ALBURTYLAB

Summary Technical Report
InnovaPrep LLC
Dry Filter Collection/Wet Elution Aspiration and Recovery Efficiency Testing

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Preface

AlburtyLab, Inc. has prepared this Summary Technical Report for InnovaPrep LLC presenting test results for aerosol aspiration and recovery efficiency (A&RE) characterization of the InnovaPrep dry-collection/wet-elution system. The dry collector was exposed to aerosolized 1 and 3 µm polystyrene microspheres (PSMs) particles and aerosolized *Bacillus anthracis* Sterne (Ba Sterne) to determine the A&RE of the dry-collection/wet-elution system.

Mr. Andrew Page was the Technical Lead for InnovaPrep. Mrs. Pam Murowchick directed laboratory operations and was the author of the original report, dated May 25, 2010. Technical questions may be directed to Mrs. Murowchick at (816) 619-3374 or via email to pmurowchick@alburtylab.com.

APPROVED FOR ALBURTYLAB, INC.

David S. Alburty
President

December 26, 2013

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Acronyms

A&RE	Aspiration and Recovery Efficiency
AD	Aerodynamic diameter
APS	Aerodynamic Particle Sizer
ATC	Aerosol Test Chamber
Ba-Sterne	*Bacillus anthracis* Sterne (aavirulent vaccine) strain
FIU	Fluorescence Intensity Unit
ft^3	Cubic feet
ft^3/min	Cubic feet per min
HEPA	High-efficiency particulate air (filter)
ID	Inner diameter
in. Hg	Inches of Mercury
km/hr	Kilometer per hour
LFI	Lateral Flow Immunoassay Strips
Lpm	Liters per minute
mg/L	Milligram per Liter
min	Minute
mL	Milliliter
mph	Mile per hour
OEM	Original Equipment Manufacturer
PBS	Phosphate Buffered Saline
psi	Pounds per square inch
RPM	Revolution per minute
S/N	Serial Number
µg	Micrograms
µg/L	Micrograms per Liter
µL	Microliter
µm	Micrometer
YG-PSM	Yellow Green Fluorescent Polystyrene Microspheres

1. Introduction

InnovaPrep LLC has developed a dry-collection/wet-elution system that provides unique capabilities and performance for biodefense applications. In addition, the system has potential as an inexpensive, high performance dry-collection/wet-elution system for first responders and as a replacement for dry filter collection/elution systems.

The system tested for InnovaPrep consisted of three components described below:

1. COLLECTION: A dry-collection/wet-elution system for highly efficient capture and elution of bioaerosols into a final volume in the range of 6 to 7 mL.
2. CONCENTRATION: An automated, rapid liquid-to-liquid concentrator for concentration of the captured bioaerosols from a 4 mL portion of the eluted sample into a definable final volume of 80 to 500 µL.
3. DETECTION: Hand-Held biological assays for direct, rapid detection of the captured and concentrated bioaerosols.

The InnovaPrep liquid-to-liquid concentrator is a rapid and fully automated system for concentrating large volume samples containing bacteria, viruses, and free DNA into volumes as small at 80 µL. InnovaPrep offers bench-top systems and OEM systems with reusable concentration cells for integration into other systems and is currently developing systems that use disposable concentration cells for zero sample-to-sample carryover, and zero-power concentration cartridges that require no separate equipment for operation.

All aerosol testing was performed by AlburtyLab personnel at the downtown engineering laboratory located in Drexel, Missouri. Medical nebulizers were used to introduce the 1 and 3 µm polystyrene microspheres (PSMs) particles and the *Bacillus anthracis* Sterne (Ba Sterne) into the aerosol test chamber (ATC).

The particle concentrations within the ATC were monitored over the course of each test using an Aerodynamic Particle Sizer (APS Model No. 3321, TSI, Inc., St. Paul, MN). In addition, during each test, paired dry filter reference samples were collected during the dry filter collector's sampling period to determine the average PSM or Ba-Sterne concentration. During the YG-PSM testing, the reference filter extracts, electret filter elution samples, and concentrated material were analyzed using a Turner® Quantech™ Digital Fluorometer. During the Ba-Sterne testing, these samples were analyzed by plating. A portion of the electret filter elution samples and the concentrate samples were also analyzed for Ba-Sterne with lateral flow immunoassay strips in order to verify that the concentrated material could be detected using these tests. Testing procedures are given in Section 2. Testing results and conclusions are presented in Section 3.

2. Testing Procedures

On Tuesday, May 4, 2010, the InnovaPrep dry collector was positioned in the ATC portion of the AlburtyLab wind tunnel. A test matrix for testing is shown below in Table 2-1.

Table 2-1. Task 1 Test Matrix

Test Particles	Test Runs	Run Nos.	Reference		Foam Extract of Electret Filters (Air-to-Liquid)	InnovaPrep© Concentrate (Air-to-Liquid-to-Liquid)
			Samples	Analysis		
1 µm polystyrene microsphere	3	1-7	2 polycarbonate filters	Fluorometer	Fluorometer	Fluorometer
3 µm polystyrene microsphere	3	16-19	2 polycarbonate filters	Fluorometer	Fluorometer	Fluorometer
Ba-Sterne	7	20-23, 25-27	2 Glass Fiber Filter	Culture	Culture & LFI	Culture & LFI

Three test runs were performed with each size of the fluorescent polystyrene microspheres and a total of seven test runs were performed with the Ba-Sterne. Run 24 was aborted due to equipment failure. No samples were analyzed from this run

2.1 Testing Setup and Procedures

The InnovaPrep dry collector was installed in the middle of the ATC. The APS inlet hose was placed at one end of the chamber. The reference filter holders were positioned on either side of the InnovaPrep dry collector inlet at a slightly higher height then the inlet slit. A schematic of the test setup is presented in Figure 2-1 and Figure 2-2 contains a photograph.

The testing procedures used are summarized in the following sections.

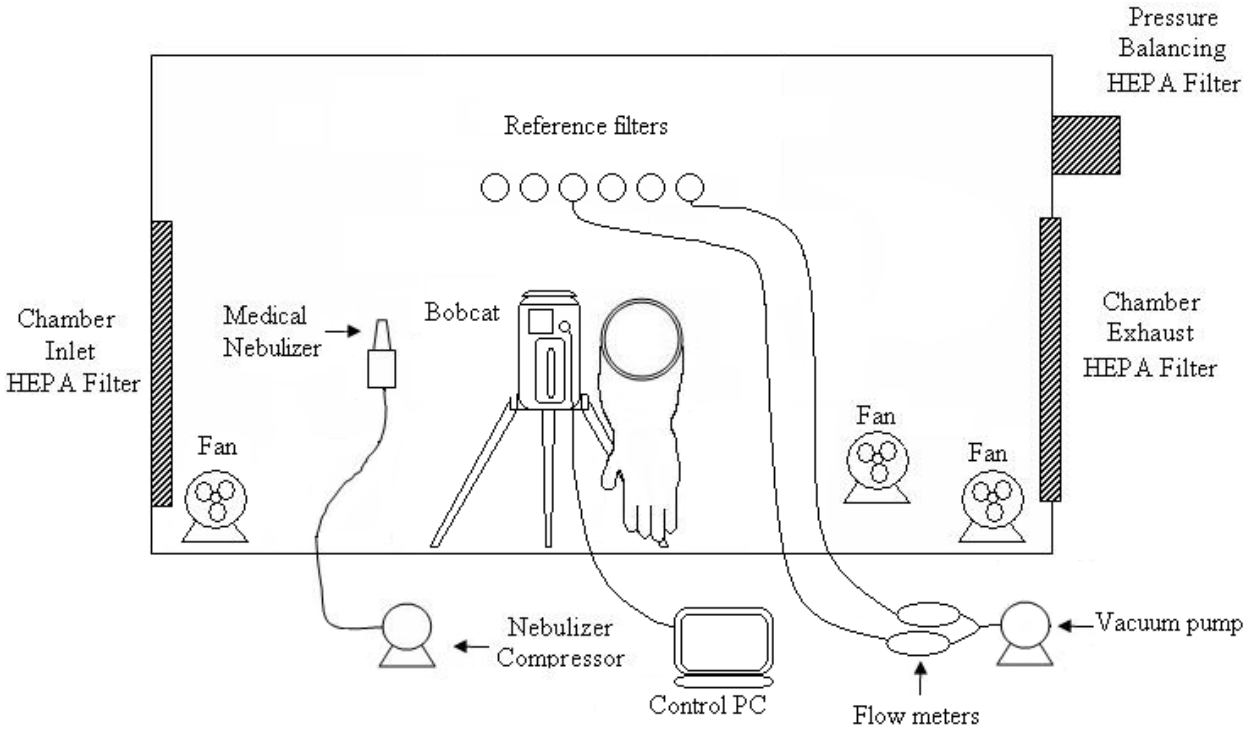


Figure 2-1. Schematic of Wind Tunnel Setup, Side View

Figure 2-2. Photograph of Test Setup, Side View

2.2 Particle Generation

Nebulization was carried out using a medical nebulizer loaded with a suspension of 1 µm Carboxylate Microspheres or a suspension of Ba-Sterne, as shown in Figure 2-3.

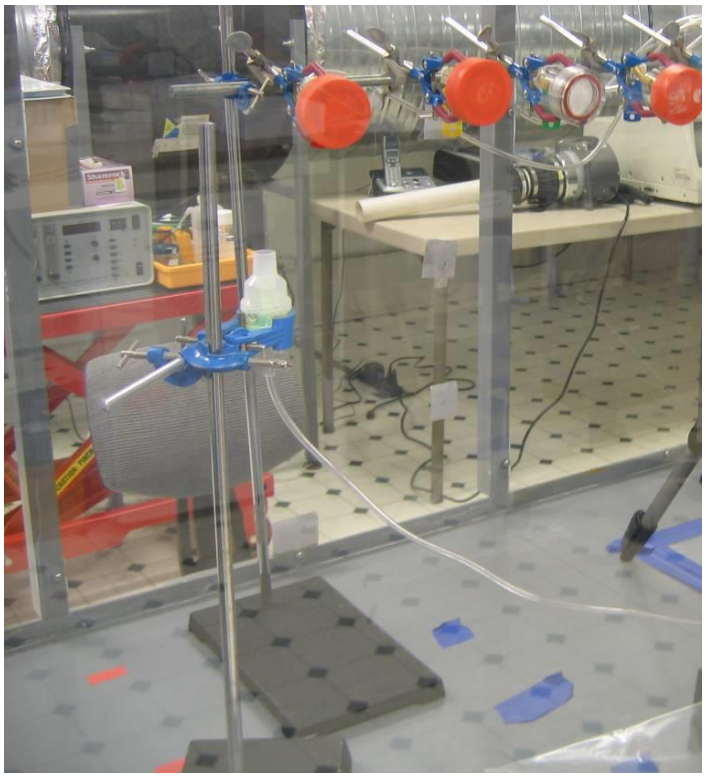


Figure 2-3. Dissemination of Test Particles using a Medical Nebulizer

During each of the tests, aerosols were generated for approximately 4 minutes, and the chamber air was mixed for approximately 1 min to achieve uniform aerosol concentration before sampling using reference filters and the dry filter collection system.

2.3 Particle Sizing and Concentration Determinations

The particle size distribution and concentrations within the ATC were monitored over the course of each test period using an Aerodynamic Particle Sizer. During each of the runs, the mode diameter of the test particles in the ATC was measured. Figure 2-4 contains the particle size distribution measured during one of the 1 µm YG-PSM test runs. Figure 2-5 contains the particle size distribution measured during one of the 3 µm test runs. Figure 2-5 contains the particle size distribution measured during one of the Ba-Sterne test runs.

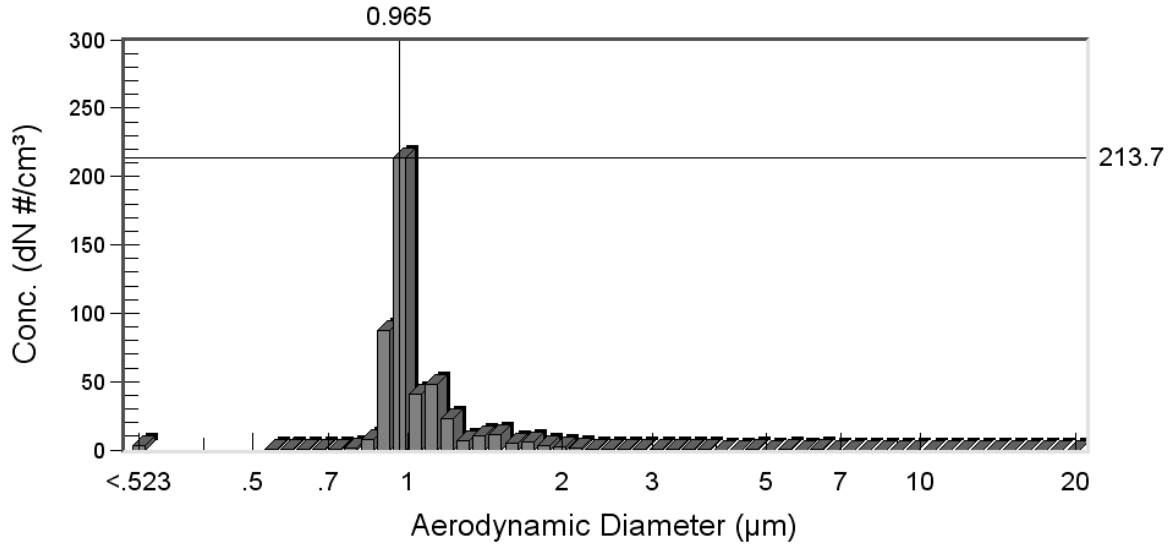


Figure 2-4. Particle Size Distribution Prior to the Start of Sampling during Run 1

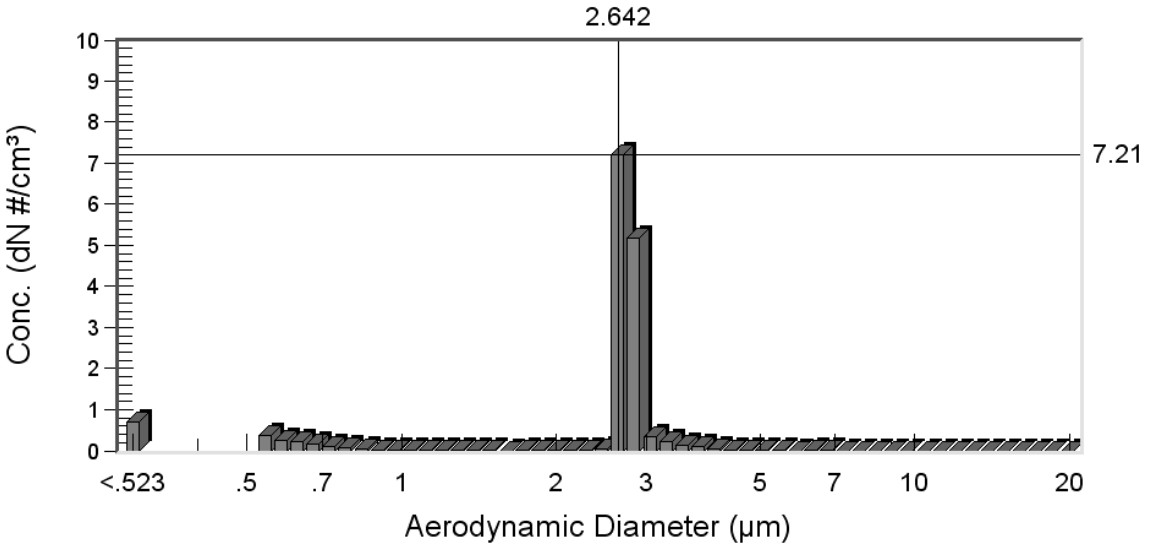


Figure 2-5. Particle Size Distribution Prior to the Start of Sampling during Run 16

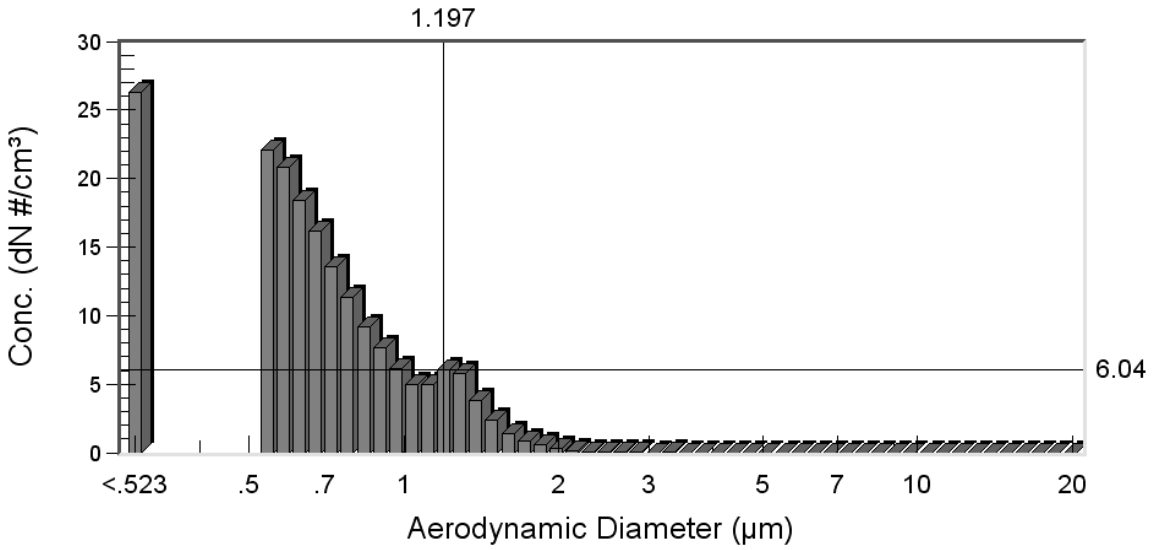


Figure 2-6. Particle Size Distribution Prior to the Start of Sampling during Run 20

2.4 Reference Sample Collection

During bead testing, two polycarbonate membrane filters were used as reference samples in order to determine the challenge aerosol concentration during each of the runs. The sampling periods coincided with the 10-minute InnovaPrep dry collector sampling periods. Each filter sampled for 10 minute, resulting in the collection of 100 Liters of aerosol.

After sampling, the reference filters were collected and processed to remove the PSMs from the filters into distilled water for fluorometric analysis.

During the Ba-Sterne testing, paired dry filter reference samples were collected for of the viable biological aerosol concentration. The sampling periods coincided with the InnovaPrep dry collector sampling periods.

Figure 2-7 summarizes the sample handling procedure for the InnovaPrep Dry Filter Collector samples during the Ba-Sterne tests. During the YG-PSM testing the same processes were used with the exception that fluorometric analysis was performed instead of plating.

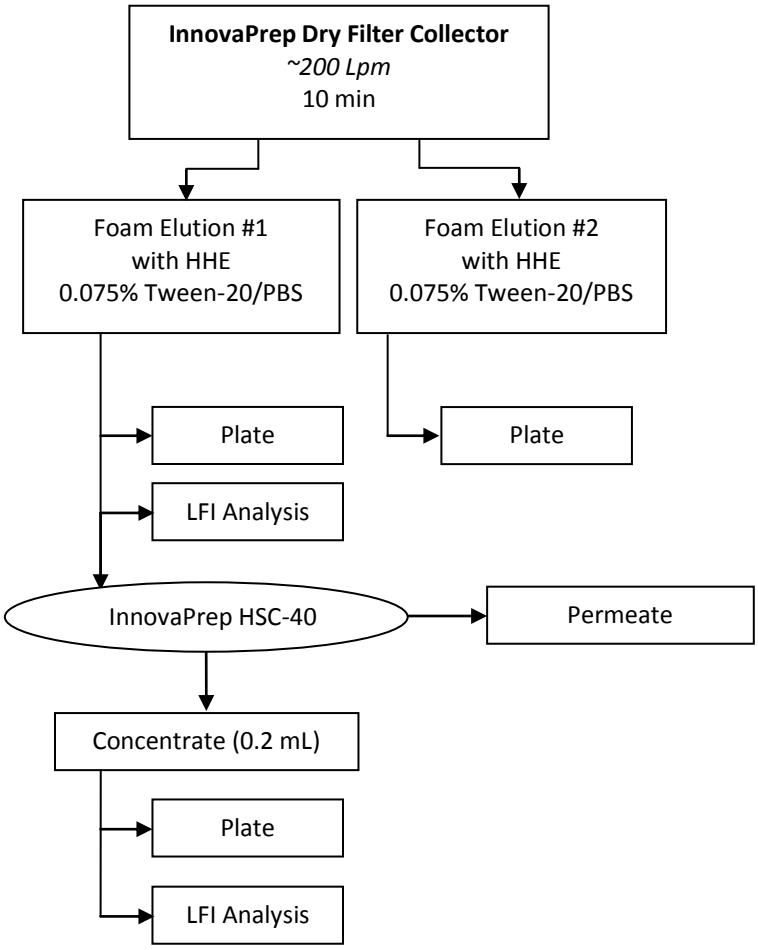


Figure 2-7. Dry Filter Collector Sample Handling Schematic

The InnovaPrep dry filter collector system consists of a medium volume, high efficiency aerosol collector which includes a replaceable dry electret filter, a simple to operate electret filter biological sample elutor, and a liquid-to-liquid concentration system. Each of these components is described in the following sections.

2.4.1 Collector

The aerosol collector is less than one-quarter of a cubic foot, is battery operated, and can be initiated via signal from a detector. The collector will draw ambient sample air at a flow rate of up to 250 liters per minute. The collector incorporates a dry electret filter as the collection media. Electret filters are produced from diaelectric polymer fibers that develop charges when air flows past them. The charges substaintially increase the efficiency of the filter and allow for the use of lower pressure drop filters. This, in turn, allows for the use of lower power blowers and battery operation. Additional advantages include low consummable costs, ease of use and replacement, and the ability to operate at low temperatures, a limitation for most wet-wall cyclone type collectors.

Figure 2-2 contains a photograph of one of the prototype dry filter collector systems in the "carry" configuration. During this tesing, collection was initiated manually using a computer

located outside of the ATC. Collection with the omnidirectional inlet and 52 mm electret filter provides high efficiency collection of biological particles over the 1 to 10 µm size range. The collection system has the added benefits of battery operation and low operating costs. During depolyment in the field, integrated tripod legs will provide an easy-to-use, stable platform while raising the inlet to 30 inches.

Following aerosol collection, the filter is removed from the Collector by unlatching the lid of the Collector, reaching in and capping the Filter Housing with the Extractor Sample Cup, lifting the assembly out of the collector, flipping it over and capping it with the Extractor Cap. The assembly was then transported to the lab bench for foam elution.

Figure 2-8. Dry Filter Collector

Limited testing was also performed to determine the effect of environmental samples on the aspiration and recovery efficiency of 1 um YG-PSMs. The environmental samples were collected by an open window at the AlburtyLab main laboratory in downtown Drexel, MO and outside near the AlburtyLab field station facility located at a rural site in Cass County. These tests were conducted as follows:

- Sampler collected an environmental sample using the "Extended Run" setting (1 min on, 5 min off at 100 Lpm) from May 28, 2010 at 16:52 until June 1, 2010 at 11:00 for a total of 90,100 L on the filter prior to the YG-PSM testing in the ATC.
- Sampler collected an environmental sample using the "Continuous" setting (200 Lpm) from May 16, 2010 at 16:45 until May 17, 2010 at 11:09 for a total of 220,800 L on the filter prior to the YG-PSM testing in the ATC.
- Following the YG-PSM testing in the ATC, the sampler collected an environmental sample using the "Continuous" setting (200 Lpm) from June 1, 2010 at 16:04 until June 2, 2010 at 9:05 for a total of 204,200 L on the filter.

2.4.2 Foam Elution

InnovaPrep has developed a Hand-Held Extraction (HHE) system as a four part assembly as shown at left. The HHE cartridge was designed to hold a volume of carbonated extraction fluid. The Extractor Cap will fit directly onto the Filter Housing used in the collector module. It is designed to direct the wet foam from the HHE cartridge evenly through the filter when the HHE is applied and the valve is pressed down. The wet foam passes through the interstitial spaces of the flat electret filter to efficiently extract any particles. Within approximately 30 seconds after extraction the foam returns to a liquid in the Sample Cup, making it available for sample processing and analysis. A push-on lid for the sample cup was also designed, so the sample can be transported dry and eluted at a laboratory.

Sample elution is achieved using a novel wet foam elution process that takes approximately 5 seconds and produces 6 to 7 milliliters of liquid sample. This wet foam elution method uses standard buffer solutions such as phosphate buffered saline (PBS) or Tris buffered saline with a surfactant or protein added to allow the solution to foam.

During this testing the HHEs used for eluting the collected test particle from the electret filter cartridges were charged with 8.0 mL of 0.075% Tween-20/PBS and were carbonated at 120 psi. Each electret filter was eluted using the method shown above. The filter was allowed to sit for 2 minutes prior to removing the extractor cap and performing a second elution of the electret filter into a second sample cup.

A 4 mL aliquot was removed from each of the first elution samples for further concentation using the InnovaPrep HSC-40 liquid-to-liquid concentator. The remaining portions of the Elution 1 samples and the Elution 2 samples were submitted for fluorometric analysis or for plating.

2.4.3 Liquid to Liquid Concentration

After the elution of the electret filter was complete, a 4 mL portion of the sample was concentrated via a liquid-to-liquid concentrator. All Elution 1 sample concentrations were performed using the InnovaPrep HSC-40 hydrosol concentration system as shown in Figure 2-9.

Figure 2-9. InnovaPrep HSC-40

A hollow fiber concentration cell was used for all liquid-to-liquid concentration cycles. Liquid throughput rates were approximately 1 mL/min and approximately 30 seconds were required for the extraction procedure. Prior to the start of testing, the extraction volume on the unit was adjusted to approximately 0.150 mL. The volumes of each Concentrate (first extract) and second extraction (Extract #2) are presented with the test results.

2.4.4 Aerosol Sampling Rate Determinations

In the InnovaPrep Dry Filter Collector the sampled air is pulled through the system using a counter-rotating axial fan. The fan speed and thus the sampling rate of the collector are controlled by microprocessor that receives hot wire anemometer velocity measurements from the exhaust of the device. This function was not included in the programming during testing. Instead the sampling rate indicator or AD value was recorded during each test period and the sampling rate was determined from a calibration curve comparing the AD value to the inlet flow rate measured using a vane anemometer.

2.5 Fluorometric Analysis and Data Reduction Procedures

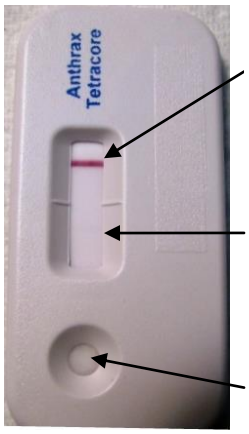
All samples collected during the PSM testing were analyzed using a Digital Fluorometer.

2.6 Ba-Sterne Titer Determinations

The Ba-Sterne titer of the reference filter extracts, Electret filter elutions, and the corresponding concentrates of each Electret filter elution from each run by was determined by preparing 10-fold serial dilutions down to the appropriate level dilution and plating 100 µL or 200 µL of the lowest dilution in duplicate onto TSA. The plated samples were incubated overnight and the colonies were then enumerated. The concentration of aerosolized Ba-Sterne in the chamber over the course of the sampling period was determined from each of the reference filters.

2.7 Lateral Flow Immunoassay Determinations

A portion of the Electret Filter Elution 1 and HSC-40 Concentrate samples were analyzed for Ba Sterne with LFIs available commercially. These are simple to use, antibody-based assays used to presumptively identify biological threat agents. An LFI has a one-time use capability designed to presumptively identify one agent.



The **Control Line** indicates whether the assay is functioning properly and is above the raised line on the plastic cassette. If the control line fails to develop, it indicates a problem with the test.

The **Test Line** is between the raised line on the plastic cassette and the Sample Port. Any visible pink or purple at the test line, even a very faint one, which appears at the 15 minute point should be considered a positive result.

150 µL of the sample is pipetted onto the **Sample Port**. It functions to filter out any large particulate matter in the sample and to hold the sample so that it can slowly wick through into the rest of the LFI.

Figure 2-10. Commercial Test Strip

In addition, an optical reader was used to verify the LFI result 15 minutes after pipetting the sample into the Sample port.

3. Results and Conclusions

The results of the testing are presented in this section. The date, test particle ID, sample collection start time, and ambient testing conditions for each of the runs are contained in Table 3-1.

Table 3-1. Ambient Testing Conditions for Each Test

Run ID	Date	Test Particle ID	ATC Sample Collection Start Time	Chamber Temperature (°F)	Chamber Relative Humidity (%)	Barometric Pressure (in. Hg)
1	5/4/10	1 µm YG-PSM	9:59 AM	72.8	41.1	29.93
2	5/4/10	1 µm YG-PSM	10:52AM	73.2	41.2	29.90
3	5/4/10	1 µm YG-PSM	11:05 AM	73.7	43.2	29.90
4	5/4/10	1 µm YG-PSM	11:26 AM	74.1	43.6	NR
5[a]	6/1/10	1 µm YG-PSM	2:44 PM	75.2	55.2	29.88
6[b]	6/1/10	1 µm YG-PSM	3:00 PM	76.1	57.0	NR
7[c]	6/1/10	1 µm YG-PSM	3:34 PM	76.2	56.0	29.85
16	5/4/10	3 µm YG-PSM	2:06 PM	75.3	41.1	29.85
17	5/4/10	3 µm YG-PSM	2:22 PM	75.7	41.0	NR
18	5/4/10	3 µm YG-PSM	2:43 PM	76.1	40.5	29.84
19	5/4/10	3 µm YG-PSM	4:07 PM	76.6	39.0	28.78
20	5/5/10	Ba-Sterne	1:29 PM	77.1	38.9	29.92
21	5/5/10	Ba-Sterne	1:48 PM	77.3	38.6	29.95
22	5/5/10	Ba-Sterne	2:06 PM	77.5	39.5	29.93
23	5/7/10	Ba-Sterne	9:58 AM	75.7	41.3	29.83
25	5/7/10	Ba-Sterne	10:25 AM	76.8	41.7	29.85
26	5/7/10	Ba-Sterne	11:16 AM	NR	42.5	29.88
27	5/7/10	Ba-Sterne	11:35 AM	NR	43.0	29.90

NR = Not Recorded.

[a] Sampler collected 90,100 L of environmental sample on the filter prior to the ATC test.

[b] Sampler collected 220,800 L of environmental sample on the filter prior to the ATC test.

[c] Sampler collected 204,200 L of environmental sample on the filter after the ATC test.

3.1 YG-PSM Testing Results

Following each run, the reference filter extracts, the Electret filter elutions, and the HSC-40 concentrate were prepared for fluorometric analysis using the methods described previously.

Table 3-2 contains a summary of the test data for the 1 µm YG-PSMs. The average aspiration and recovery efficiency of the Dry Filter Collection/Wet Elution was 105.4%. This was achieved with an average concentration factor of 41,417 $[(\#/L_{liquid})/(\#/L_{air})]$/min.

The Recovery Efficiency results and the Concentration Factor results for the 1 µm YG-PSMs in the concentrate samples recovered from the HSC-40 are also contained in Table 3-2. The extraction efficiencies ranged from 90.7% to 110.2%. The average Recovery Efficiency was

98.6%. The average concentration factor across the HSC-40 was 21.6 and the average overall concentration factor for all three steps was 864,845 [(#/L_{liquid})/(#/L_{air})]/min.

Table 3-3 contains a summary of the test data for the 1 µm YG-PSMs collected on filters that were used to collect environmental samples prior to the YG-PSM test (Run 5 and Run 6) or after the YG-PSM test (Run 7). The average aspiration and recovery efficiency of the Dry Filter Collection/Wet Elution was 73.1%. This was achieved with an average concentration factor of 41,417 [(#/L_{liquid})/(#/L_{air})]/min. The lower extraction efficiency measured during these test may be due to interference from the environmental sample during fluorometric analysis. To determine if the environmental sample impacts the fluorescence, the following test was performed. 100 uL of the S2 calibration standard was pipeted into each of 4 fluorometer cuvettes. 3 mL of an environmental sample that had been extracted from an 8 x 10 glass fiber filter using 0.05% Triton-100/PBS (with no PSLs added) was added to each of 2 of these cuvettes, labeled D1 and D2. 3 mL of filtered distilled water was added to each of the remaining cuvettes, labeled C1 and C2. All of the samples were analyzed using the fluorometer. The average bead concentration determined from the measured FIU readings from the clean samples, C1 and C2, was 7.16×10^6 YG-PSMs/mL. The average bead concentration determined from the measured FIU readings from the environmental samples, D1 and D2, was 3.84×10^6 YG-PSMs/mL. This shows a 46% reduced response in the fluorometer readings. From these results it can be concluded that there is some interference from the environmental sample.

The Recovery Efficiency results and the Concentration Factor results for the 1 µm YG-PSMs in the concentrate samples recovered from the HSC-40 are also contained in Table 3-3. The extraction efficiencies ranged from 58.0% to 100%. The average recovery efficiency was 84.7%. The average concentration factor across the HSC-40 was 9.9 and the average overall concentration factor for all three steps was 287,069 [(#/L_{liquid})/(#/L_{air})]/min.

Table 3-4 contains a summary of the test data for the 3 µm YG-PSMs. The average aspiration and recovery efficiency of the Dry Filter Collection/Wet Elution was 86.9%. This was achieved with an average concentration factor of 31,372 [(#/L_{liquid})/(#/L_{air})]/min.

The recovery efficiency results and the concentration factor results for the 3 µm YG-PSMs in the concentrate samples recovered from the HSC-40 are also contained in Table 3-4. The extraction efficiencies ranged from 89.1% to 95.8%. The average recovery efficiency was 92.1%. The average concentration factor across the HSC-40 was 16.5 and the average overall concentration factor for all three steps was 592,974 [(#/L_{liquid})/(#/L_{air})]/min.

Table 3-5 contains a summary of the test data for the Ba-Sterne. The average aspiration and recovery efficiency of the Dry Filter Collection/Wet Elution was 36.6%. This was achieved with an average concentration factor of 12,972 [(#/L_{liquid})/(#/L_{air})]/min.

The recovery efficiency results and the concentration factor results for the Ba-Sterne in the concentrate samples recovered from the HSC-40 are also contained in Table 3-5. The extraction efficiencies ranged from 89.1% to 110.9%. The average recovery efficiency was 84.8%. The average concentration factor across the HSC-40 was 16.0 and the average overall concentration factor for all three steps was 566,644 [(#/L_{liquid})/(#/L_{air})]/min. Early breadboard testing with *Bacillus atrophaeus* spores demonstrated an average efficiency of 92.7%, while this testing of the current system with vegetative *Bacillus anthracis* Sterne demonstrated lower recoveries. Preferential desiccation of vegetative *B. atrophaeus* from the electret filters, due to high face velocities, could be cited as the reason for the reduced efficiency.

Table 3-2. Summary of Results for 1 µm YG-PSM Testing

	1 um YG-Carboxy Polystyrene Microspheres					
Run Number	Run 1	Run 2	Run 3	Run 4	Average	Std Dev
Reference Filter #1						
Air Conc. Ref Filter #1, #/L	2.41E+05	2.51E+05	2.03E+05	1.88E+05		
Reference Filter #2						
Air Conc. Ref Filter #2, #/L	2.24E+05	2.29E+05	2.33E+05	2.18E+05		
Average from Reference Filters, #/L	2.32E+05	2.40E+05	2.18E+05	2.03E+05		
% Difference of Reference Filters	7.2%	9.2%	13.8%	14.8%		
APS-Total Particle Concentration (#/L)	2.38E+05	2.43E+05	2.20E+05	1.89E+05		
APS-Test Particle Concentration (#/L)	2.18E+05	2.22E+05	2.01E+05	1.70E+05		
Electret Elution 1						
Volume Extract, mL	6.1102	5.8952	5.7050	5.7039	5.8536	0.1933
Sampling Rate, Lpm	213	234	234	243	231	13
Air Conc. From Electret, #/L	2.68E+05	2.69E+05	1.85E+05	2.20E+05		
Ext #1 RE Based on Ref Filters, %	115.3%	112.0%	84.9%	108.5%	105.2%	13.8%
Ext #1 RE Based APS, %	112.7%	110.5%	84.0%	116.8%	106.0%	14.9%
Concentration Factor $[(\#/L_{liquid})/(\#/L_{air})]$/min	40,168	44,400	34,797	46,302	41,417	5,103
Electret Elution 2						
Volume Extract, mL	7.7786	8.0852	8.3818	8.5035	8.1873	0.3242
Air Conc. From Electret, #/L	1.26E+04	1.14E+04	1.30E+04	1.42E+04		
Ext #2 RE Based on Ref Filters, %	5.4%	4.8%	5.9%	7.0%	5.8%	0.9%
Cumulative RE	120.8%	116.7%	90.9%	115.5%	111.0%	13.6%
HSC-40 Feed						
Volume HSC-40 Feed, mL	3.9893	3.9724	3.9734	3.9839	3.9798	0.0082
Number of Beads Fed	3.72E+08	4.31E+08	3.01E+08	3.74E+08		
Concentrate						
Volume Concentrate, mL	0.1959	0.2267	0.2422	0.1173	0.1955	0.0556
Bead Concentration Extract #1, #/mL	1.72E+09	1.83E+09	1.37E+09	3.09E+09		
Number of Beads Recovered	3.37E+08	4.16E+08	3.32E+08	3.63E+08		
HSC-40 RE, %	90.7%	96.5%	110.2%	96.9%	98.6%	8.2%
HSC-40 Concentration Factor	18.5	16.9	18.1	32.9	21.6	7.6
Overall CF $[(\#/L_{liquid})/(\#/L_{air})]$/min	643,490	670,819	740,844	1,404,229	864,845	361,919
Extract #2						
Volume Extract #2, mL	0.1336	0.1692	0.1416	0.1488	0.1483	0.0153
Bead Concentration in Extract #2, #/mL	5.53E+07	3.94E+07	3.00E+07	2.86E+07		
Number of Beads Recovered	7.38E+06	6.67E+06	4.25E+06	4.26E+06		
HSC-40 RE, %	2.0%	1.5%	1.4%	1.1%	1.5%	0.4%
Cumulative HSC-40 RE, %	92.7%	98.1%	111.6%	98.0%	100.1%	8.1%

Table 3-3. Summary of Results for 1 µm YG-PSM/Environmental Sample Testing

	1 um YG-Plain Polystyrene Microspheres with Environmental Sample Before or After				
Run Number	Run 5	Run 6	Run 7	Average	Std Dev
Reference Filter #1					
Air Conc. Ref Filter #1, #/L	3.16E+05	2.51E+05	2.78E+05		
Reference Filter #2					
Air Conc. Ref Filter #2, #/L	3.04E+05	2.48E+05	3.08E+05		
Average from Reference Filters, #/L	3.10E+05	2.50E+05	2.93E+05		
% Difference of Reference Filters	4.0%	1.2%	10.5%		
Electret Elution 1					
Volume Elution, mL	6.7851	6.4915	7.2833	6.8533	0.4003
Sampling Rate, Lpm	200	200	200	200	0
Air Conc. From Electret, #/L	2.29E+05	1.83E+05	2.11E+05		
Ext #1 RE Based on Ref Filters, %	74.0%	73.1%	72.1%	73.1%	1.0%
Overall Concentration Factor $[(\#/L_{liquid})/(\#/L_{air})]$/min	21,821	22,521	19,796	21,379	1,415
Electret Elution 2					
Volume Elution, mL	8.2002	8.7493	8.7472	8.5656	0.3164
Air Conc. From Electret, #/L	2.69E+04	5.72E+04	5.96E+04		
Ext #2 RE Based on Ref Filters, %	8.7%	22.9%	20.3%	17.3%	7.6%
Cumulative RE	82.7%	96.0%	92.4%	90.4%	6.9%
HSC-40 Feed					
Volume HSC-40 Feed, mL	2.6400	4.0372	3.9762	3.5511	0.7897
Number of Beads Fed	8.93E+07	1.14E+08	1.15E+08		
Concentrate					
Volume Concentrate, mL	0.3099	0.2987	0.2837	0.2974	0.0131
Bead Concentration Extract #1, #/mL	2.90E+08	2.20E+08	3.87E+08		
Number of Beads Recovered	8.99E+07	6.59E+07	1.10E+08		
HSC-40 RE, %	100.7%	58.0%	95.3%	84.7%	23.3%
HSC-40 Concentration Factor	8.6	7.8	13.4	9.9	3.0
Overall Concentration Factor $[(\#/L_{liquid})/(\#/L_{air})]$/min	252,902	241,546	366,759	287,069	69,247
Extract #2					
Volume Extract #2, mL	0.1511	0.1487	0.1751	0.1583	0.0146
Bead Concentration in Extract #2, #/mL	4.89E+07	1.92E+07	5.67E+06		
Number of Beads Recovered	7.40E+06	2.85E+06	9.92E+05		
HSC-40 RE, %	8.3%	2.5%	0.9%	3.9%	3.9%
Cumulative HSC-40 RE, %	109.0%	60.5%	96.2%	88.6%	25.1%

Table 3-4. Summary of Results for 3 µm YG-PSM Testing

	3.0 um YG-Carboxy Polystyrene Microspheres					
Run Number	Run 16	Run 17	Run 18	Run 19	Average	Std Dev
Reference Filter #1						
Air Conc. Ref Filter #1, #/L	3.91E+03	2.51E+03	3.16E+03	5.66E+03		
Reference Filter #2						
Air Conc. Ref Filter #2, #/L	3.65E+03	2.46E+03	3.54E+03	5.83E+03		
Average from Reference Filters, #/L	3.78E+03	2.49E+03	3.35E+03	5.74E+03		
% Difference of Reference Filters	7.1%	2.0%	11.2%	2.8%		
APS-Total Particle Concentration (#/L)	6.76E+03	4.33E+03	5.55E+03	9.67E+03		
APS-Test Particle Concentration (#/L)	5.37E+03	3.28E+03	3.96E+03	5.90E+03		
Electret Elution 1						
Volume Extract, mL	6.2795	5.9497	6.0189	6.1980	6.1115	0.1533
Sampling Rate, Lpm	213	222	222	224	220	5
Air Conc. From Electret, #/L	3.66E+03	2.46E+03	2.56E+03	4.34E+03		
Ext #1 RE Based on Ref Filters, %	96.9%	98.8%	76.4%	75.6%	86.9%	12.6%
Ext #1 RE Based APS, %	68.2%	74.9%	64.6%	73.6%	70.4%	4.8%
Concentration Factor $[(\#/L_{liquid})/(\#/L_{air})]$/min	32,836	36,883	28,405	27,364	31,372	4,374
Electret Elution 2						
Volume Extract, mL	8.2799	8.2825	7.8257	7.7115	8.0249	0.2996
Air Conc. From Electret, #/L	8.84E+02	4.15E+02	8.53E+02	1.30E+03		
Ext #2 RE Based on Ref Filters, %	23.4%	16.7%	25.5%	22.7%	22.0%	3.8%
Cumulative RE	120.3%	115.5%	101.8%	98.3%	109.0%	10.6%
HSC-40 Feed						
Volume HSC-40 Feed, mL	3.9788	3.9739	3.9631	3.9559	3.9679	0.0104
Number of Beads Fed	4.94E+06	3.65E+06	3.77E+06	6.22E+06		
Concentrate						
Volume Concentrate, mL	0.1982	0.2240	0.2337	0.2363	0.2231	0.0174
Bead Concentration Extract #1, #/mL	2.30E+07	1.56E+07	1.44E+07	2.40E+07		
Number of Beads Recovered	4.55E+06	3.49E+06	3.36E+06	5.68E+06		
HSC-40 RE, %	92.2%	95.8%	89.1%	91.3%	92.1%	2.8%
HSC-40 Concentration Factor	18.5	17.0	15.1	15.3	16.5	1.6
Overall CF $[(\#/L_{liquid})/(\#/L_{air})]$/min	627,217	634,091	557,239	553,350	592,974	43,628
Extract #2						
Volume Extract #2, mL	0.1502	0.1389	0.1370	0.1376	0.1409	0.0062
Bead Concentration in Extract #2, #/mL	9.05E+05	2.13E+05	2.57E+05	4.44E+05		
Number of Beads Recovered	1.36E+05	2.96E+04	3.52E+04	6.11E+04		
HSC-40 RE, %	2.8%	0.8%	0.9%	1.0%	1.4%	0.9%
Cumulative HSC-40 RE, %	94.9%	96.6%	90.0%	92.3%	93.5%	2.9%

Table 3-5. Summary of Results for Ba-Sterne Testing

Run Number	Run 20	Run 21	Run 22	Run 23	Run 25	Run 26	Run 27	Average	Std Dev
InnovaPrep Dry Filter Collector Unit ID	2	2	2	1	1	1	1		
Reference Filter #1									
Air Conc. Ref Filter #1, CFU/L	3.10E+03	3.85E+03	3.75E+03	6.15E+03	5.95E+03	4.80E+03	5.35E+03		
Reference Filter #2									
Air Conc. Ref Filter #2, CFU/L	3.60E+03	3.65E+03	3.00E+03	5.15E+03	6.15E+03	7.30E+03	5.85E+03		
Average from Ref Filters, #/L	3.35E+03	3.75E+03	3.38E+03	5.65E+03	6.05E+03	6.05E+03	5.60E+03		
% Difference of Reference Filters	14.9%	5.3%	22.2%	17.7%	3.3%	41.3%	8.9%		
Electret Elution #1									
Volume Extract, mL	5.8605	6.6210	6.2451	6.4573	6.7333	7.3814	6.9746		
Sampling Rate, Lpm	246	234	236	217	195	227	236		
Ba-Sterne Titer, CFU/mL	5.30E+05	4.85E+05	4.40E+05	8.00E+05	5.70E+05	8.15E+05	6.70E+05		
Air Conc. From Electret, CFU/L	1.26E+03	1.37E+03	1.16E+03	2.38E+03	1.69E+03	2.55E+03	1.98E+03		
Ext #1 RE Based on Ref Filters, %	37.7%	36.6%	34.5%	42.1%	28.0%	42.1%	35.3%	36.6%	4.9%
CF $[(\#/L_{Liquid})/(\#/L_{air})]$/min	15,821	12,933	13,037	14,159	9,421	13,471	11,964	12,972	1,975
HSC-40 Feed									
Volume HSC-40, mL	4.0081	3.9922	4.0075	3.9803	3.9658	3.9410	4.0		
Ba-Sterne Fed, CFU	2.12E+06	1.94E+06	1.76E+06	3.18E+06	2.26E+06	3.21E+06	2.68E+06		
HSC-40 Concentrate									
Volume Extract, mL	0.2381	0.2203	0.1954	0.2362	0.2005	0.1928	0.2071	0.2129	0.0188
Ba-Sterne Titer, CFU/mL	8.75E+06	8.10E+06	6.80E+06	8.65E+06	1.25E+07	1.20E+07	1.04E+07		
Ba-Sterne Recovered, CFU	2.08E+06	1.78E+06	1.33E+06	2.04E+06	2.51E+06	2.31E+06	2.16E+06		
RE, %	98.1%	92.2%	75.4%	64.2%	110.9%	72.0%	80.6%	84.8%	16.4%
HSC-40 Concentration Factor	16.51	16.70	15.45	10.81	21.93	14.72	15.57	15.96	3.29
Overall CF $[(\#/L_{liquid})/(\#/L_{air})]$/min	692,595	589,677	584,426	363,652	737,859	471,052	527,246	566,644	127,750

3.2 LFI Results

As stated previously, during each of the Ba-Sterne tests a portion of the Electret Elution 1 samples and the HSC-40 concentrate samples were analyzed for Ba Sterne using commercially available LFI test strips.

Table 3-6. Summary of LFI Results

	LFI Response (Optical Density)	
	Electret Elution #1	HSC-40 Concentrate
Run 20	Negative (109)	Positive (138)
Run 21	Negative (95)	Positive (132)
Run 22	Negative (68)	Negative (105)
Run 23	Negative (120)	Positive (338)
Run 25	Negative (72)	Positive (307)
Run 26	Positive (131)	Positive (314)
Run 27	Positive (141)	Positive (392)

3.3 Data Archival

All supporting data is recorded electronically (APS Data), on the testing log sheets, and in the AlburtyLab Laboratory Notebook #10002.

3.4 Conclusions and Recommendations

As stated previously, early breadboard testing with *Bacillus atrophaeus* spores demonstrated an average efficiency of 92.7%, while this testing of the current system with vegetative *Bacillus anthracis* Sterne demonstrated lower recoveries. Preferential desiccation of vegetative *B. atrophaeus* from the electret filters, due to high face velocities, could be cited as the reason for the reduced efficiency. It is recommended that collection and recovery efficiency testing with *Bacillus atrophaeus* spores be performed in the future.

The HHEs developed under this program meet the requirements of first responders and other potential users.