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Effectiveness of UVGI-80 Air Sterilizer to inactivate airborne coronavirus, and reduce microbial contamination in different environmental conditions

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Abstract

Airborne infectious diseases have been a major worldwide concern for many years. The sudden and fast spread of the severe acute respiratory syndrome 2 (SARS-Cov-2), causing the coronavirus disease 2019 (COVID-19) in a pandemic form, has intensified the necessity of constant environmental disinfection. Among the possible technologies that can be used for air disinfection, there is ultraviolet germicidal irradiation (UVGI). The main mechanism involved in UVC inactivation of microorganisms such as viruses, bacteria, protozoa, fungi, yeasts, and others is mainly due to its capacity to promote dimerization of pyrimidine, disturbing the microorganism's DNA replication and transcription, therefore leading to cell death. The aim of this study was to validate the efficacy of a new UVC disinfection system to deactivate microorganisms such as viruses (including coronavirus), in different environmental conditions. The device was effective in the neutralization of airborne particles containing coronavirus genus samples, presenting > 99.99% of inactivation rate in an aerosolization test, simulating the real conditions in which this virus is most transmitted in different environments.

Keywords: UVC disinfection; UVGI; ultraviolet; air sterilizer; airborne diseases; viral inactivation.

1. Introduction

Airborne infectious diseases have been a major worldwide concern for many years. Several microbial airborne infections can be created, either in seasonal or in pandemic forms, such as influenza, tuberculosis, and most recently coronavirus infections, representing a growing challenge to global public health¹⁻³. When an infected person coughs, sneezes, or even talks, many particles are thrown in the air through small droplets (also called aerosols), and these particles can remain in the air for a long period or fall on the ground or other surfaces, carrying a high load of contaminated particles that can easily go to another person, elevating the risk of an airborne disease^{1,4}.

The sudden and fast spread of the severe acute respiratory syndrome 2 (SARS-CoV-2), causing the coronavirus disease 2019 (COVID-19) in a pandemic form, and several studies showing that the virus can be viable for a long period on different surfaces and in the air^{5.6}, has intensified the necessity of constant environmental disinfection. Especially indoor, different air disinfection systems can be used to neutralize potentially pathogenic particles^{2.7}. Among the possible technologies that can be used for air disinfection, there is ultraviolet germicidal irradiation (UVGI)⁸.

The usage of UVC light on the inactivation of microorganisms has been well studied and settled in the past few years^{2,9,10}.

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The main mechanism involved in UVC inactivation of microorganisms such as viruses, bacteria, protozoa, fungi, yeasts, and others is mainly due to its capacity to promote dimerization of pyrimidine, disturbing the microorganism's DNA replication and transcription, therefore leading to cell death^{9,11}. For this reason, the usage of UVC light disinfection systems has grown inside health-care establishments, where there is a high circulation of contaminated particles, in an attempt to reduce the transmission of diseases, including COVID-19⁷.

Previous studies and reports have shown the efficacy of UVC light (207-254nm) as germicidal agents in different conditions^{7,11-13}, even though there is still concern about the risk of human exposure to certain levels of radiation, with the possibility of hazard to skin and eyes^{7,12}.

To reduce the risk of conventional hazards attributed to UVC lights, a new device has been introduced in the global market (UVGI-80 Air Sterilizer, FagronLab, Germany). The device operates with five double high intensity germicidal lamps, with a wavelength of 254nm, but, differently from comparative devices, the lamps are enclosed in the core of the device, avoiding human exposure to the radiation. The UVGI-80 (Figure 1) promotes air circulation through a double inlet system, that drags the air with a constant airflow of 800m³/h, directing particles and droplets directly to the lamps. After this process, the air is returned to the environment through a frontal outlet, and no radiation leakage is observed from the device¹⁴.

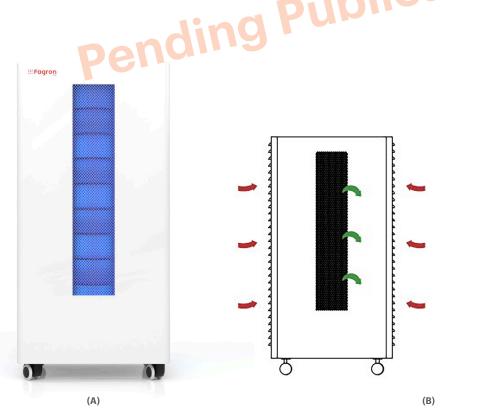
Even though previous studies have shown the efficacy of the UVC light against different microorganisms, there is poor data on this disinfection technology when it comes to SARS-CoV-2⁷. Thus, the aim of this study was to evaluate the efficacy of the UVGI-80 Air Sterilizer to deactivate microorganisms such as viruses (including coronavirus), in different environmental conditions.

2. Materials and Methods

2.1. Equipment

The tested device was the UVGI-80 Air Sterilizer, equipped with five double high-intensity UVC lamps (λ = 254nm), each lamp with 35W potency. The lamps are ozone-free (<0.1mg/m³), with less than 5µw/cm² of UV leakage. The airflow capacity is of 800m³/h, and there are no extra filters inside the device.

The UVGI-80 Air Sterilizer operates according to international standards (ISO 15858:2016).



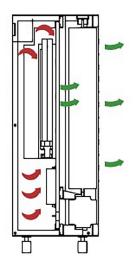


Figure 1. (A) UVGI-80 front side picture; (B) UVGI-80 front side and airflow scheme.

2.2. Preliminary Virucidal Test

To analyze the efficacy of the UVGI-80 against coronavirus, a preliminary test was performed. Swabs with Letheen broth were contaminated with synthetic SARS-CoV-2 model (Bio-Rad, Brazil), and placed in three different positions inside the chamber of the device, providing different points of contact with the UVC light.

The exposure times to be analyzed were defined to be 2 seconds, 7 seconds, and 10 seconds. After UVC treatment, the RNA was extracted and samples were analyzed by qualitative detection (presence/absence) by using the technique of Polymerase Chain Reaction (PCR) or Real-time Polymerase Chain Reaction (RT-PCR), (Bio-Rad, Brazil). Tests were performed in triplicate for each time.

2.3. Virucidal Test

After conducting the preliminary test, a validation test was performed to evaluate the efficacy of the UVGI-80 against coronavirus in aerosols¹⁵.

The test was performed in a virology laboratory, biosafety level 2, with the room temperature defined at $22^{\circ}C \pm 1^{\circ}C$. Incubation temperature was defined at $37^{\circ}C + 5\%$ of CO₂ atmosphere. The incubation period was defined as 48 hours.

The tested virus sample was a coronavirus model MHV-3 (mouse hepatitis strain 3), genus betacoronavirus (Unicamp, Brazil), from the same genus and family of the species SARS-CoV, SARS-CoV-2, Middle East respiratory syndrome (MERS-CoV), and others.

Cells were cultivated in 25 cm² cellular culture bottles, with an initial concentration of 1.5 x 10⁵ cells/mL in Dulbecco Modification of Minimum Essential Media (DMEM), Gibco[®], free from antibiotics and supplemented with 10% of fetal bovine serum (FBS). For the viral propagation, samples were inoculated in cellular culture bottles, and when the monolayer presented 70% of cytopathogenic effect (CPE), proceeded with the monolayer scraping and vigorous agitation until the dissolution of cellular agglomerates.

To evaluate the inactivation capacity of the device, samples were prepared as follow:

 Cells Preparation: 100µL of cell, diluted in DMEM culture medium with 10% of Fetal Bovine Serum (1.5 x 10⁵ cells/mL). The microplates were incubated at 37°C with 5% of CO₂ for 24 hours;

- The device UVGI-80 was attached to an air compressor to nebulize the sample (100µL) in the air inlet. With the device operating, virus samples were dragged, and sterile Petri dishes with DMEM culture medium were distributed in the air outlet, to be tested afterward for the presence or absence of the virus. Tests were made in quadruplicate, with four pre-defined exposure times (1, 5, 10, and 15 minutes);
- After the incubation period (48 hours), the microplates were read through optical microscopy for the CPE and titrates were calculated using the Spearman-Karber method¹⁶;
- 4. Results were expressed as the percentage of viral inactivation in comparison with the non-treated viral control. Tests were validated by cytotoxicity control, interference control, neutralization control, and an internal pattern of formaldehyde 0.7%.

3. Results and Discussion

3.1. Preliminary Virucidal Test

The results for the preliminary test using the UVGI-80 to reduce SARS-CoV-2 contamination are presented in Table 1. All samples presented similar behavior after the total analysis time. In the results, it was observed that the exposure time of 2 seconds was not enough to promote the absence of the virus. In the results of 7 and 10 seconds, it was observed partial efficacy among the samples, with 55% and 45% of absence, respectively. The variability in the results, since there was no apparent relation with the exposure time, might be attributed to different factors such as proximity of the samples to the lamp; mechanical stress on the sample due to the airflow speed; residues of virus sample trapped among the swab fibers, avoiding the proper direct exposure to the UVC light, among others.

An important factor to highlight is that the RT-PCR is a qualitative test, which can lead us to believe that there was a reduction in the viral load on the samples, but still in detectable levels.

The germicidal effect of UVC lamps relies on their capacity to damage the DNA and RNA of microorganisms, causing different mutations and lesions that leads to their inactivation through the inhibition of the DNA replication^{11,12}. To reach this germicidal effect, the microorganism must be directly exposed to high levels of radiation, at a low wavelength (200 to 280nm)⁴.

Sample	Exposure point	Time (s)	Detection of Synthetic SARS-CoV-2
Pre-treatment	1		Presence
	2	-	Presence
	3		Presence
	1	2	Presence
	2		Presence
	3		Presence
	1	2	Presence
	2		Presence
	3		Presence
	1	2	Presence
	2		Presence
	3		Presence
	1	7	Presence
	2		Presence
	3		Absence
	1	7	Absence
Post-treatment	2		Absence
	3		Absence
Pel	1	7	Presence
	2		Absence
	3		Presence
	1	10	Presence
	2		Absence
	3		Absence
	1	10	Presence
	2		Absence
	3		Presence
	1	10	Presence
	2		Absence
	3		Absence

3.2. Virucidal Test

It is well known that SARS-CoV-2 is mainly an airborne transmitted disease^{1,19}. For this reason, and considering the partial positive results obtained in the preliminary tests, a second test was conducted through aerosolization of coronavirus samples, to test the capacity of the UVGI-80 to neutralize the samples in a simulation of a real life condition, since aerosols are the most common transmission pathway for this virus¹⁹.

The results (Table 2) were expressed in percentual of viral inactivation, in comparison with a non-treated viral control, and showed that the device UVGI-80 was effective as a virucidal agent to the coronavirus sample in all tested time intervals, with more than 99.99% of virucidal activity.

This result is even more relevant in comparison with the virucidal control formaldehyde 0.7%, with a reduction log of 6 (reduction percentual of 99.9999%). With the obtained results, it is possible to suggest that the device UVGI-80 is effective to inactivate viral particles, therefore being a great addition to reduce the spread of viral airborne infections, including the ones caused by coronavirus genus such as MERS-CoV, SARS-CoV, and SARS-CoV-2 (COVID-19).

Table 2. Results for the virucidal test with the equipment UVGI-80.

Viral Titrate*	Exposure Time (min)	Virucidal Activity after exposure (Log 10)	Infectivity Reduction Log**	Infectivity Reduction Percentual
10 ^{8,25}	1	4.25	4	99.99%
	5	3.25	5	99.999%
	10	3.25	5	99.9999%
	15	3.25	5	99.9999%

* Average of 10 viral dilutions (10¹ to 10¹⁰) in quadruplicate.

** Average of 10 viral dilutions (10¹ to 10¹⁰) in quadruplicate.

4. Conclusions

The results presented in this study showed that the UVGI-80 Air Sterilizer was effective in the neutralization of airborne coronavirus samples, with > 99.99% of inactivation rate, showing that the device can play an important role avoiding the spread of different infections, including coronavirus, as well as reducing the level of contamination of different closed environments.

Author Contributions: Conceptualization, B.M. and H.P.; methodology, B.M. and H.P.; validation, B.M. and H.P.; data curation, B.M. and H.P.; writing original draft preparation, B.M.; writing review and editing, B.M. and H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: In this section, please provide details regarding where data sup-porting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics. You might choose to exclude this statement if the study did not report any data.

Conflicts of Interest: The authors B.M. and H.P. are employees of Fagron B.V. The funder (Fagron B.V.) had no influence on the design of the study and in the collection and analyses of data. In addition, all authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.



References

- Nordsiek F, Bodenschatz E, Bagheri G. Risk Assessment for Airborne Disease Transmission by Poly-Pathogen Aerosols. Vol 16.; 2021. doi:10.1371/journal.pone.0248004
- Welch D, Buonanno M, Grilj V, et al. Far-UVC light: A new tool to control the spread of airborne-mediated microbial diseases. Sci Rep. 2018;8(1):1-7. doi:10.1038/s41598-018-21058-w
- Yadav K, Prakash S. Tuberculosis: an airborne disease. Glob J Microbiol Res. 2017;5:225-243.
- Kalyani VL, Mathur P, Makwana N, Singhal N. Study on Coronavirus (COVID-19) and how UVC Light helps to Destroy it and its Applications. 2020;(July):3-8. doi:10.5281/zenodo.3929714
- Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020;104(3):246-251. doi:10.1016/j. jhin.2020.01.022
- Taylor D, Lindsay AC, Halcox JP. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. Published online 2020:0-2.
- Kitagawa H, Nomura T, Nazmul T, et al. Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. Am J Infect Control. 2020;(January). doi:10.1016/j.ajic.2020.08.022
- Medical Advisory Secretariat. Air Cleaning Technologies: An Evidence-Based Analysis. Vol 5.; 2005. http://www.ncbi.nlm.nih.gov/ pubmed/23074468%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3382390
- Kim D, Kang D. UVC LED Irradiation Effectively Inactivates Aerosolized Viruses, Bacteria, and Fungi in a Chamber-Type Air Disinfection System. 2018;84(17):1-11.
- Ploydaeng M, Rajatanavin N, Rattanakaemakorn P. UV-C light: A powerful technique for inactivating microorganisms and the related side effects to the skin. Photodermatol Photoimmunol Photomed. 2020;37(1):12-19. doi:10.1111/phpp.12605

- Narita K, Asano K, Morimoto Y, et al. Disinfection and healing effects of 222-nm UVC light on methicillin-resistant Staphylococcus aureus infection in mouse wounds. J Photochem Photobiol B Biol. 2018;178:10-18. doi:10.1016/j.jphotobiol.2017.10.030
- Narita K, Asano K, Naito K, et al. Ultraviolet C light with wavelength of 222 nm inactivates a wide spectrum of microbial pathogens. J Hosp Infect. 2020;105(3):459-467. doi:10.1016/j.jhin.2020.03.030
- Setlow P. Spores of Bacillus subtilis: Their resistance to and killing by radiation, heat and chemicals. J Appl Microbiol. 2006;101(3):514-525. doi:10.1111/j.1365-2672.2005.02736.x
- 14. FagronLab UVGI-80 Air Sterilizer User Manual.
- 15. British Standards Institution. BS EN 14476:2013+A2:2019 Chemical Disinfectants and Antiseptics. Quantitative Suspen-sion Test for the Evaluation of Virucidal Activity in the Medical Area. Test Method and Requirements (Phase 2/Step 1).; 2019.
- Ramakrishnan MA. Review of The Method of "Right and Wrong Cases" ('Constant Stimuli') without Gauss's Formula. World J Virol. 2016;5(2):85-86. doi:10.5501/wjv.v5.i2.85
- Pinto T de JA, Kaneko TM, Pinto AF. Controle Biológico de Qualidade de Produtos Farmacêuticos, Correlatos e Cosméticos. 4th ed.; 2015.
- United States Pharmacopeia. <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests. In: United States Pharmacopeia 43; 2020.
- Yu ITS, Li Y, Wong TW, et al. Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. N Engl J Med. 2004;350(17):1731-1739. doi:10.1056/nejmoa032867