Antimicrobial Performance of an Innovative Technology of Atmospheric Plasma Reactors against Bioaerosols: Effectiveness in Removing Airborne Viable Viruses

Jérémie Pourchez [1] , **Aurélien Peyron** [1,2], **Gwendoline Sarry** [1], **Lara Leclerc** [1] , **Paul O. Verhoeven** [2,3], **Peter Choi** [4], **Claude Pierson** [4], **Olivier Petit** [4], **Francisco Hernández** [4] **and Carmen Dumitrescu** [4,*]

1 Mines Saint-Etienne, University of Lyon, University Jean Monnet, INSERM, U 1059 Sainbiose, Centre CIS, 42023 Saint-Etienne, France
2 CIRI (Centre International de Recherche en Infectiologie), INSERM U111—CNRS UMR 5308—ENS de Lyon—UCB Lyon 1, Equipe GIMAP, Université Jean Monnet, 42100 Saint-Etienne, France
3 Laboratory of Infectious Agents and Hygiene, University Hospital of Saint-Etienne, 42270 Saint-Priest-en-Jarez, France
4 Gamma Pulse SAS, Bât 404, Ecole Polytechnique, 91128 Palaiseau, France
* Correspondence: carmen.dcu@gamma-pulse.com

Abstract: Reducing the exposure to airborne contaminants, including bioaerosols containing viruses, is a key challenge in the context of indoor air quality. This study aims to assess the effectiveness of innovative Atmospheric Plasma Reactor (APR) technology, which can be included in air cleaner devices, as an engineering control tool for reducing the concentration of viable airborne viruses. We investigated the KillViD™ APR technology that uses ultra-high electric fields and pulsed power plasma to directly electroporate living cells and produce advanced oxidizing species in situ within the micro-droplet aerosols containing the pathogens to be treated. An experimental setup was developed in order to aerosolize a high concentration of virus suspension directly into the air cleaner, containing 3 or 6 modules of 215 atmospheric plasma micro-reactors. As a virus surrogate, we used the phi11 bacteriophage which was aerosolized using a vibrating mesh nebulizer. The viability of airborne viruses after a single pass through the air cleaner was assessed by quantifying the lysis of a specific Staphylococcus aureus host strain. We were able to demonstrate that our virucidal results were robust and showed a 5-log reduction (99.999%) in terms of virucidal activity for the 3-module configuration, while we observed at least a 6-log reduction (from an initial viral load of 9.25×10^5 PFU to 0) for the 6-module configuration.

Keywords: air cleaner; pulsed power; atmospheric plasma; bioaerosol inactivation; bacteriophage; indoor air quality

1. Introduction

The crisis brought by the outbreak of the COVID-19 virus into the world made it clear that we do not have any highly efficient technological solutions for air decontamination and sterilization for the general public that are clearly identified to prevent the aerial transmission of a virulent viral infection. While the COVID-19 pandemic could represent, depending on the variation of the virus, the most important public health emergency of the century, understanding the modes of transmission of this new coronavirus is a key factor in implementing effective measures. In addition to the transmission by direct contact, indirect contact via contaminated objects, droplets, and from person to person, the diffusion of the virus by air and aerosol, long ruled out, is now evident to researchers [1,2]. While confinement was an effective strategy to prevent the devastating effect on human lives, especially to those on the front lines serving society (e.g., doctors, nurses, etc.), this was achieved at an enormous cost to the economic welfare of countries and is not sustainable in

the long term. It did not stop the pandemic from continuing to spread geographically, nor from striking back in subsequent waves. An effective solution is needed for the return to normal social and economic life where, by necessity, people gather in enclosed spaces for work, entertainment, and cultural life. Portable air cleaners with quantifiable efficiency can thus represent an interesting and promising technological strategy to reduce the risk of the airborne transmission of viruses in indoor environments by significantly decreasing the bioaerosol concentration and the potential risk of infection [3].

Airborne contamination in a continually enclosed space represents a very important route of transmission, in contrast to outdoor transmission, where a high viral load could be hard to reach since favorable air conditions (wind, turbulence, etc.) can quickly dilute the concentration of infectious particles. Decontaminating indoor air in confined places, including the home, is now advised as a preventative measure [4].

The reduction in indoor airborne pollutants can be aided by ensuring adequate ventilation with outside air [4]. However, improving ventilation on its own is insufficient to shield humans from exposure to airborne viruses. In fact, improving ventilation is only part of a strategy to reduce bioaerosol exposure when used in conjunction with other recommended practices, such as keeping a physical distance from each other and avoiding crowded indoor locations, wearing masks, and hand washing. Air purifiers may significantly reduce biological indoor air pollutants, such as airborne viruses, in this situation. An air purifier must be able to remove certain viruses that would be present in tiny airborne droplets (0.1–1 μm in size) in order to be successful at eliminating pathogens from the air. There are numerous ways in which air cleaner manufacturers report this capacity. However, as the result of a lack of thorough scientific validation of their performances, both at the lab scale and in real-life situations, commercial air cleaners are typically considered to be insufficient on their own to protect humans from bioaerosols [5].

Filters, ozone generators, ionizers, UV lights, and plasma devices (of course, some combine both types in the same unit) are the five fundamental categories into which the majority of air-cleaning technologies [6] fall and are described below.

- Filters are made to physically remove airborne particles from the surrounding air in order to enhance the indoor air quality in a specific room or region. HEPA (High-Efficiency Particulate Arresting) filters are now the most popular kind of household filter, however, certain equipment also uses fibrous media air filters. A HEPA filter's fibers are intended to capture particles with a diameter that might be as small as 0.01 microns [6]. Of course, a key consideration is how frequently air filters should be changed. Airflow across the filter can be impacted by saturation, which also has an impact on filter effectiveness.
- Ozone generators are marketed as air purifiers that purposefully release ozone gas [7]. Ozone is a highly oxidizing molecule composed of three oxygen atoms. One of the oxygen atoms can detach from it and re-attach to other molecules, changing their chemical composition. Ozone, however, is a poisonous gas. In fact, the same chemical qualities that allow ozone in high concentrations to react with organic matter outside the body also allow it to react with comparable organic matter inside the human body, leading to detrimental effects on health.
- Ionizers, often referred to as electrostatic precipitators, are electronic air cleaners that employ a high-voltage wire or carbon fiber brush to charge air particles which causes them to gravitate toward objects with the opposite electrical charge [8]. These items might be the collecting plates found inside the equipment itself or other interior surfaces found throughout the space (such as walls, carpets, etc.) to filter out airborne particles. Due to the fact that these deposited particles stay in the space, when disturbed by human actions, such as walking or cleaning, they may be resuspended from the collection surfaces. The impact of particle charging on particle deposition in the respiratory tract, which rises as particles become charged, is another aspect of ionizers to consider. Ionizer use may not, therefore, result in a reduction in the particle dosage

to the lungs. Furthermore, ionizers employ high voltage to create ionized fields, and they may purposefully or unintentionally release ozone gas as a byproduct.

- UVGI (Ultraviolet Germicidal Irradiation) air cleaners are created to employ UV irradiation to kill or deactivate microorganisms such as viruses, bacteria, and fungal spores and fragments that are airborne or growing on surfaces [9]. UVGI air cleaners use UV-A (long wave: 315–400 nm) and UV-C (short wave: 100–280 nm) radiation. UV radiation can enter a microorganism's outer cells and change its DNA, blocking replication and leading to cell death, given enough exposure time and lamp power. The UVGI cleaner in a typical airstream disinfection application has the potential to reduce the viability of vegetative bacteria and molds and to provide low to moderate reductions in viruses but little reduction in bacterial and mold spores [10].

- Plasma air cleaners use a high-voltage discharge to ionize incoming gases, causing them to lose their chemical bonds and undergo chemical changes [11]. Thermal plasma air cleaners use a high voltage and high current to create a high-temperature plasma flame. By accelerating electrons, non-thermal plasma air cleaners produce reactive ions and radicals (such as hydroxyl radicals, superoxides, and hydrogen peroxide) which oxidize substances and change their chemical composition. Plasma air cleaners have the ability to kill or inactivate airborne microorganisms [11] and can remove some gases and particles with high removal effectiveness. However, a number of hazardous byproducts, such as particulates, ozone, carbon monoxide, and formaldehyde, can also be produced.

Apart from the issues of maintenance (frequency of filter changing, for instance), energy consumption, or noise, the main problem is that consumers do not have any information on the actual air cleaning efficiency of these devices. Some standardized test methods exist [12–14], but the shortcomings are first, that these methods mostly focus on the problem of tobacco smoke or non-biological particles, and secondly, that they consider very high indoor pollution loads. Moreover, no standard procedure has been proposed to assess the performance of air cleaners focusing on airborne viruses, even if some experimental test protocol was proposed to assess the impact of air cleaners to reduce the airborne transmission risk of SARS-CoV-2 [15,16].

Thus, this study deals with the assessment of the performance of the plasma reactor technology included in the KillViD^{TM} air cleaner device in order to reduce the viable bioaerosol concentration of airborne bacteriophage viruses. In more detail, the main objective of this study is to assess the viability of airborne viruses after only a single pass through the plasma reactors of the air cleaner and thus optimize the number of modules needed to achieve air sterilization. Therefore, our objective here is not to assess the performance of an air purifier device, but to evaluate and optimize it in terms of effectiveness for removing airborne viable viruses so that this new atmospheric plasma reactor (APR) technology can be fitted to a new range of air purifiers. From these experimental results, we then compared the virucidal activity of different operating conditions of the APR by studying the viability (number of lysis aera) of the aerosolized viruses for different configurations (i.e., technology using 6 or 3 modules of APR).

2. Materials and Methods

2.1. The Atmospheric Plasma Reactor Technology Included in the KillViD^{TM} Air Cleaner Device

KillViD^{TM} is a new air cleaner device based on the innovative APR technology where the basic approach is to develop an ultra-high electric field in a plasma environment, leading to the creation of an in situ electron beam directly within the medium, resulting in the concomitant production of advanced oxidants. This is achieved by introducing a very short pulse of electricity at a high voltage into humidified air in a specific discharge geometry. The core of KillViD^{TM} exploits the discharge formation process in the Transient Hollow Cathode Discharge (THCD). THCD is a unique discharge geometry where efficient plasma production, together with the generation of an energetic electron beam, is achieved during the breakdown phase of the discharge. The unique geometry of the cathode has been found

to enhance the plasma density and the quantity of the energetic e-beam produced; the theoretical and experimental results have already been published [17–20]. The application of these research results led to the creation of a THCD under atmospheric pressure. This ATHCD (Atmospheric Transient Hollow Cathode Discharge) forms the core of the plasma reactor used in the KillViD[TM] device [21].

The heart of the KillViD[TM] air cleaner device is the atmospheric plasma reactor (APR) technology, which consists of a large number of plasma micro-reactors, approximately 1 mm^3 in size, running simultaneously at 1 kHz. One such basic module is presented while in operation in Figure 1a. The design allows the basic module to be scalable and modulable. Modules can be multiplexed and cascaded, making it possible to obtain high efficiency and versatility in various real-life applications. The air, and all the particles which are suspended in it, are "hit" several times by the plasma discharges in the few seconds needed for the air to pass through the system. The very short high voltage pulses applied to the APR make it possible to treat the aerosols in the air flowing through it. The contaminated air is then purified at an average energy consumption of 7W per module. This brings about four novel features:

1. The electrons during the discharge formation growth in the air/vapor medium surrounding the pathogen-loaded micro-droplets and aerosols create advanced oxidants, including ozone and hydroxyl radicals, in situ, among the micro-droplets and aerosols to be treated.
2. The electrons gain energy from the applied electric field, producing energetic electrons in the space among the droplets and thus act directly on each droplet to create hydroxyl ions and radicals in the droplet before a conducting plasma is created.
3. The micro-droplets create a very large surface for interaction for a given volume of air, making a highly efficient advanced oxidation reaction zone in a small chamber.
4. The THCD process leads to the creation of an ultra-high electric field zone close to the hollow cathode electrode. This ultra-high field is of the order 5×10^5 Vcm^{-1} (5V across 100 nm) and leads to direct electroporation [22] of the cellular structure of viruses and bacteria.
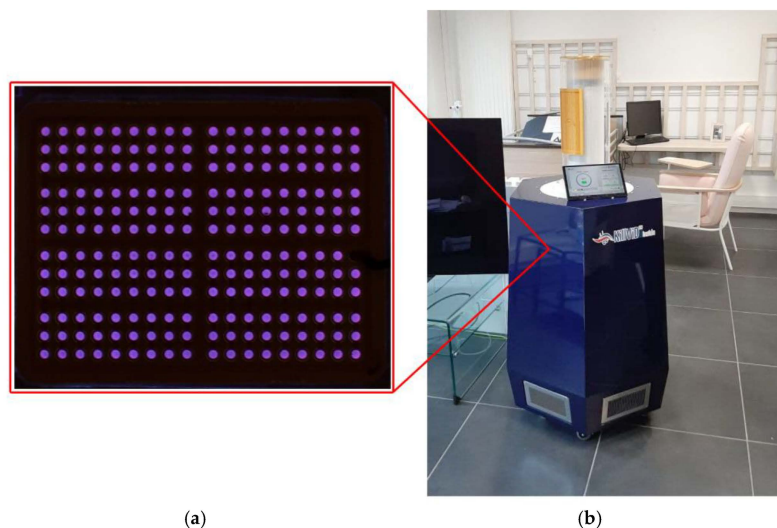

(a)   (b)

Figure 1. (**a**) One of the basic modules of APR technology inside KillViD[TM], showing 215 atmospheric micro-plasma reactors functioning simultaneously. (**b**) KillViD[TM] air cleaner validation prototype based on the APR technology.

This combined physical decontamination through electroporation and chemical reaction through direct, in situ formation of advanced oxidants, in a single energy-efficient treatment step, is the key innovation in this new technology.

The validation prototype, shown in Figure 1b, is a standalone system that applies all the destruction methods presented above to the air that flows through the machine, with the decontamination done in real-time, through a patented technology.

This prototype also includes a post-treatment stage to prevent any ozone emission, monitored with a set of sensors, to ensure that no hazardous output gases are released (such as ozone, carbon monoxide, or formaldehyde).

In this study, only the configurations of the atmospheric plasma reactors were tested, without the post-treatment stage included in the KillViD^{TM} air cleaner device. For this reason, control tests were carried out to quantify any possible virucidal activity of long-lived oxidant species (such as ozone coming out of the plasma reactors) on the sample collected during the experiment time. This would allow us to avoid any experimental bias created by the effect of the output gases and to verify that the virucidal activity is performed by the direct effect of the plasma reactors and not by the indirect effect of exhaust gases on the sample.

2.2. Virus Culture and Preparation

Bacteriophages are natural antibacterial agents that lyse a specific bacterial host strain. Based on their bacterial host specificity and bacteriolytic activity, the use of bacteriophages has been suggested as an interesting virus model to assess viable airborne viruses, such as in the viral filtration efficiency of medical facemasks [23]. In this study, we used phi11 bacteriophages. At first, they were amplified by infecting Staphylococcus. aureus RN 4220. Successive amplifications were carried out in broth in NB medium (MERCK, REF 16336) to reach a stock suspension of 10^{11} PFU/mL (PFU refers to the number of viral particles required to form one plaque). From this initial stock solution, the experiments were carried out using suspension with a constant concentration of 2×10^{10} PFU/mL (dilutions were made in Phosphate Buffer Solution—PBS).

2.3. Plating and Enumeration

The viral particles were collected by a Coriolis$^{®}$ (Bertin Instruments, Montigny-le-Bretonneux, France) biocollector, which is a biological air sampler capable of collecting viral particles into a liquid sample that can be later analyzed. The virus concentration can be detected at concentrations as low as 100 genomes copies/m3. The viral particles collected were then cascaded by a factor of 1/10 each time. The dilution was made in PBS supplemented with calcium chloride (Figure S2 in Supplementary File). The bacteriophage concentration was estimated by plaque counting, which is considered the golden standard for phage enumeration [24]. To estimate the bacteriophage concentration, COS agar (i.e., Columbia agar +5% sheep blood) was inoculated with a bacterial mat of Staphylococcus. aureus RN4220 by applying 10 μL spots of phage of increasing concentration. Incubation was carried out for 24 h at 37 °C. To estimate the concentration of phages from the agars, the following method was applied:

- The spot containing the fewest PFU was counted to determine the order of magnitude (e.g., 10^8 PFU/mL).
- A concentration correction was carried out to bring the unit of PFU/10 μL to PFU/mL (e.g., 10^8 PFU/10 μL corresponding to 1010 PFU/mL).
- The number of lysis areas was counted on the least concentrated spot to obtain the decimal part (e.g., 10^{10} PFU/mL corresponding to 4×10^{10} PFU/mL if 4 lysis areas were counted in the least concentrated spot).

2.4. Design of the Experiment, Bioaerosol Generation and Sampling

The experimental protocol (Figure 2) developed for this study to measure the virucidal activity of air cleaners consisted of:

- Cultivating bacteriophage viruses in order to prepare a suspension that filled the bioaerosol generator with 3 mL of virus suspension. The aerosol stream containing a known charge of phi11 viruses was generated using an E-flow mesh nebulizer (Pari

GmbH, Starnberg, Germany). The initial concentration of virus introduced into the nebulizer tank was always fixed at 2×10^{10} PFU/mL (see the "virus culture and preparation" section).

- Carrying out an experimental setup (Figure 3 and Figure S1 in Supplementary File) in a confined and controlled environment that consisted of aerosolizing the virus suspension directly into the air cleaner (virucidal activity quantified after a single pass of the bioaerosol in the air cleaner).
- Collecting the aerosolized viruses coming directly from the output of the air cleaner using a Coriolis® biocollector. Airborne viruses were collected in 3 mL of PBS with the Coriolis® Delta high air volume collection tool operating at 300 L/min (Bertin Instruments, Montigny-le-Bretonneux, France). This airflow of the Coriolis® biocollector, fixed at 300 L/min, is responsible for the aspiration. With the APR acting as a resistive load, the airflow at the inlet of the air cleaner was approximately 60 L/min. This airflow was measured at the inlet of the air cleaner (connected directly to the pipe that normally is connected to the nebulizer) prior to each experiment. The airflow measurement instrument used was a COPLEY DFM4 Flow Meter which has a resolution of 0.1 L/min (see Figure S3).
- Measuring the viral load collected in the Coriolis® biocollector by counting the lysis area on a confluent culture of a bacteriophage-susceptible Staphylococcus aureus strain (see the "plating and enumeration" section).
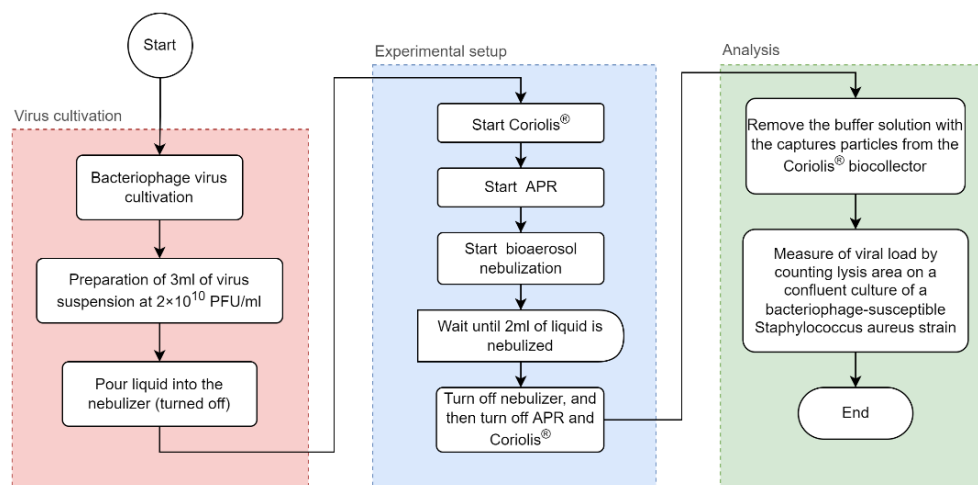

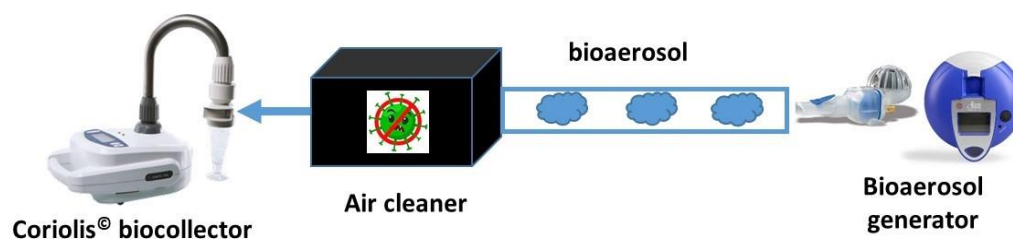**Figure 2.** Flow chart of the experimental procedure.

Figure 3. Schematic representation of the experimental setup for the evaluation of the virucidal activity of the APR technology.

Independent experiments were performed for two conditions: the "test" condition, when the air cleaner is turned on, and the "reference" condition, when the air cleaner is turned off. For the test condition, different operating configurations of the air cleaner were evaluated according to the number of active modules in the cascade (each module containing 215 atmospheric plasma micro-reactors): the 6-module configuration (using 6 reactor modules), and the 3-module configuration (using 3 reactor modules). Experiments for each

experimental condition (reference condition, test condition in 6-module configuration, and test condition in 3-module configuration) were performed at least in triplicate.

The size range of the aerosols generated during the nebulization is very important and should be compared to human respiratory aerosols. The strategy we adopted was to meet the aerosol droplet size required by the EN14683 [25] standard for the performance evaluation of medical masks. In other words, we respected the regulatory requirements for aerosol size at 3 ± 0.3 µm (simulating aerosols generated by an exhaling subject) that are required to evaluate the performance of medical masks. We have previously demonstrated that this nebulizer generates bioaerosols that are always within the range of airborne droplet size [26–30] of 3 ± 0.3 µm.

In addition, in order to validate the experimental protocol, control experiments were conducted. These consisted of evaluating a potential bias consisting in the elimination of the viruses, not by the direct action of the passage of the bioaerosol in the air cleaner, but by a potential virucidal action from several minutes of exposure to the possible oxidizing species contained in the exhaust air coming from the air cleaner outlet (for example, the presence of ozone) during the recovery of the bioaerosol in the 3 mL PBS solution at the level of the Coriolis® biocollector. To do this, a virus suspension of known concentration and volume was placed into a Coriolis® collection jar, simulating a test condition experiment with the air cleaner turned on, but without performing the bioaerosol introduction in the air cleaner inlet. Therefore, if the virus concentration remained constant in the Coriolis® collection jar after 2.5 min of exposure to the exhaust air coming from the air cleaner outlet, it will demonstrate the absence of an experimental bias. Indeed, it is important to understand if a virucidal activity is detected if it comes from the passage of the bioaerosol during a few seconds in the air cleaner, or if it comes indirectly from the air cleaner by a continuous sweep of ozone or oxidant species during several minutes at the level of the Coriolis® biocollector where the air cleaner would emit ozone or oxidizing species in the exhaust air.

2.5. Statistical Analysis

Analyses were performed on Prism 7.04 software (GraphPad, San Diego, CA, USA). Significance was established with the Kruskal–Wallis test ($p < 0.05$). Each data point represents the mean of at least three independent experiments and is presented with the arithmetic standard error of the means ($\pm$ SEM).

3. Results

To begin, the control experiment to investigate the virus removal by exposure to the possible ozone sweep induced by the operation of the air cleaner during bioaerosol collection by the Coriolis® biocollector clearly demonstrated that no experimental bias occurred. The virus concentration (concentration of virus of 10^5 PFU/mL according to the results obtained for the reference conditions, see Table 1) remained constant in the Coriolis® collection jar when simulating an experiment during 2.5 min (the same duration of the nebulization process when it occurs) with the air cleaner turned on (but without performing bioaerosol generation). Thus, we can conclude that no ozone or oxidizing species in the exhaust air were present, or present at a very low level, showing no virucidal activity on viruses collected by the Coriolis® biocollector.

Results obtained for reference experiments (i.e., experiments with APR technology turned off inside the air purifier) showed an average of 9.29×10^5 PFU with a standard deviation of 9.43×10^5 PFU (experiments conducted with $n = 5$, and $n = 3$ only for the 3-module case, see Table 1). However, it should be emphasized that these results indicate that, for five independent experiments, the value $9.29 \times 10^5 \pm 9.43 \times 10^5$ PFU is an average value obtained over a range, from a minimum value of 2.61×10^5 PFU to a maximum value of 2.77×10^6 PFU. Thus, we must interpret these results for the reference condition by saying that we were able to aerosolize 10^5 to 10^6 PFU with a maximum of 1 log deviation for a total load of air collected from the nebulizer at the exit of the air purifier with the APR

switched off. The authors support the conclusion that, in the context of the generation of high concentration viral bioaerosols, this is an excellent result in terms of reproducibility.

Table 1. Experimental results showing the different operating conditions: nebulization duration, the flow rate at the inlet of the air cleaner, total nebulized volume, viral load nebulized, bioaerosol volume collected by the Coriolis® device, concentration of virus in the Coriolis® jar, and virus load collected by the Coriolis® device.

	Nebulization Duration (s)	Flow Rate of the Air Cleaner (L/min)	Nebulized Volume (mL)	Viral Load Nebulized (PFU)	Volume Collected by the Coriolis (mL)	{Virus} In Coriolis Jar (PFU/mL)	Virus Load Collected by the Coriolis (PFU)
Reference condition (air cleaner off, n = 5)	154 ± 18	63.1 ± 0.3	2.01 ± 0.08	$4.02 \times 10^{10} \pm 1.52 \times 10^9$	2.70 ± 0.07	$3.40 \times 10^5 \pm 3.78 \times 10^5$	$9.29 \times 10^5 \pm 9.43 \times 10^5$
6-module configuration (air cleaner on, n = 5)	144 ± 19	61.7 ± 0.9	1.97 ± 0.06	$3.95 \times 10^{10} \pm 1.15 \times 10^9$	2.73 ± 0.12	0 ± 0	0 ± 0
3-module configuration (air cleaner on, n = 3)	142 ± 12	61.3 ± 0.2	1.98 ± 0.07	$3.96 \times 10^{10} \pm 1.35 \times 10^9$	2.70 ± 0.02	3.33 ± 5.77	9.07 ± 15.7

Furthermore, we must also emphasize that the global aerosolization yield, i.e., the yield between the viral load introduced in the nebulizer tank (in the order of magnitude of 10^{10} PFU, see Table 1) and the virus load collected at the outlet of the air cleaner when the air cleaner is turned off (about 10^5 to 10^6 PFU, see Table 1), may seem low a priori with a loss of 4 or 5 logs of the initial viral load introduced into the nebulizer. However, this result is quite typical for this type of experiment using airborne bacteriophages which requires very high initial concentrations to be introduced into the devices generating bioaerosol to obtain high virus airborne concentrations. Indeed, our preliminary tests (data not shown) have confirmed a point often mentioned in the literature, which is that bacteriophages have a very high affinity for plastic materials. Thus, we were able to estimate that we had a loss of 2 to 3 logs during the generation of the bioaerosol inside the nebulizer (these 2 to 3 logs are retained in the nebulizer, the bacteriophages being stuck on the walls of the plastic tank as well as on the membrane because we used the vibrating mesh nebulization technology) and 1 to 2 logs which were retained on the plates of APR micro-reactors, constituting the air purifier studied (by impaction and condensation of the airborne droplets in the geometric design of the micro-reactors which, even with the plasma turned off, allows a certain virucidy in an aerodynamic way by retaining some of the aerosolized viruses on the APR plates).

The experimental results concerning the reference and test conditions are summarized in Table 1:

- The first column shows the nebulization duration and the third column the nebulized volume for each experiment. The results demonstrated a very good reproducibility of the nebulization process, indicating the good quality of bioaerosol nebulization for each experiment. There was no significant difference in the nebulization duration in the three conditions ($p = 0.3337$) nor for the nebulized volume ($p = 0.8662$).
- The second column shows the airflow rate imposed at the inlet of the purifier which is only imposed by the inspiratory flow rate of the Coriolis® biocollector. These results also demonstrated a very good reproducibility of this flow rate, which is an important indicator of a constant passage time of bioaerosol through the air cleaner for all experimental conditions tested.
- The fifth column shows the volume remaining in the jar of the Coriolis® biocollector, given by:

$$Volume_{Remaining} = (initial \text{ PBS in the cone}) - (evaporation \text{ induced by the flow}) + (bioaerosol \text{ collected}) \quad (1)$$

where the initial PBS in the cone is 3 mL and the flow rate is 300 L/min. We can see there was no significant difference in the volume remaining in the cone in the three conditions ($p = 0. 5495$). The results also show a very good reproducibility of the collection process by

the Coriolis® device, indicating a good quality of bioaerosol recovery from the air cleaner outlet for each experiment.

- The sixth column corresponds to the measurement of the concentration of bacteriophage viruses collected by the Coriolis® device by reading the lysis area.
- The fourth and seventh columns (amount of viruses nebulized in PFU and amount of viruses collected in PFU) are not experimental measurements, but simple calculations of the experimental volume and concentration data determined elsewhere.

The virucidal activity of the different configurations of the air cleaner is illustrated in Figure 4. When the air cleaner is turned on, using the 6-module configuration, no viable airborne virus was collected by the Coriolis® device. Therefore, we demonstrated a 6-log reduction in terms of virucidal activity (i.e., a reduction of 100% of viable virus for an initial virus load in the bioaerosol of 9.25×10^5 PFU) induced solely by the operation of the air cleaner after a single pass of the bioaerosol in the device. Quite logically, the results show a higher virucidal activity in a single pass with 6 modules than with 3 modules. Indeed, we observed a 5-log reduction in terms of virucidal activity for the 3-module configuration of the air cleaner (i.e., a reduction of 99.999% of viable virus for an initial virus load in the bioaerosol of 9.25×10^5 PFU). Therefore, these conditions demonstrate a virucidal activity induced solely by the operation of the APR technology turned on inside the air cleaner of at least a viral reduction of 6 log, with the 6-module configuration, and a viral reduction of 5 log with the 3-module configuration. For the complete table with the results, please see Table S1 in the Supplementary File.
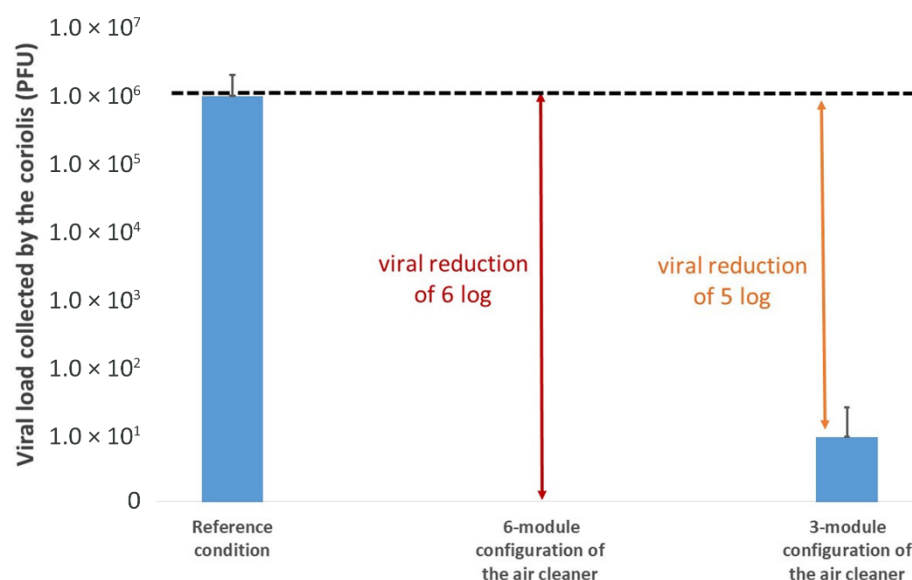

Figure 4. Viral reduction obtained for the different experimental conditions.

4. Discussion

4.1. The Proposed Experimental Setup to Assess the Virucidal Activity of the Air Cleaner Using Different Configurations of APR Technology

The experimental set-up allowed us to assess the virucidal activity, induced after a single pass of the bioaerosol in the air cleaner using a different configuration of APR technology, of at least a 6 log reduction since the viral load collected by the Coriolis® device for the reference condition was $9.29 \times 10^5 \pm 9.43 \times 10^5$ PFU (i.e., approximately 10^6 viable viruses collected after a single pass in the air cleaner when it was turned off during 2.5 min of nebulization). This result could potentially be better, but in order to measure the maximum efficiency reached by the APR, a higher initial concentration of viruses in the bioaerosol would be needed. To the best of our knowledge, this is the first time that an experimental set-up allowed for the demonstration of the potential virucidal

action of a bioaerosol up to log 6. In addition, we can also estimate the physical loss of aerosolized microorganisms. The results of the reference condition showed that the losses in the experimental setup (independent of the action of the atmospheric plasma micro-reactors) were estimated to be about a 4–5 log reduction (a mean of 9.25×10^5 PFU collected by the Coriolis® versus 4.02×10^{10} PFU nebulized by the bioaerosol generator). This loss was induced by the aerosolization efficiency of the nebulizer and from physical losses induced by aerodynamic phenomena and the impaction of the largest aerosol droplets (Mass Median Aerodynamic Diameter of the nebulizer measured at 3 ± 0.3 μm [29,30]) in the hundreds of micro-reactors. This phenomenon is important for the operation of the air purifier because it is necessary to maintain a sufficiently high flow of air through the micro-reactors to reduce the rate of condensation of these droplets onto the surfaces of the electrodes in the micro-reactors.

4.2. Limitation and Possible Extrapolation to Other Respiratory Pathogenic Viruses with the Use of the phi11 Bacteriophage as a Surrogate Virus

Phi11 is a group B [31] bacteriophage belonging to the family of Siphoviridae. Its size is about 200 nm, including a hexagonal head of about 50–60 nm in diameter and a tail measuring 150 nm [32,33]. SARS-CoV-2 belongs to the Coronavidae family and has a size of 60–140 nm [34]. The genome sizes are quite close with 30 kb and 45 kb for SARS-CoV-2 and phi11, respectively [31,34,35]. Phi11 is a non-enveloped double-stranded DNA virus with an icosahedral capsid which is characteristic of Siphoviridae [31,35], but SARS-CoV-2v-2 is an enveloped single-stranded RNA virus [34]. Basically, naked viruses are known to be more resistant than enveloped viruses [36], which makes phi11 an excellent non-pathogenic human virus that enables testing in BSL1 laboratories.

4.3. Relative Positioning of APR Technology Performances Compared to the Effectiveness Reported for Other Devices in the Literature

It is very difficult to compare results among different air cleaning technologies on the market as there is no agreed testing protocol with which to submit all technologies to assess airborne viral reduction. Few of these systems have been tested in rigorous scientific experiments and reported in peer-reviewed publications. The virucidal efficacy of such commercial air purifiers is generally unknown. Indeed, there are a small number of scientific publications that have studied the efficacy of particle removal in different technologies [37–40], concentrating mainly on HEPA filters or the association of HEPA filters with UV or cold plasma technologies [16,41], but they all refer to particles of the order of microns, while the viruses are nano-particles. The typical efficiency quoted of 99.95% (which is not sufficient to guarantee a safe reduction in the viral load in real-life conditions) refers to the removal of particles greater than 0.3 μm and contains no data concerning the virucidal efficiency. The other disadvantage of HEPA (or any other mechanical filter) is the fact that they do not destroy the viruses, as KillViD™ does, they only accumulate them in one place, making the maintenance and disposal of the filters an issue. Plasma systems can, in principle, handle any size of particle, but the technologies developed, other than the APR technology included in KillViD™, rely on cold plasma putting either ions or ozone and hydroxyl into the room air in order to deactivate the viruses. The capacity of creating ions per cm³ is very low compared with the number of air molecules per cm³, making the efficacy symbolic. KillViD™ passes the room's air through the reactors and transforms it into a short-lived, pulsed power plasma, thus destroying the viruses due to the high electric field in real-time and in one single pass. There is no mechanical filter involved to accumulate the viruses. The main advantage of KillViD™ over other technologies is the high virucidal efficacy of the reactor module (APR) in a viable form factor that can be integrated into portable air purifiers. The result of a minimum of 6 logs when passing millions of viruses in real-time and one single pass through the system is, to the best of our knowledge, a world first.

4.4. Contribution and Limitation of the APR Technology in the Management of Indoor Air Quality with Air Cleaners to Reduce Exposure to Airborne Viruses

Today, we know that there is strong evidence associating indoor air quality with the transmission of airborne respiratory viruses such as SARS-CoV-2. Indeed, many viruses can spread by exposure to droplets and aerosol particles smaller than 1 μm from respiratory fluids that are released by infected persons. Thus, the management of indoor air quality can play a major role in infectious respiratory disease transmission. In 2017, a study among 37 U.S. schools determined the effective air exchange rates during a classroom's daytime occupation. These results showed an air changes/hour ratio typically below 2.0, even if it mainly depended on the heating, ventilation, and air conditioning system configuration [42]. Thus, as the capacity for increased air exchange rates is generally limited, some researchers and public health organizations have recommended the use of portable high-efficiency particulate air cleaning units to augment the clearance of potentially infectious aerosols in the frame of the COVID-19 pandemic [43,44]. Among the possible engineering control for reducing exposure to potentially infectious respiratory aerosols, air cleaners (based on airborne virus removal) can provide a rapid and relatively cheaper solution as opposed to the option of changing the traditional ventilation system (based on the increase in air exchange rates).

However, even if several mobile air-cleaning units have been shown to be quite effective in significantly reducing bioaerosol concentrations [45], we must underline that the efficacy of portable air cleaners can be also influenced by the airflow setting, placement position, and room occupancy [46]. Indeed, air cleaner units did not provide bioaerosol exposure reductions at all locations in all scenarios. Therefore, individual exposure reductions were strongly dependent upon the relative position of the recipient to both the bioaerosol source(s) and the air cleaner(s). Furthermore, the addition of air cleaners with very variable airflow rates from a few dozen to a few hundred L/min affect room air dynamics and could impact an individual's bioaerosol exposure. All things considered, the assessment of the intrinsic virucidal performance of air cleaners is very important in this phase of new technology development (in particular, it is the main purpose of this paper devoted to the APR technology equipping KillViD^{TM} air cleaning device and its optimization through the number of atmospheric plasma micro-reactors to be incorporated), yet it is a necessary condition, but by no means sufficient to predict the effectiveness of the technology, in terms of the reduction in an individual's exposure to infectious bioaerosols in real-life applications. Based on the limitations of our study, the next step should be to move from a bench study (this paper) to a study in rooms of at least 10 m^3 in order to investigate: (i) the air cleaner's positioning to prevent the potential of drawing directed air currents from one occupant over another, and (ii) the use of multiple air cleaners spread out around the room to provide a faster and better viral cleaning of the room air, thereby reducing the bioaerosol concentrations for participants in the room and limiting the probability of airborne virus transmission.

5. Conclusions

In this article, the performance, in terms of airborne virus reduction after a single pass through, of an innovative atmospheric plasma reactor technology was assessed. To the best of our knowledge, it is the first time that an experimental set-up demonstrated a capacity to quantify the virucidal activity against a bioaerosol directly introduced into the air cleaner of at least a 6-log reduction. Concerning the optimization of the APR technology equipping the KillViD™ air cleaning device, our results led to the conclusion that the 6-module configuration of 215 atmospheric plasma micro-reactors showed the highest virucidal performance. Indeed, no viable airborne virus was detected after a single pass of the bioaerosol through the 6-module configuration of the air cleaner, demonstrating at least a 6-log reduction in terms of virucidal activity (i.e., a 100% reduction in viable virus load for an initial virus load of 9.25×10^5 PFU in the bioaerosol).

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