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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The interinstrument variability of the XMX/2L-MIL (XMX) biological air sampler was controlled by operating it with a fixed final nozzle orientation. Three XMXs were operated in a 12-m <sup>3</sup> aerosol test chamber (ATC) in which a <i>Bacillus globigii</i> (Bg) aerosol was uniformly distributed for 18 experimental trials. An analysis of variance (ANOVA) was performed on the XMX results and had an <i>F-value</i> of 0.36, compared to the <i>F-critical</i> of 3.22 for a <i>p-value</i> of 0.05. This experiment was repeated using male-specific 2 bacteriophage (MS2) viral agent surrogate for 16 experimental trials. An ANOVA was performed on the XMX results and had an <i>F-value</i> of 0.09, compared to the <i>F-critical</i> of 3.22 for a <i>p-value</i> of 0.05. Therefore, it was concluded that operating XMXs at the specified final nozzle orientation would lead to precise laboratory culture results with acceptable interinstrument variability. Three XMXs were used to determine if Remel M5® collection media would better preserve MS2 for laboratory culture analysis than the commonly used PBS solution collection media. The XMXs were operated in the ATC in which MS2 aerosol was uniformly distributed for 16 experimental trials, with 8 conducted using Remel M5® and 8 with PBS solution as collection media. An ANOVA was performed on the XMX results and had an <i>F-value</i> of 6.73, compared to the <i>F-critical</i> of 5.30 for a <i>p-value</i> of 0.05. Therefore, it was concluded that Remel M5® produced superior results, as its culture results were 2.3 times greater than those for PBS solution.					
15. SUBJECT TERMS XMX, air sampling, liquid impinger, virtual impactor, aerosol, biological aerosol, collection media, biological agent, Remel M5®, PBS solution, <i>Bacillus globigii</i> , male-specific 2 bacteriophage, MS2					
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a. REPORT	b. ABSTRACT	c. THIS PAGE			Major Jon E. Black
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**DEPARTMENT OF THE AIR FORCE**  
**USAF SCHOOL OF AEROSPACE MEDICINE (AFMC)**  
**WRIGHT-PATTERSON AFB OH**

13 July 2012

MEMORANDUM FOR AFMSA/SG3PB  
1500 WILSON BOULEVARD  
SUITE 1600  
ARLINGTON, VA 22209-2458

FROM: USAFSAM/OEHR  
2510 FIFTH STREET  
WRIGHT-PATTERSON AFB, OH 45433-7913

SUBJECT: Consultative Letter, AFRL-SA-WP-CL-2012-0059, Interinstrument Variability and Validation Study for the XMX/2L-MIL Biological Air Sampler

1. INTRODUCTION:

a. *Purpose:* From 22 August to 2 September 2011, the Risk Analysis Division of the United States Air Force School of Aerospace Medicine (USAFSAM/OEHR) performed an evaluation and validation study of the XMX/2L-MIL (XMX) biological air sampler. This study was performed using three XMXs at the Dycor Technologies, Ltd. (Dycor) aerosol test facility (ATF) in Edmonton AB, Canada. The performance of the XMX was evaluated using two biological agents, spore-forming bacteria *Bacillus globigii* (*Bg*) and viral agent surrogate male-specific 2 bacteriophage (MS2), and two liquid collection media, phosphate buffered saline (PBS) solution and Remel M5<sup>®</sup>. The interinstrument variability of the XMX was evaluated by performing an analysis of variance (ANOVA) on counts of colony forming units per milliliter (CFU/mL) of collection media resulting from sampling and plating aerosolized *Bg* in the ATF using PBS solution as the collection media. The two collection media were compared by performing an ANOVA on plated counts of plaque forming units per milliliter (PFU/mL) of collection media resulting from sampling and plating aerosolized MS2 in the ATF while alternating use of the two collection media between experimental trials.

b. *Survey Personnel:*

- (1) Maj Jon Black
- (2) SSgt George MacEachern
- (3) SSgt Christopher Thornton
- (4) Ms. Elizabeth Escamilla
- (5) Ms. Linda Armstrong

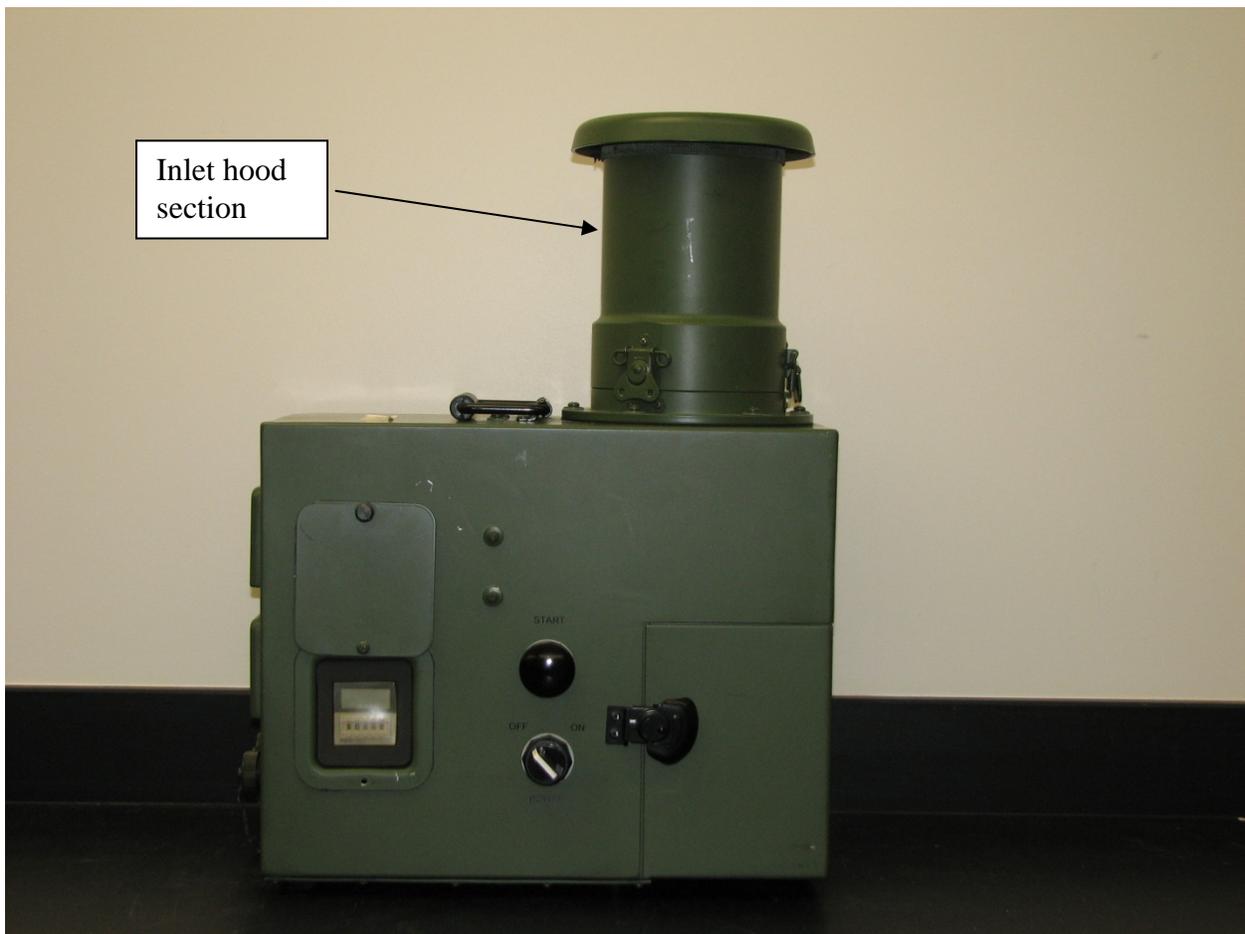
c. *Personnel Contacted:* Mr. Chris Bliss, Dycor

d. *Equipment:*

- (1) Dycor XMX1, SN X2119
- (2) Dycor XMX2, SN X2064
- (3) Dycor XMX3, SN X2337

2. BACKGROUND:

a. The XMX, shown in Figure 1, is used by Bioenvironmental Engineering personnel to collect air samples for biological analysis. The XMX combines a virtual impactor and a liquid impinger to separate, concentrate, and collect 1- to 10- $\mu$ m-diameter aerosol particles in a liquid collection media. A previous study suggested enhanced collection and preservation of MS2 for plaque assay analysis following collection when using Remel M5<sup>®</sup> compared to PBS solution as the liquid collection media for medium and high airborne agent concentrations (Ref 1). Another previous study demonstrated statistically significant interinstrument variability potentially attributable to final nozzle orientation of the virtual impactor (Ref 2).

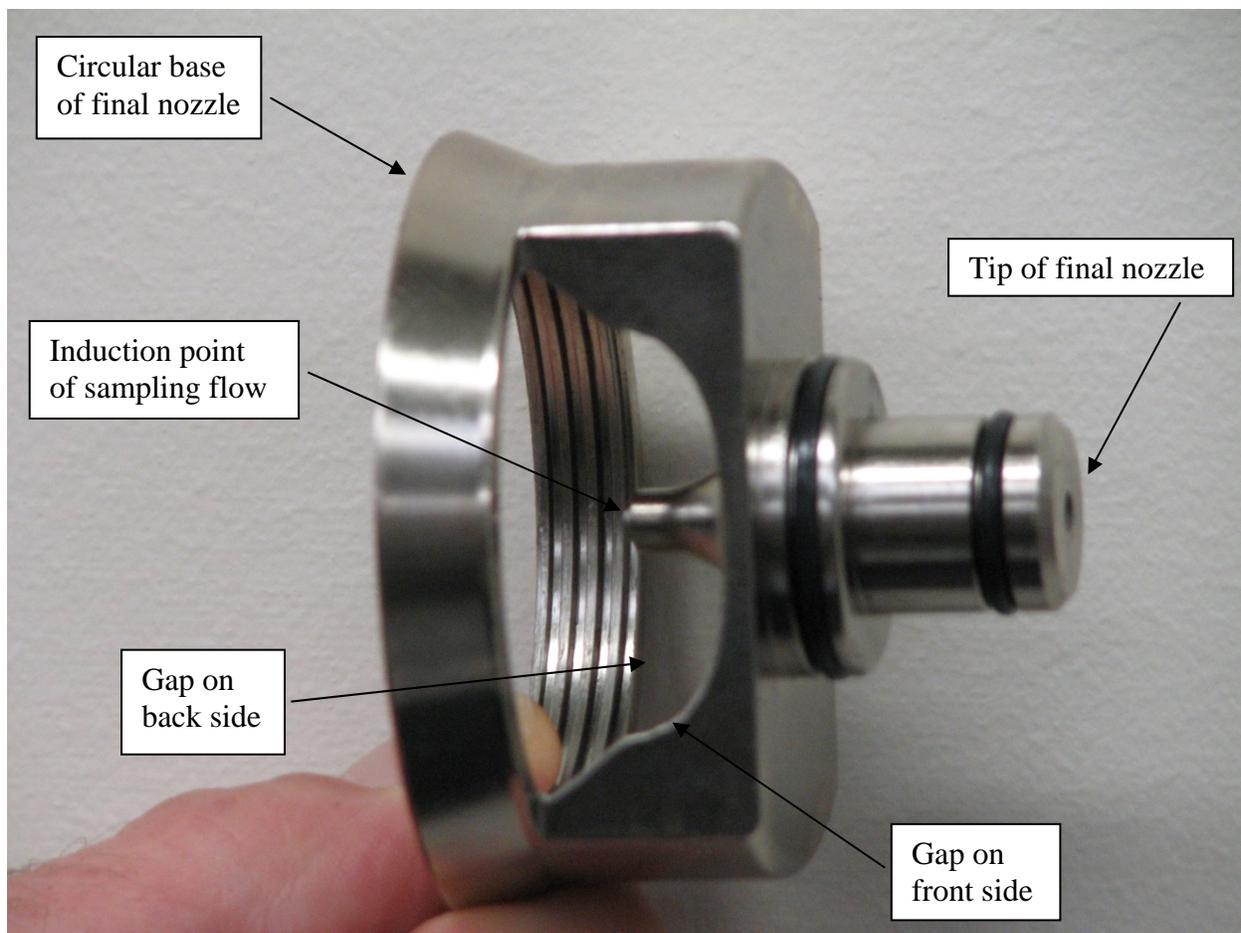


**Figure 1. XMX/2L-MIL biological air sampler**

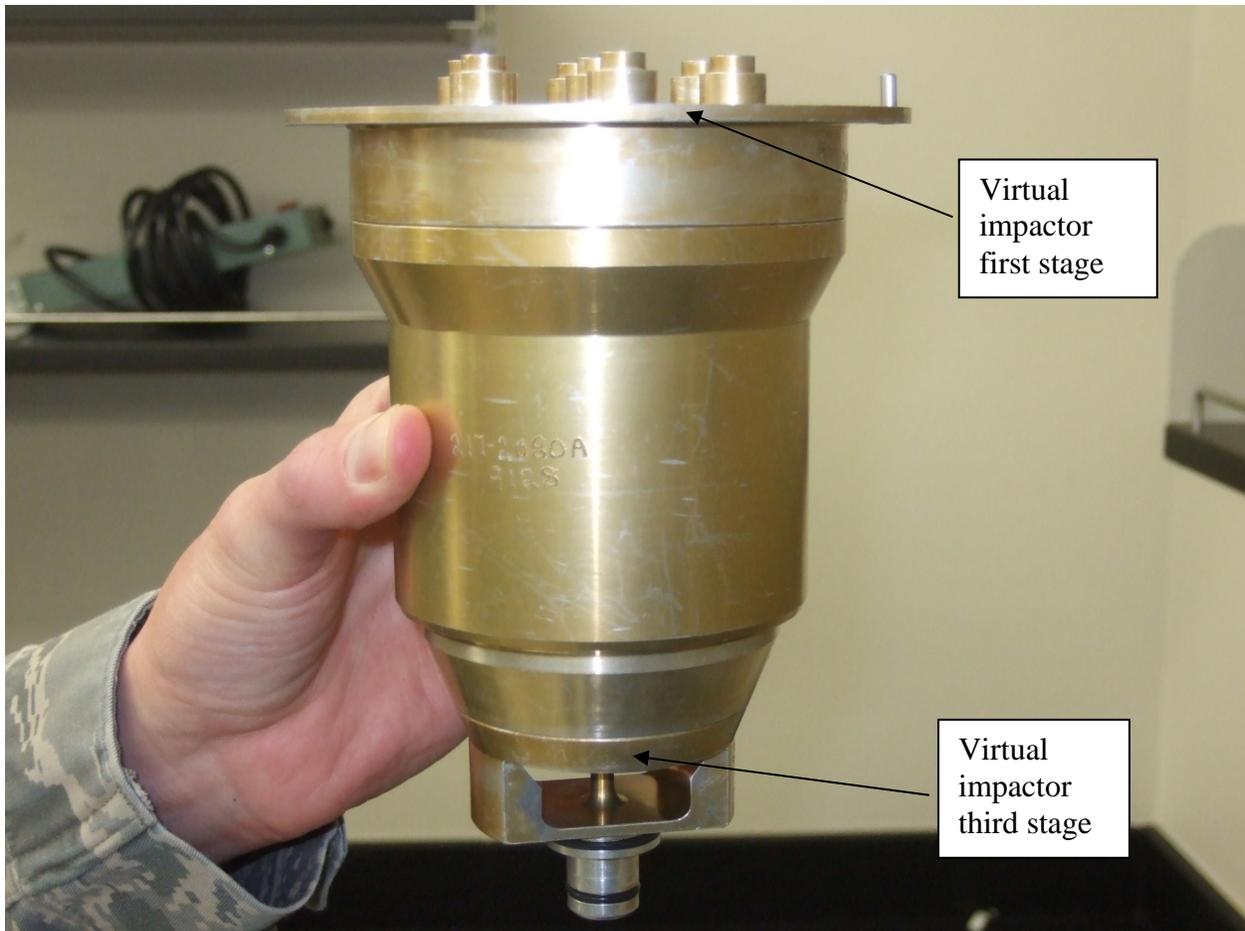
b. To better ensure consistent and reliable operation and laboratory results of cultured biological agents when using the XMX, it is important to identify operational procedures that reduce XMX interinstrument variability to a level that is not statistically significant. Additionally, if the suggested enhanced collection and preservation of MS2 by Remel M5<sup>®</sup> collection media can be shown to be statistically significant, this may suggest the use of Remel M5<sup>®</sup> collection media when using the XMX to collect multiple samples for subsequent laboratory biological analyses.

### 3. EXPERIMENTAL PROCEDURE:

a. It was hypothesized that the significant interinstrument variability witnessed in previous XMX evaluations was due to failing to operate the XMX with a fixed final nozzle orientation (Ref 2). The XMX operating manual makes no mention of assembling or operating the XMX with any specific or consistent final nozzle orientation (Ref 3). The final nozzle of the XMX is shown in Figure 2. The key features to note of the final nozzle are its circular symmetry and pair of diametrically opposed identical gaps in its sidewall. During assembly of the XMX, the virtual impactor assembly, shown in Figure 3, is inserted into the cylindrical cavity of the XMX, shown in Figure 4. As the primary flow of the XMX passes through the first and third stages of the virtual impactor assembly, the primary flow is divided into two flow streams; one stream continues on to the next successive stage of the virtual impactor while the other stream is diverted and enters the annular space between the sidewall of the cylindrical cavity and the virtual impactor assembly. The diverted flow stream proceeds downward in the annular space and turns 90 degrees as it is drawn by the XMX blower to the exhaust port inside the cavity. As this diverted flow stream turns 90 degrees and proceeds towards the exhaust port, some of this flow stream will pass through the sidewall gaps in the final nozzle and remix with the remainder of the primary flow that has passed through the third stage of the virtual impactor at the induction point of the sampling flow. The turbulent nature of this remixing of flows is dependent upon the circular positions of the gaps in the sidewall of the final nozzle. The circular positions of the gaps in the sidewall, with respect to the diverted portions of the primary flow, are set based upon the angular orientation of the final nozzle. The final nozzle orientation for all experimental trials in this study is as shown in Figure 5, with the sidewall gaps facing outward and perpendicular to the straight line drawn that bisects the circular cross-section of the XMX cavity and is parallel with the long sides of the XMX. This angular orientation of the final nozzle was selected for two reasons: (1) the ease with which it can be described and consistently duplicated during assembly and operation, and (2) perceptive intuition that it could generate the least turbulent remixing of flows, as this orientation should minimize cross-flow across the induction point of the sampling flow.

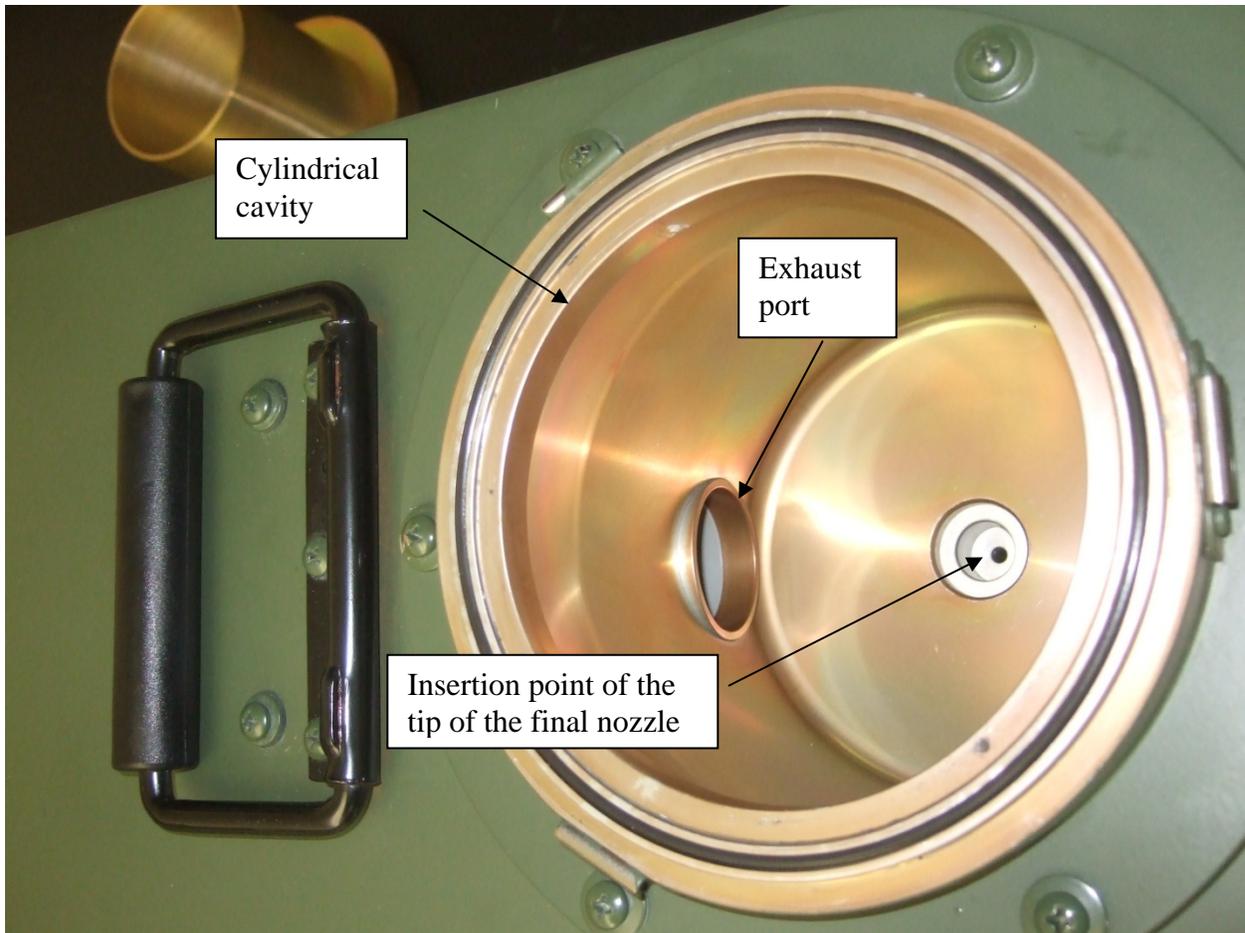


**Figure 2. Final nozzle of the XMX**



**Figure 3. XMX virtual impactor assembly**

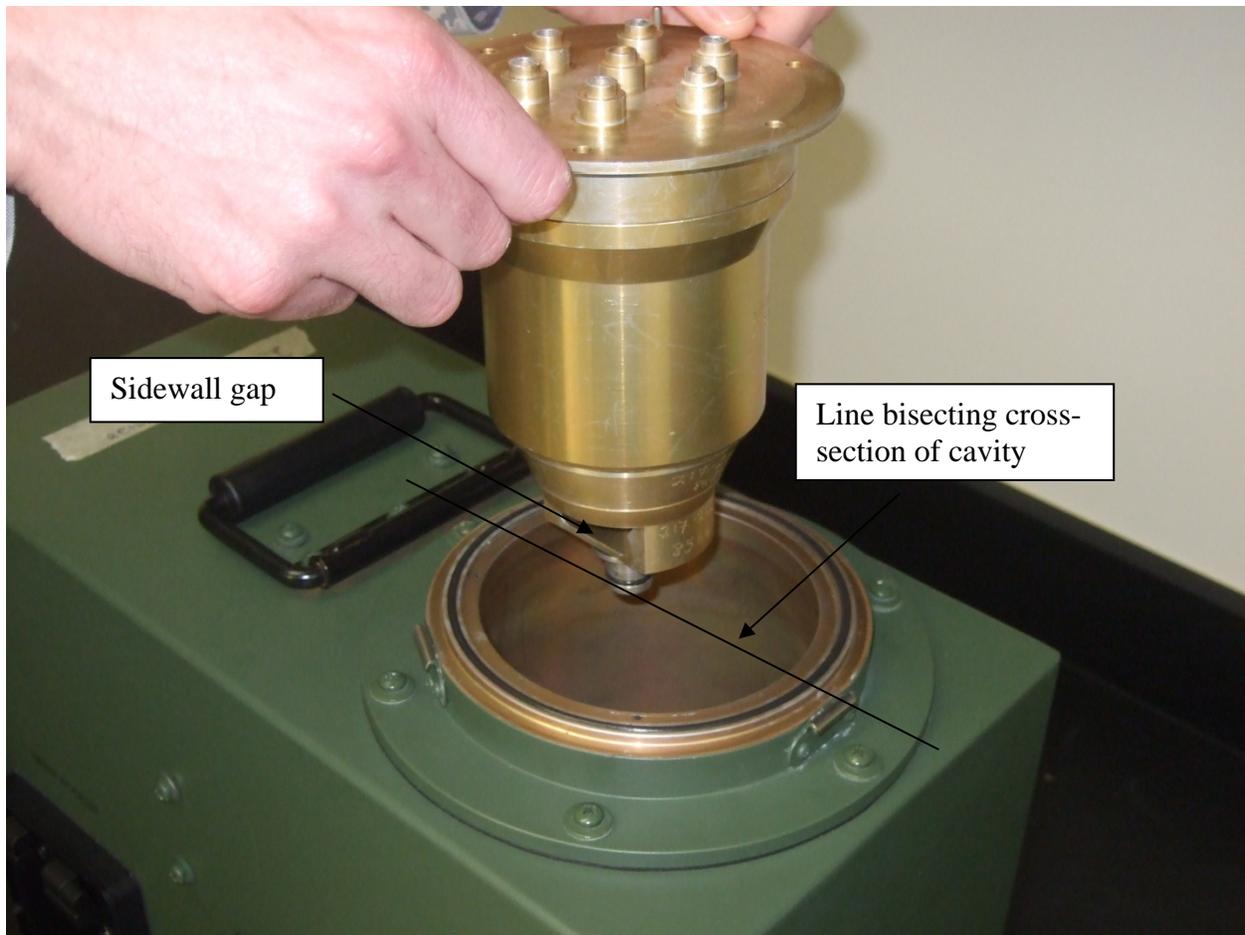
b. The three XMXs were operated per the instructions in the Dycor manual, except for using a fixed final nozzle orientation as described in paragraph 3.a. above. All sample collection periods were 5 minutes in duration. All XMXs were decontaminated between trial runs by submerging their removable virtual impactor canister components in a 5% bleach/water solution for 5 minutes, rinsing with tap water, and air drying. XMXs were operated at the standard secondary (sampling) flow of approximately 12.5 liters per minute (LPM) for all *Bg* trial runs. XMXs were then operated at a reduced secondary flow of approximately 4.5 LPM for all MS2 trial runs. The reduced secondary flow rate is achieved by inserting a flow reducer in the vacuum pump line, which draws the secondary flow through the final nozzle, between the liquid impinger module and the fluid trap. The flow reducer, provided by Dycor, is a critical orifice created by drilling a small hole lengthwise through a brass cylinder that is approximately 5 mm in diameter and 25 mm long.



**Figure 4. Cavity of the XMV where the virtual impactor assembly is inserted**

c. All experimental trials were conducted in an aerosol test chamber (ATC) at Dycor. The ATC, constructed of stainless steel, has a volume of 12 m<sup>3</sup> and is approximately 3 m long, 2 m wide, and 2 m high. The ATC has three ports in its bottom into which XMVs are raised up into such that only the XMV inlet hood section (see Figure 1) is contained within the ATC while the remainder of the XMV is outside the ATC. Rubber gaskets at each port form an airtight seal around the XMV inlet hoods. All three XMVs were operated and used to collect a sample for analysis during every trial. The ATC has two circulating fans to ensure aerosol mixing. The particle size distribution is measured by a Thermal Systems Incorporated Aerodynamic Particle Sizer, model number 3321. ATC biological agent concentration was determined using two slit-to-agar air samplers, model number STA-203 manufactured by New Brunswick Scientific and reported as agent containing particles per liter of air (ACPLA).

d. Solutions for *Bg* and MS2 aerosol generation were prepared following proprietary procedures developed by Dycor. The *Bg* solution was prepared using powder from Lot #10-124 obtained from Dugway Proving Ground and the MS2 solution was prepared using culture #15597-B1 obtained from American Type Culture Collection. The biological agent solutions were aerosolized using a Sonotek 8700-48MS ultrasonic atomizing nozzle and injected into the ATC.



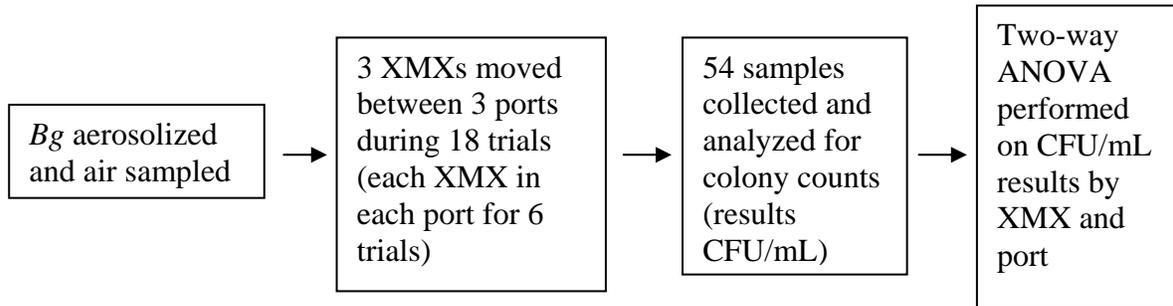
**Figure 5. Illustration of final nozzle orientation used during all experimental trials**

e. Eighteen trials were conducted with *Bg* aerosol. The three XMXs were moved to a different port for each trial such that each XMX had six trials in each of the three ports. All trials were conducted using 5 mL of PBS solution as the liquid collection media for the XMXs. The aerosol generation process was consistently operated to obtain a target airborne concentration of approximately 10 ACPLA for *Bg* in the ATC. For the 18 *Bg* trials, the mean *Bg* aerosol concentration was 10.4 ACPLA with a standard deviation of 3.5 ACPLA, and the mean count median diameter of the aerosol in the ATC was 1.4  $\mu\text{m}$  with a mean geometric standard deviation of 1.6.

f. Sixteen trials were conducted with MS2 aerosol. The three XMXs were not moved to different ports between trials; thus, each XMX had 16 trials in one port and no trials in the two other ports. Eight trials were conducted using 5 mL of PBS solution as the liquid collection media for the XMXs and eight trials were conducted using 5 mL of Remel M5<sup>®</sup> as the liquid collection media, with the collection media being alternated between trials. The aerosol generation process was consistently operated to obtain a target airborne concentration of approximately 15 ACPLA for MS2 in the ATC. For the 16 MS2 trials, the mean MS2 aerosol concentration was 15.2 ACPLA with a standard deviation of 4.0 ACPLA, and the mean count median diameter of the aerosol in the ATC was 2.5  $\mu\text{m}$  with a mean geometric standard deviation of 1.7.

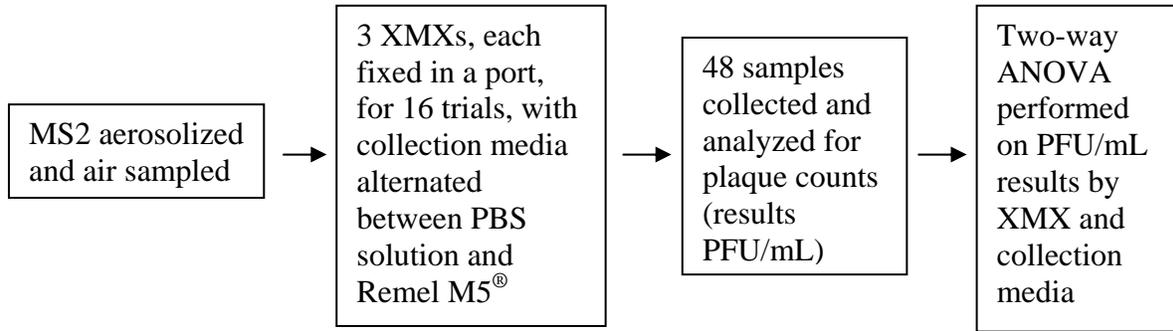
g. Laboratory procedures and analyses for samples collected for airborne *Bg* and MS2 aerosols were performed following proprietary methodology developed by Dycor. Samples collected during *Bg* trials were diluted, plated, incubated, and colony counted the next morning. *Bg* results were reported as CFU/mL of collection media by accounting for the volume of collection media pipetted and the number of serial dilutions. Samples collected during MS2 trials were diluted, plated, incubated, and plaque counted the next morning. MS2 results were reported as PFU/mL of collection media by accounting for the volume of collection media pipetted and the number of serial dilutions.

h. The *Bg* trials were designed as a two-way ANOVA experiment to evaluate the XMX interinstrument variability and ATC ports for statistical significance at a  $p$ -value of 0.05, with null hypotheses of the XMXs being equal and the ATC ports being equal. If the null hypothesis is not rejected for the XMXs, then the XMX interinstrument variability is found to not be statistically significant. Similarly, if the null hypothesis is not rejected for the ATC ports, then the test aerosol concentration distribution at the three ports is found to not be statistically significant. A diagram graphically depicting the *Bg* ANOVA experiment is shown in Figure 6.



**Figure 6. Evaluating XMX interinstrument variability and ATC ports**

i. The MS2 trials were designed as a two-way ANOVA experiment to evaluate the XMX interinstrument variability and compare collection and preservation performance for the two collection media for statistical significance at a  $p$ -value of 0.05, with null hypotheses of the XMXs being equal and the collection media being equal. If the null hypothesis is not rejected for the XMXs, then the XMX interinstrument variability is found to not be statistically significant. Similarly, if the null hypothesis is not rejected for the collection media, then the maintenance of agent viability is found to not be statistically significant. A diagram graphically depicting the MS2 ANOVA experiment is shown in Figure 7.



**Figure 7. Evaluating XMX inter-instrument variability and collection media performance**

#### 4. RESULTS:

a. The 18 *Bg* trials constitute a two-way ANOVA experiment with  $n$  equal to 54, two degrees of freedom for XMX, and two degrees of freedom for ATC port. Presented in Table 1 are the laboratory results for the *Bg* trials. The calculated  $F$ -values for the *Bg* ANOVA are 0.36 and 0.09 for the XMX and ATC port treatments, respectively, with both having an  $F$ -critical value of 3.22. The  $F$ -values are less than their respective  $F$ -critical values; therefore, neither the null hypothesis for the XMXs nor the ATC ports is rejected.

**Table 1. Laboratory Results *Bg* Experimental Trials**

Trial	XMX/Port	CFU/mL	XMX/Port	CFU/mL	XMX/Port	CFU/mL	ACPLA
1	1/1	652.5	2/2	820.0	3/3	495.0	10.6
2	2/1	1182.5	3/2	1055.0	1/3	1150.0	6.1
3	3/1	195.0	1/2	215.0	2/3	217.5	10.9
4	1/1	3425.0	2/2	3875.0	3/3	5975.0	14.7
5	2/1	270.0	3/2	250.0	1/3	415.0	9.1
6	3/1	522.5	1/2	507.5	2/3	857.5	7.8
7	1/1	327.5	2/2	360.0	3/3	477.5	11.5
8	2/1	195.0	3/2	162.5	1/3	115.0	11.4
9	3/1	1255.0	1/2	607.5	2/3	642.5	11.2
10	1/1	137.5	2/2	172.5	3/3	117.5	5.1
11	2/1	1385.0	3/2	1315.0	1/3	1227.5	13.6
12	3/1	215.0	1/2	67.5	2/3	125.0	13.7
13	1/1	52.5	2/2	45.0	3/3	50.0	4.8
14	2/1	30.0	3/2	62.5	1/3	90.0	5.5
15	3/1	542.5	1/2	742.5	2/3	1007.5	14.6
16	1/1	1550.0	2/2	1325.0	3/3	1525.0	16.7
17	2/1	145.0	3/2	2600.0	1/3	147.5	9.7
18	3/1	122.5	1/2	187.5	2/3	107.5	9.5

b. The 16 MS2 trials constitute a two-way ANOVA experiment with  $n$  equal to 48, two degrees of freedom for XMX, and one degree of freedom for collection media. Presented in Table 2 are the laboratory results for the MS2 trials. The PFU/mL per ACPLA for trials when Remel M5<sup>®</sup> was used as the collection media were 2.3 times as great as those in trials when PBS solution was used as the collection media. The calculated  $F$ -values for the MS2 ANOVA are 0.09 and 6.73 for the XMX and collection media treatments, respectively, with both having an

*F-critical* value of 3.22. The *F-value* for XMXs is less than its *F-critical* value; therefore, the null hypothesis for the XMXs is not rejected. The *F-value* for collection media is greater than its *F-critical* value; therefore, the null hypothesis for the collection media is rejected.

**Table 2. Two-Way ANOVA Values for MS2 Experimental Data**

<b>Trial</b>	<b>Collection Media</b>	<b>XMX1 (PFU/mL)</b>	<b>XMX2 (PFU/mL)</b>	<b>XMX3 (PFU/mL)</b>	<b>ACPLA</b>
1	PBS solution	2.45E+05	2.43E+05	2.83E+05	20.8
2	Remel M5 <sup>®</sup>	1.55E+05	2.23E+05	1.73E+05	19.8
3	PBS solution	1.98E+04	6.58E+04	3.88E+04	17.2
4	Remel M5 <sup>®</sup>	1.26E+05	1.29E+05	1.27E+05	20.7
5	PBS solution	7.78E+04	1.13E+04	7.53E+04	18.1
6	Remel M5 <sup>®</sup>	1.19E+05	1.18E+05	1.36E+05	17.7
7	PBS solution	5.48E+03	1.00E+01	7.23E+04	18.4
8	PBS solution	6.73E+04	7.40E+04	6.03E+04	14.1
9	Remel M5 <sup>®</sup>	9.73E+04	7.68E+04	6.23E+04	12.1
10	PBS solution	6.50E+01	1.15E+03	3.03E+02	9.8
11	Remel M5 <sup>®</sup>	9.95E+04	6.80E+04	1.27E+05	17.9
12	PBS solution	3.88E+02	5.00E+00	0.00E+00	9.9
13	Remel M5 <sup>®</sup>	5.68E+04	1.17E+05	8.53E+04	11.7
14	Remel M5 <sup>®</sup>	1.15E+05	1.03E+05	1.09E+05	13.8
15	PBS solution	0.00E+00	1.60E+02	1.80E+02	11.3
16	Remel M5 <sup>®</sup>	5.30E+04	5.30E+04	5.00E+04	10.5

## 5. CONCLUSIONS:

a. The ATC is well mixed, and the distribution of test aerosols in the ATC is sufficient for evaluating aerosol samplers because no difference was detected in the measured response due to ATC ports.

b. The three XMXs produced precise laboratory culture results for the aerosolized bacterial and viral surrogate biological agents because no differences were detected in the measured responses due to XMXs.

c. Remel M5<sup>®</sup> was superior to PBS solution in collecting and preserving an aerosolized viral surrogate agent, as there was a statistically significant difference in the measured response due to the collection media, and Remel M5<sup>®</sup> produced a 2.3 times greater cultured laboratory response than PBS solution.

## 6. RECOMMENDATIONS:

a. Bioenvironmental Engineering personnel should use the fixed final nozzle orientation described in paragraph 3.a. when operating the XMX.

b. AFMSA/SG3PB should consider a proposal to revise the existing XMX CONOPS to include use of Remel M5<sup>®</sup> when using the XMX to collect multiple samples for analyses.

c. Additional alternate collection media for use with the XMX should be experimentally evaluated based upon an informed literature review.

7. If there are questions concerning this experimental evaluation, please contact Maj Jon Black at DSN 798-3297 or via email at [jon.black@us.af.mil](mailto:jon.black@us.af.mil).

A handwritten signature in blue ink, appearing to read "David M. Sonntag". The signature is fluid and cursive, with the first name "David" being the most prominent.

DAVID M. SONNTAG, Lt Col, USAF, BSC  
Chief, Risk Analysis Division

Attachment:  
References

## References

1. Cooper, C. W. *High Volume Air Sampling for Viral Aerosols: A Comparative Approach*. MS thesis, AFIT/GES/ENV/10M-01. Graduate School of Engineering and Management, Air Force Institute of Technology (AU), Wright-Patterson AFB, OH, March 2010 (ADA519642).
2. Black, J. E. *Evaluation of XMX/2L-MIL Virtual Impactor Performance and Capture and Retention of Aerosol Particles in Two Different Collection Media*. MS thesis, AFIT/GIH/ENV/11-M01. Graduate School of Engineering and Management, Air Force Institute of Technology (AU), Wright-Patterson AFB, OH, March 2011 (ADA539055).
3. Dycor Technologies, Ltd. *XMX/2L-MIL Operator's Manual, Version 1.7*. Edmonton: Dycor Technologies, Ltd. (2007).