Investigating airborne virus transmission in public spaces using a non-pathogenic model virus phi 6

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Tiivistelmä — Referat — Abstract

The COVID-19 pandemic of 2019 has had a huge impact on the hospitality industry, decreasing production by 35.4% in Q4 of 2020. To keep the industry functional, new safety solutions have to be studied and developed for mitigation of the pandemic. In this study, airborne transmission of viruses in an indoor space was studied, and air purifiers and space dividers were tested as potential intervention methods against SARS-CoV-2 by using a non-pathogenic model virus phi 6. Filtered air purifiers were found to work as a possible solution for the mitigation of viruses spreading through aerosols in public spaces such as restaurants, however, the positioning of the devices is crucial, as the air flow to them may increase the concentration of viruses locally. Space dividers were found to increase the possibility of infection via aerosols. Other types of air purifiers were also tested: an ionizer prototype and a hydroxyl radical emitting unit, of which the ionizer prototype proved to be efficient in reducing the virus concentrations in the air. Most importantly, it was confirmed that enveloped viruses resembling coronaviruses are capable of spreading via aerosol transmission indoors.

Avainsanat — Nyckelord — Keywords

COVID-19, SARS-CoV-2, coronavirus, phi6, bacteriophage, virus transmission, aerosol transmission

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Virusten ilmateitse julkisissa tiloissa leviämisen tutkiminen käyttäen mallivirus phi 6:tta

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Tiivistelmä — Referat — Abstract

Vuoden 2019 COVID-19 pandemialla on ollut suuri vaikutus ravintola- ja matkailualaan, vähentäen sen tuottavuutta 35.4 % vuoden 2020 Q4:llä. Alan kannattavuuden säilyttämiseksi, uusia tapoja estää virusten leviämistä täytyy tutkia ja kehittää. Tässä tutkimuksessa tutkittiin mallivirus phi 6:n avulla virusten leviämistä ilmateitse sisätiloissa, sekä ilmanpuhdistimien ja tilanjakajien käyttöä mahdollisena interventiokeinona SARS-CoV-2 leviämistä vastaan. Filtterillisten ilmanpuhdistimien huomattiin toimivan mahdollisena ratkaisuna virusten ilmateitse leviämisen estämiseen julkisissa tiloissa, kuten ravintoloissa. Laitteiden sijoittelu on kuitenkin avainasemassa niiden toimivuuden kannalta, sillä ilman virtaus niitä kohti voi lisätä viruskonsentraatiota paikallisesti. Tilanjakajien todettiin lisäävän aerosolivälitteisen infektion mahdollisuutta. Myös muun tyyppisiä ilmanpuhdistimia testattiin: ionisaattoriprototyyppiä ja hydroksyyliradikaaleja tuottavaa ilmanpuhdistus-yksikköä. Näistä ionisaattorien todettiin estävän tehokkaasti virusten ilmateitse leviämistä. Tärkeimpänä tuloksena vahvistettiin, vaipallisen viruksen, kuten koronavirukset, kykenevät leviämään ilmateitse aerosoli-välitteisesti.

Avainsanat — Nyckelord — Keywords

COVID-19, SARS-CoV-2, koronavirus, phi6, bakteriofaagi, virusten leviäminen, aerosoli leviäminen

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1 Introduction

1.1 COVID-19 pandemic

The COVID-19 pandemic started in late 2019 in Wuhan, China, from where it quickly spread all over the world in the following months (WHO, 2020a). At the time of writing, there have been almost 140 million confirmed cases and almost 3 million deaths worldwide (Worldometer, 2021). WHO's response (2021a) to the pandemic has been to encourage the use of masks, maintaining social distancing, washing, and disinfecting hands often and self-quarantining if feeling unwell or after being exposed to COVID-19.

In March 17th, 2020 Finland had a total of 322 confirmed infections (Worldometer 2021) and passed a law that would allow certain safety measures to be taken to reduce the spread of COVID-19 (Hara, 2020). These safety measures included limiting the size of public events and gatherings, moving to distance learning in schools, and closing, or limiting the capacity of public spaces such as libraries, restaurants, and malls (Helsingin Sanomat 2020).

1.2 COVID-19 and the restaurant industry

One of the industries affected hardest by the pandemic has been the hospitality industry, with a 35.4 % decrease in production in Q4 when compared to that of last year, (Tilastokeskus, 2020). This has led to many restaurants and hotels having to lay off workers with no certainty of when they can return. According to an investigation by the Finnish service union PAM, around two thirds of the industry's workers were laid off in April of 2020 (Kauppalehti, 2021). A study published in March by the Finnish Tourism and Restaurant services' union, MaRa (2021), found that 24 % of the union's members estimate to be under threat of bankruptcy, and 3 % estimate to have to close by September 2021, if the restrictions are not eased or the companies compensated.

The major restrictions of the Finnish restaurant industry, specifically, have been the ones on opening hours and restaurant capacity. The restaurants were forced to stop selling alcohol at 10PM, close at 11PM and only take in half of their capacity, which was later changed to 75 % (Helsingin Sanomat, 2020, 1; Valtioneuvosto, 2021).

1.3 Phi 6 as a SARS-CoV-2 model virus

COVID-19 is caused by SARS-CoV-2 (WHO, 2021b), a member of the *Coronaviridae* family of enveloped positive-sense, single-strand RNA viruses (Steinbach et al., 2018). Coronaviruses have been known to cause the common cold in humans, as well as animal diseases. More recently though, new and more dangerous coronaviruses have emerged, causing severe acute respiratory syndrome (SARS-CoV) in 2002, Middle East respiratory syndrome (MERS-CoV) in 2012 (Flint et al., 2015) and most recently COVID-19 (SARS-CoV-2) in 2019. One of the main characteristics of coronaviruses are the spike proteins on the virus' envelope. The virus uses these spike proteins to recognize host cell's ACE2 receptors and to further fuse into the host cell's membrane (Huang et al., 2020).

The phi 6 bacteriophage belongs to the family of *Cystoviridae* and it infects certain strains of *Pseudomonas syringae*-bacteria. Like the coronaviruses, it is also an enveloped, but double-stranded, RNA virus (Vidaver et al., 1972) and has spike proteins integrated into its lipid envelope. Phi 6' spike proteins have a similar function to those of coronaviruses: they attach to the host's pilus and are necessary for the virus' successful fusion into the host cell's membrane (Daugelavicius et al., 2005).Furthermore, their virions are of similar sizes: from 60 to 140 nm for SARS-CoV-2 (Zhu et al., 2020) and 85 nm for phi 6 (Maniloff et al., 1999) These common structural elements and size factors suggest that they could spread in the air in aerosol droplets in a similar. These similarities between the viruses make phi 6 a potential candidate for usage as a SARS-CoV-2 model virus. Lastly, phi 6 is well characterized (Bamford et al., 1976; Romantschuk et al., 1988; Romantschuk and Bamford 1985) and therefore easy to work with. It is also harmless to humans, which facilitates aerosol studies.

Aerosolizing phi 6 has been studied before (Gendron et al., 2010; Turgeon et al., 2020) and it has been compared to the influenza virus (Turgeon et al 2020), a virus which is approximately of the same size as SARS-CoV-2 and phi 6, around 80-120 nm (Noda 2012). It has also already been used as a SARS-CoV-2 surrogate in a microdroplet evaporation survival study (Fedorenko et al., 2020).

1.5 Viruses and aerosols

Aerosols and droplets are generally differentiated by size, where aerosols are determined to be 5 µm or smaller and droplets larger than 5 µm (Duguid et al., 1946). The larger droplets usually fall quickly to the ground, but they can still remain in the air from seconds to minutes, depending on their size (CDC. 2020) and the ventilation conditions (Li et al., 2007). Aerosols, however, can stay in the air from minutes to hours and travel long distances, especially indoors (CDC. 2020).

There are generally three ways for respiratory tract viruses to infect humans: contact transmission, droplet transmission and aerosol transmission. In contact transmission the person is in close contact with an infected person or a surface that has been subjected to the virus from e.g. droplets. Droplets and aerosols are generated when coughing, sneezing, or talking. Droplets can infect a person if inhaled when they are still in the air or via surfaces. Lastly, aerosols can stay in the air for long periods of time and be inhaled even a long time after the source has left. They are therefore an important virus transmission mode that is difficult to control (CDC. 2020).

Viruses have been known to be able to spread through aerosols for over 50 years (Alford et al., 1966), yet, only now that the COVID-19 pandemic has emerged, has wearing masks when in public, especially if feeling ill, become a worldwide trend. Most likely it is due to aerosol spreading of certain viruses, like influenza A virus for example, being neglected, and instead the focus has been on surface or droplet transmission (Weinstein et al., 2003; McMillan Jackson et al., 1996). However, this mentality is quickly changing, and it is already widely understood that viruses do spread through aerosols (Louten 2016), as is the case with SARS-CoV-2 (Doremalen et al., 2020). One fatal example of viruses spreading through aerosols was observed by Miller et al., (2020), where SARS-CoV-2 spread in a choir practice, most likely through aerosols produced while singing loudly. 53 of the choirs 61 members were suspected of contracting COVID-19, of whom two died of the disease.

1.6 Aims of the study

Our goal was to first study how enveloped viruses spread via aerosol transmission and if they remain infective while doing so, and second to develop safety measures for restaurants, and other public spaces alike, to mitigate the aerosol transmission of viruses. These methods could be used when going forward with the current and future pandemics to relieve some of the restrictions.

At the start of this study, much less was known about aerosol transmission of viruses than is now. A great number of studies have been published in late 2020 and early 2021 on the subject (Bulfone et al., 2021; Eichler et al., 2021; Epple et al., 2021; Kwon et al., 2020; Miller et al., 2020), and the results of this study will be included in those to help us better understand how aerosol transmission of viruses occurs.

This master's thesis was a part of a bigger project, which included PCR-analysis of the samples to detect inactivated virus, air flow-modeling and aerosol measurements to study how the aerosols produced would spread to the restaurant room, and measuring harmful emissions from the equipment used. All parts of the study described in this master's thesis have been carried out by the writer of the thesis unless otherwise noted.

2 Materials & methods

2.1 Viral- and bacterial strains

Pseudomonas syringae serovar phaseolicola HB10Y (further referred to as "HB10Y") was used as the host for phi 6. The bacteria were grown at room temperature (22°C) with aeration overnight. Infective virus titer was determined by plaque assay. Phi 6 was purified by research associate Helin Veskiväli, by the previously described optimized purification protocol (Bamford et al., 1995).

2.2 Growth medias and buffers

HB10Y plates were prepared by mixing 100 µl of host bacteria in Luria-Bertani-lennox (LB)-broth, containing 0.5 % NaCl, with 3 ml LB-soft agar and pouring it on LB-plates (further referred to as "HB10Y plate") (appendix 1). The soft agar was melted in a microwave and then kept in a water bath at 42.5 °C until use. HB10Y plates were used as deposition plates to collect naturally falling aerosols (see 2.7.2), in Andersen impactors (see 2.7.1). and for finger test- surface samples(see 2.7.3). 20 mM HEPES-buffer, pH 7.2 (appendix 2), was used in replicate deposition plates, Andersen impactor plates, Biospot 300p (see 2.7.1) and surface swab sample tubes (see 2.7.3).

2.3 Simulations

The simulations were divided into restaurant and laboratory simulations and in them, different methods of intervening with the spread of aerosols and the viruses within them were tested. Virus containing aerosols were produced with a nebulizer (see 2.4). These methods included air purification units, space dividers and ionizers. Each simulation day in the restaurant consisted of one 60 min intervention- and one 60 min reference simulation. In the laboratory, two 15 min intervention, and one 15 min reference simulations were carried out on the same day. In the intervention simulations, the tested safety measures were set in the room to study their efficacy in mitigating aerosol transmission of viruses, whereas in the reference simulations these safety measures were removed. During the simulations, air (see 2.7.1) and deposition samples (see 2.7.2) were collected, and after the simulations and the nebulizing had stopped, surface swab samples and finger tests (see 2.7.3) were collected. The results of the intervention and reference simulations were then compared to see

if the safety measures reduced the amount of infective virus spread through aerosol transmission. After each simulation, the room was cleaned with a UV-C lamp (see 2.8).

2.4 Nebulization

1 × purified phi 6 was diluted with 20 mM K-phosphate buffer to a concentration of 5×10^{10} pfu/ml to be used in the nebulizer. The dilution was kept on ice until use.

Omron Ultrasonic Nebulizer Model NE-U17 was used to aerosolize the virus solution. 375 ml of sterile Milli-Q water was used in the water tank and 150 ml of virus solution was used in the aerosolization chamber at start, with another 150 ml added halfway through the simulation. Nebulization lasted for 60 minutes, the total length of the simulation. To keep the virus from inactivating from heating in the nebulizer, both the virus solution and Milli-Q water used were kept on ice before use. Additionally, the aerosolization chamber was covered with a cooling element while used to further prevent it from overheating. The produced aerosols were 6-8 µm in size and the nebulization rate was approximately 0.3 ml/min or $3\cdot10^8$ particles/s with an airflow of 14 l/min. A plastic plate was set where the nebulized aerosols would flow out from the machine to prevent them from sinking to the floor right away.

2.5 Restaurant simulations

The restaurant simulations lasted for 60 min, with seven people sitting inside the room while ongoing, simulating customers sitting at tables. FFP3-masks, hair covers, safety goggles, shoe covers, nitrile gloves and protective overalls were used for personal safety, and the wellbeing of everyone inside the simulation room was monitored with a health questionnaire after each simulation (not included in this master's thesis). The restaurant room was approximately 60 m^2 in size. The room was cleaned with a UV-C lamp (see 2.8) between the simulation.

2.5.1 Intervention methods in the restaurant simulations

Two UniqAir PRO-air purifiers with activated carbon- and HEPA filters were tested in the restaurant simulations as intervention methods. They were run at 80 % capacity, around 270 m^3 / h, because the noise they made at 100 % capacity made them impractical. The positioning of the UniqAir PRO- air purifiers is show in Figures 1 and 3.

Space dividers were also tested as an intervention method in the restaurant simulations. They were 150 cm high, 150 cm wide and made from glass panels with wooden connection parts. They were provided by Made by Choice. The positioning of the space dividers is shown in Figures 2 and 3.

2.5.2 Reference simulations in the restaurant

A reference simulation was carried out after each intervention simulation on the same day. Reference simulations were carried out the same way as intervention simulations, but the tested intervention methods were removed.

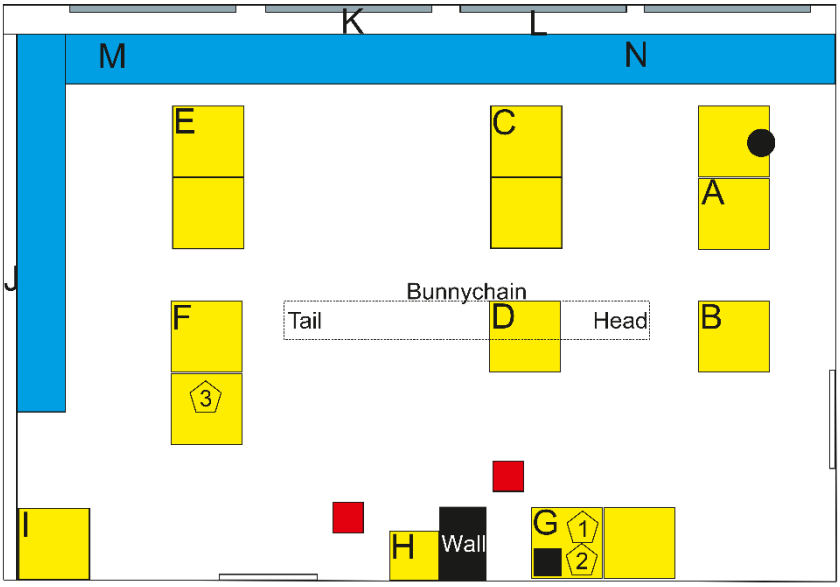

Figure 1. Restaurant layout with UniqAir PRO- air purifiers. Nebulizer is represented with a black circle, Biospot 300p with a black square, Andersen impactors with pentagons and UniqAir PRO- air purifiers with red squares. Doors to the room are marked as white rectangles and windows as grey rectangles. The Bunnychain is a decorative element hanging from the roof at 2.5 m height. Samples were taken from the positions marked with letters, from the floor and from the Bunnychain.

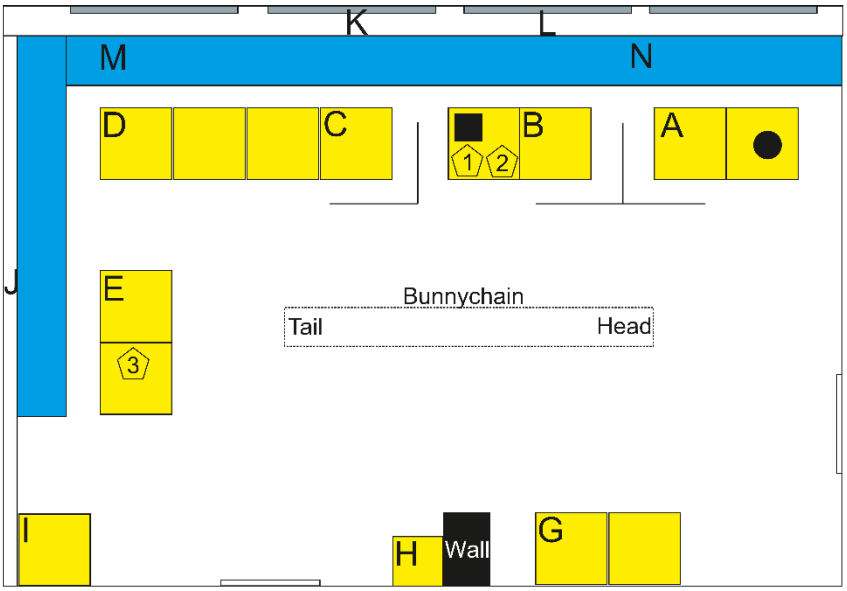

Figure 2. Restaurant layout with space dividers. Nebulizer is represented with a black circle, Biospot 300p with a black square, Andersen impactors with pentagons and space dividers with black lines. Doors to the room are marked as white rectangles and windows as grey rectangles. The Bunnychain is a decorative element hanging from the roof at 2.5 m height. Samples were taken from the positions marked with letters, from the floor and from the bunny chain.

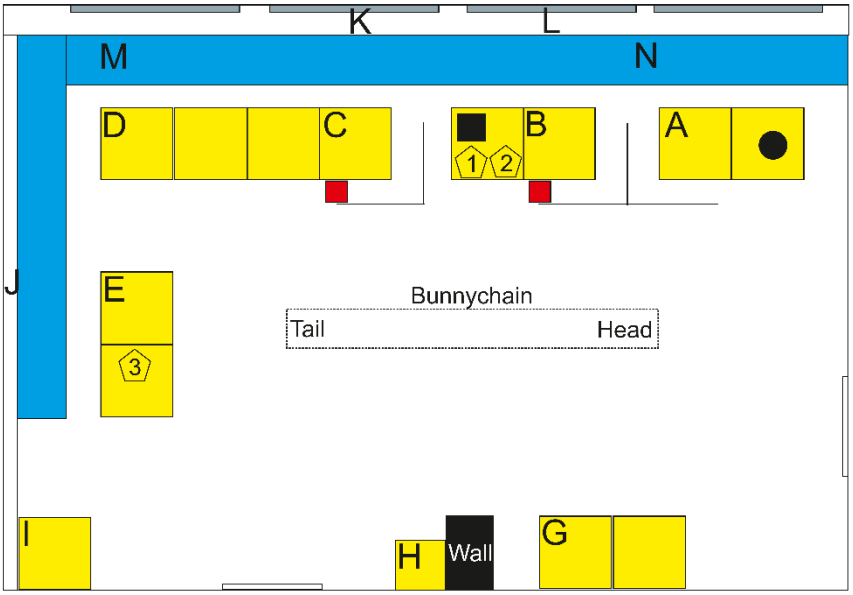

Figure 3. Restaurant layout with space dividers and UniqAir PRO- air purifiers. Nebulizer is represented with a black circle, Biospot 300p with a black square, Andersen impactors with pentagons, UniqAir PRO- air purifiers with red squares and space dividers with black lines. Doors to the room are marked as white rectangles and windows as grey rectangles. The Bunnychain is a decorative element hanging from the roof at 2.5 m height. Samples were taken from the positions marked with letters, from the floor and from the Bunnychain.

Figure 4. Restaurant simulation preparations with space dividers. Bunnychain is hanging from the roof.

2.6 Laboratory simulations

Aerte AD 2.0 and the ionizer prototype (Figure 6) were tested in laboratory environment due to time restrictions with the restaurant simulation schedule and the possibility of them releasing harmful substances, such as ozone. The emission of these substances was measured by Matti Leikas from the Finnish Institute of Occupational Health (TTL) and it is not a part of this study.

The laboratory simulations lasted for 15 min, as the room was smaller than the restaurant room, around 15 m^2, which allowed the virus to spread faster. A BSL2 laboratory at the University of Helsinki Viikki campus' EE-building was used for the laboratory simulations. No persons were inside the laboratory while simulations were running, and the electronics were operated with timers.

Ionizers produce negatively charged ions into the air, which then react with other nearby particles, such as aerosols. This process rapidly removes aerosols from the air and deposit them onto nearby surfaces (Jiang et al., 2018). In this study, an ionizer prototype was studied. Laboratory simulation layout is shown in Figure 5.

Aerte AD 2.0 is a small filterless air purification unit that releases hydroxyl radicals into the air, eliminating microbes from air and surfaces. According to the manufacturer, the apparatus covers 100 m^2 or 300 m^3 of space (Aerte, 2021). Aerte AD 2.0 was mounted on the shelf 0.6 m from the nebulizer, with the flow facing from the wall towards the room. Three ionizer prototypes were hung from the ceiling at 2 m height and approximately 0.5 m apart from each other, with the first ionizer being 0.7 m from the nebulizer. Aerosol levels were measured in the room to ensure normal conditions were present at the start of each simulation.

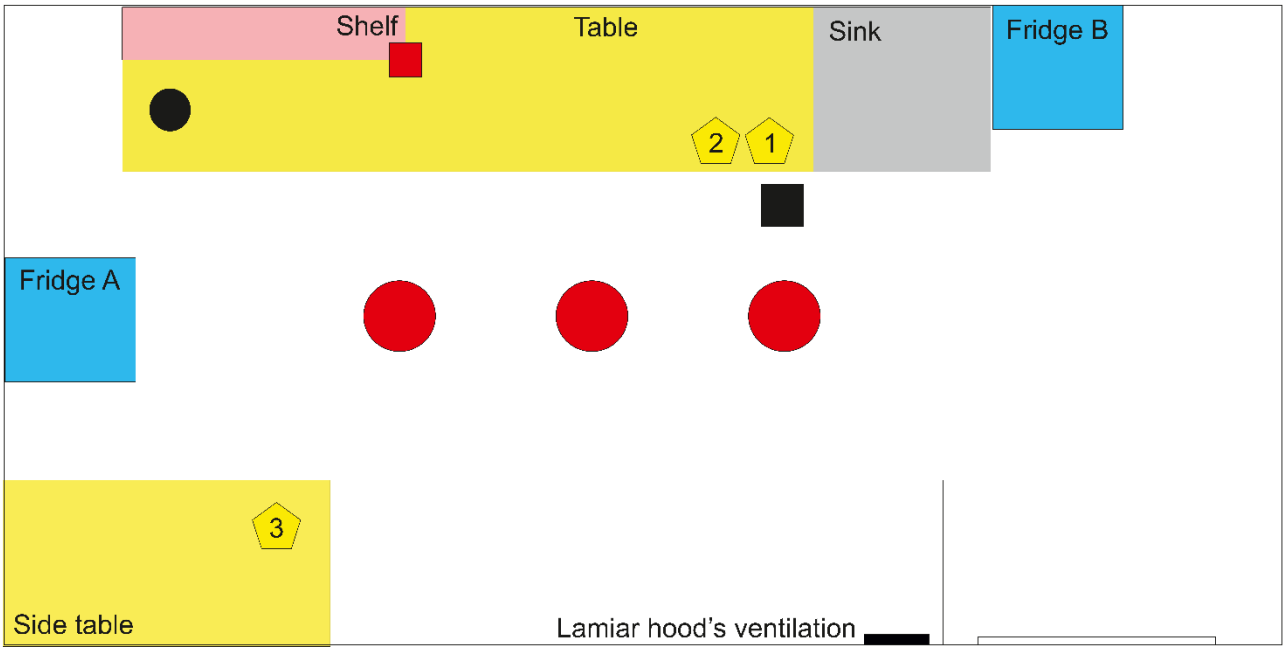

Figure 5. Laboratory simulation layout. Nebulizer is presented with a black circle, AERTE 2.0 with a red square and ionizers with red circles. Ionizers were hung from the roof at 2 m height. Biospot 300p is represented with a black square and Andersen impactors as pentagons.

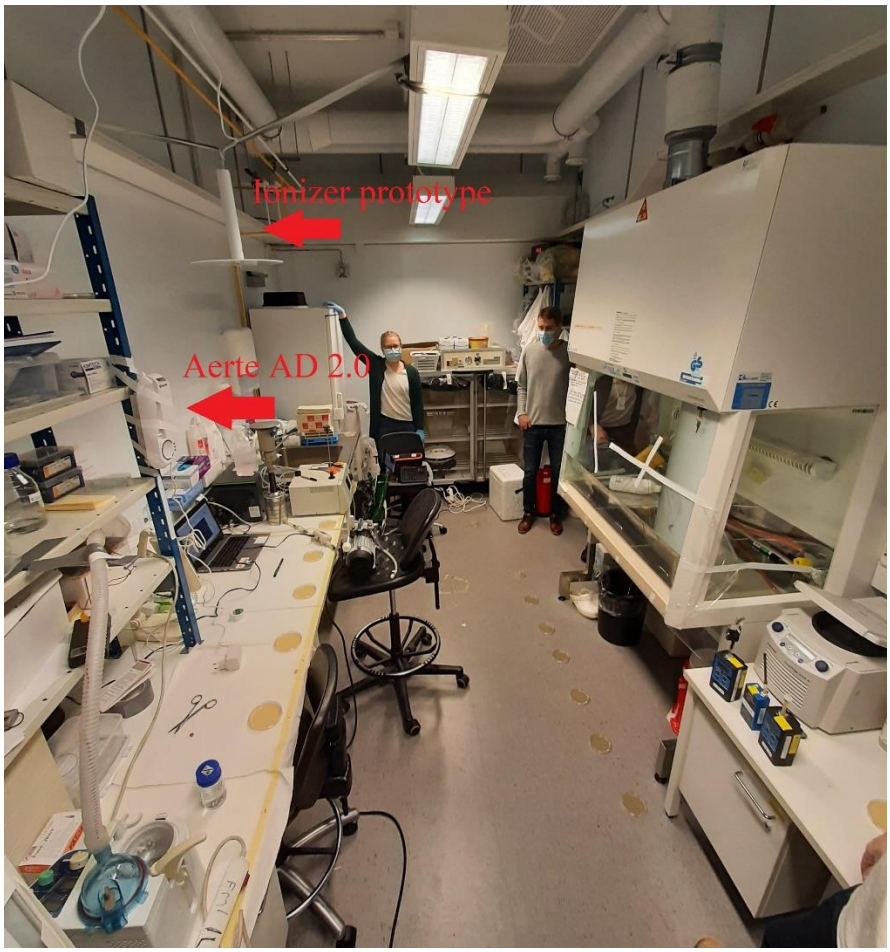

Figure 6. Laboratory simulation setup. Aerte AD 2.0 can be seen taped to the shelf and one ionizer prototype hanging from the roof. Floor plates and ionizers are not on their final places.

2.7 Sample collection

2.7.1 Aerosol sampling

Aerosols were collected onto HB10Y plates, and empty plates containing 27 ml 20 mM HEPES using Andersen cascade impactors, which consist of six levels of compartments. These compartments have decreasing sized holes on their bottoms to allow collection of different sized aerosols into different compartments. The size ranges of the aerosols collected from biggest to smallest (from close to sample inlet towards the bottom) are >7 µm, 4.7-7 µm, 3.3-4.7 µm, 2.1-3.3 µm, 1.1-2.1 µm and 0.65-1.1 µm, and they will be further referred to as compartments 1-6 with compartment 1 being >7 µm. The Andersen impactors collected aerosols for 30 min (UniqAir PRO- air purifiers and reference), 20 min (space dividers and reference) and 10 min (space dividers with UniqAir PRO- air purifiers and reference) after the first 30 min of nebulization. Time was adjusted to prevent the plates from being

overloaded by virus plaques. A flowrate of 28.3 l/min was used. HB10Y plates were analyzed by counting virus plaques. Aerosol samples in 20 mM HEPES were analyzed with plaque assay and qRT-PCR. The qRT-PCR analyzes were carried out by research associate Pavlína Gregorová and are not a part of this study.

A shorter activation time was used in the laboratory simulations, with two of the Andersen impactors being activated for the last 5 min (Andersen impactors 1 and 3) and one for 5 min after nebulizing had stopped (Andersen impactor 2). Andersen impactors 1 and 2 were set on the table at 2 m, and Andersen impactor 3 on the side table at 1.5 m from the nebulizer. Additionally, in the laboratory simulation only HB10Y plates were used in the Andersen impactors.

Aerosol Devices Inc's Biospot 300p bioaerosol sampler was also used to collect the virus (Parker et al., 2020). It collects aerosols by condensating them through a laminar-flow growth tube onto liquid on a small plate located inside the sampler. 1 ml of HEPES was used as the liquid. This method of collection should produce minimal stress to the collected viruses (Nieto-Caballero 2019). After the simulation, the plate was removed from the sampler and the liquid transferred into a sterile Eppendorf tube using a disposable plastic pipette. The tube was kept on ice until infective virus titer was analyzed with plaque assay. The Biospot 300p sampler was active for 60 min in the restaurant simulation and 15 min in the laboratory simulation, and operated with a flow rate of 8 l/min.

2.7.2 Deposition samples

Deposition samples consisted of HB10Y plates that collect naturally falling particles, such as aerosols, without any external air flows. In the restaurant room, they were used to collect aerosols at floor-, table- and head level, (approximately 40 cm above the table) (further referred to as "low", "middle" and "high") and from the Bunnychain at 2.5 m height. Samples were collected at different locations in the room on HB10Y plates, and empty plates containing 15 ml 20 mM HEPES to sample the room as widely as possible (Figures 1, 2 & 3). In the simulation with only the UniqAir PRO- air purifiers, only plates on table height were used as the idea of floor and head level plates had not yet been come up with. Plate lids were kept open for the duration of the simulation (60 min) excluding two test plates that were opened for the first and second 30 min, respectively (positioned at Couch N). This was performed in order to see does the virus concentration from the nebulizer vary during the first and second half (30 min) of the simulation. Deposition samples in 20 mM HEPES were analyzed with qRT-PCR by research associate Pavlína Gregorová and are not a part of this study.

In the laboratory simulations, only HB10Y plates were used, which were set on the table in front of the nebulizer and on the floor at 0.3 m intervals from 0.3 m to 2.2 m. Additionally, plates were placed on the fridges A (1.0 m, 2.0 m height) and B (3.0 m, 2.0 m height), on the shelf (0.5 m, 2.0 m height), on the side table (1.5 m) and on the sink (2.5 m) (Figure 4).

2.7.3 Surface samples

Surface samples consisted of swab samples and finger tests and they were taken after the simulation had ended and the nebulizing had stopped. No surface samples were taken in the laboratory simulations.

Surface swab samples were collected using sterile polyester swabs, which were cut into Eppendorf tubes containing 1 ml HEPES buffer after sampling. Couches, tables, Bunnychain (Figure 4), windowsills and the floor were sampled by swabbing an area of approximately 10 cm^2. The amount of infective virus was analyzed by plaque assay and the total amount of virus with qRT-PCR. The qRT-PCR analyzes were carried out by research associate Pavlína Gregorová and are not a part of this study. Surface swab samples were analyzed by plaque assay only in the UniqAir PRO- air purifiers simulations as they were deemed ineffective at collecting infective virus.

Finger tests were collected by swabbing the surface straight for 20 cm with two sterile gloved fingers and gently pressing them on a HB10Y plate. Tables, Bunnychain and the plate which the nebulized aerosols flowed to were sampled with finger tests. Finger tests were collected as a qualitative test for infective viruses on surfaces.

2.8 Cleaning

The restaurant room was cleaned in between and after simulations using LIT UV Elektro's Svetolit UV-C 600 W UV-lamp with the wavelength of 254 nm for 10 minutes. The power of the lamp was measured with Solar Light's Radiometer (PMA 2100) and UV Radiation Safety Sensor (PMA 2120) to be 1 W/m^2 which corresponds to 60 mJ/cm^2 in 10 minutes. The effect of the UV cleaning was analyzed by leaving three 20 µl droplets of the nebulizing solution ($5 \cdot 10^{10}$ pfu/ml) on petri dishes in the room while the UV-lamp was on. Three different scenarios were tested: one with droplets on a plate with open lid, one with a closed plate and one with a closed plate covered with aluminum foil. The closed plate was used to see if the UV could penetrate the plastic lid and the aluminum foil

covered plate was intended as a control. The droplets were then recollected into 980 µl of HEPES and virus titer determined by plaque assay. Three repeats were done.

3 Results

3.1 Virus aerosol transmission and intervention methods from the restaurant simulations

Here, the results of aerosol transmission of infective viruses are described during different intervention methods in the restaurant simulations. Each intervention simulation was followed by a reference simulation on the same day without said intervention method. In the results, plates that could not be counted due to being filled with virus plaques are marked as too many to count (TMC). TMC was determined to be more than 700 plaques on a plate.

3.1.1 Intervention method 1: UniqAir PRO- air purifiers

In this part, infective virus amounts in aerosol-, deposition-, and surface samples from the UniqAir PRO- air purifiers intervention- and reference simulations are shown and compared. Distances of the samples from the nebulizer are marked in brackets.

In the intervention, all but the smallest compartment in Andersen impactor 1 (2.0 m), were determined TMC. In the reference, compartments 1 and 2 showed significantly lower amounts of infective virus than those in the intervention (Table 1). In Andersen impactor 2 (2.0 m), infective viruses were only found in compartment 4 in intervention, whereas in the reference simulation infective virus was found in compartments 2-6 (Table 1). In Andersen impactor 3 (8.0 m), the viral load was lower in compartments 1, 2 and 6 in the intervention when compared to the reference, and equal in compartments 3-5 (Table 1). Lastly, Biospot 300p showed comparable results, though there was less infective virus collected in the intervention (Table 1).

On the deposition plates, the effectiveness of the UniqAir PRO- air purifiers depended on the position of the plates. A lowered number of infective viruses was observed in the intervention, compared to the reference, on the Windowsills K (5.5 m) and L (3.0 m), and couch N first and second 30 min (0.8 m), while the samples in between the nebulizer and the UniqAir PRO- air purifiers showed an increase in the viral load in the intervention compared to the reference (samples B (1.5 m), D (4.5 m), F (7.0 m), G (4.5 m) and H (6.5 m)) (Table 2). The far left and upper left corner showed no significant differences between the intervention and reference (samples E (6.5 m), I (9.2 m), J (10 m) and K (5.5 m)), however, in the intervention couch M (8.5 m) had almost half the viral load of the reference

(Table 2). On Couch N second 30 min, the viral load was half that of the first 30 min (Table 2). Overall, the viral load had a downward trend with distance (Figures 8 and 9).

Surface samples collected with polyester swabs were not able to collect any infective virus (Table 3), however, all of the finger tests were positive (Table 4). A positive finger test is shown in Figure 7.

Table 1. Air samples from UniqAir PRO- air purifiers simulations.

Air samples	Particle size (µm)	Distance (m)	Media	Intervention (pfu)	Reference (pfu)
Andersen impactor 1.1	>7	2.0	Agar	TMC	112
Andersen impactor 1.2	4.7-7	2.0	Agar	TMC	274
Andersen impactor 1.3	3.3-4.7	2.0	Agar	TMC	TMC
Andersen impactor 1.4	2.1-3.3	2.0	Agar	TMC	TMC
Andersen impactor 1.5	1.1-2.1	2.0	Agar	TMC	TMC
Andersen impactor 1.6	0.65-1.1	2.0	Agar	649	TMC
Andersen impactor 2.1	>7	2.0	HEPES	0	0
Andersen impactor 2.2	4.7-7	2.0	HEPES	0	30 /ml
Andersen impactor 2.3	3.3-4.7	2.0	HEPES	0	20 /ml
Andersen impactor 2.4	2.1-3.3	2.0	HEPES	20 /ml	10 /ml
Andersen impactor 2.5	1.1-2.1	2.0	HEPES	0	164 /ml
Andersen impactor 2.6	0.65-1.1	2.0	HEPES	0	64 /ml
Andersen impactor 3.1	>7	8.0	Agar	20	113
Andersen impactor 3.2	4.7-7	8.0	Agar	74	317
Andersen impactor 3.3	3.3-4.7	8.0	Agar	TMC	TMC
Andersen impactor 3.4	2.1-3.3	8.0	Agar	TMC	TMC
Andersen impactor 3.5	1.1-2.1	8.0	Agar	TMC	TMC
Andersen impactor 3.6	0.65-1.1	8.0	Agar	475	TMC
Biospot 300p	0.005-10	4.5	HEPES	3500 /ml	4500 /ml

Virus amounts represented from low to high as green to red.

Table 2. Deposition samples from UniqAir PRO- air purifiers simulations.

Deposition samples	Distance (m)	Duration (min)	Intervention (pfu)	Reference (pfu)
Couch N	0.8	1st 30	270	TMC
Couch N	0.8	2nd 30	140	TMC
Table A	0.5	60	TMC	TMC
Table B	1.5	60	706	199
Table C	4.0	60	487	564
Table D	4.5	60	423	332
Table E	6.5	60	178	207
Table F	7.0	60	316	127
Table G	4.5	60	285	171
Table H	6.5	60	309	183
Table I	9.2	60	265	251
Backwall J	10	60	137	133
Windowsill K	5.5	60	152	177
Windowsill L	3.0	60	444	TMC
Couch M	8.5	60	159	302
Couch N	0.8	60	264	TMC

Virus amounts represented from low to high as green to red.

Table 3. Swab surface samples from UniqAir PRO- air purifiers simulation.

Surface samples	Distance (m)	Medium	Intervention (pfu)	Reference (pfu)
Table B	1.5	HEPES	0	0
Couch N	0.8	HEPES	0	0
Floor next to the nebulizer	0.5	HEPES	0	0
Windowsill K	5.5	HEPES	0	0
Bunnychain above Table B	1.5, 2.5 height	HEPES	0	0
Couch next to Table D	4.5	HEPES	0	0
Floor next to Table D	4.5	HEPES	0	0
Windowsill L	3.0	HEPES	0	0
Bunnychain above table E	6.5, 2.5 height	HEPES	0	0

Table 4. Finger tests from UniqAir PRO- air purifiers simulations.

Finger tests	Distance (m)	Intervention	Reference
Table A	0.5	Positive	Positive
Table A	0.5	Positive	Positive
Table E	6.5	Positive	Positive
Table E	6.5	Positive	Positive
Nebulizer "plate"	0	Positive	Positive

Figure 7. Positive finger test HB10Y plate showing phi 6 plaques resulting from touching the plate with fingers.

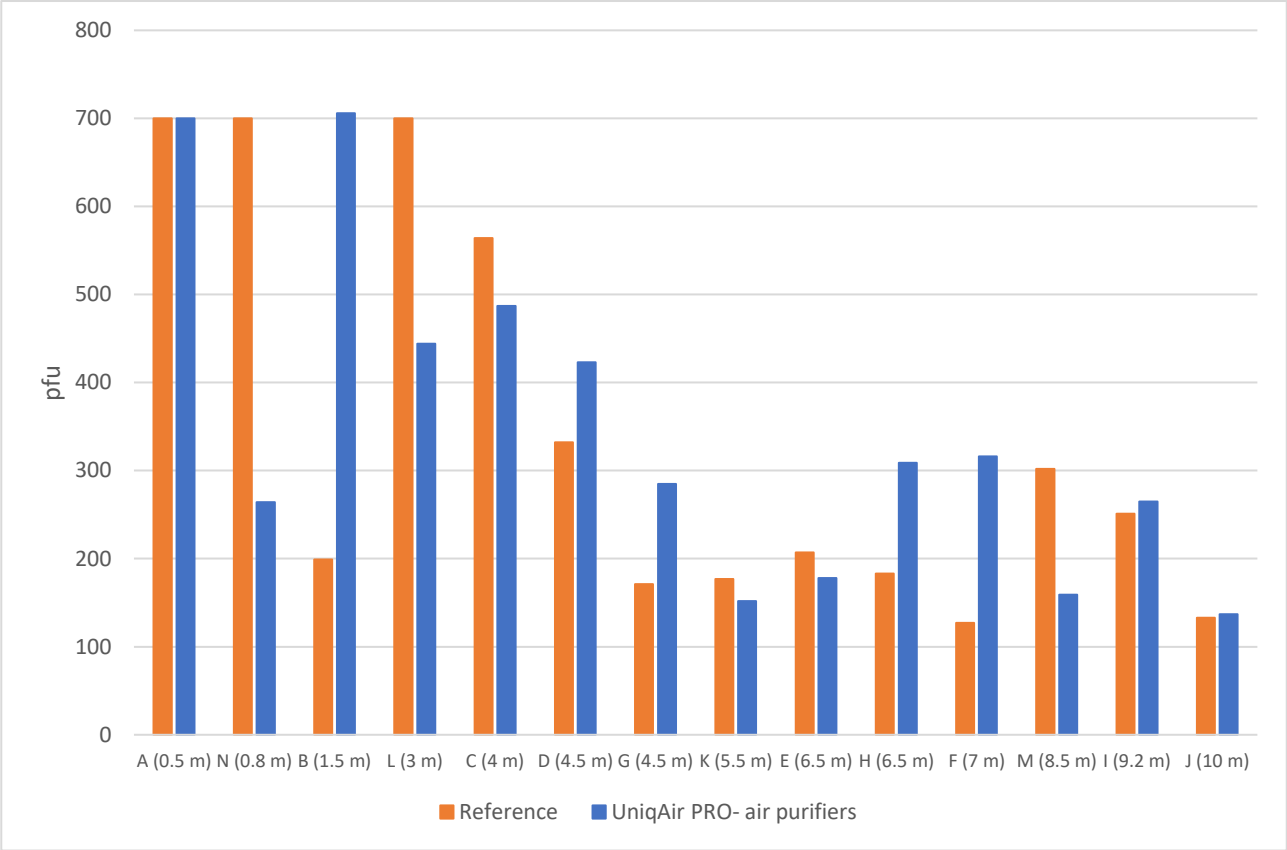

Figure 8. Viral pfu over distance from the nebulizer in UniqAir PRO- air purifiers simulation's plates.

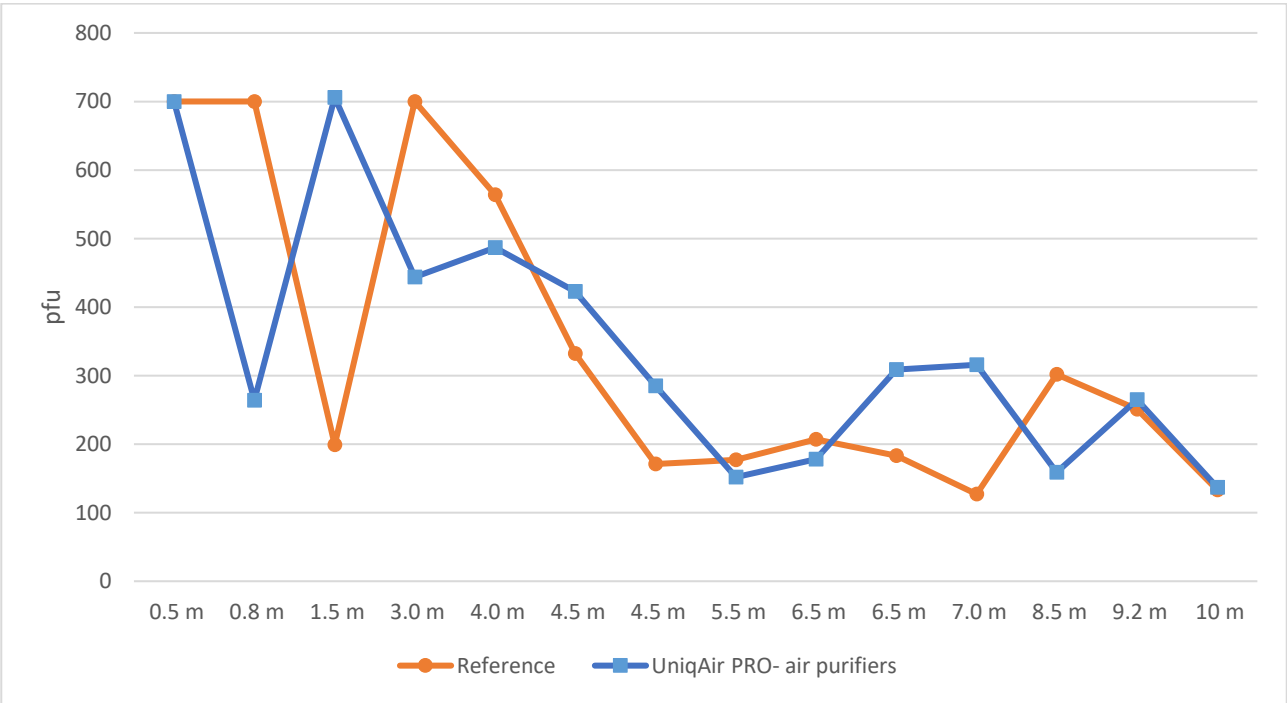

Figure 9. Viral pfu over distance from the nebulizer in UniqAir PRO- air purifiers simulation's plates.

3.1.2 Intervention method 2: space dividers

In this part, infective virus amounts in aerosol-, deposition-, and surface samples from the space dividers intervention- and reference simulations are shown and compared. Distances of the samples from the nebulizer are marked in brackets.

Andersen impactor 1 had higher amounts of virus in the intervention compared to the reference in compartments 1, 2, 3 and 6 (Table 5). In Andersen impactor 2, the samples collected had almost no infective viruses at all in either of the simulations, virus was only found in the reference compartment 1 (Table 5). Interestingly, in the intervention, Andersen impactor 3 showed highest numbers in compartments 3 and 4, while in the reference highest amounts of infective virus was found in compartments 1 and 4 (Table 5). Biospot 300p collected significantly more infective viruses during the intervention than the reference (Table 5).

On the deposition plates, the space dividers seemed to increase the number of infective viruses collected. In the intervention, the viral load was especially high, when compared to the reference, in the high plates B (3.0 m), C (5.5 m) , D (8.0 m) , G (4.5 m) and K (5.5 m), and middle plates C (5.5 m), E (8.5 m), H (6.5 m), J (10 m), K (5.5 m), L (3.0 m), M (8.5 m) and N (0.8 m) (Figures 10 and 11). The Bunnychain samples from head (1.3 m, 2.5 m height), middle (4.5 m, 2.5 m height) and tail (7.0 m, 2.5 m height), also showed an elevated number of infective viruses during the intervention when compared to the reference (Table 6). Low plates were mostly similar in the reference and intervention simulations, with some exceptions; Tables B (3.0 m), and I (9.2 m) having a higher viral load in the intervention and Tables C (5.5 m) and G (4.5 m) a lower one in the intervention (Table 6). Interestingly, Table A (0 m) high plate only had one plaque one it (Table 6). In the intervention, the virus concentration stayed constant (TMC) from 0.8 m to 3 m, (Table 6) whereas in the reference, it decreased already at 0.8 m (Figure 12). Interestingly, samples from Table A (0 m), where the nebulizer was located, had lower amounts of virus in the intervention (Table 6). A roughly 50 % increase was observed from Couch N first to the second 30 min in the reference, whereas in the intervention, a large decrease (from TMC to 130 pfu) was observed (Table 6).

Finger tests were positive in both simulations for Table A (0 m) five minutes after nebulizer had stopped, Table C (5.5 m) and Bunnychain middle (4.5 m, 2.5 m height). Interestingly, no virus was found in the reference from Couch N (0.8 m) with finger tests even though it was found in the intervention. No virus was found from Table D (8.0 m) after either simulation (Table 7)

Table 5. Air samples from space divider simulations.

Air samples	Particle size (µm)	Distance (m)	Media	Intervention (pfu)	Reference (pfu)
Andersen impactor 1.1	>7	3.0	Agar	TMC	17
Andersen impactor 1.2	4.7-7	3.0	Agar	479	135
Andersen impactor 1.3	3.3-4.7	3.0	Agar	TMC	481
Andersen impactor 1.4	2.1-3.3	3.0	Agar	TMC	TMC
Andersen impactor 1.5	1.1-2.1	3.0	Agar	401	TMC
Andersen impactor 1.6	0.65-1.1	3.0	Agar	430	353
Andersen impactor 2.1	>7	3.0	HEPES	0	16 /ml
Andersen impactor 2.2	4.7-7	3.0	HEPES	0	0
Andersen impactor 2.3	3.3-4.7	3.0	HEPES	0	0
Andersen impactor 2.4	2.1-3.3	3.0	HEPES	0	0
Andersen impactor 2.5	1.1-2.1	3.0	HEPES	0	0
Andersen impactor 2.6	0.65-1.1	3.0	HEPES	0	0
Andersen impactor 3.1	>7	8.5	Agar	234	TMC
Andersen impactor 3.2	4.7-7	8.5	Agar	97	38
Andersen impactor 3.3	3.3-4.7	8.5	Agar	TMC	166
Andersen impactor 3.4	2.1-3.3	8.5	Agar	TMC	TMC
Andersen impactor 3.5	1.1-2.1	8.5	Agar	500	376
Andersen impactor 3.6	0.65-1.1	8.5	Agar	376	363
Biospot 300p	0.005-10	3.0	HEPES	11000 /ml	4000 /ml

Virus amounts represented from low to high as green to red.

Table 6. Deposition samples from space divider simulations.

Deposition samples	Distance (m)	Duration (min)	Intervention (pfu)	Reference (pfu)
Couch N	0.8	1st 30	TMC	127
Couch N	0.8	2nd 30	130	199
Table A, low	0	60	142	137
Table A, middle	0	60	386	TMC
Table A, high	0	60	1	TMC
Table B, low	3.0	60	TMC	214
Table B, middle	3.0	60	TMC	234
Table B, high	3.0	60	436	199
Table C, low	5.5	60	95	159
Table C, middle	5.5	60	119	54
Table C, high	5.5	60	435	72
Table D, low	8.0	60	80	92
Table D, middle	8.0	60	86	61
Table D, high	8.0	60	211	74
Table E, low	8.5	60	127	127
Table E, middle	8.5	60	101	41
Table E, high	8.5	60	112	102
Table G, low	4.5	60	25	139
Table G, middle	4.5	60	59	71
Table G. high	4.5	60	392	181
Table H, low	6.5	60	82	81
Table H, middle	6.5	60	160	0
Table H, high	6.5	60	TMC	TMC
Table I, low	9.2	60	126	70
Table I, middle	9.2	60	138	94
Table I, high	9.2	60	175	136
Back wall J, low	10	60	19	17
Back wall J, middle	10	60	107	20
Back wall J, high	10	60	304	42
Windowsill K, middle	5.5	60	225	53
Windowsill K, high	5.5	60	262	26
Windowsill L, middle	3.0	60	TMC	173
Windowsill L, high	3.0	60	N/A	494

M, low	8.5	60	107	110
M, middle	8.5	60	158	0
N, low	0.8	60	98	125
N, middle	0.8	60	TMC	57
Bunnychain head	1.3, 2.5 height	60	TMC	183
Bunnychain middle	4.5, 2.5 height	60	147	85
Bunnychain tail	7.0, 2.5 height	60	TMC	69

Virus amounts represented from low to high as green to red.

Table 7. Finger tests from space divider simulations.

Finger test	Distance (m)	Intervention	Reference
Table A, 5min after nebulizer stopped	0	Positive	Positive
Table C	5.5	Positive	Positive
Table D	8.0	Negative	Negative
Couch N	0.8	Positive	Negative
Bunnychain, middle	4.5, 2.5 height	Positive	Positive

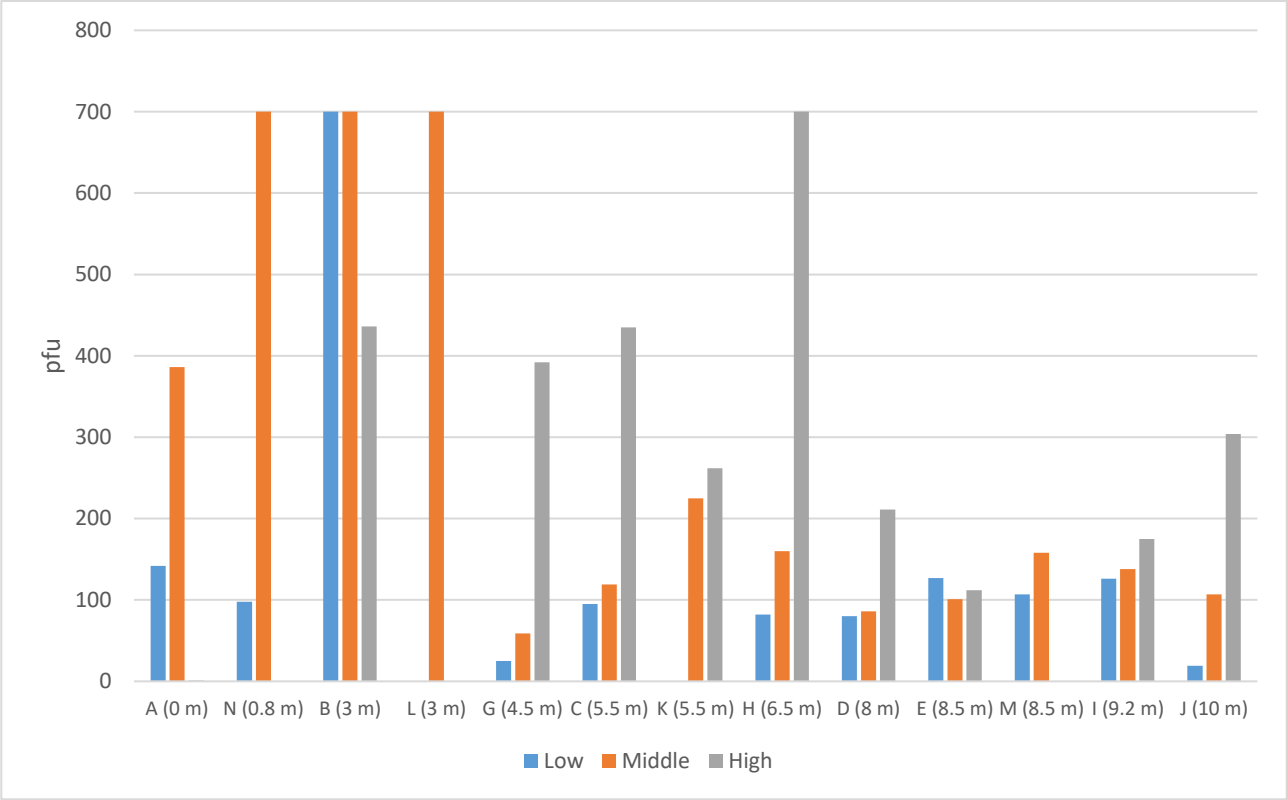

Figure 10. Viral pfu from floor (low), table (middle), and 40 cm above table (high) plates over distance from nebulizer in space dividers intervention simulation.

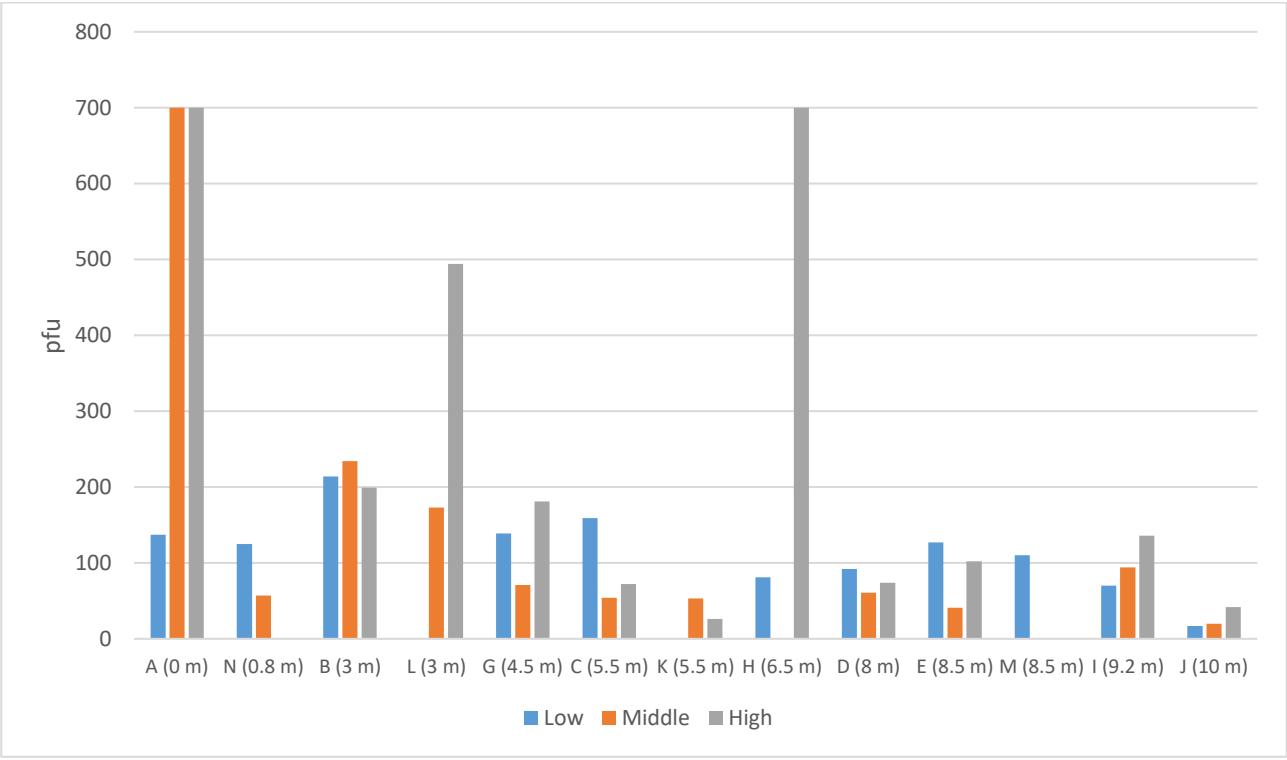

Figure 11. Viral pfu from floor (low), table (middle), and 40 cm above table (high) plates over distance from nebulizer in space dividers reference simulation.

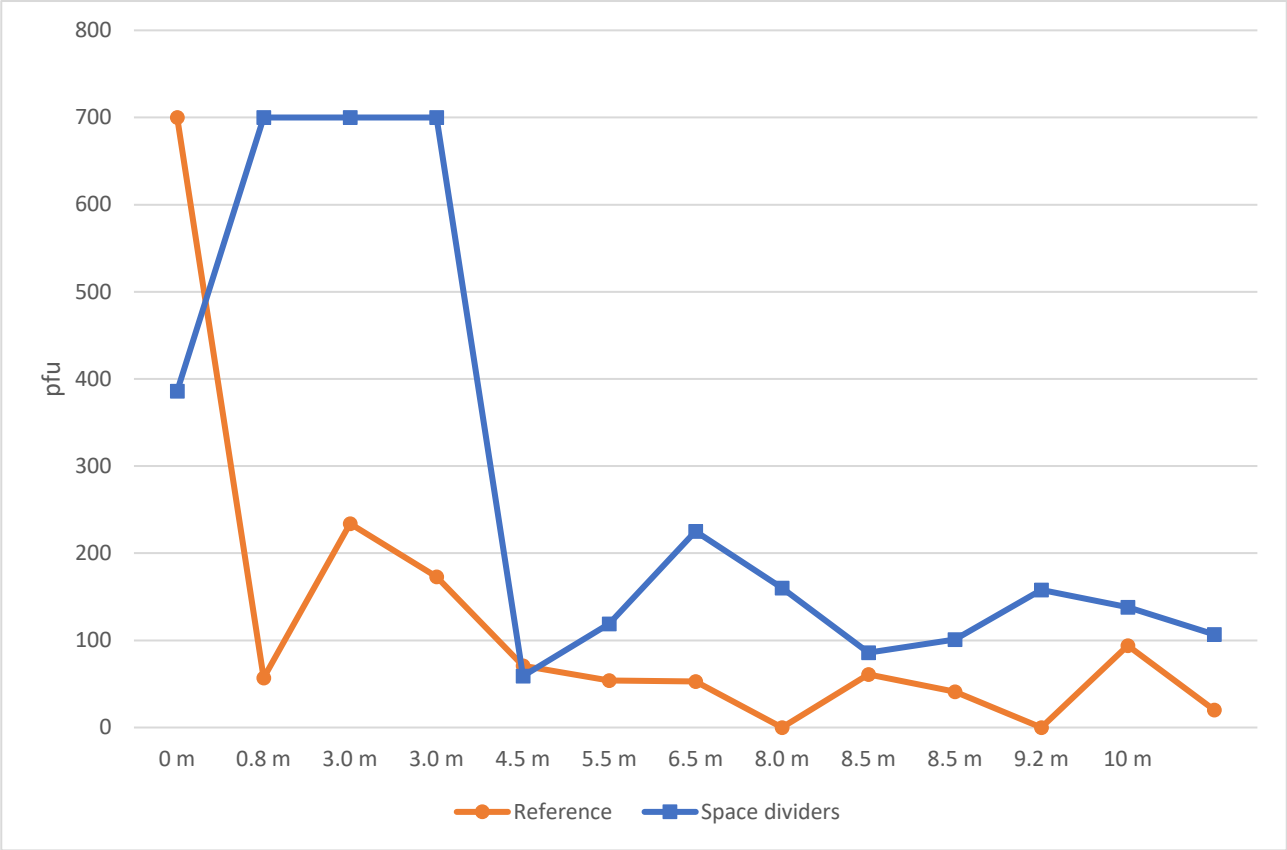

Figure 12. Viral pfu over distance from the nebulizer in space divider simulation's middle plates.

3.1.3 Intervention method 3: UniqAir PRO- air purifiers and space dividers

In this part, infective virus amounts in aerosol-, deposition-, and surface samples from the UniqAir PRO- air purifiers and space dividers intervention- and reference simulations are shown and compared. Distances of the samples from the nebulizer are marked in brackets.

In the intervention, only compartment 6 of Andersen impactor 1 was able to be counted, while in the reference compartments 1, 2 and 3 had a countable number of infective viruses (Table 8). A slightly higher number of infective viruses was collected with Andersen impactor 2 compartments 4, 5 and 6 during the intervention, than the reference (Table 8). Andersen impactor 3 had comparable results between the reference and the intervention, however, the intervention samples had slightly less infective viruses in compartments 5 and 6, and more in compartments of 1 and 2, when compared to the reference samples (Table 8). Biospot 300p results were comparable, but slightly higher in the intervention (Table 8).

The deposition samples showed an overall improvement in the number of infective viruses on the plates in the intervention, with most of the plates having less plaques, than those in the reference. All plates within 3 m of the nebulizer (N (0.8 m), A (0 m) and B (3.0 m)) collected TMC viruses in the reference simulation, except for A low (0 m). In the intervention, they were lower in N first, and second 30 min (0.8 m), A low (0 m) and N low (0.8 m) (Table 9). Further away, virus amounts were higher in the intervention than the reference only in samples A low (0 m), C middle (5.5 m), E high (8.5 m), G low (4.5 m) G middle (4.5 m), K middle (5.5 m) and Bunnychain tail (7.0 m, 2.5m height), with all the other samples showing a decreased or equivalent number of infective viruses in the intervention (Table 9). No differences were seen in samples taken from Couch N in the 1st and 2nd 30 min.

The room divided into two levels of virus concentration in the intervention, from 0-4.5 m to 5.5-10 m, with the exception of sample I (9.2 m) having a higher concentration than the other samples close to it (Figure 13). The virus concentration in the air decreased with distance from the nebulizer, but local peaks could still be seen in samples from Tables H (6.5 m) E (8.5 m) and I (9.2 m).

Out of all the simulations, the reference simulation for UniqAir PRO- air purifiers and space dividers had an exceptionally high number of TMC plates, 19 in total. TMC plates were found especially in the high (7 in total) and middle plates (7 in total, including N 1st 30 min and N 2nd 30 min) (Figure 14 and Table 9).

Finger tests showed positive results in the intervention for Table A (0 m) 5 min after nebulizer had stopped, Table C (5.5 m) and Couch N (0.8 m). In the reference, Table A (0 m) 5 min after nebulization, Couch N (0.8 m) and Bunnychain middle (4.5 m, 2.5 m height) samples were positive (Table 10).

Table 8. Air samples from UniqAir PRO- air purifiers and space divider simulations.

Air samples	Particle size (µm)	Distance (m)	Media	Intervention (pfu)	Reference (pfu)
Andersen impactor 1.1	>7	3.0	Agar	TMC	54
Andersen impactor 1.2	4.7-7	3.0	Agar	TMC	143
Andersen impactor 1.3	3.3-4.7	3.0	Agar	TMC	118
Andersen impactor 1.4	2.1-3.3	3.0	Agar	TMC	TMC
Andersen impactor 1.5	1.1-2.1	3.0	Agar	TMC	TMC
Andersen impactor 1.6	0.65-1.1	3.0	Agar	417	TMC
Andersen impactor 2.1	>7	3.0	HEPES	0	0
Andersen impactor 2.2	4.7-7	3.0	HEPES	0	0
Andersen impactor 2.3	3.3-4.7	3.0	HEPES	1 /ml	1 /ml
Andersen impactor 2.4	2.1-3.3	3.0	HEPES	13 /ml	8 /ml
Andersen impactor 2.5	1.1-2.1	3.0	HEPES	16 /ml	4 /ml
Andersen impactor 2.6	0.65-1.1	3.0	HEPES	2 /ml	0
Andersen impactor 3.1	>7	8.5	Agar	28	9
Andersen impactor 3.2	4.7-7	8.5	Agar	41	7
Andersen impactor 3.3	3.3-4.7	8.5	Agar	127	139
Andersen impactor 3.4	2.1-3.3	8.5	Agar	TMC	TMC
Andersen impactor 3.5	1.1-2.1	8.5	Agar	387	TMC
Andersen impactor 3.6	0.65-1.1	8.5	Agar	353	413
Biospot 300 p	0.005-10	3.0	HEPES	6100 /ml	5000 /ml

Virus amounts represented from low to high as green to red.

Table 9. Deposition samples from UniqAir PRO- air purifiers and space divider simulations.

Air to petridish samples	Distance (m)	Duration (min)	Intervention (pfu)	Reference (pfu)
Couch N	0.8	1st 30	268	TMC
Couch N	0.8	2nd 30	253	TMC
Table A, low	0	60	347	156
Table A, middle	0	60	TMC	TMC
Table B, low	3.0	60	TMC	TMC
Table B, middle	3.0	60	TMC	TMC
Table B, high	3.0	60	TMC	TMC
Table C, low	5.5	60	109	155

Table C, middle	5.5	60	158	66
Table C, high	5.5	60	73	TMC
Table D, low	8.0	60	109	200
Table D, middle	8.0	60	85	174
Table D, high	8.0	60	81	265
Table E, low	8.5	60	253	261
Table E, middle	8.5	60	105	154
Table E, high	8.5	60	198	123
Table G, low	4.5	60	TMC	0
Table G, middle	4.5	60	320	183
Table G. high	4.5	60	TMC	TMC
Table H, low	6.5	60	198	TMC
Table H, middle	6.5	60	174	TMC
Table H, high	6.5	60	340	TMC
Table I, low	9.2	60	162	235
Table I, middle	9.2	60	TMC	TMC
Table I, high	9.2	60	176	TMC
Back wall J, low	10	60	19	237
Back wall J, middle	10	60	44	130
Back wall J, high	10	60	96	TMC
Windowsill K, middle	5.5	60	126	0
Windowsill K, high	5.5	60	0	239
Windowsill L, middle	3.0	60	0	0
Windowsill L, high	3.0	60	TMC	TMC
M, low	8.5	60	96	313
M, middle	8.5	60	0	120
N, low	0.8	60	361	TMC
N, middle	0.8	60	TMC	TMC
Bunnychain head	1.3, 2.5 height	60	254	TMC
Bunnychain middle	4.5, 2.5 height	60	103	TMC
Bunnychain tail	7.0, 2.5 height	60	138	49

Virus amounts represented from low to high as green to red.

Table 10. Finger tests from UniqAir PRO- air purifier and space divider simulations.

Finger test	Distance (m)	Intervention	Reference
Table A, 5min after nebulizer stopped	0	Positive	Positive
Table C	5.5	Positive	Negative
Table D	8.0	Negative	Negative
Couch N	0.8	Positive	Positive
Bunnychain, middle	4.5, 2.5 height	Negative	Positive

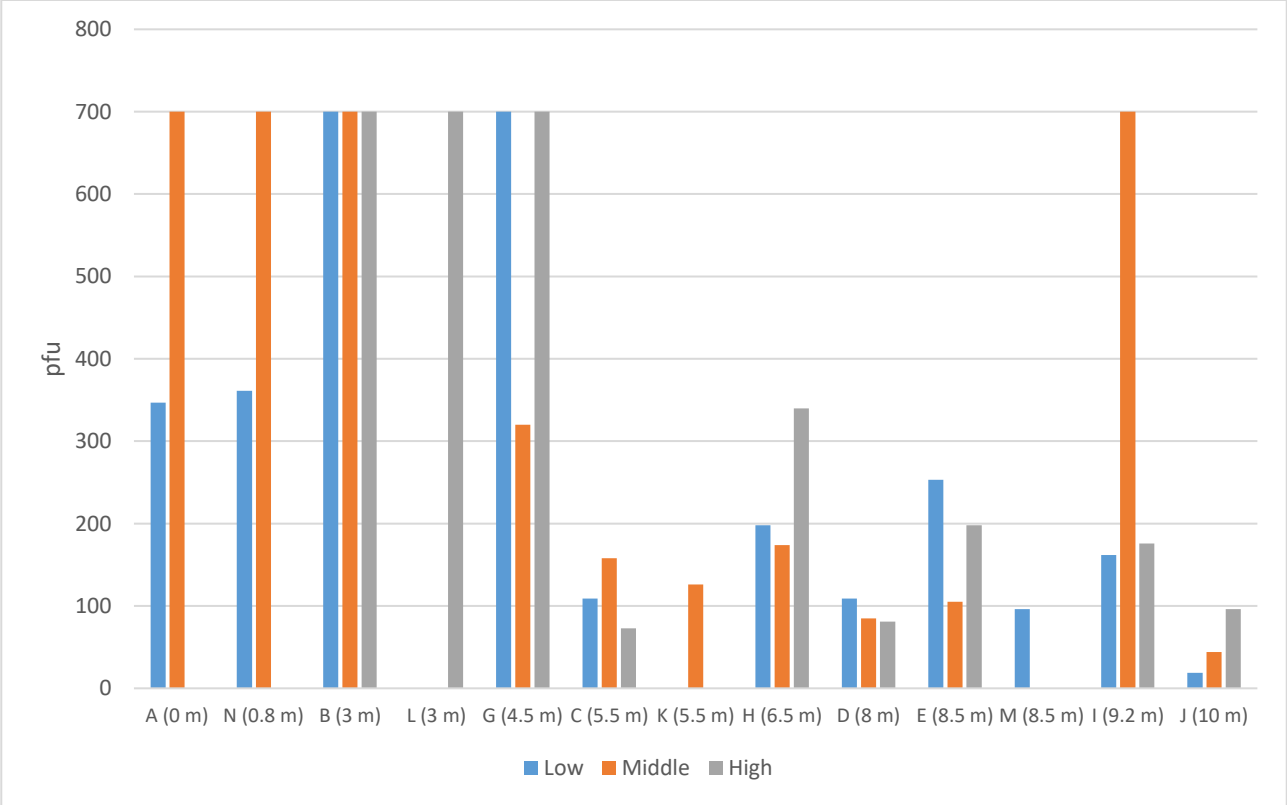

Figure 13. Viral pfu from floor (low), table (middle), and 40 cm above table (high) plates plates in UniqAir PRO- air purifiers and space dividers intervention simulation.

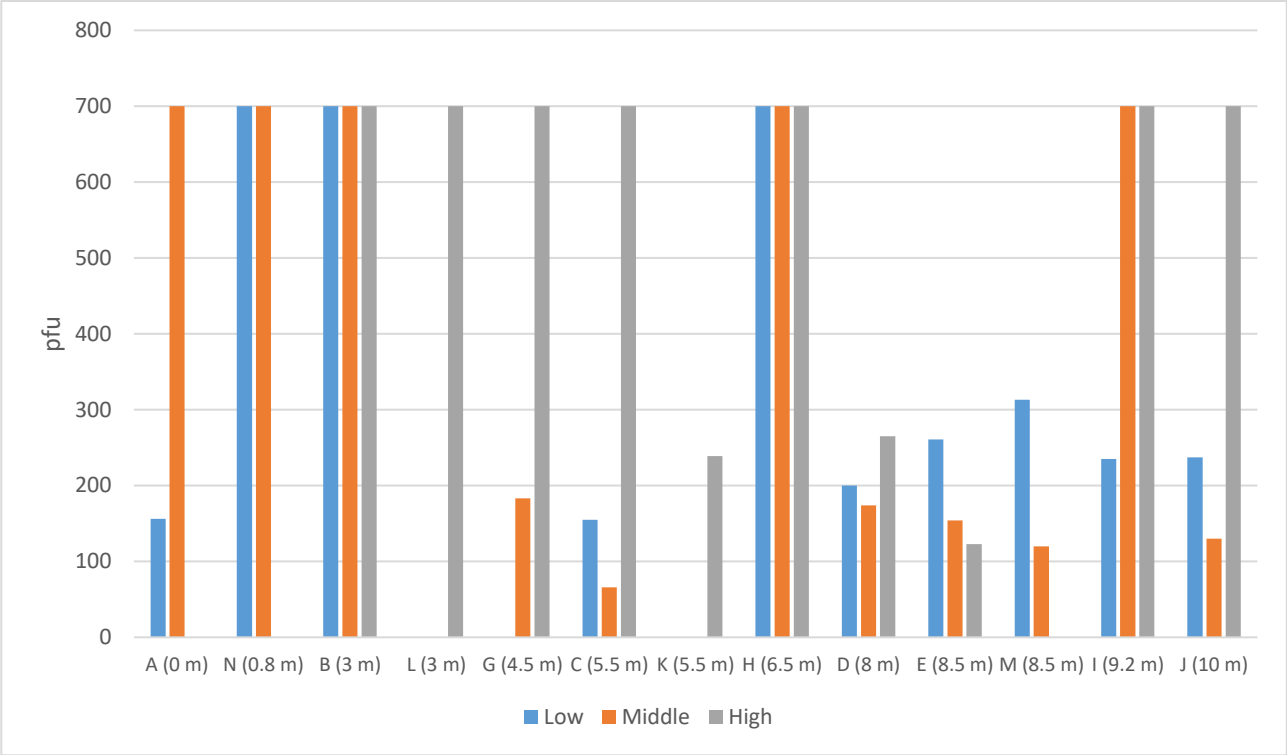

Figure 14. Viral pfu from floor (low), table (middle), and 40 cm above table (high) plates in UniqAir PRO- air purifiers and space dividers reference simulation.

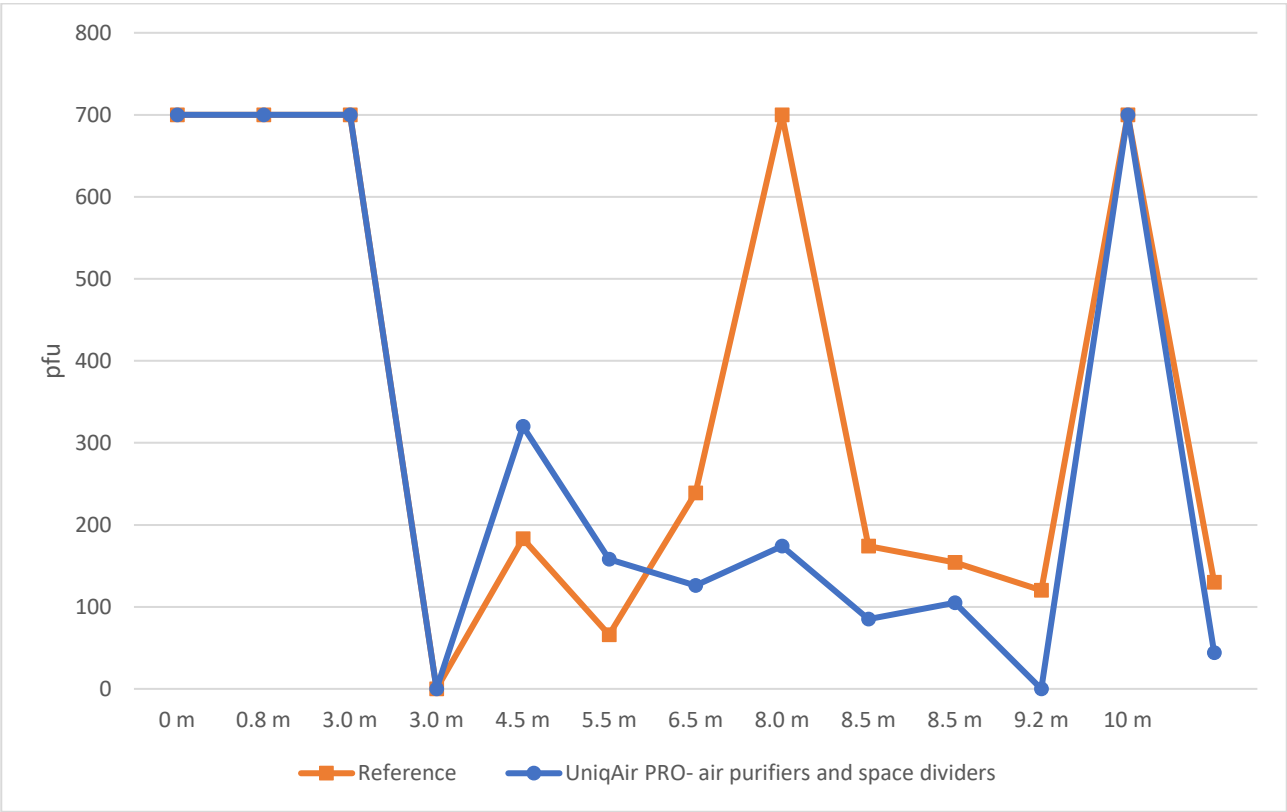

Figure 15. Viral pfu over distance in UniqAir PRO- air purifiers and space dividers simulation's middle plates.

3.2 Virus aerosol transmission and intervention methods from the laboratory simulations

Here, the results of aerosol transmission of infective viruses are described during different intervention methods in the laboratory simulations. All simulations were carried out on the same day. In the results, plates that could not be counted due to being filled with virus plaques are marked as too many to count (TMC). TMC was determined to be more than 700 plaques on a plate.

3.2.1 Intervention method 3: ionizer prototypes

Andersen impactor 1 virus amounts were higher in the intervention than the reference in compartments 1 and 6, lower in compartment 2 and comparable in 3, 4 and 5 (Table 11). In Andersen impactor 2, the virus amounts were lower in the intervention in all compartments except compartment 1, where it was slightly higher (Table 11). Virus amounts were lower or equivalent in the intervention than the reference in all Andersen impactor 3 samples (Table 11). Biospot 300p results were comparable between intervention and reference (Table 11).

In the deposition samples, a clearly lower amount of infective virus can be seen already at 0.3 m (Figure 16) when compared to the reference. The amount of infective virus continued to decrease at 0.6 m and 0.9 m, and after 1.8 m, there were less than 10 pfu collected per plate (Table 12). In the intervention, however, even at Fridge B (3.0 m) the viral load was still quite high (101 pfu), when compared to the intervention (Table 12). In the intervention, infective virus was mostly found in the samples from the table, whereas the samples on the floor only had a maximum of five plaques (0.3 m and 2.0 m) (Figure 17). In the reference, however, infective virus was found in larger amounts in many of the floor plates (Table 12).

3.2.2 Intervention method 4: Aerte AD 2.0

Andersen impactor 1 collected more or equivalent amounts of virus in the intervention than the reference in all compartments (Table 11). In Andersen impactor 2, however, all compartments but compartment 1, had less virus collected in the intervention than the reference (Table 11). In Andersen impactor 3, the intervention had slightly lower amounts of virus than the reference (Table 11). Biospot 300p results were equivalent (Table 11).

In the intervention, the number of infective viruses can be seen decreasing over distance from the nebulizer on the deposition samples after 0.9 m (Figure 16). The results after that are comparable at 1.2 m and lower in the intervention at 1.5 m. At 1.8 m, however, the intervention sample had a very high amount of virus compared to the reference (256 pfu vs 70 pfu) (Table 12). After 1.5 m, the virus amounts settled to 10-30 pfu. The amount of virus was lower on the floor than on the table in both the intervention and reference simulations (Figure 17).

Table 11. Air samples from laboratory simulations.

Sample	Particle size (µm)	Distance (m)	Reference (pfu)	Aerte AD 2.0 (pfu)	Ionizers (pfu)
Andersen impactor 1.1	>7	2.0	35	54	214
Andersen impactor 1.2	4.7-7	2.0	112	285	86
Andersen impactor 1.3	3.3-4.7	2.0	161	227	188
Andersen impactor 1.4	2.1-3.3	2.0	TMC	TMC	TMC
Andersen impactor 1.5	1.1-2.1	2.0	TMC	TMC	TMC
Andersen impactor 1.6	0.65-1.1	2.0	255	346	TMC
Andersen impactor 2.1	>7	2.0	5	27	40
Andersen impactor 2.2	4.7-7	2.0	11	8	0
Andersen impactor 2.3	3.3-4.7	2.0	80	50	41
Andersen impactor 2.4	2.1-3.3	2.0	366	260	280
Andersen impactor 2.5	1.1-2.1	2.0	303	186	271
Andersen impactor 2.6	0.65-1.1	2.0	191	65	125
Andersen impactor 3.1	>7	1.5	18	12	1
Andersen impactor 3.2	4.7-7	1.5	77	10	40
Andersen impactor 3.3	3.3-4.7	1.5	171	123	55
Andersen impactor 3.4	2.1-3.3	1.5	TMC	TMC	TMC
Andersen impactor 3.5	1.1-2.1	1.5	373	324	261
Andersen impactor 3.6	0.65-1.1	1.5	209	138	144
Biospot 300p	0.005-10	2.0	29 /ml	29 /ml	20 /ml

Virus amounts represented from low to high as green to red.

Table 12. Plate samples from laboratory simulations

Sample	Reference (pfu)	AERTE AD 2.0 (pfu)	Ionizers (pfu)
0.3 m	TMC	TMC	374
0.3 m floor	29	1	5
0.6 m	TMC	TMC	199
0.6 m floor	27	8	2
0.9 m	TMC	127	80
0.9 m floor	7	12	2
1.2 m	168	134	12
1.2 m floor	64	11	3
1.5 m	97	54	14
1.5 m floor	23	2	1
1.8 m	70	256	8
1.8 m floor	26	10	4
2.0 m	22	21	6
2.0 m floor	14	13	5
2.2 m	0	21	7
Sink (2.5 m)	230	31	3
Fridge A (1.0 m)	11	14	3
Fridge B (3.0 m)	101	31	9
Side table (1.5 m)	9	4	1
Shelf (0.5 m, 2 m height)	17	9	5

Virus amounts represented from low to high as green to red.

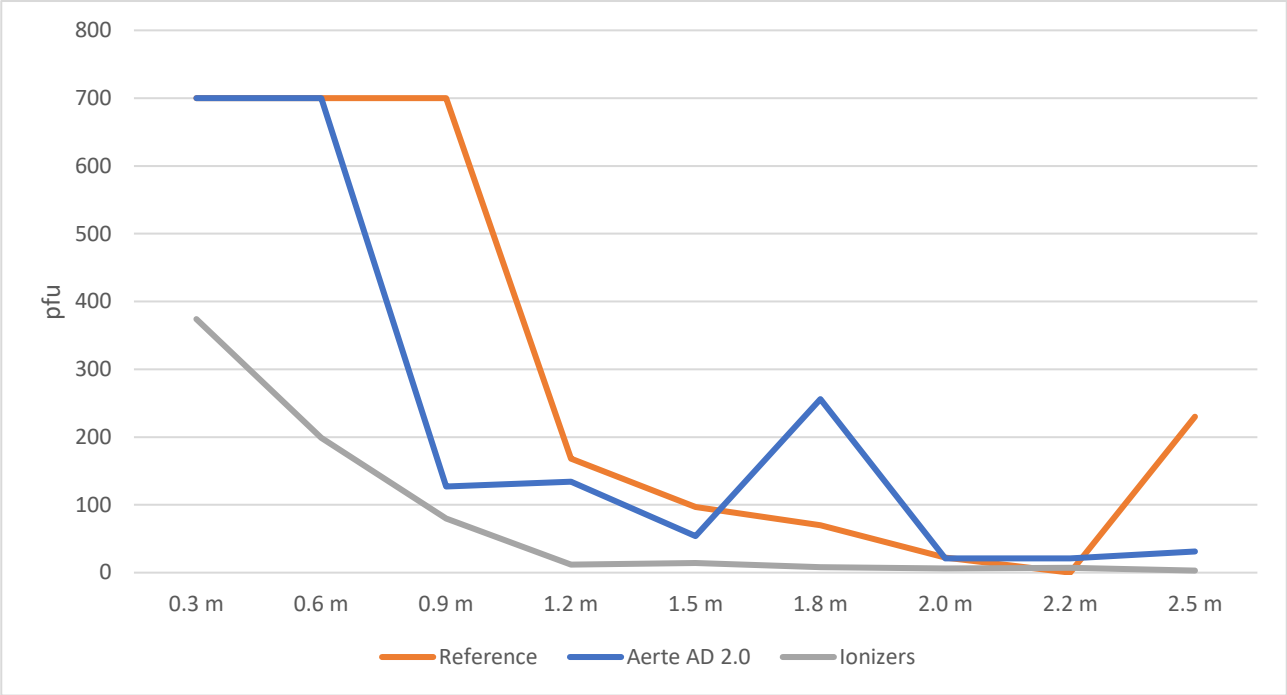

Figure 16. Viral pfu over distance from the nebulizer in laboratory simulations' table samples.

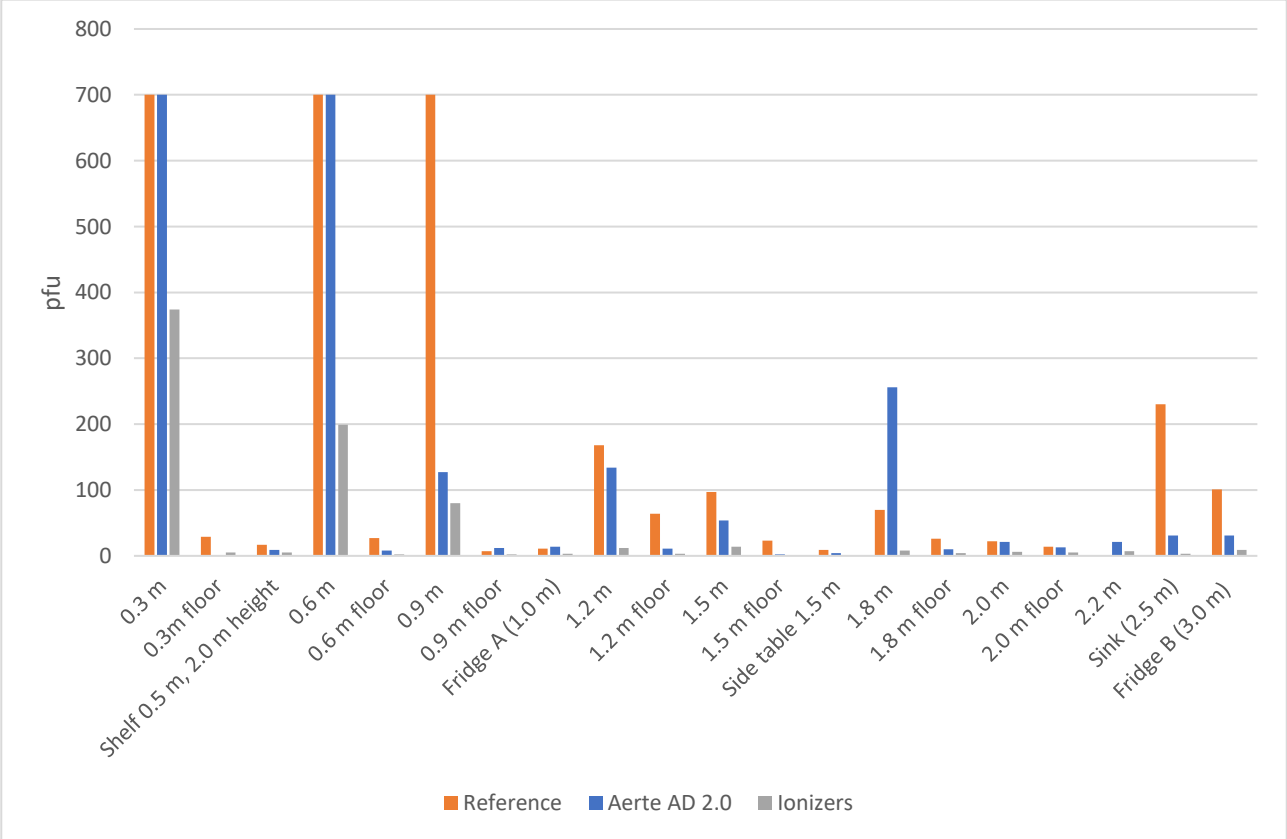

Figure 17. Viral pfu over distance from the nebulizer in laboratory simulations.

3.3 UV-C cleaning

UV-C cleaning reduced the amount of infective virus in the nebulizer solution by approximately 10^3 pfu/ml. The plastic lid was enough to block the UV-C from getting to the viruses (Table 13).

Table 13. UV-C cleaning test

UV-C cleaning sample	pfu/ml
Open lid	$9.0 \cdot 10^6$
Closed lid	$2.7 \cdot 10^{10}$
Plate covered with aluminum	$3.8 \cdot 10^{10}$

4 Discussion

The COVID-19 pandemic has had a huge impact on the restaurant industry by restricting the opening hours and the amount of people allowed inside at a time. The focus of this study was to see if enveloped viruses can spread via aerosol transmission indoors. Safety solutions to mitigate the spread of viruses via aerosol transmission were also tested to investigate how safe going to a restaurant is.

The virus concentration used in the nebulizer liquid was abnormally high, $5 \cdot 10^{10}$ pfu/ml, but necessary to be able to detect the virus with the devices used. Having a higher concentration of virus in the aerosols should not, however, affect the survival or the aerosol transmission of phi 6, as the most important factors in mitigating aerosol transmission of viruses are temperature, humidity, air flows, dehydration, and UV-radiation (Tang et al., 2006).

Phi 6 was found to spread via aerosol transmission indoors, but this was difficult to control. In a 60 m^2 restaurant room, virus was found in all sampled positions up to the other side of the room (Backwall J, 10 m) (Tables 2, 6 and 9), as well as in the laboratory (Fridge B, 3.0 m) (Table 12).

This study has helped us better understand how enveloped viruses spread through aerosol transmission. It confirms that enveloped viruses do, in fact, remain infective in the air, and surfaces, and can travel distances of at least 10 m indoors. The study also stresses the importance of ventilation and air purification in public spaces to mitigate the aerosol spread of viruses.

4.1 Restaurant simulations and virus transmission in aerosols

As the simulations went on, it became clear that they could not be considered as an overall view on virus spread indoors, but more as a case study of this particular restaurant room. Even so, there were differences between the simulation days as seen from the variation in reference simulations' results (Tables 2, 6 and 9). On the upper side of the room, there were four big windows that span almost the whole wall, which make the room's temperature fluctuate with outside temperature. Temperature changes can affect the aerosol spread of viruses, as Lowen et al., (2007) found. Cold windows can also alter the

air flow in the room, by creating draught (Wu et al., 2021). Furthermore, the ventilation plays a huge role in creating the air flow, which the viruses spread through. This is obviously different in every room and should therefore always be tested separately to ensure the safety of the customers.

4.2 UniqAir PRO- air purifiers reduce viral load locally

UniqAir PRO-air purifiers work by sucking air through a HEPA filter, altering the room's natural air flow. This could explain why some parts of the room saw an increase in the number of infective viruses found, even though the purifiers reduce the number of airborne particles (Curtius et al., 2020). These local peaks in virus concentration could be decreased by mixing the air with proper ventilation, lowering the viral dose locally. This, however, has to be done carefully and with control, as it can expose nearby people to the virus. The tables affected negatively by the air purifiers, Tables B (1.5 m), D (4.5 m), F (6.5 m), G (4.5 m) and H (6.5 m) are all in located towards the air purifiers from the nebulizer (Figure 1). Therefore, the placement of such devices is to be carefully thought to avoid the possibly hazardous air flowing onto people in between the source and the air purifiers. A study by Kwon et al., (2020) also confirms this idea: they studied a case in a restaurant in Korea, where a COVID-19 positive customer infected two other people sitting far away from them while others sitting closer were not affected. They found that in the restaurant, an air conditioner created a flow between these three tables, resulting in the two people receiving an infective amount of virus in a mere five minutes exposure time.

4.3 Space dividers may increase virus infectivity at least locally

Dividing the room with space dividers was found to be not only inefficient, but also more dangerous regarding the virus concentration. Most likely, the space dividers inhibit the air flow between these compartments, resulting in the virus aerosols staying within them for a longer time, increasing the time for exposure and increasing the infective dose. Interestingly, they did not seem to inhibit the virus from spreading between the compartments, as was thought. Instead, more virus was found in almost all parts of the room, including the samples taken from the Bunnychain hanging from the roof, suggesting that space dividers guide the aerosols upwards. This suggests that these types of space dividers should not be used in a restaurant setting to prevent infections resulting

from airborne virus transmission. Resembling results have been found in a similar study by Epple et al., (2021), where cardboard partitions were used to separate school desks and aerosol spread was measured between the compartments. They too observed an overflow of aerosols between the compartments already after 10 minutes. In our 60-minute simulation, there was therefore plenty of time for the aerosols to overflow from one compartment to another. There was also a path for the aerosols to spread from one compartment to another at the couches (Figure 2).

4.4 UniqAir PRO- air purifiers work better without space dividers

Having the UniqAir PRO- air purifiers inside the divided compartments reduced the spread of viruses, especially further away from the nebulizer (Figures 12 and 14). However, the effects of combining these two intervention methods were not as promising as having the UniqAir PRO- air purifiers alone. This suggests that having a more open room layout, without partitions, is advisable, as it does not interfere with the air flow from air purifiers or ventilation.

4.5 Ionizers work efficiently in laboratory simulations, Aerte AD 2.0 does not

The laboratory simulation proved to be an easy way to test intervention methods before possibly moving them to a less controlled environment. Interestingly, the flow from the nebulizer seemed to stay on the tables' height with very little viruses found from the plates on the floor. A low concentration of virus was also found from the side table and fridge A, indicating that the flow from the nebulizer moved straight to the other side of the room (Figure 5). A slight increase in the reference simulation's fridge B infective virus amount suggests that the flow created a gradient of aerosols in the upper right corner of the room, before exiting through the ventilation (Table 12).

The ionizer proved to be an effective device in reducing the number of infective viruses settling on the plates. Their effect has been proven before (Hagbom et al., 2015; Jiang et al., 2018; Mitchell and King 1994) and was confirmed again in our study. However, the use of ionizers in a room with people is still under debate, as they have been shown to produce harmful byproducts such as ozone, nitrogen oxides or volatile organic compounds (VOCs) oxidation intermediates (Kim et al., 2017).

In this study, Aerte AD 2.0 was found to be an inefficient device in reducing the number of infective viruses in the air. However, on the further plates at Fridge B (3.0 m) and 2.5 m, the infective virus numbers were significantly lower (Table 12), indicating that the apparatus could have potential in a bigger scale. In a small room of 15 m^2 though, the effects were neglectable. The use of highly oxidative hydroxyl radicals in rooms with humans is still under debate, as they may pose a health risk (Xu et al., 2020) or react with other airborne compounds to form air pollutants such as formaldehyde (Martínez et al., 2020).

UV-C cleaning reduced the number of infective viruses by 99.9 %

The UV-C cleaning reduced the amount of infective virus in the nebulizing solution by 3 logs or 99.9 % (Table 13), however, the plastic lid of a petri dish was enough to block its effectiveness. This suggests that not all parts of the room can be cleaned with a stationed UV-C light. Importantly, in the UV-C cleaning tests, larger droplets, when compared to aerosols, of 20 µl were used. It is possible that the droplet surface tension could protect the viruses that are located below the surface of the droplet, and that the effect would be greater when inactivating viruses in aerosols. This, however, would need to be confirmed by another study.

4.6 Infectious dose and virus release

After having these results from different intervention methods, two questions rise: how much virus an infected person spreads to their environment, and how much virus is needed for a person to get infected? A recent study by Basu (2021) estimated that on average, an infected person releases around 11 virions over five minutes. This can however rise up to 3835 at the peak of the infection. While it is important to note that even a single virion can cause infection (Zwart et al., 2009), Basu estimated that the infectious dose of SARS-CoV-2 is around 300 virions. For comparison, influenza A has an estimated infectious dose of 1950-3000 (Nikitin et al., 2014), which is up to 10 times higher than that of SARS-CoV-2.

In our study, the virus concentration of the nebulized liquid was abnormally high, $5 \cdot 10^{10}$ pfu/ml, when the peak SARS-CoV-2 concentration in human sputum has been found to be around $7 \cdot 10^{6}$ (Wölfel et al., 2020), making the nebulized solution 10^{4} more

concentrated than human sputum at its peak. Having a high virus concentration in the nebulizing solution was, however, crucial for measurement purposes as lower concentrations of phi 6 would have been hard to detect. Having a higher concentration should not affect the survival of the virus, as the most important factors in inactivating of viruses in aerosols are temperature, humidity, air flows, dehydration, and UV-radiation (Tang et al., 2006).

With the highest measurement of virus in this study being 11000 pfu/ml (Biospot 300p, Table 5), which would equal to 1.1 pfu/ml in human sputum, a clearly infective dose of SARS-CoV-2 would not have been reached in these conditions. Infection is still, however, possible, and the aerosol transmission of viruses should be mitigated with ventilation and air purification as described in this study.

4.7 Implications

Perhaps the best method of inhibiting viral spread in a restaurant setting would be to have air purifiers above each table, where the flow to them would not compromise other customers. Efficient ventilation also plays a huge role in ensuring the aerosols produced by people exit the room as quickly as possible. Space dividers that allow air passing from the sides or above them should not be used, as they seem to facilitate infection, however, they can still be used to prevent the spread of droplets from e.g. sneezing or talking (WHO, 2020b). Furthermore, if each table group would be separated with space dividers and air purifiers or suctioning ventilation set above each of the compartments, the spread of viruses could possibly be contained even better. This idea has to be studied more, but Epple et al., (2021) have already got promising results on the subject, as they noticed that a suction system constructed above an aerosol source would reduce their spread drastically. Lastly, our study stresses the importance of studying the effects of ventilation and air conditioning on the airflow indoors to design unique solutions for each individual space. The use of outdoor spaces, such as terraces, is also advisable when possible.

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7 Appendix

Appendix 1. Luria-Bertani-lennox broth (LB). For LB-plates 14 g / l of agar (Bacto) was added and for LB-soft agar 4 g / l agar was added.

Substance	Amount / l
Tryptone (Bacto)	10 g
Yeast extract (Bacto)	5 g
NaCl	5 g
Milli Q water	1 l

Autoclaved at 121 °C for 20 minutes.

Appendix 2. 20 mM HEPES-buffer

Substance	Amount / l
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	4.77 g
Milli Q water	1 l
Potassium hydroxide (KOH)	to adjust pH to 7.2

HEPES was dissolved to the water and pH adjusted to 7.2 with KOH. Water was added to 1 l and the solution was filter sterilized using a 0.2 µm filter.