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**BIOAEROSOL CHALLENGES TO
ANTIMICROBIAL SURFACE TREATMENTS:
ENHANCED EFFICACY AGAINST MS2 COLI PHAGE OF AIR
FILTER MEDIA COATED WITH POLYSTYRENE-4-
METHYLTRIMETHYLAMMONIUM TRIIODIDE**

**Brian K Heimbuch
Applied Research Associates
PO Box 40128
Tyndall AFB, FL 32403**

**Joseph D. Wander
Air Force Research Laboratory/MLQL
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403**

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**Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403-5323**

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14. ABSTRACT Two samples each of two commercial (COTS) soft respiratory protection (SRP) masks and seven SRP masks augmented with a quaternary ammonium derivative of polystyrene as the triiodide salt were sealed to an adapter and challenged for 6 hours with an aerosol containing ~10 ⁸ plaque-forming units (PFUs) of MS2 coli phage. Compared to a positive control (open path), average % reduction in PFUs for the first and second COTS and the iodinated SRP masks was 98.18%, 99.55% and 99.9945%, respectively. The two-log enhancement in removal efficiency is attributed to the available iodine's killing ~99% of penetrating coli phage particles. In a swatch test, 47-cm ² discs of the iodinated medium cut from a mask achieved 99.9999% average % reduction in PFUs.					
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Bioaerosol Challenges to Antimicrobial Surface Treatments. Enhanced Efficacy Against MS2 Coli Phage of Air Filter Media Coated with Polystyrene-4-methyltrimethylammonium Triiodide

1. Introduction

1.1 Objective: The objective of this project was to obtain an experimental measure of the enhancement of protection afforded by nominal 95% particle filter masks wrought by incorporation of a reactive antimicrobial coating.

1.2 Background: Several mechanisms for particle capture operate in different ranges of particle size. Larger infective biological aerosols are typically captured by impaction, whereas the smallest of this group in completely dispersed, unbound form will be captured by diffusion. The latter, primarily viruses, typically present as aggregates with each other and/or environmental particles of varying size.

As efficiency of capture of airborne particles experiences a minimum near 200 nm (both impaction and diffusional capture mechanisms are inefficient for particles near this size), microbes smaller than 200 nm pose the greatest potential risk to penetrate mechanical respiratory protection devices. Because formation and disaggregation of bioaerosols is a function of many conditions, it is a complex process to describe predictively. MLQL is examining empirically approaches to suppressing the rate of penetration and survival of pathogens, by comparing the quantities (in plaque-forming units [PFUs]) of viable MS2 coli phage passing through or living on existing protective materials to PFUs passing through or on analogous materials enhanced by the incorporation of reactive materials.

1.3 Scope: This document reports the results of challenges conducted during December 2005 of randomly selected samples of two commercial off-the-shelf P95 respirators, an emerging P95 respirator enhanced by the incorporation of a thin coating of an iodine-releasing polymer, and discs cut from the filter medium of another of these enhanced respirators.

2. Materials and Methods:

2.1 Facility: Testing was conducted at AFRL/MLQL, in an aerosol chamber designed specifically for applying bioaerosol challenges to candidate reactive materials. The BioAerosol Test System (BATS, Figures 1 and 2) is a port-accessible aerosolization chamber communicating with a temperature-controlled mixing plenum and thence to a sampling plenum supplying a homogeneous aerosol to six sampling ports. Three six-jet Collison nebulizers (BGI Inc, Waltham, Mass.) deliver a mist of diameter $\sim 2 \mu\text{m}$ into the mixing plenum to create the bioaerosols. Air is drawn into a central vacuum line along a path from the sampling plenum through parallel lines of PVC tubing (Excelon® RNT, US Plastics, Lima, Ohio). Each path runs through a test article and thence through one AGI-40 or two AGI-500 all-glass impingers (Chemglass, Vineland, N.J.) partially filled with a liquid collection medium. The volume of air passing in each path is controlled

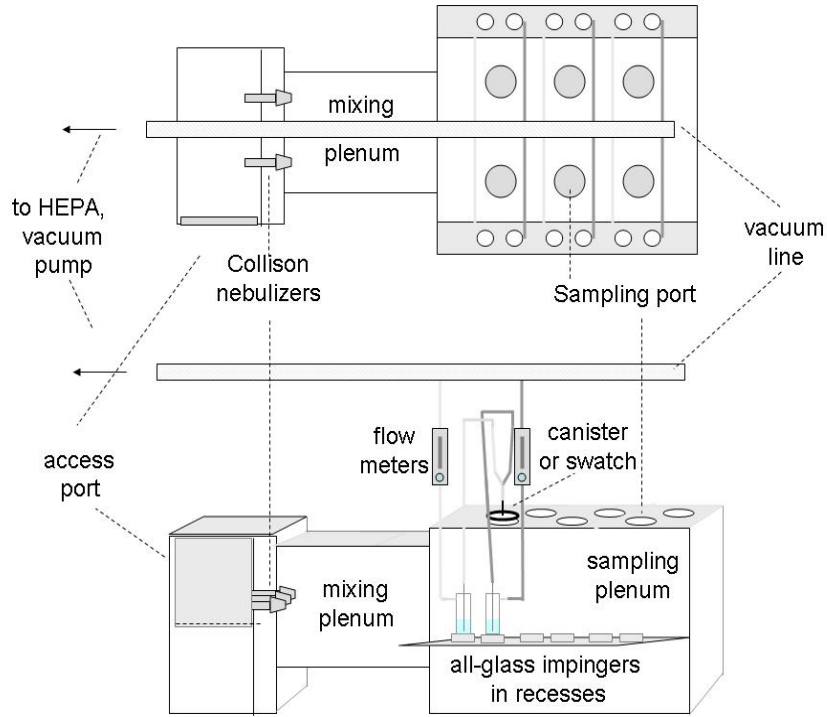


Figure 1. Top and Side View of BioAerosol Test System (BATS) Components.



Figure 2. AFRL Bio-Aerosol Test System (BATS).

by a mechanical flow meter (Blue–White 400, Huntington Beach, California, or PMR1-101346, Cole–Parmer, Vernon Hills, Illinois). At the end of the sampling path, the air exhausts through a conventional high-efficiency particle-arresting (HEPA) filter and the vacuum pump that drives the air movement. Each sampling port is able to accommodate test articles as large as 6 inches (15 cm) in diameter.

2.2 Procedure: Commercial off-the-shelf (COTS) masks were purchased. Iodinated masks and 47-cm² discs cut from an iodinated mask were provided by the manufacturer.¹ Intact protective soft masks were removed from sealed packages and, after the strings were clipped off, sealed to an empty canister with a bead of hot melt glue. The integrity of this seal was not tested. Each canister bearing a mask was sealed to the underside of a Plexiglas® plate (Figure 3), which was then seated on a rubber gasket and bolted into a sampling port. This configuration placed the external face of the mask in contact with the aerosol and caused air from the sampling plenum to flow in through the face. For the swatch tests, 47-cm² discs of filter medium cut from a treated mask were seated in an adaptor and sealed by compression into an O-ring. The adaptor was then sealed to a similar plate, which mounted into the sampling plenum. All sets of measurements included a positive control, a port which was sampled with no obstruction in the airway. The number of plaque-forming units (PFUs) measured through this port was reported as the challenge level.

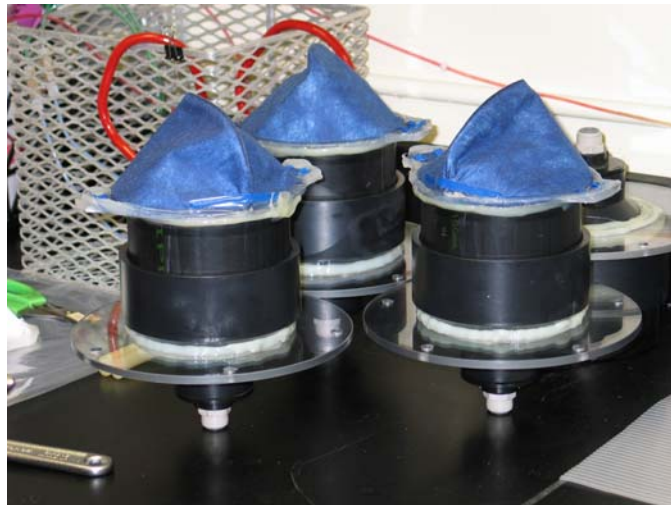


Figure 3. Masks Mounted to Adapter and Plate

In a typical mask run each of several (two to four) adapters fitted with mounted masks and one positive control were plumbed to the BATS and its effluent drawn through two AGI-500 impingers connected in parallel. The AGI-500s, which contained 100 mL each of sterile 1X phosphate buffer saline (PBS), were plumbed through a flow regulator and into a vacuum manifold. The vacuum reaching each channel was calibrated to maintain a total flow through each port of 85 LPM.

MS2 coli phage (ATCC 15597-B1) stock was diluted to $\sim 2 \times 10^8$ PFU/mL in sterile water and delivered to three six-jet Collison nebulizers. Compressed air (20 psi) was fed into the nebulizers to deliver a uniform microorganism–air aerosol that was drawn into the plenum box of the BATS and thence through the test articles and positive control. The total challenge time for each test article was 6 hours; after each hour of challenge the impingers were collected and replaced with a fresh set.

A standard plaque assay was used to determine phage concentrations of the samples and the positive control. For the test samples, three undiluted 1-mL samples from each impinger were used in the plaque assay. The positive control was serially diluted 1:10 out

to 10⁻⁵ and 1-mL aliquots of the 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions were used in the plaque assay as follows: One mL of the test solution was mixed with 1 mL of *Escherichia coli* (ATCC 15597) culture (grown to mid log-phase) and 9 mL of MS2 medium (1% tryptone, 0.1% yeast extract, 0.8% sodium chloride, and 0.1% dextrose, 1% agar), held at 55°C. The solution was mixed three times then poured into sterile Petri dishes. The Petri dishes were incubated overnight at 37°C and plaques were counted the following day. Total PFU counts were determined by averaging the PFUs determined on the triplicate sample plates, and then multiplying by the dilution factor and by the impinger volume. Percent reduction was calculated by difference, using the following formula:

$$\% \text{ Reduction} = \frac{100 (\text{Positive control PFU} - \text{Sample PFU})}{\text{Positive control PFU}}$$

To measure percent reduction of the challenge by 47-cm² circular swatches the flow through each port was adjusted to 5.4 Lpm and plumbed through a single AGI-40 impinger containing 30 mL of PBS. The remainder of the procedure was as described for the full masks.

3. Results

For all determinations except the second hour of swatch testing, the positive control (challenge) for each hour was calculated to be >10⁷ PFU. That control measurement was lost because of a handling error, and no % reduction values are reported for that set of samples.

3.1 COTS P-95 Masks: Under the conditions of testing, the two masks tested from supplier A provided an average reduction in MS2 coli phage penetration of 98.18%, passing almost 2/3 less than 5% of the challenge. (Table 1) The range of attenuation

Table 1. % Removal of MS2 Coli Phage by Two COTS P-95 Respirators (Ref. 1)

Hours	Challenge	C-A1 Count % Reduction	C-A2 Count % Reduction	C-B1 Count % Reduction	C-B2 Count % Reduction
1	1.02E+08	1.02E+06 99.00	1.94E+06 98.09	4.23E+05 99.58	1.80E+05 99.82
2	9.63E+07	1.29E+06 98.66	1.46E+06 98.49	1.98E+05 99.79	2.61E+05 99.73
3	9.36E+07	2.88E+06 96.92	1.55E+06 98.35	4.41E+05 99.53	3.15E+05 99.66
4	5.04E+07	1.59E+06 96.84	1.04E+06 97.95	6.03E+05 98.80	3.69E+05 99.27
5	8.91E+07	2.66E+06 97.02	5.40E+05 99.39	6.84E+05 99.23	2.52E+05 99.72
6	6.21E+07	1.20E+06 98.07	8.28E+05 98.67	4.86E+05 99.22	3.24E+05 99.48
Total	4.93E+08	1.06E+07 97.84	7.35E+06 98.51	2.84E+06 99.43	1.70E+06 99.66

varied from 97.84% to 98.51%. Under the conditions of testing, only one of 12 measurements of the two samples from supplier B showed reduction of less than 99%, revealing at least 80% less penetration than expected. The two masks tested from supplier B provided an average reduction in MS2 coli phage penetration of 99.55%. The range of attenuation varied from 99.43% to 99.66%.

3.2 Iodinated P-95 Masks: A total of seven samples were tested, three 6 December 2005 and four a week later (Table 2). The data from five form a reasonably tight cluster above 99.99%, and values for the first and second samples mounted are conspicuously

Table 2. % Removal of MS2 Coli Phage by Iodinated P-95 Respirators (Ref. 1)

Hours	Challenge	T-1 count % Reduction	T-2 count % Reduction	T-3 count % Reduction		
1	4.32E+07	7.23E+03 99.9833	3.92E+04 99.9094	2.70E+02 99.9994		
2	2.25E+07	1.79E+04 99.9203	7.54E+04 99.6649	9.00E+01 99.9994		
3	2.61E+07	6.60E+03 99.9747	1.99E+04 99.9239	9.00E+01 99.9997		
4	7.92E+07	7.08E+03 99.9911	1.64E+04 99.9792	1.80E+02 99.9998		
5	1.04E+08	7.65E+03 99.9926	6.39E+04 99.9383	3.00E+02 99.9997		
6	1.29E+08	1.31E+04 99.9898	5.31E+04 99.9587	4.80E+02 99.9996		
Total	4.03E+08	5.96E+04 99.9852	2.68E+05 99.9336**	1.41E+03 99.9997		
Hours	Challenge	T-4 count % Reduction	T-5 count % Reduction	T-6 count % Reduction	T-7 count % Reduction	
1	6.66E+07	3.84E+03 99.9942	3.90E+02 99.9994	3.42E+03 99.9949	5.22E+03 99.9922	
2	5.58E+07	2.25E+03 99.9960	1.50E+02 99.9997	1.92E+03 99.9966	5.91E+03 99.9894	
3	7.29E+07	3.33E+03 99.9954	3.00E+02 99.9996	4.86E+03 99.9933	3.30E+03 99.9955	
4	6.30E+07	3.21E+03 99.9949	5.10E+02 99.9992	2.91E+03 99.9954	4.17E+03 99.9934	
5	7.56E+07	3.27E+03 99.9957	3.30E+02 99.9996	4.20E+03 99.9944	4.41E+03 99.9942	
6	1.04E+08	4.50E+03 99.9957	2.10E+02 99.9998	4.26E+03 99.9959	3.09E+03 99.9970	
Total	4.37E+08	2.04E+04 99.9953	1.89E+03 99.9996	2.16E+04 99.9951	2.61E+04 99.9940	

** Sample T-2 was rejected at 99% confidence level as an outlier.

lower. Application of a Q -test to the suspected extreme outlier ($V_{out} = 99.9336\%$) reveals that the ratio of the separation from its next neighbor ($V_{adj} = 99.9852\%$) to its separation from the highest value recorded ($V_{best} = 99.9997\%$) is

$$Q = |V_{adj} - V_{out}|/|V_{best} - V_{out}| = 0.0516/0.0658 = 0.784.$$

As $Q \geq 0.680$ is the criterion² for rejection at 99% confidence level of one member from a set of seven values, we can conservatively exclude sample T-2 in Table 2 as an outlier. Because suspect results were seen for only the first two samples prepared, it is tempting to speculate that the fault lay in the hot melt glue seal, because the technique of creating the seals would be expected to improve with practice.

Under the conditions of testing, the six iodinated masks included as valid samples provided an average reduction in MS2 coli phage penetration of 99.9948%. The range of attenuation varied from 99.9985% to 99.9997%.

3.3 Swatch Tests: Under the conditions tested, two discs cut from the iodinated medium of another mask allowed zero to a few PFUs to penetrate during each sampling interval. Measured percent removal for this sample set (Table 3) averaged 99.9999% and ranged from 99.9998% to no detectable penetration during sampling intervals. Total percent removal of 99.9999% was the same for both samples within the limits of this method to discriminate.

Table 3. Measured % Removal of MS2 Coli Phage by Iodinated P-95 Medium

Hours	Challenge	S-1 count	% Reduction	S-2 count	% Reduction
1	7.28E+07	6.67E+01	99.9999	5.33E+01	99.9999
2	-----*	1.33E+01	-----*	2.67E+01	-----*
3	3.20E+07	1.33E+01	>99.9999	4.00E+01	99.9999
4	2.52E+07	4.00E+01	99.9998	< 1.3E+01	>99.9999
5	1.64E+08	1.33E+01	>99.9999	6.67E+01	>99.9999
6	2.44E+07	5.33E+01	99.9998	5.33E+01	99.9998
Total	3.18E+08	2.00E+02	99.9999	2.40E+02	99.9999

* During hour 2 the challenge sample was lost, so % Reduction was not calculated

4.0 Discussion:

From data in Table 1 both commercial masks can be seen to exclude more than the rated 95% of the bioaerosol challenge. Absent a classification of the particle sizes we cannot speculate on the significance of this observation. The purpose of gathering these data was to define a baseline against which to measure any change in the rate of penetration of viable viral particles (PFUs) at the ambient (but unmeasured) state of aggregation

produced in the BATS. The ratio of PFUs passing the test path to PFUs passing the positive control path was the criterion selected to test for enhancement of reduction in antimicrobial penetration caused by the iodinated coating. These results are consistent with data recently reported³ for N-95 respirators, by which removal of MS2 coli phage was $95 \pm 1\%$; lower performance by surgical masks was cited³ by the authors as a matter of concern for the safety of medical personnel.

Average total % penetration of the six iodinated specimens retained in Table 2 was 0.0052% (100.0000-99.9948). COTS item B experienced average total penetration of 0.45% (100.00-99.55), approximately two orders of magnitude higher. This increment is consistent with earlier observations during similar evaluations of COTS and similarly iodinated filter canisters, and the increased activity can be attributed to the reactive chemistry introduced by the halogenated polymer coating.

Consistent measurements of six 9's reduction by ideally sealed discs of the iodinated medium point up the inevitable slight compromise of integrity associated with assembling a protective item with joints connecting dissimilar materials. However, the limiting factor in realized protection is the seal between the wearer's face and the edges of the mask, which topic was not addressed in this project.

5. Conclusions:

1. The two COTS masks tested appear to be conservatively rated at P-95.
2. Under test conditions, the iodinated polymer incorporated in the test system decreased the rate of penetration of viable viral particles by a factor of approximately 10^2 (also expressed as two logs, two decades, two orders of magnitude or two 9's).
3. The iodinated polymer may accurately be classified as a reactive material, as understood in the context of protective equipment and gear.

References:

1. Sources are identified in a restricted attachment. Government agencies and their designates may request copies from AFRL/MLQO, 139 Barnes Drive, Suite 2, Tyndall AFB, FL 32403
2. D.B. Rorabacher, "Statistical Treatment for Rejection of Deviant Values: Critical Values of Dixon's "Q" Parameter and Related Subrange Ratios at the 95% Confidence Level," *Analytical Chemistry*, **1991**, *63*, 139-146.
3. A. Balaszy, M. Toivola, A. Adhikari, S.K. Sivasubramani, T. Reponen and S.A. Grinschpun, "Do N-95 respirators provide 95% protection level against airborne viruses, and how adequate are surgical masks?" *American Journal of Infection Control*, **2006**, *34*[2], 51-57.