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# Proposal for a Benchtop Chamber for SARS-CoV-2 Inactivation by Ultraviolet-C Irradiation

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## 1.Introduction

In December 2019, the world saw the emergence of a new virus, SARS-CoV-2, which causes COVID-19. The new virus spread quickly around the globe and the World Health Organization (WHO) declared the second pandemic of the 21st century on March 11, 2020. SARS-CoV-2 is the seventh documented coronavirus to cause infections in humans. The four common coronaviruses, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, cause mild respiratory tract illness and contribute up to 30% of common cold cases [1]. Therefore, with the emergence of a new disease, COVID-19, caused by the novel coronavirus, the transmission control strategies applied are those to contain respiratory diseases: the use of masks, social distancing, ventilation of indoor spaces and surface cleaning. Within this context, the use of ultraviolet C radiation (UVC) is suggested as an additional COVID-19 transmission control strategy to the established sanitary protocols.

UVC is an electromagnetic radiation with wavelengths between 200 and 280 nm, known to have a germicidal effect because it inactivates pathogens - viruses, bacteria, fungi or protozoa. The inactivation occurs mainly through the dimerization of pyrimidines: preferentially thymine dimers in DNA molecules and uracil dimers in RNA [2]. The susceptibility of a pathogen to UVC radiation is a parameter that needs to be determined experimentally: it is both a species-dependent characteristic and influenced by external factors such as temperature, air humidity and the environment in which the pathogen is found (air, water or surfaces).

Throughout the year 2020, SARS-CoV-2 proved to be a rapidly transmitting virus, with initial flu-like symptoms. Over the months, COVID-19 came to be understood as a systemic disease [3], with multiple transmission routes: direct contact, indirect contact, large droplets and aerosols – some of which are more prevalent and efficient depending on environmental conditions [4]. But it is estimated that approximately 59% of global COVID-19 transmission occurs from pre-symptomatic and asymptomatic individuals [5], indicating a prevalence of airborne SARS-CoV-2 transmission and, therefore, through aerosols. Superspreading events, such as the Skagit Valley coral outbreak in Washington, USA [6] and the outbreak in a Call Center in South Korea [7], are also supporting evidence that COVID-19 is an airborne disease. Although airborne transmission of a respiratory virus is difficult to be directly demonstrated, there is very limited evidence of SARS-CoV-2 transmission by other routes [8].

Aerosols are produced by people in all respiratory tract activities (breathing, talking, coughing, sneezing, singing, etc.). Coughing, for example, produces about 3000 droplets, while sneezing produces about 40000. Most of these droplets are small, in the range of aerosols between 1 and 10 μm in diameter. Breathing normally and speaking, in turn, produce mostly aerosols smaller than 1 μm [9]. Aerosols particles up to 10 μm can be suspended in air for hours and therefore become an important transmission route for COVID-19.

Thus, since COVID-19 has been shown to transmit more efficiently and prevalently through the air, it is necessary that the determination of virus inactivation parameters by UVC radiation be carried out in aerosolized viral samples. For this purpose, this work proposes a benchtop chamber focused on UVC irradiation at a wavelength of 254 nm of aerosolized samples of SARS-CoV-2.

## 2.Methodology

The bench chamber suggested in this article was designed with a similar configuration to the chambers built by Ko et al. [10], Lai et al. [11], McDevitt et al. [12], and Welch et al. [13], and its schematic diagram is shown in figure 1. The bench chamber model has a nebulizer through which the viral sample will be introduced into the chamber and, in addition to this inlet, there are also two others through which dry air and humidified air flow into the equipment from the desiccator and bubbler, respectively. By air current, the sample passes through micro fans so that the mixture of the aerosolized sample with the dry air and the humidified air is homogeneous. Then, the aerosol flow reaches the irradiation region in the central part of the equipment, where temperature and air humidity sensors will be placed for the proper monitoring of these variables. A sensor will also be used, in the same region, to monitor the size of aerosols circulating through the equipment.

Another variable measured in the experiment is irradiance. The mercury vapor lamps are positioned externally to the chamber and the radiation emitted by them passes through a quartz window, which presents approximately 85% to 90% transmission in the range of UVC radiation. Once the sample is irradiated, the aerosol stream moves towards an air sampling cassette filter used to collect the surviving viruses. The chamber also has a bypass channel, used when air sampling is not required. Finally, the flow goes through a rotameter and then the air leaves the equipment passing through a HEPA filter and a vacuum pump. As SARS-CoV-2 is a human pathogenic virus, the entire chamber must be placed inside a certified class II type A2 biosafety cabinet to perform the measurements.

For equipment calibration, the use of the Bacteriophage MS2 is planned, since it is a non-pathogenic virus with dose-response UVC radiation well recorded in the literature [14, 15]. The tests will be carried out according to the principles of repeatability and reproducibility of results. For data analysis it is planned to use the SPSS software for Windows.

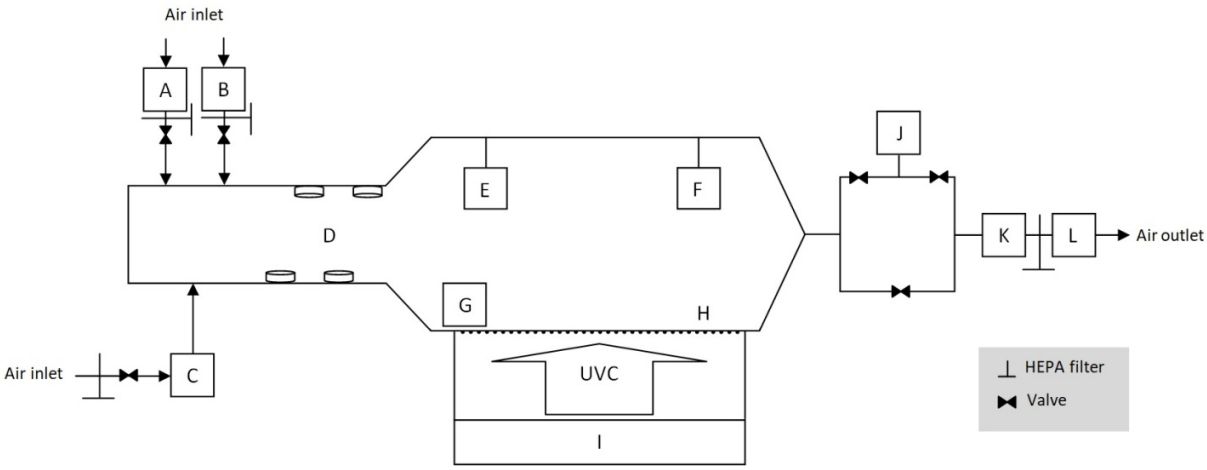


Figure 1: Schematic diagram of the benchtop ultraviolet radiation chamber. The chamber is drawn in a top-down view. Components included: Bubbler (A), air desiccator (B), nebulizer (C), micro fans (D), air temperature and humidity sensor (E), aerosol particle sensor (F), radiometer (G), UVC transmitting quartz window (H), UVC lamps (I), air sampling cassette filter (J), rotameter (K) and vacuum pump (L). The layout also features airflow control valves, HEPA filters included on all air inlets and outlets, and mirrored aluminum foil inner liner.

## 3. Results and Discussion

Viral susceptibility to UVC radiation, also called rate constant, which is an experimental parameter, is estimated according to equation (1):

$$k = \frac{\left[\ln\left(\frac{N_0}{N_t}\right)\right]}{D} \quad (1)$$

Therefore, to determine the susceptibility of SARS-CoV-2 it will be necessary to find three other variables: $N_0$, $N_t$ and D, which are respectively: the number of plaques in the absence of UVC exposure, the number of plaques after UVC irradiation and the dose applied expressed in J/cm$^2$. From the rate constant it is possible to calculate the survival rate of microorganisms from equation (2):

$$S = e^{-kD} = \frac{N_t}{N_0} \quad (2)$$

In one hand, $N_0$, $N_t$ expressed in plate-forming units, will be obtained from the collection of aerosols in the air sampling cassette filter, on the other hand, the dose is a controlled parameter since it is obtained directly from the irradiance of the mercury lamps and from the exposure time. The dose is, therefore, given according to equation (3):

$$D = it \quad (3)$$

Where $i$ is the irradiation of the UVC lamps used and $t$ is the irradiation time. And the aim of the experiment is to identify the dose value sufficient and necessary for inactivation of at least 90% of the viruses in the initial sample, also known as $D_{90}$.

## 4. Conclusions

In order for equipment that uses UVC radiation with a germicidal purpose to be properly calibrated and to work optimally, it is essential that the inactivation dose of a microorganism and its rate constant are identified. This project aims to identify these parameters for SARS-CoV-2 in aerosol samples in order to provide the scientific community with data that can be used in UVC air disinfection equipment, improving the performance and efficiency of these systems. This study also seeks to evaluate the effects of air humidity and temperature and how these two variables affect the inactivation constant k and radiation dose of SARS-CoV-2.

It is important to highlight that direct exposure to UVC radiation at a wavelength of 254 nm can cause corneal irritation and burns. But despite the risk of exposure involved, UVC radiation can be safely used in many everyday air disinfection solutions. Among these solutions, we highlight that UVC systems can be installed in ventilation ducts, together with air conditioning equipment or through a configuration of lamp systems that radiate above a certain height (upper room fixtures). These solutions can be widely applied in places such as hospitals, schools, airports, airplanes, shopping centers and offices.

Previous studies have shown that UVC radiation is effective in inactivating airborne microorganisms. Therefore, the use of UVC helps to limit seasonal influenza epidemics, tuberculosis transmission and, as expected, can be used as an additional strategy to the already established health protocols to mitigate the transmission of SARS-CoV-2.

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