaluated aerosol release into the environment as a result of aerosol generation and capture onto a test filter. This was conducted to simulate an operation in the biosafety level 3 (BSL-3) laboratory and determine whether any organisms were released into the environment. Air and surface samples were collected during blank (no aerosols) and hot (with aerosols) runs, and the numbers of organisms per sample were averaged. Statistical tests indicated that there was no statistically significant difference in the environmental contamination for the blank and hot runs for air and surface samples. The following conclusions were reached: (1) The Sono-Tek aerosol generator (Sono-Tek Corporation, Milton, NY) is easy to use, and the BSL-3 group preferred it over the inkjet aerosol generator. (2) Filters directly attached to the aerosol generator prevent aerosol release to the environment and are preferred by the U.S. Army Combat Capabilities Development Command Chemical Biological Center (Aberdeen Proving Ground, MD) safety office and the BSL-3 group. (3) It is important to ensure that the glovebox components are easy to decontaminate. (4) Noncorrosive equipment should be used in the BSC or glovebox so they can be sprayed with decontamination material to decontaminate all surfaces.

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PREFACE

The work described in this report was started in Oct 2019 and completed in May 2021. At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) was known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

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EVALUATION OF A FUTURE BSL-3 CAPABILITY: AEROSOL GENERATION AND COLLECTION ON FILTERS

1. INTRODUCTION

Risk group 3 (RG3) materials such as *Bacillus anthracis* Ames and *Burkholderia pseudomallei* are dangerous to public health because of their innate disease threat. The greatest danger from these materials comes from aerosolization because large amounts of these particles can be released accidently and thereby provide a large inhaled dose. Studies have been conducted to investigate the properties, decay, and means of decontamination for such biological organisms. Studying these materials requires aerosolization of the organisms, which increases the safety risk by exposing the scientist to inhalable aerosols. To contend with this, such studies typically incorporate the use of simulant materials to reduce the safety risk. However, aerosolization studies of RG3 materials are needed to determine whether the properties and decay characteristics of simulant materials are similar to those of the agents, so we can identify whether and which simulant materials are appropriate proxies.

Aerosol testing of RG3 organisms requires bioaerosol containment and complete capture of generated aerosols onto filters. It may also require secondary containment, such as plexiglass boxes. However, little is known regarding the success of such strategies in the biosafety level 3 (BSL-3) laboratories, and a thorough risk assessment for aerosolizing BSL-3 materials is needed. Little work has focused on how the method of particle aerosolization affects the sterility of the biosafety cabinet (BSC) and whether contamination occurs. This research would provide insight into the contamination risk associated with aerosol studies inside gloveboxes and which means of aerosolization would reduce operator risk.

In this study, the biocontainment ability of the experimental setup used with RG1 and RG2 materials was modified and evaluated for use in the BSL-3. Discussions were conducted with the BSL-3 laboratory test group and safety office of the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) to identify what is allowed in the BSL-3 laboratories to prevent organism release to the environment. Currently, aerosol testing is not conducted in the BSL-3 laboratories due to the risk of contamination; therefore, the new test setup and the validated methodology will allow experiments to be conducted with RG3 materials in the BSL-3 laboratory. In this test, a procedure for depositing aerosolized spores onto membrane filters for decontamination studies was selected for evaluation. Bacteria spores were used because vegetative cells are killed during aerosol generation and sampling (as a result of stresses and drying) and are therefore not detected by culturing.

2. METHODOLOGY

The spore-forming bacteria *Bacillus subtilis* var. *niger* (BG) was used as the test organism. These spores were aerosolized and collected onto test filters for evaluation of test methodology. A Sono-Tek aerosol generator and an inkjet aerosol generator (IJAG) were used to generate aerosols for this test. Tests were conducted in a glovebox to capture all of the aerosol that was released outside of the aerosol generator and the corresponding filter collection system. Air and surfaces were sampled to quantify the organism release. Descriptions of both aerosol generators, the glovebox, the surface sampling methodology, and the test methodology are provided herein.

2.1 Sono-Tek Aerosol Generator

Operating at a frequency of 120 kHz, the Sono-Tek aerosol generator (Sono-Tek Corporation; Milton, NY) is designed to generate high concentrations of narrowly dispersed particles. The Sono-Tek nozzle and the controller are shown in Figure 1. The Sono-Tek nozzle is connected to the controller and a syringe pump. Liquid is pushed through the Sono-Tek nozzle, and the controller is adjusted to produce a stable spray.

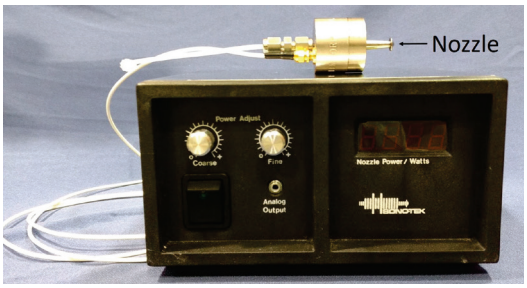

Figure 1. The Sono-Tek aerosol generator.
Spray nozzle is on top of the controller.

To deliver a dry aerosol to the filter, the Sono-Tek aerosol generator nozzle was attached to a manifold that was attached to a heated tube and an insulated tube, as shown in Figure 2. Air was pushed into the heated tube; there, the air was heated, and the heated air carried the Sono-Tek generated particles through the insulated tube. The generated test particles were dry when they exited the insulated tube and ranged in size from 1 to 4 µm aerodynamic diameter.

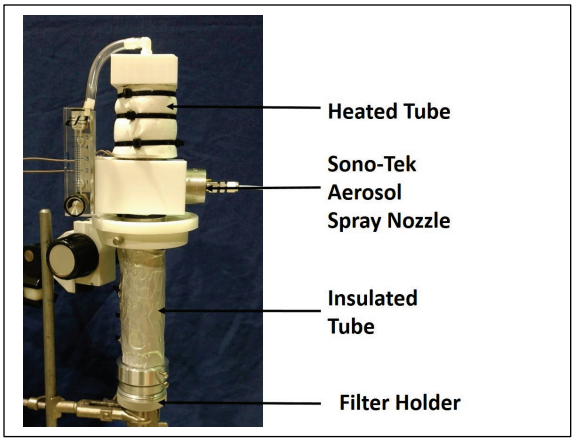

Figure 2. Sono-Tek nozzle in a manifold connected to a heated tube and an insulated tube. A filter holder is attached to the insulated tube.

2.2 IJAG

The IJAG was developed to produce a known number and a low concentration of monodispersed particles to test clean rooms and low-bioaerosol-concentration environments. The concept of using an inkjet cartridge for near-monodisperse aerosol generation was first developed at the DEVCOM CBC to enable testing of bioaerosol detection instruments in aerosol static chambers, flow-through chambers, and clean rooms. The inkjet approach was of particular interest because the nozzles are on the order of 50 µm in diameter, which accommodates transmission of liquid suspensions of bacterial spores, where the spores are typically on the order of 1 µm in diameter. An illustration of the IJAG with the aerosol output delivered to the aerodynamic particle sizer (APS; TSI, Inc.; Shoreview, MN) for particle size measurement is shown in Figure 3.

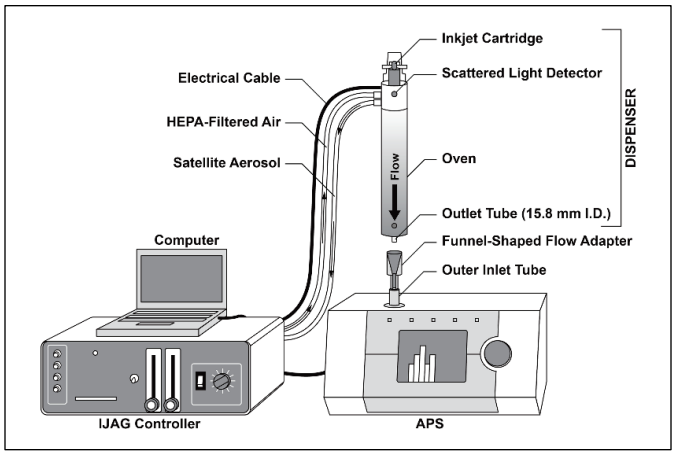

Figure 3. The IJAG connected to an APS.

The IJAG dispenser contains a liquid suspension of bacteria. Computer-generated pulses generate a push that sends a set volume of liquid through the orifice to create the primary particles. Satellite particles are also produced during this process. High-efficiency particulate air

(HEPA)-filtered airstreams aerodynamically separate and remove undesired satellite particles. The primary particles are counted by a light-scattering system that is located just below the aerosol generation area. The aerosol generation rate is adjustable and can range from 1 to 500 particles per second. Various sizes of monodisperse particles are generated by adjusting the concentration of spores in water. Generated primary particles travel through an oven (the heated tube), which evaporates the liquid and results in the final, dried primary particles. The IJAG has been used in many studies, especially to calibrate the APS with 0.95 to 13.3 µm solid and liquid particles. The aerosol output from the IJAG can be calculated directly from the particle count rate provided by the light-scattering system and the rate of airflow exiting the IJAG. Monodispersed spore clusters (1.96 and 2.4 µm) were generated using an IJAG at a rate of 100 particles per second for this study.

2.3 Glovebox

Aerosol generation and capture of the generated particles onto a filter was recreated in the glovebox, which was placed in a 64 m^3 BSL-1+ chamber. The glovebox dimensions are provided in Figure 4. There are doors on both sides of the glovebox to access the glovebox interior. The aerosol generators were placed inside the glovebox one at a time, and the generated particles were collected onto a test filter that was directly connected to the aerosol generator or placed just below the aerosol generator output. Two open-face filter holders were connected to the back wall of the glovebox to collect particles released into the environment.

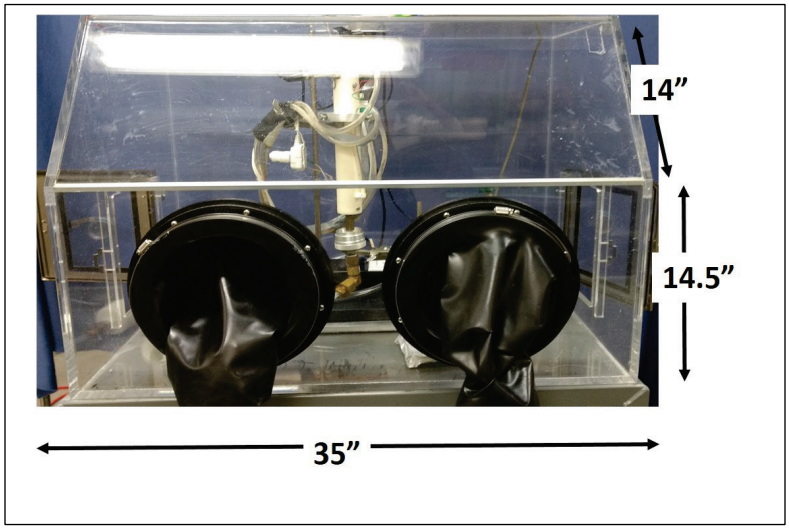

Figure 4. The glovebox has a wider base and a narrow top. The base of the box is a 35 × 24 in. rectangle, and the top is a 35 × 16.5 in. rectangle. The circular openings for attaching gloves are 10 in. in diameter.

2.4 Wipe Areas and Wipe Methods

Areas of the glovebox were wiped after each experiment to determine how many organisms had collected on these areas. Wipe areas 1, 2, and 3 (marked in Figure 5) were approximately 260, 260, and 170 $in.^2$, respectively. A surface-sampling method recommended by

National Institute for Occupational Safety and Health for collecting samples from a nonporous surface was used. A non-cotton swab (Covidien; Mansfield, MA) was cut to provide two layers of 2 × 2 in. squares to use as wipes. Swabs were first moistened with phosphate-buffered saline containing Tween 20 detergent. Swabs were then used to wipe the surface in an "S" shape in a left-to-right direction. The exposed side of the swab was folded in, and the surface was again wiped, also in an S shape, in a front-to-back direction.

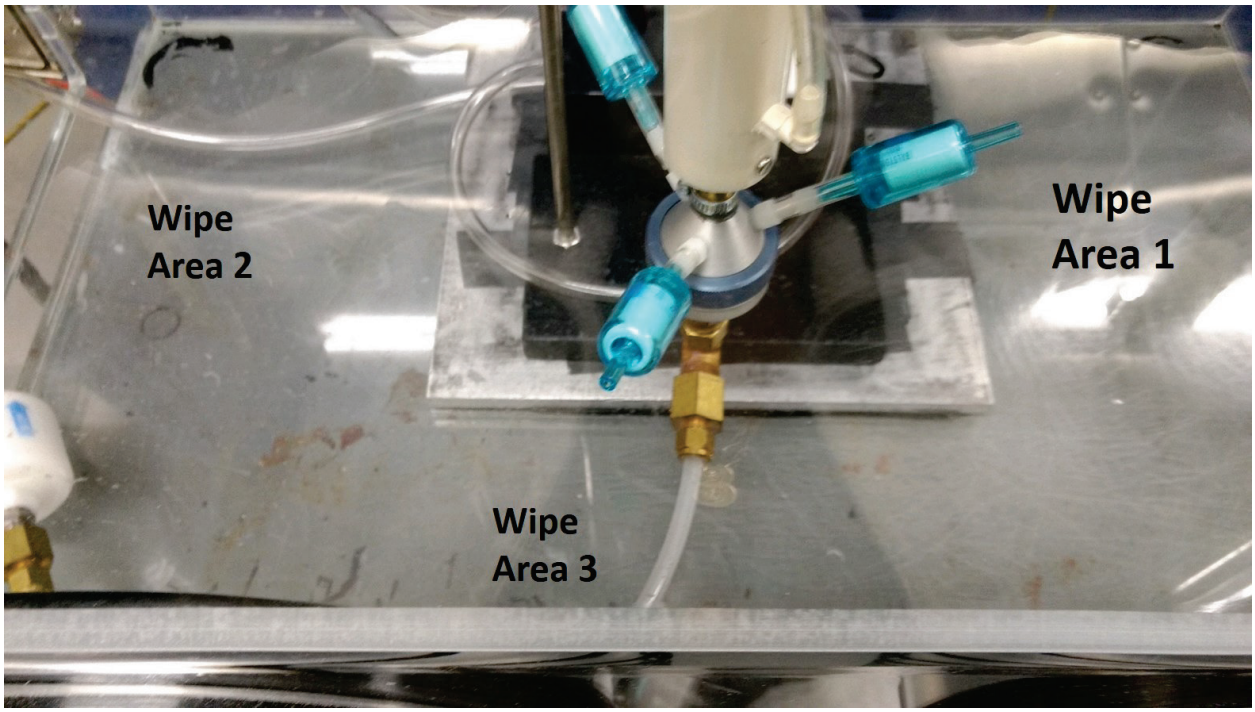

Figure 5. Locations of wipe areas 1, 2, and 3.

2.5 Evaluation of Aerosol Release to Outside the Sono-Tek Aerosol Generator with the Direct-Connection Filter

Testing was conducted to quantify the amount of aerosol released to the environment when the particles were generated and deposited onto filters. Based on discussions with the DEVCOM safety office and the BSL-3 laboratory group, only a direct-connect method with the Sono-Tek aerosol generator was used; otherwise, there would be potential for a large aerosol exposure to operators, which is unacceptable in the BSL-3 laboratories. In addition, a direct filter connect method is used for the Sono-Tek aerosol generator setup in the BSL-1 and BSL-2 laboratories; therefore, the same setup (Figure 2) was used in this test.

The glovebox and the its components were decontaminated with a 10% bleach solution before each test. New filters were installed into the filter holders, and the syringe containing solution was placed in the glovebox. The glovebox doors were closed, and the glovebox gloves were used to attach the syringe to the nozzle and place it in the syringe pump. The Sono-Tek aerosol generator was manually primed, with the controller turned off, to prevent

aerosol generation in the glovebox. Then, the aerosol was generated for 4 min, and the test filter sampled the aerosol during the aerosol generation and for an additional 2–6 min. At the end of this particle collection period, the glovebox air was sampled for 10 min by two air filters that were attached to the back wall of the glovebox. At the end of air sampling, surface sampling via wiping was conducted at locations 1 and 2. The stand that held the aerosol generator was at location 3, so that area was not wiped. Filters were removed from the filter holders, and the syringe was removed from the syringe pump. Another surface wipe was conducted to determine whether any contamination occurred during filter change and syringe removal. A blank run was conducted in between hot runs (with aerosols) to confirm that the glovebox was clean. No aerosol was generated during the blank run, but everything else was conducted the same way. Hot runs (with aerosols) and blank runs were each conducted in triplicate for this experiment. Organisms in collected samples were quantified using standard microbiological plating techniques.

2.6 Evaluation of Aerosol Release to Environment with the IJAG

IJAG-generated particles were either deposited onto a filter in an open-face filter holder that was place approximately 1 cm below the IJAG output tube or onto a filter in a closed-face filter holder and directly connected to the IJAG output. An open-face filter holder and a closed-face filter holder that were used in this test are shown in Figure 6. The closed-faced filter holder has openings with connected HEPA filters to allow air to enter but prevent aerosol from escaping. The closed-face filter holder was developed based on discussions with the DEVCOM safety office and the BSL-3 test group.

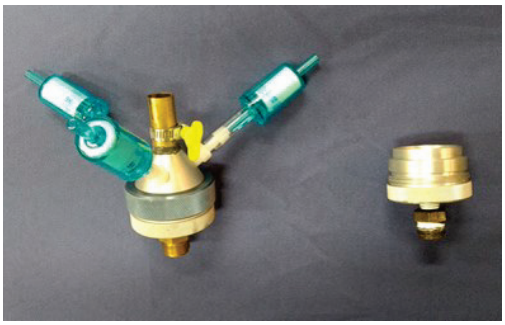

Figure 6. The 47 mm open-face filter holder (right) and closed-face filter holder (left). Three HEPA filters are attached to the closed-face filter holder to provide clean air to the system.

The IJAG was set up in the glovebox with an open-face filter holder (Figure 7) or attached to a closed-face filter holder (Figure 8) to collect the generated particles. The open-face test filter was placed approximately 1 cm away from the IJAG tube, but the closed-face filter was connected directly to the IJAG output tube.

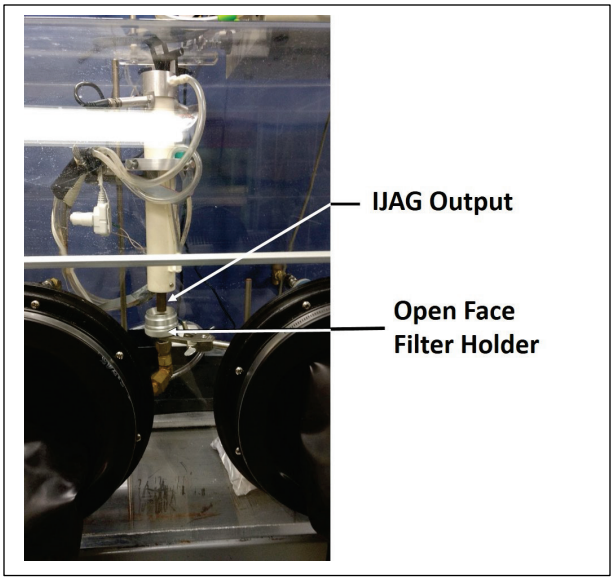

Figure 7. The IJAG output captured by an open-face filter holder.

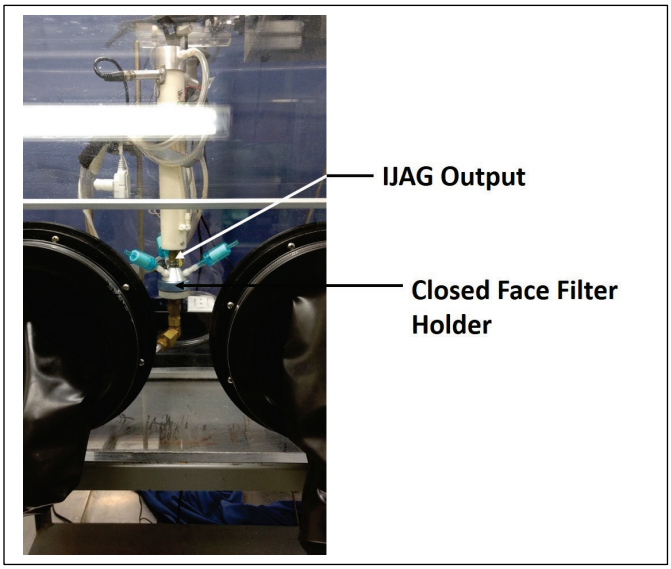

Figure 8. The IJAG output captured by a closed-face, direct-connect filter holder.

The glovebox and the components of the glovebox were decontaminated with a 10% bleach solution before each test. New filters were loaded into the filter holders, and the IJAG cartridge was placed into the glovebox. Following this, the glovebox doors were closed, and the glovebox gloves were used to load the IJAG cartridge into the IJAG. Aerosol was generated for 4 min and captured by the test filter for a total of 6 min. The additional 2 min of collection by the test filter ensured that all aerosol generated by the IJAG was captured by the test filter. The pump connected to the test filter was turned off, and the glovebox air was sampled using the two-filter setup attached to the back of the glovebox for 10 min. Next, wipe sampling of glovebox areas 1, 2, and 3 was conducted to determine whether any contamination of the box had occurred. The test

filter and the glovebox sample filters were then removed from the filter holders, and the IJAG cartridge was removed from the IJAG. A second surface wipe sampling was conducted of glovebox areas 1, 2, and 3 to capture any contamination released by the filter change and the IJAG cartridge removal.

For the direct-connect filter setup, tests were conducted with two monodispersed aerosols with mass median diameters of 1.96 and 2.4 μm. One blank test (with no aerosol generation) was conducted before each hot test (aerosol release test) to ensure that the glovebox air and surfaces were clean. The same procedures were followed for the blank and hot tests, except aerosol was not generated for the blank test, and only one set of surface samples was collected for the blank test. Tests were performed in triplicate for each particle size and each filter holder. Organisms in collected samples were quantified using standard microbiological plating techniques.

3. RESULTS AND DISCUSSION

In this study, aerosol release into the environment as a result of aerosol generation and capture onto a test filter was evaluated. This work was conducted to simulate an operation in the BSL-3 laboratory and determine whether any organisms were released into the environment. Air samples and surface samples were collected during blank (no aerosols) and hot (with aerosols) runs, and the numbers of organisms per sample were averaged. Statistical tests (*t* tests) were conducted to determine whether there were any differences between the blank and hot runs for air samples and surface samples.

The number of biological organisms collected by the air samples and surface samples for the Sono-Tek aerosol generator and direct-connect capture filter (test filter) for the blank and hot runs is provided in Table 1. Full data are provided in Appendix A. Statistical tests indicated that there was no statistically significant difference in the environmental contamination for the blank and hot runs for air and surface samples. Slightly elevated numbers of organisms were observed in the surface samples, with the blank runs showing slightly higher counts as compared with the hot runs.

The average numbers of organisms collected by air and wipe sampling after IJAG aerosol generation and particle collection with an open-face test filter placed 1 cm below the IJAG are provided in Table 2. Full data are provided in Appendix B. Statistical tests indicated that there was no statistically significant difference between the blank and hot runs; however, slightly elevated numbers of organisms were captured by the wipe samples for the hot run (33.33 vs 6.11 colony forming units [cfu] per sample).

Similarly, numbers of organisms collected by air and wipe sampling after IJAG aerosol generation and deposition onto a closed-face filter are provided in Table 3. Full data are provided in Appendix C. Statistical tests indicated that there was no statistically significant difference in environmental contamination for the blank and hot runs. Data from the two different particle sizes were grouped together, as there were no differences for the two particle sizes in organism release to the environment. Wipe results indicated that there were fewer organisms

released to the environment with the direct-connect filter to the IJAG as compared with the open-face filter. Blank test aerosol counts were lower during the IJAG tests as compared with during the Sono-Tek aerosol generator tests because the Sono-Tek aerosol generator was tested first, and the glovebox air and surface become cleaner over time as a result of repeated decontamination.

Table 1. Environmental Contamination during Use of Sono-Tek Aerosol Generator with Direct-Connect Filter for Blank and Hot Runs*

Sample	Blank Run (cfu/sample)	Hot Run (cfu/sample)
Glovebox air (average)	4.00 ± 10.52	72.67 ± 153.69
Wipes (average)	296.97 ± 458.46	105.93 ± 122.14

*Organisms in the air were collected with filters attached to the glovebox, and organisms on surfaces were collected by wipe sampling.

Table 2. Environmental Contamination during Use of IJAG Aerosol Generator with Capture Filter Placed 1 cm below IJAG Output Tube for Blank and Hot Runs*

Sample	Blank Run (cfu/sample)	Hot Run (cfu/sample)
Glovebox air (average)	0.00 ± 0.00	2.22 ± 1.93
Wipes (average)	6.11 ± 6.12	33.33 ± 57.64

*Organisms in the air were collected with filters connected to the glovebox, and organisms on surfaces were collected by wipe sampling.

Table 3. Environmental Contamination during Use of IJAG Aerosol Generator with Direct-Connect Capture Filter for Blank and Hot Runs*

Sample	Blank Run (cfu/sample)	Hot Run (cfu/sample)
Glovebox air (average)	0.56 ± 1.36	0.00 ± 0.00
Wipes (average)	7.77 ± 7.00	8.15 ± 21.34

*Organisms in the air were collected with filters attached to the glovebox, and organisms on surfaces were collected by wipe sampling.

In all tests, for both aerosol generation methods, slightly higher numbers of organisms were detected for wipe samples as compared with air samples. This may be due to the contamination of the gloves attached to the glovebox and the stands and tubing within the glovebox. These gloves were used to load the stock solution into the aerosol generator. Even though these gloves were decontaminated, the texture of the gloves may have prevented complete decontamination. In addition, the tubing and stands may not have been fully decontaminated in between tests because of the nooks and crannies on these components. To prevent contamination in the BSL-3, operations in the BSL-3 BSC will include the frequent changing of gloves. UV-C lights in the BSL-3 BSC will also reduce contamination. The use of noncorrosive equipment in the BSL or glovebox will allow for them to be sprayed with decontamination material to reach all the nooks and crannies during decontamination. In this study, the surfaces were wiped with decontamination solution-soaked paper towels, which may not have decontaminated the smallest and tightest areas.

It is not possible to get our large, 64 m^3 chamber completely free of organisms; a large amount of BG has been used in our chamber over time, and a low level of contamination exists in the chamber. However, every effort was made to decontaminate the chamber and the glovebox as thoroughly as possible.

The glovebox air and surface became cleaner over time. Tests with the Sono-Tek aerosol generator were conducted first and were followed by the IJAG tests. Air samples and wipe samples show decreased background counts over time. In situations when the glovebox was removed and placed back in the 64 m^3 chamber, the background counts increased.

The Sono-Tek aerosol generator is easy to use and provides a larger output than the IJAG. The advantage of the IJAG is that it produces a smaller number of airborne particles (1–500 particles per second), and these particles can be directly delivered to a sampler inlet or a filter; however, the IJAG is complicated to use and requires frequent cleaning (when it clogs). This may be a disadvantage in the BSL-3, but the ability to produce a small amount of aerosols is an advantage in limit of detection testing.

Based on our experience, an aerosol generator that requires a small amount of material is beneficial as this will prevent large spills. Similarly, an aerosol generator that produces a controlled amount of output is beneficial as this will prevent large aerosol release to the environment. In addition, the aerosol generator should be easy to operate with multiple layers of gloves as is done in the BSL-3, and it should function with turnkey operation. Given these criteria, studies should be performed to evaluate other aerosol generators for potential use in the BSL-3 laboratory.

4. CONCLUSION

Based on this test, the following conclusions can be made:

1. The Sono-Tek aerosol generator is easy to use, and the BSL-3 group preferred the use of the Sono-Tek aerosol generator over the IJAG in the BSL-3.
2. Filters directly attached to the aerosol generator prevent aerosol release to the environment and are preferred by the DEVCOM CBC safety office and the BSL-3 group.
3. It is important to have less equipment in the glovebox or the BSC and to make sure that these components are easy to decontaminate.
4. Noncorrosive equipment should be used in the BSC or glovebox, so that they can be sprayed with decontamination material to reach all the nooks and crannies. In this study, the surfaces were wiped with decontamination solution-soaked paper towels, which may not have decontaminated the nooks and crannies.

ACRONYMS AND ABBREVIATIONS

APS	Aerodynamic Particle Sizer
BG	*Bacillus subtilis* var. *niger*
BSC	biosafety cabinet
BSL-3	biosafety level 3
cfu	colony-forming unit
HEPA	high-efficiency particulate air
IJAG	inkjet aerosol generator
RG3	Risk group 3

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APPENDIX A:
SONO-TEK AEROSOL GENERATOR TEST RESULTS

Run	sample type	Location	Plate Counts (CFU)			Average Plate Counts (CFU)	Dilution	Extraction volume, mL	CFU/sample
	Wipe 1	Wipe of the glovebox floor - left side	1	0	1	0.7	1	20	13.33
	Wipe 2	Wipe of the glovebox floor - right side	1	3	1	1.7	1	20	33.33
Blank 1	Test filter	Test filter attached to Sono-Tek	13	15	20	16.0	1	20	320.00
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	1	2	2	1.7	1	20	33.33
	Wipe 1	Wipe of the glovebox floor - left side	1	3	1	1.7	1	20	33.33
	Wipe 2	Wipe of the glovebox floor - right side	2	0	0	0.7	1	20	13.33
Hot 1	Test filter	Test filter attached to Sono-Tek	TNTC	TNTC	TNTC	TNTC	1	20	TNTC
	Glovebox Filter 1	On the left side of the box-10 min of sampling	2	1	2	1.7	1	20	33.33
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	1	1	0	0.7	1	20	13.33
Blank 2	Wipe 1	Wipe of the glovebox floor - left side	0	1	2	1.0	1	20	20.00
	Wipe 2	Wipe of the glovebox floor - right side	0	0	1	0.3	1	20	6.67
	Wipe 1	Wipe of the glovebox floor - left side	0	0	0	0.0	1	20	0.00
	Wipe 2	Wipe of the glovebox floor - right side	0	0	0	0.0	1	20	0.00
	Wipe 3	Wipe of the glovebox floor - left side - after filter change	0	0	0	0.0	1	20	0.00
Hot 2	Wipe 4	Wipe of the glovebox floor - right side - after filter change	0	2	2	1.3	1	20	26.67
	Test filter	Test filter attached to Sono-Tek	TNTC	TNTC	TNTC	TNTC	1	20	TNTC
	Glovebox Filter 1	Wipe of the glovebox floor - left side	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	Wipe of the glovebox floor - right side	0	0	0	0.0	1	20	0.00
Blank 3	Wipe 1	Wipe of the glovebox floor - left side	41	46	41	42.7	1	20	853.33
	Wipe 2	Swipe of the floor, back wall and side wall on the right side of the box	0	0	1	0.3	1	20	6.67
	Wipe 1	Wipe of the glovebox floor - left side	1	1	TNTC	1.0	1	20	20.00
	Wipe 2	Wipe of the glovebox floor - right side	1	0	0	0.3	1	20	6.67
	Wipe 3	Wipe of the glovebox floor - left side - after filter change	3	3	2	2.7	1	20	53.33
Hot 3	Wipe 4	Wipe of the glovebox floor - right side - after filter change	2	0	3	1.7	1	20	33.33
	Test filter	Test filter attached to Sono-Tek	TNTC	TNTC	TNTC	TNTC	1	20	TNTC
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	0	0	0	0.0	1	20	0.00
	Wipe 1	Wipe of the glovebox floor - left side	31	41	47	39.7	1	20	793.33
	Wipe 2	Wipe of the glovebox floor - right side	15	23	17	18.3	1	20	366.67
	Wipe 3	Wipe of the glovebox floor - left side	5	8	10	7.7	1	20	153.33
Blank 4	Wipe 4	Wipe of the glovebox floor - right side	20	17	11	16.0	1	20	320.00
	Test filter	Test filter attached to Sono-Tek	0	0	0	0.0	1	20	0.00
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	1	0	0	0.3	1	20	6.67
	Wipe 1	Wipe of the glovebox floor - left side	5	7	11	7.7	1	20	153.33
	Wipe 2	Wipe of the glovebox floor - right side	4	11	12	9.0	1	20	180.00
	Wipe 3	Wipe of the glovebox floor - left side	1	9	8	6.0	1	20	120.00
Hot 4	Wipe 4	Wipe of the glovebox floor - right side	8	9	12	9.7	1	20	193.33
	Test filter	Test filter attached to Sono-Tek	4	4	8	TNTC	1	20	TNTC
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	0	0	1	0.3	1	20	6.67
	Wipe 1	Wipe of the glovebox floor - left side	7	4	3	4.7	1	20	93.33
	Wipe 2	Wipe of the glovebox floor - right side	4	6	6	5.3	1	20	106.67
	Wipe 3	Wipe of the glovebox floor - left side	0	2	3	1.7	1	20	33.33
Blank 5-A	Wipe 4	Wipe of the glovebox floor - right side	0	1	0	0.3	1	20	6.67
	Test filter	Test filter attached to Sono-Tek	1	1	0	0.7	1	20	13.33
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	0	0	0	0.0	1	20	0.00
	Wipe 1	Wipe of the glovebox floor - left side	86	88	95	89.7	1	20	1793.33
	Wipe 2	Wipe of the glovebox floor - right side	45	47	62	51.3	1	20	1026.67
	Wipe 3	Wipe of the glovebox floor - left side	25	31	39	31.7	1	20	633.33
Blank 5-B	Wipe 4	Wipe of the glovebox floor - right side	14	7	9	10.0	1	20	200.00
	Test filter	Test filter attached to Sono-Tek	0	0	0	0.0	1	20	0.00
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	0	0	0	0.0	1	20	0.00
	Wipe 1	Wipe of the glovebox floor - left side	20	18	21	19.7	1	20	393.33
	Wipe 2	Wipe of the glovebox floor - right side	15	17	24	18.7	1	20	373.33
	Wipe 3	Wipe of the glovebox floor - left side	5	5	11	7.0	1	20	140.00
Hot 5	Wipe 4	Wipe of the glovebox floor - right side	6	9	10	8.3	1	20	166.67
	Test filter	Test filter attached to Sono-Tek	TNTC	TNTC	TNTC	TNTC	1	20	TNTC
	Glovebox Filter 1	On the left side of the box-10 min of sampling	19	26	26	23.7	1	20	473.33
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	7	10	13	10.0	1	20	200.00
	Wipe 1	Wipe of the glovebox floor - left side	3	1	1	1.7	1	20	33.33
	Wipe 2	Wipe of the glovebox floor - right side	0	2	3	1.7	1	20	33.33
	Wipe 3	Wipe of the glovebox floor - left side	0	0	1	0.3	1	20	6.67
Blank 6	Wipe 4	Wipe of the glovebox floor - right side	0	0	0	0.0	1	20	0.00
	Test filter	Test filter attached to Sono-Tek	0	1	0	0.3	1	20	6.67
	Glovebox Filter 1	On the left side of the box-10 min of sampling	0	0	0	0.0	1	20	0.00
	Glovebox Filter 2	On the right side of the box - 10 min of sampling	0	0	0	0.0	1	20	0.00

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APPENDIX B:
INKJET AEROSOL GENERATOR (IJAG) AEROSOL TEST RESULTS:
OPEN-FACE FILTER PLACED 1 cm BELOW IJAG OUTPUT TUBE

Run	Sample Type	Location	Plate Counts (CFU)			Avg (CFU)	Dilution	Extraction Volume (mL)	CFU/sample
Blank #1	Test Filter	Test Filter 1 cm away	0	0	1	0	1	20	6.67
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	1	1	0	1	1	20	13.33
	Wipe 2	Wipe of the glovebox - right side	1	0	0	0	1	20	6.67
	Wipe 3	Wipe of the glovebox - front	1	0	0	0	1	20	6.67
Hot #1	Test Filter	Test Filter 1 cm away	508	TNTC	TNTC	508	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	1	0	1	20	6.67
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	20	17	11	16	1	20	320.00
	Wipe 5	Wipe of the glovebox - right side	0	0	1	0	1	20	6.67
	Wipe 6	Wipe of the glovebox - front	3	5	8	5	1	20	106.67
Blank #2a	Test Filter	Test Filter 1 cm away	0	0	0	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	2	0	1	1	20	13.33
	Wipe 2	Wipe of the glovebox - right side	1	0	2	1	1	20	20.00
	Wipe 3	Wipe of the glovebox - front	0	0	1	0	1	20	6.67
Blank #2b	Test Filter	Test Filter 1 cm away	0	0	0	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Hot #2	Test Filter	Test Filter 1 cm away	322	336	308	322	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	1	0	0	1	20	6.67
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	1	0	1	20	6.67
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 5	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Blank #3	Control	Test Filter 1 cm away	0	0	1	0	1	20	0.00
	Referance 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Referance 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	1	0	0	1	20	6.67
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Hot #3	Control	Test Filter 1 cm away	182	209	185	192	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	1	0	1	20	6.67
	Wipe 2	Wipe of the glovebox - right side	5	6	10	7	1	20	140.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	0	1	0	0	1	20	6.67
	Wipe 5	Wipe of the glovebox - right side	1	0	0	0	1	20	6.67
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00

Blank

APPENDIX C:
INKJET AEROSOL GENERATOR (IJAG) AEROSOL TEST RESULTS: CLOSED-FACE FILTER ATTACHED TO IJAG

TABLE C-1. Tests Conducted Using 2.4 µM Monodispersed Particles

Run	Sample Type	Location	Plate Counts (CFU)			Avg (CFU)	Dilution	Extraction Volume (mL)	CFU/sample
Blank #1	Test Filter	Test Filter 1 cm away	0	0	1	0	1	20	6.67
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	1	1	0	1	1	20	13.33
	Wipe 2	Wipe of the glovebox - right side	1	0	0	0	1	20	6.67
	Wipe 3	Wipe of the glovebox - front	1	0	0	0	1	20	6.67
Hot #1	Test Filter	Test Filter 1 cm away	TNTC	TNTC	TNTC		1	20	TNTC
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	1	0	1	20	6.67
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	20	17	11	16	1	20	320.00
	Wipe 5	Wipe of the glovebox - right side	0	0	1	0	1	20	6.67
	Wipe 6	Wipe of the glovebox - front	3	5	8	5	1	20	106.67
Blank #2a	Test Filter	Test Filter 1 cm away	0	0	0	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	2	0	1	1	20	13.33
	Wipe 2	Wipe of the glovebox - right side	1	0	2	1	1	20	20.00
	Wipe 3	Wipe of the glovebox - front	0	0	1	0	1	20	6.67
Blank #2b	Test Filter	Test Filter 1 cm away	0	0	0	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Hot #2	Test Filter	Test Filter 1 cm away	322	336	308	322	1	20	6440.00
	Glovebox Filter 1	On the left side of the glovebox	0	1	0	0	1	20	6.67
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	1	0	1	20	6.67
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 5	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Blank #3	Control	Test Filter 1 cm away	0	0	1	0	1	20	6.67
	Referance 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Referance 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	1	0	0	1	20	6.67
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Hot #3	Control	Test Filter 1 cm away	182	209	185	192	1	20	3840.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	1	0	1	20	6.67
	Wipe 2	Wipe of the glovebox - right side	5	6	10	7	1	20	140.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Wipe 4	Wipe of the glovebox - left side	0	1	0	0	1	20	6.67
	Wipe 5	Wipe of the glovebox - right side	1	0	0	0	1	20	6.67
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00

TABLE C-2. Tests Conducted Using 1.96 µm Monodispersed Particles

Run	Sample Type	Location	Plate Counts (CFU)			Avg (CFU)	Dilution	Extraction Volume (mL)	CFU/sample
Blank #7	Test Filter	Test Filter directly connected	0	0	1	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Hot #7	Test Filter	Test Filter directly connected	141	117	120	126	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Swipe 1	Wipe of the glovebox - left side	1	2	0	1	1	20	20.00
	Swipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Swipe 3	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
	Swipe 4	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Swipe 5	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Swipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Blank #8	Test Filter	Test Filter directly connected	0	0	0	0	1	20	0.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	1	0	0	0	1	20	6.67
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	1	0	2	1	1	20	20.00
Hot #8	Test Filter	Test Filter directly connected	29	47	34	37	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	10160.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	1	0	1	20	6.67
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	0	1	0	1	20	6.67
	Wipe 4	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 5	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00
Blank #9	Test Filter	Test Filter directly connected	0	0	0	0	1	20	0.00
	Referance 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Referance 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	1	0	1	1	1	20	13.33
	Wipe 2	Wipe of the glovebox - right side	24	26	22	24	1	20	480.00
	Wipe 3	Wipe of the glovebox - front	11	12	22	15	1	20	300.00
Hot #9	Test Filter	Test Filter directly connected	30	44	39	38	1	20	10160.00
	Glovebox Filter 1	On the left side of the glovebox	0	0	0	0	1	20	0.00
	Glovebox Filter 2	On the right side of the glovebox	0	0	0	0	1	20	0.00
	Wipe 1	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 2	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 3	Wipe of the glovebox - front	0	1	0	0	1	20	6.67
	Wipe 4	Wipe of the glovebox - left side	0	0	0	0	1	20	0.00
	Wipe 5	Wipe of the glovebox - right side	0	0	0	0	1	20	0.00
	Wipe 6	Wipe of the glovebox - front	0	0	0	0	1	20	0.00

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