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ActivePure Technology:

SCIENTIFIC DATA & PROOF

ACTIVEPURE® TECHNOLOGY – THE PATH TO SAFE INDOOR AIR

June 01, 2021

ABSTRACT

Airborne transmission of SARS-CoV-2 and other respiratory viruses through virus-containing aerosol particles and droplets has been established as a primary pathway for the spread of COVID-19 and similar infections.[1] *Suitable infection prevention measures are imperative, especially in high-risk environments, including where many people convene indoors, as in schools and hospitals, and where masks cannot be worn, as in restaurants and company breakrooms.*

We provide an overview of an advanced form of air purification technology called ActivePure, which is capable of safely suffusing an occupied indoor space with the same oxidative particles found to clean the atmosphere outdoors naturally.[2] *ActivePure is Advanced Photocatalysis technology, which has roots in NASA's development of Photocatalytic Oxidation (PCO); however, multi-generational improvements have materially enhanced its efficacy from its PCO origins, and its safety was validated by U.S. Food & Drug Administration (FDA) clearance of the ActivePure Medical Guardian® as a Class II Medical Device in June 2020. We cover the safety and efficacy of this technology and compare it to other air-cleaning methodologies.*

1. INTRODUCTION

Safe indoor air and surfaces are more important than ever, affecting viral transmission, student[3] and employee productivity[4], and overall health.[5] Currently, however, many indoor spaces rely on inefficient and traditional ventilation and filtration technologies, when newer technology is available that can inactivate viruses safely. By the time pathogen-containing air is passed through a filter or by an ultraviolet (UV) light, it has already spread through a room and potentially infected people.

ActivePure solves this problem by taking the fight to the source. It goes directly to the pathogens themselves and neutralizes them. The process continuously energizes the air in the room, producing the same cleaning particles naturally found in the atmosphere[6] but absent from indoor spaces.

From the moment they are emitted, particles generated in the ActivePure process begin inactivating viruses, bacteria, and mold. They clean both the air itself and any surface that the air touches, producing a real-time reduction in disease-causing airborne pathogens.

2. TECHNOLOGY OVERVIEW

ActivePure recreates the organic process that continuously occurs in outdoor air to cleanse the atmosphere. Each breath of outdoor air contains billions of oxidative particles, including hydroxyl radicals[2] and hydrogen peroxide.[7] These oxidizers are the atmosphere's primary self-cleaning mechanism. They continuously down the megatons of pollutants that humans put into the air each year.[2] By contrast, without sunlight indoors, these cleansing particles are normally missing from interior environments. ActivePure technology restores the naturally occurring oxidizer particles to levels that will break down pathogens.

To recreate this process indoors, the ActivePure system uses energetic photons from a UV-C (high-energy ultraviolet radiation) bulb to excite and release electrons from a proprietary photocatalyst, made from a base of titanium dioxide -- a naturally occurring semiconductor. Electrons are the negatively charged particles inside atoms, and once they are released, they interact with H_2O and O_2 in the ambient air, causing the same natural oxidizers to form that are produced in outdoor air when ultraviolet sunlight rays interact with the atmosphere: hydrogen peroxide (H_2O_2), hydroxide

(OH^-), hydroxyl radicals (•OH) and superoxide ($•O_2^-$).[7–9]

These oxidizers spread throughout the space at tremendous speed. When they encounter an organic molecule, they strip away an electron, beginning a series of reactions that quickly render a pathogen inert and no longer infectious.[10] After the oxidizer has altered the cell wall or viral envelope[11], the organism continues to decompose into its most basic, organic (and completely benign) components – usually CO_2 and H_2O.

3. SAFETY

ActivePure was developed with safety as its primary focus, using nature as a guide. The process is completely safe for humans. The compounds it generates are all well below[12] the safety thresholds required by OSHA and other agencies for continuous exposure.[13] Independent laboratory tests have shown that ActivePure does not increase levels of ozone[14] or Volatile Organic Compounds (VOCs)[15] in the ambient air; instead, ActivePure actually reduces ozone and VOCs. ActivePure products meet the standards of every state in which they are sold.

ActivePure devices have been shown to be safe in laboratory studies, FDA review, and worldwide installations[16,17]. The technology is used in health care settings, where it has been deployed to decrease hospital-acquired infections. ActivePure's Medical Guardian device has been cleared by the FDA and is the subject of a double-blind trial at the Cleveland Clinic to measure its impact on reducing surgical-site infections.

ActivePure's Advanced Photocatalysis technology is qualitatively different from PCO technology, which uses older, less effective photocatalyst formulas. These conventional formulas have often been shown to produce toxic byproducts such as formaldehyde[18] and ozone, but ActivePure's proprietary photocatalyst creates a more efficient reaction, using techniques that have been shown to avoid creating any of these harmful intermediaries– serving to eliminate them instead.[19] Some people confuse ActivePure's Advanced Photocatalysis technology, which is unique, with PCO-based technology.

4. EFFICACY

ActivePure has demonstrated efficacy through testing in FDA-compliant, independent laboratories where it was proven to inactivate rapidly a wide array of viruses, bacteria, and mold.

Testing by Biosafety Level 3 and 4 laboratories at the University of Texas Medical Branch in Galveston, which does extensive research for the CDC and the U.S. military, found that ActivePure destroyed at least 99.96% of a high concentration of the SARS-CoV-2 virus (the cause of COVID-19) in three minutes.* According to the report: "Since no virus was detected using the experimental device, the true percent reduction was likely greater than 99.99% in every case."[20] Additional testing has shown a greater than 99.98% reduction on surfaces within seven hours.[21]

The ActivePure device that received FDA clearance in June 2020 was found to safely reduce the concentration of the following six airborne pathogens by at least 99.9% in 30 minutes:† [22]

- MS2 bacteriophage RNA virus, which is non-enveloped and thus harder to inactivate than SARS-CoV-2 (the virus that causes COVID-19), whose envelope is more vulnerable.[23]
- PHI-X174 bacteriophage DNA virus, a surrogate for Hepatitis C and HIV
- Staphylococcus epidermis gram-positive bacteria, surrogate for Methicillin-resistant Staphylococcus aureus (MRSA), a major cause of hospital-acquired infections.
- Erwinia heribcola gram-negative bacteria, surrogate for black plague-causing bacteria.
- Aspergillus niger fungal mold, the cause of black mold disease.
- Bacillus globigii bacterial endospore, which is generally considered among the most difficult pathogens to destroy,[23] but ActivePure technology reduced

* These results have not been cleared by the FDA but have been submitted to the FDA for clearance.

† These results have been cleared by the FDA in the ActivePure Medical Guardian as a Class II Medical Device.

concentrations by 99.98% in 30 minutes. According to Aerosol Research and Engineering Laboratories, the independent testing agency, endospores like Bacillus globigii "are an extremely resilient structure…that are resistant to UV radiation, desiccation, chemical disinfectants and severe temperatures." These spores "are routinely used as a surrogate for weaponized anthrax."[24]

ActivePure technology has also been successfully tested against pollen particles, lowering allergic reactions,[25] as well as yeasts and molds.[26]

A list of pathogens against which ActivePure has been tested with success includes:

- SARS-CoV-2 – RNA Virus (Cause of COVID-19
- H1N1 Influenza (Swine Flu)
- H5N8 Influenza (Bird Flu)
- Murine Norovirus – RNA Virus
- PhiX-174 - DNA Virus
- MS2 Bacteriophage - RNA Virus
- MRSA (methicillin-resistant Staphylococcus aureus)
- Methicillin-resistant Staphylococcus epidermis
- Staphylococcus epidermidis (gram +)
- E. Coli (gram -)
- Salmonella enterica (bacterium)
- Legionella pneumophila (bacterium)
- Clostridium difficile (endospore)
- Bacillus globigii (C. difficile & anthrax surrogate)
- Erwinia herbicola (gram -)
- Listeria monocytogenes (gram +)
- Candida auris (fungus)
- Botrytis cinerea (fungus)
- Sclerotinia sclerotiorum (fungus)
- Aspergillus versicolor (fungus)
- Aspergillus niger endospores (toxic black mold surrogate)

4.1 Real-World Results

ActivePure's high inactivation rates in controlled settings have been replicated in real-world studies, with measurements and analysis conducted through independent, FDA-compliant laboratories. In occupied buildings such as hospitals, schools, retail spaces, and assisted-living facilities, ActivePure has demonstrated its ability to reduce the number of pathogens materially, both in the air and on surfaces. Some typical examples include:

- ActivePure Units installed in an assisted-living facility reduced bacteria, molds (fungi), and particulate matter by 73%-93%, an effect equivalent to increasing the ventilation in the room by three to four-fold.[27]
- Portable units installed in multiple hospital ICUs reduced bacteria, fungi, MRSA, and Staphylococcus Aureus by 73%-100%.[28]
- Units installed in another major hospital's operating room reduced airborne particulates by 90%, surface bacteria by 83%, and surface MRSA by 95%.[29]

5. SAFETY AND REGULATORY STATUS

5.1 FDA

On June 17, 2020, the Aerus Medical Guardian with ActivePure technology received FDA Class II Medical Device Clearance with 501(k) Certificate Number K201220. This multi-year testing and clearance process included a full FDA review of the safety and efficacy of the device in occupied rooms. The device demonstrated a 99.99% to 99.9999% (4-6 log) rapid reduction against all tested pathogens, including gram positive and negative bacteria, fungal and bacterial spores, and DNA and RNA viruses (for which a 99.997% reduction was achieved). The technology passed all required safety testing, including being shown not to produce harmful levels of ozone or volatile organic compounds (VOCs). The same technology used in the Aerus Medical Guardian is used in all ActivePure devices.

Additionally, ActivePure technology has been shown by the University of Texas BSL3/4 medical laboratory, cited above, to meet the FDA's guidelines[30] for effectiveness at eliminating airborne SARS-CoV-2. (ActivePure is filing for clearance by the FDA in this case, but clearance has not yet been granted.) ActivePure also demonstrated a greater than 99.99% inactivation rate against representative surrogate viruses that are harder to kill than SARS-CoV-2 and is the only technology of its kind to have

been tested against SARS-CoV-2 itself, achieving elimination from the air below detectable limits of 99.96% in under 3 minutes (inclusive of aerosols and droplets), and a 99.98% reduction from steel surfaces.

5.1 Other Regulatory Bodies

- Manufactured in an EPA-registered facility (#95839-CHN-001) and in accordance with EPA guidance regarding air cleaning devices.[31]
- Tested for compliance by a facility designated a Nationally Recognized Testing Laboratory (NRTL) by the U.S. Occupational Safety and Health Administration (OSHA), approved to perform testing for ANSI/UL Standard 867 and UL507, UL1598, UL1995 and applicable Canadian standards.
- Shown in tests at an FDA-compliant laboratory to emit gases far below all OSHA 8-hour exposure limits.[13]
- CARB (California Air Resources Board)-certified on all products sold in California. All products sold in California meet the state's requirements for ozone emissions.
- ActivePure complies with all Federal and state environmental and safety standards.

6. COMPARISONS WITH OTHER TECHNOLOGIES

6.1 Ventilation

Ventilation is meant to exchange outdoor air for indoor, in part to reduce the risk of disease transmission, but it is rarely possible to achieve enough ventilation activity to ensure proper safety.[32] Increased ventilation also comes with a direct tradeoff: energy expenditure rises as the outdoor air must be pumped, heated or cooled, and humidified. In many climates, natural ventilation through opening windows is impossible for much of the year. In most buildings, even when HVAC systems are set to their maximum fresh-air settings, most buildings will still have a surprisingly high level of risk, a level that is often underestimated because of imprecise terminology.

Both filtration (see 6.2) and ventilation systems are measured in Air Changes per Hour (ACH), the number of times per hour that a volume of fresh air equal to the room volume is brought in.[33]

The American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) recommends that a typical home be designed to receive 0.35 ACH.[34] Schools are required to have 3 ACH, but even including Energy Star and LEED buildings average closer to 2.[35] Office buildings are supposed to have 6-8 ACH, but a 2008 EPA analysis found that average levels are less than 2.[36]

These low ACH numbers would not be overly concerning if an "Air Change" meant that the room's air was entirely pristine afterward. That, however, is not what happens.

Passive systems, such as ventilation and filtration, are good at lowering initial contaminant levels, but not at eliminating them completely. They suffer from diminishing returns. Here is why:

As the fresh air comes in, it immediately begins to mix with contaminated air, diluting the entire mixture, including what leaves the room or passes through a filter. As the contaminant gets diluted, it requires increasingly large amounts of air to pass through the filter or out the window in order to remove the same amount as before. According to the CDC's calculations, a room with 4 ACH will still take more than 3 hours to eliminate >99% of an initial concentration of contaminants,[37] and that's without a constant source, such as an infectious person, in the room.

6.2 Filtration (MERV, HEPA)

Air filters such as HEPA (high-efficiency particulate air) filters, a technology developed more than 70 years ago, are designed to trap airborne particulates, either when the air is first pumped in from outside or recirculated between or within rooms. Pathogens that are continuously released in occupied rooms have plenty of time to spread and possibly infect people before finally being pulled through either a portable or duct-based filter.

This danger was clearly demonstrated in a study where filters in a hospital had little impact on protecting construction personnel from airborne Aspergillus.[38] The CDC states that for a space treated with a portable HEPA filter that provides the

recommended 2 ACH, it would take over *2* hours to eliminate 99% of an initial concentration of airborne virus. This number becomes 6-10 hours when the air in the room is relatively stagnant, as in most classrooms, offices, and homes.[37]

Other limitations of filtration include:

- Filters have no effect on pathogens on surfaces.
- MERV filters (minimum efficiency reported value) rated 13 and below trap fewer than half of virus-sized particles.[39]
- The greater the filtration efficiency, the more stress is placed on the HVAC system. As a result, these systems need to be upgraded in size and power, at a high cost and producing damage to the environment.

6.3 Ionization

Ionizers use electricity to produce ions that impart an electrical charge to nearby particles. Over 90% of these ions cause particles to stick together and fall to the ground or other exposed surfaces, where the pathogens continue to live, rather than neutralizing them.[40–42] Only superoxide ions work to puncture the outer membranes of viruses like SARS-CoV-2, as well as fungi and bacteria, and inactivate them. But only a very small proportion of the ions produced by ionizers are superoxides.[41,43] Advanced Photocatalysis, by contrast to ionizers, uses a precise process to produce only the highly reactive compounds that will rapidly neutralize pathogens.[44] These are the same compounds used in the natural self-cleaning processes of the atmosphere.[2]

The large number of charged particles produced by ionizers has been linked to excessive sympathetic nervous system activation in humans (the "fight or flight" response) [45], a factor which has also been shown to play a role in "long-COVID" sufferers.[46]

6.4 Ultraviolet Germicidal Irradiation (UVGI)

A dose of UV-C strong enough to neutralize SARS-CoV-2 viruses was shown to range from 3.7 mJ/cm^2 - 16.9 mJ/cm^2.[47] For a standard bulb with 245 μW/cm2, it would take more than 14 seconds of continuous and focused exposure to achieve the lowest range of this dose. However, UV-C is fundamentally limited when it comes to air purification, as it cannot be used in occupied spaces safely–except when hidden, which limits its efficiency. Instead it must be placed in the ducts, where air moves at speeds of hundreds of feet per minute, allowing only a fraction of a second of exposure time to the UV bulb and thus can only achieve an incomplete inactivation.[48]

UV-C light robots have become commonly used in hospitals, where they clean rooms between patient occupancy. These robots can operate only in unoccupied spaces because the light can cause irreparable damage to the retina. As a result, UV-C is only an episodic remedy and does nothing to address continuous risk arising from the reintroduction of pathogens into occupied spaces in between cleanings. In addition, UV-C light cannot reach pathogens in shadows.

6.5 PCO Cleaners

Nearly all PCO-type cleaners are based on early versions of the technology. They have a simple titanium dioxide catalyst and an inefficient UV reactor. These cleaners typically have significantly reduced reaction rates, limiting their effectiveness. As a result, the cleaners have to use fans to pull air into the reaction chamber, which renders them little better than any other passive filtration technology.[49] ActivePure's Advanced Photocatalysis technology has solved these limitations with a new photocatalyst formula and reactor shape, resulting in an FDA-cleared technology qualitatively different from PCO.

It is important to note as well that early-generation PCO-type air cleaners create ozone. They also generate dangerous Volatile Organic Compounds (VOCs). These deficiencies preclude their use in occupied settings, such as hospitals and schools. It was precisely these limitations that led to the creation of Advanced Photocatalysis technology, used today by governments, educators, retailers, and consumers.

4. CONCLUSION

The COVID-19 pandemic has highlighted the need for advanced strategies to combat not just the current widespread virus, but tomorrow's pathogens as well. Public health experts have urged a greater understanding of the danger of airborne spread of the SARS-CoV-2 virus in both droplets and tinier

aerosols and of the relationship between viral load and disease severity.[50]

It has become clear that the world needs layered defense strategies that combine distancing, masking, and environmental controls. While the first two mitigations are dependent on individual behavior and will become less commonplace as the COVID-19 vaccination rollout progresses, the third can be implemented en masse and maintained indefinitely.

We conclude that ActivePure's Advanced Photocatalysis technology provides a safe and effective answer for places where masks cannot always be worn – for example, hospitals, schools, breakrooms, dentist offices, and restaurants. Even after the majority of the population has been vaccinated, the combination of imperfect vaccine efficacy, the waning of immunity over time, and the emergence of variants will create an environment of persistent community transmission requiring the mitigation that a technology like ActivePure provides. More important, such a technology allows us to defend against other airborne pathogens, including the flu and viruses to come.

As schools and businesses continue to plan for their reopening, increasing attention has been given to the importance of HVAC systems, air filtration, and surface disinfection. Much of this focus has been misplaced. MERV 13 filters and UVGI applications must wait for airborne virus particles to come to them before being partially filtered or killed. Surface disinfection technologies are partially useful, but are limited by infrequent application, high costs, and the health concerns posed by toxic chemicals.

By contrast, an active purification technology such as Advanced Photocatalysis that can continuously and safely inactivate virus particles and other pathogens both in the air and on surfaces will have an immediate impact on limiting transmission in occupied public spaces.

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ActivePure®
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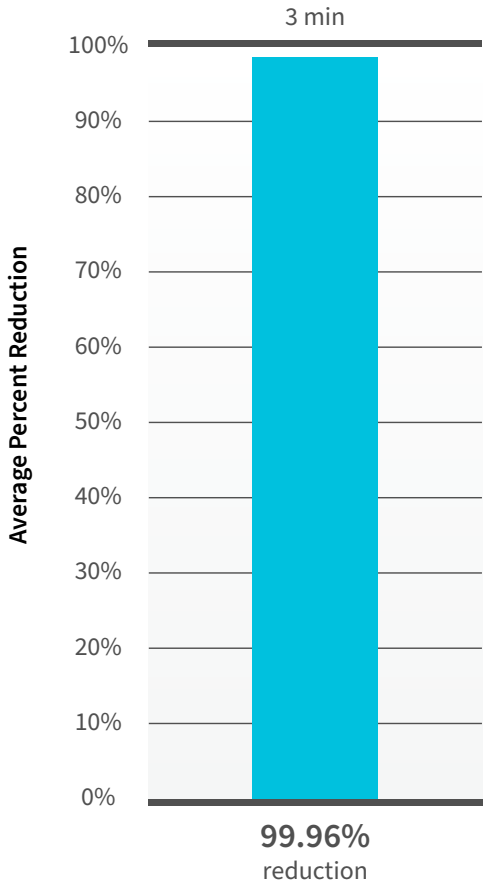
EFFICACY OF MEDICAL GUARDIAN AGAINST VARIOUS AEROSOLS

ACTIVEPURE TECHNOLOGY® **RAPIDLY ELIMINATES 99.9% OF AIRBORNE SARS-COV-2 VIRUS** IN FDA-COMPLIANT MILITARY LAB TESTS

- ActivePure Technology eliminated over 99.9% of highly concentrated “live” airborne SARS-CoV-2 virus in just 3 minutes
- Lab testing was conducted by one of the world’s top biosafety testing facilities, the University of Texas Medical Branch (UTMB), which primarily tests for the U.S. Military and the Centers for Disease Control (CDC)
- Tests were done in triplicate, according to FDA guidelines and protocols.
- Live SARS-CoV-2 virus was sprayed into a test chamber at extremely high concentrations (7+ logs, or over 10 million particles per milliliter)
- Within 3 minutes, the ActivePure Technology reduced the concentration of the virus to no more than 1.6 logs, a reduction of 99.96%



% Reductions measured incrementally over natural degradation of SARS-CoV-2. Outside of control group - over 99.9% reduction of SARS-CoV-2.

“These results demonstrate the effectiveness of the technology”

– **Dr. William S. Lawrence, PhD**
Director of the Aerobiology Services Division
UTMB’s Galveston National Laboratory

UTMB®
The University of Texas Medical Branch

VERIFICATION OF THE EFFECTIVENESS OF ACTIVEPURE® TECHNOLOGY IN **DECONTAMINATION OF SARS-COV-2 ON SURFACES**

Preface

This final report was prepared at MRIGlobal (MRIGlobal) for the work performed under MRIGlobal Task No. 311624.01.001, "Verification of the Effectiveness of ActivePure® Technology in Decontamination of SARS-CoV-2"

Test devices were supplied to MRIGlobal by Aerus, LLC for the conduct of the program. The experimental phase of this task was initiated by MRIGlobal on May 18, 2020 and ended on June 19, 2020.

The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Carl Gelhaus, Ph.D., Luca Popescu, Ph.D., Kristen Solocinski, Ph.D., Sam Humphries, and managed by William Sosna.

The studies were performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal or approved laboratory procedures, and any deviations were documented.

Approved by:

Ed Sistrunk
Division Director
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MRIGLOBAL

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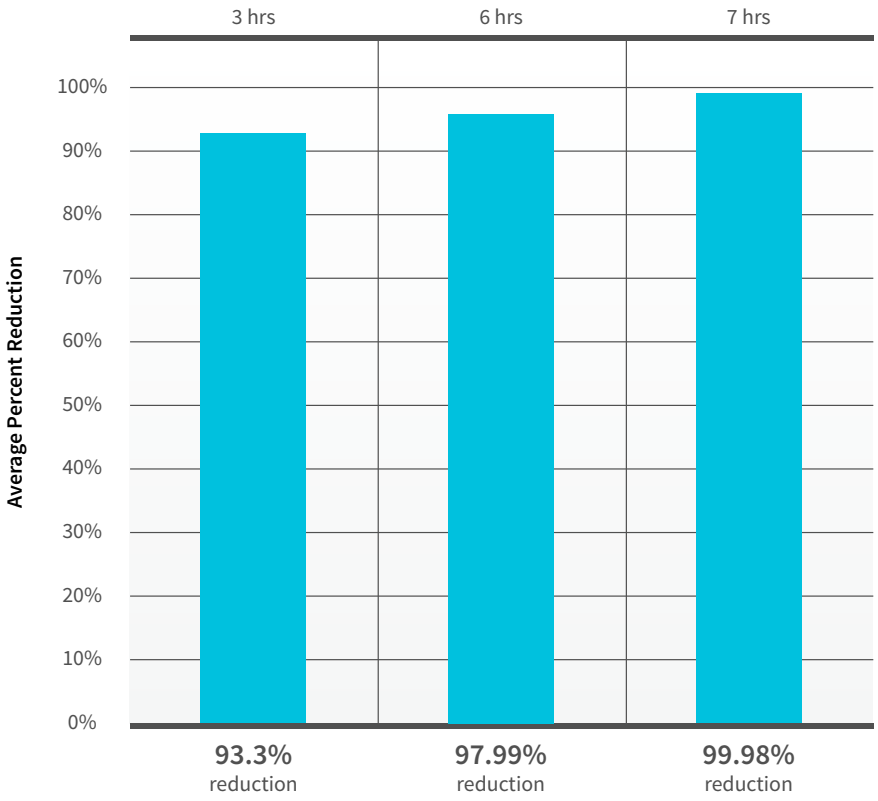
Study Director

Test Results for ActivePure® SARS-CoV-2 on Surfaces



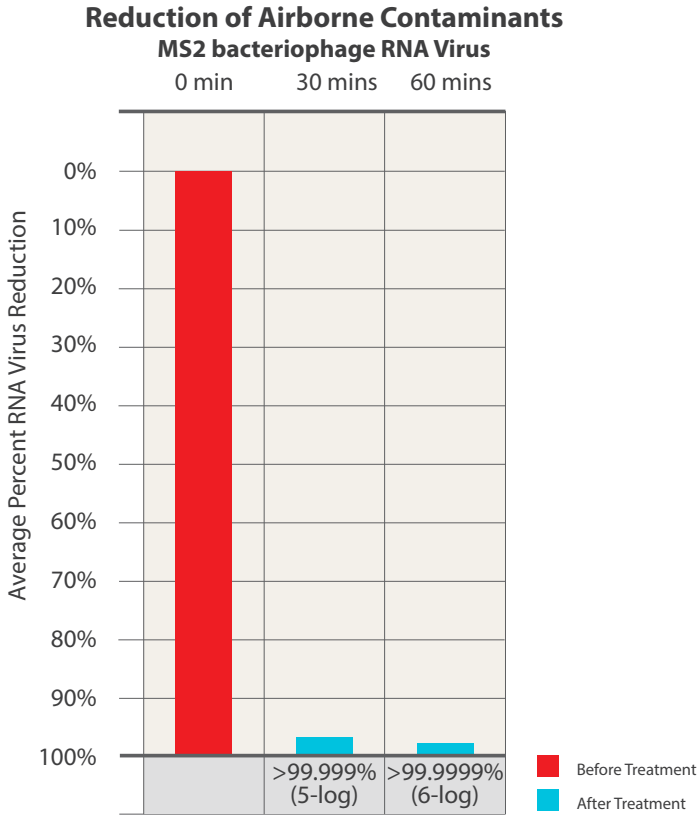
% Reductions measured incrementally over natural degradation of SARS-CoV-2.Outside of control group - over 99.9% reduction of SARS-CoV-2.

NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

Net Log Reduction Of MS2 Bacteriophage Virus Bioaerosol

RNA Virus

> OVER 99.9999% REDUCTION OF MS2 BACTERIOPHAGE VIRUS IN ONLY 60 MINUTES!



Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.

NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

Net Log Reduction Phi-X147 Of Bacteriophage Virus Bioaerosol

DNA Virus

> OVER 99.995% REDUCTION OF PHI-X147 BACTERIOPHAGE VIRUS IN ONLY 60 MINUTES!

Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.

NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

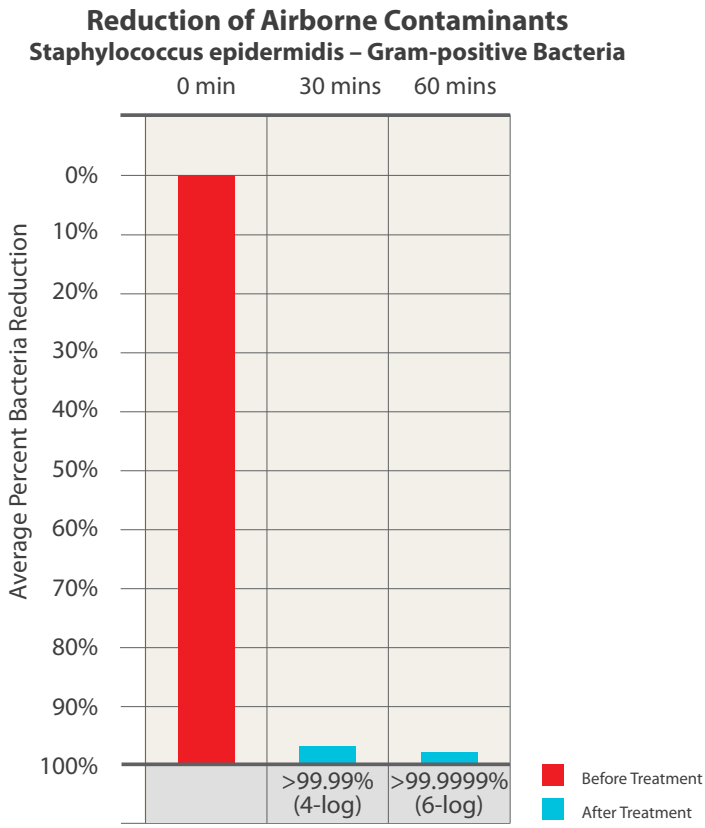
Net Log Reduction Of Staphylococcus Epidermidis Bioaerosol

Gram-positive Bacteria

> OVER 99.9999% REDUCTION OF STAPHYLOCOCCUS EPIDERMIDIS IN ONLY 60 MINUTES!

Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.



NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

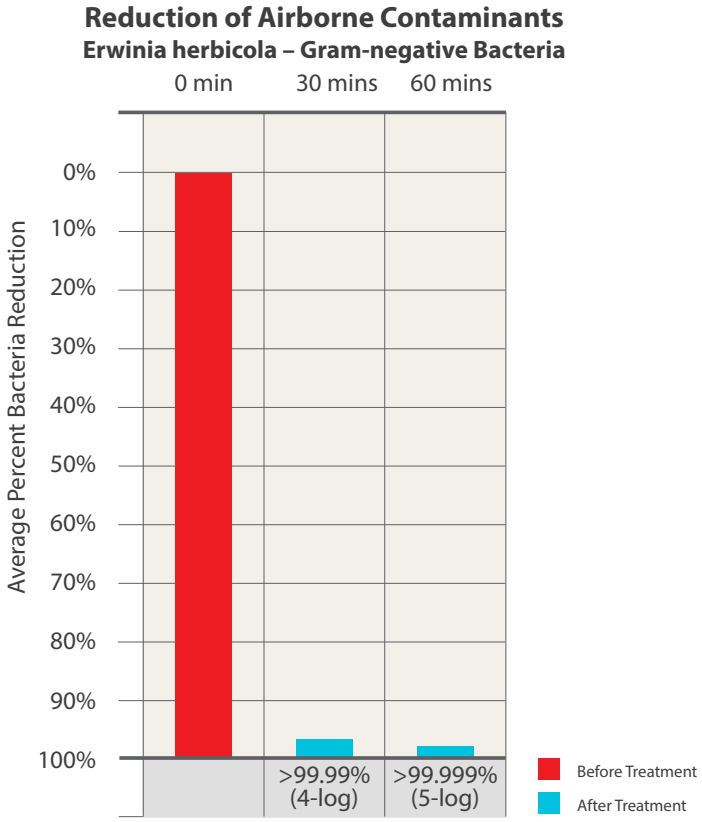
Net Log Reduction Erwinia Herbicola Bioaerosol

Gram-negative Bacteria

> OVER 99.999% REDUCTION OF ERWINIA HERBICOLA IN ONLY 60 MINUTES!

Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.



NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

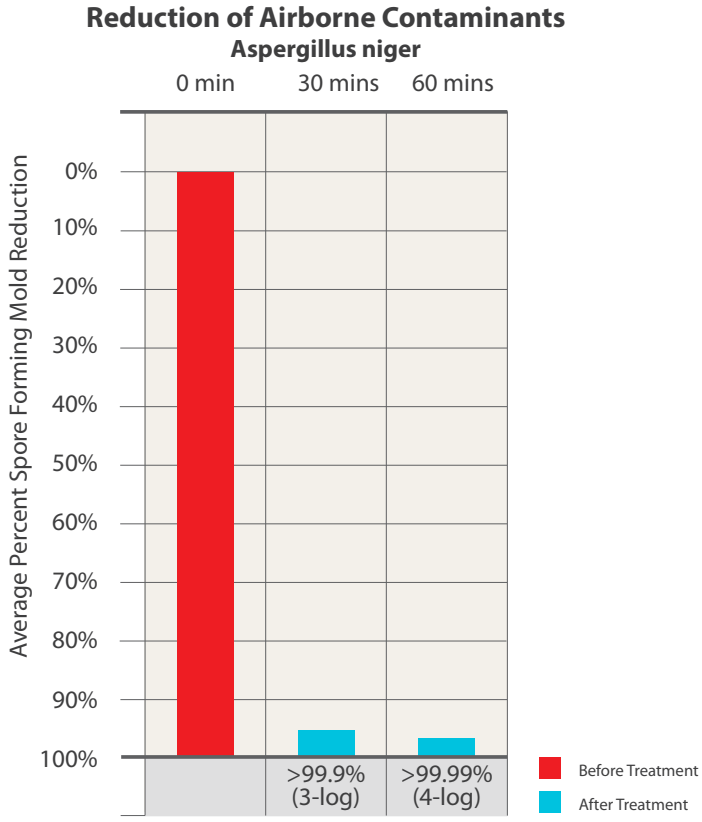
Net Log Reduction Of Aspergillus Niger (fungal spores)

Mold

> *OVER 99.99% REDUCTION OF ASPERGILLUS NIGER IN ONLY 60 MINUTES!*

Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.



NEW AERUS MEDICAL UNIT TESTING – Complying with FDA Protocols in a FDA Certified Compliant Laboratory

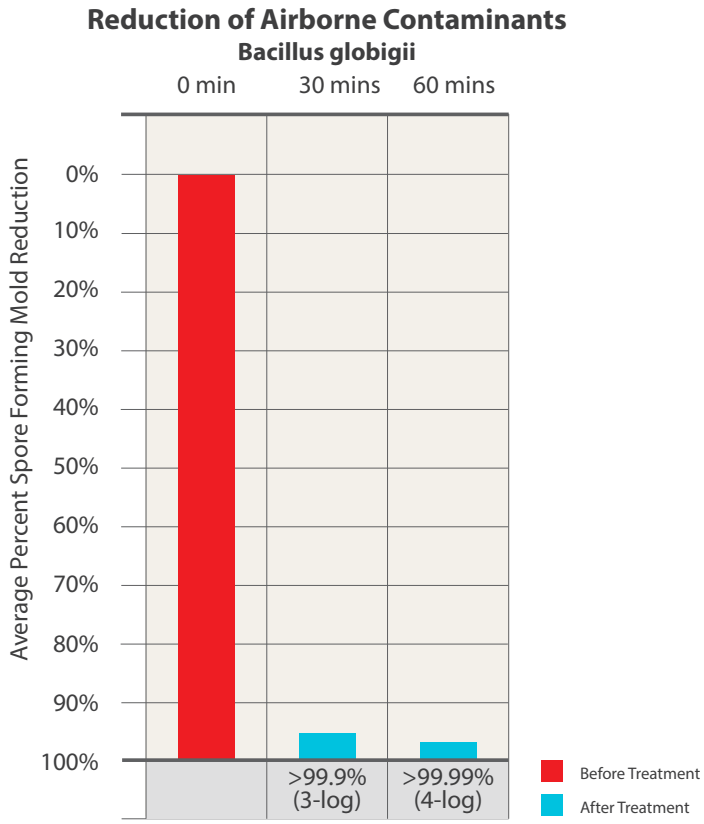
Net Log Reduction Of Bacillus Globigii (bacterial spores)

Mold

> OVER 99.99% REDUCTION OF BACILLUS GLOBIGI IN ONLY 60 MINUTES!

Results based on laboratory testing

Scientific testing has demonstrated the use of ActivePure® Technology to substantially reduce airborne and surface contaminants.
Field results may vary based on environmental conditions. These results have not been certified by the FDA.



Efficacy of Aerus Medical Guardian Air System against Various Bioaerosols

Jamie Balarashti [a], Sylvester Marshall III [a], Dan Merchant [a]

[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Background: This in-vitro study characterized the decontamination efficacy of the Aerus Medical Guardian against various aerosolized biologicals. Aerus Medical LLC's (Bristol, VA) Medical Guardian unit is an indoor air purification system that is designed to neutralize airborne microorganisms. The effectiveness of the system was assessed for six (6) aerosolized biologicals: two vegetative bacteria: *Staphylococcus epidermidis (Gram +)* and *Erwinia herbicola (Gram -);* two viruses: MS2 (RNA bacteriophage) and Phi X174 (DNA bacteriophage); a bacterial endospore; *Bacillus globigii*, and a mold spore; *Aspergillus niger*. The testing consisted of a single control trial plus triplicate decontamination trials for each organism tested.

Methods: Each of the six (6) organisms used for testing were nebulized into a sealed environmental bioaerosol chamber containing the Aerus Medical Guardian air system. AGI impingers were used to capture chamber bioaerosol concentrations at set sampling times. Viable cascade impactors were utilized with some organisms for higher resolution sample collection. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Chamber control trial data was subtracted from Medical Guardian trial data to yield net LOG reduction in the chamber for each tested bioaerosol challenge.

Results: *Staph* had an average Net LOG reduction of 5.46 +/- 0.34 for the triplicate trials. *Erwinia's* average Net LOG reduction was 5.36 +/- 0.37. The Medical Guardian's efficacy against the MS2 virus was 5.58 +/- 0.43. The performance against Phi X174 showed 4.05 +/- 0.27 net LOG reduction. The endospores, *Bacillus*, had an average net LOG reduction of 4.23 +/- 0.31, and the mold spores, *A. niger*, had a net LOG reduction of 4.12 +/- 0.10. The two test viruses, MS2 and Phi X174, organisms showed no viability after t=75minute (MS2) and T=60minutes (PhiX174), in order to calculate the net LOG kill for these organisms a fictitious single colony count was used to calculate the limits of detection for each trial and to show the minimum Net LOG reduction achieved for the Medical Guardian.

Summary: Overall, the Medical Guardian showed an average Net LOG reduction for all organisms tested of 4.80 +/- 0.74. The unit seemed most effective at removing vegetative bacteria from an environment, with an average net LOG reduction of the two vegetative bacteria tested of 5.41 +/- 0.07. However both viruses were below detectable limits due there being zero plaques observed on plates after a certain time point, meaning that the net LOG reduction for those organisms only represents a minimum value. Spores are known to be tough and highly resilient, and therefore it is not unexpected that their net LOG reductions, average 4.18 LOG, are not quite as high as the other organisms. Overall the Medical Guardian showed a high efficacy against of a broad range of viable bioaerosol.

This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Overview

This study was conducted to evaluate the efficacy of the Aerus Medical Guardian produced by Aerus Medical LLC's (Bristol, VA), to decontaminate viable airborne bioaerosols. Testing was conducted in a controlled stainless steel aerosol chamber designed to simulate a small room environment. The Medical Guardian's effectiveness was tested against six (6) Bio-safety level 1 (BSL1) organisms in order to evaluate the system's Net LOG reduction of the viable bioaerosols.

All Bioaerosol challenge testing for this study used an Aerus Medical Guardian (Model # F170A) developed by Aerus Medical LLC (Bristol, VA). A picture and details of the Medical Guardian air system is shown in **Figure 1.**

The effectiveness of the Medical Guardian was evaluated against vegetative bacterium, endospores, viruses, and mold spores. Testing with each of the six distinct organisms was completed in triplicate trials plus a control trial to demonstrate the capability of reducing viable bioaerosol concentrations. There were a total of twenty-four (24) independent trials in this study.

Each organism was examined during a set process involving a control trial and three test trials. During the control trials the Medical Guardian would remain inside the testing chamber, but would never be activated. The organisms were aerosolized into the controlled chamber, and air samples were collected at set time points throughout each trial. Comparisons of the number of living organisms between the control trials and the test trials allowed for determination of the Medical Guardian's efficacy at removing viable bioaerosols from an enclosed room environment.

AERUS	Aerus Medical Guardian
Picture:	
Device Features	
Manufacturer:	Aerus Medical LLC
Model:	F170A
Notes:	Ion Generator, Photocatalytic Oxidizer

Figure 1: *Aerus Medical Guardian air system*

Bioaerosol Testing Chamber

A large sealed aerosol test chamber was used to replicate a potentially contaminated room environment and to contain any potential release of aerosols into the surrounding environment.

The aerosol test chamber is constructed of 304 stainless steel and is equipped with three viewing windows and an air-tight lockable chamber door for system setup and general ingress and egress. The test chamber internal dimensions are 9.1ft x 9.1ft x 6.8ft, with a displacement volume of 562 cubic feet, or 15,914 liters. **Figure 2** shows a diagram of the testing chamber configuration. Testing conditions inside the chamber were consistently 72.0 F with 50% relative humidity.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber was equipped with four 3/8 inch diameter stainless steel probes for aerosol sampling each sampling about 18" from the nearest sidewall. A stainless 1 inch diameter port was used for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer for the bacteriophages, vegetative cells and bacterial spores, or a dry powder eductor for the fungal spores.

A ¼ inch diameter probe was used for continuous aerosol particle size monitoring via a TSI Aerodynamic Particle Sizer (APS) model 3321. All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects and at a height of approximately 40 inches from the floor.

The aerosol sampling and aerosol dissemination probes are stainless steel and bulk headed through the chamber walls to provide external remote access to the aerosol generator and samplers during testing.

The test chamber is equipped with two high-flow HEPA filters for the introduction of filtered purified air into the test chamber during aerosol evacuation/purging of the system between test trials and a HEPA filtered exhaust blower with a 500 ft^3/min rated flow capability for rapid evacuation of remaining bioaerosols.

A magnehelic gauge with a range of 0.0 +/- 0.5 inch H_2O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

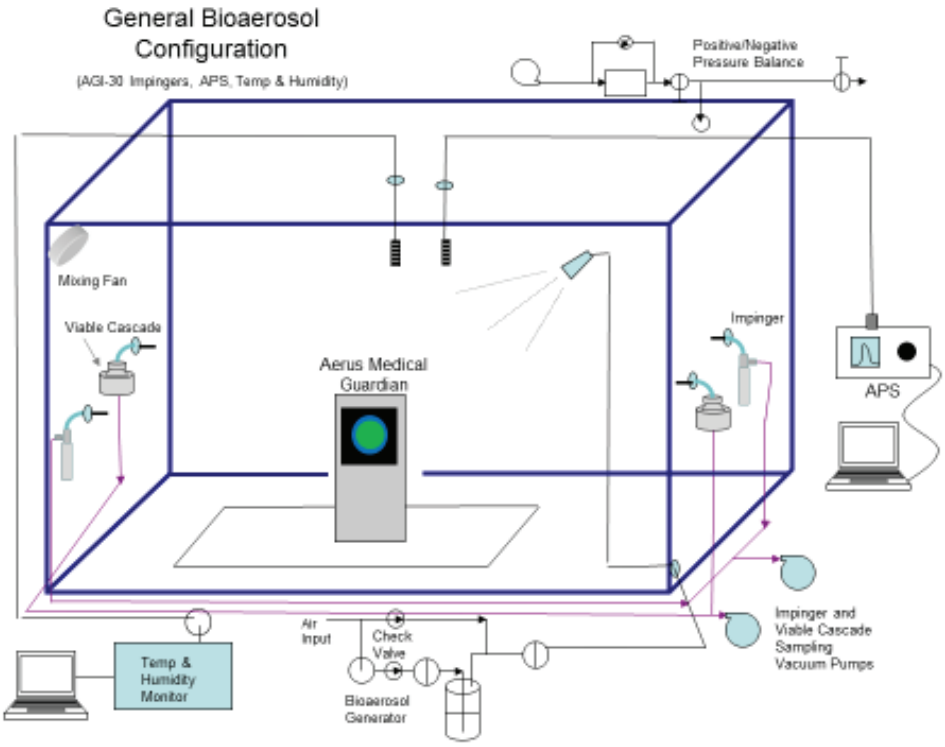


Figure 2: *Bio-Aerosol Test Chamber Flow Diagram.*

Test Location and Conditions

Testing was conducted at Aerosol Research and Engineering labs located at 15320 S. Cornice Street in Olathe, Kansas 66062. Laboratory conditions were approximately 70.6 F with 36% relative humidity.

Bioaerosol Sampling and Monitoring System

Two AGI-30 impingers (Ace Glass Inc., Vineland NJ) were used for bio-aerosol collection of biological aerosols to determine the chamber concentration. These impingers were connected to the bioaerosol chamber via sample ports located at opposite corners of the chamber.

The AGI-30 impinger vacuum source was maintained at a negative pressure of 18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The AGI-30 sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and Aerus trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., St Paul, MN). The APS sampled for the entire duration of all trials (90 minutes) with 1 minute sampling intervals.

The impingers were filled with 20 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms.

During testing with some organisms, sample collections were also obtained using a pair of viable cascade impactors. A viable cascade impactor (SKC Inc., Valley View, PA) comprises an inlet cone, precision-drilled 400-hole impactor stage, and a base that holds a standard-size agar plate (**Figure 3**). A high flow pump pulls microorganisms in air through the holes (jets) where they are collected on the agar surface.

Figure 3: SKC BioStage Viable Cascade Impactor.

Trial	Run	Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Total Trial Time (min)	Impinger Sample Time (min)	Sampling	Plating and Enumeration
1 2	Control Challenge	*Stapilococcuss epidermidis* (+, vegetative)	12228	2.5-3.0μm	10^4-10^6	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers & Viable Cascade	all samples in triplicate
3 4	Control Challenge	*Erwinia herbicola* (-, vegetative)	27155	2.5-3.0μm	10^4-10^6	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers & Viable Cascade	all samples in triplicate
5 6	Control Challenge	*MS2 bacteriophage* (E. coli phage)	15597-B1	<50 nm	10^4-10^6	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers	all samples in triplicate
7 8	Control Challenge	*Aspergillus niger* (mold, spore forming)	13835	<5.0μm	10^4-10^6	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers	all samples in triplicate
9 10	Control Challenge	*Bacillus globigii* endospore *(Bacillus* Spores)	16404	<3.5 μm	10^4-10^6	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers & Viable Cascade	all samples in triplicate
11 12	Control Challenge	Phi X174 bacteriophage (E. coli *phage)*	13706-B1	<50 nm	10^3-10^5	90	0, 15, 30, 45, 60, 75, 90	APS, Impingers & Viable Cascade	all samples in triplicate

Table 1: *Bioaerosol Test Matrices for all trials.*

This method of bioaerosol collection was chosen as the most sensitive and accurate sampling process for the quantification of bioaerosols with this testing configuration. With viable collection enumeration detection at one colony forming unit (cfu), direct collection onto nutrient agar, and immediate incubation after sample collection, it provides the highest sensitivity for low concentration viable aerosol collection and measurement. This method of collection was not a feasible option for all organisms due to the plating methods of viruses and growth patterns of mold spores. **Table 1** shows the complete test matrix for this study.

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc. Waltham MA) driven by purified filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the Collison nebulizer. The mold spore organisms were disseminated using a dry powder aerosolization unit.

Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 60 psi, which obtained an output volumetric flow rate of 50-80 lpm with a fluid dissemination rate of approximately 1-2 ml/min. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN).

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for a wide range of BSL3 pathogenic organisms. It is routine in the bioaerosol field to use surrogate species to test performance against BSL3 organism decontamination due to the high cost and limited lab space associated with aerosol BSL3 testing. For this reason a broad range of viable bioaerosol surrogates were selected to specifically test the Guardian's efficacy against various types of pathogenic bioaerosols encounter in hospital and other environments.

Staphylococcus epidermidis (Gram +, ATCC 12228) acts as a surrogate for its cousin, *Staph aureus,* which has developed multi drug resistance (MRSA) and is one of the major leading causes of hospital-acquired infections.

Erwinia Herbicola (Gram -, ATCC 27155) is a Gram-negative bacterium and has been used historically as a surrogate for black plague causing bacterium, *Yersinia pestis*.

MS2 (ATCC 15597-B1) is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus and the norovirus.

PhiX-174 (ATCC 13706-B1) is a small, single-stranded DNA virus that is often used as a surrogate for HCV, HCB, and HIV in research studies.

Aspergillus niger (ATCC 13835) endospores are used as a surrogate for several toxic black mold species such as *Stachybotrys chartarumand*.

Bacillus globigii (now named *Bacillus subtillis*) endospores (ATCC 16404) are routinely used as a surrogate for weaponized anthrax, *Bacillus anthracis*. Phi X174

Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). Working stock cultures were prepared using sterile techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 100ml of *Staph* and *Erwinia Herbicola* stock was prepared in tryptic soy liquid broth media, and incubated for 24 hours with oxygen infusion (1cc/min) at 37°C. Biological stock concentrations were greater than 1 x 10^9 cfu/ml for *Staph* and *Erwinia* using this method.

Stock cultures were centrifuged for 12 minutes at 4000 rpm in sterile 50mL conical tubes, growth media was decanted, and the cells re-suspended in sterile PBS buffer for aerosolization. Aliquots of these suspensions were enumerated on tryptic soy agar plates (Hardy Diagnostics, Cincinnati OH) for viable counts and stock concentration calculations. For each organism, test working stocks were grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the specific bacteriophage. After an appropriate incubation time the cells were lysed and the cellular debris discharged by centrifugation. MS2 stock yields were greater than 1 x 10^9 plaque forming units per milliliter (pfu/ml) with a single amplification procedure. Phi X174 stock yields were less than MS2 yields, and ranged between 1 x 10^8 to 1 x 10^9 pfu/ml after two amplifications.

Endospore Culture & Preparation

Bacillus globigii spores were growth, sporelated, spray dried and stored in a pure dry powder (2.41 x 10^{11} cfu/gr.) form ahead of time. Master stocks were prepared using the pure spore and were kept stored in a 30% ethanol : 70% PBS + tween solution to maintain their endospore state and to prevent contamination by other vegetative cells. Nebulization proceed directly using this master stock.

Aspergillus niger fungal spores were also cultured, purified and stored in purified bulk powder form with a concentration of 3.4 x 10^9 cfu/gr. Due to the size of the spore (5µm diameter) nebulization was not used for dissemination of the bioaerosol, instead a custom dry eductor and feeder was used dispersed into the chamber using a ARE Labs dry powder aerosolization unit.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-48 hours (species dependent) and enumerated and recorded.

Inert Particle Characterization

In order to calculate the dissemination efficiency, stability and to pinpoint impinger/viable cascade sample times pre-testing was conducted using polystyrene latex beads (PSL miscrospheres) prior to bioaerosol testing. PSL microspheres were used to characterize the various aspects of the chamber and Aerus system.

Polydispersed PSL beads with aerodynamic diameters of 1.0µm were nebulized and chamber concentrations were recorded using the APS. The control trials were used to calculate nebulization efficiencies, particle stability and AGI-30 collection efficiencies were used to estimate generation efficiencies, dissemination times, sample times and aerosol persistence prior to bioaerosol testing. Live trials with the Medical Guardian system were also performed to measure particle removal rates of the system.

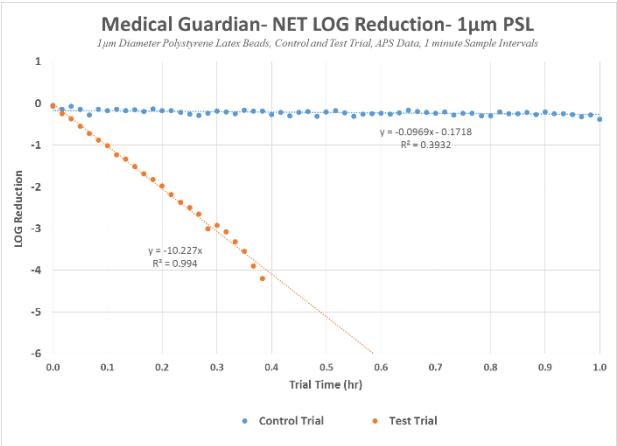


Figure 4: *LOG Reduction of 1.0um PSL Beads*

Figure 4 shows the LOG reduction of 1.0um PSL beads both with the Medical Guardian unit on (Test Trial) and off (Control Trial). The figure shows the

General Timeline for Bioaerosol Chamber Testing

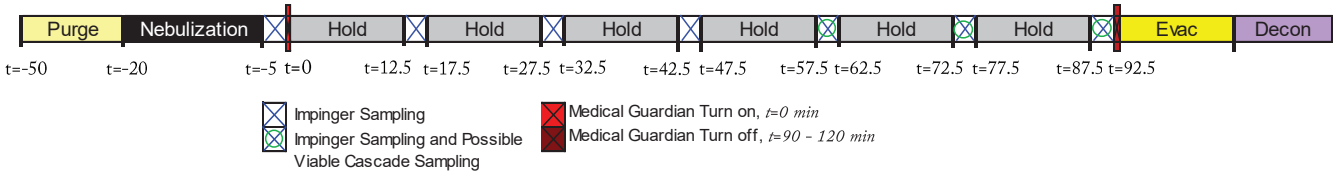


Table 2*:* General *Trial Timeline for Aerus Medical Guardian Decontamination Trials.*

comparison between the reduction with and without the Medical Guardian. When the Guardian is turned on the LOG reduction of 1.0um PSL beads follows a precise logarithmic function. It is notable that this size (1um) is smaller than the vegetative bacteria, bacterial endospores, and mold spores, but larger than the two viruses.

Control Testing

To accurately assess the Aerus Medical Guardian unit, test chamber pilot control trials were performed with all Bioaerosols from 90 to 120 minute periods without the Medical Guardian in operation to characterize the biological challenge aerosol for particle size distribution, aerosol delivery/collection efficiency, decay rate and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual reduction from Medical Guardian challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on for the duration of trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control runs and was turned off during Medical Guardian decontamination trials. The two impingers used for bacteriophage, vegetative, fungal and bacterial endospore test sampling were pooled and mixed prior to plating and enumeration.

Medical Guardian Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 50 mL of biological stock and operated at 50 psi for a period of 20 or 25 minutes (organism dependent). For control and Medical Guardian trials, the impingers were filled with 20 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms.

The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Guardian test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 2, 5, or 10 minutes (organism dependent) at intervals of 15 minutes throughout the entire period. **Table 2** above shows the general timeline for each Medical Guardian live bioaerosol challenge trial.

Collected impinger chamber samples were pooled and mixed at each sample interval for each test. Aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval, and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

For Medical Guardian biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (up to 90 minutes). Subsequent impinger samples were taken at intervals of 15 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the Medical Guardian system over time. **Table 2** outlines the general timeline for the testing procedure with the Medical Guardian.

Test chamber temperature and humidity were recorded at the initiation and completion of each test.

All samples were plated in triplicate on tryptic soy agar media over a minimum of a 2 log dilution range.

Plates were incubated for viable plaque forming units (pfu) formation for the viral phase of the study, and colony forming units (cfu) for fungal spore, and bacterial endospore phases of the study. Plates were incubated and enumerated for viable counts to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test. The chamber was decontaminated between live microorganism trials with aerosol/vaporous hydrogen peroxide (35%). The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

Data Analysis

Shows the results of the quadruplicate trials for each biological tested for this study. All results indicate the calculated viable bioaerosol concentration both upstream and downstream of the Clean Air System and the Net LOG reduction provided by the system.

All trials show individual and group average +/- standard deviations for Net LOG reduction on a per challenge organism basis.

Results by Organism

Staphylococcus epidermidis

The *S. epidermidis* are a gram positive, aerobic bacteria that grow in trypticase soy at 37°C and were chosen to act as surrogates for *Staph aurus.* Infections from *S. aurus* are a leading cause of hospital-acquired infections and are linked to 50,000 deaths in the USA per year.

Staph cultures were initiated the day prior to their testing, and grew to a concentration greater than $1e^{10}$ cfu/ml. After nebulization, initial concentrations of *Staph* averaged $2.6e^{5}$ cfu/L of atmosphere inside the testing chamber. The control trial experienced only a 0.62 LOG reduction of *Staph* after 90 minutes of sample collections using the AGI-30 impingers.

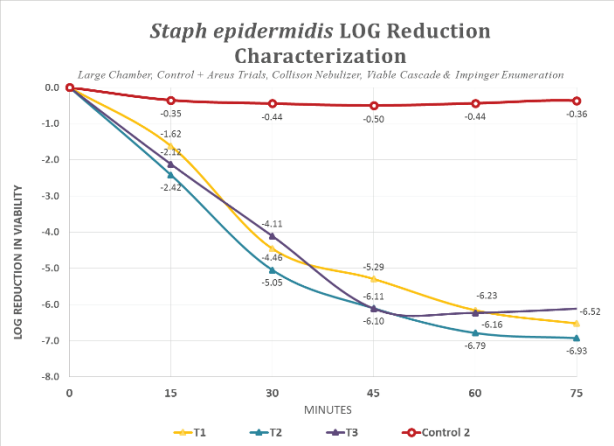


Figure 5: LOG Reduction of *S. epidermidis* in control and Medical Guardian test trials.

The Medical Guardian trials saw a drastic increase in reduction with the unit activated. In 15 minutes, over the three test trials, an average of only 1.183% of the viable *Staph* still remained inside the testing chamber. **Figure** 5 shows the LOG reduction of *Staph* for the control and test trials over the 90 minute testing periods. At 60 minutes there was an average net LOG reduction of *Staph* of 5.95 +/- 0.34 with a limit of detection of 8.15. **Figure 6** shows the LOG reductions for each *Staph* trial and their average +/- standard deviation.

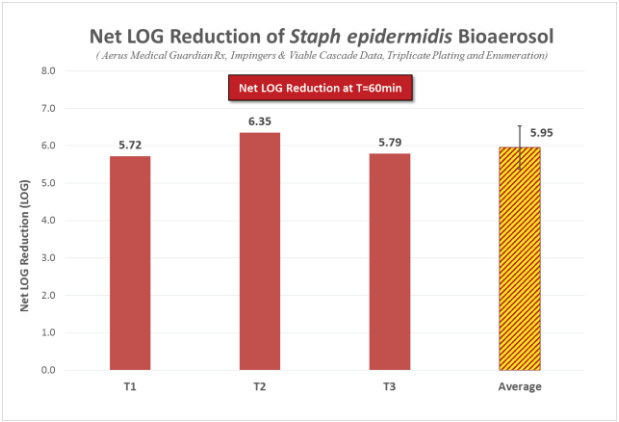


Figure 6 Net LOG Reduction of *Staphylococcus epidermidis*

Erwinia herbicola

The black plague surrogate, *Erwinia herbicola,* was the second and final vegetative bacterium tested in this study. The gram-negative bacterium were grown overnight to an average concentration of $8.4e^{9}$ cfu/ml,

and after nebulization had an average concentration of 1.9e^5 cfu/L of atmosphere inside the testing chamber.

The *Erwinia* control trial saw a 0.70 LOG reduction after 90 minutes of sample collection. After 15 minutes of being activated, the Medical Guardian removed an average of 99.992% of the viable *Erwinia* from the chamber atmosphere. The average net LOG reduction of *Erwinia* by the Medical Guardian at 75 minutes was 5.36 +/- 0.37 with a limits of detection of 7.64.

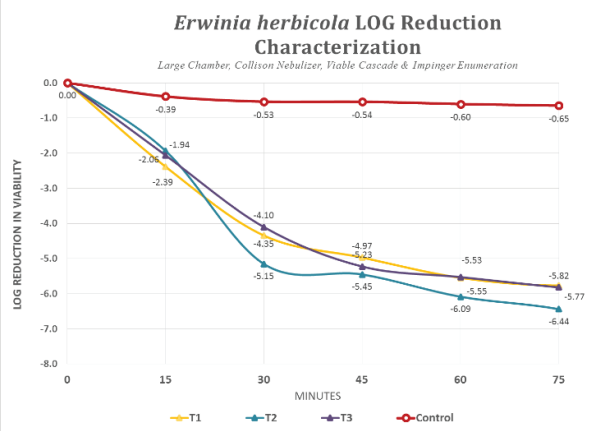


Figure 7: LOG Reduction of *Erwinia herbicola* in control and Medical Guardian test trials.

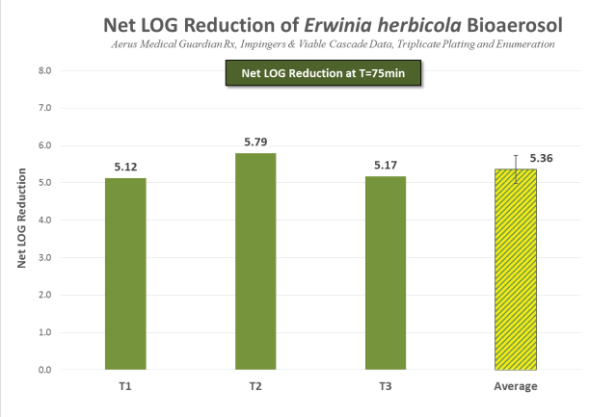


Figure 8: Net LOG *Reduction of Erwinia herbicola.*

MS2 Virus

The bacteriophage MS2 is a single stranded RNA virus that often serves as a surrogate for noroviruses in studies of disease transmission. MS2 infects *E. coli*, which was used as its mechanism of reproduction for this study. Average concentrations of MS2 reached 5.4e^9 pfu/mL in culture pre testing, and chamber concentrations after nebulization averaged 8.1e^5 pfu/L of atmosphere.

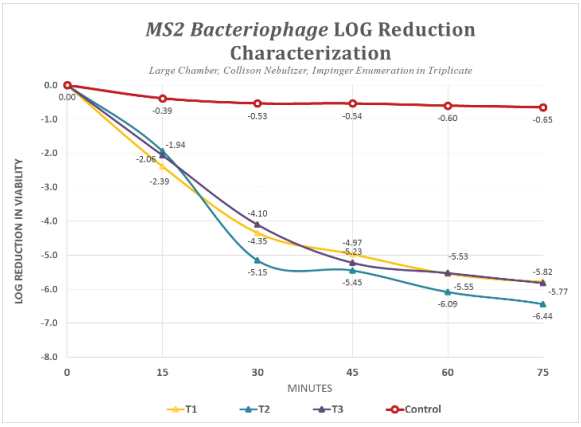


Figure 9: LOG Reduction of MS2 bacteriophage in control and Medical Guardian test trials.

The single control trial saw a slightly increased reduction in viable organisms overtime compared with the vegetative bacterium, reaching a LOG reduction of 0.94 in 90 minutes. With the Medical Guardian activated, an average of 99.999% of the virus had been removed from the chambers atmosphere in 15 minutes. At 60 minutes, there was an average net LOG reduction of MS2 of 5.58 +/- 0.43 with a limits of detection of 6.37. The Net LOG reduction is considered a minimum value because a single pfu was artificially added at 60 minutes in order to show detection limits. There were in reality, no observed viable growth collected at 60 minutes. However, if zeros were to be marked for the plates the logarithmic math does not calculate.

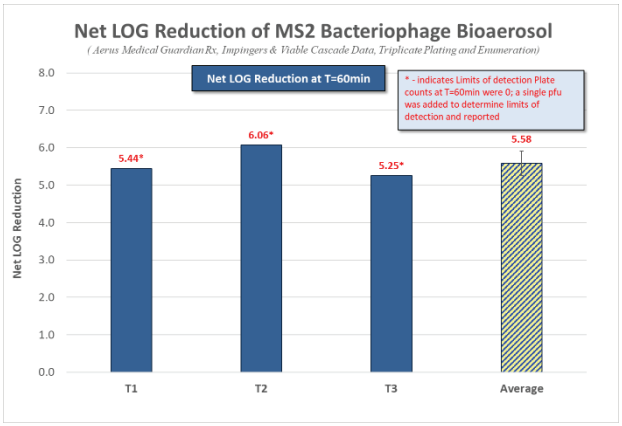


Figure 10: Net LOG Reduction of MS2 bacteriophage

***Bacillus globigii* endospores**

Endospores are an extremely resilient structure formed by some bacteria under unfavorable conditions that are resistant to UV radiation, desiccation, chemical disinfectants and severe temperatures. The *Bacillus*

globigii spores tested served as surrogates for a similar species that causes Anthrax.

Unlike the vegetative bacterium and viruses, the spores did not need to be cultured before testing. Large quantities of spores stored in alcohol can be purchased and are ready for immediate testing. Pre nebulization stock of *B. globigii* was at a concentration of $2.7e^9$ cfu/mL, and chamber concentration post nebulization had an average concentration of $7.95e^5$ cfu/L.

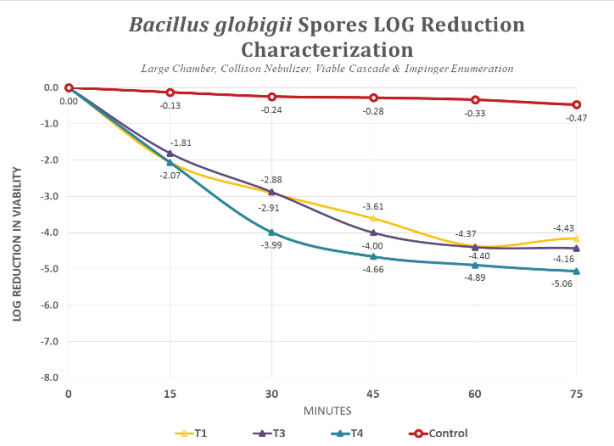


Figure 11: LOG Reduction of *Bacillus globigii* endospores in control and Medical Guardian test trials.

With the Medical Guardian off, the control trial saw a LOG reduction after 90 minutes of 0.49. After 15 minutes with the Medical Guardian activated during the test trials, 1.09% of the spores remained in the chambers atmosphere. After 90 minutes there was a net LOG reduction of 4.23 +/- 0.31 with a limits of detection of 5.70.

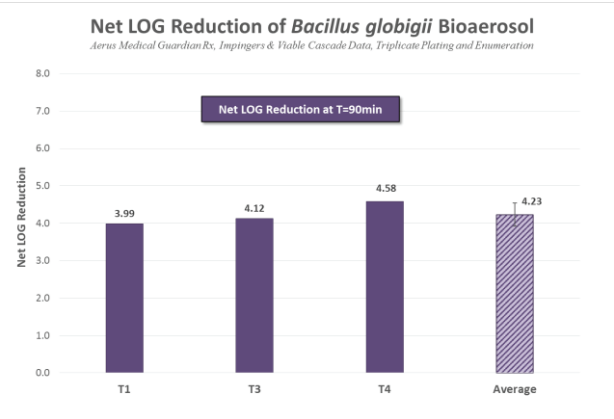


Figure 12: Net LOG Reduction of *Bacillus globigii* endospores

***Aspergillus niger* Mold Spores**

Aspergillus niger, the second spore based organism tested is a fungus rather than bacterial species. It is known for causing black mold disease in fruits and vegetables and is closely related to *Aspergillus fumigatus,* the most common airborne fungal pathogen. Like *B. globigii*, the *A. niger* spores did not require cultivation, but were commercially acquired ready for testing.

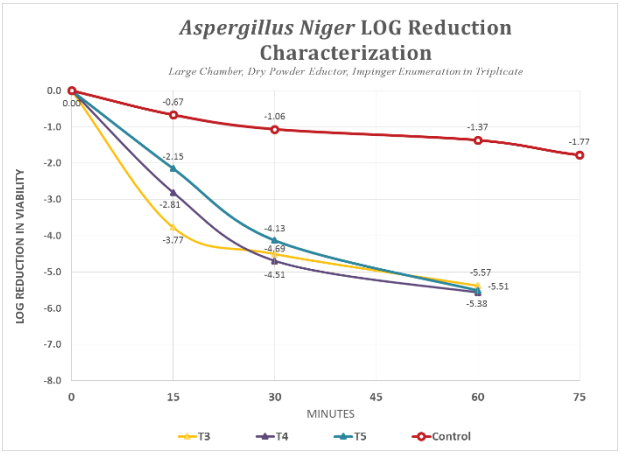


Figure 13: LOG Reduction of *Aspergillus niger* spores in control and Medical Guardian test trials.

Concentrations of *A. niger* inside the chamber after nebulization was less than previous organisms, with an average of $2.61e^4$ cfu/L. The control trial revealed a LOG reduction after 90 minutes of 1.77, which was higher than had been observed previously. With the Medical Guardian activated for 15 minutes during testing trials, an average of only 0.29% of *A. niger* remained inside the chamber. After 60 minutes there was an average net LOG reduction of 4.12 +/- 0.10 with a limits of detection of 5.59.

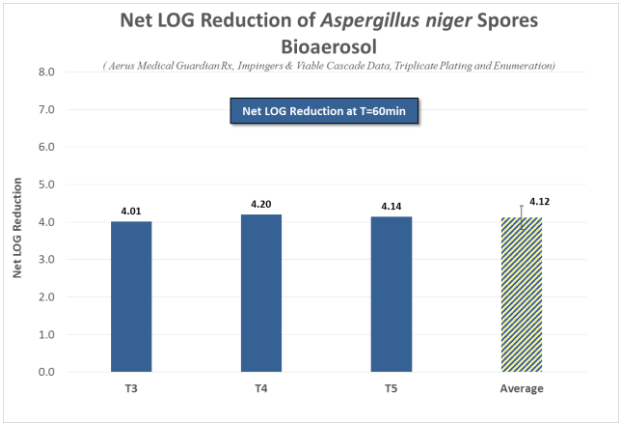


Figure 14: Net LOG Reduction of *Aspergillus niger* spores.

Phi X174 Virus

The Phi X174 bacteriophage testing was planned immediately following MS2 testing, however obstacles arose with culturing the virus to desirable concentrations. The testing was pushed back to the end of the study to give time to develop slight alterations to the growth protocols. Like MS2, Phi X174 infects *E. coli,* however it is a DNA virus with a small genome.

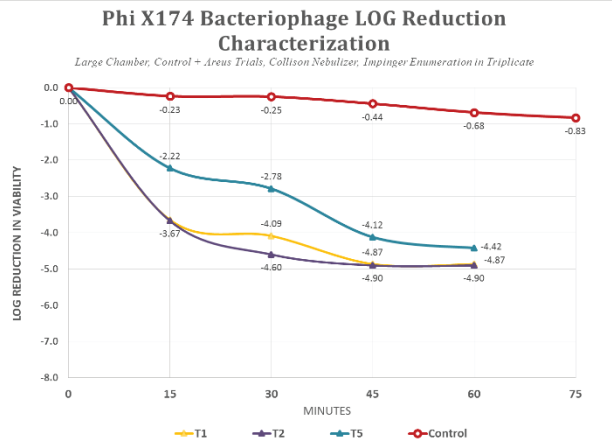


Figure 15: LOG Reduction of Phi X174 bacteriophage in control and Medical Guardian test trials.

Pre-testing cultures of Phi X174 had an average concentration of $4.4e^8$ pfu/mL, which was significantly lower than any other organisms tested. Once nebulized, the concentration of the atmosphere inside the testing chamber averaged $7.63e^3$ pfu/L. There was a LOG reduction of Phi X174 of 0.89 at 90 minutes during the control trial, and an average net LOG reduction of 4.05 +/- 0.27 with limits of detection of 4.73 during testing trials. There were no plaques observed beyond 30 minutes and a single pfu was added to determine detection limits.

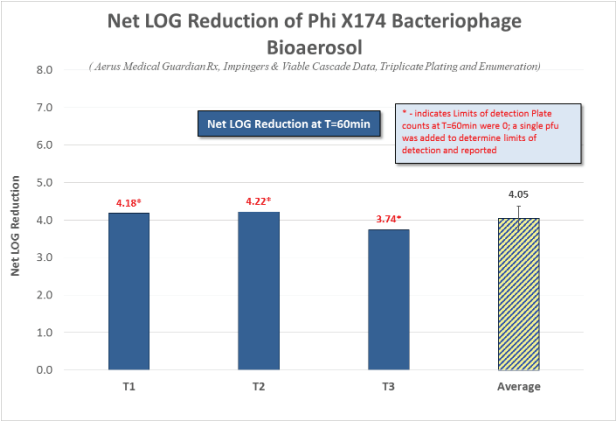


Figure 16: Net LOG Reduction of Phi X174 bacteriophage.

Aerus Medical Guardian Bioaerosol Challenge Summary					Net LOG Reduction Results						Limits of Detection
	Challenge Organism	Surrogate for	ATCC #	Gram	Time (min)	T1	T2	T3	Avg Net LOG Reduction	Standard Deviation	
1	*Staphylococcus epidermidis*	Methicillin Resistant *Staph aureus* (MRSA)	12228	pos	60	*5.72*	*6.35*	*5.79*	*5.95*	*0.34*	*8.15*
2	*Erwinia herbicola*	*Yersinia pestis* (Black Plague)	27155	neg	75	*5.12*	*5.79*	*5.17*	*5.36*	*0.37*	*7.64*
3	MS2 virus	Influenza, Norovirus	15597-B1	NA	60	*5.44*	*6.06*	*5.25*	*5.58*	*0.43*	*6.37*
4	Phi X174 virus	HCV, HBV, HIV	13706-B1	NA	60	*4.18*	*4.22*	*3.74*	*4.05*	*0.27*	*4.73*
5	*Bacillus globigii* endospore	*C. difficile* (spore) & Anthrax (spore)	16404	pos	90	*3.99*	*4.12*	*4.58*	*4.23*	*0.31*	*5.70*
6	*Aspergillis niger* mold spore	Black Mold	13835	NA	60	*4.01*	*4.20*	*4.14*	*4.12*	*0.10*	*5.59*

*Red text = at detection limits, Calculated LOG Reduction values represent a minimum

Table 3: *Net LOG reduction summary for the Medical Guardian air system.*

Summary of Results

Overall, the Medical Guardian's showed an average Net LOG reduction for all organisms tested of 4.80 +/- 0.74. The unit was more effective at removing vegetative bacterium from the testing chambers atmosphere than either the viruses or spores, with an average net LOG reduction for just the two vegetative bacteria of 5.41 +/- 0.07. However testing of both viruses were below detectable limits due there being zero plaques observed on plates after a certain point, meaning that the net LOG reduction for those organisms only represents a minimum value. Spores are known to be tough and highly resilient, and therefore it is not unexpected that their net LOG reductions are not quite as high as the other organisms.

The **Figure 17** below shows each average net LOG reduction for the organisms tested +/- their standard deviations. **Table 3** shows a final summary table of the net LOG reduction results for each organism tested in this study.

The Medical Guardian had an overall Clean Air Delivery Rate of 94.63 cubic feet per minute (cfm), which is explained in detail in **Appendix A.**

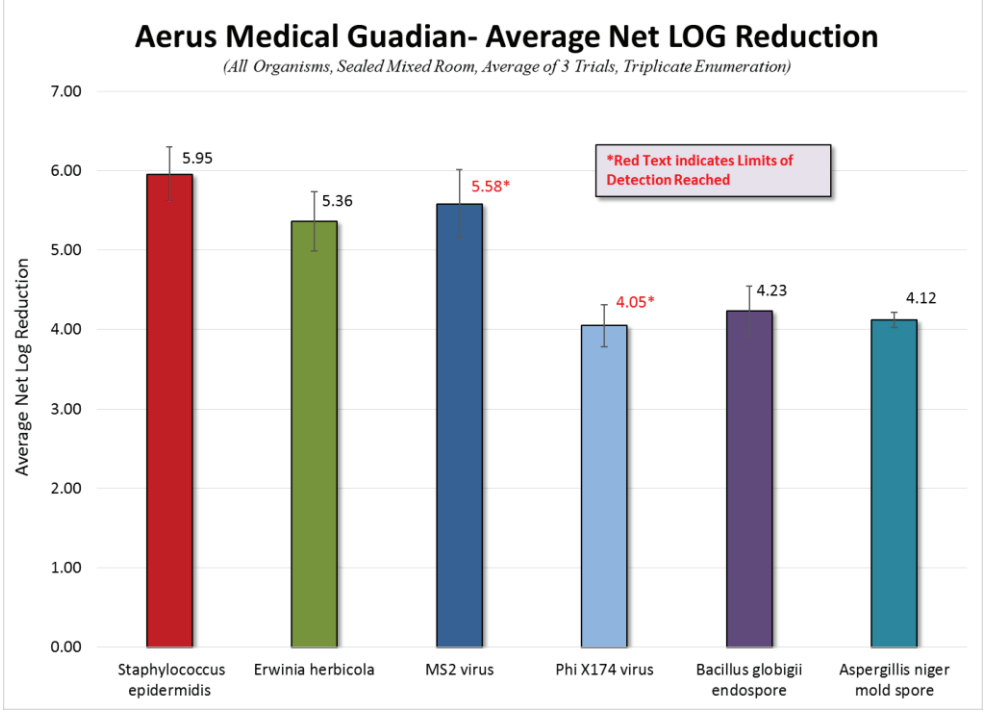


Figure 17: *Average net LOG reduction for each bioaerosol organism tested, average +/- standard deviation for the triplicate trials.*

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P Hyman et al. *Practical Methods for Determining Phage Growth Parameters*. Bacteriophages: Methods and Protocols, Vol. 1*: Isolation, Characterization and Interactions.* vol. 501. 2009. pp. 175-201.

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GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

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Airborne Inactivation of the Novel Coronavirus SARS-CoV-2 by Aerus Technology

Aerus Medical, LLC contract

Final Report Date of 22 December 2020

To

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Objective

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has had a profound, detrimental effect across the globe. The disease is associated with flu-like symptoms, persistent chest pain, respiratory complications/distress, confusion, and death. To date, there has been over 68 million cases of COVID-19 and over 1.5 million COVID19-related deaths worldwide, while in the United States alone, there has been approximately 15 million COVID19 cases and 284,000 COVID19-related deaths. In attempts to prevent the spread of the virus, several countries have implemented mandatory shutdowns of businesses and schools. While these measures have proven effective in controlling the spread of infection, they unfortunately have crippled several economies and are therefore unsustainable.

SARS-CoV-2 is believed to be transmitted by various means which includes direct, indirect, or close contact with infected people; aerosol transmission; and fomite transmission. In the case of aerosol transmission, SARS-CoV-2 virions become suspended in respiratory secretions or droplets expelled into the air by infected individuals. These droplets, due to their extremely small sizes, remain aerosolized for extended periods of time and are potentially inhaled by other individuals in the vicinity. Due to this route of aerosol transmission, the need for air purification devices capable of inactivating the virus within indoor environments is paramount.

Aerus Medical, LLC, has developed technology that can eliminate microbial contaminants by creating powerful oxidizers that can inactivate microbes. Aerus technology has demonstrated in lab tests the ability to eliminate surface SARS-CoV-2 (coated in a bio-film) by 99.98% in just 7 hours. In tests resulting in FDA Class II Medical Device Clearance, Aerus technology demonstrated 99.999% reductions in airborne MS2 Bacteriophage in just 30 minutes. These findings suggest Aerus technology would also be effective at eliminating airborne SARS-CoV-2.

The objective of this study was to test the ability of a device manufactured by Aerus Medical, LLC, to inactivate airborne SARS-CoV-2.

Experimental Procedure

Virus
SARS-CoV-2, strain USA_WA1/2020, was provided by the World Reference Center for Emerging Viruses and Arboviruses.

Plaque assay
Vero E6 cells were seeded in 6-well or 12-well plates to a confluency of 90-100%. Samples were serially 10-fold diluted in PBS. Diluted samples were plated onto the cells and left to incubate at 37ºC with 5% CO2 for one hour. After incubation, samples were overlaid with a mixture of 2X MEM (supplemented the 8% FBS and 2% pen/strep) and1.6% LE agarose. The plates were incubated overnight at 37ºC at 5% CO2. Plaques

were visualized with neutral red stain and a light box. The lower limit of detection (LLD) for the assay was 40 pfu/ml.

Aerosol generation and sampling

The aerosolization was performed using a Biaera aerosol control platform (Aero3G, Biaera Technologies, LLC) fitted with a custom-made 150-liter chamber manufactured by Biaera Technologies, LLC (**Figure 1**). The viral aerosol was generated using a 6-jet Collison nebulizer (flowrate set to 14.0 LPM), and it was combined with a standard volume of air to deliver a given concentration of virus to the chamber. For each nebulization, 10 ml of the viral inoculum at concentrations of 1-5 x 10^7 pfu/ml was used. The duration of aerosolization was 15 minutes. Aerosol samples were collected at 12.5 LPM for 5 minutes immediately following aerosolization and at various testing time-points using BioSamplers (SKC, Inc.) containing 20 ml of collection medium to determine the chamber viral concentrations. The total airflow to the aerosol setup was 50.0 LPM. Temperature and humidity were monitored during nebulization and sampling.

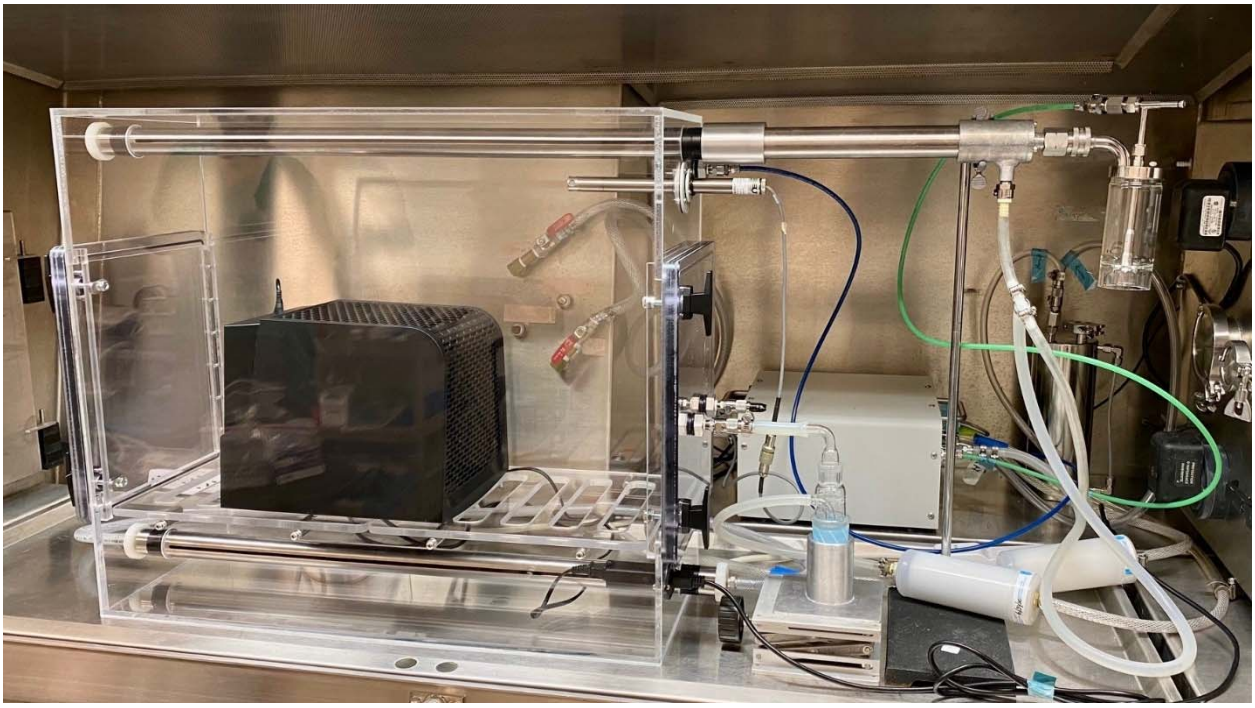

Figure 1. Aerosol testing setup

Aerus devices

Two devices manufactured by Aerus Medical were used in the study, an experimental device and a control device. These were the Aerus Pure & Clean and the Vollara Air & Surface Pro. The experimental device was equipped with the Aerus ActivePure technology capable of inactivating airborne viruses, as well as a fan used for air intake and output (all other technologies within the devices were either removed or deactivated). The control device was essentially identical to the experimental device, but the viral

inactivation ActivePure technology was removed from the control device. For operation, both devices were plugged into a 110V outlet within the chamber.

Aerosol testing timeline

The time and duration of aerosolization, device on-time, and sampling were performed according to **Figures 2A-C**. The device on-times tested were 3 and 10 minutes (**Figure 2A**), 15 and 30 minutes (**Figure 2B**), and 60 minutes (**Figure 2C**). These timelines were used for both the experimental (using the experimental device) and control (using the control device) runs. A test run with a timeline similar to **Figure 2A** was also performed wherein the control device was not turned on at t=0. This was done to ascertain the degree at which the aerosol droplets settle without air circulation being generated by the fan within the devices.

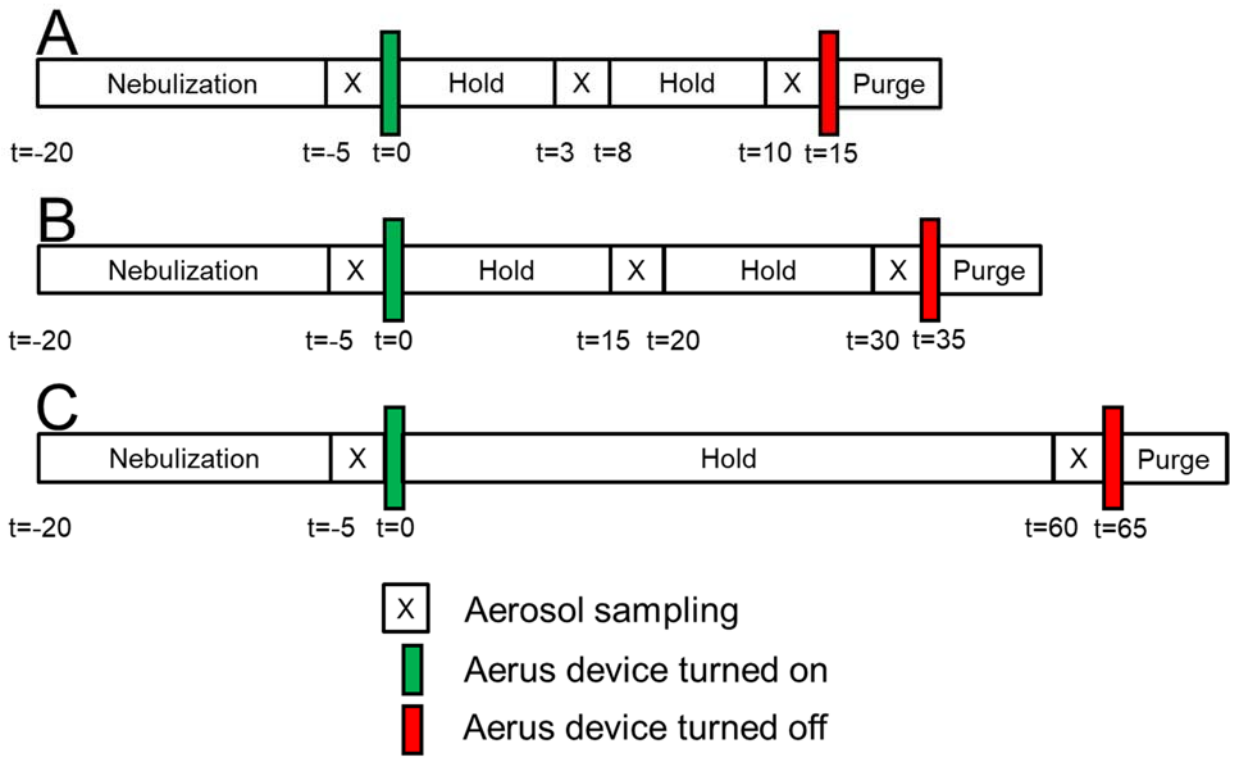

Figure 2. Testing timelines

Results

During the aerosol and sampling runs, the average humidity level was approximately 70%, and the temperature was approximately 22ºC. **Table 1** provides the log reductions (relative to the viral concentration at t=0) in viral concentration for the control and experimental devices with the various on-times. All device on-times were tested in triplicate except for the 3-minute (unit off) control, 10-minute (unit off) control, 30-minute control, and 60-minute control. In the case of the experimental device, the measured viral concentrations were below the plaque assay LLD (40 pfu/ml or 1.6 logs) for all but one

sample collection. Therefore, the range in log reduction was ≥2.87 to ≥3.38 which equates to ≥99.87 to ≥99.96%. However, since no plaque were detected in nearly every case, the percent inactivation could be ≥99.99% (≥4 logs). The log reductions in viral concentration for the control runs ranged from 0.61 to 2.91; however, the viral concentration of one sample collection (60-minute control) did fall below the assay LLD therein giving a log reduction of ≥3.18. The true net reduction in viral concentration could not be determined since the experimental device inactivated the virus to undetectable levels. Moreover, the true net log reduction for the runs over 3 minutes would be misleading since a greater portion of the virus settled in the test chamber as time passed. Because of this, it is likely that more of the virus was inactivated than could be measured. The study raw data is provided in the Appendix.

Table 1. Log reduction in viral concentration

Test unit	Device on-time	Log reduction from t=0	Test unit	Device on-time	Log reduction from t=0
Control device	3 minutes	0.84	Experimental device	3 minutes	3.38
		0.68			≥2.98
		0.61			≥2.87
	10 minutes	1.49		10 minutes	≥3.38
		1.27			≥2.98
		1.15			≥2.87
	15 minutes	1.49		15 minutes	≥3.38
		1.15			≥2.91
		1.18			≥2.92
	30 minutes	2.91		30 minutes	≥3.38
		1.91			≥2.91
		----			≥2.92
	60 minutes	2.42		60 minutes	≥3.22
		≥3.18			≥2.97
		----			≥2.87
	3 minutes (unit off)	0.61			
	10 minutes (unit off)	1.18			

Summary

The Aerus technology inactivated airborne SARS-CoV-2 to undetectable levels. The results show that, when accounting for the LLD, the percent reduction in virus was ≥99.87 to ≥99.96%; however, since no virus was detected after using the experimental device, the true percent reduction was likely greater than 99.99% in every case. The true net reduction could not be determined due to the LLD of the quantitation assay, but this too was likely greater than 99.99%.

Appendix

UTMB/Aerus Medical, LLC

Raw data

Plate #	Sample	Sample Description	Run Date	Plaque Assay Date	Stain & Read Date	Dilution Read (10^{-x})	PFU Count	µl/Well	PFU/ml	Log_{10} PFU/ml
1	post-nebulization	Nebulizer #1	11/16/2020	11/16/2020	11/18/2020	4	442	250	1.77E+07	7.25
2	post-nebulization	Nebulizer #2	11/16/2020	11/16/2020	11/18/2020	4	389	250	1.56E+07	7.19
3	post-nebulization	Nebulizer #3	11/16/2020	11/16/2020	11/18/2020	4	402	250	1.61E+07	7.21
4	post-nebulization	Nebulizer #4	11/16/2020	11/16/2020	11/18/2020	4	370	250	1.48E+07	7.17
5	post-nebulization	Nebulizer #5	11/16/2020	11/16/2020	11/18/2020	4	270	250	1.08E+07	7.03
6	post-nebulization	Nebulizer #6	11/16/2020	11/16/2020	11/18/2020	4	297	250	1.19E+07	7.07
7	0-minute experimental	BioAnalyzer #1-1	11/16/2020	11/16/2020	11/18/2020	3	24	250	9.60E+04	4.98
8	3-minute experimental	BioAnalyzer #1-2	11/16/2020	11/16/2020	11/18/2020	1	1	250	4.00E+01	1.60
9	10-minute experimental	BioAnalyzer #1-3	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
10	0-minute experimental	BioAnalyzer #2-1	11/16/2020	11/16/2020	11/18/2020	3	24	250	9.60E+04	4.98
11	15-minute experimental	BioAnalyzer #2-2	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
12	30-minute experimental	BioAnalyzer #2-3	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
13	0-minute experimental	BioAnalyzer #3-1	11/16/2020	11/16/2020	11/18/2020	2	166	250	6.64E+04	4.82
14	60-minute experimental	BioAnalyzer #3-2	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
15	0-minute control	BioAnalyzer #4-1	11/16/2020	11/16/2020	11/18/2020	2	104	250	4.16E+04	4.62
16	3-minute control	BioAnalyzer #4-2	11/16/2020	11/16/2020	11/18/2020	1	151	250	6.04E+03	3.78
17	10-minute control	BioAnalyzer #4-3	11/16/2020	11/16/2020	11/18/2020	1	34	250	1.36E+03	3.13
18	0-minute control	BioAnalyzer #5-1	11/16/2020	11/16/2020	11/18/2020	2	80	250	3.20E+04	4.51
19	15-minute control	BioAnalyzer #5-2	11/16/2020	11/16/2020	11/18/2020	1	26	250	1.04E+03	3.02
20	30-minute control	BioAnalyzer #5-3	11/16/2020	11/16/2020	11/18/2020	1	1	250	4.00E+01	1.60
21	0-minute control	BioAnalyzer #6-1	11/16/2020	11/16/2020	11/18/2020	2	79	250	3.16E+04	4.50
22	60-minute control	BioAnalyzer #6-2	11/16/2020	11/16/2020	11/18/2020	1	3	250	1.20E+02	2.08
23	post-nebulization	Nebulizer #1	11/17/2020	11/17/2020	11/19/2020	5	70	250	2.80E+07	7.45
24	post-nebulization	Nebulizer #2	11/17/2020	11/17/2020	11/19/2020	5	62	250	2.48E+07	7.39
25	post-nebulization	Nebulizer #3	11/17/2020	11/17/2020	11/19/2020	5	68	250	2.72E+07	7.43
26	post-nebulization	Nebulizer #4	11/17/2020	11/17/2020	11/19/2020	5	37	250	1.48E+07	7.17

27	post-nebulization	Nebulizer #5	11/17/2020	11/17/2020	11/19/2020	5	91	250	3.64E+07	7.56
28	post-nebulization	Nebulizer #6	11/17/2020	11/17/2020	11/19/2020	5	79	250	3.16E+07	7.50
29	0-minute experimental	BioAnalyzer #1-1	11/17/2020	11/17/2020	11/19/2020	2	96	250	3.84E+04	4.58
30	3-minute experimental	BioAnalyzer #1-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
31	10-minute experimental	BioAnalyzer #1-3	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
32	0-minute experimental	BioAnalyzer #2-1	11/17/2020	11/17/2020	11/19/2020	2	81	250	3.24E+04	4.51
33	15-minute experimental	BioAnalyzer #2-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
34	30-minute experimental	BioAnalyzer #2-3	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
35	0-minute experimental	BioAnalyzer #3-1	11/17/2020	11/17/2020	11/19/2020	2	93	250	3.72E+04	4.57
36	60-minute experimental	BioAnalyzer #3-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
37	0-minute control	BioAnalyzer #4-1	11/17/2020	11/17/2020	11/19/2020	2	115	250	4.60E+04	4.66
38	3-minute control unit off	BioAnalyzer #4-2	11/17/2020	11/17/2020	11/19/2020	2	28	250	1.12E+04	4.05
39	10-minute control unit off	BioAnalyzer #4-3	11/17/2020	11/17/2020	11/19/2020	1	75	250	3.00E+03	3.48
40	0-minute control	BioAnalyzer #5-1	11/17/2020	11/17/2020	11/19/2020	2	155	250	6.20E+04	4.79
41	3-minute control	BioAnalyzer #5-2	11/17/2020	11/17/2020	11/19/2020	2	32	250	1.28E+04	4.11
42	10-minute control	BioAnalyzer #5-3	11/17/2020	11/17/2020	11/19/2020	1	83	250	3.32E+03	3.52
43	0-minute control	BioAnalyzer #6-1	11/17/2020	11/17/2020	11/19/2020	2	152	250	6.08E+04	4.78
44	60-minute control	BioAnalyzer #6-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
45	post-nebulization	Nebulizer #1	11/19/2020	11/19/2020	11/21/2020	4	232	250	9.28E+06	6.97
46	post-nebulization	Nebulizer #2	11/19/2020	11/19/2020	11/21/2020	4	192	250	7.68E+06	6.89
47	post-nebulization	Nebulizer #3	11/19/2020	11/19/2020	11/21/2020	4	202	250	8.08E+06	6.91
48	post-nebulization	Nebulizer #4	11/19/2020	11/19/2020	11/21/2020	4	236	250	9.44E+06	6.97
49	post-nebulization	Nebulizer #5	11/19/2020	11/19/2020	11/21/2020	5	18	250	7.20E+06	6.86
50	post-nebulization	Nebulizer #6	11/19/2020	11/19/2020	11/21/2020	4	141	250	5.64E+06	6.75
51	0-minute experimental	BioAnalyzer #1-1	11/19/2020	11/19/2020	11/21/2020	2	74	250	2.96E+04	4.47
52	3-minute experimental	BioAnalyzer #1-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
53	10-minute experimental	BioAnalyzer #1-3	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
54	0-minute experimental	BioAnalyzer #2-1	11/19/2020	11/19/2020	11/21/2020	2	82	250	3.28E+04	4.52
55	15-minute experimental	BioAnalyzer #2-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
56	30-minute experimental	BioAnalyzer #2-3	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
57	0-minute experimental	BioAnalyzer #3-1	11/19/2020	11/19/2020	11/21/2020	2	74	250	2.96E+04	4.47

58	60-minute experimental	BioAnalyzer #3-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
59	0-minute control	BioAnalyzer #4-1	11/19/2020	11/19/2020	11/21/2020	2	52	250	2.08E+04	4.32
60	3-minute control	BioAnalyzer #4-2	11/19/2020	11/19/2020	11/21/2020	1	128	250	5.12E+03	3.71
61	10-minute control	BioAnalyzer #4-3	11/19/2020	11/19/2020	11/21/2020	1	37	250	1.48E+03	3.17
62	0-minute control	BioAnalyzer #5-1	11/19/2020	11/19/2020	11/21/2020	2	71	250	2.84E+04	4.45
63	15-minute control	BioAnalyzer #5-2	11/19/2020	11/19/2020	11/21/2020	1	22	250	8.80E+02	2.94
64	0-minute control	BioAnalyzer #6-1	11/19/2020	11/19/2020	11/21/2020	2	49	250	1.96E+04	4.29
65	15-minute control	BioAnalyzer #6-2	11/19/2020	11/19/2020	11/21/2020	1	32	250	1.28E+03	3.11
66	30-minute control	BioAnalyzer #6-3	11/19/2020	11/19/2020	11/21/2020	1	6	250	2.40E+02	2.38

ActivePure®
TECHNOLOGY

MRI GLOBAL TESTING ON PRODUCT EFFICACY

MRIGlobal

The science you expect.
The people you know.

Verification of the Effectiveness of ActivePure® Technology in Decontamination of SARS-CoV-2

Final Report

For

Aerus, LLC

14841 Dallas Parkway
Aberdeen Bldg., Suite 500
Dallas, Texas 75254

MRIGlobal Project No. 311624.01.001

August 13, 2020

Preface

This final report was prepared at MRIGlobal (MRIGlobal) for the work performed under MRIGlobal Task No. 311624.01.001, "Verification of the Effectiveness of ActivePure® Technology in Decontamination of SARS-CoV-2"

Test devices were supplied to MRIGlobal by Aerus, LLC for the conduct of the program. The experimental phase of this task was initiated by MRIGlobal on May 18, 2020 and ended on August 2, 2020.

The Study Director of the program was Rick Tuttle. Execution of the study was assisted by Carl Gelhaus, Ph.D., Luca Popescu, Ph.D., Kristen Solocinski, Ph.D., Sam Humphries, and managed by William Sosna.

The studies were performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal or approved laboratory procedures, and any deviations were documented.

MRIGLOBAL

Richard Tuttle

Rick Tuttle
Study Director

Approved by:

Claire L Cantrell

for Ed Sistrunk
Division Director
Medical Countermeasures

August 13, 2020

Contents

Figures

Tables

Section 1.
Objective

The emergent threat of COVID-19 infection originating from SARS-CoV-2 and the high rate of transmission associated severe illness and fatalities, has created a needed response for rapid development and evaluation of effective countermeasures. In response to testing for Aerus, LLC, MRIGlobal conducted testing and evaluation of Aerus, LLC's whole room air disinfection system with ActivePure® Technology. The ActivePure® room disinfection system uses free oxygen and water molecules in the air that are pulled through a honeycomb matrix. The technology creates powerful oxidizers that are released back into the room which destroy biological bacterial and viral contaminants. The ActivePure® whole room disinfection system was evaluated in independent surface destruction tests of SARS-CoV-2 (Washington Isolate Strain) in laboratory trials at MRIGlobal.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor

Aerus, LLC
14841 Dallas Parkway
Aberdeen Bldg., Suite 500
Dallas, TX 75254

2.2 Sponsor's Representative

Andrew Eide
Vice President, Product Development and Manufacturing

2.3 Testing Laboratories

MRIGlobal
425 Volker Boulevard
Kansas City, MO 64110
Phone: (816) 753-7600
Fax: (816) 753-8823

2.4 Personnel Responsibilities

Study Director—MRIGlobal

Rick Tuttle
(816) 753-7600, Ext. 5752
Email: rtuttle@mriglobal.org

Section 3.
Test Systems and Methods

3.1 Equipment

Test Equipment

The ActivePure whole room disinfection system uses ActivePure® Technology and is a portable air purification system with dimensions of approximately 13" D × 13" W × 22" H. The system generates powerful oxidants that destroy bacteria, virus and odors without the use of chemicals. The system recirculates air in room environments at desired volumetric flow settings of 300 cfm using fan forced air flow. The system is designed to purify air and surfaces in rooms up to 3000 ft^3. As air enters the system, oxygen and water molecules in the air enter the unit through a honeycomb matrix which converts the molecules to powerful oxidizers that are released back into the room which destroy biological bacterial and viral contaminants. The ActivePure® unit was provided to MRIGlobal by Aerus, LLC and was set for single speed air recirculation flow operation (300 cfm). The unit was also equipped with an on/off power switch and an ActivePure® on/off switch for selection between blower only operation, or combined blower and ActivePure® operation for testing.

SARS-CoV-2 (USA-WA1/2020) was obtained from The University of Texas Medical Branch (UTMB) from an isolate of a patient who traveled to an infected region of China and developed the clinical disease (COVID-19) January 2020 in Washington, USA. The complete genome of USA-WA1/2020 has been sequenced. The Isolate-GenBank: MN985325 and after one passage in in Vero cells GenBank: MT020880. The complete genome of SARS-CoV-2 strain USA-WA1/2020 has been sequenced after four passages in collaboration with Database for Reference Grade Microbial Sequence (FDA-ARGOS; GenBank: MT246667). Each vial used on study contains approximately 0.5 mL of cell lysate and supernatant from Cercopithecus aethiops kidney cells infected with SARS-CoV-2 isolate USA-WA1/2020.

3.2 Methods

Testing Description

MRIGlobal conducted testing characterization of a single ActivePure® portable air purification system in surface decontamination trials to evaluate the log reduction destructive kill effectiveness against an envelope virus (SARS-CoV-2) strain USA-WA1/2020. All tests were conducted in a biological class 3 facility at MRIGlobal, Kansas City, MO. The biological safety cabinet has internal dimensions of 6'W × 4'D × 4'H, with a displacement volume of approximately 96 ft^3. The cabinet is annually pressure decay tested for leak free integrity, and certified for safety. For testing of the ActivePure® unit, the cabinet was sealed with the exhaust, and filter air inlet vents capped with gasketed steel plates for isolation and testing under static conditions without cabinet flow. The ActivePure® unit was positioned in the center of the biosafety cabinet with position marks drawn on the bottom of the cabinet for proper placement and alignment preceding each test. The unit was tested for viral surface destruction efficacy using sterile 1" × 3" × 1 mm stainless steel test coupons inoculated with SARS-CoV-2. Test coupons were each inoculated with a 200 µL of SARS-CoV-2 stock suspension in a sterile class 2 biological safety cabinet. Individual test coupons were placed in test identification labeled

sterile petri dishes and inoculated from a standard stock viral suspension with 200 mL of SARS-CoV-2 virus using a calibrated micropipette. The viral suspension was then evenly coated over the test coupons surface using sterile cell spreaders. Coated test coupons were air dried at standard laboratory conditions in the biological level 2 safety cabinet prior to exposure tests. Additional positive control coupons were similarly prepared and were subjected to the same environmental conditions and time course as test coupons without being subjected to ActivePure® Technology exposure. The positive control coupons served as viral concentration standards to define the efficacy of the system in deactivating the SARS-CoV-2 virus from test coupons.

For each test, a set of three SARS-CoV-2 inoculated test coupons were placed on the floor of the biosafety cabinet at the exhaust outlet of the ActivePure® unit, and at a 45° offset angle from the outer housing of the blower to avoid direct air flow turbulence. A diagram of the test setup is shown in Figure 1.

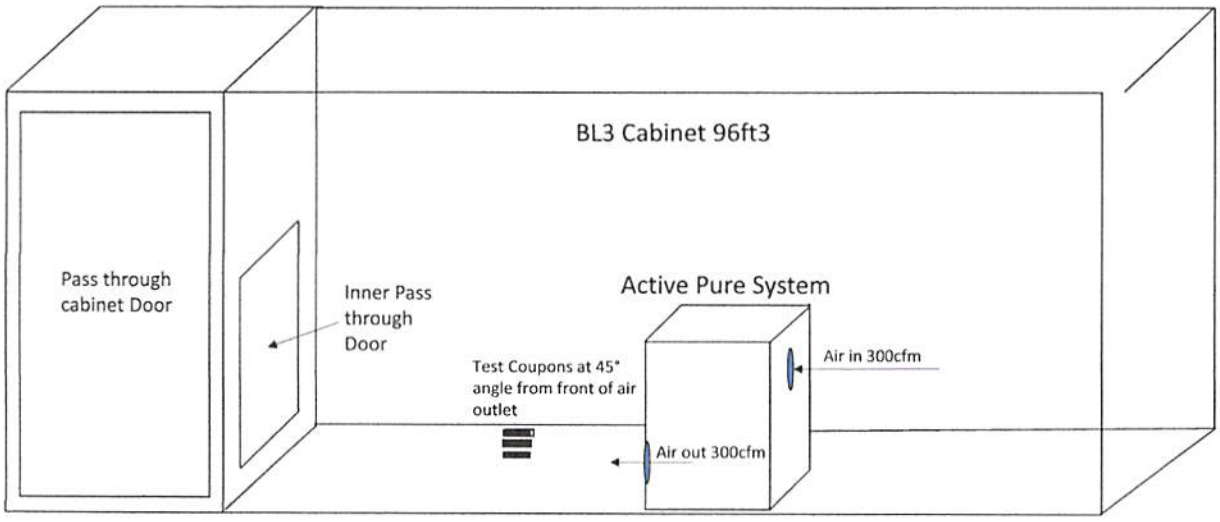

Figure 1. Diagram of ActivePure® Test System

Tests were conducted over four (4) exposure times of 1, 3, 6 and 7 hours. Test coupons were subjected to the ActivePure® technology operation during the exposure process. Positive control coupons were subjected to the same exposure time course with only the unit blower flow operational without ActivePure® technology operational. This provided a common and standardized test control for all system tests over each timecourse, and accurate assessment of the test units ActivePure® technology viral deactivation efficacy. A test matrix is shown in Table 1.

Table 1. ActivePure® Test Matrix

System operating condition	System Flow Volume (cfm)	Test virus	Exposure time (hours)	Number of coupons	Coupon viral inoculation volume (µl)	Coupon DMEM extraction vol (ml)	Total samples/ Test	Number of tests	Total Sample assays
With ActivePure	300	SARS - CoV – 2	1	3	200	2	3	1	3
Without ActivePure	300	SARS - CoV – 2	1	3	200	2	3	1	3
With ActivePure	300	SARS - CoV – 2	3	3	200	2	3	1	3
Without ActivePure	300	SARS - CoV – 2	3	3	200	2	3	1	3
With ActivePure	300	SARS - CoV – 2	6	3	200	2	3	1	3
Without ActivePure	300	SARS - CoV – 2	6	3	200	2	3	1	3
*Without ActivePure	300	SARS - CoV – 2	*7	3	200	2	3	1	3
*With ActivePure	300	SARS - CoV – 2	*7	3	200	2	3	1	3

* Added additional humidity above ambient lab humidity

Section 4.
Sample Analysis and Results

Stock virus used for test and control coupon inoculation (SARS-CoV-2, strain USA-WA1/2020) were concentration titered by serial dilution to obtain the 50% tissue culture infectious dose ($TCID_{50}$). This was conducted to ensure that sufficient concentration and quantity of virus were available for testing. For cell and virus cultures, sterile DMEM (Mediatech) supplemented with 7% fetal bovine serum (HyClone), GlutaMax (Gibco), and penicillin-streptomycin-neomycin antibiotic mixture (Gibco) were utilized. Vero E6 cells (monkey kidney cells obtained from ATCC (CRL-1586) were used for assays with ASFV. All cells were maintained at 36°-38°C and 5% CO_2 in a humidified atmosphere, and cells were seeded into flasks for propagation and expanded into 96 well plates for titration of SARS-CoV-2 virus. Cells were infected with viral coupon sample extractions at 70% confluence and observed for the presence of cytopathic effect (CPE) for four (4) to five (5) days post-infection. A 10X serial dilution of coupon sample viral extractions were applied to cell assay plates at up to an 8 log dilution factor for the presence of viral growth into the plate host cells. Plates were inoculated with 5 replicate samples at each dilution level, with each row of replicates 10x more dilute than that used in the preceding row for viral cell infectivity detection. Viral propagation plate readings were conducted under high intensity magnification of each plate cell for viral host cell infectivity and recorded on a sample test log for positive (+) or negative (-) viral propagation. Data was entered into a Reed Muench calculation for sample concentration measurement and determination of the $TCID_{50}$ (50% tissue culture infectious dose of a virus).

Test Results:

Coupon preparation including SARS-CoV-2 inoculation, drying, exposure testing, extractions, and cell assay plating were conducted in a sterile class 2 biological safety cabinet. Testing for 1, 3, and 6 hour tests with the ActivePure® technology were conducted in the BL3 cabinet without the addition of humidity. An additional test over a 7 hour ActivePure® technology exposure period was conducted on 8/2/2020, with the humidity maintained in the test cabinet using a Honeywell digital humidity monitor with humidity level setpoint control that provided a continuous and regulated humidity level of 63% RH (relative humidity) during the test. Following a 4 day plate assay viral incubation period, plates were read for viral infectivity and data recorded on $TCID_{50}$ test logs. Results were entered into a Reed Muench data analysis program for results and comparison of positive test control sample viral titer coupon concentrations to ActivePure®exposed test coupon results. A plot showing the averaged log reduction efficacy of the ActivePure® Technology in deactivating a set of three (3) SARS-CoV-2 infected stainless steel coupons. The log reduction data shows the averaged test coupon viral deactivation in relation to the averaged positive control coupons (non- ActivePure® exposured) over each exposure period, and is shown in Figure 2.

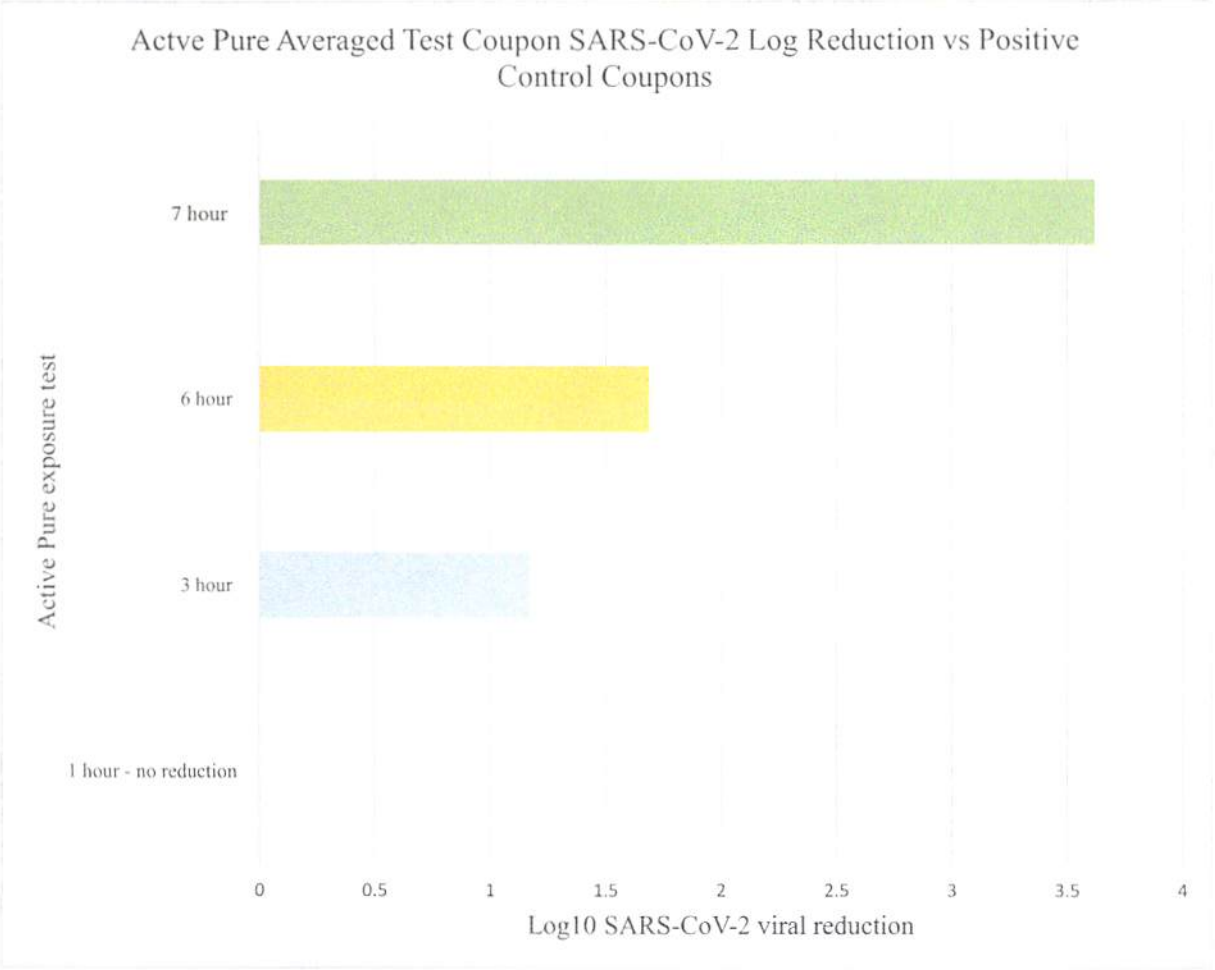

Figure 2. Test Results for ActivePure® SARS-CoV-2 log reduction Efficacy

Laboratory environmental conditions in MRIGlobals BSL3 laboratories are monitored continually and data logged using an Amegaview data capture system. The laboratory temperature and humidity conditions for each test, and test cabinet %RH (if applicable) as well as the log and percent reduction efficacy of the ActivePure® unit are shown in Table 2.

Table 2. Tabulated Test Results, Test Parameters and Environmental Conditions

Test Date	Active Pure exposure time (hr)	Lab Temperature (°F)	Lab Humidity (%RH)	BL3 Test Cabinet Humidity (%RH)	Averaged SARS-CoV-2 Log viable reduction	Averaged SARS-CoV-2 viable reduction (%)
5/17/2020	1	72	47	NA	0	0
6/15/2020	3	71.5	55	NA	1.17	93.27
6/15/2020	6	71.5	55	NA	1.69	97.95
8/2/2020	7	72	52	63	3.62	99.98

Testing of the ActivePure® unit showed substantial viral reduction of SARS-CoV-2 on test coupons for the 3 and 6 hour tests with results of 93.27%, and 97.95% respectively. The 7 hour test included controlled humidification of the test cabinet at 63% RH throughout the test with a viable reduction of 99.98% of SARS-CoV-2 on test coupons. It is theorized that the humidity levels in the test cabinet (air tight seal), may have reduced over the ActivePure® operation and the ActivePure® production of powerful oxidizers may have reduced or depleted during 1, 3, and 6 hour testing, thus reducing effectiveness against SARS-CoV-2. The 7 hour test, with the addition of humidity control regulated at 63% showed almost a 2 log viral kill increase over the 6 hour test, and approximately a 2.5 log increased virus kill in relation to the 3 hour test. 1 hour exposure test showed no viral deactivation efficacy of the ActivePure® system in reducing test coupon viral concentrations in relation to positive control coupons.

Section 5.
Quality Assurance

5.1 Type of Study

This study was non-GLP, however all work was executed using established SOPs, at MRIGlobal in Kansas City, MO and all procedures utilized were technically valid in accordance with MRIGlobal Standard Operating Procedures and/or laboratory procedures.

5.2 Standard Operating Procedures

The study was performed according to the relevant standard operating procedures and/or laboratory procedures of MRIGlobal.

Section 6.
Location of Study Data

Exact copies of all raw data, correspondence, records, final protocol, amendments, and deviations, and any other study documentation necessary for reconstruction of the study will be archived at MRIGlobal. All raw data (including original study records, data sheets, work sheets, and computer printouts) will be archived by MRIGlobal.

ActivePure® TECHNOLOGY

What to Look for When Purchasing an Air Quality Solution:

Are you committed to providing the safest air possible for those in your care? ActivePure is the only technology that meets **ALL** of the Surgeon General's requirements.

SAFETY

1. Has the FDA cleared any devices with this technology?
2. Is it safe to use in spaces occupied by people and pets?

CONVENIENCE & QUALITY

3. Can the technology be configured to work in occupied areas, including hard-to-treat spaces such as elevators, stairwells, and restrooms?
4. Will the manufacturer guarantee product claims and back them up with field testing?
5. Is the cost of installing and using the technology competitive with other options?

EFFECTIVENESS

6. Is the technology proven to inactivate, not just filter, the virus that causes COVID?
7. Is the technology effective against viruses, bacteria, and other pathogens in the air and on surfaces?
8. Does the technology work continuously 24/7, providing protection as infected people enter a space?
9. Were the tests proving its efficacy done by independent, FDA-compliant labs?
10. Has the technology been proven in challenging real-world settings?

Choosing the right technology to protect against the COVID-19 virus and other pathogens is not easy.

Unlike other technologies, ActivePure has been extensively tested and is proven to fight the spread of disease by proactively inactivating pathogens on surfaces and in the air, including the virus that causes COVID-19. With both portable and installed products, you can adapt ActivePure to any space: from a small unit for home or office to larger capacity solutions that can cover auditoriums covering tens of thousands of square feet.

Learn more at **activepure.com/10questions**