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# **Cold Plasma Bipolar Ionization Device**

May 24 Results for Aerosol Treatment Technology Evaluation with Cold Plasma Bipolar Ionization Device

### Background

EPA researchers are building on an expansive body of biological wide-area remediation research by applying that knowledge to reduce the risk of exposure to SARS-CoV-2, the virus that causes COVID-19. This research will help state, tribal, local, and territorial governments—including public health agencies—and guide homeowners, business owners, and workplace managers to reduce the risk of exposure to SARS-CoV-2.

<u>Recent studies have indicated that exposure to aerosolized SARS-CoV-2 can facilitate the spread of COVID-19</u>. As an emphasis on aerosol transmission of COVID-19 and a desire to repopulate indoor environments (e.g., schools, businesses, mass transit) continues to grow, more attention is being focused on technologies that claim to reduce or eliminate virus transmission via aerosols. Although the number and types of aerosol treatment devices on the market continues to grow, few independent assessments exist to evaluate the efficacy of these technologies. Additionally, many of these technologies are considered pesticide devices, which are regulated by EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), but vendor's efficacy claims are not reviewed or verified by EPA as part of a registration process. Critical research is needed to better understand what benefits these aerosol treatment technologies may provide as part of a holistic strategy to reduce airborne transmission of COVID-19 and other diseases in enclosed spaces.

# Challenge

The risk of airborne disease transmission can be reduced in enclosed spaces by increasing <u>ventilation</u> and/or <u>air filtration</u>, physical distancing, and wearing well-fitting masks, all of which work to reduce the concentration of virus in the air we breathe. These strategies can be difficult to implement in certain settings, so there has been a growing interest in technologies that are intended for use in occupied spaces and can potentially reduce the amount of infectious virus in the air. There are many different types of these technologies designed to be operated either in-room or in-duct, including chemical products, ultraviolet (UV) light devices, ionization devices, photocatalytic devices, and mechanical air filtration. Different technologies are intended to operate at different scales – for example, <u>portable air cleaners are designed to filter air in a single room or area</u>, while filters or other technologies installed in HVAC systems operate throughout an entire home or <u>HVAC zone</u>.

The efficacy of many of these emerging technologies has not been well characterized against viruses and other airborne pathogens, as there are often few or no independent (non-commissioned) efficacy assessments that have been conducted. The testing that has been completed is often conducted at a small scale relative to the space in which the technology would be deployed, or other conditions during the testing are not representative of real-world settings (e.g., artificially high air exchange rates). Without standardized testing methods or protocols, comparing efficacy across technologies is difficult. The current limitations on data availability and test design prohibit comprehensive evaluation of how effective different types of technologies will be in reducing concentrations of airborne pathogens in real-world settings.

# **Research Objective**

The overall objective of this research is to evaluate the efficacy of different types of aerosol treatment technologies in reducing airborne virus concentrations using a large-scale test chamber and a standardized testing approach. Conducting this research at a sufficiently large scale with a recirculating HVAC system provides EPA and the public with an independent source of efficacy information that can be more reliably translated to real-world settings. This research also seeks to establish protocols for aerosol treatment product and device efficacy studies, which facilitates cross-study and cross-technology comparisons. The results from this study will inform evaluation of the added benefit that aerosol treatment

technologies may have as part of a strategy to reduce airborne disease transmission in various settings, such as in mass transit or schools. In addition, the testing design and results inform the potential development of standardized test protocols in evaluating different types of aerosol treatment technologies.

This study may identify products and devices that demonstrate efficacy in reducing airborne virus concentrations. The results of this research do not supplant data required for product registration, nor for adding additional claims to product labels or pesticide devices. Products and devices must be used in accordance with their label claims under FIFRA. EPA does not endorse the use of any products tested in this study.

### Disclaimer

EPA is conducting several studies to support the response to the COVID-19 pandemic, the results of which may be useful to the Nation. The summary here is intended to provide a simple representation of the results of on-going testing; therefore, only a brief description of the purpose of the study, methods, and interim results are provided. This study is being conducted in accordance with an approved Quality Assurance Project Plan. The interim results have been reviewed by internal EPA technical experts, quality assurance staff, and management. No interpretation of the interim results is provided. Once complete, the study and its results will be described in detail in a publication subjected to external, expert peer review. EPA does not endorse the purchase or sale of any commercial products or services.

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#### SEPA United States Environmental Protection

# **Results for Aerosol Treatment Technology Evaluation with Cold Plasma Bipolar Ionization Device**

# May 24, 2021 Report

# **Experiemental Approach**

A standard EPA, ASHRAE, or ASTM-approved method does not currently exist for evaluating efficacy of aerosol treatment products or devices against bioaerosols. Utilizing EPA's specialized Aerosol Test Facility in Research Triangle Park, NC and experience in conducting research with aerosolized biological agents, a standardized approach that can be applied across multiple technology types was developed to achieve the objectives of this project. An objective of the test conditions was to maintain a high enough concentration of viable aerosolized test virus in the air for 90 minutes in control conditions (without technologies active) to be able demonstrate a 3-log (99.9%) reduction in airborne virus concentration during test conditions (with technologies active) relative to the time-matched control tests. For these tests, MS2, a nonpathogenic bacteriophage that infects *Escherichia coli*, was used as the test virus.

A large test section of the aerosol wind tunnel in EPA's Aerosol Test Facility was sectioned off from the recirculating wind tunnel to serve as the test chamber utilized for this research. The 12 ft x 10 ft x 25 ft chamber provides a 3,000 ft<sup>3</sup> conditioned space for dissemination and sampling of bioaerosols and for evaluating different aerosol treatment strategies (Figure 1). The chamber is temperature and relative humidity (RH) controlled, and its air is high-efficiency particulate air (HEPA)-filtered prior to each test. A mock heating, ventilation, and air conditioning (HVAC) system was designed and constructed in the test chamber to simulate air circulation and exchange rates typical of a wide range of general application settings. An Omni-Aire 1000V (Omnitec, Mukilteo, WA) negative air machine (NAM), with the HEPA filter removed, serves to represent a cold air return, recirculating air through the HVAC system and test chamber. A rectangular section downstream of the return serves as the installation section for aerosol treatment technologies. Air passing by the installed technology then flows through the 8-inch main duct before passing through six evenly spaced 6-inch branches that distribute air back to the chamber through 10-inch round diffusers with butterfly dampers at the distribution points. The system was constructed from galvanized steel materials, and the NAM airflow rate is set to 350 cubic feet per minute (CFM), resulting in approximately 7 air changes per hour (ACH) in the chamber. No outside air is introduced during testing. Two metal fans (LASKO 2265 QM, West Chester, PA) placed in opposite corners of the chamber facilitate mixing, operating at 1448 ft/min. In between tests, the chamber is opened, and recirculated HEPA-filtered air is run through the chamber until negligible particle counts are present in the air in preparation for the next test.

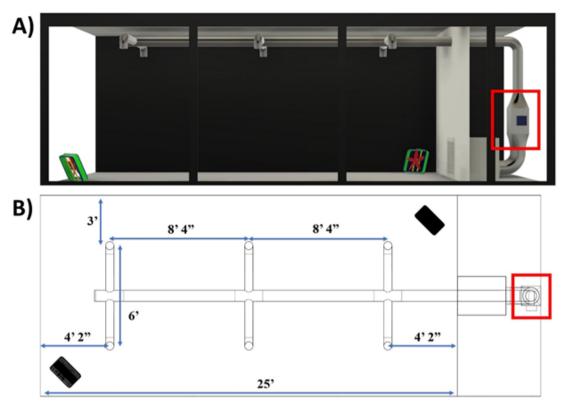


Figure 1. Test chamber schematics of A) side view and B) top-down view. The tested technology is installed in the rectangular section of the ductwork outlined in the red box.

The bacteriophage MS2 (ATCC 15597-B1), a non-enveloped virus that infects the host cell *Escherichia coli* (ATCC 15597), was used in this current study. Conducting research with aerosolized viruses at these large scales necessitates the use of safer, e.g., Biosafety Level (BSL)-1 potential surrogate viruses, versus using more pathogenic agents, such as SARS-CoV-2 (BSL-3). As a non-enveloped virus, MS2 is expected to be more resistant to chemical inactivation than enveloped viruses (e.g., SARS-CoV-2). MS2 stock is prepared using a top agar overlay technique, and samples are analyzed using a plaque assay with the *E. coli* host.

In this study, four 6-jet Collison Nebulizers (CH Technologies, Westwood, NJ) are used to aerosolize MS2 into the test chamber. In each nebulizer, a 10 mL mixture of 1:4 parts MS2 stock (MS2 in SM Buffer) to 0.22 µm filter-sterilized deionized water with 6 drops of Antifoam A (Sigma-Aldrich, St. Louis, MO) is nebulized over a period of 10 minutes prior to the first bioaerosol sampling period. Aerosolized MS2 is sampled using SKC BioSamplers (SKC Inc., Eighty-Four, PA) connected to air sampling pumps that draw air at a rate of 12.5 L/min. The sampling period for each aerosol sample is 10 minutes, resulting in a total volume of 125 L of air sampled during each sampling period. Bioaerosol samples are taken in duplicate from a 5 ft "breathing zone" height during each sampling period. The first aerosol sample in each test is taken immediately following the 10-minute aerosolization period (time = 0 sample). The count median diameter of aerosolized particles is 46 nm at the beginning of each test (time = 0 minutes), and it increases over the duration of the test to 100 nm at the end of 120 minutes, as measured by a Scanning Mobility Particle Sizer (Model 3080 Electrostatic Classifier/TSI 3010 Condensation Particle Counter, TSI Inc., Shoreview, MN).

Deposition of viable MS2 on surfaces and inactivation of MS2 on surfaces was also evaluated using uniform pieces of 2 cm x 4 cm stainless-steel material (coupons), which were prepared, cleaned, rinsed, and sterilized prior to testing. The deposition coupons were placed into the test chamber blank (i.e., clean, with no virus present prior to testing), and the inactivation coupons were inoculated with MS2 inoculum made in 5% Fetal Bovine Serum (FBS) with 1X phosphate-buffered saline (PBS) prior to being placed in the chamber. The two sets of coupons were co-located at five different locations on the chamber floor. The deposition coupons were placed inside the chamber to determine if the reduction of MS2 in the air was due to airborne inactivation or due to settling of viable virus particles.

# Cold Plasma Bipolar Ionization Device Results - May 24, 2021

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With the input of research stakeholders, a cold plasma bipolar ionization device (PuriFi Labs, Phoenix, AZ) that is sized to treat 2000-4000 ft<sup>2</sup> of living space was selected for evaluation. Bipolar ionization generates positively and negatively charged particles that react with airborne contaminants, including virus aerosols. The device was installed in-duct and operated continuously during tests with the technology active. The device remained installed, but not powered, during control tests. The device was operated for 30 minutes in the sealed chamber prior to the aerosolization of MS2 for tests conducted on 4/22/2021 and 4/27/2021 and for 90 minutes prior to tests conducted on 4/29/2021, 5/4/2021, and 5/13/2021. The 30-minute and 90-minute ion buildup times resulted in ion counts of approximately 1000-2000 ions/cm<sup>3</sup> and 2500-6000 ions/cm<sup>3</sup>, respectively, measured using three Air Ion Counter Model AIC2 (AlphaLab, Inc., Salt Lake City, UT) ion meters located in the test chamber at the height of the bioaerosol sampling ports (5'). All testing was conducted at  $22 \pm 2 \,^{\circ}$ C and a RH of 30-35%[1]. This RH was selected because virus viability is reduced at intermediate RH[2], and an objective of the test design is to maintain sufficiently high concentration of viable aerosolized MS2 after 90 minutes that will allow for demonstration of a 3-log reduction in tests with technologies active relative to control conditions throughout the duration of each test.

Figure 2 shows the concentration of MS2 from aerosol samples, normalized to the initial (time = 0 minutes) sampled concentration at the beginning of each test. Two control tests (without the cold plasma bipolar ionization device active) were run on 4/15/21 and 4/20/21 for a 90-minute duration with 10-minute bioaerosol sampling periods starting at time = 0, 15, 30, 60, and 90 minutes. Three corresponding 90-minute tests with the PuriFi device active were run on 4/22/21, 4/27/21, and 4/29/21. Tests with a duration of 120 minutes were run on 5/6/21 and 5/12/21 for the control tests with sampling periods starting at time = 0, 30, 60, 90, and 120 minutes, with the corresponding PuriFi tests run on 5/4/21 and 5/13/21.

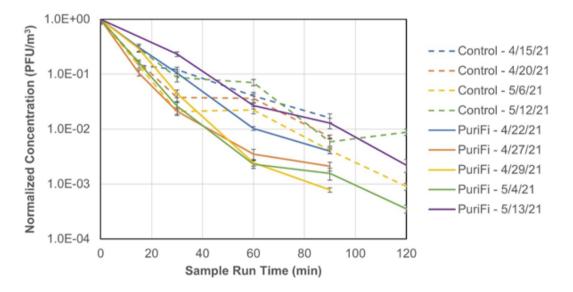


Figure 2. Normalized MS2 recoveries at each timepoint throughout control and PuriFi tests with the device active. Each timepoint represents recovery of MS2 from duplicate bioaerosol samples as determined by plaque assay, and the error bars represent pooled standard deviation for each sampling timepoint. Data from each sampling timepoint is normalized to the sample recovery at time = 0 for each respective test.

Figure 3 shows A) the concentration of MS2 recovered at each sampling timepoint during testing, averaged over each of set of the PuriFi and control tests, and the corresponding average percent reduction in  $\log_{10}$  recoveries at each sampling timepoint, ranging from -44% (time = 15 min) to 86% (time = 60 min). Figure 3B) shows the calculated  $\log_{10}$  reduction in MS2 recovery between the mean of the control and the PuriFi test samples at each sampling timepoint.

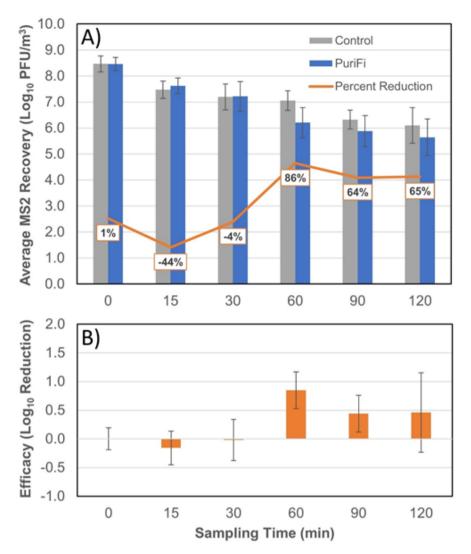


Figure 3. A) Concentration of MS2 at each sampling timepoint during the control and PuriFi test experiments, averaged over each respective test condition (PuriFi tests vs. controls), where error bars represent the standard deviation in averaged recoveries, and the average percent reduction in  $log_{10}$  recoveries from PuriFi tests vs. controls. B)  $Log_{10}$  reduction between the averaged recoveries of MS2 samples from each sampling period of the control tests relative to the PuriFi tests, where error bars represent pooled standard error from the test and control experiments.

Figure 4 shows the average concentration of MS2 recovered from the deposition coupons that were located on the floor throughout the chamber for the tests with the PuriFi device active and average recoveries from the control tests. The deposition coupons were placed inside the chamber to determine if the reduction of MS2 in the air was due to airborne inactivation or due to settling of viable virus particles. The average recovery of MS2 from the inoculated coupons from the PuriFi tests was  $7.0 \pm 0.1 \log_{10}$  PFU/coupon, and the average recovery from the control tests was  $6.9 \pm 0.1 \log_{10}$  PFU/coupon.

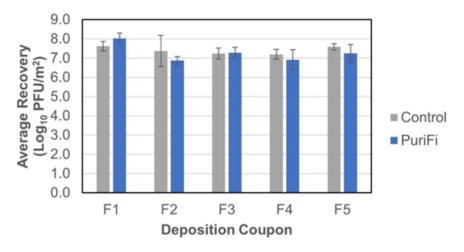


Figure 4. Average concentration of MS2 recovered from coupons initially inserted into the test chamber blank, averaged over the control and PuriFi test conditions at each location, representing the amount of viable MS2 deposited on each coupon during testing. Error bars represent standard deviation in the averaged recoveries.

[1] EPA recommends that indoor relative humidity be maintained between 30% and 50% (https://www.epa.gov/iaq-schools/moisture-control-part-indoor-air-quality-design-tools-schools).

[2] Lin, Kaisen, and Linsey C. Marr. 2020. "Humidity-Dependent Decay of Viruses, but Not Bacteria, in Aerosols and Droplets Follows Disinfection Kinetics." Environmental Science & Technology 54 (2):1024-32. doi: 10.1021/acs.est.9b04959.

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