

# AD Series Ultraviolet Germicidal Irradiation System:

# Testing and Estimated Efficacy Against the SARS-CoV-2 Coronavirus

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## Abstract

The Wayclear AD Series Ultraviolet Germicidal Irradiation System is designed for disinfection of air in a variety of settings. It is a continuous-use device that uses fans to move air through the device where it is exposed to high-intensity UVC light generated by an array of energy-efficient LEDs. UVC light is an effective disinfectant of many pathogens, and the COVID-19 pandemic has generated a particular interest in this type of disinfection system. This document provides background information about SARS-CoV-2 virus, the causative agent of the COVID-19 pandemic, and a technical description of the germicidal effect of UV light. An efficacy study was designed to examine the disinfection capability of AD Series Systems against virus contaminated aerosols. This study used the MS2 virus as a surrogate in place of SARS-CoV-2, due to biosafety concerns. The results indicate that the device provides highly effective disinfection of aerosolized virus. After 30 minutes, >99% inactivation rates were achieved. This increased to >99.99% after 120 minutes. Because the MS2 virus used as a surrogate in the study is predicted to be much more resistant to UV inactivation than SARS-CoV-2, the effectiveness of the AD Series in disinfection of SARS-CoV-2-contaminated air was estimated. Using these estimates, it is predicted that AD Series Systems would result in >99.999% inactivation in as little as 10 minutes, under conditions similar to the efficacy study, greatly reducing the potential transmission risk of this dangerous infection.

# The Wayclear AD Series Ultraviolet Disinfection System Products

Wayclear AD Series UV Disinfection Systems are wall-mounted, scalable, ultraviolet (UV) disinfection devices for inactivating aerosolized pathogens. The devices use several small fans to circulate air through it, where it is exposed to UV light. As air passes through the AD system, it is exposed to high-intensity UV light to disinfect aero-solized pathogens. The system uses energy-efficient light-emitting diodes (LEDs) to generate light in the UV-C range at a 275nm wavelength. AD Series Systems are designed for continuous use and to apply an inactivation dose to the majority of the air column as it passes through the device. The UV light produced is contained within the unit, providing protection to any occupants of the room in which it is installed. Units can be used to disinfect rooms up to 10,000 ft.<sup>2</sup>

While UV-C light produced by this system is effective at inactivating a wide array of aerosolized pathogens, there is a particular interest in the use of this technology to reduce the risk of transmission of SARS-CoV-2, the causative agent of the COVID-19 pandemic.

## **COVID-19 Background**

The Coronavirus Disease 2019 (COVID-19) pandemic is a major health emergency unlike any other public health crisis we have faced in the past 100 years. By early September 2020, approximately nine months after the disease was identified, there have been almost 900,000 reported deaths worldwide, including nearly 190,000 in the United States (JHCRC 2020). It is estimated that between 2 and 4 million people worldwide will die from COVID-19 before the end of 2020 (UWIMHE 2020). In addition to the devastating public health aspects of this pandemic, the disease has had serious consequences in the lives of most people and significant economic impacts, both globally and at the local level. It is likely that COVID-19 will continue to affect us for years to come (Scudellari 2020).

The virus that causes COVID-19 has been officially designated as SARS-CoV-2. It belongs to the Coronavirodae family of viruses, so named because the spherical virus particles resemble the solar corona (Yang 2020). They are single-strand RNA (ssRNA) viruses, as compared to double-stranded RNA (dsRNA) or single/double-stranded DNA (ssDNA/dsDNA) viruses (Astuti 2020). The ssRNA genome is encased in a protein shell, known as the nucleocapsid. The capsid is then encompassed by lipid bilayer, similar to the cell membrane present in cellular organisms (Astuti 2020). This lipid bilayer contains viral proteins and glycoproteins that bind to receptors on host cells, which initiates invasion and the start of the viral life cycle (**Figure 1**).

Coronaviruses, like all other viruses, have a unique life cycle compared to other pathogens where replication is reliant upon infecting a host cell for replication (**Figure 2**).







Coronaviruses are well known and are often responsible for mild respiratory illnesses, such as the common cold (Mesel-Lemoine 2012). SARS-CoV-2 is a novel strain that causes the much more severe disease seen in some COVID-19 patients. The common symptoms of COVID-19 include fever and chills, a dry cough, shortness of breath, fatigue, headache, muscle and body aches, loss of taste and smell, nasal congestion, nausea or vomiting, and diarrhea. Less common symptoms can include encephalitis, liver or kidney failure, and heart or other cardiovascular issues (CDC 2020). Symptomology varies greatly, with some people having very mild illness or no symptoms at all while others suffer severe disease that may require hospitalization or result in death. Generally, older patients suffer from more severe disease than younger patients, but it is difficult to predict how the disease will progress in an individual patient.

Preventing the transmission of the virus is the key to keeping the pandemic under control and avoiding localized outbreaks. This takes a multi-faceted approach, which includes social distancing, face coverings, and disinfection procedures to directly inactivate viral particles. Disinfection is particularly important in healthcare settings, workplaces, schools, and other facilities where there is a high risk of disease transmission and where social distancing may face logistical issues and other challenges. Ultraviolet Germicidal Irradiation (UVGI) is an effective method of disinfection for both surfaces and aerosolized virus present in air.

#### **Ultraviolet Germicidal Disinfection**

Ultraviolet (UV) light is a form of electromagnetic radiation at wavelengths from 100nm to 400nm. It falls between X-ray and visible light on the electromagnetic spectrum (**Figure 3**). Within the UV spectrum, it can be divided into three groups: UV-A (320–400nm), UV-B (280–320nm), and UV-C (100–280nm). As with all electromagnetic radiation, the shorter the wavelength, the greater the energy. UV light, across a range of wave-

lengths, has damaging biological effects. Taking advantage of this, UV has been used extensively to disinfect surfaces, air, and water (Brickner 2003, Cutler 2011) - otherwise known as UVGI.

UV disinfection is is an attractive method, as it does not involve harsh and dangerous chemicals and leaves no residue behind after disinfection. Generally, UVC light is used for UVGI applications, as it has the greatest effectiveness (**Figure 4**).





Sources of light for UVGI have historically included various types of mercury and metal halide vapor lamps and, more recently, light-emitting diodes (LEDs). LEDs are an attractive option for this purpose, as they are more energy efficient, have a much longer service life than traditional arc lamps, and do not contain hazardous materials.

The damaging effects of UV light have been well characterized and are universal across all forms of life. Bacteria and viruses are particularly susceptible, owing to their small size, simple genomes, the lack of protective physical barriers to exposure, and the absence of complex mechanisms to repair damage (Yu 2017).



The main driver of UVGI is direct damage of the pathogen genome, and both RNA and DNA are affected. This process has been well characterized (Mullenders 1993, Friedburg 2003, Pfeifer 1997). The most effective wavelength (approximately 254nm) for UVGI matches the maximum absorption of DNA and RNA, demonstrating that damage of these nucleic acids is the most important factor for germicidal activity (Anderson 2000). The structure of DNA and RNA is compared in **Figure 5**. The main UV-induced effect is a covalently bonded linkage between adjacent Cytosine (C) or Thymine (T) / Uracil (U) nucleobases (Mullenders 1993, Friedburg 2003, Cutler 2011). Possible sequence combinations are C-C, C-T, and T-T in DNA and C-C, C-U, and U-U, in RNA. These linkages are called dimers, and two types commonly result from UV exposure, cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PP) (Pfeifer 1997, Quinet 2018). They are formed when a photon of UV light (essentially a packet of electromagnetic radiation), strikes the nucleic acid structure, and the transferred energy is used in the creation of new chemical bonds that link the adjacent nucleobases. An example of the molecular structure of UV-induced dimers is shown in **Figure 6**.



Figure 5. Comparison of DNA and RNA. DNA is double helix molecule composed of A, C, G, and T nucleobases. RNA is a single helix composed of A, C, G, and U nucleobases.

The exact structure of this UV-induced nucleic acid damage is different depending on the bases involved; the overall molecular structure, however, is the same. The biological effect of this dimerization is a change in the three-dimensional structure of the nucleic acid (Figure 7). This change in the threedimensional structure renders the affected microbe or virus biologically inactive. In order for a microbe or virus to cause infection, it must be able to replicate nucleic acids, which allows it to infect new tissues or cells and spread to new hosts. Enzymes called polymerases move along the nucleic acid strand to create a copy that is assembled in a new virus particle or to encode viral proteins. The conformational change shown in Figure 7 interferes with the ability of polymerase enzymes to move along the nucleic acid strand, preventing replication, thereby "killing" the microbe or virus (Cutler 2011).

Additionally, high intensity UV light exposure can introduce nucleic acid strand breaks and produce free radicals that cause oxidative damage (Freidberg 2003, Panikkanvalappil 2013a, Panikkanvalappil 2013b).

Proteins can also be affected by UV light exposure, resulting in unfolding and decay of the protein structure (Abaskharon 2016, Balanikas 2020). This can result in interruption of cellular processes and destabilization of the membrane or lysis when embedded proteins become unfolded. Generally, this type of damage requires exposure to UV light intensities and exposures that are not necessary for effective disinfection.

The effects caused by UV light on microbes and viruses is universal, but the dose required for effective disinfection is dependent on the organism itself and the carrier (aerosols, surfaces, solutions, etc.) (Tseng 2005, Tseng 2007).



**Figure 6.** CP and 6-4 PP dimers formed after UV exposure between adjacent thymine bases. Before UV exposure, each nucleobase is independent. After exposure the bases are linked together. (Adapted from Quinet 2018).

Viruses require a smaller dose than bacteria, although the required dose for inactivation varies among different viruses. In general, single-stranded DNA/RNA viruses (such as SARS-CoV-2) are more susceptible than double-stranded viruses (Tseng 2005, Tseng 2007). Bacteria have a wide-ranging susceptibility to UV disinfection, with spores being the most highly resistant (Chang 1985). Aerosols require a smaller dose than solutions or surfaces, likely due to scattering and absorption of the light (Brickner 2003). Similarly, the relative humidity of aerosols affects the required dose for inactivation, with higher humidity aerosols requiring a larger dose for equal disinfection, which may be related to absorption of photons by water molecules (Ko 2000, Peccia 2001, Tseng 2005).

The concept of UVGI is best measured at the population level, as there are technological challenges to measuring the effect of UV on an individual bacterial cell or virus particle, where the effects are at the molecular level and not easily observed. Because of this, studies of UVGI rely upon detecting the reduction in a population, which is much easier to directly examine. This brings up the concept of a UV inactivation dose, which is the UV irradiance dose required to reduce a microbe or virus population by a set amount, 90% or 99%, for instance. This allows for a standard comparison of the UV dose required to inactivate different microbes or viruses.

The current COVID-19 pandemic has generated much interest in UVGI applications where health care facilities, schools, business, etc., need effective disinfection measures to help prevent spread of the SARS-CoV-2 virus. Many recent academic publications have been focused on determining the effectiveness of UVGI for SARS-CoV-2 inactivation, particularly for aerosols (Beggs 2020, Nardell 2020, Ragan 2020). While these studies are difficult to conduct using a Biosafety level 3 pathogen, preliminary studies and those conducted using related viruses suggest that SARS-CoV-2 is likely highly susceptible to UV inactivation (Beggs 2020).

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#### Laboratory Testing of the AD Series System

The antiviral activity and efficacy of the Wayclear AD Series System was tested at the Microchem Laboratory in Round Rock, TX - specifically, an AD-1 model. A custom testing procedure was developed by Microchem for this device. In this study, purified Escherichia virus MS2 (ATCC 15597-B1) was used as the test organism. The MS2 virus is commonly used in laboratory testing of disinfectants. Both SARS-CoV-2 and the MS2 virus are ssRNA viruses, making MS2 an appropriate surrogate for this experiment. A laboratory strain of Escherichia coli (ATCC 15597) was used as the host for viral infection. In this type of virus assay, the host bacteria is added directly to the molten agar prior to pouring the plates, provid-

ing for uniform distribution of the host throughout the medium. When a virus sample is applied to the surface of the medium, the virus infects the host bacteria, which results in the inhibition of bacterial growth, visualized as an area of clearing on the plate where host is unable to grow. These areas of clearing are known as plaques. By counting plaques and multiplying by the dilution factor, the number of plaque-forming units (PFU) in the original sample can be quantified. Antiviral activity is determined by comparison of the number of PFUs in test samples collected at various time points to the control samples.

The testing procedure was performed in a sealed chamber representing a small room, with two AD-1 Systems installed. A sample of purified MS2 virus was mixed with phosphate-buffered saline (PBS) and nebulized in the sealed testing chamber over the course of 60 minutes. After nebulization, the AD-1 Systems were powered on and allowed to operate. Air samples at time zero and at 30, 60, 80, and 120 minutes post nebulization were collected using an impinger to capture airborne virus particles and suspend them in PBS. A dilution of the suspended samples was plated on 50% tryptic soy agar (TSA) with E. coli added to the media and incubated at 36°C for 12–18 hours. PFUs were counted and compared to samples obtained from a control run. The results for the AD-1 testing study are shown below in **Table 1**:

		PFU/CUBIC METER	MEAN PFU/ CUBIC METER	PERCENT REDUCTION
Time Zero	Replicate 1	6.25E+08	5.57E+08	0.000%
	Replicate 2	4.88E+08		
30 Minutes	Replicate 1	4.03E+06	4.34E+06	99.220%
	Replicate 2	4.64E+06		
60 Minutes	Replicate 1	1.57E+06	1.43E+06	99.740%
	Replicate 2	1.28E+06		
90 Minutes	Replicate 1	3.36E+05	2.59E+05	99.950%
	Replicate 2	1.82E+05		
120 Minutes	Replicate 1	4.66E+04	4.34E+04	99.992%
	Replicate 2	4.01E+04		

Table 1. Raw data and calculated percent reduction obtained from the AD-1 efficacy study.



Results indicate that after 30 minutes of exposure time, >99% of the virus has been inactivated. This increases incrementally to 99.99% after 120 minutes.

Biologists often use log reduction for easier visualization of the effectiveness, especially as the reduction approaches 100%. On a log scale, a 90.000% reduction is equal to 1 log, 99.000% is equal to 2 logs, 99.900% is equal to 3 logs, 99.990% is equal to 4 logs, 99.999% is equal to 5 logs, and so forth. A graph showing the log reduction for the AD-1 System across time points is shown in **Figure 6**.



The data shows that after 30 minutes of operating the AD-1 Systems in the confined testing space, the log reduction value was 2.11. This continues at an approximate linear rate across the subsequent timepoints, eventually resulting in a 4.11 log reduction at the 120-minute time point. Taken together, the results show that the AD-1 System is highly effective at inactivating aerosolized MS2 virus.

Additional testing of the AD-1 System will investigate the role of gravitational settling, natural inactivation of virus particles, and the role of humidity in the performance of the system.

# **Extrapolation of Testing Results for SARS-CoV-2**

As mentioned above, different viruses and microbes have varying susceptibilities to UVGI. This can be related to physical size, molecular weight, DNA conformation, propensity toward clumping, presence of repair enzymes, surface properties, index of refraction, and their individual DNA sequence (Kowalski 2009). By experimental determination of microbe or virus survival at a given time and at a known UV irradiation dose, the UV susceptibility constant (*k*) can be determined. The (*k*) constant is reported in cm2/mJ. The equation for this is shown below:

$$k = -1/H \times \ln(f)$$

Where *H* is the UV irradiation dose in mJ/cm<sup>2</sup> and f is the fraction of surviving microbe or virus at a given time.

The larger the (k) constant is, the more susceptible the microbe or virus is to UV inactivation. The (k) constant for the MS2 virus has been reported as 0.0064 cm<sup>2</sup>/mJ (Tseng 2007). Biosafety concerns make UVGI studies on SARS-CoV-2 difficult to perform directly, especially with aerosols, which carry the greatest risk of transmission. Studies have inferred the (k) constant of SARS-CoV-2 through examination of other coronaviruses. In Beggs et al. 2020, the estimated k value for SARS-CoV-2 was 3.770 cm<sup>2</sup>/mJ, based on earlier work with coronaviruses (Walker 2007). The authors, however, recommended using 0.3770 cm<sup>2</sup>/mJ for theoretical calculations as a worst-case scenario, because the actual (k) constant for SARS-CoV-2 was unknown and it is best to err on the side of caution (Beggs 2020).

By comparing these estimates, even in the worst-case scenario, the SARS-CoV-2 virus would be orders of magnitude more susceptible to UV inactivation than the MS2 virus. This indicates that the AD-1 system would be extremely effective against the SARS-CoV-2 virus, even in a highly concentrated environment.



The theoretical reduction of any virus or microbe after UVGI can be described using the following decay equation (McDevitt 2012):

$$N_{\tau} = N_0 \times e^{-k \times H}$$

Where N<sub>0</sub> is the initial number of active virus particles, N<sub>T</sub> is the number of active virus particles at a given time, k is the UV susceptibility constant of the virus, and H is the UV irradiation dose in mJ/cm<sup>2</sup>.

Using this equation, the inactivation dose required for a given reduction in SARS-CoV-2 would be approximately 60 times less than that required for the MS2 virus. **Figure 7**, shown below, demonstrates the comparison of the theoretical reduction of SARS-CoV-2 compared to the MS2 virus as the UV dose increases. In this comparison, the viral concentration starts at the same level and then shows a dramatic decline in the SARS-CoV-2 virus concentration as the dose increases, while the MS2 virus declines much less rapidly.

#### Conclusions

The results shown in this report indicate that the AD-1 Series is highly effective at inactivating aerosolized MS2 virus, which is suggested to be much more resistant to UV inactivation than the SARS-CoV-2 virus, the causative agent of the COVID-19 pandemic. Additionally, the viral concentrations tested in this study (~5.6E+08/m3) were much greater than SARS-CoV-2 viral concentration obtained from isolation wards housing COVID-19 patients, which were only ~4.0E+03/m3 (Guo 2020).



These results suggest that the AD Series would show increased effectiveness against the SARS-CoV-2 virus, especially in real-world scenarios, such as non-isolation healthcare settings, schools, and workplaces where the actual airborne viral load may be quite small. Considering the estimated susceptibility of SARS-CoV-2, a >99.999% (5-log) reduction would be expected after UV exposure times as little as 10 minutes with the AD-1 system. This high level of inactivation would greatly reduce the SARS-CoV-2 transmission risk, helping to protect individuals from this dangerous infection.

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