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Virucidal Efficacy of Chlorine Dioxide Interventions on MS2 Phage Bioaerosol in a Laboratory Chamber

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Abstract

The ongoing SARS-CoV-2 outbreak has rapidly increased the desire to manage bioaerosol exposures in indoor settings. Studies using chlorine dioxide gas (ClO_2) at low concentrations have shown this intervention to be an effective mitigation strategy against viral, bacterial, and fungal elements in ambient air. There is an array of available products for generating ClO_2 gas however most involve the use of expensive or sophisticated technology that makes their applicability limited to specialized consumers. The purpose of this study was to determine the virucidal efficacy of three pragmatic and affordable, ClO_2 generating products using an aerosolized MS2 surrogate in a sealed chamber room under five different scenarios. The products tested included: Ultrashock—a ClO_2 releasing pod (30 ppmv), Filter Media—a ClO_2 impregnated zeolite media made to fit into an air blower housing (<0.01 ppmv) and Flow Stick—a smaller ClO_2 impregnated media filled air reactor tube (<0.01 ppmv). Testing scenarios included product deployment post MS2 bioaerosol introduction (Ultrashock and Filter Media), during MS2 bioaerosol introduction (Filter Media and Flow Stick) and prior to MS2 bioaerosol introduction (Filter Media). MS2 surface samples were collected using sterile petri-dishes and MS2 and ClO_2 air samples were collected from sampling ports on the outer chamber wall at 0, 90 and 180 minutes. The Ultrashock and Filter Media with air flow in the rapid sweep scenario showed the greatest reduction in air MS2 (T_{180} = 99.992% and T_{180} = 99.996% respectively) compared to the control (T_{180} = 99.6%). When compared to the control results, the filter media with air flow engaged prior to the introduction of MS2 yielded reductions of 99.87% and 99.93% in air and on surfaces respectively at T_0, demonstrating the protective effect residual ClO_2 has against air and surface contamination. These product formats have potential uses as remedial

and preventative interventions against viral constituents in air and should undergo further evaluation to determine efficacy and human health risk.

Keywords

Virucidal Activity, Chlorine Dioxide, MS2 Phage, Bioaerosols

1. Introduction

The ongoing SARS-CoV-2 outbreak has increased the desire to manage bioaerosol exposures in indoor settings. In occupational settings, many businesses were able to successfully continue operations virtually after workers transitioned to work from home policies. However, businesses and services where large crowds once congregated closed entirely, reduced operations, or limited the number of patrons entering the premise until mask ordinances were in effect and vaccines rolled out. In response, there have been numerous recommendations and technologies developed to reduce indoor air bioaerosol concentrations as a protective measure for occupational and general population health. These range from increased air exchange and exhaust rates, adding additional filtration like HEPA filters and UV ionization in HVAC units, to adding chemical based treatments like ozonation and chlorine dioxide [1] [2].

Of the methods listed, chlorine dioxide gas (ClO_2) offers unique benefits in that it can reduce bioaerosols in the air while also providing a reduction in microbes on contact surfaces depending on the concentration used [3] [4]. Chlorine dioxide gas (ClO_2) is a free radical molecule with a powerful oxidizing capability while also maintaining the ability to quickly diffuse through an enclosed space ensuring contact with hard-to-reach areas and surfaces [3]. It has successfully been used as a method for decontamination at high concentrations but there is growing evidence that even at low concentrations it provides a reduction in bioaerosol constituents.

In 2008, Ogata and Shibata demonstrated the protective effects of ClO_2 at low concentrations against influenza A in mice. The team exposed the mice to a 0.032 ± 0.026 ppm time weighted mean of ClO_2 and introduced the virus to determine potential protective effects. The study concluded a low concentration ClO_2 intervention introduced concurrently with virus reduced mortality by 100% compared to controls and that introducing ClO_2 five minutes post virus introduction yielded a 90% reduction in mortality [5]. Current human occupational exposure limits for ClO_2 are set by NIOSH and OSHA at a time weighted average of 0.1 ppm (0.3 mg/m^3) with a STEL of 0.3 ppm (0.83 mg/m^3) [6]. Ogata and Shibata's work used ClO_2 concentrations almost ten-fold below the OSHA TWA. Furthermore, another study investigated 6-month continuous exposures to ClO_2 at 0.1 ppm using rats and concluded no harmful effects were observed based on a series of toxicology examinations [7]. In a 2016 follow-up study, Ogata and Shibata demonstrated significant reductions in airborne bioaerosols

using ClO_2 at 0.1, 0.02 and 0.01 ppm in a contact chamber and surgical suite. This research suggests there is a high potential for using low concentration ClO_2 as a method to protect humans from bioaerosol exposure without incurring additional risk from inhalation of the chemical disinfectant [8].

However, the number of studies where ClO_2 is applied at low concentrations in public spaces remains the least explored application. This creates an opportunity to explore applying this technology in real world or simulated laboratory settings where COVID negatively impacted normal business operations in industries such as restaurants and food service, retail, education, entertainment, airlines, and mass transit [9] [10] [11]. Studies to date have primarily used room volume to calculate the appropriate dose necessary to stay under the OSHA PEL when releasing ClO_2 vapor by fumigation [12]-[19]. Fumigation via vapor requires the users to perform complex calculations to dose the room with ClO_2 placing this application out of the comfort zone of many users. On the other hand, Akamatsu, Ogata and Shibata utilized custom ClO_2 generating equipment that required electricity and likely substantial capital investment in the technology that make these methods impractical or out of reach to many end users.

ICA Trinova, LLC has developed three products that attempt to address these barriers to use.

1) Ultrashock Fast Release Pod (Ultrashock)—a two-part media system that when activated rapidly releases a high concentration of ClO_2 gas targeted at 30 ppmv.

2) Filter Media—impregnated zeolite media, integrated into standard fan and blower housings designed for a slow linear release of low-level ClO_2 gas targeted at <0.1 ppmv.

3) Flow Stick—an air reactor tube designed to rotate air in a vortex pattern around the impregnated zeolite media while providing a slow, linear release of low-level ClO_2 gas targeted at <0.1 ppmv.

The purpose of this study is to understand the efficacy of these products in an indoor setting as preventative and corrective measures to reduce bioaerosols in air and on surfaces. In this study, three ICA Trinova product formats were tested in five protocols to evaluate applicable deployment scenarios and inform future study designs to assess efficacy and human health risk.

2. Methods

2.1. Chamber and Environmental Conditions

Experiments were conducted in a 742 cubic foot sealed chamber room constructed of plexiglass paneling and aluminum supports. The chamber contained a single-entry door sealed during all experiments and three self-closing sampling ports only opened briefly to retrieve petri-dishes placed on the inside shelves of the chamber. The shelves were placed approximately 3 feet above the floor and approximately four feet apart from each other as a manner to determine adequate dispersion of MS2 bioaerosol throughout the chamber. Air was recircu-

lated within the chamber at 200 SCFM using a small fan and temperature was maintained at 24˚C - 26˚C with humidity at 40% - 50% for all experiments performed.

2.2. MS2 Preparation, Sample Collection and Enumeration

Virucidal efficacy of tested products was determined using Escherichia coli bacteriophage MS2 (ATCC: 15597-B1) (MS2) as a conservative surrogate for human viruses. MS2 was diluted in sterile Phosphate Buffered Saline (PBS) and injected into the chamber using a Single Jet Atomizer 9302 (TSI Incorporated, USA) pressurized to 35.0 PSI. Surface deposition/reduction was quantified using sterile open to air petri-dishes located on the shelves and floor of the chamber. Three sterile petri-dishes were retrieved from each sampling port shelf at intervals specified under each protocol listed below. Sterile petri-dishes located on the floor were retrieved at the end of each protocol's designated post exposure time point. Following the retrieval of each open to air petri dish, 20 mL of PBS containing sodium thiosulfate (final conc. 0.01%) was added to each dish, covered, homogenized by gentle swirling for 5 minutes, and then poured into a sterile 50-mL centrifuge tube containing 0.1 mL of 10× TSB. A positive control consisting of directly inoculated petri plate and negative control consisting of an uninoculated exposed petri plate were processed similarly. Positive and negative controls were performed to provide quality control and reference data as per laboratory standard accredited ISO17025:2017 methodology.

Air sampling for MS2 was performed using a BioSampler liquid impinger (SKC Ltd.) containing 20 mL of sterile PBS with sodium thiosulfate (final concentration 0.01%) to sample 120 liters of the chamber air through an air sampling port located midway through the side of the chamber.

All collected samples were analyzed on the day of the study undiluted and at various dilutions in replicates of at least two. MS2 was analyzed and enumerated as Plaque Forming Units (PFU) as per EPA 1602.

2.3. Chlorine Dioxide Air Sampling

Chlorine dioxide samples were collected using an AirChek Sampler (SKC Inc.) connected to two Midget Impingers with fritted nozzle (SKC Inc.) containing 25 mL of 0.02% potassium iodide solution. Chamber air was sampled for 30 minutes at a flowrate of 1 LPM through an air sampling port located at the front side of the chamber. The potassium iodide solution was transferred into two separate sterile containers and analyzed for chlorine dioxide concentration using ion chromatography by ATS laboratories (Marietta, GA), titrations or derived based on media mass used and release coefficients.

2.4. Protocols

Six protocols were tested, one control and five tests using three different ICA TriNova, LLC products of which one, the filter media, was tested in three appli-

cation scenarios.

Control: A control protocol was established to validate sample methods previously described and to determine natural degradation of MS2 across time for comparison against product interventions. MS2 samples were retrieved after MS2 bioaerosol introduction at T_0 (control), T_{90} and T_{180} measurements respectively.

Ultrashock: Protocol testing the Ultrashock product followed the same workflow as the control. However, after the T_0 samples were collected following the MS2 aerosolization step, the two-part Ultra Shock media was mixed and added to the room on shelf A using the sampling port. Wetted ClO_2 litmus strips were placed on the shelves at T_{90} to confirm gas generation. At the conclusion of the protocol the room was opened and vented outside.

Filter media: The filter media was installed on an air scrubber and negative air machine (Global IndustrialTM) and used in three different scenarios. The first protocol tested the filter media product while running the air machine continuously for 19 hours with MS2 introduced at the 16th hour mark for 75 minutes. Samples were collected immediately after MS2 cessation, followed by samples 90 and 180 minutes later. The second filter media protocol tested the filter media under recirculated air from a small fan without the air machine running. The third protocol simulated sweeping a contaminated room where the filter media was deployed post exposure. In this scenario, the air machine was off during the 75-minute MS2 application and turned on with a switch after application.

Flow Stick: The Flow Stick device was installed on the outer wall of the chamber and connected to the room through an inlet. The device's small fan was engaged at the start of aerosol introduction into the chamber. Aerosol introduction was conducted for 75 minutes, and the Flow Stick device remained on for 180 minutes. Sampling followed the same collection timeline as the control protocol.

2.5. Analysis

Exploratory Data Analysis in Tableau software was performed to validate mixing of the room within each experiment and to compare results between products. All MS2 values were converted from PFU to log values using Equation (1) to confirm room the was well mixed.

$$\text{Log} = \text{Log}_{10}\left(\text{PFU}\right) \tag{1}$$

After confirming adequate air mixing was achieved by comparing floor and shelf MS2 results within each protocol, the mean of shelf A, B and C were generated within each time step sampled and used as the basis for determining and plotting percent reductions.

3. Results

3.1. Reduction of MS2 in the Air

Room mixing was determined to be adequate as demonstrated by the analysis

performed shown in **Appendix** and summarized MS2 air concentrations are shown in Table 1. All products tested and the control showed reductions across time. The Ultrashock and Filter Media under the rapid sweep scenario (T_{90} = 99.994%, T_{180} = 99.992% and T_{90} = 99.996%, T_{180} = 99.996% respectively) showed the greatest reduction over time compared to the control (T_{90} = 96.4% and T_{180} = 99.6%) shown in Figure 1. The Filter Media and Flow Stick device showed only

Table 1. MS2 sample results in air and surfaces for each protocol.

MS2 Air Results (Plaque Forming Units/Liter)*						
Time	Control	Ultrashock	Filter Media Running	Filter Media OFF	Filter Media Rapid Sweep	Flow Stick
0	5833	3850	7.5	8000	21,583	8000
90	208	0.2	0.1	153	0.1	153
180	21	0	0.1	46.4	0.1	46.4
MS2 Surface Results (Plaque Forming Units/Petri Dish)**						
Time	Control	Ultrashock	Filter Media Running	Filter Media OFF	Filter Media Rapid Sweep	Flow Stick
0	25033.3 ± 2640.7	3440 ± 1732.2	18.2 ± 9.1	20666.7 ± 5363.1	38400 ± 10905.5	20666.7 ± 5363.1
90	1427.7 ± 151.9	56.7 ± 23.1	9.1 ± 0	1139.3 ± 502.8	821.3 ± 232.3	1139.7 ± 503.2
180	881.7 ± 644.8	36 ± 4	9.1 ± 0	366.7 ± 125.3	185 ± 60.8	366.7 ± 125.3

*Single sample; **Mean and SD of three samples.

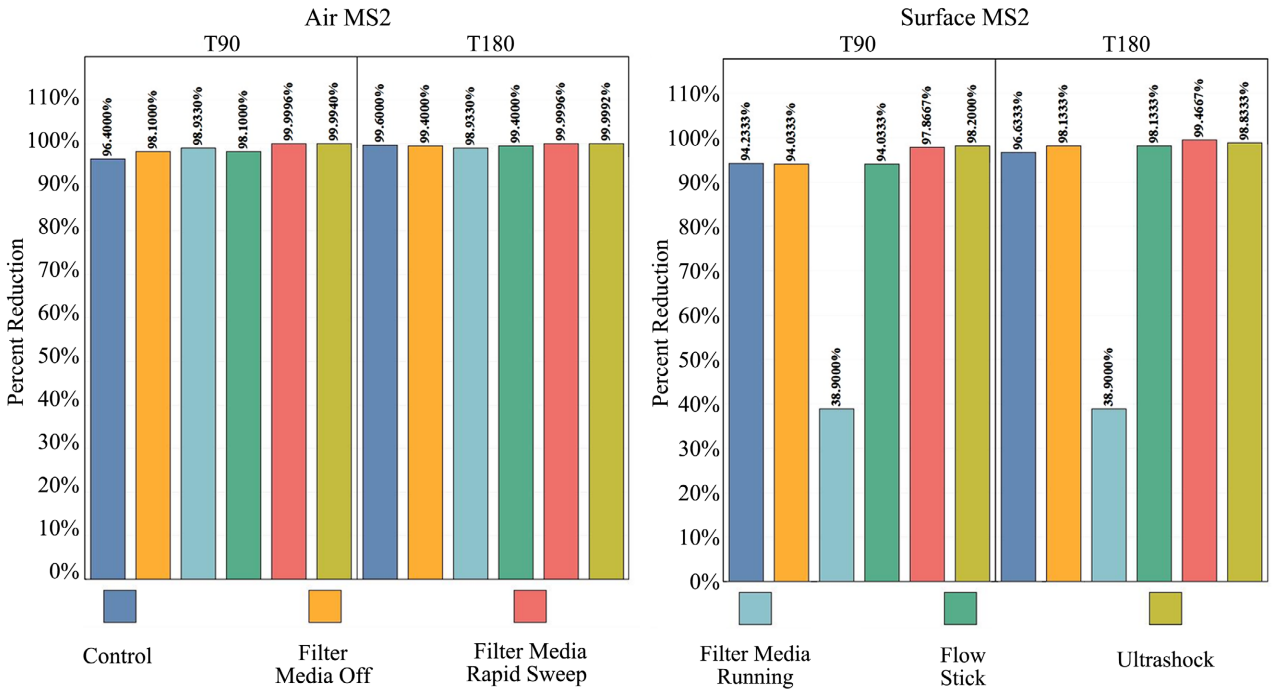

Figure 1. MS2 Percent reduction in surface and air samples at 90 and 180 minutes.

marginal reductions compared to the control. The Filter Media running scenario yielded the lowest MS2 air concentration (7.5 PFU/L air) at T_0, a 99.87% reduction when compared to the control at T_0 and percent reductions above 98% at T_{90} and T_{180} when compared to the protocol's T_0 result. These results indicate that MS2 introduced into the chamber during the 19-hour filter run were absorbed by the filter media or inhibited the residual ClO_2 in air immediately after application. Evidence of this is also seen in the petri dish results discussed in the next section.

3.2. Reduction of MS2 on the Surfaces

Ultrashock and Filter Media under the Rapid Sweep scenario (T_{90} = 98.2% and T_{180} = 98.833%; T_{90} = 97.8667% and T_{180} = 99.4667% respectively) showed the greatest reduction on surfaces compared to the control (T_{90} = 94.233% and T_{180} = 96.633%). Marginal surface reductions were seen among the Filter Media and Flow Stick device. These results suggest that airflow through the filter media is the most important factor in reducing bioaerosols in the air from depositing on surfaces. This is evident by the high levels of MS2 still found in the air during these test runs shown in Table 1. In contrast, the Filter Media running scenario produced the lowest surface results of all protocols tested at 18.2 ± 9.1 PFU/L consequently generating the lowest log reductions (T_{90} = 38.9% and T_{180} = 38.9%) as illustrated by Figure 1. When compared to the control the filter media running yielded reductions of 99.93% on surfaces respectively at T_0. These results suggest that removal of MS2 from the air, ultimately reduced the opportunity for surface deposition to occur.

3.3. Chlorine Dioxide Gas Concentrations

Table 2 provides the calculated and measured ClO_2 concentrations under each scenario. The Ultrashock pod provided the highest and longest sustained concentration at 25 PPMV. The Filter Media and Flow Stick products are designed for linear low-level release of ClO_2. The Filter Media Off sustained a 0.3 ppmv concentration at 180 minutes. During the Filter Media Running scenario, prior to the MS2 introduction, the room started with an unmeasured background ClO_2 level released from the media as air was circulated. While there's no way to

Table 2. Chlorine dioxide gas concentrations measured in each protocol.

Chlorine Dioxide Concentrations in Air (in Parts Per Million by Volume)						
Time	Control	Ultrashock*	Filter Media Running**	Filter Media OFF***	Filter Media Rapid Sweep	Flow Stick***
90	NA	25	0.31	-	0.05	-
180	NA	25	0.26	0.3	0.05	0.01

*Potassium Iodide Titration method; **Background ClO_2 present at T_0 before MS2 introduction; ***Estimated from media mass and release.

determine the initial ClO_2 demand generated before the MS2 introduction for this protocol, gas concentrations measured at 90 minutes show the room was close to the 0.3 ppmv targeted baseline. Under the Rapid Sweep scenario, the MS2 introduced rapidly consumed the ClO_2 released resulting in a concentration 0.05 ppmv across all time in the study. Finally, the Flow Stick residual gas concentration was inferred at 0.01 ppmv.

4. Discussion

This study was intended to determine the validity of the test protocol design and sampling devices used while inferring efficacy of the products tested under the scenarios applied. Chlorine dioxide gas interventions have been utilized in numerous applications at high concentrations. As more studies come to light showing the efficacy of ClO_2 at very low concentrations, questions regarding human health risks will continue to arise. The need for temporal monitoring of ClO_2 at lower concentration ranges is vital for inferring human health risks and determining room reentry rates at higher gas concentrations.

Additionally, the sampling method and limitation in quantification used in these tests favors using extremely high concentrations of dispersed MS2 that may not be representative of real-world and much lower concentrations of bioaerosols found in real-world settings. This is an important consideration when interpreting these results because the Flow Stick and Media Filter alone may have greater efficacy in lower concentration ranges where the residual ClO_2 released is enough to reduce a lighter pathogen load. The Filter Media Running protocol is evidence that reducing air burden leads to a reduction in surface deposition. However, test protocols need to be designed to determine this in laboratory settings to promote applicability to real-world settings and exposure scenarios. Future work should experiment with bioaerosols containing surrogate bacteria and fungi in addition to viral surrogates and at air concentrations commonly found in ambient and occupational settings for which this technology could protect public health.

5. Conclusion

The aim of this study was to determine reliable protocols for testing the efficacy of products using chlorine dioxide gas as an intervention to reduce pathogens in the air and on surfaces. The results of this study concluded that experimental design and methods used are reliable and repeatable for future scenario planning. Chlorine dioxide interventions can be effective as a standalone product and in combination with other filter technologies as a means of reducing bioaerosol threats. However, product application is highly dependent on the use scenario characteristics such as the space needing treatment, pathogen load, room characteristics and designated use, and the human population at risk. Future work should be done to further assess efficacy in real-world scenarios using refined laboratory settings that mimic external conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix. Determination of Room Mixing and MS2 Dispersal Using Petri-Dishes

MS2 log transformed concentrations of surface petri-dishes are shown in Table A1. Initial T_0 show consistent plate counts across dishes stored on shelves within each protocol tested and across different product protocols except for the Filter Running protocol. This result was anticipated because the T_0 samples for this protocol occurred while the ClO_2 intervention was active and the other five protocols T_0 samples were retrieved before introduction of the ClO_2 intervention. Similar magnitude reductions were observed for all shelves within the same monitoring time step in each protocol.

Table A1. Initial MS2 surface results for each protocol in log plaque forming units.

Location	Minutes	Control			Ultrashock			Filter Media Running			Filter Media Off			Filter Media Rapid Sweep			Flow Stick		
		(Log Plaque Forming Units)																	
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Shelf	0	4.36	4.45	4.39	3.39	3.74	3.38	0.96	1.26	1.44	4.32	4.41	4.18	4.69	4.59	4.44	4.32	4.41	4.18
	90	3.19	3.1	3.16	1.48	1.85	1.85	0.96	0.96	0.96	3.21	2.78	3.08	2.97	2.99	2.74	3.21	2.78	3.08
	180	2.62	3.21	2.78	1.51	1.6	1.56	0.96	0.96	0.96	2.71	2.44	2.5	2.16	2.41	2.19	2.71	2.44	2.5
Floor	180	2.86	2.59	2.63	1.2	1.3	1.86	0.96	0.96	0.96	2.04	2.6	2.04	2.42	1.86	2.28	2.04	2.6	2.04

In addition to the similar trends among shelf results, all final floor and shelf plate counts retrieved at 180 minutes showed similar results. These results indicated room air was properly mixed, and MS2 was evenly dispersed throughout the chamber. This justified summarizing the results further using the mean of shelf counts for each time step. Floor results were not included in further analysis.