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Comparative Analysis of Two Biological Warfare Air Samplers Using Live Surrogate Agents

James C. Enderby

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THESIS

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AFIT/GIH/ENV/12-M01

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Abstract

The United States Air Force has several high-volume biological air samplers, including the XMX/2L-MIL (Dycor Technologies Ltd, Edmonton AB, Canada) and the Biocapture 650 (FLIR, Arlington VA, USA). Limited information is available on each in its collection of viruses and bacteria. Previous research on the XMX/2L-MIL determined that modifications to the secondary flow rate and using a virus preserving collection media may provide improved virus collection rates. Here these modifications were investigated to determine the impact on the collection of viral and bacterial aerosols. Additionally, relative collection rates were compared against those for the Biocapture 650. MS2 bacteriophage was the viral surrogate and *Bacillus thuringensis kurstaki* (Biologics Process Development Inc, Poway, CA, USA) the bacterial surrogate. Aerosolized particles were released into a wind tunnel where three of each samplers were exposed simultaneously. Samples were analyzed using plaque assay, cell culture on growth media, and real-time polymerase chain reaction (RT-PCR). Viability issues made it difficult to discern the impact of virus preserving media. Research showed that secondary flow rate reduction provided a statistically lower collection of viable bacteria compared to the standard secondary flow rate. The Biocapture 650 generally performed on par or better than the XMX/2L-MIL in collection of both bacterial and viral aerosols. However, longer sampling periods with the Biocapture 650 for viruses resulted in statistically inferior results.

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Finally, I am grateful to be blessed with my wife and children who without their unwavering support and love throughout this journey, this project would not have been completed.

James C. Enderby

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COMPARATIVE ANALYSIS OF TWO BIOLOGICAL WARFARE AIR SAMPLERS USING LIVE SURROGATE AGENTS

I. Introduction

Background

Biological warfare agents pose one of the most significant threats to military personnel and civilians. In the United States Air Force (USAF), the Bioenvironmental Engineering (BEE) and Civil Engineering Emergency Management (CEX) communities are tasked with providing detection, identification, and quantification of biological warfare agents. To accomplish this mission, several high-volume air samplers were purchased by the respective disciplines. The BEE community acquired the XMX/2L-MIL (Dycor Technologies Ltd, Edmonton AB, Canada) while the CEX community purchased first, the Dry Filter Unit (DFU)-1000 (Lockheed Martin Integrated Systems, Gaithersburg, MD, USA) followed by, more recently, the Biocapture 650 (FLIR, Arlington VA, USA). While each piece of equipment had its benefits, the equipment had not been thoroughly evaluated and compared to one another. As such, the USAF School of Aerospace Medicine initiated studies to compare the collection efficiencies of these samplers to one another.

The first study compared the DFU-1000 to the XMX/2L-MIL in the collection of viral aerosol particles (Cooper, 2010). The study found that the XMX/2L-MIL was significantly better than the DFU-1000; however, several modifications were made to the XMX/2L-MIL, specifically the collection media and reduction of the secondary flow rate, which made it uncertain exactly why the XMX/2L-MIL was superior in performance. A follow-on study furthered the evaluation of the XMX/2L-MIL in collection of inert particles looking at secondary flow rates, collection media impact, and inter-instrument variability (Black, 2011). The purpose

of this thesis is to evaluate the XMX/2L-MIL further by determining the impact of secondary flow rate reduction on the collection of bacterial containing aerosol particles and the impact of different collection media for virus containing aerosol particles. Furthermore, it seeks to compare the relative collection efficiency of the Biocapture 650 to the various configurations of the XMX/2L-MIL and help identify what might be gained or lost by switching to the Biocapture 650 as the primary high-volume air sampler of the USAF.

Problem Statement

Extensive literature exists summarizing air sampling of viral aerosols in laboratory and field settings. Studies utilizing high-volume air samplers for viruses are more limited, however. High-volume air samplers are on the Air Force's first-line of defense for response, detection, and risk assessment of biological warfare agents. Based on this, further study of the Air Force's high-volume air sampler arsenal is critical. Experimental evaluation of the XMX/2L-MIL for collection of viruses has been conducted to a limited degree and evaluation of the collecting bacteria is not significantly available in published literature. Experimental evaluation of the Biocapture 650 is not available for the collection of viruses or bacteria in published literature. Additionally, no studies have been identified that directly compare collection efficiencies of the XMX/2L-MIL and the Biocapture 650. Evaluating air samplers when exposed to equivalent viral or bacterial loads could provide information regarding sampling effectiveness which would be critical in a field response. Side-by-side comparison has not been conducted for these samplers. A comparative analysis of the XMX/2L-MIL and the Biocapture 650 would provide important information on implementation procedures for these devices by Air Force (AF) personnel.

XMX/2L-MIL viral studies have been done extensively for Phosphate Buffer Saline (PBS) solution and water, and, to a very limited degree in AF evaluations, for virus preserving media, specifically Remel M5 (Dycor Technologies Ltd, Edmonton AB, Canada). Further evaluation is needed to determine if Remel M5 is a viable alternative media to PBS or water.

Furthermore, viral studies have been conducted comparing the effectiveness of utilizing the reduced secondary flow rate on the XMX/2L-MIL with Remel M5 compared to PBS, finding that Remel M5 is more effective. The comparison did not study the use of the full secondary flow rate against the reduced secondary flow rate using PBS media. The flow rate comparison has not been done for bacterial studies. Therefore, additional research is warranted to determine if the reduced secondary flow modification is effective for bacteria.

Additionally, the Biocapture 650 has not been evaluated in the collection of viral or bacterial particles in current literature. The Biocapture 650 has several options for sampling collection time, ranging from 5-60 minutes. Current AF procedures call for a sample collection time of 30 minutes, regardless of suspected agent. An evaluation of collection efficiency by varying collection times could prove useful in determining the best procedures for various sampling situations.

In summary, a comparative analysis of the effectiveness of current Air Force high-volume air samplers is necessary. The study should be conducted in a controlled laboratory equipment exposure chamber to provide stable aerosol conditions and controllable external variables, such as aerosol particle size, relative humidity, and temperature. Equipment evaluation should include resources that would be applicable and useful to a field response, such as use of virus preserving media, and evaluation of viable and non-viable bacterial and viral

particles. This analysis would aid in equipment selection for response as well as provide a baseline for future comparative studies.

Research Questions

The purpose of this research is to validate the effectiveness of previously tested modifications, using inert particles, to the XMX/2L-MIL using viable surrogate organisms for both spore-forming bacterial and viral agents. The study will also evaluate the bioaerosol collection efficiency of the Biocapture 650 and its feasibility as either a supplemental capability of the XMX or the XMX's eventual successor. The following performance characteristics are to be evaluated:

1. Which XMX configuration, virus preserving media or standard media, results in the highest collection efficiency of the surrogate viral agent?
2. Which XMX configuration, full secondary flow or reduced secondary flow, results in the highest collection efficiency of the surrogate spore-forming bacterial agent?
3. How does the Biocapture 650 collection efficiency compare to the various XMX configurations for both viral and spore-forming bacterial agents?
4. What would be gained/lost by switching from the XMX to the Biocapture 650?

Scope and Approach

This research seeks to evaluate the collection efficiencies of two high-volume air samplers, the XMX/2L-MIL and the Biocapture 650. This will be done through a comparative analysis of the samplers when exposed simultaneously to viral and bacterial aerosols. The analysis will compare the results for both viable and total (viable and non-viable) particles both as total plaque or colony forming units (PFU or CFU) collected and PFU/CFU per liter of air

sampled by the air sampler. The experiment will also evaluate the difference between using a virus preserving media in the XMX/2L-MIL, usage of full secondary flow rates compared to reduced secondary flow rates in the XMX/2L-MIL for bacterial sampling, and varying collection times for the Biocapture 650.

Significance

The significance of this research is that it will provide a side-by-side analysis of two of the AF's primary biological warfare air samplers. The comparison will provide insight into which sampler and configuration, if any, is significantly better for viral or bacterial air sampling. This comparison may help determine best approaches for employment of the two air samplers in various field responses.

Preview

This thesis uses the scholarly article format. The following chapter is the article produced from the research, which was submitted to *Aerosol Science and Technology* journal. The article serves as the body of this thesis and its layout is prescribed by the above peer-reviewed journal. As an independent chapter (Chapter 2), it includes an abstract, introduction, experimental methodology, results and discussions, and conclusions. Chapter 3 offers a final discussion of the article's conclusions, along with pertinent findings specific to the AF employment of the equipment and future research not specifically discussed in Chapter 2.

II. Scholarly Article

Abstract

The XMX/2L-MIL (Dycor Technologies Ltd, Edmonton AB, Canada) and the Biocapture 650 (FLIR, Arlington VA, USA) are two high-volume air samplers designed to sample biological aerosols. Limited information has been published on their performance with respect to collection of viruses and bacteria. Previous research on the XMX/2L-MIL found that modifications to the secondary flow rate, as well as using a virus preserving collection media, may provide improved virus collection rates. Here, we investigated the impact of these modifications on the collection of viral and bacterial aerosols. Additionally, we compared these relative collection rates against those for various sampling times for the Biocapture 650. MS2 bacteriophage was used as the viral surrogate and *Bacillus Thuringensis Kurstaki* (Biologics Process Development Inc, Poway, CA, USA) as the bacterial surrogate. Aerosolized particles were released into a wind tunnel where three of each sampler was exposed simultaneously. Samples were analyzed using plaque assay for MS2 and cell culture on growth media for BTK, as well as real-time polymerase chain reaction (RT-PCR). Secondary flow rate reduction provided a statistically lower collection of viable bacteria compared to the standard secondary flow rate. Lowering the secondary flow rate resulted in no significant difference in collection of viable/non-viable bacteria or concentrations. The Biocapture 650 generally performed on par or better than the XMX/2L-MIL in collection of both bacterial and viral aerosols. However, longer sampling periods with the Biocapture 650 for viruses resulted in statistically inferior results.

Introduction

Viral and bacterial diseases are among the largest contributors to recent disease pandemics, with significant outbreaks of H5N1 avian influenza and measles outbreaks in 2011

and early 2012 alone. Since the start of 2011, the current outbreak of avian influenza has resulted in 582 illnesses with 343 deaths in six countries as of 20 January 2012 (WHO, 2012), a 58.9% mortality rate. Additionally, the World Health Organization had received reports of over 155,000 measles cases in 2011 (WHO, 2011). Both of these diseases are spread by airborne transmission of biological aerosol, thus increasing the potential for increased incidence rates and rapid spread of disease. Based on these and similar outbreaks, as well as the appeal of biological agents as weapons of mass destruction, rapid detection and identification of biological aerosols is critical (Black, 2011).

There is a wide-range of air sampling equipment capable of collecting biological aerosols. The Edgewood Chemical Biological Center, operated by the United States Army, has tested at least 29 different aerosol air samplers (Kesavan et al., 2010). As noted by Kesavan et al., air samplers are vital to the detection and identification of airborne biological agents and protection of human life. This study focuses on comparing two high-volume air samplers, the XMX/2L-MIL (Dycor Technologies Ltd, Edmonton AB, Canada) and the Biocapture 650 (FLIR, Arlington VA, USA), operated under different configurations, in their ability to collect surrogate biological and viral aerosols. The XMX/2L-MIL and Biocapture 650 are shown in Figure 1.

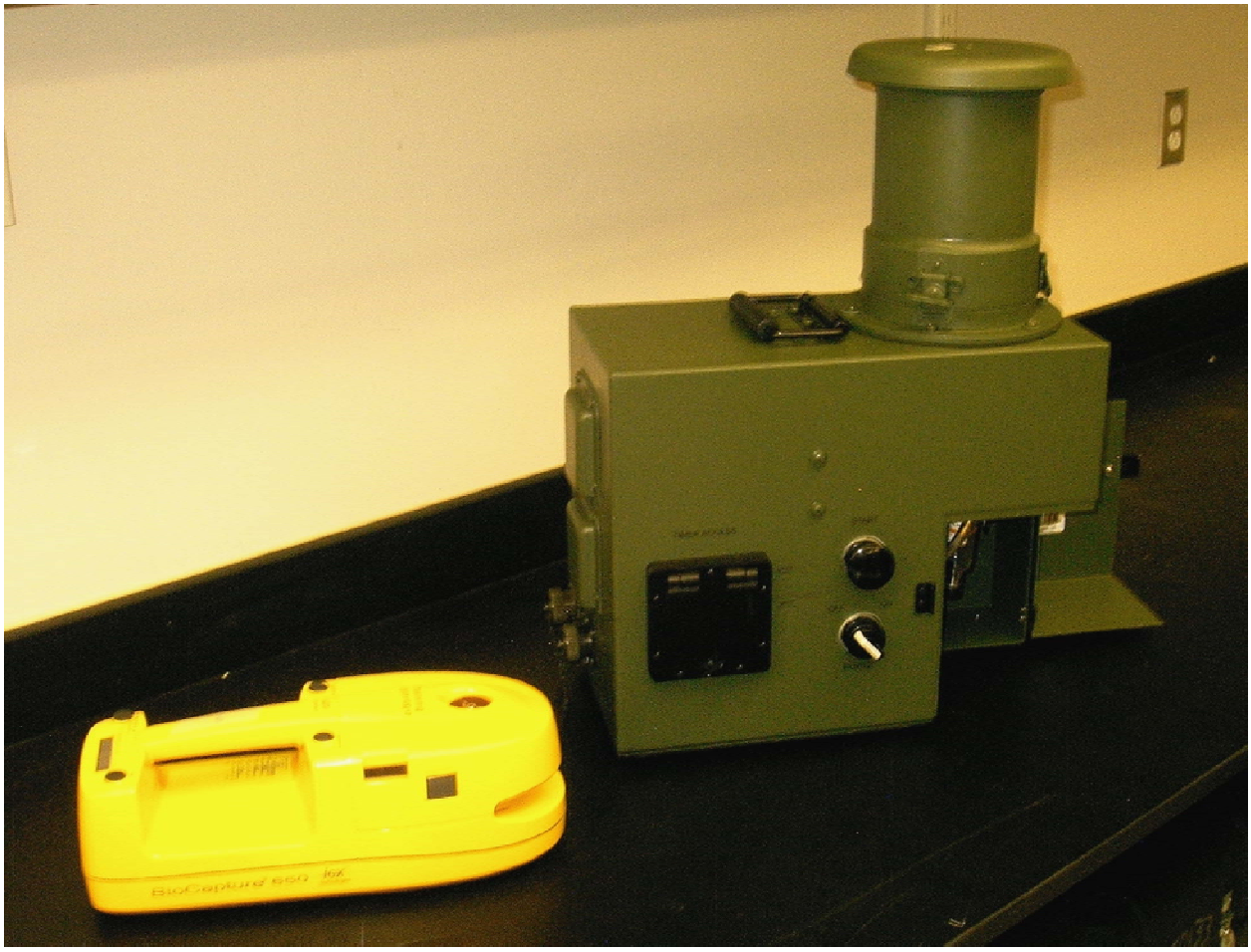

Figure 1: Biocapture 650 and XMX/2L-MIL

Collection methods for aerosol samplers have been detailed in previous studies but can be split into three broad categories: liquid impaction, solid impaction, and filter collection (Verreault et al., 2008). As outlined in studies by Cooper (2010) and Black (2011), the XMX/2L-MIL uses virtual impaction followed by liquid impingement to collect particles into liquid collection media. The Biocapture 650 collects particles through use of a rotating impactor that captures particles on a dry surface, and then rinses them with a buffer solution into the collection vial (Kesavan and Schepers, 2007). The particle size collection ranges are similar

with the XMX/2L-MIL limited to the 1 to 10 micron range (Tucker, 2005) and the Biocapture 650 to 0.5 to 10 microns (Kesavan and Schepers, 2007). Studies by Black found that XMX/2L-MIL concentrated sub-micron particles in the secondary flow (Black, 2011).

In a study comparing the XMX/2L-MIL against the Dry Filter Unit (Lockheed Martin Integrated Technologies, Gaithersburg, MD, USA) the use of both a reduced secondary flow rate and a virus preserving media, Remel MicroTest M5 Multi-Microbe Media (Remel M5), resulted in improved collection efficiency of viral particles (Cooper, 2010). Unfortunately, it could not be determined whether the improvement was attributed to only one or both modifications. Follow-up studies with viral particles showed that the improvement could be directly attributed to use of Remel M5 collection media (Black, 2011). However, the impact of using Remel M5 compared Phosphate Buffer Solution (PBS) was not thoroughly evaluated. Neither study tested the collection efficiency of bacterial particles.

This study was conducted to answer three specific questions:

1. Which XMX configuration, virus preserving media or standard PBS media, results in the highest collection efficiency of the surrogate viral agent?
2. Which XMX configuration, full secondary flow or reduced secondary flow, results in the highest collection efficiency of the surrogate spore-forming bacterial agent?
3. How does the Biocapture 650 collection efficiency compare to the various XMX configurations for both viral and spore-forming bacterial agents?

Experimental Methodology

Experimental studies were performed in an aerosol test chamber (ATC) operated by the United States Environmental Protection Agency (EPA), Research Triangle Park, North Carolina. The ATC has a volume of 547.4 cubic feet, measuring 20 feet (ft) long, 5.75 ft wide, and 4.75 ft

high. It is comprised of two main sections, the main exposure chamber and the equipment exposure chamber. Since the XMX has a high primary flow rate, is relatively large, and generates a large degree of heat, the ATC needed to be sufficiently large otherwise it may negatively impact the aerosol distribution, ATC environmental conditions, and the experiment data collection. The wind speed in the ATC was maintained at 8 kilometers per hour (kph) $\pm$ 0.2 kph; temperature at 20.6 degrees Celsius ($\pm$ 0.2 degree C); and humidity at 56% ($\pm$ 1.0 %).

Surrogates of viable biological agents were used to avoid risks associated with using bioagent aerosols rated BSL-2 or higher. Male Specific Coliphage 2 (MS2), American Type Culture Collection (ATCC) 15597-B1, was selected as the surrogate viral agent and was prepared in house. Various studies (Cooper, 2010; Riemann and Cliver, 2006) have shown why MS2 is a viable viral agent surrogate. *Bacillus thuringensis kurstaki* (BTK), ATCC 33679 (Biologics Process Development Inc, Poway, CA, USA), was selected as the bacterial agent for this study. Greenberg et al. (2010) has demonstrated that *Bacillus thuringensis* provides the best overall non-pathogenic surrogate for a spore-forming bacteria such as *Bacillus Anthracis*.

Test aerosols were generated in-house by EPA personnel. MS2 was prepared by using MS2 bacteriophage. First, a 1L sterile Nalgene® bottle was used then 20 mL of MS2 fitrate (3 x 10^9) was mixed with 500 mL of Luria-Bertani broth media. Two collison bottles were created each containing 250 mL of the mixture. For the BTK, two centrifuge bottles of BTK solid spores were re-suspended using 2L of sterile distilled water in a sterile 2L Nalgene® bottle. Two collison bottles were created each containing 250 mL of the solution.

The two collison bottles were placed in the main exposure chamber and a collison nebulizer was utilized to generate the aerosol. The aerosol passed through an interface separating the main exposure chamber and the equipment exposure chamber. Once passing

through the interface, the exposure equipment of concern was exposed to the aerosols for collection.

The layout of the equipment exposure chamber is shown in Figure 2. Three SKC Biosamplers® (SKC Inc., Eighty Four, PA, USA) were placed side-by-side 42" downwind of the honeycomb screen, mounted with the inlets 22.5" above the floor. Three Biocapture 650s were placed on an open metal shelf 117" downwind of the honeycomb screen and were mounted 10.5" above the floor. A collection nozzle for the Ultraviolet-Aerodynamic Particle Sizer (UV-APS) (TSI, Shoreview, MN, USA) was placed near the inner Biocapture 650. Three XMX/2L-MILs were placed 197" downwind of the honeycomb screen. All samplers were 15" apart across the width of the chamber.

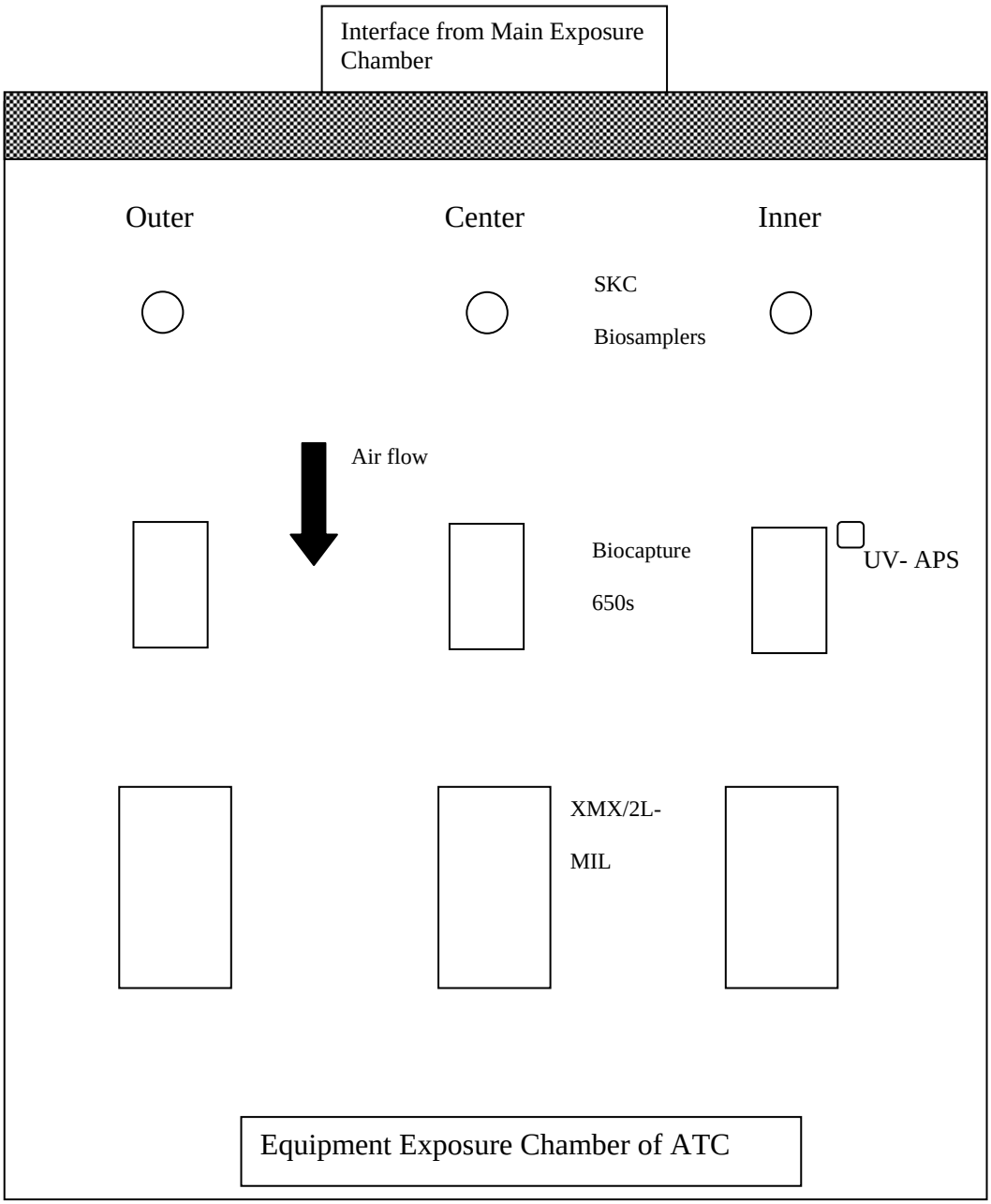

Figure 2: Diagram ATC Layout

Depending on the test aerosol being used, the high-volume air samplers (HVSs) were operated with different collection media, different secondary flow rates, or for different sample

collection periods. The XMX/2L-MIL had a sample collection time of 5 minutes whereas the Biocapture 650 varied between 5 and 30 minutes. Different sampling periods were tested to evaluate the impact of sampling time on collection efficiency. Table 1 shows the eight test configurations. The Biosamplers® sampled for a time period equal to the longest sampling period between the two HVSs.

Table 1: Test Configurations

Equipment	Surrogate	Configuration	Collection Media	Sample Time	Number of Test Runs
XMX/2L-MIL	Viral (MS2)	Reduced Secondary Flow	Remel M5	5 minutes	8
XMX/2L-MIL	Viral (MS2)	Reduced Secondary Flow	PBS	5 minutes	8
Biocapture 650	Viral (MS2)	NA	PBS	5 minutes	6
Biocapture 650	Viral (MS2)	NA	PBS	15 minutes	5
Biocapture 650	Viral (MS2)	NA	PBS	30 minutes	1
XMX/2L-MIL	Bacterial (BTK)	Full Secondary Flow	PBS	5 minutes	6
XMX/2L-MIL	Bacterial (BTK)	Reduced Secondary Flow	PBS	5 minutes	6
Biocapture 650	Bacterial (BTK)	NA	PBS	30 minutes	8

For several test runs, a flow reducer was used with the XMX/2L-MIL to reduce the secondary flow rate from approximately 12.5 lpm to 4 lpm. Two studies with MS2 (Cooper, 2010; Black, 2011) have demonstrated that this modification results in a higher collection efficiency compared to using the full secondary flow rate.

Additionally, several tests were conducted to compare the collection efficiency of different collection media, specifically the commonly used Phosphate Buffer Solution (PBS) and a virus preserving media, Remel MicroTest M5 Multi-Microbe Media (Remel M5). The procedures for preparing the collection media were consistent with those documented in previous

studies (Cooper, 2010; Black, 2011; Black, 2011). Operation and decontamination of the XMX/2L-MIL have been discussed in detail elsewhere (Cooper, 2010; Black, 2011). The Biocapture 650 was operated consistent with manufacturer instructions. Decontamination was done by removing the expendable sampling cartridge and then wiping the casing with a 10% solution of bleach and water before installing a new sampling cartridge.

For each sampling run, the collison nebulizer was turned on and allowed to generate aerosol for 5 minutes. After 5 minutes and confirmation from the UV-APS that an adequate aerosol was present in the test chamber, the Biosamplers®, Biocapture 650s, and XMX/2L-MILs were turned on near simultaneously. The samplers were then allowed to run for their specified time periods and then turned off. After sampling was complete, the aerosol generator was turned off and the sample containers were removed consistent with manufacturer instructions. The sample vials were immediately taken to the EPA microbiology lab where analysis could be initiated.

Viable analysis was conducted by EPA personnel. Viable analysis for BTK was completed by using cell culture on growth media. For MS2, viable analysis was done using plaque assay. The validated and appropriateness of these methods have been outlined in other literature (Adams, 1959; Fatah et al., 2005; Cooper, 2010). Total analysis (viable and non-viable) was done by the Research Triangle Institute using RT-PCR, a process covered in previous studies (O'Connell, et al., 2006; Cooper, 2010). The RT-PCR DNA extraction process for BTK involved the addition of zirconia beads and milling for about 60 seconds, followed by centrifugation and filtering to remove debris. RNA extraction of MS2 was done using the ZR Viral RNA Kit from Zymo Research and the manufacturer's protocol. RT-PCR amplifications were done using the iScript one-step RT-PCR kit with SybrGreen for MS2 and the SybrGreen

Sso Fast EvaGreen Supermix for BTK (Bio-Rad, Hercules, CA, USA). Reactions were set up in a 96-well format using 2.5 µl of each extract and plates were run on a Bio-Rad iCycler. Each plate included a standard curve with known concentrations generated by serial dilutions of a stock sample. Total analysis was done for one Biosampler®, two Biocapture 650s, and one XMX/2L-MIL per test run.

Results of laboratory analysis were provided by EPA personnel and analyzed in several ways. First, analysis was done of the Total Count, T_c, the total number of plaque forming units (PFU) or colony forming units (CFU), collected by each sampler. Second, analysis was done of the Total Concentration, the total count divided by the total volume of air sampled. The secondary flow rate of the XMX/2L-MIL was used for this calculation. These results were determined for both the viable analysis and the total (RT-PCR) analysis.

The results were then statistically evaluated to determine if there was a significant difference in the values between samplers, sampling times, configurations, or collection media. Statistical analysis was done using Minitab statistical software. Both a one-way analysis of variance (ANOVA) and a Kruskal-Wallis (K-W) ANOVA were calculated. Additionally, for the one-way ANOVA, the Anderson-Darling statistic was determined to test for normality. As will be described in the results section, either the one way ANOVA result or the K-W ANOVA result was used for analysis depending on the results of the normality test.

Results

Air Sampler Flow Rate

Flow rates for the XMX/2L-MIL using a HI-Q Flow Calibrator (HI-Q Environmental Products, San Diego, CA, USA). Three flow rates were measured for each XMX/2L-MIL: total flow, full secondary flow (FF), reduced secondary flow (RF). The flow rates are the mean of ten

measurements and are given in SLPM. For the Biocapture 650, the manufacturer advertised flow rate of 200 lpm was used. Table 2 below summarizes the flow rates for the XMX/2L-MILs.

Table 2: XMX/2L-MIL Flow Rates

XMX Position	Total Flow Rate (slpm)	Full Secondary Flow Rate (slpm)	Reduced Secondary Flow Rate (slpm)
Inner	545.5	13.727	3.761
Center	443.7	13.705	3.971
Outer	579.9	14.034	3.814

Comparison of Air Sampling Equipment:

Data sets for each surrogate were split into four total categories. First was based on laboratory analysis technique. The categories were Viable and Total (viable and non-viable), Total representing the results of the RT-PCR. The other categories were defined as Operational Performance, based on total count, T_c, and Viability Maintenance, based on concentration per liter of air sampled. As such, there were four data sets evaluated for each surrogate: Viable-Operational Performance, Total-Operational Performance, Viable-Viability Maintenance, and Total-Viability Maintenance. Statistical comparisons were done first for all configurations, time periods, and collection media against each other, then individual head-to-head comparisons of each. For all statistical comparisons, a $\alpha = 0.05$ was used.

Analysis of the SKC biosampler viable/non-viable T_c suggests that the aerosol generation was consistent on each day of the test trials. However, this is based on a small data set of 3-4 samples per day. Additionally, while the aerosol generation process remained consistent throughout the process, minor differences, such as height difference within the collisons between the air flow inlet and liquid mixture, could have resulted in different aerosol generation amounts

from run to run. Analysis of the UV-APS data showed a geometric mean (GM) particle diameter between 0.88 -1.18 μm for BTK with a geometric standard deviation between 1.61-1.7. For MS2, the GM was between 0.82-1.05 um with a GSD between 1.33-1.54. This met the goal of a GM particle diameter between 1-2 μm.

Comparison of Collection of BTK Aerosol:

Viable-Viability Maintenance:

The K-W ANOVA statistic was used for all cases, except the comparison by equipment location for the Biocapture 650 and the XMX/2L-MIL (FF), which was determined to be normally distributed and thus the one-way ANOVA statistic was used. It should be noted that in all cases, the K-W ANOVA statistic and the one-way ANOVA statistic provided similar results. Evaluation of each Biocapture 650 and XMX/2L-MIL by configuration showed there was no significant difference based on equipment location. Combined with studies by Black (2011) that showed a fixed final nozzle orientation within the XMX/2L-MIL statistically minimized inter-instrument variability, this would suggest that the aerosol in the ATC was well-mixed. Comparative analysis between all samplers showed a significant difference ($p = 0.001$) in the median standardized BTK collection. A head-to-head comparison by pairs of each of the three configurations showed a significant difference between the Biocapture 650 vs. XMX/2L-MIL (FF) ($p = 0.003$) and the Biocapture 650 vs. XMX/2L-MIL (RF) ($p = 0.001$). In both cases, the Biocapture 650 had a higher median collection of CFU/L air as shown in Figure 3 below.

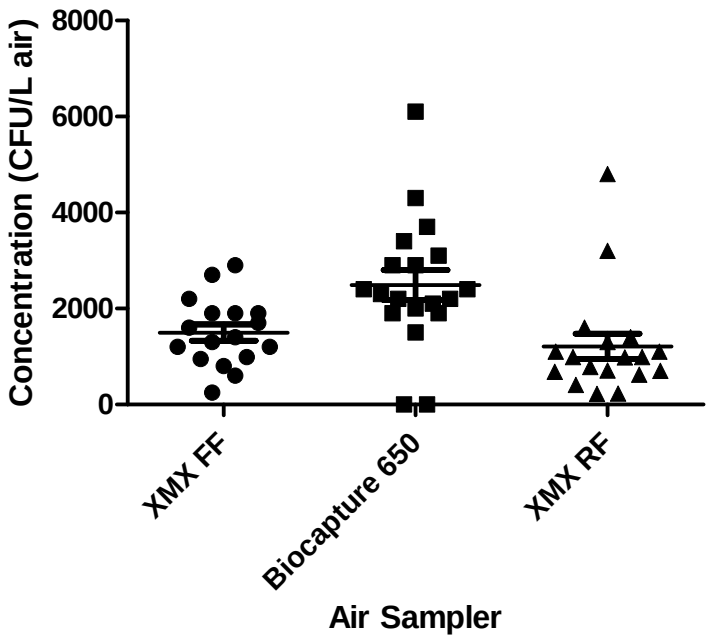

Figure 3: BTK Viable Concentration by Air Sampler

Total-Viability Maintenance:

Again, the K-W ANOVA statistic was predominantly used. As before, the K-W ANOVA and one-way ANOVA provided similar results. When evaluated together, the three tested configurations showed to have no significant difference in sampled concentration; despite a one order magnitude difference in median concentrations between the XMX/2L-MIL (RF) and the other two configurations. However, when compared head-to-head, the K-W ANOVA (α = 0.05) showed a possible significant difference ($p = 0.037$) between the XMX/2L-MIL (RF) and the Biocapture 650. The apparent conflicting results will be discussed later in the Discussion section. The median concentrations are shown in Figure 4 below.

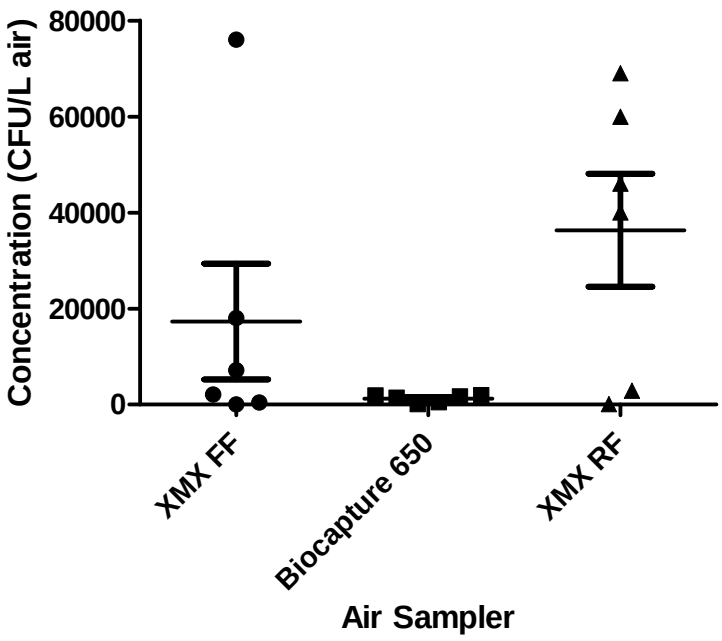

Figure 4: BTK Total Concentration by Air Sampler

Viable- Operational Performance

For comparison of T_c (total CFUs) for the viable analysis, the K-W ANOVA statistic was used. The comparison between XMX/2L-MIL (RF) vs. XMX/2L-MIL (FF) had differing results for the K-W ANOVA and the one-way ANOVA. However, the distribution of the data was not normal, thus suggesting the K-W ANOVA statistic was more appropriate. Overall, there was a significance difference between the Biocapture 650 and the XMX/2L-MIL in either configuration ($p = 0.000$ for all comparisons), confirmed by a Dunn's post test rank sum. A plot of the median total counts is shown in Figure 5.

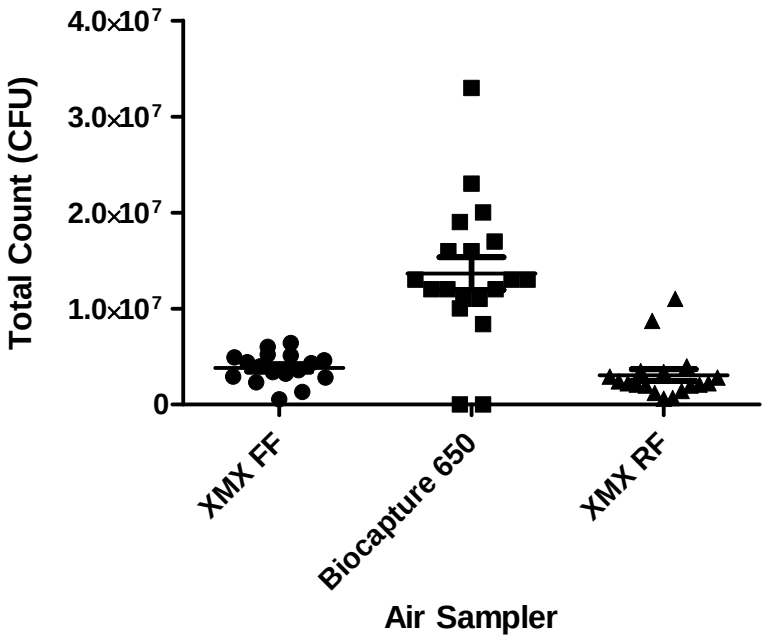

Figure 5: BTK Viable T_c by Air Sampler

Total- Operational Performance

The results of the K-W ANOVA showed no significant difference between the median values for the total count from the RT-PCR analysis. The graph of median counts is presented in Figure 6 below.

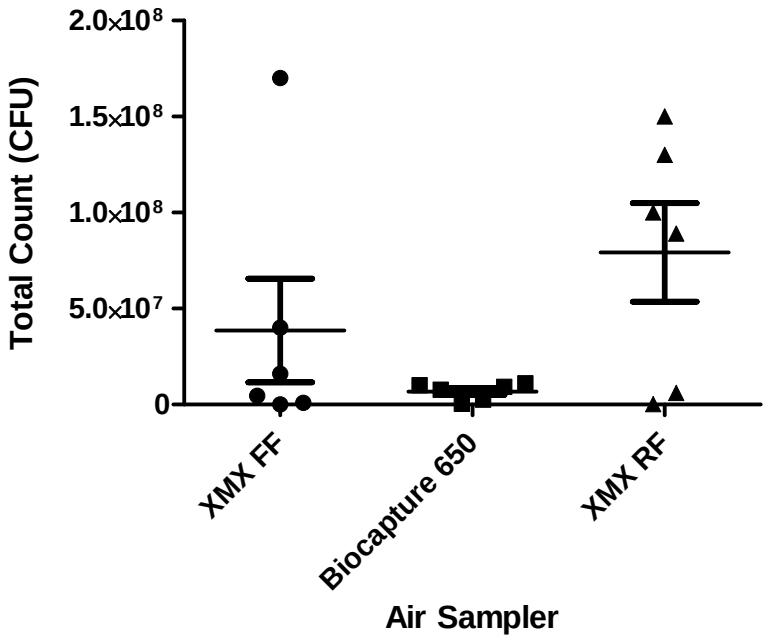

Figure 6: BTK Total T_c by Air Sampler

Comparison of Collection of MS2 Aerosol:

Before providing the results of the MS2 analysis, it was observed during laboratory analysis that the viability of the MS2 varied greatly during the sampling test runs. It was determined that the viability decreased over time if the same stock solution was used on subsequent days. As such, there were several days were the MS2 was not viable and plaque assays provided total counts of zero. On other days, the viability results were classified as "too numerous to count" when undiluted, but zero when diluted. Therefore a total count of 300 PFUs, the standard convention for EPA lab personnel for cultures "too numerous to count", was used for those events. Clearly, this would impact the analysis of the viability results which will be discussed later.

Viable-Viability Maintenance:

As previously discussed, there were several days where culturable analysis was not possible. The majority of these samples were for the Biocapture 650 15-minute exposure samples. Since there were few data available for the Biocapture 650 15-minute samples, they were not included in the statistical analysis. The K-W ANOVA was used for all results. For the results that were available, it was again determined that the location of samplers did not result in a significant difference, again suggesting the wind tunnel was well-mixed. When the three configurations, Biocapture 650- 5-minutes (BC 5 min), XMX/2L-MIL with PBS media (XMX-PBS), and XMX/2L-MIL with Remel M5 media (XMX-M5) were compared, they were found to be statistically the same. However, when compared head-to-head in pairs, a statistical difference was found between the BC 5 min and the XMX-PBS results. Despite this, the head-to-head comparison of BC 5 min to XMX-M5 and XMX-M5 to XMX-PBS showed them to be statistically the same. Furthermore, a Dunn's post test showed no significant difference between the three data sets. Possible causes of the conflict will be addressed in the Discussion section. The plot of median collected concentrations is shown in Figure 7.

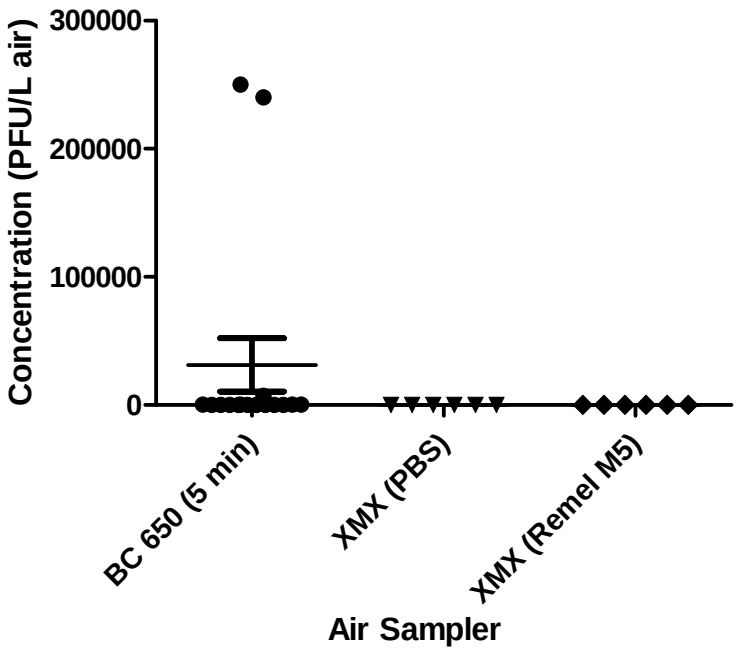

Figure 7: MS2 Viable Concentration by Air Sampler

Total-Viability Maintenance:

For the Total analysis, the Biocapture 650 15 minute (BC 15 min) samples were included in the statistical analysis. When all configurations were compared together as a group, the K-W ANOVA found that at least one data set was significantly different ($p = 0.006$). A Dunn's rank sum determined that XMX-PBS results were significantly different from both exposure periods for the Biocapture 650 with the XMX-PBS collecting a greater concentration. Head-to-head comparison by pairs confirmed a significant difference between the XMX-PBS and BC 15 min ($p = 0.007$) though one between the XMX-PBS and BC 5 min ($p = 1.000$) suggested they were statistically similar. Additionally, the head-to-head comparison between BC 15 min with XMX-M5 ($p = 0.030$) and BC 5 min ($p = 0.006$) also showed the collected concentration of BC 15 min to be significantly less. Figure 8 depicts the median concentrations for each of the air samplers.

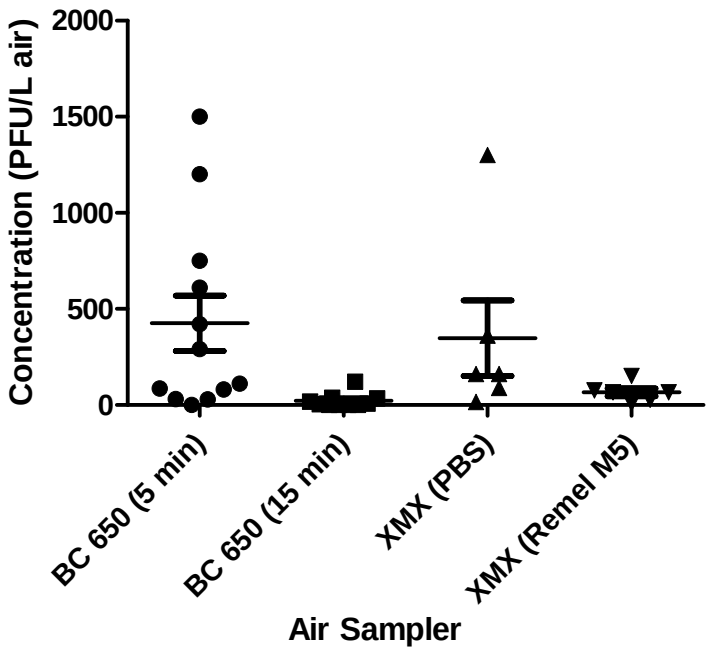

Figure 8: MS2 Total Concentration by Air Sampler

Viable- Operational Performance:

As with the viability maintenance, the BC 15 min results were not included in the analysis. Utilizing the K-W ANOVA, the various comparisons, both as a group and in pairs found no significant statistical difference in Total PFU Counts. Figure 9 shows the median counts.

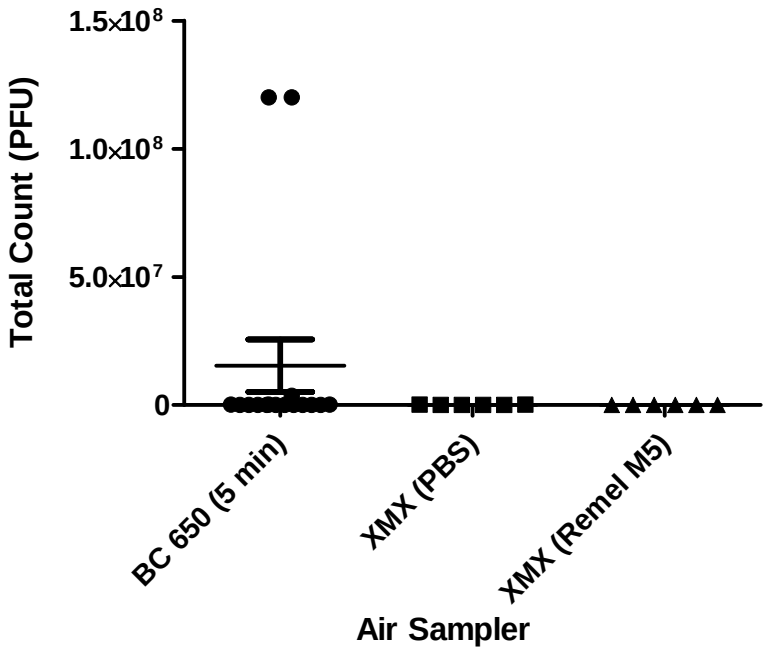

Figure 9: MS2 Viable T_c by Air Sampler

Total- Operational Performance:

As with the viability maintenance, when all data sets were compared together, at least one data set ($p = 0.025$) was found to be significantly different from the rest. The Dunn's rank sum for the group determined the XMX-PBS result to be significantly greater than the total PFU counts for the BC 15 min. The head-to-head K-W ANOVA also showed that the XMX-PBS total count was statistically greater than that of the BC 15 min ($p = 0.007$). Furthermore, the head-to-head comparisons of BC 15 min with the XMX-M5 ($p = 0.030$) also showed it collected a significantly less amount of bacteriophage. The total counts for each air sampler configuration is shown in Figure 10.

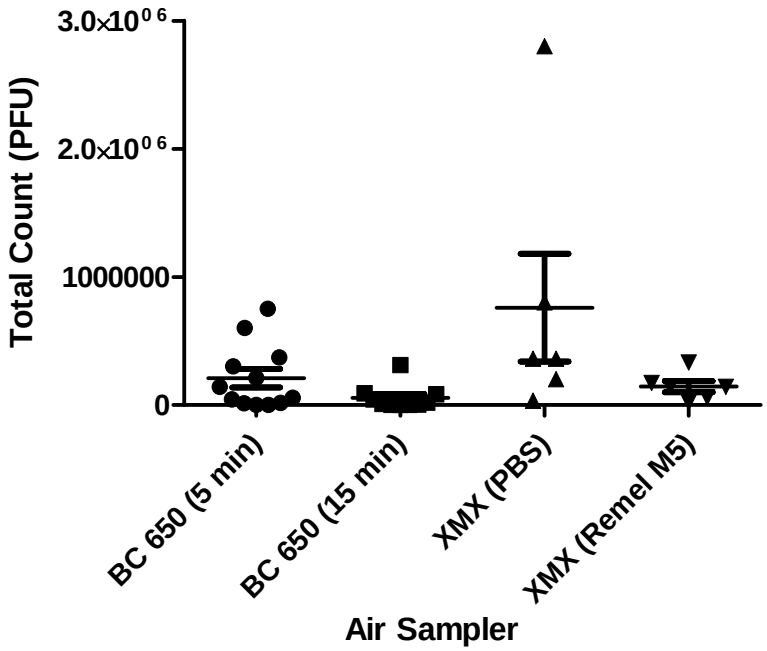

Figure 10: MS2 Total T_c by Air Sampler

Discussion

As previously mentioned, two parameters of collection results were identified for analysis. The first was viability maintenance and was represented by the collected concentration, PFU or CFU per liter of air sampled (PFU/L air or CFU/L air). As discussed, the different samplers each have different flow rates and amounts of air sampled. This parameter was meant to standardize the results based on the amount of air sampled to provide for a more direct comparison of results. The second parameter was deemed operational performance. This parameter looked only at the total count, T_c, (PFU or CFU), independent of how much air was sampled. This parameter has value as in various cases, such as a response to a terrorist use of biological warfare agents, where simply collecting enough particles to provide a positive identification of the threat agent is the goal.

Comparison of Collection of BTK Aerosol:

Viability Maintenance:

Based on the results, the Biocapture 650, when operated for a 30-minute sampling period, collected a significantly greater concentration of viable bacteria than the XMX/2L-MIL regardless of the secondary flow rate used. When compared to one another, there was no significant difference in the collected viable concentration between the two configurations of the XMX/2L-MIL. The XMX/2L-MIL results are especially interesting as studies by Cooper (2010) suggested that a reduced secondary flow rate could have been responsible for a higher concentration ratio when sampling MS2 bacteriophage. However, that study had limited data points. Regardless, the results of this study suggest that XMX/2L-MIL secondary flow rate is inconsequential in collection of viable aerosolized bacteria.

While the Biocapture 650 outperformed the XMX/2L-MIL in concentration collection of viable particles, it did not do so for total collection of bacteria. In this case, the group comparison found no significant difference between the median concentrations for the two XMX/2L-MIL configurations and the Biocapture 650. However, a direct comparison between the XMX/2L-MIL (RF) vs. the Biocapture 650 did find a significant difference. One explanation for why the significant difference was noted in a pairs comparison, but not group comparison, is that the inclusion of the data for the XMX/2L-MIL (FF) created statistical noise that made it more difficult to discern the difference between the XMX/2L-MIL (RF) and the Biocapture 650. Once removed, the statistical difference was more clearly identified. Regardless, in collection of total concentration, the Biocapture 650 performed on par with the two XMX/2L-MIL collection methods at best.

Operational Performance:

As with viability maintenance, the Biocapture 650 collected a significantly greater number of particles compared to either configuration of the XMX/2L-MIL. This is not particularly surprising as the Biocapture 650 sampled twice the amount of air as the XMX/2L-MIL, 5500 liters vs. 2218-2900 liters, thus it would be anticipated to have a higher T_c. However, whereas the collected concentration for the two XMX/2L-MIL configurations was statistically similar, the XMX/2L-MIL (FF) had a statistically greater total count compared to the XMX/2L-MIL (RF). Regardless, each configuration provided an adequate sample for analysis so while the results may be statistically significant they may not be operationally significant. For total (viable and non-viable) particle count, there was no significant difference between any of the air samplers.

Summary (BTK):

Overall, the best air sampler is dependent on the situation and objectives of the air sampling. The Biocapture 650 would appear to be the preferred equipment choice as it performed better, or at least even with the XMX/2L-MIL in both viability maintenance and operational performance. However, this is based on 30 minute sampling periods for the Biocapture 650 compared to 5 minutes sampling periods with the XMX/2L-MIL. It would be of interest to determine if the Biocapture 650 continued to perform on par with the XMX/2L-MIL using its shorter sampling periods. Furthermore, the XMX/2L-MIL performed adequately at the exposed concentrations and did so sampling for one-sixth of the time. If the length of sampling time is a consideration, the XMX/2L-MIL may be a more applicable choice.

Comparison of Collection of MS2 Aerosol:

Viability Maintenance:

With regards to viable particles, the Biocapture 650 may have collected a higher concentration than the XMX/2L-MIL when using PBS collection media. However, because of the highly varied viability of the stock solution, this analysis is unreliable. For total (viable/non-viable particles), the XMX/2L-MIL operated with either media and the Biocapture 650 when operated for 5 minutes all performed better than the Biocapture 650 when operated for 15 minutes. The statistical difference between the shorter and longer collection periods for the Biocapture 650 is especially noteworthy. As mentioned previously, while the Biocapture does provide a liquid sample for analysis, it is truly a dry collection method. Previous studies (Downie, 1965; Tseng & Li, 2005) have shown that dry collection methods recover significantly less aerosolized virus or MS2 compared to wet collection methods. The Biocapture 650's shorter sampling period being statistically better than the longer sampling period suggests that operating the Biocapture 650 longer could result in the loss of aerosolized virus particles. This could be due to physical loss of particles because of the operational collection method or degradation to the virus caused by the collection method.

Operational Performance:

For the total count of viable particles, the results of the analysis are again unreliable because of the variability in viability of the stock solution. While no statistical difference was identified, these results cannot be conclusively supported. With regards to total (viable/non-viable) particle counts, it again appears that the XMX/2L-MIL, with either collection media, and the 5-minute collection period for the Biocapture 650 outperformed the 15-minute collection period of the Biocapture 650.

Summary (MS2):

Analysis of results for viable particles was made unreliable by the high variation in viability of the stock MS2 solution. For total particles, the Biocapture 650, when operated for the longer collection period, was inferior to all other configurations. It would be of interest to determine if total (viable/non-viable) particles and concentration continued to decrease for longer collection periods of the Biocapture 650, specifically the 30 minute sampling mode.

Conclusions

This study evaluated and compared two high-volume air samplers under different configurations in their collection of an aerosolized biological agent. The XMX/2L-MIL was evaluated using different collection media for viruses and different secondary flow rates for bacterial sampling. Because of the variability in the MS2 stock solution's viability, it cannot be determined with any confidence whether the virus preserving media was more effective than the standard PBS solution. The results of the RT-PCR analysis showed no statistical difference in the collection of viable and non-viable particles.

The secondary flow modifications to the XMX/2L-MIL appear to have little influence on the collection of aerosolized bacteria. There was no significant difference in the collected concentrations between the two configurations. Results suggest that the full secondary flow results in a larger viable particle count, but there was no significant difference in the collection of total particles.

The Biocapture 650 outperformed the XMX/2L-MIL configurations in collection of viable bacteria, but performed on par with the XMX/2L-MIL's reduced secondary flow configuration in collection of viable and non-viable particles. This is based on a longer sampling period and amount of air sampled by the Biocapture 650. For viral particles, a longer sampling

period for the Biocapture 650 appears to be less efficient than a shorter sampling period as well less than the XMX/2L-MIL. Although the results presented are limited to two surrogate biological agents, the relative collection efficiencies, including the possible impact on virus degradation, could be relevant and applicable to other biological aerosols.

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III. Conclusion

Chapter Overview

This chapter discusses the research findings as they pertain to the research questions originally posed in Chapter 1. The scholarly article submitted to *Aerosol Science and Technology* communicates the prominent findings of the research. However, due to certain restrictions such as manuscript length and specific questions applicable to the United States Air Force (USAF), the article does not answer the fourth research question, nor does it address certain concerns of the Biocapture 650 found during experimentation. This chapter first briefly covers a review of findings of the study pertaining to the unaddressed research question then will address the significance of the research. Recommendations for future research and a summary of the thesis will conclude this thesis.

Review of Findings

Four research questions were posed at the beginning of this thesis. Three of those questions were addressed in the previous chapter. The fourth question was "What would be gained/lost by switching from the XMX/2L-MIL to the Biocapture 650". There are several factors that need to be considered when answering this question.

Before answering this question, additional information not provided in the article is necessary. The USAF medical and Civil Engineering (CE) communities are tasked with conducting detection, identification, and presence/absence of biological hazards to personnel and the general public. The USAF inventory currently includes three pieces of equipment, the XMX/2L-MIL which is used by the medical responders and the DFU and Biocapture 650

utilized by the CE responders. As noted by Hermann and co-workers (2006), detection and quantification of an airborne virus "is dependent on three primary factors; the concentration of the airborne virus, the collection efficiency of the air sampling system, and the analytical sensitivity of the diagnostic assay". However, the USAF's laboratory methods do not lend themselves toward quantification of infectivity. As such, the primary concern of biological air sampling is to obtain a large enough total count of viable/non-viable particles to get a positive identification on either the Hand-Held Assays (HHAs) or laboratory equipment.

The first factor requiring consideration is the reliability of the Biocapture 650. A total of 62 samples were taken with the Biocapture 650, including trial runs. Of these 62 samples, the Biocapture 650 failed to provide an adequate liquid sample 15 times (24.2%). An adequate sample was defined as either enough liquid to conduct laboratory analysis. Thirteen times the Biocapture 650 failed to provide any liquid sample and twice it provided amounts that were inadequate for laboratory testing. Of these 15 failures, only one can be clearly contributed to improper seating of the sampling cartridge by technicians. All sampling cartridges were within their expiration dates and two of the three Biocapture 650s used in the experiment were undergoing their first uses, the third Biocapture was approximately one year old and rarely used. It was also not consistent that one Biocapture failed more often than another suggesting a poor piece of equipment. The 15 failures were distributed with 7 by the center Biocapture, 6 by the outer, and 2 by the inner.

After the study, a call was made to FLIR (Bazzell, 2012), the Biocapture 650 manufacturer. The manufacturer's representative stated that they had identified a similar problem during a research study in Europe around the same time period as this study. They found that the failure of the cartridges to provide a liquid sample was attributed to a mismatch in

flow rates set into the sample cartridge and the main body of the Biocapture 650. Apparently, the error has been fixed and there have been no additional complaints of failing cartridges since the repair implementation.

With regards to sample collection, the Biocapture 650, 5 minute sampling period, performed on par with the XMX/2L-MIL when sampling MS2, but results were significantly less when operated for a 15 minute sampling period. The Biocapture 650, 30 minute sampling period, was more efficient at collecting viable BTK than the XMX/2L-MIL, but less so in total (viable/non-viable) collection. The current USAF CE plan of operation (CONOPs) for the Biocapture 650 calls for a standard 30-minute sampling period regardless of agent, situation, or location.

Considering only collection efficiency and operational modes, very little is gained by switching from the XMX/2L-MIL to the Biocapture 650. While the Biocapture 650 did collect more viable BTK, the HHAs used by the military for presumptive identification of biological agents are based on antibody response to antigens (the agent). This response is largely independent of whether the agent is viable or non-viable. Additionally, the USAF employs the Joint Biological Agent Identification and Diagnostic System (JBAIDS) to provide PCR analysis of biological warfare samples. As such, it detects both viable and non-viable agent without distinguishing between the two, making a higher viable collection rate less significant.

There are several disadvantages to switching to the Biocapture 650. First amongst these is time. Utilizing a standard collection time of 30 minutes is six times longer than that used by the XMX/2L-MIL. If time is of the essence, as it may be in an emergency response, this increased sampling time could delay the identification of the agent. Second, as suggested by the results for the MS2 collection, a longer sampling period may actually result in worse collection

efficiency for viruses. This could lead to a sample below the sensitivity for the HHA or the JBAIDS but may still be present and infective. If the suspected agent is an unknown, the Biocapture 650 may not be the best "first-use" air sampler.

Significance of Research

This research is significant in that it could impact how the USAF implements usage of these air samplers. Based on the results for the Biocapture 650, changes to the CE CONOPS may be necessary. If sampling an unknown agent, it may be prudent to start with a shorter sampling time and then work up to longer sampling times based on results of HHAs. The same may also be appropriate if the agent is known or suspected to be of a virus. This would be applicable to follow-up sampling after presumptive identification, additional sampling in other locations to determine areas impacted, or clearance sampling after mitigation and clean-up. If sampling is being done of a bacteria, particularly a spore-forming bacteria, the longer sampling period may be appropriate as it is not anticipated that the bacteria would be degraded in the same way as a virus because of the bacteria's resistive properties. The final disadvantage of switching to the Biocapture 650 is control of the sampling media. Currently, the Biocapture 650 comes pre-loaded with PBS solution with no possibility for change. If future studies determine that Remel M5 or another collection media is more efficient than PBS, those changes could be implemented when using the XMX/2L-MIL, but not with the Biocapture 650.

The results of the study pertaining to the XMX/2L-MIL suggest that secondary air flow modifications are not significant for bacteria. However, the modification was not statistically worse. As most potential uses of the equipment will pertain to sampling unknown agents, making the modification a standard practice could significantly improve collection efficiency for viruses, while not reducing it for bacteria. The study was unable to answer whether the virus preserving

media is more effective for viable particles. While there was no statistical difference in total (viable/non-viable) particles, being able to identify a higher viable concentration may aid in determining the relative infectivity of the agent present.

Recommendations for Future Research

There are several potential future research opportunities. The first of which is conducting additional experimentation to determine the collection efficiency of viable particles when using Remel M5 compared to PBS. Similarly, a laboratory study of different preservative collection media could prove useful in determining which possible medias may warrant further investigation. Additionally, it would be of interest to conduct further studies on the Biocapture 650 on its collection of aerosolized bacteria, when sampling for shorter time periods, such as 5 and 15 minutes. It would also be interesting to determine if degradation of viral samples continues when the Biocapture 650 is operated for 30 minutes. For future aerosol tests, it is recommended that a single chamber ATC that provides for precise calculation of generated aerosol be used as opposed to a multi-stage ATC. Finally, additional research could be done on possible bacterial preserving media as several have been identified in literature.

Appendix A: Trial Matrix and Schedule

Table 3: Sample Matrix and Schedule

Date	Phase	Number of Sample Runs	XMX Configuration	Biocapture Sampling Time
12-Sep-11	MS2 Testing	3	Reduced Secondary Flow w/ PBS media	5 minutes
13-Sep-11	BTK Testing	4	Reduced Secondary Flow w/ PBS media	30 minutes
14-Sep-11	BTK Testing	4	Full Secondary Flow w/ PBS Media	30 minutes
15-Sep-11	BTK Testing	2	Reduced Secondary Flow w/ PBS media	Did Not Run
15-Sep-11	BTK Testing	2	Full Secondary Flow w/ PBS Media	Did Not Run
19-Sep-11	Blank	1	Reduced Secondary Flow w/ PBS media	30 minutes
19-Sep-11	MS2 Testing	3	Reduced Secondary Flow w/ PBS media	30 minutes (1 run), 15 minutes (2 runs)
20-Sep-11	MS2 Testing	3	Reduced Secondary Flow w/ Remel M5 media	5 minutes
21-Sep-11	MS2 Testing	3	Reduced Secondary Flow w/ Remel M5 media	15 minutes
22-Sep-11	MS2 Testing	2	Reduced Secondary Flow w/ PBS media	Did Not Run
22-Sep-11	MS2 Testing	2	Reduced Secondary Flow w/ Remel M5 media	Did Not Run

Appendix B: Raw Data for Sample Analysis

Table 4: MS2 Raw Data for Sample Analysis

Device/Configuration	Viable Concentration (PFU/L air)	Viable Total Count (PFU)	RT-PCR Concentration (PFU/L air)	RT-PCR Total Count (PFU)
Biocapture (5-min)	61	30000	420.0	210000
Biocapture (5-min)	2	950	80.0	400
Biocapture (5-min)	53	26000	1200.0	600000
Biocapture (5-min)	56	28000	750.0	370000
Biocapture (5-min)	51	26000	85.0	43000
Biocapture (5-min)	21	10000	1500.0	750000
Biocapture (5-min)	30	15000	29.0	14000
Biocapture (5-min)	32	16000	290.0	140000
Biocapture (5-min)	250000	120000000	110.0	55000
Biocapture (5-min)	7200	3600000	31.0	16000
Biocapture (5-min)	240000	120000000	610.0	300000
Biocapture (5-min)	310	150000	0.6	290
Biocapture (5-min)	280	140000	NA	NA
Biocapture (5-min)	400	200000	NA	NA
Biocapture (5-min)	250	120000	NA	NA
Biocapture (5-min)	190	94000	NA	NA
Biocapture (15-min)	0	0	7.2	18000
Biocapture (15-min)	0	0	34.0	84000
Biocapture (15-min)	22	55000	120.0	310000
Biocapture (15-min)	21	53000	17.0	44000
Biocapture (15-min)	Not Culturable	Not Culturable	0.8	1900
Biocapture (15-min)	Not Culturable	Not Culturable	0.7	1700
Biocapture (15-min)	Not Culturable	Not Culturable	1.6	4100
Biocapture (15-min)	Not Culturable	Not Culturable	37.0	92000
Biocapture (15-min)	Not Culturable	Not Culturable	3.4	8600
Biocapture (15-min)	Not Culturable	Not Culturable	0.3	760
XMX (PBS Media)	77	170000	1300.0	2800000
XMX (PBS Media)	48	140000	15.0	33000
XMX (PBS Media)	23	51000	360.0	800000
XMX (PBS Media)	15	42000	89.0	200000
XMX (PBS Media)	27	60000	160.0	360000
XMX (PBS Media)	24	69000	160.0	360000
XMX (Remel M5)	56	120000	65.0	140000
XMX (Remel M5)	43	120000	65.0	140000

XMX (Remel M5)	56	120000	150.0	330000
XMX (Remel M5)	37	110000	76.0	170000.0
XMX (Remel M5)	56	120000	27.0	59000.0
XMX (Remel M5)	43	120000	9.6	21000.0

Table 5: BTK Raw Data for Sample Analysis

Device/Configuration	Viable Concentration (CFU/L air)	Viable Total Count (CFU)	RT-PCR Concentration (CFU/L air)	RT-PCR Total Count (CFU)
XMX (Full Flow)	1200	3200000	NA	NA
XMX (Full Flow)	2700	6000000	18000	40000000
XMX (Full Flow)	800	2300000	NA	NA
XMX (Full Flow)	1900	5200000	NA	NA
XMX (Full Flow)	600	1300000	7200	16000000
XMX (Full Flow)	950	2800000	NA	NA
XMX (Full Flow)	1300	3600000	NA	NA
XMX (Full Flow)	1900	4300000	76000	170000000
XMX (Full Flow)	990	2900000	NA	NA
XMX (Full Flow)	1600	4400000	NA	NA
XMX (Full Flow)	250	540000	48	110000
XMX (Full Flow)	1900	5100000	NA	NA
XMX (Full Flow)	2200	4900000	2100	4600000
XMX (Full Flow)	1200	3400000	NA	NA
XMX (Full Flow)	1700	4600000	NA	NA
XMX (Full Flow)	2900	6400000	400	880000
XMX (Full Flow)	1400	4000000	NA	NA
Biocapture	2900	16000000	NA	NA
Biocapture	2300	13000000	NA	NA
Biocapture	2000	11000000	1400	7700000
Biocapture	4300	23000000	480	2600000
Biocapture	1500	8400000	NA	NA
Biocapture	2400	13000000	NA	NA
Biocapture	3100	17000000	NA	NA
Biocapture	1900	10000000	NA	NA
Biocapture	0.5	2700	NA	NA
Biocapture	6100	33000000	NA	NA
Biocapture	2200	12000000	1900	10000000
Biocapture	2900	16000000	79	430000

Biocapture	3400	19000000	2000	11000000
Biocapture	2100	12000000	1700	9200000
Biocapture	1900	11000000	NA	NA
Biocapture	2200	12000000	NA	NA
Biocapture	0.3	1400	NA	NA
Biocapture	2400	13000000	NA	NA
Biocapture	3700	20000000	NA	NA
XMX (Reduced Flow)	1100	2900000	NA	NA
XMX (Reduced Flow)	990	2200000	69000	150000000
XMX (Reduced Flow)	700	2000000	NA	NA
XMX (Reduced Flow)	3200	8700000	NA	NA
XMX (Reduced Flow)	4800	11000000	60000	130000000
XMX (Reduced Flow)	680	2000000	NA	NA
XMX (Reduced Flow)	780	2100000	NA	NA
XMX (Reduced Flow)	1100	2400000	46000	100000000
XMX (Reduced Flow)	710	2100000	NA	NA
XMX (Reduced Flow)	1400	4000000	NA	NA
XMX (Reduced Flow)	990	2200000	40000	89000000
XMX (Reduced Flow)	410	1200000	NA	NA
XMX (Reduced Flow)	1300	3500000	NA	NA
XMX (Reduced Flow)	1600	3400000	86	180000
XMX (Reduced Flow)	980	2800000	NA	NA
XMX (Reduced Flow)	220	600000	NA	NA
XMX (Reduced Flow)	620	1400000	2900	6000000
XMX (Reduced Flow)	230	670000	NA	NA

Appendix C: Statistical Analysis

BTK Statistical Results

Table 6: One-Way ANOVA Analysis of Viable Concentration by Biocapture Location

```
Source                DF        SS       MS     F      P
Biocapture Location    2    675614   337807  0.16  0.852
Error                 16  33318597  2082412
Total                 18  33994211

S = 1443   R-Sq = 1.99%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N  Mean  StDev  ------+---------+---------+---------+---
Center  5  2620   2222    (----------------*----------------)
Inner   7  2643    905       (-------------*-------------)
Outer   7  2243   1201  (-------------*-------------)
                          ------+---------+---------+---------+---
                             1600      2400      3200      4000

Pooled StDev = 1443
```

Table 7: K-W Analysis of Viable Concentrations (All Samplers)

```
Device               N  Median  Ave Rank      Z
Biocapture          19  2300.0      37.9   3.58
XMX (full flow)     17  1400.0      25.6  -0.61
xmx (reduced flow)  18   985.0      18.4  -3.02
Overall             54              27.5

H = 14.63  DF = 2  P = 0.001
H = 14.65  DF = 2  P = 0.001  (adjusted for ties)
```

Table 8: Dunn's Multiple Comparison Test Viable Concentrations

Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P < 0.05?**
XMX FF vs Biocapture 650	-12.34	No
XMX FF vs XMX RF	7.198	No
Biocapture 650 vs XMX RF	19.53	Yes

Table 9: K-W Analysis of Viable Concentrations (BC vs. XMX FF)

```
Device            N  Median  Ave Rank      Z
Biocapture       19    2300      23.4   2.93
XMX (full flow)  17    1400      13.1  -2.93
Overall          36              18.5

H = 8.59  DF = 1  P = 0.003
H = 8.62  DF = 1  P = 0.003  (adjusted for ties)
```

Table 10: K-W Analysis of Viable Concentrations (BC vs. XMX RF)

```
Device               N  Median  Ave Rank      Z
Biocapture          19  2300.0      24.5   3.19
xmx (reduced flow)  18   985.0      13.2  -3.19
Overall             37              19.0

H = 10.18  DF = 1  P = 0.001
H = 10.19  DF = 1  P = 0.001  (adjusted for ties)
```

Table 11: K-W Analysis of Viable Concentrations (XMX RF vs. XMX FF)

```
Device               N  Median  Ave Rank      Z
XMX (full flow)     17  1400.0      21.5   1.96
xmx (reduced flow)  18   985.0      14.7  -1.96
Overall             35              18.0

H = 3.86  DF = 1  P = 0.050
H = 3.86  DF = 1  P = 0.049  (adjusted for ties)
```

Table 12: K-W Analysis of Viable Concentration by XMX RF Location

```
XMX (RF)
Location   N  Median  Ave Rank      Z
Center     6  1045.0      11.9   1.36
Inner      6  1200.0      11.3   0.98
Outer      6   690.0       5.3  -2.34
Overall   18               9.5

H = 5.53  DF = 2  P = 0.063
H = 5.54  DF = 2  P = 0.063  (adjusted for ties)
```

Table 13: One-Way ANOVA of Viable Concentrations by XMX FF Location

```
Source             DF       SS      MS     F      P
XMX (FF) Location   2  1393531  696765  1.46  0.266
Error              14  6681563  477255
Total              16  8075094

S = 690.8   R-Sq = 17.26%   R-Sq(adj) = 5.44%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean   StDev  --+---------+---------+---------+-------
Center  6  1758.3  1097.5               (-----------*-----------)
Inner   6  1600.0   296.6            (-----------*-----------)
Outer   5  1068.0   234.2  (------------*-------------)
                          --+---------+---------+---------+-------
                          500       1000      1500      2000

Pooled StDev = 690.8
```

Table 14: K-W Analysis of Total Concentrations (All Samplers)

Device	N	Median	Ave Rank	Z
biocapture	6	1550	6.2	-1.87
XMX (full flow)	6	4650	9.7	0.09
xmx (reduced flow)	6	43000	12.7	1.78
Overall	18		9.5	

H = 4.46 DF = 2 P = 0.108

Table 15: K-W Analysis of Total Concentration (BC vs. XMX FF)

Device	N	Median	Rank	Z
biocapture	6	1550	5.3	-1.12
XMX (full flow)	6	4650	7.7	1.12
Overall	12		6.5	

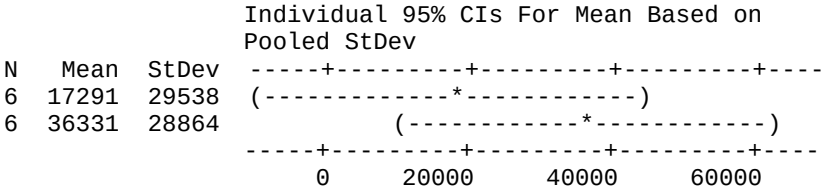
H = 1.26 DF = 1 P = 0.262

Source	DF	SS	MS	F	P
Device	1	1087526720	1087526720	1.28	0.285
Error	10	8528239083	852823908		
Total	11	9615765804			

S = 29203 R-Sq = 11.31% R-Sq(adj) = 2.44%

Table 16: One-Way ANOVA Analysis of Total Concentration (XMX RF vs. XMX FF)

Level	N	Mean	StDev
XMX (full flow)	6	17291	29538
xmx (reduced flow)	6	36331	28864



Pooled StDev = 29203

Table 17: K-W Analysis of Total Concentration (BC vs. XMX RF)

Device	N	Median	Rank	Z
Biocapture	6	1550	4.3	-2.08
xmx (reduced flow)	6	43000	8.7	2.08
Overall	12		6.5	

H = 4.33 DF = 1 P = 0.037

Table 18: One-Way ANOVA of Viable Total Count by Biocapture Location

```
Source                DF           SS           MS     F      P
Biocapture Location    2  1.68440E+13  8.42202E+12  0.14  0.872
Error                 16  9.73111E+14  6.08195E+13
Total                 18  9.89955E+14

S = 7798683   R-Sq = 1.70%   R-Sq(adj) = 0.00%


                                 Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level   N      Mean     StDev    -----+---------+---------+---------+----
Center  5  14200540  12049101      (------------------*-----------------)
Inner   7  14485714   4738294           (--------------*---------------)
Outer   7  12428771   6553364    (---------------*---------------)
                                 -----+---------+---------+---------+----
                                8000000  12000000  16000000  20000000

Pooled StDev = 7798683
```

Table 19: K-W Analysis of Viable Total Count (All Samplers)

```
Device               N    Median  Ave Rank      Z
Biocapture          19  13000000      41.1   4.68
XMX (full flow)     17   4000000      24.1  -1.09
xmx (reduced flow)  18   2200000      16.4  -3.67
Overall             54                27.5

H = 24.00  DF = 2  P = 0.000
H = 24.02  DF = 2  P = 0.000  (adjusted for ties)
```

Table 20: Dunn's Multiple Comparison Test Viable Total Count

Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P < 0.05?**
XMX FF vs Biocapture 650	-17.05	Yes
XMX FF vs XMX RF	7.67	No
Biocapture 650 vs XMX RF	24.72	Yes

Table 21: K-W Analysis of Viable Total Count (BC vs. XMX FF)

```
Device            N    Median  Ave Rank      Z
Biocapture       19  13000000      25.2   4.04
XMX (full flow)  17   4000000      11.0  -4.04
Overall          36                18.5

H = 16.32  DF = 1  P = 0.000
H = 16.34  DF = 1  P = 0.000  (adjusted for ties)
```

Table 22: K-W Analysis of Viable Total Count (XMX FF vs. XMX RF)

Device	N	Median	Ave Rank	Z
XMX (full flow)	17	4000000	22.1	2.28
xmx (reduced flow)	18	2200000	14.2	-2.28
Overall	35		18.0	

H = 5.19 DF = 1 P = 0.023
H = 5.19 DF = 1 P = 0.023 (adjusted for ties)

Table 23: K-W Analysis of Viable Total Count (BC vs. XMX RF)

Device	N	Median	Ave Rank	Z
Biocapture	19	13000000	25.9	3.98
xmx (reduced flow)	18	2200000	11.7	-3.98
Overall	37		19.0	

H = 15.85 DF = 1 P = 0.000
H = 15.88 DF = 1 P = 0.000 (adjusted for ties)

Table 24: K-W Analysis of RT-PCR Total Count (All Samplers)

Device	N	Median	Ave Rank	Z
Biocapture	6	8450000	7.7	-1.03
XMX (full flow)	6	10300000	9.0	-0.28
xmx (reduced flow)	6	94500000	11.8	1.31
Overall	18		9.5	

H = 1.91 DF = 2 P = 0.385

Table 25: K-W Analysis of RT-PCR Total Count (BC vs. XMX FF)

Device	N	Median	Rank	Z
Biocapture	6	8450000	6.0	-0.48
XMX (full flow)	6	10300000	7.0	0.48
Overall	12		6.5	

H = 0.23 DF = 1 P = 0.631

Table 26: K-W Analysis of RT-PCR Total Count (XMX RF vs . BC)

Device	N	Median	Rank	Z
Biocapture	6	8450000	5.2	-1.28
xmx (reduced flow)	6	94500000	7.8	1.28
Overall	12		6.5	

H = 1.64 DF = 1 P = 0.200

Table 27: K-W Analysis of RT-PCR Total Count (XMX RF vs. XMX FF)

Device	N	Median	Rank	Z
XMX (full flow)	6	10300000	5.5	-0.96
xmx (reduced flow)	6	94500000	7.5	0.96
Overall	12		6.5	

H = 0.92 DF = 1 P = 0.337

MS2 Statistical Results

Table 28: K-W Analysis of Viable Concentration by Biocapture Location

Biocapture Location (5 min)	N	Median	Ave Rank	Z
Center	6	120.5	7.2	-0.87
Inner	6	155.5	9.2	0.43
Outer	4	226.5	9.5	0.49
Overall	16		8.5	

H = 0.76 DF = 2 P = 0.682

Table 29: K-W Analysis of Viable Concentration (All Samplers)

Device	N	Median	Ave Rank	Z
Biocapture	16	125.50	17.3	2.11
XMX PBS	6	25.50	8.2	-2.13
XMX Remel M5	6	49.50	13.3	-0.42
Overall	28		14.5	

H = 5.61 DF = 2 P = 0.061
H = 5.62 DF = 2 P = 0.060 (adjusted for ties)

Table 30: K-W Analysis of Viable Concentration (BC vs. XMX PBS)

Device	N	Median	Ave Rank	Z
Biocapture	16	125.50	13.3	2.14
XMX (PBS)	6	25.50	6.7	-2.14
Overall	22		11.5	

H = 4.57 DF = 1 P = 0.033

Table 31: K-W Analysis of Viable Concentration (BC vs. XMX Remel M5)

```
Device          N  Median  Ave Rank      Z
Biocapture     16  125.50      12.5   1.22
XMX Remel M5    6   49.50       8.8  -1.22
Overall        22               11.5

H = 1.48  DF = 1  P = 0.224
H = 1.49  DF = 1  P = 0.222  (adjusted for ties)
```

Table 32: K-W Analysis of Viable Concentration (XMX PBS vs. XMX Remel M5)

```
Device          N  Median  Rank      Z
XMX PBS         6   25.50   5.0  -1.44
XMX Remel M5    6   49.50   8.0   1.44
Overall        12           6.5

H = 2.08  DF = 1  P = 0.150
H = 2.11  DF = 1  P = 0.146  (adjusted for ties)
```

Table 33: K-W Analysis of Total Concentration (All Samplers)

```
Device                 N   Median  Ave Rank      Z
Biocapture (15 min)   10    5.300       9.4  -3.28
Biocapture (30 min)    2   47.000      15.3  -0.50
Biocapture (5 min)    13  110.000      24.6   2.31
XMX (PBS)              6  160.000      26.3   1.81
XMX (Remel M5)         6   65.000      16.8  -0.54
Overall               37               19.0

H = 14.55  DF = 4  P = 0.006
H = 14.56  DF = 4  P = 0.006  (adjusted for ties)

* NOTE * One or more small samples
```

Table 34: Dunn's Multiple Comparison Test of Total Concentration

Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P < 0.05?**
BC 650 (5 min) vs BC 650 (15 min)	13.78	Yes
BC 650 (5 min) vs XMX (PBS)	-1.083	No
BC 650 (5 min) vs XMX (Remel M5)	6.917	No
BC 650 (15 min) vs XMX (PBS)	-14.87	Yes
BC 650 (15 min) vs XMX (Remel M5)	-6.867	No
XMX (PBS) vs XMX (Remel M5)	8	No

Table 35: K-W Analysis of Total Concentration (BC 5-min vs XMX PBS)

Device	N	Median	Rank	Z
Biocapture (5 min)	12	200.0	9.5	0.00
XMX PBS	6	160.0	9.5	0.00
Overall	18		9.5	

H = 0.00 DF = 1 P = 1.000
H = 0.00 DF = 1 P = 1.000 (adjusted for ties)

Table 36: K-W Analysis of Total Concentration (BC 5-min vs. XMX Remel M5)

Device	N	Median	Ave Rank	Z
Biocapture (5 min)	12	200.00	11.1	1.78
XMX Remel M5	6	65.00	6.3	-1.78
Overall	18		9.5	

H = 3.17 DF = 1 P = 0.075
H = 3.17 DF = 1 P = 0.075 (adjusted for ties)

Table 37: K-W Analysis of Total Concentration (BC 15-min vs. XMX Remel M5)

Device	N	Median	Ave Rank	Z
Biocapture (15 min)	10	5.300	6.5	-2.17
XMX (Remel M5)	6	65.000	11.8	2.17
Overall	16		8.5	

H = 4.71 DF = 1 P = 0.030
H = 4.71 DF = 1 P = 0.030 (adjusted for ties)

Table 38: K-W Analysis of Total Concentration (XMX PBS vs. XMX Remel M5)

Device	N	Median	Rank	Z
XMX (PBS)	6	160.00	8.5	1.92
XMX (Remel M5)	6	65.00	4.5	-1.92
Overall	12		6.5	

H = 3.69 DF = 1 P = 0.055
H = 3.72 DF = 1 P = 0.054 (adjusted for ties)

Table 39: K-W Analysis of Total Concentration (BC 15-min vs. XMX PBS)

Device	N	Median	Ave Rank	Z
Biocapture (15 min)	10	5.300	6.0	-2.71
XMX (PBS)	6	160.000	12.7	2.71
Overall	16		8.5	

H = 7.35 DF = 1 P = 0.007
H = 7.36 DF = 1 P = 0.007 (adjusted for ties)

Table 40: K-W Analysis of Total Concentration (BC 15-min vs. BC 5-min)

Device	N	Median	Ave Rank	Z
Biocapture (15 min)	10	5.300	7.3	-2.77
Biocapture (5 min)	12	200.000	15.0	2.77
Overall	22		11.5	

H = 7.67 DF = 1 P = 0.006

Table 41: K-W Analysis of Viable Total Count (All Samplers)

Device	N	Median	Ave Rank	Z
Biocapture (5 min)	16	62000	13.6	-0.70
XMX (PBS)	6	64500	14.6	0.03
XMX (Remel M5)	6	120000	16.9	0.81
Overall	28		14.5	

H = 0.73 DF = 2 P = 0.695
H = 0.73 DF = 2 P = 0.693 (adjusted for ties)

Table 42: K-W Analysis of Viable Total Count (BC 5-min vs. XMX PBS)

Device	N	Median	Ave Rank	Z
Biocapture (5 min)	16	62000	11.1	-0.48
XMX (PBS)	6	64500	12.6	0.48
Overall	22		11.5	

H = 0.23 DF = 1 P = 0.632
H = 0.23 DF = 1 P = 0.632 (adjusted for ties)

Table 43: K-W Analysis of Viable Total Count (BC 5-min vs. XMX Remel M5)

Device	N	Median	Ave Rank	Z
Biocapture (5 min)	16	62000	11.0	-0.63
XMX (Remel M5)	6	120000	12.9	0.63
Overall	22		11.5	

H = 0.39 DF = 1 P = 0.531
H = 0.40 DF = 1 P = 0.527 (adjusted for ties)

Table 44: K-W Analysis of Viable Total Count (XMX PBS vs. XMX Remel M5)

Device	N	Median	Rank	Z
XMX (PBS)	6	64500	5.5	-0.96
XMX (Remel M5)	6	120000	7.5	0.96
Overall	12		6.5	

H = 0.92 DF = 1 P = 0.337
H = 0.99 DF = 1 P = 0.319 (adjusted for ties)

Table 45: K-W Analysis of RT-PCR Total Count (All Samplers)

```
Device                N  Median  Ave Rank      Z
Biocapture (15 min)  10   13300      11.0  -2.46
Biocapture (5 min)   12   97500      17.5   0.00
XMX (PBS)             6  360000      26.5   2.44
XMX (Remel M5)        6  140000      19.3   0.50
Overall              34              17.5

H = 9.36  DF = 3  P = 0.025
H = 9.37  DF = 3  P = 0.025  (adjusted for ties)
```

Table 46: Dunn's Multiple Comparison Test of RT-PCR Total Count

Dunn's Multiple Comparison Test	**Difference in rank sum**	**Significant? P < 0.05?**
BC 650 (5 min) vs BC 650 (15 min)	6.5	No
BC 650 (5 min) vs XMX (PBS)	-9	No
BC 650 (5 min) vs XMX (Remel M5)	-1.833	No
BC 650 (15 min) vs XMX (PBS)	-15.5	Yes
BC 650 (15 min) vs XMX (Remel M5)	-8.333	No
XMX (PBS) vs XMX (Remel M5)	7.167	No

Table 47: K-W Analysis of RT-PCR Total Count (BC 5-min vs. XMX PBS)

```
Device                N  Median  Ave Rank      Z
Biocapture (5 min)   12   97500       8.1  -1.59
XMX (PBS)             6  360000      12.3   1.59
Overall              18                9.5

H = 2.54  DF = 1  P = 0.111
H = 2.54  DF = 1  P = 0.111  (adjusted for ties)
```

Table 48: K-W Analysis of RT-PCR Total Count (BC 15-min vs. XMX PBS)

```
Device                N  Median  Ave Rank      Z
Biocapture (15 min)  10   13300       6.0  -2.71
XMX (PBS)             6  360000      12.7   2.71
Overall              16                8.5

H = 7.35  DF = 1  P = 0.007
H = 7.36  DF = 1  P = 0.007  (adjusted for ties)
```

Table 49: K-W Analysis of RT-PCR Total Count (BC 15-min vs. XMX Remel M5)

```
Device                N  Median  Ave Rank      Z
Biocapture (15 min)  10   13300       6.5  -2.17
XMX (Remel M5)        6  140000      11.8   2.17
Overall              16                8.5

H = 4.71  DF = 1  P = 0.030
H = 4.71  DF = 1  P = 0.030  (adjusted for ties)
```

Table 50: K-W Analysis of RT-PCR Total Count (BC 5-min vs. XMX Remel M5)

Device	N	Median	Ave Rank	Z
Biocapture (5 min)	12	97500	9.3	-0.28
XMX (Remel M5)	6	140000	10.0	0.28
Overall	18		9.5	

H = 0.08 DF = 1 P = 0.779
H = 0.08 DF = 1 P = 0.778 (adjusted for ties)

Table 51: K-W Analysis of RT-PCR Total Count (XMX Remel M5 vs. XMX PBS)

Device	N	Median	Rank	Z
XMX (PBS)	6	360000	8.5	1.92
XMX (Remel M5)	6	140000	4.5	-1.92
Overall	12		6.5	

H = 3.69 DF = 1 P = 0.055
H = 3.72 DF = 1 P = 0.054 (adjusted for ties)

Table 52: K-W Analysis of RT-PCR Total Count (BC 15-min vs. BC 5-min)

Device	N	Median	Ave Rank	Z
Biocapture (15 min)	10	13300	9.5	-1.32
Biocapture (5 min)	12	97500	13.2	1.32
Overall	22		11.5	

H = 1.74 DF = 1 P = 0.187

Appendix D: EPA Protocols

Standard Operating Procedure for the Viable Count of Microorganisms

SOP-WDE-09-06
May 2009

Prepared for

National Homeland Security Research Center
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Contract EP-D-05-065

Aligned with your

P.O. Box 12313
Research Triangle Park, NC 27709

Standard Operating Procedure for the Viable Count of Microorganisms

Prepared by: April Corbett Date: 5/22/09

Reviewed by: William O. Ellenson Date: 5/22/09

Approved by: Paulette A. Yongue Date: 5/22/09

Prepared for

National Homeland Security Research Center
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

ALION
SCIENCE AND TECHNOLOGY
Aligned with your

P.O. Box 12313
Research Triangle Park, NC 27709

Contents

1.0 Scope and Application

The viable count method is used to determine the viability of a liquid culture.

2.0 Summary of Method

The viable count method is used to obtain microorganism counts of samples by diluting the samples and plating to enumerate the number of colony-forming units present. This SOP describes how to estimate the number of viable organisms present in a sample.

3.0 Definitions

BHI agar	brain heart infusion agar
BHI broth	brain heart infusion broth
CFU	colony-forming units
IPA	isopropyl alcohol
LBA	luria-bertani agar
LBB	luria-bertani broth
PBS	phosphate buffer saline
PFU	plaque-forming units
TNTC	too numerous to count
TSA	tryptic soy agar
TSAB	TSA w/ 5% sheep blood
TSB	tryptic soy broth
Viability	ability of an organism to reproduce under favorable conditions

4.0 Health and Safety Warnings

Standard laboratory personal protective equipment, including safety glasses and lab coats, should be worn at all times during the operation in accordance with the Health and Safety Protocol.

5.0 Cautions

5.1 All safety precautions for working with live microorganisms should be followed. The preparation and the plating of microorganisms should be performed inside a biosafety cabinet.

5.2 If a sample is spilled in the biosafety cabinet, clean the spill with 70% IPA or 1–3% sodium hypochlorite.

6.0 Personnel Qualifications

Personnel should be knowledgeable of the information in this SOP.

7.0 Apparatus and Materials

7.1 Equipment

Biosafety cabinet

Incubator (35 ± 2 ºC)

Vortex mixer

Calibrated micropipette

7.2 Supplies

Disposable sterile test tubes

Disposable sterile micropipette tips

Disposable sterile spreaders

Gloves

70% IPA

1–3% sodium hypochlorite

Sterile PBS

Top Layer agar

Agar plates (TSA, TSAB, BHI, LB, etc.)

Liquid medium (TSB, BHI broth, LB, etc.)

8.0 Sample Storage

All samples should be stored in a sterile test tube or sterile Whirl-Pak bag. Samples that cannot be processed the same day as collected must be stored at 2–8 ºC for a maximum of 24 hours.

9.0 Procedure

9.1 Preparation of Biosafety Cabinet and Items Used

All testing of samples should be done in the biosafety cabinet. To prepare, clean the biosafety cabinet with 70% IPA or 1–3% sodium hypochlorite. In addition, all items that will be used in the biosafety cabinet should be sanitized with 70% IPA or 1–3% sodium hypochlorite.

9.2 Spread Plate Method (Bacteria)

1. Label test tubes with sample identification and dilution factor.
2. Label duplicate plates with sample identification, dilution being plated, and date and initials.
3. Aseptically add the proper amount of PBS to each sterile test tube using a micropipette. The amount is determined by the analyst according to the desired final volume.

4. Perform a negative control of the diluent that is being used by plating 0.5 mL onto an agar plate and spreading evenly using a sterile spreader. The type of agar plate used will be the same as that used in the test session.
5. Perform a 1:10 serial dilution of the sample. Vortex the sample between each dilution. Continue the 1:10 dilutions to achieve a plate count of 30–300 cfu. The analyst may estimate the needed dilutions of an unknown sample by performing preliminary plating and counts of the neat sample and a 1:10 dilution.
6. Mix the sample prior to plating. Use a micropipette to transfer 0.1 to 0.5 mL of sample to the surface of an agar plate. Use a sterile spreader to evenly spread the sample on the agar plate to obtain isolated colonies.
7. Repeat steps 5 and 6 for each dilution to be plated.
8. Allow the surface of the agar plates to dry, invert the plates, and place them into the incubator.
9. Incubate bacterial plates at 35 ± 2 ºC for a maximum of 3 days.

9.3 Double Agar Plate Method (Bacteriophage)

1. Label test tubes with sample identification and dilution factor.
2. Label duplicate plates with sample identification, dilution being plated, and date and initials.
3. Aseptically add the proper amount of diluent to each sterile test tube using a micropipette. The amount is determined by the analyst according to the desired final volume.
4. Perform a negative control of the diluent that is being used by plating 0.5 mL onto an agar plate and spreading evenly using a sterile spreader. The type of agar plate used will be the same as that used in the test session.
5. Perform a 1:10 serial dilution of the sample. Vortex the sample between each dilution. Continue the 1:10 dilutions to achieve a plate count of 30–300 pfu. The analyst may estimate the needed dilutions of an unknown sample by performing preliminary plating and counts of the neat sample and a 1:10 dilution.
6. In a sterile tube, mix 1 mL of the host cells with 1 mL of the sample. Add the host cells first to avoid cross-contamination of the phage between samples.
7. Incubate the host/sample mixture at room temperature for 5 min to allow the phage in the sample to adsorb to the host (shaking is not necessary).
8. Add 3 mL of molten top-layer agar (no warmer than 47 °C) to the tube containing the host and sample, vortex briefly, and immediately pour the contents of the tube onto an agar plate. Spread the top-layer agar evenly by gently swirling the plate.
9. Allow the plate to sit undisturbed on a level surface for a few minutes until the top agar layer solidifies, and then incubate the plate at 35 ± 2 ºC a maximum of 3 days.

10.0 Troubleshooting

Refer to section 13 of this method.

11.0 Data Analysis and Calculations

1. After the incubation period, enumerate all colonies on countable plates (30–300 cfu).
2. Plates that are greater than the countable range are considered too numerous to count (TNTC).
3. Record results to two significant digits (e.g., 1.7×10^n) onto the viable count worksheet for the dilutions plated (see Appendix A).
4. Use the middle range countable plates to determine the cfu/mL by using the following formula:

$$\text{cfu/mL} = \frac{(\text{average cfu/plate})(\text{dilution factor})}{\text{volume plated}}$$

$$\text{Average cfu/plate} = \frac{\text{count of plate1} + \text{count of plate2}}{2}$$

5. Use the middle range countable plates to determine the pfu/mL by using the following formula:

$$\text{pfu/mL} = \frac{(\text{average pfu/plate})(\text{dilution factor})}{\text{volume plated}}$$

$$\text{Average pfu/plate} = \frac{\text{count of plate1} + \text{count of plate2}}{2}$$

6. Record calculations in the laboratory notebook and the viable count worksheet.

12.0 Data and Records Management

Record all data promptly and legibly in instrument logbooks and a personal logbook using permanent ink. Completed logbooks will be archived in the general archives.

13.0 Quality Control and Quality Assurance

13.1 The manufacturer's Certificate of Analysis will be accepted for the QC/QA of purchased media.

13.2 A negative control will be performed for each test session per section 9.2. No growth on the negative control validates results. If there is growth on the negative control, results are invalid and the test session will need to be repeated.

13.3 If any of the test results are contaminated, the results are invalid and the test session will need to be repeated.

Appendix A

Viable Count Worksheet

Date of test/by: _____ Sample ID: _____

Media/diluent lot #:_____

Date off test/by: _____ Negative Control Result: _____

Dilution	Amt. Plated	Plate 1 Count	Plate 2 Count	Average	cfu/mL or pfu/mL

$$\text{cfu/mL} = \frac{(\text{average cfu/plate})(\text{dilution factor})}{\text{volume plated}}$$

$$\text{pfu/mL} = \frac{(\text{average pfu/plate})(\text{dilution factor})}{\text{volume plated}}$$

Comments:

Standard Operating Procedure for Operation of the BGI Collison Nebulizer (1, 3, 6, or 24 Jets)

SOP-ZED-11-02
June 2011

Prepared for

National Homeland Security Research Center
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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Standard Operating Procedure for Operation of the BGI Collison Nebulizer (1, 3, 6, or 24 Jets)

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Contents

1.0 Scope and Application

The Collison nebulizer is used to generate aerosols in the Aerosol Test Facility. This SOP describes how to operate the Collison nebulizer to produce aerosols by atomizing liquids and suspensions.

2.0 Summary of Method

Nebulization is a second, or refined, atomization of a fluid. In an atomizer, a gas is used to aspirate the liquid into a sonic velocity gas jet, wherein it is sheared into droplets. In a nebulizer, this liquid/gas jet is then impacted against a barrier (the inside of the jar) to remove the larger fraction of the droplets.

3.0 Health and Safety Warnings

3.1 Standard laboratory personal protective equipment including safety glasses and lab coats should be used at all times during the operation in accordance with health and safety protocol.

3.2 ***The glass jar is not to be subjected to pressures above 1 psig under any circumstances.*** While the recommended pressure to be applied to the nebulizer may vary from 20 to 100 psig, this pressure is expanded to just above atmospheric inside the jar. Great care must be exercised at all times when connecting the nebulizer's output to other apparatus to ensure that the flow of gas out of the nebulizer is not restricted in any manner that will cause significant back pressure.

4.0 Cautions

4.1 Do not immerse the tip of the nozzle more than 3/8 inch below the surface.

4.2 The glass jar should not be subjected to pressures above 1 psig under any circumstances.

5.0 Personnel Qualifications

Personnel should be knowledgeable of the information in this SOP.

6.0 Equipment and Materials

Collison nebulizer

Clean, dry compressed air

Compression fittings to connect the equipment

Diffusion dryer

Aerosol neutralizer

7.0 Operation of the Collison Nebulizer

7.1 Setting up the System

Select an appropriate size nebulizer and jar based on the experimental design, output requirements, and sampling time.

Fit a precision pressure gauge between the source of clean, dry compressed air (house air or cylinder) and the plugged end of the "T". This is done by removing the plug screw by hand.

A schematic diagram of the basic setup is shown in Figure 1.

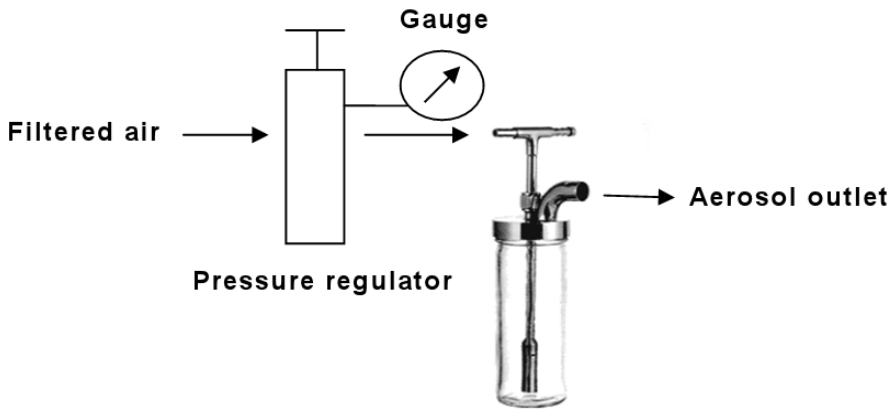

Figure 1. Schematic diagram of the Collison nebulizer setup.

Note: To produce a dry aerosol that is free of static charge, a diffuser dryer and aerosol neutralizer can be connected to the aerosol outlet of the Collison nebulizer.

7.2 Aerosol Liquid

No specific liquid level is recommended because the T may be pulled up or pressed down to a variety of positions. It is important to begin with the tip of the nozzle immersed no more than 3/8 of an inch below the surface. Deeper immersion will cause the surface of the liquid to interfere with the formation of the jet.

Figure 2. Cutaway view of the precious liquids jar.

For very small amounts of liquid, a precious liquids jar, made of fabricated Pyrex with a 5-mL well in the bottom, can be used as a replacement for the standard jar. It must be used in conjunction with the extension sleeve added to the bottom of the nozzle**.** The purpose of the sleeve is to permit the nozzle to operate in a position sufficiently elevated such that the spray does not interact with the bottom of the jar. The sleeve is slotted to permit bending the tabs

inwards for the purpose of achieving a firm fit between the sleeve and the nozzle. A cutaway picture of the precious liquids jar is shown in Figure 2.

7.3 Operation

Turn on the compressed air source and set the pressure gauge for volumetric output according to Table 1.

Table 1. Collison Nebulizer Volumetric Requirements

Pressure (psig)	Volume of Free Air (L/min)			
	1 Jet	3 Jets	6 Jets	24 Jets
20	2	6	12	48
40	3.3	10	20	80
60	4.5	13.5	27	NR
80	5.8	17.3	34.5	NR
100	7	21	42	NR

NR – not recommended

8.0 Troubleshooting

If the Collison nebulizer does not perform as described in section 7.0, refer to the nebulizer instructions for assistance.

9.0 System Maintenance

Basic cleaning functions can be performed by disconnecting the apparatus from the air hose and removing and emptying the jar. All components can then be immersed in a cleaning solution appropriate to the material being aerosolized. Ultrasonic cleaning is highly recommended. The nozzle may be removed from the T stem by hand. A small, custom-made O-ring is fitted to the stem in a groove above the threads to seal the juncture. If an internal jet becomes plugged, it may be cleared with the cleanout drill.

10.0 Data and Records Management

All data will be recorded promptly and legibly in instrument logbooks using permanent ink. Completed logbooks will be archived in the general archives.

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Vita

Major James Enderby is a 1998 graduate of the University of Colorado-Boulder where he received a Bachelor of Science degree in electrical engineering and a Masters of Business Administration, with an emphasis on computer resources/information management from Webster University (2011). Prior to his assignment at the AF Institute of Technology, he served as Bioenvironmental Engineering flight commander at Pope AFB, NC and occupational health, later readiness, element chief at Ramstein AB, Germany. Following completion of his studies at AFIT, his next assignment is projected to be the United States Air Force School of Aerospace Medicine located at Wright-Patterson AFB, OH.

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The United States Air Force has several high-volume biological air samplers, including the XMX/2L-MIL and the Biocapture 650. Limited information is available on each in its collection of viruses and bacteria. Previous research on the XMX/2L-MIL determined that modifications to the secondary flow rate and using a virus preserving collection media may provide improved virus collection rates. Here these modifications were investigated to determine the impact on the collection of viral and bacterial aerosols. Additionally, relative collection rates were compared against those for the Biocapture 650. MS2 bacteriophage was the viral surrogate and Bacillus thuringensis kurstaki the bacterial surrogate. Aerosolized particles were released into a wind tunnel where three samplers each were exposed simultaneously. Samples were analyzed using plaque assay, cell culture on growth media, and real-time polymerase chain reaction (RT-PCR). Viability issues made it difficult to discern the impact of virus preserving media. Research showed that secondary flow rate reduction provided a statistically lower collection of viable bacteria compared to the standard secondary flow rate. The Biocapture 650 generally performed on par or better than the XMX/2L-MIL in collection of both bacterial and viral aerosols. However, longer sampling periods with the Biocapture 650 for viruses resulted in statistically inferior results

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