



Performance of Aura Air™ Purification System against Anti-Infectious Bronchitis Virus (IBV)

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Abstract

The COVID-19 pandemic caused by the SARS-CoV-2 novel coronavirus has had severe economic and public health impacts worldwide. Public health officials have advocated for limiting indoor activities, wearing masks in public, and hand washing to control the spread of the virus. Given the closing of schools and businesses, teleworking, and public fear of contracting infections in indoor spaces, in May 2020, Aura Air developed and tested a smart air device to potentially purify indoor air of viral and other pathogenic particles. The company initiated a study with The Chaim Sheba Medical Center to test the effectiveness of the smart air's disinfection capabilities on the coronavirus as part of our strategic, long-term collaboration. The study aimed to measure the device's ability to purify air contaminated by and to inactivate anti-IBV (Infectious Bronchitis Virus), an avian coronavirus that does not cause infections in humans and is similar in size to the SARS-CoV-2. The system uses a multi-cascade air disinfection principle, and the study was performed in two stages. The first stage tested each disinfection element's antiviral performance, and the second tested the ability to disinfect virus-contaminated air environments by virus aerosol. Results showed that each separate module within the filtration device had strong antiviral properties. Furthermore, the filtration system effectively reduced virus amount in an aerosol contaminated environment in both high and low aerosol concentrations. Together with vaccination programs and other measures to limit viral spread, this device may allow for the reopening of global economies by providing some measure of confidence that indoor air spaces are safe to resume normal activities.

Keywords: Aura Air; Air purification; Multi-cascade air disinfection; Coronavirus

Introduction

In early 2000, a novel virus began causing widespread disease and deaths in Wuhan, China. DNA sequencing identified the etiological agent as a coronavirus that was subsequently named the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the World Health Organization (WHO) [1]. The disease caused by the virus was called COVID-19 (previously known as "2019 novel coronavirus") [2]. According to John's Hopkins, to date, the SARS-CoV-2 pandemic has caused close to 106 million confirmed infectious cases and more than 2.4 million deaths globally [3]. The sheer number of infections has overwhelmed healthcare systems worldwide, leading to hasty reorganizations to care for COVID-19 patients. One of the casualties of such reorganizations is less attention on Hospital-Acquired Infections (HAIs).

HAIs have become a growing concern in the healthcare community. The American Centers for Disease Control and Prevention (CDC) estimates that HAIs account for almost 100,000 deaths and 1.7 million infections each year [4]. Worldwide, hospital-wide prevalence rates range from 5% to 10%, depending on the country [5]. One approach to curb this phenomenon is to decrease the number of airborne pathogens using filtration devices [6,7]. In August 2019, Aura Smart Air installed air purifiers to test their efficacy in reducing HAIs in collaboration with the department of general and oncological surgery at The Chaim Sheba Medical Center in Israel. Initial results indicate that the purification device can reduce levels of various bacteria.

Following the outbreak of the COVID-19 pandemic, the global medical community's focus

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switched from preventing hospital-acquired infections to preventing the spread of SARS-CoV-2 within the hospital setting [8]. The first COVID-19 case in Israel was documented on February 27th, 2020, and by March, all schools and non-essential work facilities were closed as part of the effort to control the outbreak [9,10]. As the outbreak progressed, the Sheba Medical Center was elected to be the national center for the development of COVID-19 treatment and prevention guidelines. Sheba Medical Center is a tertiary referral center in a university hospital setting. It is the largest in Israel and serves an area with a population of over 1.5 million [11]. Several units were converted into a Corona Hospital that contained 400 beds and 214 ventilators, including maternity, pediatric, surgical, and psychiatric wards. A 70-bed Corona Intensive Care Unit was built, including full ventilation capacities, life support, and Extracorporeal Membrane Oxygenation (ECMO) machines. The strain on the healthcare system led to approaches to alleviate that burden. One of the approaches was developing an air purification and disinfection device by Aura Air[™], a company that a smart air management platform that cleanses and disinfects indoor air while monitoring its quality in real-time.

The Aura Air[™] purification and disinfection system, Smart Air[™], uses multi-cascade air disinfection to filter and neutralize pathogens. The apparatus comprises a Pre-filter, a Ray filter[™] that includes a HEPA filter, a carbon filter and smart fabrics infused with copper, a UVC (ultraviolet C) LED, and a Sterionizer[™]. The polymer mesh pre-filter removes large contaminants from the air, such as large particles of dust, pollen, insects, and animal hair [12]. The High-Efficiency Particulate Air (HEPA) filters consist of randomly arranged fibers and retain particles >0.3 µm [13]. The carbon filter uses a bed of activated carbon to remove contaminants using adsorption in which the pollutant molecules are trapped inside the carbon's porous structure [14]. The Smart Copper Fabric is a patented and US Environmental Protection Agency (EPA) approved technology made from cotton impregnated with copper oxide [12]. The smart copper fabric is integrated into the Ray Filter[™] to enhance the filter's ability to neutralize bacteria, viruses, fungus, and mold successfully [15]. The UVC LED uses the short-wavelength UV-C light to neutralize microorganisms by inducing nucleic acid damage and is effective against bacteria, viruses, and parasites [16,17]. Finally, the Sterionizer is based on bipolar ionization technology and creates positively and negatively charged ions that damage the outer membrane and disrupt its normal function [18,19].

Previous results show that the device successfully filters a series of high-risk pathogens, including viruses, such as Influenza H1N1 and H5N1 [20]. This study aimed to test the device's efficacy in slowing down the spread of COVID-19 within a hospital setting. Our results show that the air purification and disinfection device and its elements were able to inactivate a coronavirus of the same size as SARS-CoV-2. The ability of the multi-cascade air disinfection device to purify contaminated indoor air environments could potentially allow for increasing public confidence to safely reopen global economies in conjunction with vaccination programs and other public health safety measures.

Materials and Methods

Virus propagation

The Avian coronavirus Infectious Bronchitis Virus (IBV) was obtained from MIGAL, Galilee Research Institute Ltd (Lab of Prof. J. Pitcovski). Virus propagation was performed in chicken eggs (embryos) [21]. Allantoic fluid was harvested 48 h Post-Inoculation

(PI) and stored at – 80°C until RNA extraction.

Virus detection: Real-time RT-PCR assay

RNA was extracted from the allantoic fluid using QIAamp. Viral RNA Mini Kit (QIAGEN) was reversed transcribed using SuperScript[™] III Reverse Transcriptase (Invitrogen). A conserved region of 336 bp located at nucleotide position 741 to 1077 of the H120 strain N gene sequence (Gen Bank accession no. AM260960) was used to design primers for Real-Time (RT)-PCR assay. RT-PCR was performed using TaqMan[™] Fast Advanced Master Mix (Applied Biosystems) on a StepOnePlus[™] system (Applied Biosystems). Results were recorded and analyzed with StepOne software. Amplification plots were recorded, analyzed, and the threshold cycle (Ct) determined with StepOne plus RT-PCR System, A and B applied Bio-Systems.

Viral content after the experiments were compared samples that were not amplified (Cycle "0") and to control non-treated samples (Aura Air device not activated). RT-PCR was performed on viruses during the exponential replication phase. Cycle 32 was determined as the last point in which control sample measurements did not show detector saturation and was used for the remainder of the experiments. To determine the initial concentration of the virus (Cycle "0"), the data were processed according to no. of cycles using a simplified exponential equation:

$$N_0 = N_k / 2^{k-1}$$

Where: N_0 : Initial concentration; N_k : Measurement at Cycle No. 32; K: Number of cycles (32)

Aura Air[™] test description

The Aura Air devices are manufactured by Beth-El Zikhron Yaaqov Industries Ltd., a leading designer and manufacturer of CBRN Air Filtration and Air Treatment Products.

System elements testing

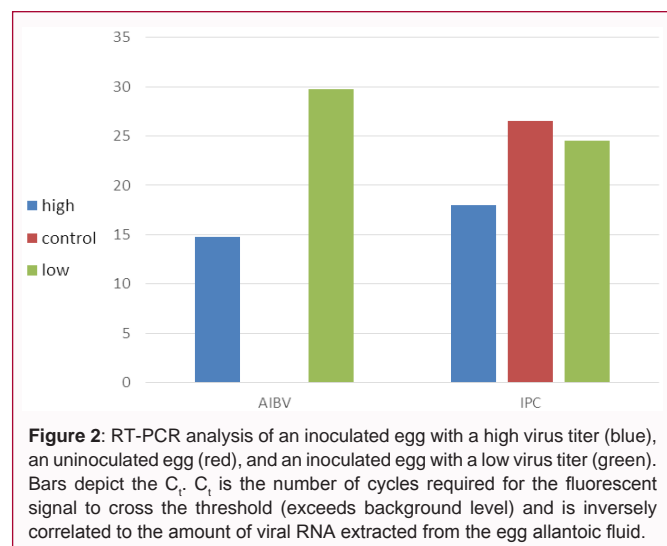
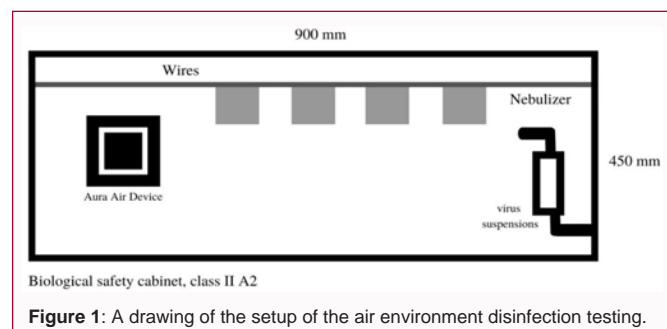
IBV, known to replicate well in chicken eggs [22], was cultivated in chicken eggs, extracted, and suspended in PBS (Phosphate-buffered saline). All experiments used a dilution of 1:100. The Aura Air HEPA filters and smart copper fabric were cut to squares of 0.5 cm × 0.5 cm and contaminated by 10 µl of the viral suspension. After 10 min, samples were rinsed with 0.1 ml dPBS, and the residual virus was extracted.

To test the Sterionizer[™] and UVC LED, 10 µl of the virus suspension was added to a neutral polycarbonate surface (Figure 1), Sterionizers[™] (High-Sterionizer D6[™] and Low power- Sterionizer D5[™]) were positioned 30 cm from viral suspension and the ion flow was directed to the suspension using an additional fan. UVC LEDs were placed 1 cm from the suspension. After 10 minutes of exposure, the viral suspension was transferred to 90 µl of dPBS and residual viral RNA was extracted.

Air environment disinfection testing

The Aura Air[™] device was placed inside an acrylic test chamber (450 mm × 500 mm × 900 mm, approx. 0.2 m³) within a Biological Safety Cabinet Class II A2 (Figure 1).

During the tests, viral air contamination was achieved by spraying virus suspensions (diluted 1:10 and 1:100) into the chamber using compressor-type nebulizer InnoSpire Essence at an airflow speed of ~0.2 mL/min. The filtration system was activated for 30 min following the contamination by aerosol. Control runs were performed in the same manner without system activation. The



procedure was repeated with high and low viral concentrations. To test for air virus concentrations, salt-soaked pads were placed inside the chamber at the height of ~40 cm. Sampling was performed during time ranges of 1 min to 10 min, 11 min to 20 min, and 21 min to 30 min with or without (control) activation of the filtration system. After completion, the pads were placed in cold dPBS, and residual viral RNA was extracted.

Results

Biological system control

Virus propagation in chicken eggs: RNA was extracted from the allantoic fluid of infected chicken eggs 48 h post-inoculation. RT-PCR assay analysis indicated the presence of the IBV RNA in the inoculated eggs (Figure 2).

Direct PCR measurements

Testing of separate elements of the filtration system: Separate elements of the filtration system were contaminated with the virus and exposed for 10 min. After the treatment, RT-PCR analysis of the residual virus particles showed a noticeable reduction in the virus's detection relative to control (A regular fabric with no filtration properties).

Testing the filtration system's ability to handle virus aerosols: Wires were placed into the test chamber, and virus aerosols were sprayed into the chamber. RT-PCR analysis of the wires post-treatment showed a significant reduction of residual viral particles on the wires when the filtration system was activated for 10, 20, or 30 min, relative to the control where the filtration system was not activated.

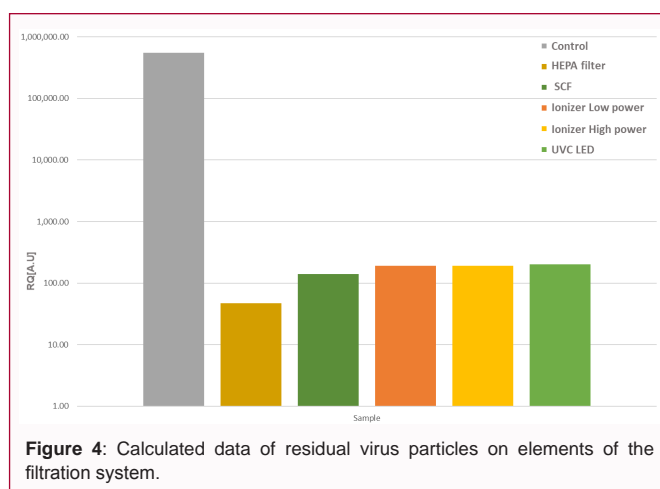
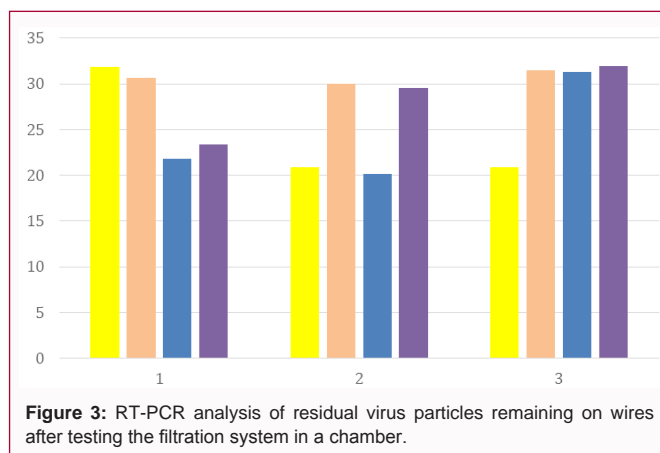


Table 1: IBV Reduction Ratio.

Sample	Coronavirus Reduction Ratio [%]
Ref. 1	
HEPA rep1	99.7243
SCF rep.1	99.9744
Sterionizer™ D5 rep.1	99.9651
Sterionizer™ D6 rep.1	99.9429
UVC LED rep.1	99.9631

RT-PCR analysis of residual virus particles remaining on wires after testing the filtration system in a chamber: Yellow bars represent wires sprayed with an aerosol containing a low concentration of virus particles without activating the filtration system, orange bars represent wires sprayed with an aerosol comprising a low concentration of virus particles while activating the filtration system, blue bars represent wires sprayed with an aerosol containing a high concentration of virus particles without activating the filtration system, and purple bars represent wires sprayed with an aerosol comprising a high concentration of virus particles while activating the filtration system. The filtration system was activated, or not activated, for 10 (1), 20 (2), or 30 (3) min, as shown in the Figure 3.

Viral content in test materials (Time, cycle "0")

System elements testing: Separate elements of the filtration system were exposed to the virus for 10 min. RT-PCR data were

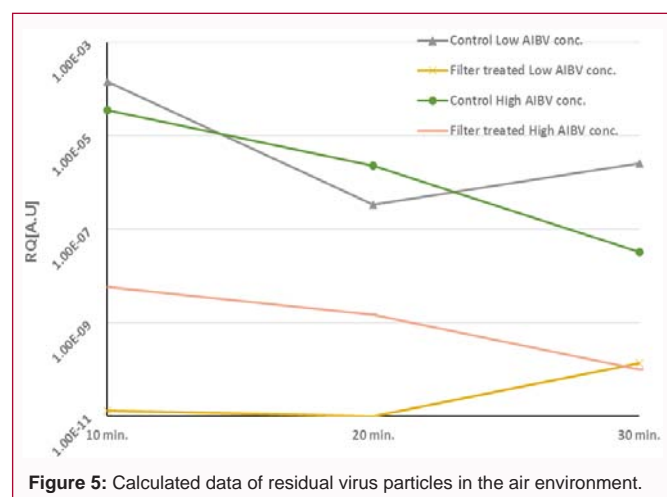


Figure 5: Calculated data of residual virus particles in the air environment.

processed as previously described. RT-PCR analysis of the residual virus particles remaining after being sprayed onto the different filters showed a noticeable reduction of virus levels relative to a control fabric with no antiviral properties (Figure 4 and Table 1). Normalization of calculated data shows that most of the system's elements can reduce viral content by over 99.9%.

Grey bar (control) represents fabric with no disinfection properties. Elements with disinfection properties (See legend) show a significant reduction of residual virus levels when the content is calculated according to RT-PCR cycle "0".

Air environment disinfection testing

Air viral contamination was achieved by spraying virus suspensions during filtration system activation. Viral air concentrations were measured using pads placed within the chamber. Pads were collected into tubes containing iso-normal dPBS (10 ml/tube), from which residual virus RNA was extracted, and RT-PCR was performed. The RT-PCR measurements were taken at cycle 32 (see experimental methods) and used to determine the samples' viral content. Processed results showed a significant reduction of residual viral particles on the pads taken when the filtration system was activated compared to samples taken from untreated air (Figure 5). A normalized comparison shows that the Aura Air system reduced original virus levels by over 99.9% (Table 2).

Discussion

Infectious diseases caused by viruses, bacteria, or parasites are a major global economic and health issue. Since many of these pathogens are airborne, the ability to efficiently disinfect the air in enclosed spaces may substantially reduce infection rates. Such filtration systems can also decrease the number of allergens and other chemicals, thus reducing pollution-related respiratory disorders.

With the outbreak of the COVID-19 pandemic worldwide, health authorities face a significant challenge in containing the viral spread. This is especially true in medical settings such as hospitals and hospices. An effective air filtration method can prove vital in mitigating COVID-19, and other viral, spread. Previously, results have shown that the Aura Smart Air device successfully managed to filter a series of high-risk pathogens, including various viruses, such as Influenza H1N1 and H5N1 [20]. As part of the changing reality and arising need, we wanted to test the device's ability to filter a Coronavirus. Therefore, we built an experimental setup that could

Table 2: Coronavirus Reduction Ratio (normalized results).

Sample	Duration of treatment		
	10 min.	20 min.	30 min.
Aura Air treated Low AIBV conc.	99.99999	99.99975	100
Aura Air treated High AIBV conc.	99.98325	99.93515	99.6845

quantify the Aura Smart Air device's capacity, both as separate parts and as a whole, to purify air contaminated by a coronavirus similar in size to the SARS-CoV-2.

A SARS-CoV-2 viral particle is 60 nm to 140 nm large, making them easily transmitted by larger respiratory droplets and air pollution (>1 m). Viral particles can be removed from the air using filtration, which relies on air movement [6,23,24]. The High-Efficiency Particle Air (HEPA) filters have been recommended as suitable for viral removal from the air [13]. HEPA filters are ideal because they have the correct balance between efficient particle removal and the ease at which air moves through them [24]. The United States Center for Disease Control and Prevention (CDC) has previously suggested using portable HEPA purifiers as an adjunct to infection control measures for SARS-CoV-1, the infectious agent behind the 2003 SARS epidemic [25].

An enhanced ventilation system may be essential to limiting the spread of SARS-CoV-2 indoors until vaccines are readily available. If a ventilation system is designed correctly and kept clean, it should be efficient in removing airborne infectious agents. When separate rooms share ventilation systems and have partial air recirculation, the recirculation path should be closed to prevent cross-contamination [26].

The Aura Air device utilizes a smart and innovative purification and disinfection technology to decontaminate air. The system circulates the air in a closed room to enhance ventilation by filtering 206 Cubic Feet per Minute (CFM). The technology is based on four stages: Pre-Filter, Ray-Filter, UV-C LED, and Sterionizer. The ray filter is based on three layers: HEPA (equivalent to MERV-16), which efficiently filters particles of 0.3 μ m by >95%, a carbon filter that absorbs Volatile Organic Compounds (VOCs) and foul odors, and a zinc-lined Smart Copper Fabric, which further filters the air. Additionally, copper is effective in inactivating viruses such as HIV-1 [27].

Short-wavelength Ultraviolet Radiation (UV-C) can bind to RNA and DNA nucleic acids, resulting in covalently linked dimers of adjacent pyrimidines that often induce DNA mutations [28,29]. In addition, UVC radiation has been shown to neutralize coronaviruses in the air or on surrounding surfaces [30], as well as COVID-19 specifically [31]. UV-C LED can also inactivate pathogens by directly damaging membranal proteins [32]. The Aura Air system uses four UV-C LEDs at a wavelength of 275 nm, which are effective in neutralizing different pathogens by destroying cell surface proteins.

The Sterionizer, based on bipolar ionization technology, utilizes specialized tubes that convert oxygen molecules from the air into charged atoms that cluster around microparticles, surrounding and deactivating airborne pathogens [18,19,33]. Indeed, studies have shown that ionization reduces the airborne spread of Influenza A in poultry farms [18]. Previous clinical trials tested the Sterionizer in the Kitasato Research Center for Environmental Science in Japan [34]. The Sterionizer reduced influenza virus H1N1 levels in the air by 92% after 30 min of exposure and 98.9% after 60 min of exposure.

The Aura Air devices are manufactured by Beth-El Zikhron Yaaqov Industries Ltd., a leading designer and manufacturer of CBRN Air Filtration and Air Treatment Products.

We were able to propagate the IBV coronavirus in eggs. RT-PCR assay demonstrated the presence of the IBV virus in the inoculated eggs relative to the negative control. The filtration system's different elements showed a significant reduction of virus residuals relative to a control fabric with no disinfection properties. The air environment disinfection testing resulted in a noticeable decrease of residual viral particles on the pads taken when the filtration system was activated compared to samples taken from untreated air. A normalized comparison shows more than a 99.9% reduction of the virus during experiment duration.

Therefore, the Aura Air holds great promise in reducing the airborne spread of pathogens, in general, and of coronaviruses, in particular. For optimal use, Aura Air units should be installed where airborne virus exposure is most likely such as diagnostic laboratories, rooms for staff that treat virus patients, operating rooms where patients or carriers of the virus will be operated on, and rooms of patients with weakened immune systems. Following installation, a clinical trial will assess the Aura Air system's efficiency in reducing airborne infections.

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