HIGH VOLUME AIR SAMPLING FOR VIRAL AEROSOLS: A COMPARATIVE APPROACH

THESIS

Casey W. Cooper, Captain, USAF, BSC

AFIT/GES/ENV/10-M01

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

AFIT/GES/ENV/10-M01

HIGH VOLUME AIR SAMPLING FOR VIRAL AEROSOLS: A COMPARATIVE APPROACH

THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Environmental Engineering and Science

Casey W. Cooper, BS

Captain, USAF, BSC

March 2010

AFIT/GES/ENV/10-M01

HIGH VOLUME AIR SAMPLING FOR VIRAL AEROSOLS: A COMPARATIVE APPROACH

Casey W. Cooper, BS
Captain, USAF, BSC

Approved:

_____/signed/_____	22 Feb 2010
Maj Jeremy M. Slagley, PhD (Chairman)	Date
_____/signed/_____	04 Feb 2010
Lt Col David A. Smith, PhD (Member)	Date
_____/signed/_____	04 Feb 2010
Charles A. Bleckmann, PhD (Member)	Date
_____/signed/_____	04 Feb 2010
Daniel L. Felker, PhD (Member)	Date

Abstract

The United States Air Force has high volume biological air sampling equipment available including the XMX/2L-MIL and DFU-1000. Neither system has been evaluated for effectiveness in the collection of viruses. Furthermore, decontamination methods have not been evaluated for these systems after use in sampling for a viral agent.

MS2 bacteriophage was used as a surrogate virus. Aerosolized MS2 was released into a 12 m^3 exposure chamber. High and moderate airborne concentrations of MS2 were evaluated. Low volume impingers were used for comparative purposes as well. Samples were analyzed using plaque assay and polymerase chain reaction (PCR).

At high viral loads the XMX/2L-MIL and DFU-1000 achieved collection effectiveness equal to or greater than the low volume impingers. At moderate levels of airborne viral load, the XMX/2L-MIL was capable of collecting viral quantities within 25% of the quantities collected by the low volume impingers. The DFU-1000 achieved marginal collection effectiveness of virus at moderate concentrations compared to the XMX/2L-MIL and is considered to be unreliable in the quantification of viral agent at moderate levels and below. The DFU-1000 and XMX/2L-MIL were capable of collecting detectable MS2 with PCR analysis at all concentrations. Ten percent sodium hypochlorite (commercial bleach) solution effectively decontaminated MS2.

Acknowledgments

I would like to thank my advisor, Maj. Jeremy Slagley, who made numerous contributions to the completion of this project. I would also like to thank Maj. James Lohaus who assisted with the project conception and data collection in this study. Ms. Elizabeth Escamilla and the staff of Applied Technology Center laboratory also greatly contributed to the data collection, laboratory analysis, and study conception of this project; as did Chris Bliss and Diana Semler of Dycor Technologies. Dr. Joe Wander, Air Force Research Laboratory, contributed to my understanding of the experimental use of viral surrogates. Maj. Jon Black provided numerous contributions in the final editing process of this thesis. Finally, this project would not have been possible without the dedication of the entire thesis committee and the support of the United States Air Force School of Aerospace Medicine.

Casey Cooper

Table of Contents

Page

Page

Page

Page

List of Figures

Page

Page

List of Tables

Page

Page

Page

HIGH VOLUME AIR SAMPLING FOR VIRAL AEROSOLS: A COMPARATIVE APPROACH

I. Introduction

Viral Disease and Biowarfare Overview

Viral disease outbreaks have been the largest contributor to recent disease pandemics including the Severe Acute Respiratory Syndrome (SARS) virus, the H5N1 avian influenza, and most recently the H1N1 swine flu. These disease outbreaks have caused widespread concern and often weigh heavily on public health resources. The 2009 outbreak of the H1N1 strain alone has resulted in 9,079 hospitalizations and 593 deaths worldwide as of September 4, 2009 (CDC, 2009). In a nine month period from November 2002 to July 2003, over 8,000 people were infected with the SARS virus. Of those infected, 774 died, giving SARS a mortality rate of 9.6 percent (WHO, 2004). Each of these recent viral disease outbreaks is capable of airborne transmission by viral aerosol, thus greatly increasing the incidence of new cases and rapidly facilitating their global spread. Localized viral disease epidemics have also resulted in severe impacts to Air Force training and operations. Outbreaks of Adenovirus Subtype 14 have persistently affected the basic training operations at Lackland Air Force Base, Texas. These outbreaks resulted in two fatalities and numerous hospitalizations since 2007 and continue to cause illness in the training population. New viruses and mutations of existing viruses are expected to present a challenge for the foreseeable future.

In addition to the emergence of naturally occurring viral disease causing agents, numerous potential bioterrorism agents are also capable of dissemination by viral aerosol. These agents include smallpox, viral hemorrhagic fever agents, and Venezuelan Equine Encephalitis (VEE). The use of smallpox virus as a biological warfare agent can be traced back as early as the eighteenth century when blankets and clothing items from smallpox patients were given to Native Americans during the French and Indian War (Martin, Christopher, & Eitzen, 2007). Consideration of smallpox and other viral agents as potential biological weapons continued into the modern era. The Soviet Union pursued a large clandestine program during the cold war that included weapons research on numerous viral agents including smallpox, Marburg, and VEE. These agents were prepared in quantities sufficient for use in intercontinental ballistic missiles to disseminate the aerosolized virus over a large geographical area (Alibek, 1999). Al-Queda and other terrorist organizations also have expressed interest in developing, acquiring, and using biological weapons. An Iraqi Al-Queda website expressed such interest with the following post in 2005:

> Biological weapons are considered the least complicated and the easiest to manufacture [of] all weapons of mass destruction. All the information concerning the production of these weapons is readily available in academic books, scholarly publications and even on the internet….In addition to the ease of production, these weapons are also considered to be the most affordable. With $50,000 a group of amateurs can possess a biological weapon sufficient to threaten a superpower. It is for this reason that biological weapons are called the poor man's atomic weapon (Salama & Bursac, 2009).

Disease Life-Cycle Models, Transmission, and Control

The life cycle of all infectious disease can be evaluated through the use of the Host-Agent-Environment (HAE) triad model. This model provides the basic framework for preventing the spread of infectious disease. The host is defined as the susceptible population to a particular disease causing pathogen or agent. Agents can be altered and concentrated from their natural form to make the host more susceptible or to overcome the host resistance. Particularly virulent strains of Marburg virus and VEE were selected in the Soviet biological weapons program for these purposes (Alibek, 1999). Use of the HAE framework might also include strengthening the resistance of a host through immunization. The environment portion of the HAE model is defined as the medium that the agent can be transferred from host to host, typically through a vector or fomite. Chlorination of water supplies to create unsuitable conditions for waterborne agents is a method in which the environmental portion of the HAE framework is used. Quarantine and isolation can also be used in the context of the HAE model by restricting the infected host from an environment where infection of other susceptible hosts could occur. Another important disease model in understanding the behavior of infectious disease is the Natural History of Disease, which divides all infectious disease into two basic phases: prepathogenesis and pathogenesis. The prepathogenesis period begins with the agent in its environment and before a host is exposed. The prepathogenesis period continues after exposure during a period where the agent is adapting to the host. In this phase, infection can be prevented as the host's immune response may prevent the agent from fully adapting to the host. Once the host is infected, the pathogenesis period begins. Symptoms do not appear at first during latency or incubation. The early portion of the

pathogenesis period is very critical in preventing the spread of disease since the infected host shows no appearance of infection, but can often introduce the agent to new hosts. Once infected hosts are identified or infectious pathways in the environment are known, methods can be devised to isolate the infected hosts or prevent the interaction between susceptible hosts and disease pathways in the environment. If conducted properly these quarantine and isolation procedures are shown to be very effective. Meltzer et al. modeled potential response strategies to a release of smallpox as a biowarfare agent. Quarantine and isolation was shown to be capable of eliminating an outbreak of smallpox. The authors assumed that 50 percent of an infected population could be isolated starting 30 days after agent release and projected that by 240 days post release no new cases would occur (Meltzer, Damon, LeDuc, & Millar, 2001). To ensure effective isolation, the infectious agent must be unable to transfer from infected hosts to a susceptible population outside of the isolation areas. Effective levels of isolation and quarantine can be particularly difficult for airborne agents that can be quickly spread on aerosolized droplets or particles. The use of an aerosolized viral agent in biowarfare could drastically increase the complexity of protecting potential hosts since the viral agent would be widely disseminated prior to the initiation of a response. Determining the size of a cordon or initial quarantine area would be a necessary step in initial response to such an incident. This step could be accomplished with an effective air sampling methodology.

Direct environmental controls have also been proposed for localized outbreaks of viral disease. The Air Force has proposed using ultraviolet radiation inside the ventilation

systems in high risk areas at Lackland AFB to control the airborne dissemination of Adenovirus. However; there is no field deployable sampling methodology available for viruses that would be able to determine if these controls would be effective or necessary. Quarantine, isolation, protective equipment, and environmental controls must be able to reduce host exposure to a level that is below the infectious dose for a specific viral agent. This infectious dose varies according to the agent in question and the route by which exposure occurs. To be effective, a sampling method for viral aerosols must be capable of detection at levels at or below the minimum infectious dose.

Biological Sampling and Detection Equipment

A wide range of equipment is marketed for the detection and quantification of biological aerosols. Much of this equipment is also advertised as being capable of collecting airborne viruses. Collection methods and devices for the sampling of viral aerosols can be broadly grouped into two categories: high volume and low volume. High volume methods typically collect air samples at rates of over 40 liters per minute, and some are capable of sampling rates of up to 1,000 liters per minute. Because of the low airborne concentrations expected in an outdoor environment, air sampling devices intended for use in response to biological warfare agents are almost exclusively high volume. The quick collection of a large volume of sample allows for fast confirmatory analysis for immediate response personnel. Low volume devices operate at airflows below 40 liters per minute and are more frequently used in laboratory or field experimental analysis where collection and quantification of aerosolized biological specimens is necessary. Sampling devices for bioaerosols can also be grouped by their means of collection.

These broad categories include liquid impaction, solid impaction, and filter collection (Verreault, Moineau, & Duchaine, 2008).

Solid impaction methods include Andersen samplers, slit samplers (slit to agar samplers are an example), and cyclone samplers. While the efficiency of solid impaction samplers is typically higher for larger particles; some, such as the Andersen sampler, can be used for particle sizes as small as 0.65 micrometers (Verreault, Moineau, & Duchaine, 2008). Some solid impaction samplers also provide additional capabilities not available for liquid or filter collection, such as particle sizing (Andersen sampler) and time resolution (Slit to agar). Illustrations of these solid impaction devices are shown in Figure 1.

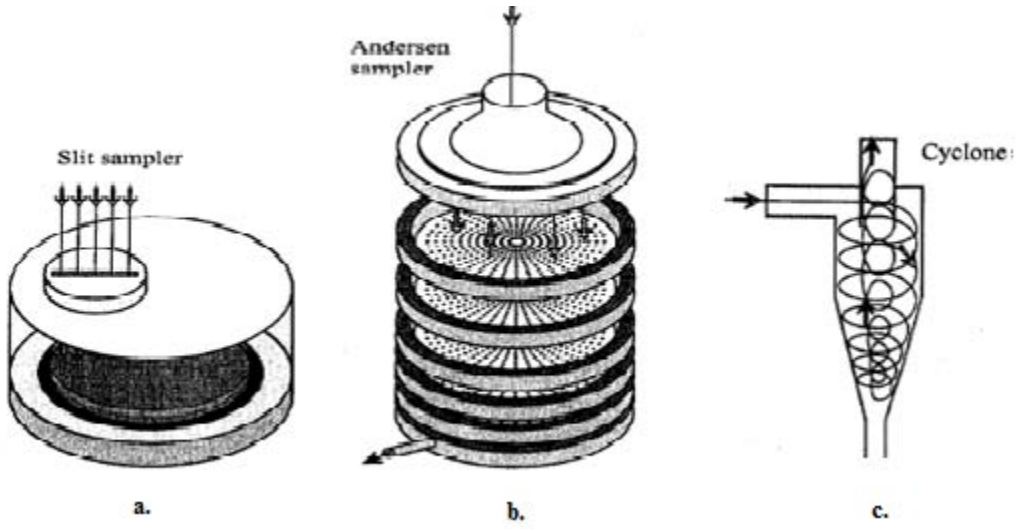

Figure 1: Solid Impaction Aerosol Collection
(Verreault, 2008. Reproduced with Permission from American Society of Microbiology)

Liquid collection methods have been used for bioaerosol collection since the 1950's, and involve the use of an impinger to capture viruses. Impingers are the most commonly used type of sampler in the collection of airborne viruses. Classic impinger systems such as

the All Glass Impinger (AGI) place an airflow exit at a set distance of 4 mm for the AGI-4 and 30 mm for the AGI-30 from the bottom of a fluid vessel. This placement creates a sharp turn in the streamline of the airflow that traps particles in the collection fluid. More recently, a new form of liquid impinger, the swirling aerosol collector, came into use for the collection of viral aerosols. The swirling aerosol collector is expected to retain more viability in the collected sample than other impinger methods. This impinger is manufactured by SKC, Inc. and marketed under the trade name Biosampler. An illustration comparing the swirling aerosol collector with a conventional impinger, the AGI-30 is shown in Figure 2.

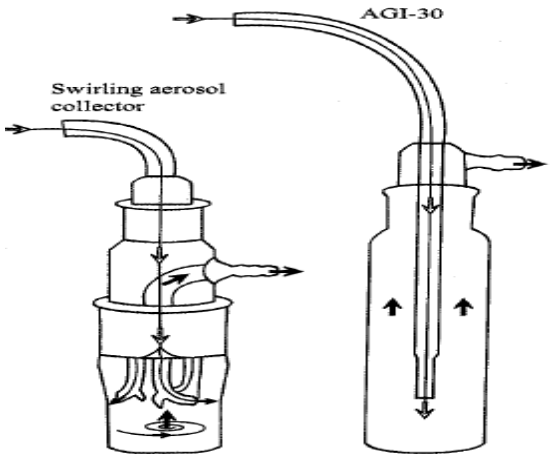

Figure 2: Liquid Impinger Models
(Verreault, 2008. Reproduced with Permission from American Society of Microbiology)

Filter aerosol collection is often more efficient than other sampling methodologies for collecting small particles with aerodynamic sizes less than 500 nanometers (Verreault, Moineau, & Duchaine, 2008). However; filter collection presents several limitations for their use in sampling for airborne viruses. Filters are known to cause structural damage

to the collected virus and desiccate the sample, thus reducing the amount of viable virus available for culture analysis (Verreault, Moineau, & Duchaine, 2008). Additionally, the use of filters for collection can pose difficulties in sample analysis. Many sample analysis methodologies require a liquid medium; therefore, the sample must be extracted from the filter into the liquid media. The tendency of the filter to retain the sample (Cox & Wathes, 1995) can increase the challenges of this extraction. This extraction process could significantly reduce the amount of sample available for analysis.

Air sampling for biological agent identification exclusively employs high volume air sampling equipment. This requirement for high volume sampling equipment is driven by the relatively low air concentrations expected in a response area and the need for fast collection of a sufficient quantity of sample for analysis. Many low volume systems, such as those described above, have been evaluated for collection of viral aerosols in a wide range of studies. So far, most high volume systems have not been evaluated for effectiveness in the collection of viral aerosols. This lack of evaluated effectiveness creates a major obstacle to the reliable and confident use of these systems in response to an aerosolized viral agent.

Air Force Use of Biological Sampling Equipment

The United States Air Force has employed biological agent detection at high risk sites for over 10 years. This capability is still in place with the Air Force use of the Portal Shield monitoring system. The Portal Shield, deployed in high risk areas throughout the world, provides continuous agent monitoring at a fixed site. In the period immediately

following the attacks on September 11, 2001, and the anthrax letter incidents in September and October 2001, the Air Force scrambled to acquire air sampling equipment capable of mobile response to an incident involving biological weapons. The two systems purchased by the Air Force for this purpose included the XMX/2-MIL, manufactured by Dycor Technologies, Inc., and the DFU-1000 manufactured by Lockheed Martin Integrated Technologies. The XMX was selected by the Bioenvironmental Engineering community as their primary field portable sample method for viral aerosols, while Civil Engineering Emergency Management personnel selected the DFU-1000. These two instruments were purchased with the intent of responding to an incident involving biological weapons. Very little investigation was made into other potential uses, capabilities, or limitations associated with these samplers. Organizations outside of the Air Force have used the XMX/2L-MIL and DFU-1000 for purposes other than biological warfare response with varying degrees of success. This expanded use prompted the United States Air Force School of Aerospace Medicine (USAFSAM) to consider the using the XMX/2L-MIL and DFU-1000 to monitor for adenovirus and for other responses involving other viral agents.

The XMX/2-MIL is a multiple stage collection method that pairs virtual impaction with liquid impingement. Virtual impaction differs from conventional aerosol impaction in that conventional impaction removes particles below a certain cutoff size, while virtual impaction separates a single airflow stream into two airflows that are differentiated by particle size. After passing through virtual impaction, airflow is divided into major and minor flow streams. The major flow stream primarily contains particles smaller than a

certain cutoff size, while the minor flow stream primarily contains particles larger than the cutoff size (Loo & Cork, 1988). The XMX/2L-MIL uses multiple virtual impaction stages to concentrate a high volume of airflow into a low volume airflow consisting primarily of particles in the respirable size range of 1 to 10 micrometers. The flow rate entering the XMX is greater than 500 liters per minute, but is reduced into a secondary flow of approximately 12.5 liters per minute. This concentrated secondary flow is then transferred via a liquid impinger into a collection media. Typically sterile water, phosphate buffered saline, or a user specified collection media, such as Remel M5, is used for this final sample collection step.

The DFU-1000 is a high volume air sampler that employs filter collection as an aerosol collection mechanism. The DFU-1000 is capable of sampling for long periods of time at high rates of airflow up to 800 liters per minute. The DFU-1000 utilizes a standard 47 millimeter diameter polyester felt filter with a 1.0 micrometer pore size. This filter has been evaluated for particle sizes as low as 100 nanometers and was shown to have a 75 percent collection efficiency for particle sizes of 100 nanometers (Lawrence, 2003). The DFU-1000 is intended for indoor air sampling only however an updated version, the DFU-2000, was developed to allow outdoor sampling. The DFU-2000 is a DFU-1000 with a protective housing, a mast extending up to 3 meters in height, and a pre-separator to remove large particles or debris. These modifications allow for the DFU-2000 to sample in harsh ambient conditions (JPEO-CBDX, 2008).

While the XMX/2-MIL and DFU-1000 are widely deployed at most domestic and overseas Air Force bases, most bases typically maintain only one or two of each system. For this reason, the ability to use these devices multiple times at multiple sample collection points is critical and multiple use capability is a major comparison criteria. In order to provide this capability, the collection system used must be capable of decontamination under field operating conditions. No study employing infectious viral agent has been published to determine if either the XMX/2-MIL or DFU-1000 is able to be decontaminated in the field after exposure to a viral agent.

Biological Analysis Methods

Analysis methods for viral samples include plaque assay and polymerase chain reaction (PCR) analysis. Plaque assay was originally developed for the measurement of bacteriophage stock, but has since been applied to the study of mammalian virus (Adams, 1959). Plaque assays function by culturing a virus sample on a plate consisting of cell nutrient agar and cells that are susceptible to infection. The cell material utilized depends on the virus for which the analysis is intended. The plaque assay method provides a quantitative measure of viable infectious virus present in a sample. However; several limitations prevent plaque analysis from being used as the preferred analysis method for biological response to a viral agent. These limitations include the time lapse required to culture the plates and the availability of infectious virus to perform analysis. In order to use plaque assay as a primary or confirmatory analysis method, recovery of viable viruses from an aerosol collector is required. These limitations severely restrict the use of plaque

assay in a biological agent response. Nevertheless, plaque assays can still be effective for confirmation analysis when a positive test result is obtained using other methods.

PCR analysis provides a solution to the limitations presented by plaque assay. PCR allows for the detection of viral DNA, and functions by replicating a small amount of DNA in a sample to make multiple copies which then allows for detection to occur. This technique requires the addition of "short DNA strands called primers to target specific sequences in sample DNA" (Ty, 2007). The addition of the primers often requires that some sequence of the sample DNA be known (Bermingham & Luettich, 2003). This limits the use of PCR to applications where a specific agent or group of specific agents is suspected. The Air Force employs the Joint Biological Agent Identification and Diagnostic System (JBAIDS) to provide PCR analysis capability to its Laboratory Response Teams (LRT). JBAIDS is manufactured by Idaho Technology Incorporated, and is capable of identifying 10 known biological agents. Results can be provided in a period of 40 minutes (Wilson, 2006). The primary limitation of PCR is that it detects only the viral DNA or RNA present rather than the infectivity of the virus. Therefore; PCR cannot distinguish between deactivated virus and infectious virus. This limitation makes PCR analysis unsuitable to evaluate the effectiveness of environmental controls for viral aerosols such as those proposed for the control of adenovirus.

Laboratory Evaluation of Sampling Methodologies

Most laboratory studies for airborne viruses are conducted using an exposure chamber. The use of an exposure chamber for initial evaluation of viral aerosol collection methods

allows for the study of aerosols under controlled atmospheric conditions for extended periods and over multiple trials. The use of an exposure chamber also allows the aerosol to be continuously mixed and prevents the gravitational settling of particles during a trial (Verreault, Moineau, & Duchaine, 2008). These laboratory evaluations can then be followed with field testing in order to comprehensively evaluate a sampling methodology under less controlled conditions.

Thesis Objectives and Limitations

This study seeks to determine if the XMX/2-MIL and DFU-1000 meet the criteria required for their use in response to the malicious release of a viral agent or during an outbreak of a viral disease. These criteria include recovery of viable virus, limits of detection or quantification, and field decontamination. These sampling systems will be evaluated simultaneously and compared based on these criteria. This will provide information to responders in selecting the equipment and sampling methods to conduct air sampling for viral agents. This study will be limited to evaluating the XMX/2-MIL and DFU-1000 only. Other high volume air sampling devices are not widely available to Air Force personnel. The XMX/2L-MIL and DFU-1000 will be evaluated in a laboratory exposure chamber. Field testing will not be conducted as part of this project. This study will test the XMX/2L-MIL and DFU-1000 using a single sample duration and collection media. While the use of different sample durations and collection media could affect the performance of the equipment, time and resources did not allow these parameters to be evaluated in this study. Ambient factors during sampling were not varied during analysis. Ambient factors such as temperature and humidity have been widely studied in

previous viral studies and found to have significant impact on the infectivity of viruses (Verreault, Moineau, & Duchaine, 2008). Further laboratory studies and field testing will be necessary to fully evaluate the effectiveness of these sampling methodologies under all conditions. In the decontamination portion of this study, only field decontamination was evaluated. Laboratory grade decontamination such as autoclaving or heat sterilization is likely to be more effective. In practice, these laboratory grade methodologies are unlikely to be available for field use in a response. Analysis was limited to quantification by viral plaque assay with presence/absence analysis using the JBAIDS. Quantitative and semi-quantitative PCR analysis was not performed as part of this study. These quantitative PCR methods are unavailable to Air Force LRTs for analysis of samples collected from the XMX/2L-MIL and DFU-1000 in response to a biological agent, and would not be representative of Air Force field capabilities.

The Air Force does not typically evaluate biological sampling equipment acquired from commercially available sources. Information is provided by the manufacturer and evaluated by the organization considering the purchase. This acquisition method is classified as commercial off the shelf (COTS) (Wilson, 2006). While this process is considerably faster and less involved than other acquisition processes, it fails to fully assess the capabilities and limitations of the equipment. Furthermore, the Air Force is reliant on the accuracy of manufacturer information. This introduces the potential for biased or incomplete information to be used during purchasing and equipment application. This study provides a limited, independent evaluation of two biological sampling systems and should provide insights on the effectiveness of using Air Force

resources to independently evaluate future biological equipment acquisitions and deployment applications of existing equipment.

II. Literature Review

Overview

This section seeks to review scientific literature pertinent to sampling for aerosolized viral agents. Many focus areas are presented including historical studies on viral agents of interest, factors related to aerosol particle size, external factors that affect sampling, use of viral surrogates, previous studies involving high and low volume viral air sampling, and decontamination of sampling equipment. Studies conducted both in the field and in laboratory environments will be reviewed. Currently, Air Force biological air sampling equipment employs dry filtration, virtual impaction, and collection by liquid impinger. This review will provide more emphasis on these sample collection methods, and on factors influencing their use. The literature reviewed will further the objective of applying laboratory methods to comparatively evaluate operational equipment for the sampling of viral aerosols. The literature review for decontamination methods will focus on hypochlorite solution use for decontamination, and emphasize studies previously conducted by USAFSAM.

Air Sampling Background

Air sampling is a critical component of an evaluation to support making a health risk assessment following the release of a biological, radiological, or chemical agent. Air sampling is the primary method to determine the "nature, concentration, and pathogenesis" of airborne microorganisms (Verreault, Moineau, & Duchaine, 2008). However; the science of air sampling for viruses is developing, particularly with regard to

the use of high volume air sampling methods. Numerous studies have been conducted on a variety of sampling methodologies and analytical techniques resulting in the identification of many "advantages and pitfalls" (Verreault, Moineau, & Duchaine, 2008). Detection and quantification of an airborne virus "is dependent on three primary factors: the concentration of airborne virus, the collection efficiency of the air sampling system, and the analytical sensitivity of the diagnostic assay" (Hermann, Hoff, Yoon, Burkhardt, Evans, & Zimmerman, 2006). Unfortunately, many of these techniques have not been fully explored for many viral agents of interest or for high volume air samplers currently in operational use by the Air Force. Due to the limited breadth and depth of work on the subject of air sampling for viruses, "there are currently no standard methods for the recovery and detection of specific pathogens… The lack of guidance requires that sampling methods be optimized and validated for each target pathogen. (Hermann, Hoff, Yoon, Burkhardt, Evans, & Zimmerman, 2006). Such optimization and validation is necessary for all pathogens of interest, including viral agents, and for all sampling equipment currently in the Air Force inventory, including the XMX/2L-MIL and the DFU-1000.

Historical Studies of Interest

Air Sampling for Variola

Disease researchers have attempted air sampling in cases involving airborne viruses for over 60 years. Most of these early attempts involved sampling in areas where airborne viruses were considered likely to be found, such as in the immediate vicinity of a patient confirmed to have a viral disease. Two early studies with implications in the study of

biological warfare agent sampling involved air sampling at a smallpox hospital in Madras, India. These studies, conducted in 1960 and 1963, involved air sampling in close proximity to smallpox patients at various stages of disease progression and at various points throughout the hospital and patient wards. The 1960 study, conducted by Meiklejohn et al., involved the collection of 38 separate air samples collected using a crude filter consisting of a glass tube packed with dry cotton. Sample analysis was conducted by inoculating chicken embryos with the collected sample. This analysis method allowed for the detection and crude quantification of infectious virus (in pock forming units), assuming the presence of sufficient quantities. Both the air sample collection and analysis methods had been tested experimentally in a laboratory environment and shown to be effective for the detection of aerosolized vaccinia (Meiklejohn, Kempe, Downie, Berge, St. Vincent, & Rao, 1961). Laboratory studies indicated that liquid impinger collection were four times more efficient in the collection of virus than using dry cotton. Aggressive sampling techniques were used in many of the air samples, such as locating the sampler 12 inches from the mouth of an acutely ill patient and sweeping scabs directly underneath the sample collection point (Meiklejohn, Kempe, Downie, Berge, St. Vincent, & Rao, 1961). Despite these aggressive techniques, variola recovery from air samples was relatively ineffective. In the 1960 study, only one sample of the 38 collected produced a positive result for variola and even then in small quantities (Meiklejohn, Kempe, Downie, Berge, St. Vincent, & Rao, 1961). The 1963 study used an all glass impinger with liquid collection media instead of a dry filter, but was only modestly more successful. Ten impinger samples out of 52 collected resulted in the detection of Variola major (Downie, Meiklejohn, St. Vincent, Rao, Sundara Babu, &

Kempe, 1965). The overall low level of detection in the air samples was attributed to the lack of sensitivity of the sampling methodology and potentially due to environmental conditions, such as rapid air dilution in the hospital. Variola recovery from surface swabs on pillows, bed sheets, and from settling plates was more effective with a majority of these samples yielding infectious variola. These results indicate that swabbing can be an effective recovery technique for the detection of viral contamination. Additionally, swabbing could also allow for the quantification of virus containing particles that have settled from the air.

Air Sampling for Adenoviruses

Air sampling was also attempted for adenovirus in 1967. This study, conducted by Artenstein et al. on military patients with confirmed adenovirus infections, used a high volume electrostatic precipitator with a cyclonic pre-impactor system capable of sampling approximately 10,100 liters per minute (Artenstein, Miller, Lamson, & Brandt, 1968). Similar to the smallpox studies, Artenstein et al. used aggressive sampling techniques in this study. Aggressive techniques included having an infected patient cough directly over the sampling orifice and having the patient stand in a room and cough frequently for 5 minutes while the air sampler was running. Of the four adenovirus sample runs, three produced viable virus. This study can be applied to the Air Force requirement for a system capable of sampling for viable adenovirus because it demonstrates that in sufficient concentrations, environmental recovery of viable adenovirus from an air sample is possible.

Implications of Historical Studies to Modern Air Sampling Equipment

Many of the modern high volume air sampling systems for bioaerosols employ collection methods similar to those found in the historical studies. For example, wet-walled cyclones are used in high volume systems to collect respirable bioaerosol particles. Wet-walled cyclone collection has many similarities to the pre-impactor used by Artenstein eliminate large, irrespirable, particles from final sample collection. Furthermore, many of the sampling methods used in the smallpox field studies form the basis for much of the biological air sampling equipment used today. For example, the dry cotton packed tubes used by Meiklejohn to collect samples for smallpox are a crude dry-filter sampling system similar in principle to the current DFU-1000 used by the Air Force. These early studies demonstrate that the recovery of viable airborne virus is possible provided the concentration of virus present is sufficient. Some limitations of these early studies have been addressed today. For example, sample analysis methods for both the smallpox studies and the adenovirus studies required infectious virus to be recovered by the air sampling method. Detection techniques for viral DNA and RNA such as polymerase chain reaction (PCR) were unavailable in the 1960's and do not require infectious virus for detection. The smallpox studies also demonstrate the difficulties in applying sampling techniques to field studies even when the sampling techniques are found effective in a laboratory setting. Many of these difficulties persist in field applications of sampling methodologies today. Studies on smallpox could not be repeated today since research on active variola is strictly controlled. Therefore these studies provide the only direct information available on air sampling for this important viral agent. Probably the most important observation of the air sampling studies for smallpox was the effectiveness

of liquid collection compared to dry filter collection. The liquid impinger collected viable smallpox virus in 12 out of 52 samples (Downie, Meiklejohn, St. Vincent, Rao, Sundara Babu, & Kempe, 1965), while the dry filter method collected viable smallpox virus in only 1 out of 38 samples (Meiklejohn, Kempe, Downie, Berge, St. Vincent, & Rao, 1961). This disparity suggests that liquid collection for some viral agents could be more effective than dry collection methods. The limited detection of virus in both of these smallpox studies also reveals the difficulties in conducting air sampling for Variola, and the relatively high airborne concentrations of viable virus required to generate a detectable, much less quantifiable, result. These details underscore the need for biological air sampling systems to be robustly evaluated for their effectiveness in the collection of viral agents. This need should extend to the sampling equipment currently used by the Air Force as well.

Particle Characteristics and Viral Aerosols

Particle size has been shown to be one of the most important characteristics related to the airborne residence time of the viral aerosol, its potential for infection, and the efficiency at which the virus can be recovered during sampling. Therefore the particle size of an aerosol containing a biological agent must be fully considered in both laboratory and field evaluation of sampling equipment.

Health Assessment Considerations Regarding Particle Size on Air Sample Collection

The health impact of aerosolized particles “is size dependent”, and measurement of particle size distribution should be included in field studies and “controlled in laboratory

studies of virus particles" (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). The nose and upper airways prevent airborne particles greater than 10 micrometers from entering the respiratory system. For this reason, most air sampling equipment for bioaerosols strip out particles larger than this 10 micrometer cutoff. The study of aerosolized biological agents has largely centered on particle sizes in the "respirable" size range of less than 10 micrometers to provide a proper representation of human exposure. Less is known or understood at the submicrometer end of the particle size distribution. Submicrometer sized particles likely play a larger role in morbidity than previously considered (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005), and are not efficiently collected by most high volume air sampling equipment. The respiratory deposition probability of various particle sizes is shown in Figure 3. It is important to note that even a 0.3 micrometer sized particle has a 15 percent probability of deposition in the human respiratory tract (Maynard & Kuempel, 2005). For this reason, air sampling equipment for viral agents should be able to include a reasonable collection efficiency of submicrometer particles to better characterize human exposure to a viral aerosol. Preferably, the collection efficiency for submicrometer particles would be at least equal to the deposition probability in the respiratory tract.

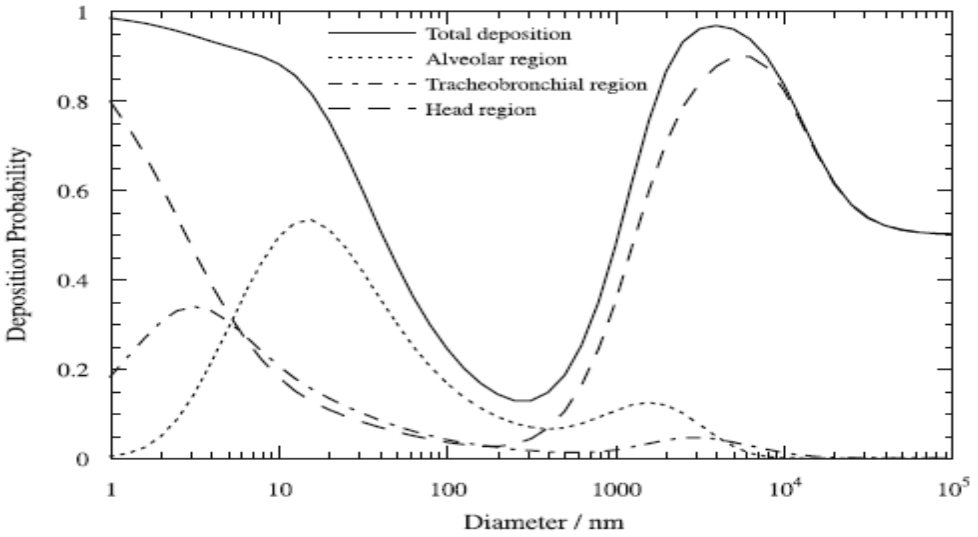

Figure 3: Respiratory Deposition Probability
(From Maynard & Kuempel, 2005. Reprinted with kind permission from Springer Science)

Air Sampling and Particle Size Considerations

Development of biological detection equipment has centered on the ability of the equipment to limit air sampling to the respirable size range. This includes the equipment currently inventoried by the Air Force. The XMX/2L-MIL limits sample collection primarily to the 1 to 10 micron size range (Tucker, 2005), and has very limited collection effectiveness in the collection of submicrometer particles. Specific information on the collection efficiencies of the XMX/2L-MIL is available through USAFSAM. The DFU-1000 is a filter collection system and collects particles above the minimum collection size of the filter. However, particle collection can extend to particle size ranges significantly below the pore size of the filter. The standard filter provided with the DFU-1000 is a polyester felt filter with a 1 micron pore size and has a reported efficiency of approximately 75 percent for 100 nanometer sized particles (Lawrence, 2003). Although

it is unclear if these efficiencies reported by Lawrence consider the high velocity air flow conditions produced across the filter by the DFU-1000 during sample collection. Regardless, high efficiencies would not be unexpected for the collection of submicrometer sized particles. Filters are commonly used in the sampling of fine viral particles, particularly for particle sizes less than 500 nanometers (Verreault, Moineau, & Duchaine, 2008). Additionally, low volume liquid impinger sampling systems have also been evaluated for their collection efficiency of micrometer and submicrometer particles, including the AGI-30 and the Biosampler. The reported collection efficiencies for these systems are included in Table 1.

Particle Size (Microns)	AGI-30 Collection Efficiency (%)	Biosampler Collection Efficiency (%)
0.3	69	78
0.6	71	88
0.8	72	91
1.1	82	92
1.7	93	93
2.0	95	95

Table 1: Particle Collection Efficiency of AGI-30 and Biosampler From Willeke et al., 1998

Particle Composition of Viral Aerosols

Virus containing particles consist of a variety of constituents, much of which is inorganic (Verreault, Moineau, & Duchaine, 2008). For this reason, the virus accounts for very little of the agent containing particle and a negligible portion of the aerosol. Also, the addition of virus to experimental aerosols has little impact on the size characteristics of

the aerosol when compared to the suspension media or the method used to generate the aerosol (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Free viruses are uncommon in the environment since particles tend to form aggregates very rapidly when airborne (Verreault, Moineau, & Duchaine, 2008). This tendency, along with the binding of virus within droplets and particles, allows for the air sampling of virus particles without the need to collect the ultrafine individual virons. The importance of submicrometer sized viral particles in natural viral aerosols is not well understood, but is likely to have a strong affect on the health risks relating to human exposure. Furthermore, the majority of airborne virus containing particles may be submicrometer sized (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Hogan's observation has been verified in other viral particle studies as well. In a study of exhaled influenza virus particle sizes conducted by Fabian et al. on patients infected with influenza A and B, approximately 70 percent of particles in exhaled air were less than 0.5 micrometers in size and 87 percent were less than 1 micrometer in size. Exhaled influenza containing particles greater than 5 micrometers were very rarely observed. Fabian's study indicates that fine particles of less than 1 micrometer may be a major contributor in disease transmission for influenza (Fabian, et al., 2008). Studies on other viral diseases have yielded different particle size information. For example, livestock viruses such as Foot and Mouth virus and Aujeszky's disease virus were mostly contained in particles greater than 3 micrometers in size (Verreault, Moineau, & Duchaine, 2008). Particle or droplet size may significantly impact the airborne viability of virus in a aerosol. As shown in Figure 4, the droplet nucleus, including the viruses in an aerosol droplet, become more exposed to environmental factors as the droplet dries

out. The final size of the droplet nucleus may also be directly related to the relative humidity. This is particularly true in the study of artificially produced viral aerosols in an exposure chamber.

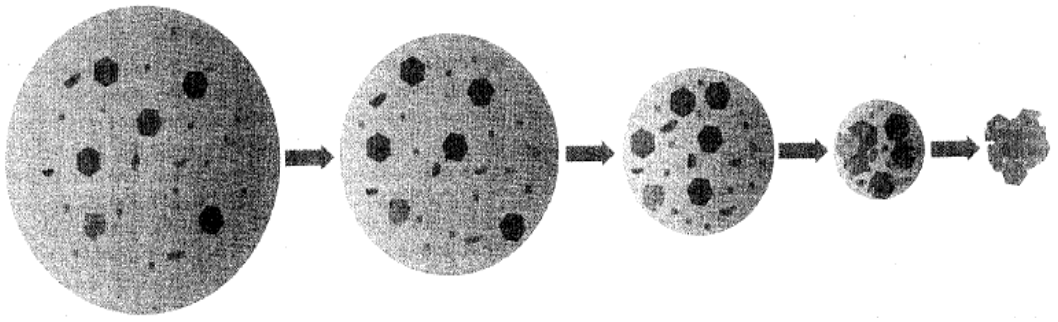

Figure 4: Droplet Evaporation in a Viral Aerosol
From Verreault et al., 2008. Reproduced with permission from the American Society of Microbiology.

Artificially produced aerosols, such as those used in controlled chamber studies, are not studied in the presence of other aerosols that may be present in a natural environment. This condition prevents "binding of the nebulized particles", and allows particle size to be "influenced only by the size of the droplet created by the nebulizer and the solute concentration" (Verreault, Moineau, & Duchaine, 2008), which may not represent behavior in a natural environment. This factor may limit field use of aerosol collection data gathered in the laboratory chamber setting. For this reason, air sampling studies conducted in a chamber environment should consider these limitations before applying laboratory generated data for field use. This limitation should be applied to laboratory evaluation of Air Force high volume air sampling equipment as well.

Particle Size and Settling Characteristics

The particle size of a viral aerosol is the key parameter in determining the settling rate and, consequentially, their duration in the airborne environment. Particle sizes of 100 micrometers settle from a drop height of 8 feet in approximately 8 seconds, while 1 micrometer particles require 19 hours to settle. Submicrometer sized particles can remain airborne for several months or longer (Utrup & Frey, 2004). The slow settling characteristics of 1 micrometer and smaller particles allows for airborne exposure to occur long after the aerosol is disseminated in an area. This feature also allows aerosols comprised of submicrometer particles to be carried for longer distances after dissemination and leads to larger areas of exposure risk. Dilution and environmental degradation of the aerosol may reduce these health risks; nonetheless, a high volume air sampler would need to collect a reasonable fraction of these micrometer and submicrometer sized particles in order to fully assess health risk.

Measurement of Particle Size Distribution of Viral Aerosols

A variety of equipment is available to measure particle size during aerosol studies, including real time measurement devices such as particle spectrometers and gravimetric devices such as cascade impactors. Cascade impactors use a series of impaction stages with descending particle size cutoff points. The cutoff size points are "determined by the velocity of air through the nozzle and the distance of the nozzle from the collection surface" (Cox & Wathes, 1995). These cascade impactors pre-date the real time methodologies and are typically used as the "gold standard" in the development of real time methods. An evaluated method is the aerodynamic particle sizer (APS)

manufactured by TSI. The APS measures particle size by "determining the time-of-flight of individual particles in an accelerating flow field" and is capable of measuring particle size distributions in a particle size range of 0.5 microns to 20 microns (Peters & Leith, 2003). The APS, model 3321, was evaluated by Peters and Leith for collection efficiency over a range of particle sizes from 1 micron to 4 microns. These efficiency evaluations were conducted by comparing particle mass data obtained from the APS with the results of particle collection using a cascade impactor. Counting efficiency for particle size ranges of 1 to 3 microns was approximately 45% for the TSI 3321 and approximately 60% for particles in the 4 micron size range. Despite these lower overall counting efficiencies, the particle size distribution produced by the TSI 3321 was similar to the size distribution generated using data from a cascade impactor. This is primarily due to the relatively consistent efficiency over the range of particle sizes evaluated (Peters & Leith, 2003). This study demonstrates that the APS data can be used to determine a particle size distribution, but data on total particle load likely requires adjustment. Another commonly used instrument for particle size analysis is the Grimm Technologies portable aerosol spectrometer (PAS) model 1.108. The PAS divides a particle size distribution into 16 size channels, while the APS divides the particle size distribution into 52 size channels (Peters, Ott, & O'Shaughnessy, 2006). Both the PAS and APS can count particles as large as 20 microns. One key advantage of using the PAS for particle size analysis in the study of a viral aerosol is that the Grimm provides particle size distribution data for particles as small as 0.3 microns. The APS distribution data has a lower particle size counting limit of 0.5 microns (Peters, Ott, & O'Shaughnessy, 2006). The accurate measurement of the particle size distribution is essential in the laboratory

evaluation of air sampling equipment. These measurements ensure that artificially generated aerosols used in a chamber sufficiently represent particle sizes that may be present in the environment. The evaluation of Air Force high volume air sampling equipment should incorporate particle size measurements that are as accurate as possible for both micrometer and submicrometer particles.

External Factors on the Collection of Viral Aerosols

Many environmental factors can impact the behavior and sampling characteristics of aerosolized viruses. Factors that are particularly important to the behavior of aerosolized virus include relative humidity and temperature. Levels of ultraviolet radiation can significantly impact the viability of aerosolized viruses in an outdoor environment. As discussed earlier, the particle size and composition of a virus containing droplet can be greatly affected by the relative humidity (Verreault, Moineau, & Duchaine, 2008). This allows the size of particles in a chamber to be increased or decreased through the adjustment of relative humidity. Many viruses also exhibit different infectivity characteristics as the relative humidity is changed, which will be discussed further in the surrogate viruses section. "The stability of certain infectious airborne viruses" is also influenced by temperature, with certain viruses exhibiting greater infectivity at lower temperatures (Verreault, Moineau, & Duchaine, 2008). This effect on virus stability was shown to be minimal for certain bacteriophages, such as MS2, when aerosols were generated using tyrptone broth instead of salt solution (Dubovi & Akers, 1970). For these reasons, proper control of environmental conditions must be maintained in air chamber studies during the evaluation of Air Force air sampling equipment.

Furthermore, the impact of relative humidity on viral stability could be minimized by impropriating tyrptone broth in the aerosol solution.

Infectious Concentration of Viral Aerosols

There is a dearth of published information available on the minimum aerosol concentration required to produce an infection with viral agents of interest. One study conducted by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) exposed non-human primates to an aerosol of Ebola-Zaire, a CDC category A agent. Two rhesus monkeys were exposed to a 400 PFU aerosol dose of Ebola-Zaire. Both monkeys died or were euthanized after morbidity was observed (Leffel & Reed, 2004). Although these lethal viral exposure levels would apply only to an aerosol containing Ebola-Zaire, aerosol studies attempting to evaluate a limit of detection for high volume air sampling equipment should attempt to achieve viral concentrations below the lethal doses used in the Leffel study.

Viral Surrogates

The evaluation of air sampling methodologies for viral agents requires the aerosolization of infectious virus. Since these agents can be very pathogenic to humans and capable of airborne transmission, any study aerosolizing active pathogen typically requires operating under bio-safety level (BSL) three or four. According to the American Biological Safety Association, Smallpox and Marburg both require a BSL 4 level laboratory, while any studies using VEE require a BSL 3 lab (ABSA, 2004). Providing this level of protection, in addition to purchasing the agent itself, is very resource intensive and

impractical under most circumstances (Foarde, Hanley, Ensor, & Peter, 1999). To overcome these limitations, many studies use a surrogate virus to simulate a pathogenic virus with similar characteristics.

Viral Surrogate Use in Previous Studies

Bacteriophages are often used as viral surrogates, including male specific 2 (MS2) and T3 bacteriophage. Bacteriophages, as their name implies, use bacteria as their only host organism, and represent no risk to humans, providing that the host bacteria are not pathogens. MS2 has been used in studies to represent a variety of viral agents. Langlois used MS2 as a surrogate for smallpox virus in the development of field deployable biological detection equipment (Langlois, 2002). Another study by Foarde et al. used MS2 as a surrogate for a variety of similar viral agents, including retroviruses and pox viruses. Foarde noted that bacteriophages have aerosol characteristics similar to many human viruses (Foarde, Hanley, Ensor, & Peter, 1999). Like to agents of interest, viral surrogates can also be very persistent and hardy under experimental conditions. Utrup and Frey used MS2 as a viral surrogate when they studied the fate of bioterrorism-relevant organisms in an indoor environment. Utrup and Frey observed that 52 percent of the MS2 aerosolized in an exposure chamber remained viable in an aerosol form during the 45 minute time frame of the study (Utrup & Frey, 2004). These successful previous studies make bacteriophages a reasonably vetted and economical choice for the evaluation of high volume air sampling equipment by the Air Force.

Limitations to the Use of Viral Surrogates

The use of viral surrogates has many limitations. Hogan et al. observed that generalizations should not be made between different virus types and that viability during sampling can only be accurately determined when viruses are tested individually (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Different viral types and subtypes exhibit a broad range of structures and composition of nucleic acids. MS2, despite its common use as a surrogate, cannot accurately represent the behavior of all viral agents of interest (Tseng & Li, 2005). Many studies have found that even viruses of similar size and shape exhibit different behavior in aerosolization and collection. Hogan et al. evaluated MS2 and T3 separately and found large differences in collection efficiency performance between sampling for MS2 and sampling for T3. These differences were observed for all three sampling methodologies evaluated including the AGI-30 manufactured by Ace Glass Inc., the Biosampler swirling aerosol collector manufactured by SKC, Inc., and the Fritted Bubbler. For example, the lower limit of virus collection after 30 minutes of sampling for the AGI-30 using MS2 was approximately 19 percent, while for T3 under the same conditions, the lower limit of virus collection was observed to be approximately 1 percent (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Tseng and Li evaluated sampling characteristics for four different bacteriophages including MS2, Phi X174, Phi 6, and T7. These bacteriophages represented a broad range of nucleic acid structure including single strand RNA, single strand DNA, double strand RNA, and double strand DNA, respectively. Viruses with a lipid envelope, such as Phi 6, are generally hydrophobic, while viruses without a lipid envelope such as MS2, Phi X174, and T7, exhibit

hydrophilic behaviors. This condition is particularly important during sample collection since viruses with lipid envelopes are much more sensitive to stresses during sampling. Tseng and Li also found that viruses with tail fibers, such as T7, exhibit greater sensitivity to relative humidity than untailed viruses (Tseng & Li, 2005). Their studies show that it is important to consider more than viral size and aerosol characteristics in the selection of a surrogate virus. Surrogate virus selection should also consider the physical structure of the virus and the nucleic acid type and structure. Similar to MS2, the Marburg virus is also a single strand RNA virus, although significantly larger than an MS2 bacteriophage (Elliott, McCormick, & Johnson, 1982). Poliovirus, similar in size to MS2, is a single strand RNA virus, and is non-enveloped (Hogle, 2002). Verreault observed that "structure of a virus alone cannot be used to predict the survival of the virus under different environmental conditions" however (Verreault, Moineau, & Duchaine, 2008). For example, the stability of the St. Louis Encephalitis virus is not affected by relative humidity (Verreault, Moineau, & Duchaine, 2008). Structurally similar surrogates may not show this tolerance over broad humidity ranges. Therefore, even a well chosen surrogate may not exhibit similar characteristics to the target virus despite the similarity in size and structure. For this reason, evaluations of Air Force sampling equipment should use multiple viral surrogates to ensure that bias is not introduced due to characteristics specific only to a single agent.

Alternatives to the Use of Viral Surrogates

The broad range of studies using surrogate viruses in place of a pathogen show that the use of surrogate viruses has value primarily because there is little alternative available at

a reasonable cost and acceptable level of safety. The only alternative to using surrogate virus is to use an inert particle of similar size, such as polystyrene latex beads. Polystyrene latex beads were used by Hogan to evaluate the collection of submicrometer particles by bioaerosol sampling equipment (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). While these inert particles can provide data to evaluate a sampling method for a particle size range of interest, it is not possible to determine the effect of the sampling method on virus viability. Air Force evaluations on air sampling equipment should evaluate particle size collection efficiencies using inert particles separately from studies on viral aerosols.

Low Volume Sampling Methods

Several low-volume sampling methodologies have been evaluated and described in current literature for the collection of airborne virus. While low volume air samplers have many limitations, their development and evaluation is necessary to determine the relative effectiveness of other sampling methods, such as high volume samplers. Three evaluated technologies include the AGI-30, the Biosampler, and the Fritted Bubbler.

Collection Mechanisms for Low Volume Air Sampling

The AGI-30 and Biosampler utilize a right angled collection tube to limit particle collection sizes to those below a respirable size of approximately 10 micrometers. Liquid collection devices, such as these, have an advantage over other collection methods since most biological analytical methods require liquid media (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). The use of a liquid collection device prevents

the need to transfer a sample into a liquid media after collection thus preserving more of the sample for analysis. The disadvantage of liquid aerosol collection is loss of sampling media due to evaporation. This fluid loss can result in the reaerosolization of virus and potentially reduce the collection efficiency (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). The AGI-30 uses a 1 mm diameter nozzle that is placed 30 mm above the bottom of the collection vessel and 10 mm above the surface of the collection media. This placement produces a sharp turn in the streamline of the airflow at the outlet, forcing particles with higher inertia to penetrate the liquid and become trapped. The Biosampler is a "swirling aerosol collector" that uses three 0.63 mm nozzles to create a swirling action in the media which also causes particles to penetrate the fluid and become trapped (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Fritted bubblers are typically used in gas capture as opposed to bioaerosol particle collection. Bubblers use a glass frit at the end of the collection tube where the air stream contacts the media. This porous frit causes bubble formation that allows the interception of particles through an impaction mechanism with the bubble. Very small particles will also diffuse to the air-liquid interface of the bubbles thus allowing capture.

Evaluation of Low Volume Air Sampling Methodologies

A study conducted by Hogan et al. attempted to compare the collection efficiencies of different bioaerosol collection devices, including the AGI-30, Biosampler, and Fritted Bubbler. This study used MS2 and T3 bacteriophage solutions and atomized the viral solution to create ultrafine particulate aerosols. This study is relatively novel in that it compared the collection efficiencies for submicrometer particles and ultrafine particles

with diameters of less than 100 nanometers (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Prior studies had focused on aerosol particle sizes of approximately one micrometer in diameter or larger. There is a concern that ultrafine particles could transfer by diffusion through the alveolar membrane thus providing a route of entry into the blood stream. The Hogan et al. study found that all three evaluated samplers were inadequate in the sampling of ultrafine particles with collection efficiencies below 10%. The Fritted Bubbler was the least efficient in the collection of ultrafine particles and the AGI-30 was found to be the most efficient although still below 10%. All three samplers demonstrated sharp increases in collection efficiency as particle diameter increases above 100 nm. Extremely small particles with diameters less than 30 nm showed an increase in collection efficiency as particle diameter decreases due to tendency of these particles to diffuse into the media (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). However, most viruses are larger than this diffusive size range. Hogan et al. also evaluated collection efficiencies of the samplers at different flow rates. The manufacturer specified flow rate for all three samplers is 12.5 liters per minute (lpm). The study found that found that for flow rates less than 2.5 lpm, very little media depression occurs in the AGI-30 and the fluid remains motionless in the Biosampler, thus preventing the desired swirling motion. Turbulent liquid motion is observed in the AGI-30 at flow rates higher than 2.5 lpm and in the Biosampler at flow rates higher than 8.7 lpm. The collection efficiencies were evaluated at flow rates of 6.25 lpm and 12.5 lpm. The AGI-30 and Biosampler were more efficient at 12.5 lpm, while the Fritted Bubbler had higher efficiencies at 6.25 lpm. The three samplers were also evaluated for particle collection efficiency and virus viability as a function of sampling time. The AGI-30 and

Biosampler demonstrated relatively steady particle collection efficiency over time, while the Fritted Bubbler shows an increase over time due to the deposition of particles in the frit. The Fritted Bubbler also has a high rate of media loss resulting in a higher virus concentration simply due to the evaporation of the fluid. MS2 loses viability in the AGI-30 for sampling periods greater than 30 minutes, indicating that viral collection with the AGI-30 should be limited to less than 30 minutes. There was very little correlation in the viability of MS2, as compared to T3 indicating that generalizations cannot be made across different viruses. Similar to previously conducted studies, such as those by Tseng and Li, this demonstrates that viral surrogates may not accurately simulate pathogenic viruses (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). Hogan's study evaluates many of the potential variations associated with using liquid collection devices for viral aerosol. Comparing Hogan's results to those obtained in studies of aerosol particles greater than 1 micrometer could prove valuable in assessing the entire collection of a viral aerosol across the entire range of particle sizes. The paper did not account for potential variation from different collection media since a single media, phosphate buffered saline (PBS), was used as the liquid collection media for all sample runs. The study did not account for collection efficiency variation due to changes in temperature and humidity. The Hogan study is also limited in that it does not explore the processes responsible for the low collection efficiencies observed. A study commissioned by the Air Force Research Laboratory and conducted by Riemenschneider et al. sought to determine the impact of reaerosolization in the sampling of viral aerosols (Riemenschneider, et al., 2009). This study examined the AGI-30 and the Biosampler. Similar to the Hogan study, flow rates and sampling time were varied, but used to assess

the impact of reaerosolization on sampling efficiency. Sample evaluations were also conducted with an inert aerosol tracer, poly styrene latex (PSL), and live MS2 virus. Similar to Hogan et al., the Riemenschneider study found that collection efficiency was lower when flow rates differ significantly from the recommended 12.5 lpm, with higher flow rates greatly increasing the reaerosolization of both virus and PSL. The Riemenschneider study also used a single collection fluid, deionized water, as a collection media. Riemenschneider was more thorough than the other authors in explaining this selection over other collection media, such as phosphate buffered saline (PBS), which is commonly used in liquid collection systems. The use of saline solutions can create salt aerosols that can make it difficult to distinguish the reaerosolization of virus (Riemenschneider, et al., 2009). The Biosampler was also found to have a significantly lower reaerosolization rate as compared to the AGI-30; although sampling durations greater than 30 minutes were found to increase the reaerosolization in the Biosampler. Further studies should be conducted to determine the impact that longer sampling durations have on virus viability, in addition to the affects of the collection fluid selected. This observation also indicates that initial studies on air sampling equipment should select a lower sampling time to minimize the effects of sampling durations on liquid collection systems, and thus remove this potentially significant variable. Of the three low volume samplers evaluated in these reviewed studies, only the AGI-30 and Biosampler are widely described in published literature relevant to the sampling of viral aerosols. Therefore, evaluation of high volume air sampling equipment should include either an AGI-30, Biosampler, or both as a standard reference to compare collection of virus particles.

Collection Media

The collection media used in the air sampling of viral aerosols can also impact the collection of virus. A study by Hermann et al. compared various additives to PBS. This comparison was made to optimize a sample collection process for porcine reproductive and respiratory syndrome virus (PRRSV). PBS is a media commonly used for impinger collection methods. Hermann et al. compared the addition of 1% activated carbon, 0.5% bovine serum albumin, and 20% ethylene glycol to PBS. Combinations of these additives were evaluated as well. None of the additives tested had a significant impact of the collection efficiency of virus, and all solutions and additives were within 10% of the PBS results without additives. Ethylene Glycol and PBS was shown to be slightly more effective than the baseline PBS for the collection of PRRSV, and could warrant further study. Ethylene Glycol, commonly used in antifreeze, has a lower freezing point than PBS and water and could be used where operations in cold environments were required (Hermann, Hoff, Yoon, Burkhardt, Evans, & Zimmerman, 2006). The Hermann et al. study was limited due to the use of PCR for quantification of sample results. Therefore, maintenance of viable virus during sampling was not considered by Hermann.

Remel M5 Solution as a Collection Media

Most of the reviewed studies on the liquid collection of viruses used either PBS solution or sterile water. One limitation of these collection media is that they are unable to preserve the virus for extended time periods (Escamilla, 2009). This could be a particularly important limitation in many military operating environments, where sample transport over a long distance could be required for laboratory analysis. Fortunately,

transport media is available for the extended preservation of viruses including Remel M5. Remel M5 media has been shown to be effective at preserving virus for up to 48 hours after specimen collection (Remel, 2005). Remel M5 has not been described in published literature for the collection of air samples, and no literature is available to support its use for this purpose. Liquid impingers also produce a foaming effect, especially when the collection media contains proteins or carbohydrates. Remel M5 media contains both of these constituents in its formulation, which could be a key limitation in selecting Remel M5 as a collection media in impingers due to re-aerosolization of the collected virus in the impinger. Antifoam solutions can be used to reduce this foaming effect. Six antifoamants were evaluated for their impact on viral infectivity by Hermann and none were shown to have a significant impact on viral infectivity. Four of the six antifoamants did significantly affect the host cells however (Hermann, Hoff, Yoon, Burkhardt, Evans, & Zimmerman, 2006). If further foaming reduction is required, the air flow rate into the impinger can be reduced, although doing so could have a significant impact on sampler performance, as discussed earlier in both the Hogan and Riemenschneider studies. Dycor Technologies has developed a flow reducer for the XMX/2L-MIL for this purpose, but it has not been evaluated for performance in the sampling of a viral aerosol. Therefore, it is evident from the existing data that a virus preserving collection media, such as Remel M5, should be evaluated for use in Air Force sampling equipment to improve the sampling and analysis of viral aerosols, despite the inherent limitations due to the foaming. The selection of an effective virus preserving sample media would enhance the use of Air Force air sampling equipment in deployed environments.

High Volume Sampling

The urgency required for field sampling in response to a suspected biological agent release typically does not allow for the use of low volume sampling devices. Additionally, due to the dilutive effects of an open atmosphere, sampling in response to an outdoor release of a biological agent requires a large sample volume to better ensure the ability to assess the potential human environmental exposure. To meet these urgent detection and analysis requirements, high volume sampling methodologies should be used. High volume sampling enables the collection of dilute aerosols, such as those in an outdoor environment (Cox & Wathes, 1995). This capability comes with limitations, however. Cox and Wathes describe these limitations in their *Bioaerosols Handbook* (Cox & Wathes, 1995). Aerosol integrity can be compromised by high volume air sampling. Use of high volume air samplers in an indoor environment "may strip the biological particles faster than the regeneration rate." This stripping effect of this can result in the "misrepresentation of the bioaerosol concentration" (Cox & Wathes, 1995). This deficiency could be very significant for sampling in a laboratory exposure chamber. The rapid stripping of the aerosol may create particular difficulties in the evaluation of high volume samplers using an exposure chamber with limited volume and aerosol regeneration. Therefore it is necessary to properly monitor the aerosol concentration during the evaluation of high volume sampling systems using a chamber.

Laboratory Studies of XMX/2L-MIL Air Sampling for Viral Aerosols

Dycor Technologies conducted an unpublished study to determine if the XMX/2L-MIL effectively recovers aerosolized virus. These studies were conducted using a standard

configuration XMX/2L-MIL with PBS collection media in a 12 cubic meter exposure chamber. This study consisted of 5 trials using MS2 bacteriophage with an airborne viral target load of 25 agent containing particles per liter of air. Results are shown in Table 2. The MS2 collected in this study by the XMX/2L-MIL are approximately equivalent to an aerosol concentration measurement of 891 PFU/Liter of Air.

Trial	ACPLA	Sample Concentration (PFU/ml)
1	23.6	485000
2	23.0	445000
3	28.1	490000
4	25.3	472500
5	23.2	447500
Average	24.6	468000

Table 2: MS2 Collection with XMX/2L-MIL
(Dycor Technologies, 2009)

These results demonstrate the feasibility of using the XMX/2L-MIL to recover infectious virus. Dycor did not evaluate multiple viral loads or decontamination of the XMX/2L-MIL. Additionally, these results were collected with a single XMX/2L-MIL per trial run. Therefore, intra-instrument variability could not be assessed using the data obtained in this study. Published studies concerning the collection of viruses in a laboratory setting are not available for the DFU-1000; therefore, the overall effectiveness of DFU-1000 cannot be assessed from existing literature. This data on collection of MS2 using PBS as

the collection media, could be used as a baseline to which other sample media, such as Remel M5, could be compared.

Field Studies using the XMX/2L-MIL for Viral Air Sampling

High volume air sampling for viral aerosols in the field has been performed with the XMX/2L-MIL, or similar systems, in at least two separate studies. The first study conducted in 1988 by Brenner et al. used a prototype XM2 with functions similar to the XMX/2L-MIL (Brenner, Scarpino, & Clark, 1988). The XM2 used a combination of virtual impaction followed by liquid impingment, and was used for air sampling during the land application of wastewater. Brenner et al. was able to recover active bacteriophage from the XM2 sampling media. Bacteriophage concentrations measured by the XM2 ranged from 0 to 9 plaque forming units (PFU) per cubic meter of air (Brenner, Scarpino, & Clark, 1988). The XMX/2L-MIL was used during an H7N3 avian flu investigation conducted by Schofield et al. in 2005. Schofield collected samples inside a barn where infected birds were present and in a nearby command post. Downwind samples and random samples in the local area were collected as well. PCR detection and semi-quantitative PCR were used for analysis. The two samples collected inside the barn yielded positive results and relatively high estimates of viral load using the semi-quantitative analysis (Schofield, Ho, Kournikakis, & Booth, 2005). Additionally, live virus was extracted from these samples. The four samples collected near the command post were found to be positive for H7N3 by PCR detection; however, the less sensitive semi-quantitative PCR was not able to detect H7N3. The authors concluded that these results were false positives caused by residual material associated

with previous sample collection performed inside the barn. One sample taken downwind in the local area was found to be positive for PCR detection, but was negative using semi-quantitative PCR analysis. However, the possibility of cross contamination from other samples was ruled out. This sample was thus considered a true positive (Schofield, Ho, Kournikakis, & Booth, 2005). The studies by Dycor and Brenner et al demonstrate that viable virus is capable of surviving XMX/2L-MIL collection under field sampling conditions. The residual contamination present between samples in the Schofield study demonstrates the need for an effective strategy to decontaminate the XMX/2L-MIL after exposure following any sample event.

Field Studies using the DFU-1000 for Viral Air Sampling

The DFU-1000 was used in one published field study for viral air sampling. This study conducted by Russell in 2006, involved environmental sampling for adenovirus subtype 4 in a Marine Corps training area in San Diego, California. Air samples were collected in squad bays using both the DFU-1000 and an electrostatic precipitator. A total of 20 samples were collected with the electrostatic precipitator and 19 with the DFU-1000. Forty-two percent of the samples collected with the DFU-1000 were positive for adenovirus and 50 percent were positive for the electrostatic precipitator (Russell, et al., 2006). Samples were analyzed using PCR only; although some samples were randomly selected for viral culture analysis. Because the samples were analyzed using PCR, the ability of the DFU-1000 to recover viable virus from field samples cannot be determined; however, this study does demonstrate the potential for detection of virus under field conditions using the DFU-1000. The Russell study does not provide results on the

recovery of viable virus; therefore, the effectiveness of the DFU-1000 in a situation where viable quantification of airborne is needed, or where confirmatory sample analysis by culture is desired. These studies indicate that the DFU-1000 may be suitable for the field collection of viral agents of interest and should be evaluated with other equipment currently inventoried by the Air Force. The existing data from both field and laboratory studies indicate that Air Force high volume air sampling equipment can be used to recover both viable and non-viable air samples and should be comparatively evaluated to optimize their use in the field.

Virtual Impaction

Some air sampling systems with applications to bioaerosols, such as the XMX/2L-MIL, use a virtual impaction process to split an incoming air flow in two fractions of minor and major flows, with the "minor flow containing larger particles above a certain cutoff size" (Cox & Wathes, 1995). This splitting allows the production of a concentrated aliquot of flow rich in particles relative to the ambient concentration. Often, several of these virtual impactors are used in a multistage series to further increase the concentration of the particle rich airflow. Such a multistage virtual impaction system was described by Romay in which a three stage virtual impaction system was evaluated. This system reduced an initial flow of 300 lpm to 1 lpm, while maintaining 50 to 90 percent of the total particle load in the desired 2.3 to 8.4 micron size range (Romay, Roberts, Marple, Liu, & Olson, 2002). One potential disadvantage of using virtual impaction in the collection of viral containing particles is the high efficiency at which particle sizes of 2 microns and less are eliminated from the minor flow stream. The multistage virtual

impaction bioaerosol sampler used by Romay had a 62 percent capture efficiency for the 2.31 micron sized particles, but only a 28 percent efficiency for the 1.95 micron sized particles (Romay, Roberts, Marple, Liu, & Olson, 2002). These results could indicate very low particle collection efficiencies for sizes less than the 1 micrometer cutoff used in the design of the XMX/2L-MIL. This may cause the XMX/2L-MIL to significantly under represent a viral aerosol consisting largely of submicrometer particles. Further laboratory analysis could confirm if these limitations are a significant contributor to the ability of the XMX/2L-MIL to fully characterize a virus containing aerosol.

Filter Sample Collection and Extraction

Collection by filtration offers many benefits in the sampling of viral aerosols, including greater collection efficiencies of submicron viral particles (Verreault, Moineau, & Duchaine, 2008). An evaluation of filter performance in the collection of nano sized particles and viruses was conducted by Burton et al. and found relatively high collection efficiencies. Polytetrafluoroethylene (PTFE) filters with 0.3, 0.5, 1, and 3 micron pore sizes, polycarbonate (PC) filters with 1 and 3 micron pore sizes, and gelatin filters were simultaneously evaluated. Physical collection efficiency was evaluated using MS2 virons and inert sodium chloride particles. For MS2 collection, the PTFE filters were all similar in performance, with the 0.3 and 1 micron pore size filters recovering approximately 100% of the MS2 viral particles, and the 3 micron pore size filters recovering approximately 95% (Burton, Grinshpun, & Reponen, 2007). The PC filters demonstrated significantly lower MS2 collection efficiencies compared to the PTFE. The 1 micron PC filter had a physical collection efficiency of approximately 70% and an approximately

30% efficiency for the 3 micron PC filters. For inert sodium chloride particle collection, the 0.3 micron and 3 micron sized PTFE filters demonstrated a near 100% collection efficiency for all particle sizes in the test range of 10 to 500 nanometers. The collection efficiency of the 1 micron PC filters ranged from 50% to 100% over the test range and 30% to 60% for the 3 micron filters (Burton, Grinshpun, & Reponen, 2007). These collection efficiencies compare very favorably to the collection efficiencies measured by Hogan for the AGI-30, Biosampler and the Fritted Bubbler. The physical collection for these liquid samplers was 10% or less over the 25 to 100 nanometer particle size range (Hogan, Kettleson, Lee, Bamaswami, Angenent, & Biswas, 2005). The Burton study has some important limitations in its application however. First, the flow rate for the filter samplers was 4.0 lpm, therefore the data from this low volume collection may not be applicable to the high flow rates used by the DFU-1000. Second, the data for the MS2 recovery was obtained using only real time quantitative PCR, plaque assays were not taken to determine viral infectivity after collection. These studies show that filter collection of viral aerosols with the DFU-1000 may be effective. A further study could determine if the conditions produced by high volume collection negate the advantages of filter collection shown in these studies, however.

Limitations to the Use of Filters in Air Sampling

The advantages filter collection offers in fine and ultrafine particle size collection offset by some critical limitations. The primary limitation to the use of filters for viral air sampling is the extraction of the sample from the filter. This extraction is typically required for analysis, and often a large amount of sample cannot be recovered from the

filter. In studies conducted by the Occupational Safety and Health Administration (OSHA), a 68 percent sample removal efficiency was obtained during filter extraction from the polyester felt DFU-1000 filters (Lawrence, 2003). A study conducted by the United States Environmental Protection Agency (EPA) evaluated the extraction characteristics of MS2 bacteriophage from a filter surface. Filters were seeded with "medium" concentrations of MS2 (6.7×10^4 PFU/Filter) and high concentrations of MS2 (5.2×10^5 PFU/Filter). Extraction was accomplished by placing the seeded filter in a tube containing PBS solution and then shaking the tube for 30 seconds, followed by 30 seconds of vortexing (EPA, 2009). For the medium seeded filters, extractions of between 21 and 52 percent of culturable MS2 were observed. The high seeded filters achieved extraction efficiencies of around 100 percent. Although the results from these seeded filters may not be an exact representation of MS2 in a filter after air sampling, these results indicate that as the MS2 concentration of the filter of the DFU-1000 increases, extraction effectiveness will also likely increase. The lower extraction efficiencies for medium seeded filters likely limits the effectiveness of using the DFU-1000 when a low concentration of viral agent is present.

Improvements to Filter Extraction

Methods to improve the retrieval of bioaerosol samples from a filter have been researched as well. Burton et al. compared filter extraction methods, vortex with ultrasonic agitation and vortex with shaker agitation, for bacterial surrogates for *B. anthracis*. Both methods involved vortexing the filter and sample for 2 minutes, followed by agitation for 15 minutes. This study found that both methods were capable

of extracting culturable sample from the filter, but that vortex with shaker agitation produced "significantly higher physical extraction efficiency for both mixed cellulose ester (MCE) and polytetrafluoroethylene (PTFE) filters" (Burton, Grinshpun, & Hornung, 2005). Residual sample remaining on the DFU-1000 filter is expected to significantly reduce the collection effectiveness of this method; however, the degree to which this impacts the ability to accurately assess the presence and magnitude of a viral agent is unknown.

Analytical Methods as Used in Literature

Technological developments have greatly advanced laboratory and field analytical methods since the early studies on viral agents conducted in the 1960's. These early studies required active viral cultures using a variety of culture methodologies, including inoculation into chicken embryos and plate cultures. While active viral cultures can still provide confirmation of a positive result, and must still be used to study viral infectivity, they are of little use in a response environment. In addition to the time requirements, viral isolation and culture is very resource intensive, as well as requiring large amounts of consumable supplies and laboratory support equipment along with sterile working conditions (Fatah, Arcilesi, Chekol, Lattin, Shaffer, & Davies, 2005). Fortunately, other analysis methods are available today that are able to provide fast detection and presumptive identification for viral agents to base level first responders. These methods include immunochemical based techniques and polymerase chain reaction (PCR).

Viable Culture Methods

In order to detect or quantify viable virus, a laboratory based culture method must be used. The primary method of quantifying viruses is through the use of a plaque assay. A plaque assay involves mixing a certain dilution of a virus preparation with susceptible host cells, and spreading this mixture on a nutrient plate. After incubation, the host cells, or bacteria in the case of plaque assay for bacteriophages, form a film on the plate with clearings. These clearings in the film are plaques and are representative of the virus in the sample (Adams, 1959). The number of plaques counted is directly propotional to the number of viruses present in the sample. For bacteriophages, each plaque can "be understood as a phage colony containing the decendants of a single phage particle" (Adams, 1959). Every phage present in the sample will not produce a plaque; therefore, plaque counts should be used as a relative method of quantification as opposed to an absolute quantification (Adams, 1959). A relative quantification method is all that is required for the comparison of air sampling equipment since the limitations to quantification described by Adams would be present in each plaque assay conducted as part of the comparative analysis.

Immunochemical Analysis

Immunochemical based techniques include hand held assays (HHA) and enzyme linked immunosorbent assays (ELISA). These analysis methods provide quick turnaround times to response personnel. HHAs are regularly employed by Bioenvironmental Engineering and Civil Engineering Emergency Management for on scene detection. These simple devices can be used to detect a variety of biological agents including variola (Peruski &

Peruski, 2003). Immunochemical processes have significantly high detection limits, however. For detection of bacterial agents, such as *B. anthracis*, detection limits of approximately 10,000 colony forming units per milliliter of sample (CFU/ml) were required (Peruski & Peruski, 2003). ELISA methods were also developed and evaluated for the rapid detection of Ebola virus and for "Ebola like particles" by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in a study conducted by Kallstrom et al. The Kallstrom study found that ELISA could be used in the detection of Ebola virus, with detection limits of approximately 500 to 1000 plaque forming units in an analyzed sample. Similar to PCR, ELISA does not require infectious virus for detection. For example the Kallstrom study used an irradiated Ebola virus sample in their analysis (Kallstrom, et al., 2005).

PCR Analysis

Although immunochemical based techniques provide a rapid detection method that is reasonably effective, the limits of detection are still a concern for these methods. The Air Force utilizes PCR as its presumptive detection methodology in response to a biological agent release. PCR provides a much lower limit of detection than immunochemical methods. For detection of *B. anthracis,* PCR techniques provide a limit of detection in the range of 1 to 100 CFU/ml of sample, which is much lower than the immunochemical limit of 10,000 CFU/ml (Peruski & Peruski, 2003). PCR allows for a small amount of DNA in a sample to be multiplied through a series of cycles that effectively doubles the amount of target sequence DNA per cycle. RNA viruses such as MS2 do not contain DNA, therefore an additional step known as reverse transcriptase, is required. This

process uses the enzyme reverse transcriptase to synthesize complementary DNA from RNA. This synthesis process then allows the complementary DNA to be used in PCR for detection. Real-time reverse transcriptase fluorogenic PCR was developed by O'Connell et al. for the analysis of MS2 bacteriophage. Fluorogenic PCR uses a fluorogenic probe that increases in florescence in the presence of the target DNA sequence (O'Connell, et al., 2006). This florescence provides a measurable metric and a threshold of the change in florescence is set to denote the presence of the DNA in a sample. O'Connell developed and tested five separate primer and probe sequences for MS2 (O'Connell, et al., 2006). For biological agent detection using the JBAIDS, sample reagents containing these primer and probe sequences are prepared specifically for each agent of interest. O'Connell evaluated each sample for up to 45 cycles, and results were provided as the number of cycles required for the target sequence to reach a threshold level of detection. There was some variation in the number of cycles required for detection based on the probe and primer sequences used. This variation led to a change in the limit of detection. For example, one of the five probe/primer sequences was capable of detecting 0.4 picograms (pg) of MS2 in a sample. Two of the five probe/primer sequences were capable of detecting 4 pg of MS2 in a sample, while all five sequences were capable of detecting 400 pg of MS2 in the sample (O'Connell, et al., 2006). This study demonstrates the importance of properly selecting the probe/primer sequences for viral sample analysis using PCR and the low levels of detection available in PCR analysis. The O'Connell study also highlights some of the many difficulties in using PCR as a quantitative analysis method, since just the selection of probe and primer sequences greatly affected the sensitivity of the analysis.

Limitations of PCR Analysis

A critical issue associated with any laboratory analysis method for biological agent detection is the potential for false positives. This limitation was considered in the selection of the JBAIDS. The acquisition testing specifications for the JBAIDS required a false positive rate for variola of no more than 10%, or specificity of 90% (Wilson, 2006). Actual contractor validation testing of the JBAIDS obtained a specificity of 99%. This specificity testing evaluated the ability of the JBAIDS to distinguish variola from vaccinia, which is a closely related virus. Sensitivity requirements were also included in the JBAIDS evaluation criteria. One pass or fail criteria for sensitivity required that the JBAIDS be capable of identifying vaccinia virus 85% of the time. Actual operational testing results for vaccinia obtained a sensitivity of 90.4%. Similar testing was conducted for Ebola and Venezuelan Equine Encephalitis virus, and sensitivity of 100% was obtained (Wilson, 2006). Some viral analysis using the JBAIDS did result in lower sensitivities. For example, the operational testing sensitivity for Marburg was 78.5% (Wilson, 2006), or a false negative rate of more than 1 in 5. While limited in its application, the high levels of sensitivity and specificity allow the JBAIDS to provide a standalone presumptive detection methodology during response to a biological agent release. Due to the widespread use of PCR analysis and its proven capabilities, all evaluations on Air Force biological air sampling equipment should incorporate PCR analysis as an assessment criterion.

Decontamination

The limited availability of air sampling equipment during a response to biological agent release requires that effective decontamination methods and materials be available to allow for multiple samples to be taken with a single piece of equipment. Ideally, one decontamination methodology would be applicable to all biological air sampling equipment. The manufacturer of the XMX/2L-MIL, recommends that removable canister components be submerged in a 10% bleach (sodium hypochlorite) and water solution for 5 to 10 minutes then rinsed with tap water followed by rinsing with distilled water (Dycor Technologies, 2001). The manufacturer does not provide additional guidance on decontamination procedures for specific microbial agents, requiring the user to develop these procedures. As shown by Schofield et al., eliminating cross contamination between samples collected using the XMX/2L-MIL is very important (Schofield, Ho, Kournikakis, & Booth, 2005).

Decontaminating Agents

A variety of chemical and physical processes have been used for decontamination of air sampling equipment. The adenovirus and meningitis study conducted by Artenstein used a single high volume sampler, and thus required decontamination between trial runs. The decontamination method used was a dual wash and rinse process involving a wash in a solution of 70% alcohol and a rinse with distilled water (Artenstein, Miller, Lamson, & Brandt, 1968). Decontamination effectiveness was not separately evaluated in the Arttenstein et al. study. USAFSAM has evaluated decontamination procedures and methods for the XMX/2L-MIL. These prior studies on the XMX/2L-MIL were limited to

bacterial agents, and focused only on decontamination for the XMX/2L-MIL. These studies used *Bacillus atrophaeus*, a spore forming organism commonly used as a surrogate for *Bacillus anthracis*, and *Bacillus subtilis* as a surrogate for *Yersinia pestis* (LaRoche, 2009). The first study compared two methods of decontamination using a 10% bleach solution. The first method evaluated involved wiping the canisters of the XMX/2L-MIL with the bleach solution, while the second method submerged the canisters in the bleach solution. The study found that there was no statistically significant difference between the two methods, and confirmed prior studies, which found that hypochlorite is an effective decontaminating agent. Operator error can be a significant factor for the wipe decontamination method however; therefore, the study recommended that field decontamination use the submersion method (LaRoche, 2009). A second study conducted by USAFSAM evaluated the effectiveness of three solutions for decontamination; 5% bleach, 10% bleach, and 3% hydrogen peroxide (Lohaus, et al., 2009). All three solutions were found to be effective for the decontamination of bacterial agents. Decontamination by hypochorite bleach was selected by USAFSAM for further evaluations due to its widespread commercial availability and anti-microbial effectiveness.

Sensitivity of Viruses to Decontamination

While viral agents are less resistant to decontamination than bacterial spores and mycobacteria, small non-enveloped viruses are still more resistant than both gram-negative and gram-positive bacteria. Large non-enveloped viruses and lipid enveloped viruses are some of the least resistant organisms to decontamination (LaRoche, 2009).

The relative sensitivities of different classes of microbes to decontamination is shown in Figure 5.

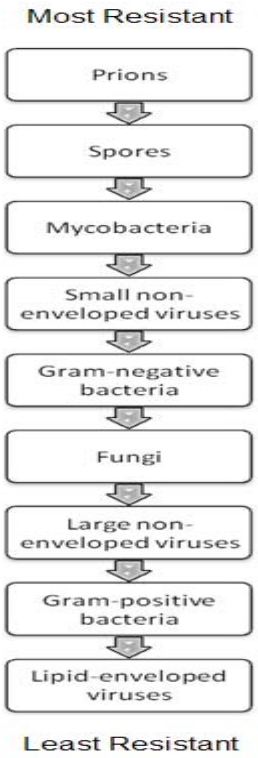

Figure 5: Resistance of Microorganisms to Decontamination (Adapted from LaRoche, 2009)

Surface Decontamination of Air Sampling Equipment

The non-expendable surfaces subject to contamination on the XMX/2L-MIL are unpainted and painted metal, while the surfaces on the DFU-1000 are primarily plastic, foam plastic, and coated plastic. This difference in surfaces introduces two potential limitations to applying previous studies; the ability of the decontaminant to penetrate the surface and the sensitivity of the material surface to corrosion and degradation from the

decontaminant. Sodium hypochlorite is very corrosive and can impact the operation of sensitive equipment; however, the LaRoche study noted that corrosion was unlikely to be a significant risk to the performance of the XMX/2L-MIL (LaRoche, 2009). LaRoche measured the presence of contamination on the surfaces of the air sampling equipment by using cotton swabs soaked in PBS solution (LaRoche, 2009). While swabbing can be an effective technique in identifying contamination, it has several limitations. Rose et al. evaluated the recovery of *Bacillus anthracis* (BA) from a steel surface using swabs. Four different swab materials were used, including cotton, macrofoam, polyester, and rayon. Extraction effectiveness and the effectiveness of pre-moistening the swab prior to sample collection were also evaluated. The authors found that macrofoam and cotton produced the greatest recovery of BA and that pre-moistening the swab increased sample collection. Vortexing was more effective in extracting sample from the swab than sonication. However, recovery was still relatively low, with the cotton swabbing only recovering 27.7 percent of the BA on the surface (Rose, Jensen, Peterson, Banerjee, & Arduino, 2004). A follow-on study conducted using macrofoam swabs found that the percentage of sample recovered on the swab decreased significantly when lower concentrations of BA were present on the surface (Hodges, Rose, Peterson, Noble-Wang, & Arduino, 2006). These lower sensitivities at low concentrations could be a significant limitation to using surface swabs for post-decontamination evaluation when there are very low concentrations of surface contamination. Furthermore, it is of interest to note that the studies conducted by Rose and Hodges used BA, a spore-forming bacteria. As shown in Figure 5, spore forming bacteria are more resistant than viruses. Therefore,

using swabs in the evaluation of viral contamination may be even less effective than using swabs to measure bacterial contamination.

Limitations of Previous Decontamination Studies

One key limitation to the decontamination studies conducted by USAFSAM was that decontamination effectiveness was not evaluated for equipment reuse when using PCR analysis methods (LaRoche, 2009). Since PCR does not require a viable organism for detection, DNA and RNA residuals from previous sampling could result in a positive result during PCR analysis, despite culture analysis indicating the absence of contamination. PCR analysis using the JBAIDS system is the presumptive method of identifying biological agents for the Air Force and should be included in all studies involving the sampling or decontamination of biological agents.

Problem Statement and Summary

Previous literature provides a reasonably thorough background to laboratory and field studies on the air sampling of viral aerosols. Previous studies involving the use of high volume air sampling for viruses are more limited however. Since these high volume sampling methods are the primary means of response, detection, and risk assessment for the Air Force; additional study is necessary. Experimental evaluations of the XMX/2L-MIL and the DFU-1000 for the collection of viruses are not available in published literature. Furthermore, no studies comparing performance at different levels of airborne viral load have been performed for the XMX/2L-MIL or the DFU-1000. Evaluating air sampling equipment at different levels of airborne viral load could provide information

on the effectiveness of viral samplers at lower levels of detection which would be critical in a field response. Side by side comparison of existing high volume methodologies has also not been conducted. Simultaneous comparison of high volume sampling methodologies and low volume sampling has also not been completed. Since low volume collection methods such as the AGI-30 and Biosampler are widely evaluated in literature, low volume methodologies could be considered a "gold standard" with which to compare the XMX/2L-MIL and DFU-1000. A comparative evaluation of the XMX/2L-MIL and the DFU-1000 would provide valuable information on the feasibility of conducting laboratory evaluations to guide future purchase decisions of Air Force sampling equipment for bioaerosols.

XMX/2L-MIL viral studies have been limited to PBS and water collection media. Effectiveness of other solutions such as Remel M5 has not been evaluated for performance in viral collection. This evaluation is necessary to determine if Remel M5 could be used for field conditions where the use of PBS and water media may not be appropriate.

PCR detection has not been evaluated in the previous decontamination studies for the XMX/2L-MIL. Furthermore, no decontamination studies have been completed for the DFU-1000. USAFSAM is performing a complete decontamination evaluation on viral agents for the XMX/2L-MIL that includes PCR analysis. Decontamination effectiveness for viral agents should be evaluated as a comparative measure of equipment performance for both the XMX/2L-MIL and the DFU-1000. This comparative evaluation of

decontamination should include JBAIDS analysis to ensure that both systems are capable of being decontaminated to a level below what the JBAIDS is capable of detecting.

In summary, a comparative evaluation on the effectiveness of Air Force high volume air sampling equipment currently present in the inventory is necessary. This study should be based in a controlled laboratory exposure chamber to provide stable aerosol conditions and quantify external variables such as aerosol particle size, relative humidity, and temperature. Equipment evaluation should include resources that would be applicable and beneficial to a field response, such as the use of virus preserving sample media, and detection with a field analysis tool such as the JBAIDS. This evaluation would not only aid in equipment selection for current response use, but would also form a baseline for future comparative studies.

III. Methodology

Objective

This section describes the methodology used for the comparison of the XMX/2L-MIL and DFU-1000 high volume air samplers. Procedures for the comparison of viral aerosol sampling effectiveness and field decontamination are developed and described.

Study Design Overview

The primary means of evaluating sampling equipment for viral aerosols is through the use of an aerosol test chamber (ATC), where aerosol concentrations and conditions can be measured and monitored. Due to the high volumetric flow rates of air sampled by most high volume samplers, a sufficiently large chamber must be provided. Laboratory analysis methods, surrogate agent selection, and decontamination equipment and methodologies must be established as well. These procedures and selection methods are described in this section.

Surrogate Virus and Host Cell Selection

Male Specific Coliphage 2 (MS2), American Type Culture Collection (ATCC) 15597-B1, was chosen as the surrogate viral agent for this study. MS2 offers several advantages that are not offered by other agent choices. Male specific coliphages such as MS2 are able to be controlled in the laboratory and cannot propogate in conditions outside the laboratory. The reasons for these qualities of MS2 were described by Riemann as the manner that MS2 attaches to and infects the *E.-coli* bacterium. "Male specific coliphage

strains infect 'male' strains of *E.-coli* via the pilus." The pilus only forms in *E.-Coli* at temperatures exceeding 30 degrees Celsius, thus requiring laboratory incubation for MS2 to propogate (Riemann & Cliver, 2006). Numerous prior studies have shown that MS2 can be effectively recovered during air sampling using a variety of methodologies. MS2 is an un-enveloped, single strand RNA virus with an approximate size range of 20 to 30 nanometers. Un-enveloped viruses have been shown to have greater resistance to decontamination and thus represent a suitable worst case for evaluation. The evaluation of viral collection was conducted in conjunction with a decontamination study on the XMX/2L-MIL conducted by USAFSAM, and was, therefore, the most suitable agent available. Additionally a virus that is more resistant to environmental conditions, such as un-enveloped viruses, is more likely to survive sampling and provide results to which equipment comparisons can be made. Prior studies on high volume air sampling in the laboratory setting is limited; therefore using a more resistant virus increases the likelihood of obtaining data and being able to compare results between sampling systems.

Chamber Setup and Layout

Aerosolization studies were conducted in an aerosol test chamber (ATC) provided by Dycor Technologies Ltd. in Edmonton, Alberta. The ATC is 12 cubic meters in volume and approximately 3 meters in length, 2 meters wide, and 2 meters high. A layout of the chamber is shown in Figure 6 below.

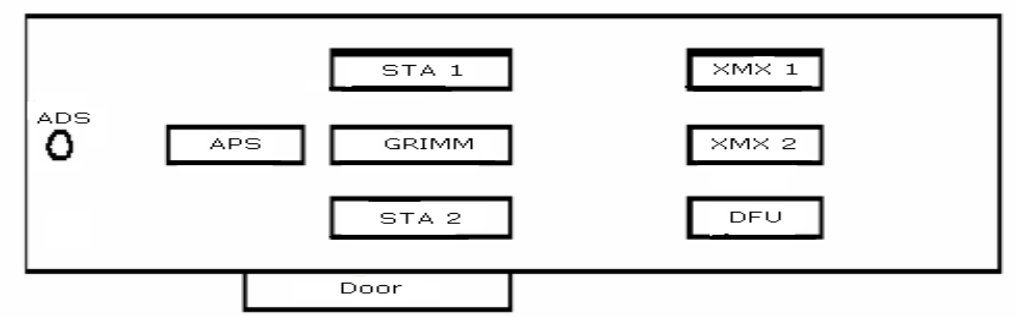

Figure 6: Aerosol Test Chamber Layout

Three high volume ports were located approximately 1.9 meters from the point of aerosol dispersal. A schematic showing the full layout of the ATC with component locations and measurements of interest is available from USAFSAM. The chamber was equipped with two circulating fans for aerosol mixing. Particle sizing was measured using a TSI Aerodynamic Particle Sizer (APS), model number 3321. Real-time particle concentrations were monitored and maintained using a Grimm Optical Particle Counter model 1.108. The vent hood of the XMX was raised into the ATC to a height of approximately 16 centimeters. The DFU-1000 was attached to a 5 cm diameter copper pipe that was raised into the chamber to a height of approximately 16 centimeters, consistent with the two XMX/2L-MIL samplers. Intake height was kept constant among instruments to eliminate bias in sampling that might occur by differences in sample collection height. The adaptation of the copper pipe to the DFU-1000 is similar in configuration to the adaptation of the DFU-2000 assembly which allows the DFU-1000 to be used in outdoor environments. A DFU-2000 is shown in Figure 7 and consists of a DFU-1000, a plastic housing unit, boom extension, and particle pre-separator.

Figure 7: DFU-2000 (Left) and DFU-1000 (Right)

Low Volume Air Sampling

Two low volume air sampling methods were included in each exposure trial; the AGI-30 and the Biosampler. These low volume samplers were chosen due to their widespread use in prior published studies and proven effectiveness in the collection of particle sizes of 1 micron and larger. These low volume samplers can be used as a comparison benchmark for the high volume methods. The AGI-30 and Biosampler were attached to a stand inside the chamber near the DFU-1000 sample port and placed at a height below the inlet points of the XMX/2L-MIL and DFU-1000. This setup is shown in Figure 8. Air flow to the AGI-30 was provided by a vacuum line attached to the test chamber.

Figure 8: Biosampler and AGI-30 in Aerosol Test Chamber

The Biosampler was operated using an SKC Vac-U-Go non-compensating vacuum pump. The Vac-U-Go pump was attached to the biosampler through an apparatus that included a water trap (Erlenmeyer flask) and field rotameter. The apparatus connected to the Biosampler is shown in Figure 9. Low volume air sampling equipment was calibrated before and after sampling each day using a Bios DryCal DC-2 Air Flow Calibrator. The Bios DryCal DC-2 served as the primary flow calibrator for both the AGI-30 and Biosampler. An SKC field rotameter was used with the biosampler as a secondary flow standard. The AGI-30 was operated at a calibrated flow rate of 12.75 to 12.80 lpm, and the Biosampler at a calibrated flow rate of 12.5 lpm. Flow rates for the AGI-30 and Biosampler were converted to standard liters per minute (SLPM) using temperure and ambient pressure data. Temperature was monitored during each sample collection period. Ambient pressure data was obtained hourly for Edmonton, AB from the Canadian Weather Service.

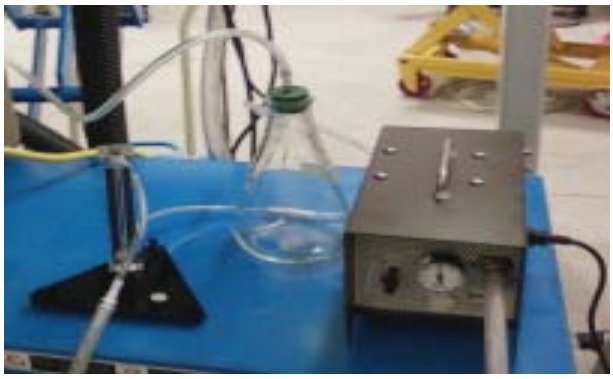

Figure 9: Biosampler Apparatus

Aerosolization of Viral Solution

Aerosol Introduction

MS2 aerosol solution was prepared by diluting a 4.4×10^{10} plaque forming units per milliliter (PFU/ml) stock solution with Luria broth. The MS2 stock solution was diluted by a factor of 10 for the high and medium airborne viral loads and by a factor of 100 for low airborne viral loads. Airborne viral loads are presented in terms of agent containing particles per liter of air (ACPLA). The final concentration of aerosol solution is shown in Table 3 for each target level of viral load.

Airborne Viral Load	Target ACPLA	Dilution Factor	Concentration of Aerosol Solution
High	100	10	4.4×10^{9}
Medium	10	10	4.4×10^{9}
Low	1	100	4.4×10^{8}

Table 3: MS2 Aerosol Solution

MS2 solution was aerosolized using a Sonotek 8700-48MS ultrasonic atomizing nozzle, mixed with HEPA filtered air and introduced into the chamber. The MS2 aerosol solution was placed on an automated rocker platform (Maddell ZD-9550) and supplied to the aerosol generator described above. The rocker and aerosol generator are shown in Figure 10 and Figure 11. MS2 solution was supplied to the aerosol generator at a rate of 1 ml/min when additional aerosol injection was triggered by the Grimm measurements.

Figure 10: Rocker Platform

Figure 11: Aerosol Generator

Maintenance of Viral Aerosol Concentration

Aerosol particle concentrations were monitored using a Grimm optical particle counter. A target concentration for each level of viral load was specified by Dycor. These levels were extrapolated using data from prior exposures conducted in the exposure chamber. MS2 aerosol regeneration was provided via automatic injection, when the particle count dropped below the target concentration level. Particle counting during the five minute air purge was controlled in a similar fashion and continued until the Grimm optical particle counter registered a sustained particle concentration of less than 1 particle (0.5 to 2.0 microns in size) per 0.12 liters of air.

Final Determination of Aerosol Concentration

Final aerosol concentration was determined using slit to agar plaque count. Two slit to agar biological air samplers, model number STA-203 manufactured by New Brunswick Scientific, were employed for this purpose. These slit to agar samplers operated at a flow rate of 30 lpm. The slit to agar samplers were operated for two minutes per plate during the high and moderate viral load trials and for five minutes per plate during the low viral load trials and for the chamber blanks. Multiple plates were necessary during the high and moderate viral load conditions to allow the plates to be counted after incubation. Excessive exposure on a single plate would result in a “too numerous to count” (TNTC) result that would invalidate the plate count.

Equipment Preparation

Preparation of Collection Media

Remel MicroTest M5 Multi-Microbe Media (Remel M5) was selected as the collection media for all instruments in this study. Remel M5 is commercially available in three milliliter vials only, thus requiring multiple three ml vials to be combined in order to produce the lot sizes required for the sampling equipment. Prior studies conducted by USAFSAM noted excessive foaming when Remel M5 was used in an impinger collection system due to turbulence produced in the media. This foaming was partially addressed by the addition of 0.1% anti-foam, Y-30 aqueous emulsion, manufactured by Sigma Aldridge, to the media. This was done after the Remel M5 had been combined into 40 milliliter lots by adding 40 microliters of anti-foam to each lot. Remel M5 with anti-foam was used as the collection media for all samples, including for the preservation of DFU-1000 sample filters. Use of similar collection media for all applications eliminated the variability that would have been introduced by the use of multiple media types.

Preparation of XMX/2L-MIL

XMX/2L-MIL canister components were sterilized overnight in an autoclave prior to each day of sampling. Removal of contamination was verified by swabbing each canister prior to use. Swabs were taken around the air flow points of each canister. The canister assembly was then inserted into the XMX/2L-MIL per manufacturer instructions. In order to further reduce the foaming of the Remel M5 observed in prior testing, a flow reducer was designed by Dycor for USAFSAM. This flow reducer consists of a brass

cylinder with a small hole that is placed between the liquid impingement module and the fluid trap to reduce secondary flow into the impinger module. This reduces airflow into the collection media from approximately 12.5 lpm to 4 lpm and reduces the agitation and evaporation of the collection media (Bliss, 2009).

The XMX/2L-MIL was prepared with an expendable impinger nozzle for each test per the manufacturer's instruction. A 50 milliliter sample collection tube was filled with 5 milliliters of Remel M5 media. This was then inserted into the XMX/2L-MIL per the manufacturer's instructions. The liquid impinger module of the XMX/2L-MIL is shown in Figure 12.

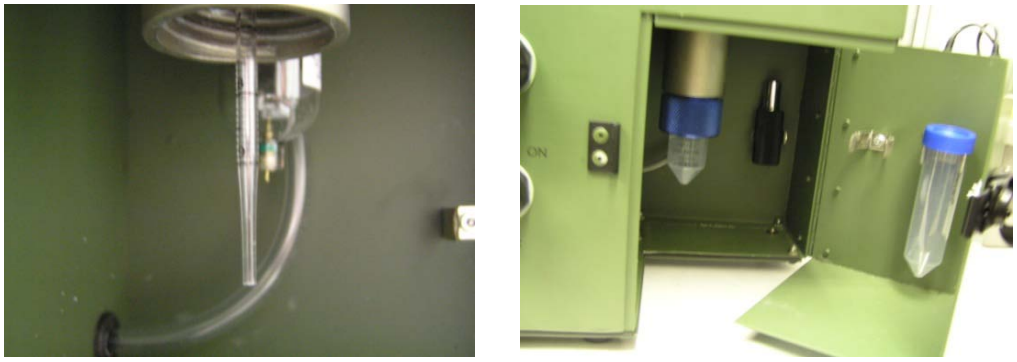

Figure 12: XMX Impinger Module Shown Without Tube (Left) and With Tube (Right)

Preparation of DFU-1000

The DFU-1000 was prepared according to Air Force Technical Order 11H1-11-2 using a standard sampling expendables kit. A new expendables kit was used for every sample trial and includes 3 filters (1 spare), 2 pairs of latex gloves, 1 pipette, plastic whirl bags, paraffin film, and a zip-lock bag. The kit and its contents are shown in Figure 13.

Figure 13: DFU Sampling Kit Contents

The filter cartridges were then inserted into the intake module and attached to the pump module and casing. The DFU-1000 filter cartridges and intake module are shown in Figure 14.

Figure 14: DFU Filter Cartridge (Left) and Intake Module (Right)

Test Cycles

XMX/2L-MIL Decontamination Trials

Two test days were devoted to the evaluation of field decontamination effectiveness following XMX/2L-MIL exposure to viral agent during sampling. Each of these sample days consisted of an initial swabbing to verify absence of contamination, five minutes of sampling during exposure to a purged or "clean" chamber, five minutes of sampling under high levels of viral load, decontamination using hypochlorite solution, a 15 minute air purge, and five minutes of sampling from a purged chamber following air purge. Three XMX/2L-MIL high volume air samplers were simultaneously operated during these trials. Sampling data obtained from these XMX/2L-MIL decontamination trial days is included in this study only for evaluating response variability among multiple XMX/2L-MIL air samplers.

Comparison Trials

Three test days were allocated to the comparison of the XMX/2L-MIL and the DFU-1000. One day was allotted for studies under each level (High, Medium, and Low) of viral load. For each sampling day, two, five minute chamber exposures to the appropriate level of viral load were made for each high volume sampler. For each five minute exposure period sampling using an AGI-30, and Biosampler was included as well. Additionally, five minutes of sampling with the XMX/2L-MIL and DFU-1000 were completed for two chamber blanks each day. Decontamination evaluation was accomplished during these test days, as well, and is discussed in the "Decontamination

comparison" section. A complete test matrix for the comparison trials is included in Appendix 1.

Retrieval and Preparation of Sample

AGI-30 and SKC Biosampler

After each viral exposure run, collection media from the Biosampler and AGI-30 was removed from the impinger assembly and transferred to a 50 ml collection tube. A final volume of sample was measured and recorded for each sample. An example of the 50 ml collection tubes used for all equipment throughout the study is shown in Figure 15

Figure 15: 50 ml Conical Collection Tube
(From Lawrence, 2003)

XMX/2L-MIL

Following each test run, the expendable impinger tube was discarded and the 50 ml sample tube removed from the impinger module. Standard laboratory practices were followed to ensure that any MS2 contamination present on the XMX/2L-MIL unit was unable to come in contact with the sample. Once the sample tube was removed from the

XMX/2L-MIL, it was capped, sealed with parafilm and taken to the laboratory for analysis by plaque assay.

DFU-1000

After air sampling was complete, both dry filters were removed from the DFU-1000 filter inserts and placed in a 50 ml collection tube with 15 ml of Remel M5 collection media. Collection media was allowed to make complete contact with the filter material by hand agitating the collection tube. Once received in the laboratory working area, the filters and sample media were vortexed for 10 seconds at a speed of approximately 3200 revolutions per minute. Sample media was then collected for analysis by plaque assay.

Laboratory Analysis

Preparation and Incubation of Plates

Differently sized plates were prepared for the plaque assay and STA collection system. The plaque assay required 100 mm plates, while the STA required 150 mm plates for the slit to agar. Plaque assay plates were prepared by using 10 ml of MS2 growth media with an overlay of 200 µl of *E.-coli*, ATCC 15597, (8.75×10^8 CFU/ml) and 200 µl of sample, or diluted sample, as appropriate. The overlay was mixed by gently hand swirling the prepared plate. Slit to agar plates were prepared using 25 ml of MS2 growth media, with 500 µl of *E.-coli* as an overlay. This preparation technique for plaque assay plates is described by Adams in *Bacteriophages* (Adams, 1959). The MS2 growth media consisted of 5.6 grams bacto-agar, 6 grams of sodium chloride, 5 grams of proteose

peptone, 2 grams of yeast extract, and 5.2 ml of glycerol added to 1 liter of distilled water. After sample was added plates were incubated overnight, for a minimum of 12 hours, at a temperature of 37 degrees Celsius.

Quantification of Virus in Sample

Plaque Assay

Virus quantity was determined through the use of plaque assay. Plaque assay provides a relative quantification of bacteriophage in a sample (Adams, 1959). Serial dilutions were necessary for the proper quantification of these assays. This was accomplished by vortexing the collected sample in the 50 ml collection tube to homogenize the sample. Vortexing was accomplished using a Vortex Genie 2 manufactured by Scientific Industries, Inc. Samples were vortexed at a speed of approximately 3200 revolutions per minute (RPM). An aliquot of sample (50 µl) was then pipetted into 450 µl of sterile Remel M5 media. The dilution was vortexed for five seconds to homogenize the sample with the sterile M5 media. For further dilutions of 10^{-2}, 10^{-3}, and 10^{-4}, this process was repeated until the proper dilution had been achieved. Figure 16 shows the serial dilution process in detail (LaRoche, 2009). The sample plates referenced above were incubated at least 12 hours. Plaque forming units were counted visually for each plate, as shown in Figure 17, and the count was scaled to the proper order of magnitude by dividing the count of plaque forming units by the dilution factor. For example, the number of plaque forming units on a 10^{-3} dilution would be divided by 0.001 to obtain the final count present in the undiluted sample. Once an approximate range of expected plaque forming

units per ml of sample was known, a single dilution was plated as opposed to plating each of the serial dilutions. This reduced the time, resources, and manpower required for duplicate plating and analysis of multiple dilutions. Two plaque assay plates were prepared for each specified dilution, as well as the undiluted samples.

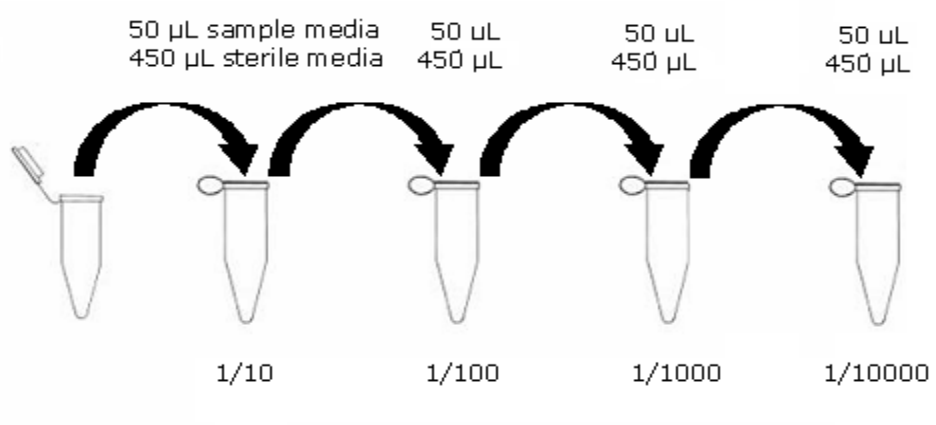

Figure 16: Serial Dilution Procedure
(Adapted From LaRoche, 2009)

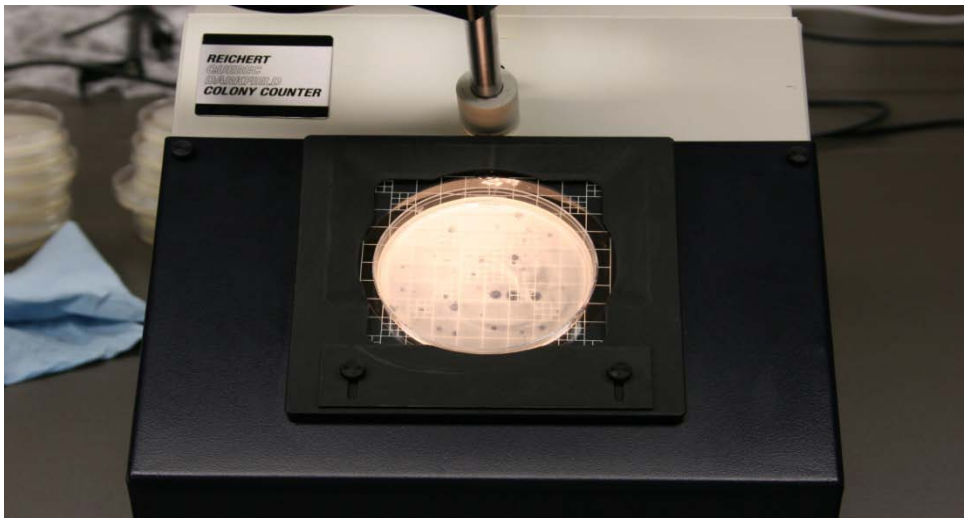

Figure 17: Visual Counting of Plaque Assay

PCR Analysis

Evaluation of decontamination capabilities to levels below the limits of detection for PCR analysis requires that samples be analyzed by PCR equipment used by Air Force laboratory personnel during response to a biological incident. Samples were shipped to the Applied Technology Center of USAFSAM located at Brooks City-Base, Texas for PCR analysis. This analysis was performed for MS2 presence/absence using the JBAIDS system. Reagents, probe, and primer sequences for MS2 are proprietary products obtained from Idaho Technologies.

Decontamination Comparison

Selection of Swab Surfaces for Contamination Detection

Prior decontamination studies on the XMX/2L-MIL analyzed swabs taken from surfaces directly in contact with the sample airflow (LaRoche, 2009). These surfaces are the likely pathways for previous sampling events to contaminate succeeding samples. The virtual impactor of the XMX/2L-MIL consists of the five components shown in Figure 18, with each having surfaces in direct contact with sample airflow.

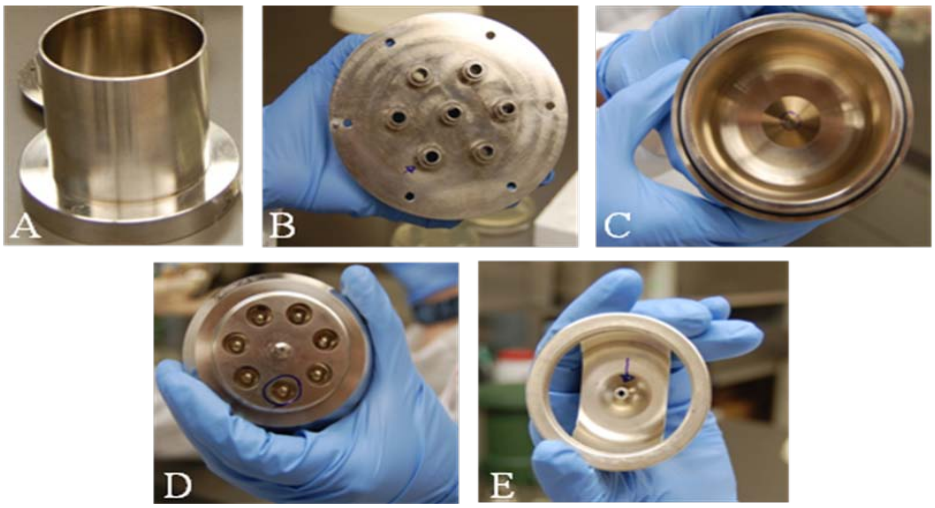

Figure 18: Components of XMX/2L-MIL Virtual Impaction Module
A - Primary Inlet, B - Primary Nozzle Plate, C - Upper Canister, D - Lower Canister
E - Final Nozzle (LaRoche, 2009)

Similarly, surfaces in direct contact with the sample air flow on the DFU-1000 were selected for contamination detection. These surfaces are located on the air intake housing and on the plastic filter inserts and are shown in Figure 19. After the surfaces were swabbed, the swab was placed in 5 ml of Remel M5.

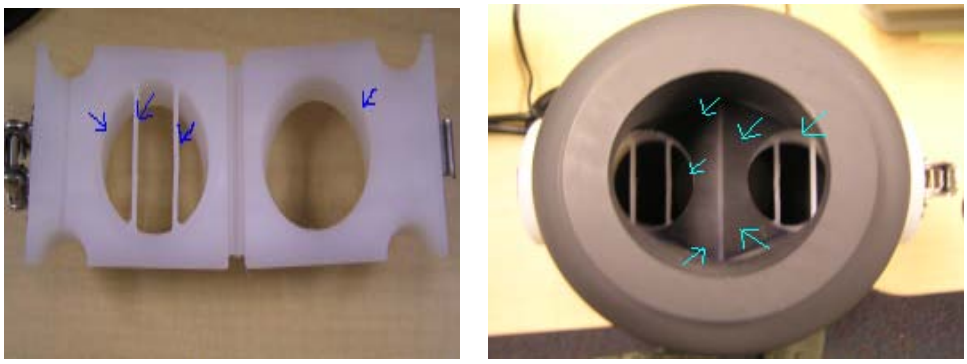

Figure 19: Swab Surfaces on DFU-1000

Hypochlorite Decontamination

A decontamination solution was prepared in a 5 gallon container (shop bucket) using 1 liter of commercially available, 5.25% sodium hypochlorite bleach mixed with 9 liters of tap water. A rinse container was prepared with 10 liters of tap water. These materials were similar to those used in previous decontamination studies on bacterial agents conducted by the USAFSAM (LaRoche, 2009). Each component for both the XMX/2L-MIL and DFU-1000 was submerged in the container with hypochlorite solution for 5 minutes. This was followed by five minutes of submersion in the rinse container. Each component was then hand dried using paper shop towels. Throughout the decontamination process, separate working stations and containers were used for each instrument. This procedure reduced the possibility of cross contamination between instruments during the decontamination process.

Post Decontamination Swabbing

After the completion of the hypochlorite decontamination process and drying, surface swabs were taken again using the previously described procedure. This allowed for the quantification of residual surface contamination following decontamination. Swabs were analyzed by vortexing for 10 seconds at approximately 3200 rpm, similar to the filter extraction used for the DFU-1000 samples. This procedure allowed for the extraction of MS2 from the swab into the M5 media. The collection media was then plated using the plaque assay technique described earlier.

Post Decontamination Air Sample Collection

To evaluate the extent that MS2 residual from previous sample runs contaminated subsequent sample attempts, a 5 minute air sample from a purged chamber was collected following the first MS2 exposure and field decontamination for each of the three comparison trial days. This sampling was performed using the 2 XMX/2L-MILs and the DFU-1000 used in the MS2 exposure and field decontamination. Samples were collected as described in the previous sections.

Decontamination Station Ambient Air Monitoring

Results from prior studies evaluating the decontamination of bacterial agents indicated the need to monitor for ambient air contamination in the decontamination work stations. This is necessary since the decontamination stations at the Dycor facility are co-located in the same room as the aerosol test chamber. This condition creates the potential for airborne agent released during the removal of the high volume air samplers to pose a contamination risk during portions of the decontamination processes such as drying or surface swabbing. A single XMX/2L-MIL was operated to determine the ambient level of MS2 contamination present in the work area. This XMX/2L-MIL was placed near the location of the high volume air sampler test ports on the Dycor exposure chamber. This location was considered representative of a worst case exposure to ambient MS2 contamination at the location of the decontamination stations. The decontamination stations were located on the opposite side of the test chamber room. Undiluted plaque

assays were prepared to analyze the samples collected from this ambient air sampling for MS2.

Data Analysis

Calculation of Plaque Forming Units in Sample

Plaque forming units per milliliter of collection media was used to determine the air concentration collected by the air sampling system. First the total PFU collected was calculated by multiplying the liquid plaque concentration (PFU/ml) by the total volume of collection media remaining after collection was complete. Use of the remaining volume of collection media, as opposed to initial media volume, is particularly important for impinger collection systems that tend to lose sample media during sampling. This total PFU collection was then divided by the total air volume sampled during the five minute trial. PFU data for the DFU-1000 and surface swabs were similarly determined, except that the volume of media added to the collection swab or filter was the volume used as the total media volume.

Particle Size Distribution Analysis

To determine the extent that the particle size distribution had as a performance factor in the effectiveness of the sampling systems, particle size distributions were analyzed for similarity using their Count Median Diameter (CMD). A CMD distribution of 51 six second Grimm samples was prepared for each trial. Comparison of CMD distributions between trials was conducted using a Kruskal-Wallis one way ANOVA (K-W ANOVA),

with a Dunn's rank sum post-test when significant differences were identified by the K-W ANOVA. Non-parametric comparisons of the median were needed once it was determined that an assumption of normality or log-normality could not be made. This determination was made using results from the Kolmogorov-Smirnov test for normality. Additionally, non-parametric analysis methods, such as K-W ANOVA, are considered to provide a more conservative analysis of variance. Kruskal-Wallis and Kolmogorov-Smirnov analysis was performed using Minitab version 15. The Dunn post-test analysis was performed using Prism Graphpad software, version 5.

MS2 Sample Data Analysis

The small number of samples taken for both the air sampling and decontamination comparisons do not allow for the reliable use of parametric statistics for data analysis. For a small data set, a determination of normality cannot be made or assumed. Additionally a comparison of means is more likely to be influenced by the presence of outlying data points. For these reasons, the non-parametric K-W ANOVA was used for the analysis of these results as well. The Dunn post test was conducted once the K-W ANOVA identified significant differences between data sets.

IV. Results and Analysis

Air Sampler Flow Rate

Flow rate measurements for the XMX/2L-MIL were conducted by Dycor Technologies using a proprietary method. Measurements were made at the exhaust point of each instrument after three minutes of operation and 20 minutes of operation. The results for the measurements taken after three minutes of operation are presented in Table 4. Each sample presented in this study was collected for five minutes; therefore, the air flow rate at three minutes is considered to be a representative average air flow rate during the five minutes of sample collection.

Instrument	Temperature (Celsius)	Pressure (kPa)	Flow Velocity (m/s)	Flow (slpm)
XMX 1	46.4	93.49	6.60	667
XMX 2	42.6	93.43	6.15	620
XMX 3	44.6	93.42	6.15	620
XMX 4	43.3	93.42	5.94	599
DFU	28.3	93.90	7.67	778

Table 4: High Volume Equipment Flow Rate Measurements Provided by Dycor Technologies, 2009

The measurements provided by Dycor were calculated using a proprietary methodology and presented as flow rate in standard liters per minute (SLPM) or air flow at 25 degrees Celsius and 101.325 kilopascals (kPa). These flow rates were then converted to liters per

minute using ambient temperature and pressure conditions. The ambient flow rates for each trial are presented in Appendix 4. Flow rates measured after three minutes of operation are used in the remainder of the data analysis. These ambient flow rates are considered to be the most representative measurement of the actual air flow through the high volume sampler. This conversion allows for sample data from the high volume air samplers to be compared with data obtained from the low volume air sampling equipment on a standardized unit of volume (1 liter) basis. Flow rates calculated using the Dry-Cal for the AGI-30 and SKC Biosampler are presented in Table 5. These flow rate measurements were confirmed before and after each day of air sampling. Both the AGI-30 and Biosampler act as a critical orifice, thus maintaining constant flow during sampling.

Instrument	Ambient Flow Rate (lpm)	Pump Method Used
AGI-30	12.75	Vacuum Line
Biosampler	12.5	Vac-U-Go Sonic Flow Pump

Table 5: Low Volume Equipment Flow Rate Measurements

Particle Analysis of Viral Aerosol in Chamber

Particle Loading in Chamber

Particle loading in the chamber was measured by the Grimm Optical Particle Counter and was observed to vary widely throughout the five minutes for each trial run. An example

of particle loading variation is shown in Figure 20 below. Figure 20 shows a time elapsed plot of the particle count present in the chamber using 18 second moving averages collected from the Grimm OPC.

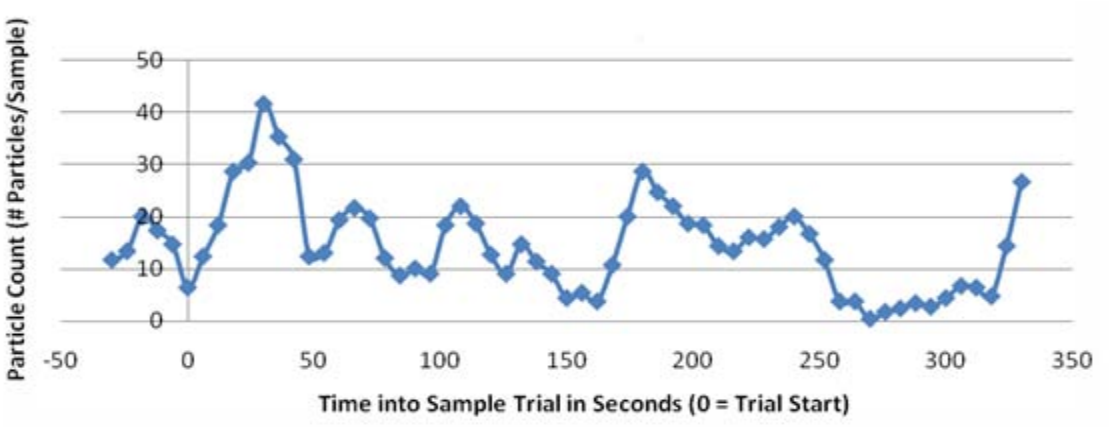

Figure 20: Particle Load in Chamber as a Function of Time, Trial 06

The results shown in Figure 20 are representative of the variation observed for all trials in the particle loading throughout each trial. A significant drop in aerosol particle concentration in the chamber was frequently observed after the air sampling equipment began operating for the majority of trials. This drop in particle concentration demonstrates the tendency of high volume air sampling equipment to strip particles from the aerosol faster than they can be regenerated. Chamber particle concentration plots for all sample trials are included in Appendix 3

Overall Particle Size Distribution during Sample Runs

Particle size distributions (as dN/dLogDp) for both high and low ACPLA sample runs are shown in Figure 21 and Figure 22. These figures are presented as examples of the particle size data recorded by each instrument during an air sampling trial. Geometric mean particle size for each trial run is shown in Table 6. Geometric mean particle size and count median diameter (CMD) are the same for lognormal particle size distributions.

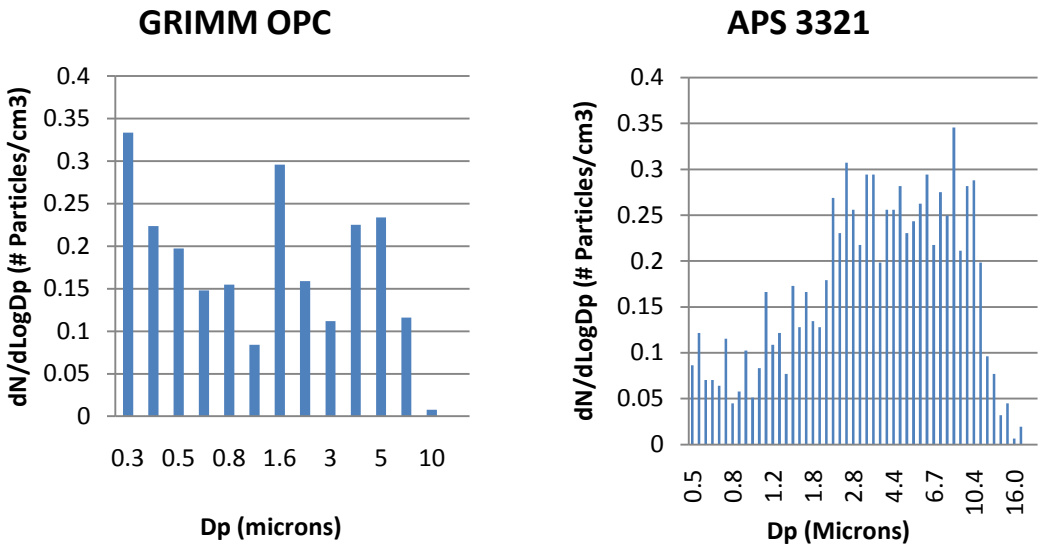

Figure 21: Particle Size Distributions for Example High ACPLA Trial 06, ACPLA = 93.2

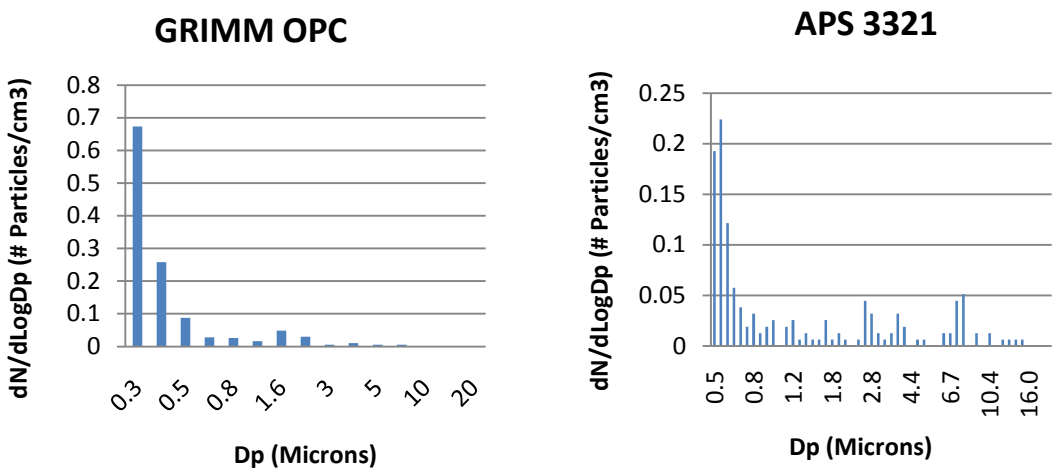

Figure 22: Particle Size Distributions for Example Low ACPLA Trial 18, ACPLA = 9.3

As shown by both the visual comparison of the particle size distribution plots and by comparison of the geometric mean particle size, there is a substantial difference in the particle size data reported by the APS 3321 and the Grimm OPC. This requires that the most representative size distribution be selected for further analysis and comparison. Particle size distributions plotted using data from the Grimm OPC tend to represent smaller particle sizes more than particle size distributions plotted using APS data. The most likely reason for this difference is the particle size sorting limit for each instrument. The Grimm OPC has a lower particle size sorting limit of approximately 0.3 microns, while the APS has a lower sorting limit of approximately 0.5 microns. For this reason, the Grimm OPC is considered to be the most representative instrument for the evaluation of particle size distribution and further analysis will be limited to data obtained from the Grimm OPC.

Trial	ACPLA	APS 3321 Geometric Mean Diameter (Microns)	Grimm OPC Geometric Mean Diameter (Microns)
TR 06	93.2	3.588	1.410
TR 09	74.4	3.376	1.227
TR 18	13.9	1.420	0.489
TR 21	21.0	2.264	0.588
TR 25	9.3	N/A[a]	0.642
TR 28	18.4	2.513	0.624

[a] Particle size distribution for trial run not collected due to equipment error

Table 6: Comparison of Geometric Mean Diameter From APS 3321 and Grimm OPC

Comparison of Particle Size Distribution Between Sample Runs

Count median diameter (CMD) was calculated for each 6-second particle size distribution measured using the Grimm optical particle counter. Particle size distributions between sample trial runs were compared using a Kruskal-Wallis one way ANOVA of the count median diameters for each 6 second sample. Six second CMDs for each trial run are presented as a in Figure 23 below.

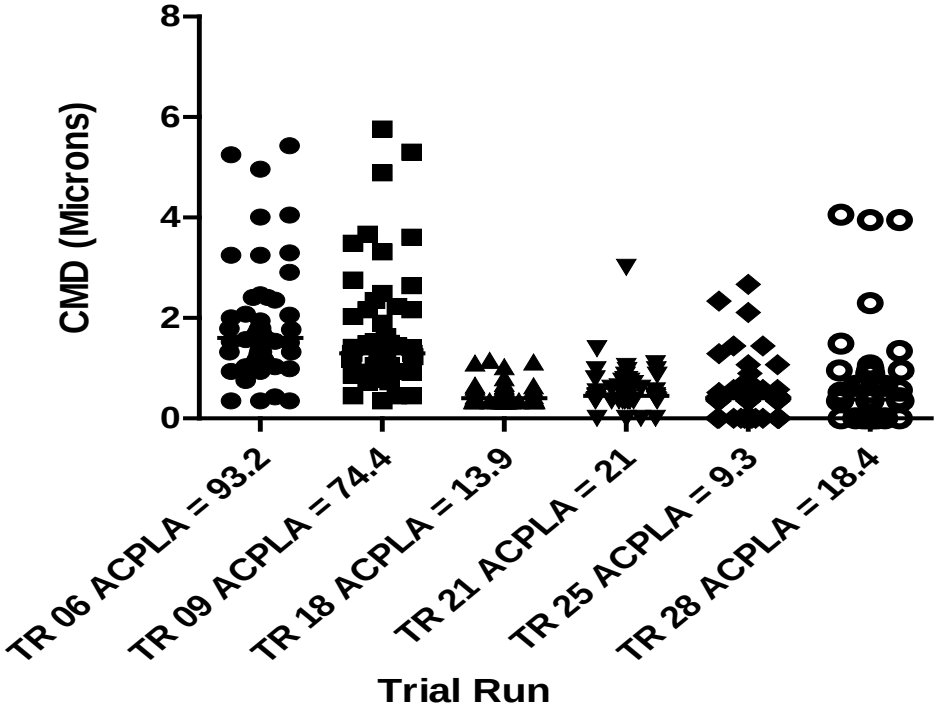

Figure 23: Six Second Count Median Diameter (CMD) Measured by Trial Run

Comparison of particle size between sample runs using a Kruskal-Wallis one-way ANOVA ($\alpha = 0.05$) revealed a significant difference ($P < 0.001$) in the median CMDs between the 6 trial runs. A Dunn's rank sum post test identified that the high ACPLA median CMDs were significantly different from the low ACPLA median CMDs. No other significant differences, such as CMDs within high ACPLA or within low ACPLA trials, were identified. Median CMD for each trial is shown in Table 7.

Trial	ACPLA	Median CMD (Microns)	Range of CMD (Microns)
TR 06	93.2	1.60	0.35 – 5.43
TR 09	74.4	1.30	0.35 – 5.76
TR 18	13.9	0.40	0.35 – 1.16
TR 21	21.0	0.45	0.00[a] – 3.01
TR 25	9.3	0.40	0.00[a] – 2.67
TR 28	18.4	0.38	0.00[a] – 4.06

[a]Numerous six second samples taken with the Grimm OPC failed to detect particles, therefore a 0.00 CMD was used for statistical representation.

Table 7: Comparison of CMD Between Trials

Comparison of Air Sampling Equipment in the Collection of MS2 Aerosol:

Performance Comparison of MS2 Collection for all Evaluated Air Sampling Equipment

Overall results for the collection of aerosolized MS2 bacteriophage as a function of viral load present in the chamber are shown in Figure 24. Viral load in the chamber is expressed in terms of agent containing particles per liter of air (ACPLA).

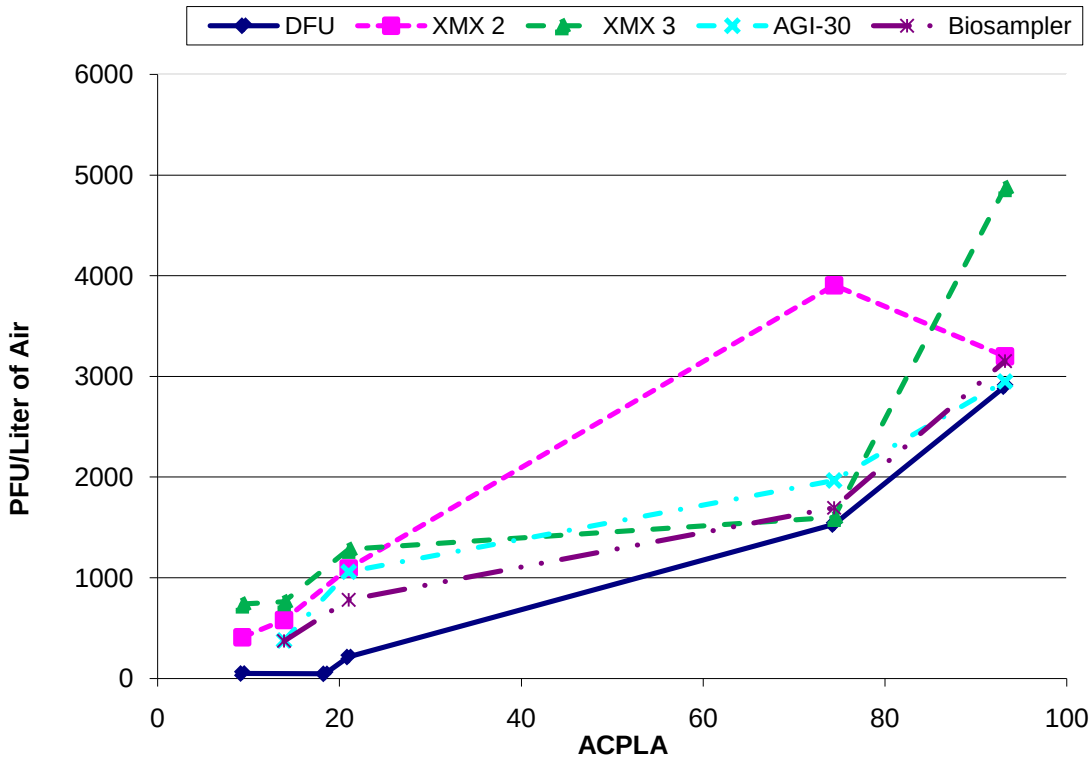

Figure 24: MS2 Air Sample Collection Relative to ACPLA Present in Chamber

As indicated by the results shown in Figure 24, the XMX/2L-MIL was shown to be as effective as both low volume air samplers to which it was compared. A large degree of intra-instrument variability was observed in the XMX at high ACPLA levels. The DFU-1000 appears to have similar MS2 collection performance to the other evaluated air samplers when high ACPLA levels are present. At lower ACPLA levels, the DFU-1000 appears to underperform the XMX/2L-MIL, AGI-30, and Biosampler on a concentration basis.

Several data points are excluded from these presentations including 1 data-point for the DFU-1000, 2 data-points for the AGI-30, 2 data-points for the Biosampler, and 1 data-point from each XMX unit. Upon initial evaluation of the data from the plaque assays, it was apparent that serial dilutions were probably mis-recorded either during the plating itself or when the plates were being counted and data assigned to a particular dilution. This probable error may have resulted in a shift in the plaque count of an order of magnitude from the actual plaque count had the plates been accurately recorded. A plot incorporating all data points is included in Appendix 7, Figure 33. Plots containing all data points corrected to the serial dilution that the author considers to be correct are included in Appendix 9.

Air sample results were standardized by the viral load (ACPLA) present in the exposure chamber to PFU/liter of air per ACPLA. This standardization allows for a median MS2 air sample concentration to be determined using data collected from multiple levels of airborne viral load present in the chamber. Standardized results are presented in Figure 25.

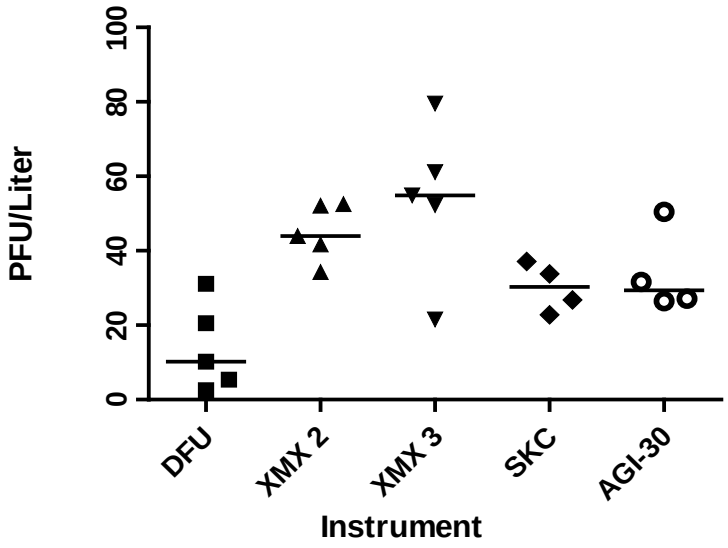

Figure 25: Air Sample Results Standardized to 1 ACPLA of Viral Load

The median results and range for each instrument are presented in Table 8. The reduced MS2 collection by the DFU-1000 at lower ACPLA levels significantly decreases the median standardized MS2 concentration measured by for the DFU-1000 as shown in Table 8.

Number of Trials	Instrument	Median Sampled MS2 Concentration per ACPLA Present in Chamber PFU/(Liter Air*ACPLA)	Range of Sampled Concentration per ACPLA Present in Chamber PFU/(Liter Air*ACPLA)
5	XMX 2	43.98	34.33 – 52.50
5	XMX 3	54.89	21.51 – 79.57
5	DFU	10.19	2.55 – 31.12
4	AGI-30	29.39	26.44 – 50.43
4	Biosampler	30.29	22.78 – 37.14

Table 8: MS2 Collection by Instrument Standardized to 1 ACPLA of Viral Load

Comparative analysis between all evaluated samplers using Kruskal-Wallis one way ANOVA ($\alpha = 0.05$) revealed a significant difference ($p = 0.009$) in the median standardized MS2 collection. A Dunn's rank sum post test revealed that the standardized MS2 collection obtained using the DFU-1000 is significantly different from the MS2 collection obtained using the XMX/2L-MIL. This significant difference was found for both XMX 2 and XMX 3. Other comparison results using the Dunn's post-test reveal no significant difference between the XMX/2L-MIL, the AGI-30, or the Biosampler or between the DFU-1000 and the AGI-30 or Biosampler. Kruskal-Wallis one way ANOVA results were also performed on the corrected data-set in Appendix 9 and confirm the findings of the data analysis presented with the data points excluded.

Quantification Limitations of Air Sampling Instruments

Low levels of airborne viral load, less than 5 ACPLA, were not successfully produced during any of the six trials. The lowest level of viral load produced was 9.3 ACPLA. Plots of MS2 sample collection relative to ACPLA present in chamber for the high volume air samplers with linear trendlines are included in Figure 26. As indicated by the linear trendline, the DFU-1000 is unable to reliably deliver quantifiable results at ACPLA levels lower than approximately 14 ACPLA. MS2 collection using the XMX/2L-MIL suggests a possible linear relationship relative to ACPLA present in the chamber. This allows the XMX to reliably produce quantifiable results for MS2 collection at the levels of airborne viral load evaluated in this project. The similarity of the linear trendlines for

XMX 2 and XMX 3 appear to be caused by chance. A high degree of variability was observed between XMX 2 and XMX 3.

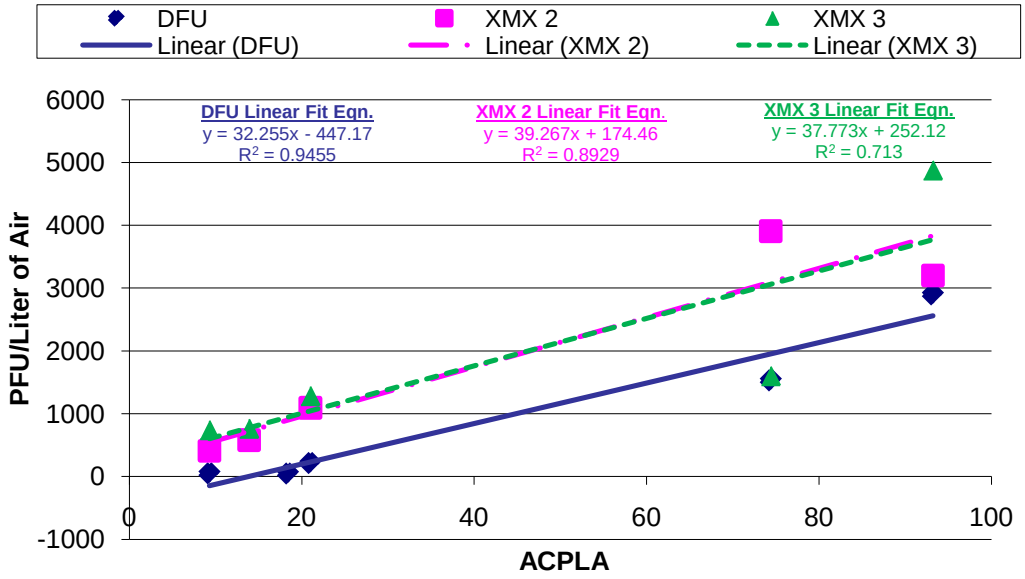

Figure 26: MS2 Collection by High Volume Air Sampling Equipment with Linear Trendlines

MS2 sample collection trendlines for low volume sampling equipment are shown in Figure 27. Similar to the XMX/2L-MIL, the AGI-30 and Biosampler produce reliable results at all trial ACPLA levels. This indicates that quantifiable measurements of airborne viral load can be obtained using the AGI-30 and Biosampler at the levels of airborne MS2 evaluated in the trials presented in this study.

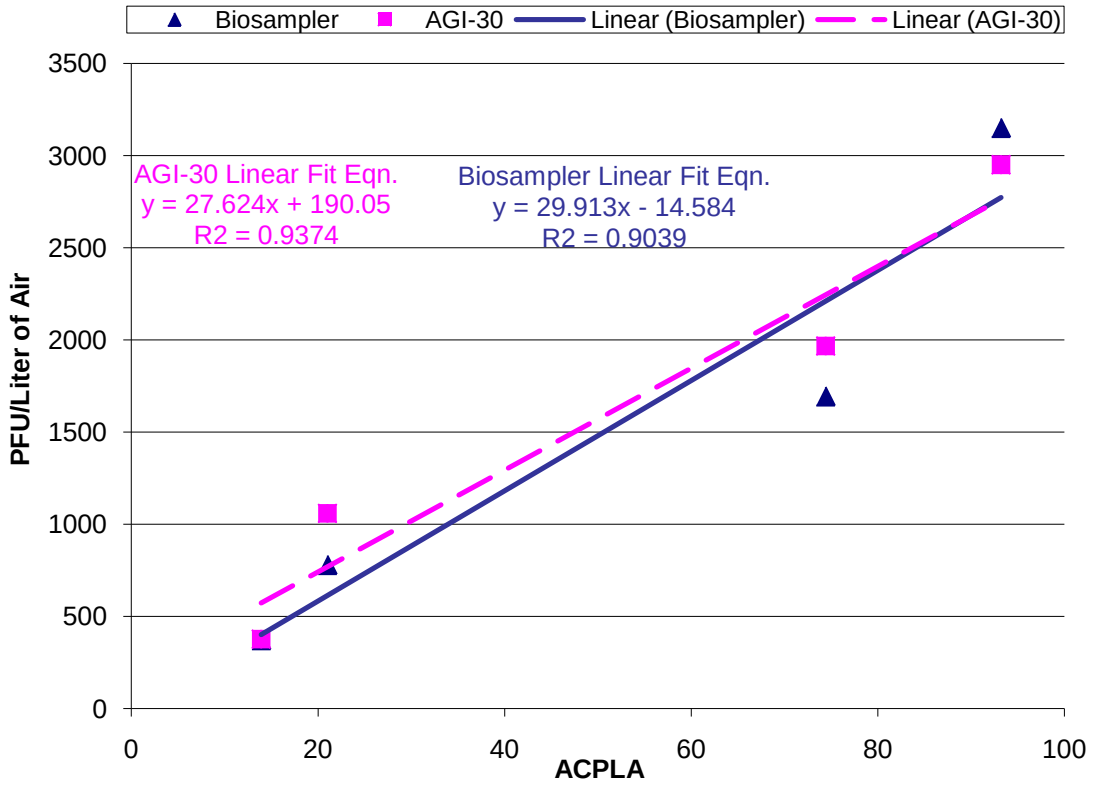

Figure 27: MS2 Collection by Low Volume Air Sampling Equipment with Linear Trendlines

PCR Results from Air Sampling

PCR results for the air sampling are shown in Table 9. All high volume air sampling systems were capable of collecting a sample resulting in PCR detection for MS2. XMX 2 produced one false negative PCR result and one inconclusive result during a trial with a very high MS2 plaque assay count. While the Biosampler and AGI-30 were capable of collecting an MS2 sample above the detection limits of the JBAIDS in a majority of trials, as expected, the low flow rates result in difficulty collecting sufficient MS2 for a relatively high proportion of the samples. This effect was particularly pronounced with

the Biosampler, where only 60 percent of samples resulted in a detection of the presence of MS2. PCR analysis includes one replicate per sample. An inconclusive result occurs in the event of difference between the primary analysis and the replicate. An absent result occurs when the detection threshold is not reached after a large number of PCR cycles. The number of cycles is typically established by the manufacturer, with 45 cycles being the typical value used for the JBAIDS. Raw results from the PCR analysis, including the number of cycles required to reach the detection threshold, is included in Appendix 2, Table 25.

Instrument	Number of Trials with PCR Analysis	JBAIDS-PCR Result for MS2 (# Present/#Absent/#Inconclusive)
XMX 2	6	4/1/1
XMX 3	6	6/0/0
DFU	6	6/0/0
AGI-30	5	4/0/1
Biosampler	5	3/2/0

Table 9: JBAIDS PCR Results for Sample Analysis Following Exposure to MS2

Intra-instrument Variability for the XMX

Results for the trials that collected MS2 using 3 XMX/2L-MIL samplers only are presented in Table 10. These results also indicate a large degree of variation between multiple XMX/2L-MIL operating in high viral load conditions and confirm the large intra-instrument variability found during trials TR06 and TR09.

Trial	ACPLA	MS2 Concentration XMX 1 (PFU/Liter of Air)	MS2 Concentration XMX 2 (PFU/Liter of Air)	MS2 Concentration From XMX 3 (PFU/Liter of Air)
TR 3	80.8	8109	9597	18879
TR 13	82.2	3274	3311	2282

Table 10: MS2 Concentration Sampled during High ACPLA XMX/2L-MIL Decontamination Trials

Comparison of MS2 Collection by XMX Apparatus and Sample Media

A previous study conducted by Dycor Technologies collected MS2 bacteriophage using an unmodified XMX/2L-MIL with phosphate buffered saline collection media. As previously described, the XMX/2L-MIL systems employed in the current study were modified by reducing the air flow to the impinger and by using Remel M5 media instead of phosphate buffer solution. In the previously conducted study by Dycor, five samples were collected using a single XMX/2L-MIL over a relatively narrow viral chamber load range of 23.0 to 28.1 ACPLA. The results from this study in PFU/liter of air were standardized PFU/liter of air per ACPLA present in the chamber. This allows for comparison with the results from the current study. The side by side comparison of the two studies is shown in Table 11.

Instrument	Number of Trials	Median Sampled MS2 Concentration per ACPLA Present in Chamber PFU/(Liter Air*ACPLA)	Range of Sampled Concentration per ACPLA Present in Chamber PFU/(Liter Air*ACPLA)
XMX Dycor (12.5 lpm secondary flow and PBS Collection Media)	5	36.30	32.90 – 38.78
XMX SAM (Secondary Flow Reduction and Remel M5 Collection Media)	10	56.55	37.21 – 79.57

Table 11: Comparison of XMX/2L-MIL using PBS Media and No Secondary Flow Reduction (XMX Dycor MS2 Study) with XMX/2L-MIL using Remel M5 Media and Secondary Flow Reduction

Data analysis using a Kruskal-Wallis one-way ANOVA ($\alpha = 0.05$) revealed a significant difference ($p = 0.027$) in the MS2 collection effectiveness between the two XMX operating methodologies. Full results from this Kruskal-Wallis one-way ANOVA are included in Appendix 6. These results indicate a significant difference between the two sampling apparatuses, but are not able to identify the factors responsible for the difference. Either of the two modifications, or the combination of modifications, could have been responsible for the differences in viral collection observed between the two studies.

Decontamination Comparison of Air Sampling Equipment

Decontamination effectiveness for the XMX/2L-MIL and DFU-1000 was evaluated by comparing sample results to initial background results and by comparing the degree that

contamination was reduced when field decontamination is performed on the XMX/2L-MIL and DFU-1000.

Comparision of Equipment Field Decontamination Between Air Samples

As shown in Table 12, residual contamination remaining in air samples taken from a purged chamber after decontamination was significantly reduced to levels comparable with the initial background levels in the chamber before MS2 was introduced. The residual contamination level detected in the post decontamination was compared to the initial background level in the chamber using a Kruskal-Wallis one-way ANOVA ($\alpha = 0.05$) and found not to be significantly different for XMX unit 2 ($p = 0.268$), XMX Unit 3 ($p = 0.077$), or the DFU ($p = 0.275$). Full results from this ANOVA analysis are included in Appendix 6. The analysis presented here is limited by the small number of trials (n=3) with which the comparisons are made.

Instrument	Initial Background Median MS2 Concentration (PFU/Liter of Air)	Post Decontamination Median MS2 Concentration (PFU/Liter of Air)
XMX 2	0.000	0.019
XMX 3	0.004	0.067
DFU	0.027	0.009

Table 12: MS2 Air Sample Concentrations Measured using the XMX/2L-MIL and DFU-1000 before Exposure (Background) and after Decontamination

Median reduction for contamination between sampling events is presented in Table 13 below. Raw data for each trial is included in Appendix 5. A minimum reduction of 99.9% in MS2 was achieved for each sample run and for all evaluated equipment. Percent reduction is obtained by comparing the sample result collected from exposure to MS2 in the chamber to the result obtained after sampling in an air purged chamber with the decontaminated instrument.

Instrument	Number of Trials	Median Reduction in MS2 Contamination %	Range of Reduction in MS2 Contamination %
XMX 2	3	99.994	99.935 - 99.999
XMX 3	3	99.999	99.983 - 99.999
DFU	3	99.999	99.996 – 100.000

Table 13: Reduction in MS2 Contamination between Air Samples Measured by Plaque Assay

Raw data, including results from the plaque assay, from which the mean reduction was generated is included in Appendix 5. Analysis conducted with a Kruskal-Wallis ANOVA ($\alpha = 0.05$) established no significant difference ($p = 0.304$) in the median reduction of MS2 decontamination obtained from the two XMX/2L-MIL and the DFU-1000 indicating that both instruments can be sufficiently decontaminated with hypochlorite bleach. The results from this Kruskal-Wallis ANOVA are shown in Appendix 6. PCR results for analysis with the JBAIDS are presented in Table 14.

Instrument	Number of Trials	Post-Exposure Sample JBAIDS Results #Present/#Absent/#Inconclusive	Post-Decontamination Sample JBAIDS Results #Present/#Absent/#Inconclusive
XMX 2	3	3/0/0	0/2/1
XMX 3	3	3/0/0	0/3/0
DFU	3	3/0/0	0/3/0

Table 14: Evaluation of MS2 Contamination between Air Samples by JBAIDS-PCR Analysis

Effective reduction of residual contamination between air samples to levels below the detection limits of the JBAIDS was observed for both the DFU-1000 and XMX/2L-MIL. One post-decontamination sample was inconclusive for the presence of MS2.

Comparison of Surface Removal of Decontamination

Mean reduction in surface contamination is shown in Table 15, with data for individual trials included in Appendix 5. The mean reduction in surface decontamination for the DFU-1000 was very similar to the mean reductions observed for the XMX/2L-MIL. Analysis using Kruskal-Wallis one-way ANOVA ($\alpha = 0.05$) revealed no significant difference ($p = 0.209$) between the DFU-1000 and XMX/2L-MIL in mean reduction of MS2 surface contamination after decontamination with hypochlorite. The range of values for mean reduction in surface contamination for the DFU-1000 is slightly higher than reductions observed in the XMX/2L-MIL, however.

Instrument	Number of Trials	Median Reduction in MS2 Contamination %	Range of Decontamination %
XMX 2	5	99.589	99.091 - 99.966
XMX 3	5	99.615	99.583 - 99.999
DFU	4	99.702	97.753 – 99.711

Table 15: Reduction in Surface Contamination Measured by Plaque Assay

Similar to the results obtained in the evaluation of residual contamination between air samples, surface decontamination was also reduced to a level below the JBAIDS limit of detection. These results are shown in Table 16 below. Pre-decontamination detection of surface MS2 contamination was shown to be inconsistent with several samples showing absence for MS2 or inconclusive results. Possible explanations for these false negative results are explained in the discussion section. All samples and duplicates for post-decontamination samples were found to be absent of surface contamination with MS2. Complete laboratory results from the JBAIDS-PCR analysis, including cycles required to reach detection threshold, are included in Appendix 2, Table 25.

Instrument	Number of Trials	Post-Exposure Surface Sample JBAIDS Result #Present/#Absent/#Inconclusive	Post-Decontamination Surface Sample JBAIDS Result #Present/#Absent/#Inconclusive
XMX 2	6	4/2/0	0/6/0
XMX 3	6	5/0/1	0/6/0
DFU	5	2/2/1	0/5/0

Table 16: Evaluation of Surface Contamination by JBAIDS-PCR Analysis

Background Contamination in Work Area

Results for the XMX/2L-MIL air samples taken in the decontamination work area are included as a daily average in Table 17. Four 30 minute samples were collected each day during the time periods in which the equipment was removed from the chamber, decontaminated, swabbed, and prepared for the next sample being collected. A single spike in the number of plaque forming units was noted in the final sample taken on 30 July 2009. As with the air samples collected inside the exposure chamber, results are reported as PFU/Liter of Air and as a daily average.

Date	Mean MS Air Concentration in Work Area (PFU/Liter of Air)	Range of MS Air Concentration in Work Area (PFU/Liter of Air)
28 July 09	0.008	0.006 – 0.009
30 July 09	0.021	0.003 – 0.058
31 July 09	0.006	0.004 – 0.009

Table 17: MS2 Air Sample Concentration in Work Area

The airborne concentration of MS2 in the work area was very low. These results from the plaque assay are also supported by PCR analysis of the work area samples. Of the 12 samples collected in the work area and analyzed by JBAIDS-PCR, 11 failed to detect the presence of MS2 and one sample was inconclusive for the presence of MS2. The inconclusive sample was taken on 30 July 2009 and corresponded to the highest sample concentration detected by plaque assay out of the 12 samples taken in work area. Based on these results, some contamination could have potentially entered the work area from

the chamber on 30 Jul 2009; however, any impact on post-decontamination sample results appears to be negligible.

V. Discussion and Conclusions

Discussion Overview

This thesis comparatively evaluated the performance of currently inventoried high volume air sampling equipment in response to a surrogate viral agent, MS2 bacteriophage. Criteria evaluated included overall effectiveness of virus recovery over a range of airborne agent concentrations and the decontamination characteristics of the equipment using methods available during fielded operations. Overall limitations of this research included: the inability to achieve low agent concentrations representative of minimally infectious doses, the relatively small number of samples collected, the limited variation of environmental conditions evaluated, and the inherent limitations in the use of a surrogate viral agent.

Aerosolized MS2 Collection by Instrument

Comparison of MS2 Collection by High Volume Collection Systems

This study demonstrated that the XMX/2L-MIL was capable of significantly ($p = 0.009$) greater collection of MS2 than the DFU-1000 under the sampling conditions evaluated for low ACPLA conditions. The collection of MS2 bacteriophage by the XMX/2L-MIL was also statistically similar to the AGI-30 and SKC Biosampler. Previous studies, such as the Indian smallpox study during the 1960's conducted by Downie, demonstrated that methods employing dry media recovered less viable virus than liquid collection methods (Downie, 1965). Tseng et al. also demonstrated significantly higher relative recoveries of aerosolized MS2 using a liquid collection method, the AGI-30, than the relative recovery

obtained using dry nuclepore filters (Tseng & Li, 2005). Therefore, it was not surprising that the overall collection was significantly less for high volume sampling methods employing dry media than high volume methods employing liquid collection. One interesting finding in this study was the large variation in collection effectiveness of the DFU-1000 relative to different levels of MS2 concentration in the chamber. The DFU-1000, on a per unit of air volume basis, demonstrated similar viable collection performance at higher ACPLA to the XMX/2L-MIL. At lower ACPLA, the DFU-1000 significantly underperformed compared to the XMX/2L-MIL. This finding demonstrates the potential that recovery of viable viral agent from dry media is more limited by the separation of the collected agent from the media than from the actual maintenance of viable agent on the filter during sampling. These suggestions are discussed further in other areas of this section. Relatively low air concentration levels would be expected during a response to a viral agent and this study demonstrates that the XMX/2L-MIL would likely outperform the DFU-1000 under such conditions. The linear regression of the plaque assay results for the MS2 collected by the DFU-1000 suggests that MS2 concentrations could not reliably be quantified at levels below 14 ACPLA. The XMX/2L-MIL and the DFU-1000 both recovered detectable quantities of viable MS2 bacteriophage at all evaluated concentrations of MS2 containing particles. This was further confirmed by the JBAIDS-PCR analysis of the collected samples, which mostly reported detectable results for MS2 samples taken during the trials. Interestingly, one of the XMX/2L-MIL systems, XMX 2, produced one sample that failed to detect MS2 and one inconclusive sample for MS2 when analyzed with the JBAIDS-PCR. These results

occurred despite very high viable MS2 concentrations for these samples when analyzed using plaque assay. This non-detect result would be considered a false negative result for the JBAIDS-PCR analysis and there are several explanations for this occurrence. First, as stated earlier, the JBAIDS analysis was performed at Brooks City Base, Texas, which required significant transit times. These transit times were particularly long, up to 16 days, from the time that the samples were actually collected. The effect that these longer hold times would have had on the integrity of viral RNA in the sample is not known. Second, the sensitivity of the JBAIDS, as reported by Wilson varies among different viral agents with some viral agents having sensitivities as low as 78.5 percent. The sensitivity of the JBAIDS for non-pathogenic surrogates, such as MS2 is unknown, but this could explain the occurrence of a false negative like that observed here. Third, technical error during laboratory analysis could also result in a false negative. For example, during analysis the technician may have failed to place the extracted RNA into the capillary tube for analysis (Escamilla, 2009). Although a technical error such as this would be rare, such an error could nonetheless explain a false negative in both the primary and duplicate sample. Technical laboratory errors, such as those mentioned above, could indicate the need to take duplicate air samples for each area during a response. Collecting and analyzing duplicate samples would increase confidence that a negative result obtained from the laboratory is truly a negative result.

Comparison of High Volume Liquid Collection to Low Volume Liquid Collection

This study demonstrated similar collection performance between the XMX/2L-MIL high volume liquid collection method and both of the low volume liquid collection methods. However, several limitations in applying the results of the AGI-30 for MS2 collection must be noted in this discussion that could have underrepresented the reported results. First, during the analysis of the AGI-30 samples, serial dilutions were only performed to a factor of 10^{-1}. This resulted in a plate count that was TNTC for one of the samples, trial run 06, taken during a high ACPLA trial. Results were reported in the data analysis at the minimum threshold at which a TNTC result is obtained. These results could significantly underestimate the actual collection of MS2 by the AGI-30. Secondly, the excessive foaming by the Remel M5 collection media resulted in a significant loss of sample during collection. Including a liquid trap, similar to the Erlenmeyer flask used for the Biosampler apparatus, between the sampler and the hose could have prevented this problem. Since a trap was not included in the AGI-30 apparatus, there was no way to prevent sample loss, and MS2 collection by the AGI-30 may be underreported as a result. The XMX/2L-MIL demonstrated a large degree of variability between the two instruments at high MS2 containing particle concentrations. This large degree of variability was also confirmed during the trials in which three XMX/2L-MIL systems were exposed to a high concentration of MS2 containing particles for the purposes of evaluating viral decontamination methods. Variability between different XMX/2L-MIL systems was also noted by LaRoche during trials involving collection of bacterial agents. This large degree of variability could be a significant limitation if the XMX/2L-MIL

were used in a situation where quantification of high concentrations of viral agent was the goal. LaRoche also attributed observed XMX/2L-MIL variation to inconsistent mixing within the exposure chamber. Since the same exposure chamber used by LaRoche was used for this study, similar mixing inconsistencies may have occurred in these trials as well. High concentrations such as those created during the high agent concentration trials are very unlikely during a response to an environmental release of a viral agent or as a consequence from a natural disease outbreak. Intra-instrument variability cannot be assessed for the DFU-1000, AGI-30, or Biosampler since multiple samplers were only included in this study for the XMX/2L-MIL. Variability may have an important impact on the performance of these other sampling systems as well. Future studies should include multiple simultaneous trials of all equipment being evaluated to fully consider the effect of intra-instrument variability on system performance.

Factors Impacting Air Sampling Performance

The particle size analysis between the high particle concentration trials and the low particle concentration trials revealed a significant difference in the count median diameter of the MS2 test aerosols. Each air sampling system has a specific collection efficiency that varies by particle size. As shown in chapter two, detailed information on collection efficiency is available for the XMX/2L-MIL, the AGI-30, and the Biosampler. Only limited information was available for the DFU-1000. Collection efficiencies for the AGI-30 and Biosampler were extrapolated using data from a study conducted by Willeke comparing the capture efficiencies of low volume air sampling methods (Willeke,

Xuejun, & Grinshpun, 1998). Table 18 and Table 19 show theoretical particle capture for the XMX/2L-MIL, AGI-30, and Biosampler for high and low ACPLA trials. The example trials used in this comparison are TR06 for the high ACPLA run and TR18 for the low ACPLA run. These theoretical particle capture tables were generated using particle size distributions measured by the Grimm optical particle counter for the entire trial period of 5 minutes and reflect the particles counted from a 6 liter aggregate sample.

Particle Size Range (Microns)	**Total Particles in Size Range (#Particles/Liter)**	**XMX/2L-MIL Theoretical Particle Collection (# Particles/Liter)**	**Biosampler Theoretical Particle Collection (# Particles/Liter)**	**AGI-30 Theoretical Particle Collection (# Particles/Liter)**
0.3 – 2.0	960	134	844	745
2.0 – 4.0	383	280	365	364
4.0 – 7.5	334	234	316	316
7.5 - 20	8	2	7	7
Total	1685	650	1532	1432

Table 18: Theoretical Particle Collection by Sampling System for High ACPLA Trial

Particle Size Range (Microns)	Total Particles in Size Range (#Particles/Liter)	XMX/2L-MIL Theoretical Particle Collection (# Particles/Liter)	Biosampler Theoretical Particle Collection (# Particles/Liter)	AGI-30 Theoretical Particle Collection (# Particles/Liter)
0.3 – 2.0	793	41	659	568
2.0 – 4.0	42	30	41	40
4.0 – 7.5	10	6	10	10
7.5 - 20	0	0	0	0
Total	845	77	710	618

Table 19: Theoretical Collection by Sampling System for Low ACPLA Trial

More detailed information on the XMX/2L-MIL particle collection efficiency for the high and low ACPLA trials is available through the USAFSAM. XMX/2L-MIL collection efficiency for particles less than 1 micron in size was not available. The theoretical particle capture presented in Table 18 and Table 19, assumes a low collection efficiency of 3 percent for these small particles. As discussed in chapter two, the AGI-30 and Biosampler have superior particle size capture efficiencies when compared with the XMX/2L-MIL for all particle sizes up to 20 micrometers. These collection efficiencies and the theoretical particle captures shown in Table 18 and Table 19 indicate that the XMX/2L-MIL would be expected to have significantly lower collection performance if particle capture is the primary contributing factor to viable virus recovery. The capture efficiencies used for Table 18 and Table 19 were determined using an unmodified

XMX/2L-MIL with either phosphate buffered solution or sterile water as the collection media. The modifications made to the XMX/2L-MIL for viral collection in this study included lowering the flow rate into the liquid impinger and the use of Remel M5 media. As discussed, flow rates into the impinger were reduced from approximately 12.5 lpm to approximately 4 lpm. Previous studies conducted by Hogan, as discussed in chapter two, address the effect of flow reduction on impinger collection efficiency. These studies indicate that significantly lowering the air flow rate into the impinger significantly reduces the collection efficiency. Lowering the flow rate of the AGI-30 and Biosampler from 12.5 lpm to 4 lpm reduced the observed particle collection efficiency for 0.3 micrometer particles by approximately 80 percent (Hogan, 2005). Similar effects should be expected for the impinger module on the XMX/2L-MIL. For these reasons, particle collection efficiency using the modified XMX/2L-MIL is an unlikely cause of the high performance in comparison to the low volume sampling methods observed in this study. Particle collection for the DFU-1000 filters were described by Lawrence as being very efficient for particle collection as low as 0.1 micron in size; however, as described in chapter two, the conditions under which this reported efficiency was determined are unknown. Therefore, the effect that the smaller particle size distributions in the low concentration trials had on the performance of the DFU-1000 cannot be assessed. Other factors potentially leading to the observed MS2 collection performance of the XMX/2L-MIL could include the effect that decreased foaming may have on MS2 activity. The excessive foaming observed in the AGI-30, when Remel M5 was used as the collection media, may have significantly lowered the culturable virus recovered in the sample.

While these observed turbulence effects were significantly lower for the Biosampler, increased foaming from using Remel M5 without flow reduction may have reduced MS2 activity in the samples collected using the Biosampler as well. Further studies should be conducted to determine the particle capture efficiency for the modified XMX/2L-MIL, the AGI-30, and the Biosampler with Remel M5 collection media. Use of quantitative PCR analysis techniques in future studies could possibly determine the impact, if any, that increased foaming has on the viability of MS2 in liquid media. Finally, while the AGI-30 and Biosampler have remarkably better particle collection for small particle sizes, these smaller particles may contribute less to the total quantity of viable MS2 in the aerosol. Small particles would dry significantly faster than larger particles, thus exposing the virus in the particles more to the environmental factors in the chamber. This drying of liquid viral containing particles was described by Verreault and discussed in chapter two. If the larger particles present in the test aerosols contained a significantly larger proportion of viable MS2, this could explain the high performance of the XMX/2L-MIL.

Ambient Conditions and Air Sampling Performance

As discussed in chapter three, ambient conditions including relative humidity and temperature were kept relatively consistent between trials. The relative humidity levels of 30 to 32% percent would not be expected to significantly impact the activity of the aerosolized MS2. This was deomonstrated in studies conducted by Dubovi et al., showing that MS2 collection using an AGI-30 was not affected by variations in relative humidity when the MS2 was suspended in a tryptone broth. The Luria broth used for the

MS2 aerosol solution in this study contains tryptone in similar amounts to the broth used by Dubovi. The Dubovi study suggests that changes in relative humidity would not be expected to significantly impact the collection of our MS2 test aerosol using liquid collection methodologies. Similar suggestions about the effect of relative humidity on the collection of MS2 aerosol using the DFU-1000 cannot be made, however. Further studies should determine if significant changes in relative humidity can effect the collection of MS2 viral aerosol by either the DFU-1000, XMX/2L-MIL or any other system under consideration by the Air Force.

Surrogate Virus Use and Sampler Performance

As discussed in chapter two, the use of surrogate virus in aerosol studies cannot be used to precisely predict the behavior of aerosolized viruses capable of human pathogenesis. This study, therefore, can only be accurately used to predict the effectiveness of the collection of MS2 aerosol in a controlled environment. Further studies should compare collection of other aerosolized viral surrogates and, if possible, live viral agents.

Media Selection and Sampler Performance

As demonstrated by Hermann and discussed in chapter two, collection media for liquid impinger sampling systems can have a significant influence on overall collection performance. The results from this study showed that the Remel M5 media used in an XMX/2L-MIL with impinger flow reduction was very capable at collecting aerosolized MS2. While a statistically significant difference in MS2 collection between the modified

XMX/2L-MIL apparatus used in the study and the fielded XMX/2L-MIL configuration was shown, there are several limitations to this comparison. First, the significance of the individual modifications within the systems cannot be assessed. Either the reduction in flow rate to the liquid impinger or the use of Remel M5 media instead of PBS solution could have been responsible for the increased MS2 collection. Second, the previous work conducted by Dycor evaluated the collection of MS2 by the XMX/2L-MIL over a very narrow ACPLA range. The results from this study were obtained over a wide range of ACPLA in the chamber. Although results were standardized by the ACPLA present, some difference may be attributable to the variation in ACPLA between the two studies, such as the difference in particle size distribution discussed previously. Future studies should assess the performance of collection media through independent, simultaneous, and side by side evaluations.

DFU-1000 Performance

The two most likely causes of the relatively low MS2 collection by the DFU-1000 at low to moderate ACPLA levels are problems with filter extraction and desiccation, or drying of the MS2 during collection. While desiccation of the collected MS2 may significantly contribute to a reduction in MS2 viability, this would be expected for both high ACPLA trials and low ACPLA trials. The only difference between collection of MS2 during high and low ACPLA trials is the amount of collected MS2 on the filter. The presence of additional MS2 on the filter would not be expected to preserve the viability of the MS2 from the desiccation caused by the rapid flow of air over the filter. Therefore, filter

extraction is left as the most likely contributor to significantly reduced DFU-1000 performance at lower ACPLA levels. As discussed in chapter 2, relative recovery of MS2 from a filter significantly increases when large amounts of MS2 are seeded on the filter as compared with more moderate amounts.

Decontamination

The field use of 10 percent sodium hypochlorite bleach was shown to have similar effectiveness for the decontamination of the XMX/2L-MIL and the DFU-1000. This comparison is applicable for both surface decontamination and for reduction in residual contamination between air samples. As expected, decontamination of viral agents was shown to be more effective than the decontamination of bacterial agents conducted by LaRoche. This observation also confirms previous studies demonstrating the greater susceptibility of viral agents to decontamination than spore-forming bacterial agents. The results of this study demonstrate that both the XMX/2L-MIL and DFU-1000 can be used to obtain multiple samples for JBAIDS-PCR analysis in response to a viral agent. Further enhancements to the bleach solution, such as the reduction of the pH as suggested by LaRoche, are not necessary to properly decontaminate the equipment after exposure to viral agents. Furthermore, while only a single viral surrogate was used to compare the decontamination characteristics of the equipment, non-enveloped viruses are more resistant to decontamination than enveloped viruses. It would be expected that the decontamination characteristics for other viral agents on both the XMX/2L-MIL and the DFU-1000 would be similar to or better than those observed in this study.

Recommendations

Laboratory Evaluation of Air Sampling Equipment

Further acquisition of commercially produced biological detection equipment should be evaluated independently for all biological agents of interest. This study shows that a reasonable evaluation of air sampling equipment can be performed using a limited number of trials in a laboratory environment. Additionally, acquisition decisions could be aided by the implementation of the limited comparison evaluation conducted in this study.

Field Use of Existing High Volume Air Sampling Equipment Inventory

Laboratory evaluation of high volume air sampling equipment demonstrated the effectiveness of the equipment in the collection of aerosolized MS2. This shows that in the presence of a sufficient aerosol concentration, both the DFU-1000 and XMX/2L-MIL are capable of recovering viral samples for both viable and non-viable analysis. Therefore, both the XMX/2L-MIL and DFU-1000 should continue to be used for field detection of viral agent in air samples. This study indicates, however, that the XMX/2L-MIL should be used, in preference to the DFU-1000, for field uses where relative viable quantification of a viral agent is desired. Since an effective concentration during the release of a viral agent would likely be much lower than the concentrations studied in this comparison, the XMX/2L-MIL would probably be the best choice to ensure viable collection. Furthermore, if airborne virus concentrations were lower than those used in

this study, the XMX/2L-MIL would likely be the best choice for PCR detection as well.

Field use of Hypochlorite Decontamination

Decontamination using immersion in 10 percent hypochlorite bleach solution is recommended for both the XMX/2L-MIL and DFU-1000. Alterations to the bleach solution, such as the adjustment of pH, should not be necessary to adequately decontaminate either the XMX/2L-MIL or the DFU-1000 in response to an aerosolized viral agent.

Future Research Opportunities

Evaluation of Future Air Force Air Sampling Equipment Acquisitions

The Air Force is in the process of considering further additions to the inventory of air sampling equipment for biological agents. One system under consideration is the Biocapture 650 manufactured by ICX Technologies: Albuquerque, NM. Civil Engineering Emergency Management has already purchased this system to enhance response capability. Collection performance characteristics for agents of interest have not been compared with the existing inventory. Future studies similar to the work presented here could be conducted to evaluate the capabilities and limitations provided by this equipment and to determine if the Biocapture 650 is a reliable substitute for the existing systems. Furthermore, future equipment under consideration for purchase should be comparatively evaluated with existing inventory to verify the effectiveness of new procurements prior to their use as a replacement for existing systems.

Limit of Detection for XMX/2L-MIL and DFU-1000

The results of this current study were unable to determine a limit of detection for MS2 sampling using the DFU-1000 and the XMX/2L-MIL. Therefore, the possibility still exists for a high false negative rate on both instruments. Further studies comparing the DFU-1000, XMX/2L-MIL and future Air Force system acquisitions at minimally achievable aerosol concentrations should be completed.

Evaluation and Optimization of Collection Media

The importance of sample media selection was shown in this study and by previous studies conducted by Hermann. More definitive studies should be conducted to evaluate the application of Remel M5 as a collection media for both low and high volume viral aerosol collection. Furthermore, optimization of sample media should be considered for future research. The Air Force operates in a variety of different environments and locations and sample media should be selected and optimized to meet the needs of the varied environmental conditions.

Performance Evaluation for Other Agents and Surrogate Agents

One major limitation of this study is that a single surrogate agent, MS2, was used for the evaluation. The use of MS2, an non-enveloped RNA bacteriophage, was a reasonable choice for the initial comparison of air sampling equipment since un-enveloped viruses have more resistance to environmental conditions and decontamination. Some viral agents of interest, however, such as Variola and Marburg, are enveloped viruses and may

not exhibit similar collection characteristics. Further research should be conducted using multiple viral surrogates to evaluate the applicability and performance of the air sampling equipment.

Conclusions

Comparison of Air Sampling Equipment Effectiveness

This study evaluated and compared numerous characteristics relevant to the field application of high volume air sampling equipment in response to a biological agent. The XMX/2L-MIL modified apparatus used in this study appears to have the most promising capability in terms of collection of aerosolized MS2. This was shown for all ranges of ACPLA used in this study. While the XMX/2L-MIL was shown to be more effective than the DFU-1000 in the collection of aerosolized MS2, this study did not provide an evaluation of the effectiveness of the XMX/2L-MIL at very low levels of airborne agent concentration. It cannot be inferred from this study that a non-detection result obtained from an air sample collected using the XMX/2L-MIL could be used to definitively determine the absence of airborne virus following an aerosol release. A key question remains for biological air sampling equipment: What does a result of non-detection really mean? PCR detection was successful on samples collected by the DFU-1000 and XMX/2L-MIL for all evaluated concentrations of MS2 in this study; however, lower concentrations may not produce reliable detection by the PCR. Furthermore, this study identifies many factors that should be considered throughout the selection and evaluation process of biological sampling equipment including selection of surrogate agent, ambient

testing conditions, physical aerosol characteristics, equipment alterations, and decontamination characteristics. Each of these characteristics should be considered independently in order to fully evaluate any air sampling system. Finally, minimum airborne concentrations of viral agent necessary to obtain quantitative measurements from the XMX/2L-MIL cannot be assessed from the results in this project since very low levels were not obtained during any of the sample trials.

Comparison of Decontamination Characteristics

This study demonstrates that decontamination of air sampling equipment by immersion in hypochlorite bleach solution is effective for both the XMX/2L-MIL and the DFU-1000. The results of this study indicate that both the XMX/2L-MIL and DFU-1000 can be properly decontaminated using techniques and materials available in field conditions and effectively reused for additional sampling during a response. These findings should provide greater confidence to responding personnel in the validity of multiple sample results taken using a limited number of collection systems. The high relative performance of hypochlorite decontamination for the XMX/2L-MIL and DFU-1000 does not imply that decontamination by hypochlorite should be assumed for all equipment. Decontamination should continue to be evaluated as a portion of equipment selection and use by the Air Force.

Final Considerations

Biological agents continue to be a key vulnerability in the field response and detection of weapons of mass destruction. Virtually all detection equipment for chemical, nuclear, and radiological response has an established limit of detection and a reasonable ability to quantify the hazard level for exposed personnel. The same cannot be said for the equipment used in a biological response. At the same time, biological agents would often be the lowest cost choice for a terrorist organization or rogue state intending to use unconventional weapons. This study contributes to the knowledge necessary to effectively conduct sampling for a viral agent. Although limited to a single class of biological agent and also limited to the equipment currently used by the Air Force, the author hopes that this study will guide the equipment selection for response personnel who may face a biological agent head on. Further work is necessary to increase the knowledge and confidence level associated with the use of this equipment.

Appendix 1: Trial Matrix and Schedule

Date	Trial Identification	Description	Air Sampling Equipment Used
27-Jul	Background 01	XMX Surface Swab	3 XMX
	Background 02	Chamber Background Air Sample	3 XMX
	Trial Run 03	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	3 XMX
28-Jul	Background 04	XMX Surface Swab	2 XMX
	Background 05	Chamber Background Air Sample	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 06	MS 2 High ACPLA Air Sample	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 07	Post MS2 Exposure Surface Swab	2 XMX, 1 DFU
	Background 08	Post Decontamination Surface Swab and Chamber Background Air	2 XMX, 1 DFU
	Trial Run 09	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 10	Post Decontamination Surface Swab	2 XMX, 1 DFU
29-Jul	Background 11	XMX Surface Swab	3 XMX
	Background 12	Chamber Background Air Sample	3 XMX
	Trial Run 13	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	3 XMX
	Trial Run 14	Post Decontamination Surface Swab	3 XMX
	Trial Run 15	15 Minute Air Purge	3 XMX
30-Jul	Background 16	XMX Surface Swab	2 XMX
	Background 17	Chamber Background Air Sample	2 XMX, 1 DFU
	Trial Run 18	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 19	Post Decontamination Surface Swab	2 XMX, 1 DFU
	Background 20	Chamber Background Air Sample	2 XMX, 1 DFU
	Trial Run 21	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 22	Post Decontamination Surface Swab	2 XMX, 1 DFU
31-Jul	Background 23	XMX Surface Swab	2 XMX
	Background 24	Chamber Background Air Sample	2 XMX, 1 DFU
	Trial Run 25	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 26	Post Decontamination Surface Swab	2 XMX, 1 DFU
	Background 27	Chamber Background Air Sample	2 XMX, 1 DFU
	Trial Run 28	MS 2 High ACPLA Air Sample and Post MS2 Exposure Swab	2 XMX, 1 DFU, 1 AGI-30, 1 Biosampler
	Trial Run 29	Post Decontamination Surface Swab	2 XMX, 1 DFU

Table 20: Sample Matrix and Schedule

Appendix 2: Raw Data for Sample Analysis

7/28/2009: Target ACPLA = 100				
Trial	Run Time (min)	Count (PFU)	ACPLA	Ave. ACPLA for Trial
Bkg05	5	1	0.0	0.0
TR06-1	2	5861	97.7	93.2
TR06-2	2	invalid plate		
TR06-3[a]	1	2530	84.3	
Bkg08	5	0	0.0	0.0
TR09-1	2	5543	92.4	74.4
TR09-2	2	3230	53.8	
TR09-3[a]	1	2382	79.4	

7/30/2009: Target ACPLA = 10				
Trial	Run Time (min)	Count (PFU)	ACPLA	Ave. ACPLA for Trial
Bkg17	5	0	0.0	0.0
TR18-1	2	877	14.6	13.9
TR18-2	2	720	12.0	
TR18-3	2	901	15.0	
Bkg20	5	0	0.0	0.0
TR21-1	2	1481	24.7	21.0
TR21-2	2	923	15.4	
TR21-3	2	1382	23.0	

7/31/2009 Target ACPLA = 1				
Trial	Run Time (min)	Count (PFU)	ACPLA	Ave. ACPLA for Trial
Bkg24	5	1	0.0	0.0
TR25	5	1397	9.3	9.3
Bkg27	5	0	0.0	0.0
TR28	5	2753	18.4	18.4

[a]Counted first half of plate only

Table 21: Calculation of ACPLA by Trial

AGI-30

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg5 (CT)	1	0	0	5	0	2.5
TR06 (CT)[a]	301	301	0	1505	1505	1505
TR06 (CT)[a]	301	301	1	15050	15050	15050
Bkg8 (CT)	1	3	0	5	15	10
TR09 (CT)[a]	301	301	0	1505	1505	1505
TR09 (CT)	216	240	1	10800	12000	11400

DFU-1000

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg5 (CT)	3	6	0	15	30	22.5
TR06 (CT)[a]	301	301	0	1505	1505	1505
TR06 (CT)[a]	301	301	1	15050	15050	15050
TR06 (CT)[a]	301	301	2	150500	150500	150500
TR06 (CT)	152	174	3	760000	870000	815000
TR06 (CT)	3	19	4	150000	950000	550000
TR06 (S)	301	301	0	1505	1505	1505
TR07 (S)	3	2	0	15	10	12.5
Bkg8 (CT)	2	3	0	10	15	12.5
TR09 (CT)[a]	301	301	0	1505	1505	1505
TR09 (CT)[a]	301	301	1	15050	15050	15050
TR09 (CT)[a]	301	301	2	150500	150500	150500
TR09 (CT)	85	87	3	425000	435000	430000
TR09 (CT)	6	16	4	300000	800000	550000
TR09 (S)[a]	301	301	0	1505	1505	1505
TR10 (S)	2	3	0	10	15	12.5

Biosampler

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg5 (CT)	2	2	0	10	10	10
TR06 (CT)[a]	301	301	0	1505	1505	1505
TR06 (CT)	203	223	1	10150	11150	10650
Bkg8 (CT)	0	1	0	0	5	2.5
TR09 (CT)[a]	301	301	0	1505	1505	1505
TR09 (CT)	130	93	1	6500	4650	5575

XMX - Unit 2

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg4 (S)	4	6	0	20	30	25
Bkg5 (CT)	4	3	0	20	15	17.5
TR06 (CT)[a]	301	301	3	1505000	1505000	1505000
TR06 (CT)	77	54	4	3850000	2700000	3275000
TR06 (S)	56	62	2	28000	31000	29500
TR06 (S)	9	9	3	45000	45000	45000
TR07 (S)	2	2	0	10	10	10
Bkg8 (CT)	2	3	0	10	15	12.5
TR09 (CT)	301	286	3	1505000	1430000	1467500
TR09 (CT)	49	56	4	2450000	2800000	2625000
TR09 (S)	38	45	2	19000	22500	20750
TR09 (S)	3	6	3	15000	30000	22500

XMX - Unit 3

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg4 (S)	1	0	0	5	0	2.5
Bkg5 (CT)	0	2	0	0	10	5
TR06 (CT)[a]	301	301	3	1505000	1505000	1505000
TR06 (CT)	53	33	4	2650000	1650000	2150000
TR06 (S)[b]	95	66	2	47500	33000	40250
TR07 (S)	1	2	0	5	10	7.5
Bkg8 (CT)	12	6	0	60	30	45
TR09 (CT)	275	222	3	1375000	1110000	1242500
TR09 (CT)	22	21	4	1100000	1050000	1075000
TR09 (S)[b]	186	194	2	93000	97000	95000

XMX - Unit 4

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/ml b	PFU/ml ave
Bkg16 (S)	1	0	0	5	0	2.5
Sample1 (CT)	1	13	0	5	65	35
Sample2 (CT)	3	6	0	15	30	22.5
Sample3 (CT)	6	7	0	30	35	32.5
Sample4 (CT)	8	6	0	40	30	35

[a] Sample Plaques were "Too Numerous to Count". 301 was Used as a Representative Value

[b] Sample was "Replated" 24 hours after Collection due to a Mislabeling of Original Sample Plates

S: Denotes Surface Swab Sample

CT: Denotes Air Sample from "Collection Tube"

Table 22: Plate Counts for High ACPLA Trials

AGI-30

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
TR18 (CT)	34	43	1	1700	2150	1925
TR21 (CT)	111	114	1	5550	5700	5625

DFU-1000

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg17 (CT)	2	1	0	10	5	7.5
TR18 (CT)	119	117	3	595000	585000	590000
Bkg20 (CT)	0	1	0	0	5	2.5
TR21 (CT)	130	111	2	65000	55500	60250
TR21 (S)	162	184	0	810	920	865
TR22 (S)	1	0	0	5	0	2.5

Biosampler

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
TR18 (CT)	27	22	1	1350	1100	1225
TR21 (CT)	49	51	1	2450	2550	2500

XMX - Unit 2

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg16 (S)	6	5	0	30	25	27.5
Bkg17 (CT)	0	0	0	0	0	0
TR18 (CT)	76	80	3	380000	400000	390000
TR18 (S)	104	64	1	5200	3200	4200
TR19 (S)	3	2	0	15	10	12.5
Bkg20 (CT)	1	0	0	5	0	2.5
TR21 (CT)	144	150	3	720000	750000	735000
TR21 (S)	60	52	1	3000	2600	2800
TR22 (S)	5	1	0	25	5	15

XMX - Unit 3

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg16 (S)	3	3	0	15	15	15
Bkg17 (CT)	0	0	0	0	0	0
TR18 (CT)	87	118	3	435000	590000	512500
TR18 (S)	47	65	1	2350	3250	2800
TR19 (S)	0	2	0	0	10	5
Bkg20 (CT)	0	2	0	0	10	5
TR21 (CT)	168	177	3	840000	885000	862500
TR21 (S)	76	101	1	3800	5050	4425
TR22 (S)	2	1	0	10	5	7.5

XMX - Unit 4 Work Area Samples

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg16 (S)	6	5	0	30	25	27.5
Sample1 (CT)	1	4	0	5	20	12.5
Sample2 (CT)	15	13	0	75	65	70
Sample3 (CT)	3	5	0	15	25	20
Sample4 (CT)	50	36	0	250	180	215

S: Denotes Surface Swab Sample CT: Denotes Air Sample from "Collection Tube"

Table 23: Plate Counts for Moderate ACPLA Trials

AGI-30

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
TR25 (CT)	37	51	0	185	255	220
TR28 (CT)	41	53	0	205	265	235

DFU

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg24 (CT)	1	1	0	5	5	5
TR25 (CT)	16	40	2	8000	20000	14000
TR25 (S)	24	37	0	120	185	152.5
Bkg27 (CT)	0	0	0	0	0	0
TR28 (CT)	21	32	2	10500	16000	13250
TR28 (S)	70	108	0	350	540	445
TR29 (S)	2	2	0	10	10	10

Biosampler

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
TR25 (CT)	24	23	0	120	115	117.5
TR28 (CT)	28	22	0	140	110	125

XMX - Unit 2

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg23 (S)	0	0	0	0	0	0
Bkg24 (CT)	0	0	0	0	0	0
TR25 (CT)	55	55	3	275000	275000	275000
TR25 (S)	10	12	1	500	600	550
TR26 (S)	1	1	0	5	5	5
Bkg27 (CT)	32	40	0	160	200	180
TR28 (CT)	81	127	2	40500	63500	52000
TR28 (S)	13	13	1	650	650	650
TR29 (S)	1	0	0	5	0	2.5

XMX - Unit 3

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg23 (S)	4	1	0	20	5	12.5
Bkg24 (CT)	0	1	0	0	5	2.5
TR25 (CT)	99	100	3	495000	500000	497500
TR25 (S)	9	15	1	450	750	600
TR26 (S)	1	0	0	5	0	2.5
Bkg27 (CT)	9	24	0	45	120	82.5
TR28 (CT)	68	53	2	34000	26500	30250
TR28 (S)	10	14	1	500	700	600
TR29 (S)	1	0	0	5	0	2.5

XMX - Unit 4

Trial	Count a	Count b	Dilution (1e-X)	PFU/mL a	PFU/mL b	PFU/ml ave
Bkg23 (S)	0	1	0	0	5	2.5
Sample1 (CT)	6	3	0	30	15	22.5
Sample2 (CT)	6	8	0	30	40	35
Sample3 (CT)	4	2	0	20	10	15
Sample4 (CT)	3	3	0	15	15	15

S: Denotes Surface Swab Sample CT: Denotes Air Sample From "Collection Tube"

Table 24: Plate Counts for Low ACPLA Trials

Cap #	Sample	CP
101	101 -Bkg4 U2 Swabs	45.00
101	Repl. Of 101	45.00
102	102 -Bkg4 U3 Swabs	45.00
102	Repl. Of 102	45.00
103	103 -Bkg4 U4 Swabs	45.00
103	Repl. Of 103	45.00
104	104 -Bkg5 U2 CT	45.00
104	Repl. Of 104	45.00
105	105 -Bkg5 U3 CT	45.00
105	Repl. Of 105	45.00
106	106 -TRG U2 CT	29.71
106	Repl. Of 106	29.74
107	107 -TR06 U2 Swabs	34.95
107	Repl. Of 107	35.82
108	108 -TR06 U3 CT	24.58
108	Repl. Of 108	24.58
109	109 -TRG U3 Swabs	32.76
109	Repl. Of 109	32.84
110	110 -Predecon TR06 DFU	37.86
110	Repl. Of 110	45.00
111	111 -Bkg 07 Post decon DFU	45.00
111	Repl. Of 111	45.00
112	112 -Post decon swabs - XMX U2	45.00
112	Repl. Of 112	45.00
113	113 -Post decon 743 Swabs	45.00
113	Repl. Of 113	45.00
114	114 -Bkg 8 U2 CT	45.00
114	Repl. Of 114	45.00
115	115 -Bkg 8 U3 CT	45.00
115	Repl. Of 115	45.00
116	116 - Pre decon DFU TR 09 PM	35.77
116	Repl. Of 116	34.65
117	117 -Post TR9 Bkg DFU Post clean pm	45.00
117	Repl. Of 117	45.00
118	118 -TR9 U2 CT	45.00
118	Repl. Of 118	45.00
119	119 -TR9 U2 Swabs	31.65
119	Repl. Of 119	31.55
120	120 -TR9 U3 CT	24.93
120	Repl. Of 120	25.05
121	121 -TR9 U3 swabs	30.88
121	Repl. Of 121	30.79
122	122 -Post decon 10 U2 Swabs	45.00
122	Repl. Of 122	45.00
123	123 -Post decon 10 U3 Swabs	45.00
123	Repl. Of 123	45.00
124	124 -AGI Bkg 05	45.00
124	Repl. Of 124	45.00
125	125 -AGI TR 06	32.11
125	Repl. Of 125	32.06

126	126 -AGI Bkg 08	45.00
126	Repl. Of 126	45.00
127	127 -AGI TR09	33.08
127	Repl. Of 127	33.27
128	128 -Biosampler TR06	32.6
128	Repl. Of 128	32.59
129	129 -Biosampler Bkg 05	45.00
129	Repl. Of 129	45.00
130	130 -Biosampler BKg08	45.00
130	Repl. Of 130	45.00
131	131 -Biosampler TR09	32.48
131	Repl. Of 131	32.82
132	132 -DFU Chamber Blank Bkg5	45.00
132	Repl. Of 132	45.00
133	133 -DFU TR6	25.48
133	Repl. Of 133	25.66
134	134 -DFU BKG8	45.00
134	Repl. Of 134	45.00
135	135 -DFU TR09 release	28.61
135	Repl. Of 135	28.64
136	136 -U4 CT1 28July TR 6-10	45.00
136	Repl. Of 136	45.00
137	137 -28july TR6-10 U4 CT2	45.00
137	Repl. Of 137	45.00
138	138 -TR6-10 U4 CT3	45.00
138	Repl. Of 138	45.00
139	139 -28july TR6-10 U4 CT4	45.00
139	Repl. Of 139	45.00
140	140 -BKG 16 U2 Swabs	45.00
140	Repl. Of 140	45.00
141	141 -BKG 16 U3 Swabs	45.00
141	Repl. Of 141	45.00
142	142 -BKG 16 U4 Swabs	45.00
142	Repl. Of 142	45.00
143	143 -BKG 17 U2 CT	45.00
143	Repl. Of 143	45.00
144	144 -BKG 17 U2 CT	45.00
144	Repl. Of 144	45.00
145	145 -TR18 U2 CT	28.52
145	Repl. Of 145	28.62
146	146 -TR18 U2 Swabs	32.88
146	Repl. Of 146	33.67
147	147 -TR18 U3 CT	27.21
147	Repl. Of 147	27.51
148	148 -TR18 U3 Swabs	33.68
148	Repl. Of 148	34.46
149	149 -DFU Bkg 17	45.00
149	Repl. Of 149	45.00
150	150 -DFU TR18	30.46
150	Repl. Of 150	30.31

151	151 -AGI BKG17	45.00
151	Repl. Of 151	45.00
152	152 -AG1TR18	35.8
152	Repl. Of 152	35.38
153	153 -SKC BKG 17	45.00
153	Repl. Of 153	45.00
154	154 -SKC TR18	35.04
154	Repl. Of 154	35.17
155	155 -PD19 U2 Swabs	45.00
155	Repl. Of 155	45.00
156	156 -PD 19 U3 Swabs	45.00
156	Repl. Of 156	45.00
157	157 -Bkg20 U2 CT	45.00
157	Repl. Of 157	45.00
158	158 -Bkg 20 U3 CT	45.00
158	Repl. Of 158	45.00
159	159 - TR21 U2 CT	28.64
159	Repl. Of 159	28.5
160	160 -TR21 U3 CT	29.28
160	Repl. Of 160	29.19
161	161 -TR21 U2 Swabs	34.00
161	Repl. Of 161	34.72
162	162 -TR21 U3 Swabs	33.12
162	Repl. Of 162	33.05
163	163 -TR21 Pre-decon DFU 30July pm	34.91
163	Repl. Of 163	34.76
164	164 -Post decon 27 (22)	45.00
164	Repl. Of 164	45.00
165	165 -PD 2d U2 Swabs	45.00
165	Repl. Of 165	45.00
166	166 -PD22 U3 Swabs	45.00
166	Repl. Of 166	45.00
167	167 -PD22 U3 Swabs	45.00
167	Repl. Of 167	45.00
168	168 -BKG 20 SKC	38.14
168	Repl. Of 168	40.00
169	169 -BKG 20 AGI	45.00
169	Repl. Of 169	45.00
170	170 -TR21 AGI	36.18
170	Repl. Of 169	45.00
171	171 -DFU Bkg 20	45.00
171	Repl. Of 171	45.00
172	172 -DFU TR21	32.28
172	Repl. Of 172	32.02
173	173 -U4 #1 30Jul	45.00
173	Repl. Of 173	45.00
174	174 -U4 #2 30Jul	45.00
174	Repl. Of 174	45.00
175	175 -U4 #3 30 Jul	45.00
175	Repl. Of 175	45.00

176	176 -U4 #4 30Jul	40.00
176	Repl. Of 176	45.00
180	180 -Bkg 23 U2 Swabs	45.00
180	Repl. Of 180	45.00
181	181 -Bkg 23 U3 Swabs	45.00
181	Repl. Of 181	45.00
182	182 -Bkg 23 U4 Swabs	45.00
182	Repl. Of 182	45.00
183	183 - TR25 U2 CT	36.37
183	Repl. Of 183	34.44
184	184 - TR25 U3 CT	34.80
184	Repl. Of 184	34.82
185	185 -TR25 U2 Swabs	45.00
185	Repl. Of 185	45.00
186	186 -TR25 U3 Swabs	36.65
186	Repl. Of 186	37.10
187	187 -PD26 U3 Swabs	45.00
187	Repl. Of 187	45.00
188	188 - PD26 U2 Swabs	45.00
188	Repl. Of 188	45.00
189	189 - TR 25 SKC	45.00
189	Repl. Of 189	45.00
191	191 -DFU Post Decon TR25	45.00
191	Repl. Of 191	45.00
192	192 -DFU Pre Decon TR25	37.24
192	Repl. Of 192	45.00
193	193 -DFU	45.00
193	Repl. Of 193	45.00
194	194 -DFU TR25	33.56
194	Repl. Of 194	34.82
195	195 -DFU Bkg 24	45.00
195	Repl. Of 195	45.00
196	196 -BKG 27 U2 CT	36.16
196	Repl. Of 196	45.00
197	197 -BKG 27 U3 CT	45.00
197	Repl. Of 197	45.00
198	198 -TR28 U2 CT	45.00
198	Repl. Of 198	37.45
199	199 -TR28 U3 CT	31.2
199	Repl. Of 199	30.98
200	200 -TR28 U2 Swabs	45.00
200	Repl. Of 200	45.00
201	201 -TR28 U3 Swabs	36.39
201	Repl. Of 201	45.00
202	202 -PO 29 U2 Swabs	45.00
202	Repl. Of 202	45.00
203	203 -PO29 U3 Swabs	45.00
203	Repl. Of 203	45.00
204	204 - AGI TR28	36.8
204	Repl. Of 204	37.00
205	205 -SKC TR28	45.00
205	Repl. Of 205	45.00

206	206 -DFU TR28	35.8
206	Repl. Of 206	35.89
207	207 -DFU Bkg 27	45.00
207	Repl. Of 207	45.00
208	208 - DFU TR28 swab Pre-Decon	45.00
208	Repl. Of 208	45.00
209	209- DFU TR29 Post-Decon	45.00
209	Repl. Of 209	45.00
210	210 -U4 #4 31July	45.00
210	Repl. Of 210	45.00
211	211 -U4 #3 31July	45.00
211	Repl. Of 211	45.00

Table 25: JBAIDS PCR Analysis of MS2 Samples (From Applied Technology Center)

Appendix 3: Particle Loading in Chamber

(All Plots are 3 Sample, 18 Second Moving Averages)

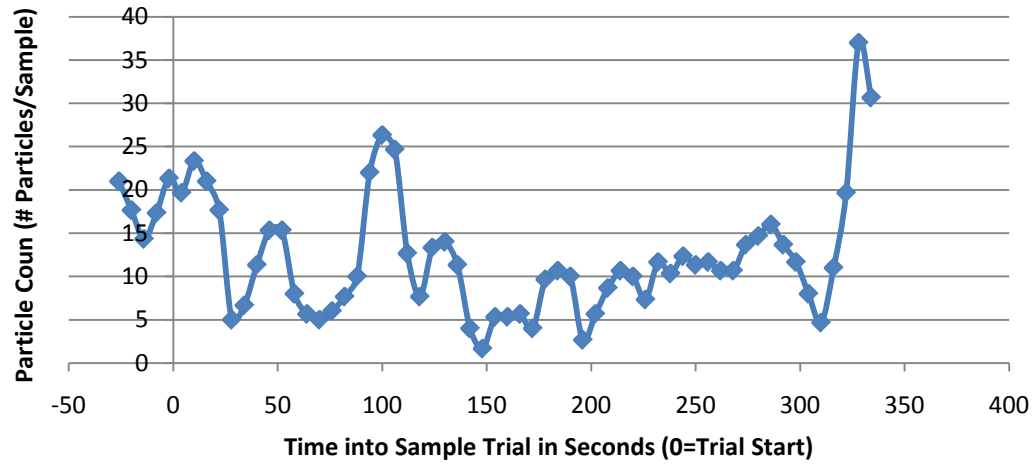

Figure 28: Particle Load in Chamber as a Function of Time, Trial 09

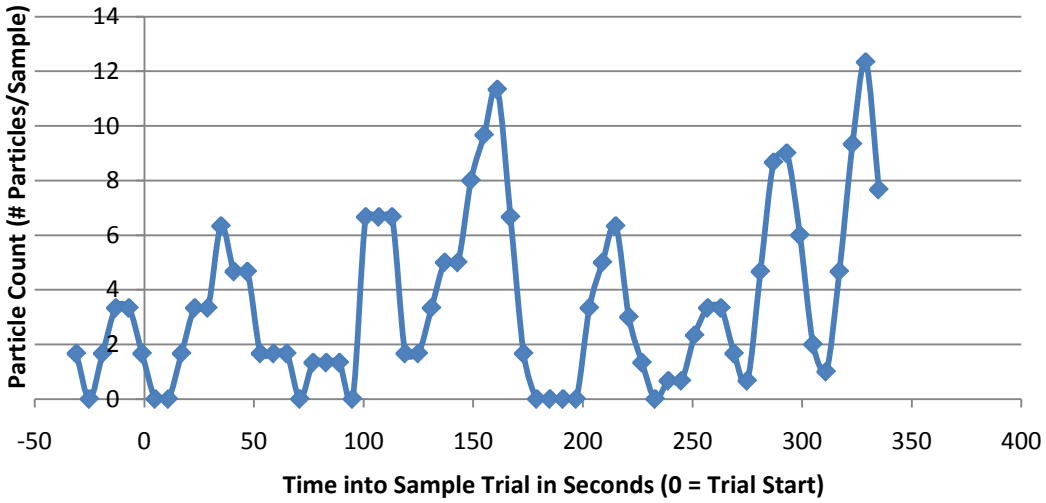

Figure 29: Particle Load in Chamber as a Function of Time, Trial 18

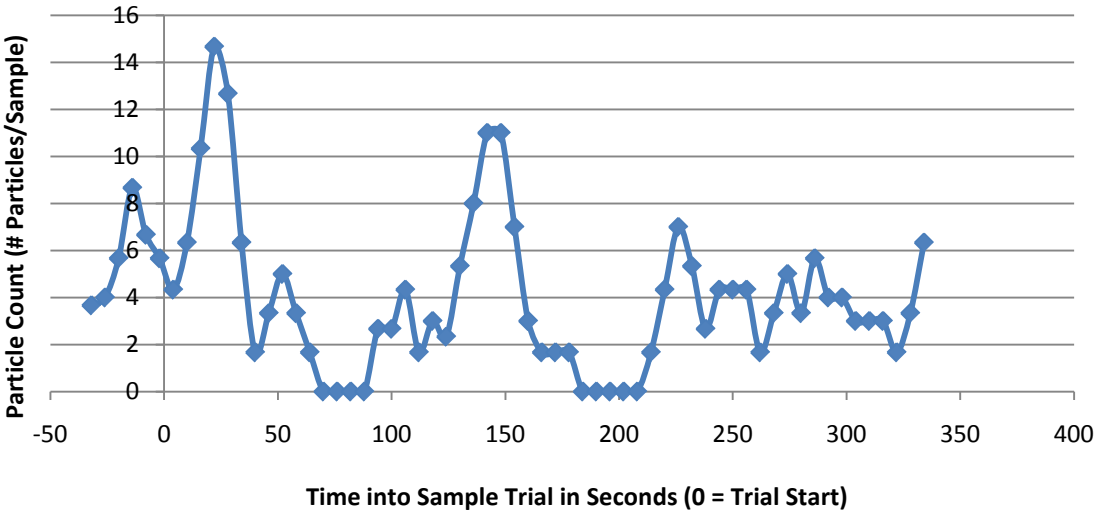

Figure 30: Particle Load in Chamber as a Function of Time, Trial 21

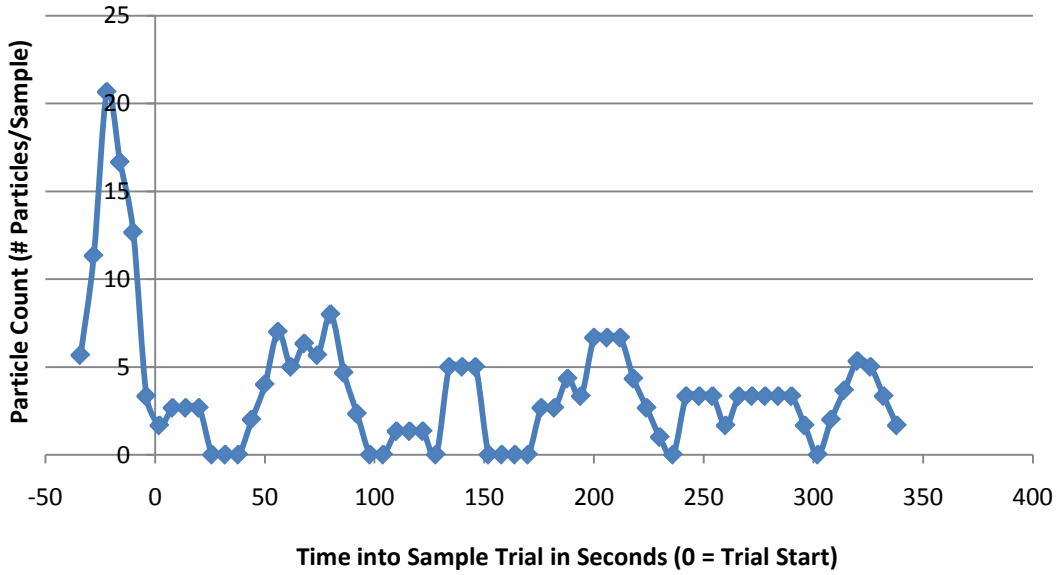

Figure 31: Particle Load in Chamber as a Function of Time, Trial 25

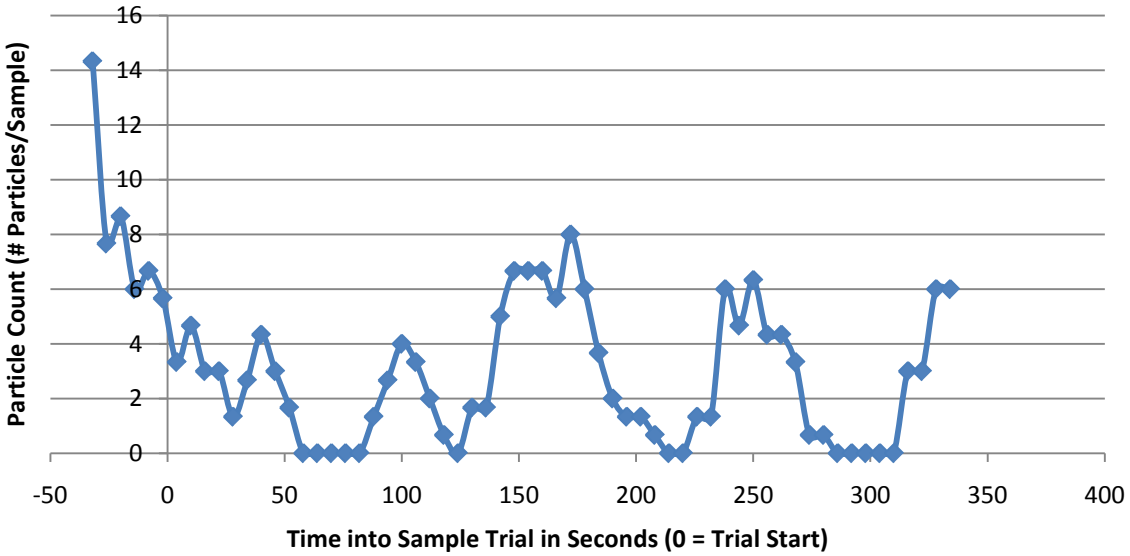

Figure 32: Particle Load in Chamber as a Function of Time, Trial 28

Appendix 4: Air Sample Comparison Data

TR 06 -- High ACPLA (Target ACPLA = 100 , Actual ACPLA =93.2)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	93.83
Relative Humidity (%)	34.3

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	2150000	5	5	3199
XMX Unit 3	3275000	5	5	4874
DFU	815000	15	15	2900
SKC	10650	20	18.5	3152
AGI-30[a]	15050	20	12.5	2951

TR 09 -- High ACPLA (Target ACPLA = 100, Actual ACPLA = 74.4)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	25.7
Temp (Deg K)	298.7
Pressure (kPa)	93.77
Relative Humidity (%)	33.6

Instrument	Viral Media Conc (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	2625000	5	5	3906
XMX Unit 3	1075000	5	5	1600
DFU	430000	15	15	1530
SKC	5575	20	19	1695
AGI-30	11400	20	11	1967

[a]Plate was "Too Numerous to Count". Minimum Plate Value of 301 PFU used for Media Conc. and Air Conc.

TR 18 -- Moderate ACPLA (Target ACPLA= 10, Actual ACPLA = 13.9)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26.6
Temp (Deg K)	299.6
Pressure (kPa)	92.93
Relative Humidity (%)	30.8
ACPLA	**13.9**

Instrument	Viral Media Conc (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	390000	5	5	580
XMX Unit 3	512500	5	5	763
DFU[b]	590000	15	15	2100
SKC	1225	20	19	372
AGI-30	1925	20	12.5	377

TR 21 -- Moderate ACPLA (Target ACPLA = 10, Actual ACPLA = 21)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	92.92
Relative Humidity (%)	30.5
ACPLA	**21**

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	735000	5	5	1094
XMX Unit 3	862500	5	5	1283
DFU	60250	15	15	214
SKC	2500	20	19.5	780
AGI-30	5625	20	12	1059

[b]Plate Sample Dilution was Recorded One Order of Magnitude too Low. Results Excluded from Analysis

TR 25-- Low ACPLA (Target = 1 ACPLA, Actual ACPLA = 9.3)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	25.5
Temp (Deg K)	298.5
Pressure (kPa)	93.45
Relative Humidity (%)	34.3
ACPLA	**9.3**

Instrument	Viral Media Conc. (PFU/ml)	edia Volume Initi	Media Volume Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	275000	5	5	409
XMX Unit 3	497500	5	5	740
DFU	14000	15	15	50
SKC[c]	118	20	19	36
AGI-30[c]	220	20	14	48

TR 28 -- Low ACPLA (Target ACPLA = 1, Actual ACPLA = 18.4)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	93.3
Relative Humidity (%)	37.6
ACPLA	**18.4**

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2[c]	52000	5	5	77
XMX Unit 3[c]	30250	5	5	45
DFU	13250	15	15	47
SKC[c]	125	20	18.5	37
AGI-30[c]	235	20	13	48

[c]Plate Sample Dilution was Recorded One Order of Magnitude too High. Results Excluded from Analysis

Appendix 5: Decontamination Comparison Trial Data

Decontamination Trial 1: Background 5 (Pre-Exposure Background)

Time (min) 5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Background 05 **ACPLA =** **0**

Instrument	Media Conc (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX 2	17.5	0.026
XMX 3	5	0.007
DFU	22.5	0.080

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX 2	Negative	45	Negative	45
XMX 3	Negative	45	Negative	45
DFU	Negative	45	Negative	45

Decontamination Trial 1: Trial 06 (Exposure)

Time (min) 5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Trial Run 06 **ACPLA =** **93.2**

Instrument	Media Conc. (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX 2	2150000	3199
XMX 3	3275000	4874
DFU	815000	2900

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX 2	Positive	29.71	Positive	29.74
XMX 3	Positive	24.58	Positive	24.58
DFU	Positive	25.48	Positive	25.66

Decontamination Trial 1: Background 08 (Post Decontamination)

Time (min)	5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Background 08 **ACPLA =** **0**

Instrument	Media Conc (PFU/ml)	Measurement (PFU/Liter of Air)
XMX 2	12.5	0.019
XMX 3	45	0.067
DFU	12.5	0.044

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX 2	Negative	45	Negative	45
XMX 3	Negative	45	Negative	45
DFU	Negative	45	Negative	45

% Reduction w/ 10% Bleach	
XMX 2	99.999
XMX 3	99.999
DFU	99.998

Decontamination Trial 2: Background 17 (Pre-Exposure Background)

Sample Run Tim	5
Collector	**Q (L/min)**
XMX 2	672
XMX 3	672
DFU	843

Background 17 **ACPLA =** **0**

Collector	Media Conc (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX Unit 2	0	0.000
XMX Unit 3	0	0.000
DFU	7.5	0.027

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX Unit 2	Negative	45	Negative	45
XMX Unit 3	Negative	45	Negative	45
DFU	Negative	45	Negative	45

Decontamination Trial 2: Trial 18 (Exposure)

Time (min) 5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Trial Run 18 **ACPLA =** **13.9**

Collector	Media Conc (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX Unit 2	390000	580
XMX Unit 3	512500	763
DFU[a]	59000	210

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX Unit 2	Positive	28.52	Positive	28.62
XMX Unit 3	Positive	27.21	Positive	27.51
DFU	Positive	30.46	Positive	30.31

Decontamination Trial 2: Background 20 (Post Decontamination)

Time (min) 5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Background 20 **ACPLA =** **0**

Instrument	Media Conc. (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX Unit 2	2.5	0.004
XMX Unit 3	5	0.007
DFU	2.5	0.009

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX Unit 2	Negative	45	Negative	45
XMX Unit 3	Negative	45	Negative	45
DFU	Negative	45	Negative	45

% Reduction w/ 10% Bleach	
XMX Unit 2	99.999
XMX Unit 3	99.999
DFU	99.996

[a]Corrected Data

Decontamination Trial 3: Background 24 (Pre-Exposure Background)

Time (min)	5
	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Background 24 **ACPLA =** **0**

Instrument	Media Conc. (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX Unit 2	0	0.000
XMX Unit 3	2.5	0.004
DFU	5	0.018

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX 2[b]	N/A		N/A	
XMX 3[b]	N/A		N/A	
DFU	Negative	45	Negative	45

[b]PCR Results Not Available for this Sample

Decontamination Trial 3: Trial 25 (Exposure)

Time (min)	5
	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Trial Run 25 **ACPLA =** **9.3**

Instrument	Media Conc. (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX Unit 2	275000	409
XMX Unit 3	497500	740
DFU	14000	50

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX Unit 2	Positive	36.37	Positive	34.44
XMX Unit 3	Positive	34.8	Positive	34.82
DFU	Positive	33.56	Positive	34.82

Decontamination Trial 3: Background 27 (Post Decontamination)

Time (min)	5

	Q (L/min)
XMX 2	672
XMX 3	672
DFU	843

Background 27 **ACPLA =** **0**

Instrument	Media Conc. (PFU/ml)	Air Conc. (PFU/Liter of Air)
XMX 2	180	0.268
XMX 3	82.5	0.123
DFU	0	0.000

Instrument	PCR A Result	Cycles A	PCR B Result	Cycles B
XMX 2	Positive	36.16	Negative	45
XMX 3	Negative	45	Negative	45
DFU	Negative	45	Negative	45

% Reduction w/ 10% Bleach	
XMX 2	99.935
XMX 3	99.983
DFU	100

Appendix 6: Statistical Analysis

Instrument	N	Median	Ave Rank	Z
AGI	4	29.39	11.0	-0.32
Biosampler	4	30.29	10.0	-0.65
DFU	5	10.19	4.0	-2.98
XMX 2	5	43.98	16.4	1.64
XMX 3	5	54.89	18.0	2.24
Overall	23		12.0	

H = 13.41 DF = 4 P = 0.009

* NOTE * One or more small samples

Table 26: Kruskal-Wallis Analysis of Air Sampling Equipment Comparison for MS2

Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?
DFU vs XMX 2	-12.4	Yes
DFU vs XMX 3	-14	Yes
DFU vs SKC	-6	No
DFU vs AGI-30	-7	No
XMX 2 vs XMX 3	-1.6	No
XMX 2 vs SKC	6.4	No
XMX 2 vs AGI-30	5.4	No
XMX 3 vs SKC	8	No
XMX 3 vs AGI-30	7	No
SKC vs AGI-30	-1	No

Table 27: Dunn's Multiple Comparison Test of Air Sampling Equipment for MS2

Instrument	N	Median	Ave Rank	Z
DFU	4	99.17	4.8	-1.56
XMX 2	5	99.62	7.5	0.00
XMX 3	5	99.70	9.7	1.47
Overall	14		7.5	

H = 3.11 DF = 2 P = 0.211
H = 3.13 DF = 2 P = 0.209 (adjusted for ties)

* NOTE * One or more small samples

Table 28: Kruskal-Wallis Analysis of Surface Decontamination by Instrument

Instrument	N	Median	Ave Rank	Z
DFU	3	99.99	6.7	1.29
XMX 2	3	99.99	4.0	-0.77
XMX 3	3	99.99	4.3	-0.52
Overall	9		5.0	

H = 1.69 DF = 2 P = 0.430
H = 2.38 DF = 2 P = 0.304 (adjusted for ties)

* NOTE * One or more small samples

Table 29: Kruskal-Wallis Analysis of Contamination Reduction between Air Samples

Instrument	N	Median	Ave Rank	Z
XMX Dycor	5	36.51	4.4	-2.20
XMX SAM	10	56.55	9.8	2.20
Overall	15		8.0	

H = 4.86 DF = 1 P = 0.027

Table 30: Kruskal-Wallis Analysis of XMX Dycor Apparatus and XMX SAM Apparatus

Trial Phase	N	Median	Ave Rank	Z
Background	3	0.000000000	2.7	-1.09
Post Decontamination	3	0.019000000	4.3	1.09
Overall	6		3.5	

H = 1.19 DF = 1 P = 0.275
H = 1.23 DF = 1 P = 0.268 (adjusted for ties)

* NOTE * One or more small samples

Table 31: Kruskal-Wallis Analysis of Post-Decon Air Sample vs. Background for XMX 2

Trial Phase	N	Median	Ave Rank	Z
Background	3	0.004000	2.2	-1.75
Post Decontamination	3	0.067000	4.8	1.75
Overall	6		3.5	

H = 3.05 DF = 1 P = 0.081
H = 3.14 DF = 1 P = 0.077 (adjusted for ties)

Table 32: Kruskal-Wallis Analysis of Post-Decon Air Sample vs. Background for XMX 3

Trial Phase	N	Median	Ave Rank	Z
Background	3	0.027000	4.3	1.09
Post Decontamination	3	0.009000	2.7	-1.09
Overall	6		3.5	

H = 1.19 DF = 1 P = 0.275

* NOTE * One or more small samples

Table 33: Kruskal-Wallis Analysis of Post-Decon Air Sample vs. Background for DFU

Trial	N	Median	Ave Rank	Z
TR 06	51	1.5987	239.6	7.61
TR 09	51	1.2987	228.4	6.62
TR 18	51	0.3969	111.9	-3.68
TR 21	51	0.4500	123.2	-2.68
TR 25	51	0.3969	102.8	-4.48
TR 28	51	0.3806	115.2	-3.39
Overall	306		153.5	

H = 128.38 DF = 5 P = 0.000
H = 129.08 DF = 5 P = 0.000 (adjusted for ties)

Table 34: Kruskal-Wallis Analysis of CMD between Trial Runs

Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?
TR 06 vs TR 09	10.86	No
TR 06 vs TR 18	127.8	Yes
TR 06 vs TR 21	116.5	Yes
TR 06 vs TR 25	136.9	Yes
TR 06 vs TR 28	124.6	Yes
TR 09 vs TR 18	117	Yes
TR 09 vs TR 21	105.6	Yes
TR 09 vs TR 25	126	Yes
TR 09 vs TR 28	113.8	Yes
TR 18 vs TR 21	-11.35	No
TR 18 vs TR 25	9.059	No
TR 18 vs TR 28	-3.196	No
TR 21 vs TR 25	20.41	No
TR 21 vs TR 28	8.157	No
TR 25 vs TR 28	-12.25	No

Table 35: Dunn's Multiple Comparison Test of CMD's between Trial Runs

Appendix 7: Air Sampling Results with Excluded Data

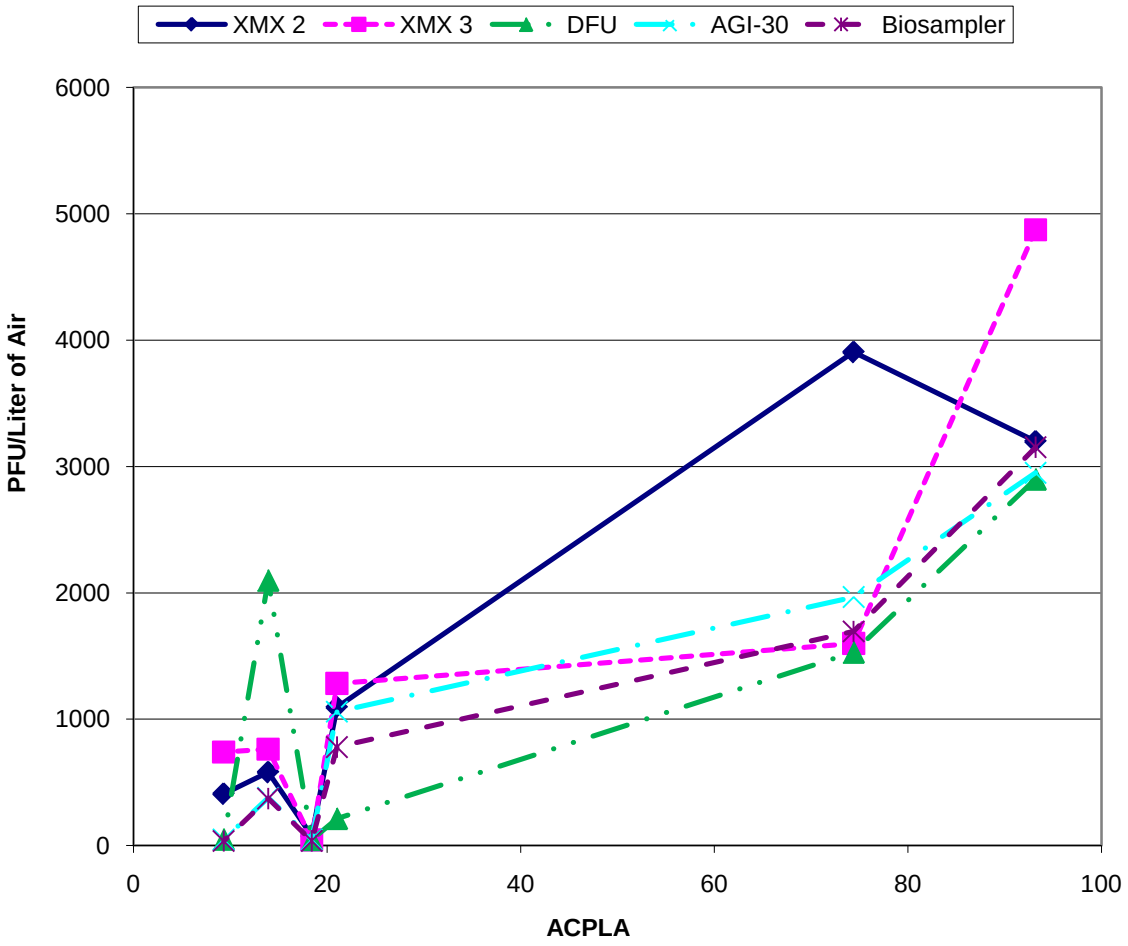

Figure 33: MS2 Air Sample Collection Relative to ACPLA Present in Chamber with Excluded Data

Appendix 8: Air Sample Concentration with Corrected Data

TR 06 -- High ACPLA (Target ACPLA = 100 , Actual ACPLA =93.2)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	93.83
Relative Humidity (%)	34.3

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	2150000	5	5	3199
XMX Unit 3	3275000	5	5	4874
DFU	815000	15	15	2900
SKC	10650	20	18.5	3152
AGI-30[a]	15050	20	12.5	2951

TR 09 -- High ACPLA (Target ACPLA = 100, Actual ACPLA = 74.4)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	25.7
Temp (Deg K)	298.7
Pressure (kPa)	93.77
Relative Humidity (%)	33.6

Instrument	Viral Media Conc (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	2625000	5	5	3906
XMX Unit 3	1075000	5	5	1600
DFU	430000	15	15	1530
SKC	5575	20	19	1695
AGI-30	11400	20	11	1967

[a]Corrected

TR 18 -- Moderate ACPLA (Target ACPLA= 10, Actual ACPLA = 13.9)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26.6
Temp (Deg K)	299.6
Pressure (kPa)	92.93
Relative Humidity (%)	30.8

Instrument	Viral Media Conc (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	390000	5	5	580
XMX Unit 3	512500	5	5	763
DFU[a]	59000	15	15	210
SKC	1225	20	19	372
AGI-30	1925	20	12.5	377

TR 21 -- Moderate ACPLA (Target ACPLA = 10, Actual ACPLA = 21)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	92.92
Relative Humidity (%)	30.5

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	735000	5	5	1094
XMX Unit 3	862500	5	5	1283
DFU	60250	15	15	214
SKC	2500	20	19.5	780
AGI-30	5625	20	12	1059

[a]Corrected

TR 25-- Low ACPLA (Target = 1 ACPLA, Actual ACPLA = 9.3)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	25.5
Temp (Deg K)	298.5
Pressure (kPa)	93.45
Relative Humidity (%)	34.3

Instrument	Viral Media Conc. (PFU/ml)	Media Volume Initia	Media Volume Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2	275000	5	5	409
XMX Unit 3	497500	5	5	740
DFU	14000	15	15	50
SKC[a]	1180	20	19	359
AGI-30[a]	2200	20	14	483

TR 28 -- Low ACPLA (Target ACPLA = 1, Actual ACPLA = 18.4)

Sample Run Time (min)	5
	Q (L/min)
XMX Unit 2 Flow Rate	672
XMX Unit 3 Flow Rate	672
DFU Flow Rate	843
Biosampler Flow Rate	12.5
AGI-30 Flow Rate	12.75
Temp (Deg C)	26
Temp (Deg K)	299
Pressure (kPa)	93.3
Relative Humidity (%)	37.6

Instrument	Viral Media Conc. (PFU/ml)	Media Vol. Initial	Media Vol. Final	Air Conc. (PFU/Liter of Air)
XMX Unit 2[a]	520000	5	5	774
XMX Unit 3[a]	302500	5	5	450
DFU	13250	15	15	47
SKC[a]	1250	20	18.5	370
AGI-30[a]	2350	20	13	479

[a]Corrected

Appendix 9: Corrected Air Sampling Data Analysis

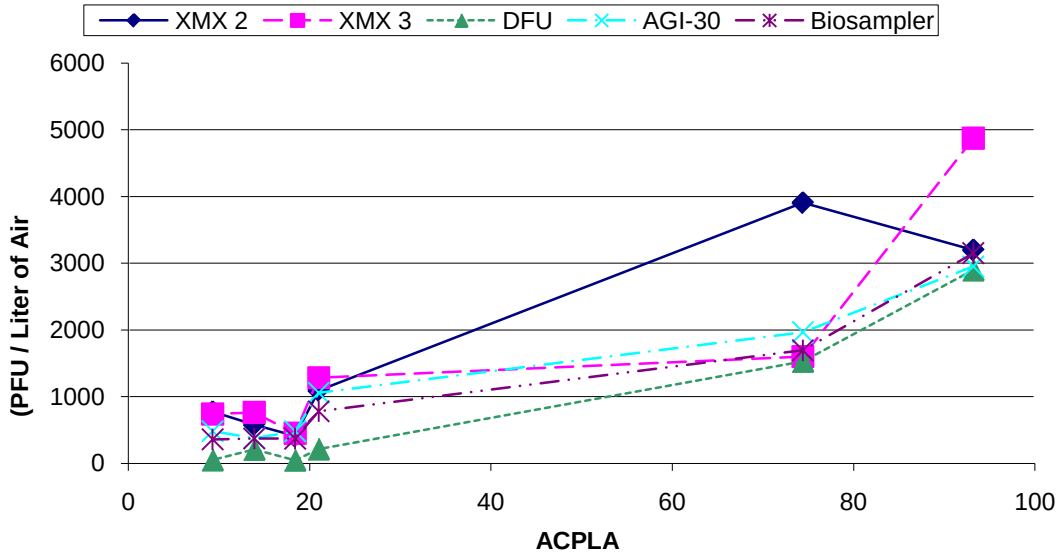

Figure 34: MS2 Air Sample Collection Relative to ACPLA Present in Chamber with Corrected Data

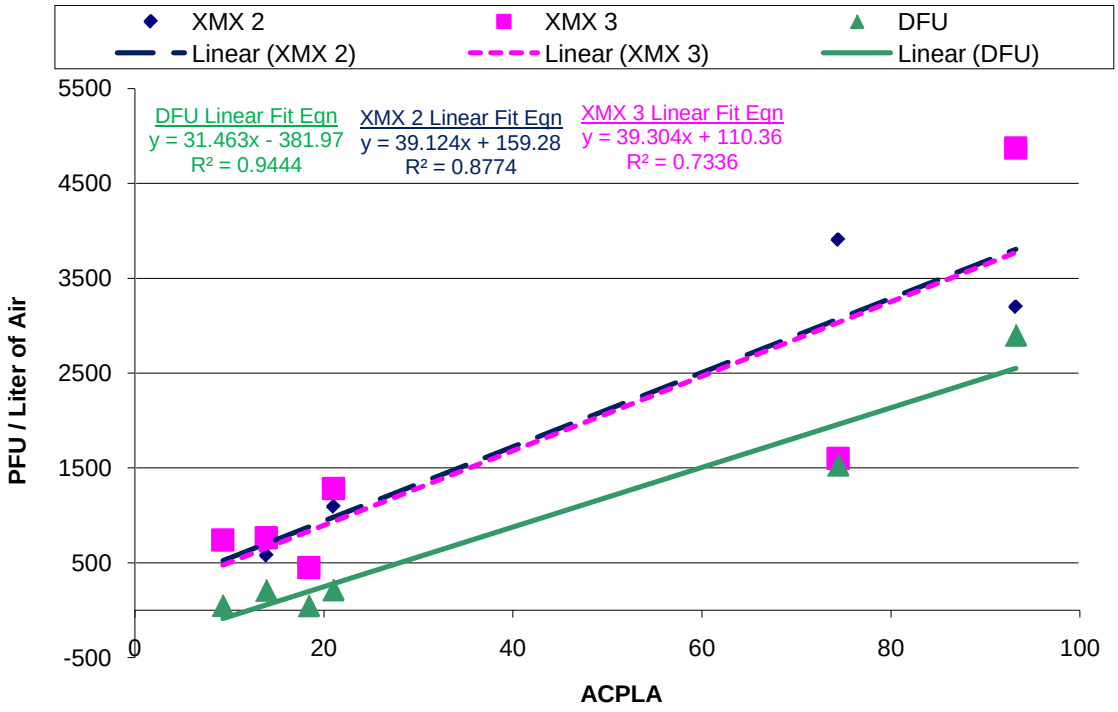

Figure 35: MS2 Collection by High Volume Air Sampling Equipment with Linear Trendlines (Corrected Data)

Instrument	N	Median	Ave Rank	Z
AGI	6	29.39	16.3	0.26
Biosampler	6	30.29	13.8	-0.52
DFU	6	12.65	5.2	-3.21
XMX 2	6	46.91	21.2	1.76
XMX 3	6	53.59	21.0	1.71
Overall	30		15.5	

H = 13.36 DF = 4 P = 0.010

Table 36: Kruskal-Wallis Analysis of Air Sampling Equipment Comparison for MS2 (Corrected Data)

Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?
DFU vs XMX 2	-16	Yes
DFU vs XMX 3	-15.83	Yes
DFU vs Biosampler	-8.667	No
DFU vs AGI-30	-11.17	No
XMX 2 vs XMX 3	0.1667	No
XMX 2 vs Biosampler	7.333	No
XMX 2 vs AGI-30	4.833	No
XMX 3 vs Biosampler	7.167	No
XMX 3 vs AGI-30	4.667	No
Biosampler vs AGI-30	-2.5	No

Table 37: Dunn's Multiple Comparison Test of Air Sampling Equipment for MS2 (Corrected Data)

Bibliography

ABSA. (2004). *Risk Group Classification for Infectious Agents*. Retrieved January 10, 2010, from American Biological Safety Association: http://www.absa.org/riskgroups/viruses.html

Adams, M. H. (1959). *Bacteriophages.* New York: Interscience Publishers.

Alibek, K. (1999). *Biohazard.* New York: Dell Publishing.

Artenstein, M. S., Miller, W. S., Lamson, T. H., & Brandt, B. L. (1968). Large Volume Sampling for Meningococci and Adenoviruses. *American Journal of Epidemiology , 87* (3), 567-577.

Bermingham, N., & Luettich, K. (2003). Polymerase Chain Reaction and its Applications. *Current Diagnostic Pathology* , 159-164.

Bliss, C. (2009, September 28). Flow Reducer for the XMX and effect on the Impinger Operation. (C. W. Cooper, Interviewer)

Brenner, K. P., Scarpino, P. V., & Clark, C. S. (1988). Animal Viruses, Coliphages, and Bacteria in Aerosols and Wastewater at a Spray Irrigation Site. *Applied and Environmental Microbiology , 54* (2), 409-415.

Burton, N. C., Grinshpun, S. G., & Hornung, R. R. (2005). The Effect of Filter Material on Bioaerosol Collection of *Bacillus subtilis* Spores Used as a *Bacillus anthracis* Simulant. *Journal of Environmental Monitoring , 7*, 475-480.

Burton, N. C., Grinshpun, S. G., & Reponen, T. (2007). Physical Collection Efficiency of Filter Materials for Bacteria and Viruses. *The Annals of Occupational Hygiene , 51* (2), 143–151.

CDC. (2009, September 18). Update: Influenza Activity --- United States, April--August 2009. *Morbidity and Mortality Weekly Report , 58* (36), pp. 1009-1012.

Cox, C. S., & Wathes, C. M. (1995). *Bioaerosols Handbook.* Boca Raton, FL: CRC Press.

Downie, A. W., Meiklejohn, M., St. Vincent, L., Rao, A. R., Sundara Babu, B. V., & Kempe, C. H. (1965). The Recovery of Smallpox Virus from Patients and their Environment in a Smallpox Hospital. *Bulletin of the World Health Organization , 33*, 615-622.

Dubovi, E. J., & Akers, T. G. (1970). Airborne Stability of Tailless Bacterial Viruses S-13 and MS-2. *Journal of Applied Microbiology , 19* (4), 624-628.

Dycor Technologies. (2009). *Determination of Viral Load in Exposure Chamber.* Edmonton, AB: Dycor Technologies.

Dycor Technologies. (2001). *XMX/2L-MIL Users Guide.* Edmonton, AB: Dycor Technologies.

Elliott, L. H., McCormick, J. B., & Johnson, K. M. (1982). Inactivation of Lassa, Marburg, and Ebola Viruses by Gamma Irradiation. *Journal of Clinical Microbiology , 16* (4), 704-708.

EPA. (2009). *Aerosol Wind Tunnel Testing Task 2: Measurement of Spore Extraction Efficiency.* Environmental Protection Agency, National Homeland Security Research. Research Triangle Park, NC: RTI International.

Escamilla, E. (2009, July 22). Molecular Biologist. (C. Cooper, Interviewer)

Fabian, P., McDevitt, J. J., DeHaan, W. H., Fung, R., Cowling, B. J., Chan, H. C., et al. (2008). Influenza Virus in Human Exhaled Breath: An Observational Study. *Public Library of Science One , 3* (7), 1-6.

Fatah, A. A., Arcilesi, R. D., Chekol, T., Lattin, C., Shaffer, E., & Davies, M. (2005). *Guide for the Selection of Biological Agent Detection Equipment for Emergency First Responders.* Washington, DC: Department of Homeland Security.

Foarde, K. K., Hanley, J. T., Ensor, D. S., & Peter, R. (1999). Development of a Method for Measuring Single-Pass Bioaerosol Removal Efficiencies of a Room Air Cleaner. *Aerosol Science and Technology , 30*, 223–234.

Hermann, J. R., Hoff, S. J., Yoon, K. J., Burkhardt, A. C., Evans, R. B., & Zimmerman, J. J. (2006). Optimization of a Sampling System for Recovery and Detection of Airborne Porcine Reproductive and Respiratory Syndrome Virus and Swine Influenza Virus. *Applied and Environmental Microbiology , 72* (7), 4811-4818.

Hodges, L. R., Rose, L. J., Peterson, A., Noble-Wang, J., & Arduino, M. J. (2006). Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface. *Applied and Environmental Microbiology , 72* (6), 4429-4430.

Hogan, C. J., Kettleson, E. M., Lee, M. H., Bamaswami, B., Angenent, L. T., & Biswas, P. (2005). Sampling Methodologies and Dosage Assessment Techniques for Submicrometre and Ultrafine Virus Aerosol Particles. *Journal of Applied Microbiology , 99*, 1422-1434.

Hogle, J. M. (2002). Poliovirus Cell Entry: Common Structural Themes in Viral Cell Entry Pathways. *Annual Review of Microbiology , 56*, 677–702.

JPEO-CBDX. (2008, September 23). *Dry Filter Unit (DFU).* Retrieved January 10, 2010, from Joint Program Executive Office for Chemical and Biological Defense: http://www.jpeocbd.osd.mil/packs/Default.aspx?pg=1002

Kallstrom, G., Warfield, K. L., Swenson, D. L., Mort, S., Panchal, R. G., Ruthel, G., et al. (2005). Analysis of Ebola Virus and VLP Release Using an Immunocapture Assay. *Journal of Virological Methods , 127*, 1-9.

Langlois, R. (2002, January/February). Rapid Field Detection of Biological Agents. *Science and Technology Review* .

LaRoche, B. C. (2009). *Analysis of Expedient Field Decontamination Methods for the XMX/2L-MIL High-Volume Aerosol Sampler.* Wright-Patterson AFB, OH: Air Force Institute of Technology.

Lawrence, R. (2003). *Bacillus anthracis* Spores (Etiologic Agent of Anthrax) in Air. *The National Occupational Research Agenda* (pp. 81-106). Salt Lake City, Utah: The University of Utah.

Leffel, E. K., & Reed, D. S. (2004). Marburg and Ebola Viruses as Aerosol Threats. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science , 2* (3), 186-191.

Lohaus, J. H., Semler, D. D., Escamilla, E., Semler, K. A., Llyod, A. J., Nicol, S. L., et al. (2009). *Effectiveness of Field Bacterial Decontamination Methods for a Biological Agent Collection System.* Brooks City Base, TX: USAF School of Aerospace Medicine.

Loo, B. W., & Cork, C. P. (1988). Development of High Efficiency Virtual Impactors. *Aerosol Science and Technology , 9*, 167-176.

Martin, J. W., Christopher, G. W., & Eitzen, E. M. (2007). History of Biological Weapons: From Poisoned Darts to Intentional Epidemics. In *Medical Aspects of Biological Warfare* (pp. 1-20). Washington, DC: Borden Institute.

Maynard, A. D., & Kuempel, E. D. (2005). Airborne Nanostructured Particles and Occupational Health. *Journal of Nanoparticle Research , 7*, 587-614.

Meiklejohn, G., Kempe, C. H., Downie, A. W., Berge, T. O., St. Vincent, L., & Rao, A. R. (1961). Air Sampling to Recover Variola Virus in the Environment of a Smallpox Hospital. *Bulletin of the World Health Oranization , 25*, 63-67.

Meltzer, M. I., Damon, I., LeDuc, J. W., & Millar, J. D. (2001, November-December). Modeling Potential Responses to Smallpox as a Bioterrorist Weapon. *Emerging Infectuous Diseases , 7* (6), pp. 959-969.

O'Connell, K. P., Bucher, J. R., Anderson, P. E., Cao, C. J., Akbar, S. K., Gostomski, M. V., et al. (2006). Real-Time Fluorogenic Reverse Transcription-PCR Assays for Detection of Bacteriophage MS2. *Applied and Environmental Microbiology , 72* (1), 478-483.

Peruski, L. F., & Peruski, A. H. (2003). Rapid diagnostic assays in the genomic biology era: detection and identification of infectious disease and biological weapon agents. *BioTechniques , 35* (4), 840-846.

Peters, T. M., & Leith, D. (2003). Concentration Measurement and Counting Efficiency of the Aerodynamic Particle Sizer 3321. *Journal of Aerosol Science , 34*, 627-634.

Peters, T. M., Ott, D., & O'Shaughnessy, P. T. (2006). Comparison of the Grimm 1.108 and 1.109 Portable Aerosol Spectrometer to the TSI 3321 Aerodynamic Particle Sizer for Dry Particles. *The Annals of Occupational Hygiene , 50* (8), 843–850.

Remel. (2005). *Microtest M5 Multi-Microbe Media Instructions for Use.* Retrieved January 24, 2010, from Remel : http://www.remelinc.com/Support/MyRemelOnline/IFU.aspx

Riemann, H. P., & Cliver, D. O. (2006). *Foodborne infections and intoxications* (3rd Edition ed.). San Diego, CA: Elsevier Academic Press.

Riemenschneider, L., H, W. M., Y, W. C., Lundgren, D., J, W., H, L. J., et al. (2009). Characterization of Reaerosolization from Impingers in an Effort to Improve Airborne Virus Sampling. *Journal of Applied Microbiology , 108* (1), 314-324.

Romay, F. J., Roberts, D. L., Marple, V. A., Liu, B. Y., & Olson, B. A. (2002). A High-Performance Aerosol Concentrator for Biological Agent Detection. *Aerosol Science and Technology , 36*, 217-226.

Rose, L., Jensen, B., Peterson, A., Banerjee, S. N., & Arduino, M. J. (2004). Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces. *Emerging Infectious Diseases , 10* (6), 1023-1029.

Russell, K. L., Broderick, M. P., Franklin, S. E., Blyn, L. B., Freed, N. E., Moradi, E., et al. (2006). Transmission Dynamics and Prospective Environmental Sampling of Adenovirus in a Military Recruit Setting. *Journal of Infectuous Disease , 194*, 877-885.

Salama, S., & Bursac, E. (2009). Jihadist Capabilities and the Diffusion of Knowledge. In *Jihadists and Weapons of Mass Destruction* (pp. 101-125). Boca Raton, Florida: CRC Press.

Schofield, L., Ho, J., Kournikakis, B., & Booth, T. (2005). *Avian Influenza Aerosol Sampling Campaign in the British Columbia Fraser Valley, 9–19 April 2004.* Defense Research and Development Canada.

Tseng, C.-C., & Li, C.-S. (2005). Collection Efficiencies of Aerosol Samplers for Virus-Containing Aerosols. *Journal of Aerosol Science , 36*, 593–607.

Tucker, T. (2005). *The Preparation of Guidence Documents and Optimization of the Use of Weapons of Mass Destruction (WMD) Equipment Brooks City Base, TX.* Brooks City Base, TX: Air Force Institute of Operational Health.

Ty, A. R. (2007). *Aerosolization and Taqman PCR Detection of Bradyrhizobium japonicum USDA 110 as a Biowarfare Simulant.* Wright-Patterson AFB, OH: Air Force Institute of Technology.

Utrup, L. J., & Frey, A. H. (2004). Fate of Bioterrorism-Relevant Viruses and Bacteria, Including Spores, Aerosolized into an Indoor Air Environment. *Experimental Biology and Medicine , 229*, 345-350.

Verreault, D., Moineau, S., & Duchaine, C. (2008, September). Methods of Sampling for Airborne Viruses. *Microbiology and Molecular Biology Reviews , 72* (3), pp. 413-444.

WHO. (2004, April 21). *Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003.* Retrieved January 5, 2010, from World Health Organization: http://www.who.int/csr/sars/country/table2004_04_21/en/index.html

Willeke, K., Xuejun, L., & Grinshpun, S. A. (1998). Improved Aerosol Collection by Combined Impaction and Centrifugal Motion. *Aerosol Science and Technology , 28* (5), 439-456.

Wilson, S. A. (2006). *A Novel Approach for the Development and Acquisition of a Diagnostic Medical Device from Concept to Fielding: The Joint Biological Agent Identification and Diagnostic System (JBAIDS).* PhD Thesis, George Mason University, Department of Molecular and Microbiology, Fairfax, VA

Vita

Captain Casey Cooper is a 2004 graduate of the United States Air Force Academy where he received a bachelors of science in environmental engineering. Prior to his assignment at the Air Force Institute of Technology, he served as chief of industrial hygiene at Scott AFB, IL. 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, Ohio.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From – To)*
25-03-2010	Master's Thesis	May 2009 - Mar 2010

4. TITLE AND SUBTITLE

High Volume Air Sampling for Viral Aerosols: A Comparative Approach

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Cooper, Casey W., Captain, USAF, BSC

5d. PROJECT NUMBER
N/A

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(S)
Air Force Institute of Technology
Graduate School of Engineering and Management (AFIT/EN)
2950 Hobson Way
WPAFB OH 45433-7765

8. PERFORMING ORGANIZATION REPORT NUMBER

AFIT/GES/ENV/10-M01

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Ott, Darrin K., Lt Col., USAF, BSC
US Air Force School of Aerospace Medicine, Department of Occupational and Environmental Health
2413 Kennedy Circle
Brooks City-Base, TX 78235
DSN: 674-4652

10. SPONSOR/MONITOR'S ACRONYM(S)
USAFSAM/OEHR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The United States Air Force has high volume biological air sampling equipment available including the XMX/2L-MIL and DFU-1000. Neither system has been evaluated for effectiveness in the collection of viruses. Furthermore, decontamination methods have not been evaluated for these systems after use in sampling for a viral agent. MS2 bacteriophage was used as a surrogate virus. Aerosolized MS2 was released into a 12 m^3 exposure chamber. High and moderate airborne concentrations of MS2 were evaluated. Low volume impingers were used for comparative purposes as well. Samples were analyzed using plaque assay and polymerase chain reaction (PCR). At high viral loads the XMX/2L-MIL and DFU-1000 achieved collection effectiveness equal to or greater than the low volume impingers. At moderate levels of airborne viral load, the XMX/2L-MIL was capable of collecting viral quantities within 25% of the quantities collected by the low volume impingers. The DFU-1000 achieved marginal collection effectiveness of virus at moderate concentrations compared to the XMX/2L-MIL and is considered to be unreliable in the quantification of viral agent at moderate levels and below. The DFU-1000 and XMX/2L-MIL were capable of collecting detectable MS2 with PCR analysis at all concentrations. Ten percent sodium hypochlorite (commercial bleach) solution effectively decontaminated MS2.

15. SUBJECT TERMS
Bioaerosol, Air Sampling, Health Protection, High Volume, Dry Filter Unit, Virtual Impaction, Bioterrorism, MS2 Bacteriophage, Virus, Particle Size, Decontamination, Polymerase Chain Reaction, Plaque Assay, Joint Biological Agent Identification and Detection System

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Major Jeremy M. Slagley (AFIT/ENV)
U	U	U	UU	177	**19b. TELEPHONE NUMBER** *(Include area code)* 937-785-3636 ext. 4511

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39-18

Form Approved
OMB No. 074-0188