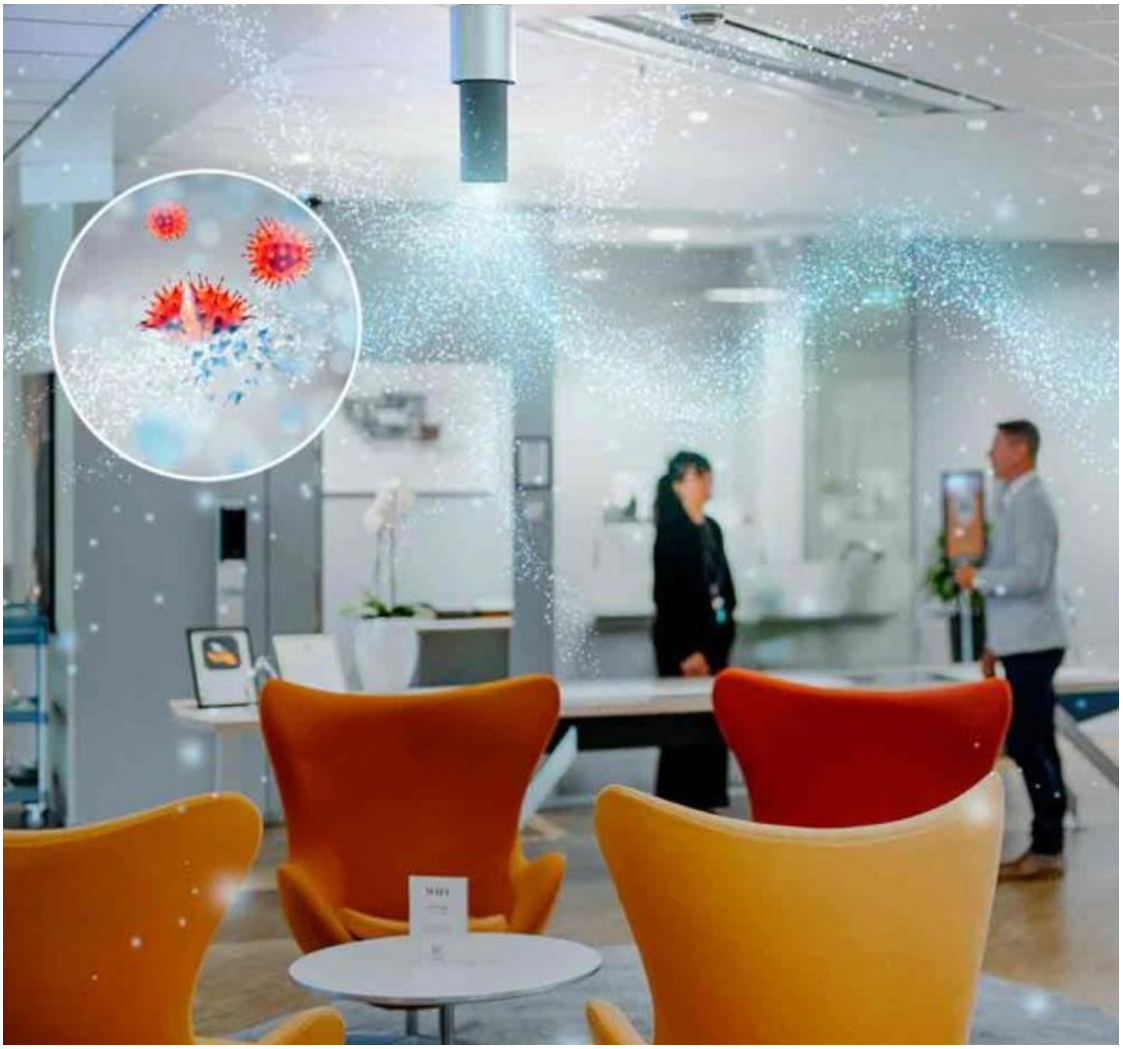
LightAir



The impact of LightAir IonFlow technology on viruses

In particular the viruses underlying COVID-19 and SARS, influenza virus, calicivirus and rotavirus

LightAir

Contents

1. Executive summary

The purpose of this document is to summarize how LightAir IonFlow ultrahigh-density ionization technology affects viruses, based on the results from two separate and independent studies, using different test methods and different viruses.

The viruses tested in the studies are pseudoviruses for COVID-19 (SARS-CoV-2) and SARS (SARS-CoV-1), both part of the coronavirus family, as well as influenza virus, calicivirus and rotavirus. These viruses are all widely spread around the world. They have had serious short-term and long-term effects on individuals, families and societies. The economic effects of these viruses run into billions of dollars each year.

Destroys viruses while they're still in the air and on surfaces

Both the study from IrsiCaixa on COVID-19 and SARS and the study from the Karolinska Institute on influenza virus, calicivirus and rotavirus clearly show that LightAir IonFlow ultrahigh-density ionizer effectively neutralizes viruses and make them harmless to human cells both while still lingering in the air and when attached to surfaces.

This is vital as these viruses are known to spread both in the air where we breathe them in, and in droplets on surfaces that we touch with our hands. Thus there is a strategic role for technology to fill in helping to eliminate these viruses.

LightAir makes a difference

LightAir's ionization technology is built into a range of products offering solutions to households as well as the commercial and public sectors, thereby effectively decreasing the spread of infections and making a difference to people's health and the cost of virus epidemics to society.

The world is still grappling with a COVID-19 pandemic. It has had terrible effects on our health, civil and professional life, and economy. It is still far from over and the threat of mutations and new viruses are on the horizon.

LightAir can make a real difference in the fight against COVID-19, by offering an effective device that destroys viruses, and thus stops the spread of infections.

2. The seven-year study at the Karolinska Institute

Founded in 1810 originally as an "academy for the training of skilled army surgeons", the Karolinska Institute today is a leading medical university, and one of the largest in Europe. Since 1901 the Nobel Assembly at Karolinska has had the honor of selecting the Nobel laureates in Physiology or Medicine.

The objective of the Karolinska study was to investigate how effective ionization is at collecting, eliminating and identifying viruses in the air using the LightAir IonFlow ultrahigh-density ionizer. This seven-year study was carried out by renowned scientists and was published in November 2014 in Nature Scientific Reports, the world's most cited scientific journal.

Method

Influenza virus, calicivirus and rotavirus were chosen for the study. These viruses were thought to be airborne, which was confirmed in the study. The concentration of viruses was evaluated by collecting samples and studying them through a scanning electron microscope. Aerosol-transmitted viruses were also used to infect guinea pigs, and the results of an infected group treated with LightAir IonFlow ultrahigh-density ionizer were compared with an untreated infected group.

Voice of the scientist

"This device enables unique possibilities to analyze air and prevent the spread of infectious diseases, which provides a wide medical and clinical application."

Lennart Svensson, professor of molecular virology and project leader of the Karolinska study

Results

3 out of 4 (75%) animals without protection from the LightAir ionizer were infected by the virus, while 0 out of 4 (0%) animals were infected when the ionizer was used as a protection.

The test showed significantly higher numbers of rotavirus and calicivirus gathered on the active ionizer compared to the inactive ionizer (approx. 1500-3000 times), which led to the conclusion that this technique can actively and effectively collect viral particles from the air.

The infectivity of aerosolized viruses was significantly reduced by more than 97%, indicating that ionization of the aerosol accounts for the vast majority of infectivity reduction, and not the exposure of the charged collector plate. This result proves that the LightAir IonFlow ultrahigh-density ionizer neutralizes viruses while they are still in the air.

3. The study at IrsiCaixa

IrsiCaixa is a leading institute with over 25 years of research in HIV and AIDS situated in Barcelona, Spain. They are currently working with other institutions to develop a coronavirus vaccine and are participating in numerous clinical trials to reduce transmission and treat the progression of the disease.

In March 2021, IrsiCaixa tested under biosafety level 2 conditions whether the LightAir IonFlow ultrahigh-density ionizer neutralizes engineered SARS-CoV-1 and SARS-CoV-2 viral viruses, as it had earlier been proven to do on influenza virus, calicivirus and rotavirus.

Method

The objective of this study was to evaluate the capacity of LightAir IonFlow high-density ionization technology to inhibit SARS-CoV-1 and SARS-CoV-2 viral entry into human target cells. Pseudoviral entry was tested on HEK-293 cells expressing ACE2.

Different concentrations of the viruses were placed in droplets and added to the human cells on a surface. The samples were then subjected to ionization for either 30 or 60 minutes and compared with untreated controls. The test was also repeated to confirm the test results.

How do pseudoviruses enable testing?

Pseudoviruses, also called *engineered viruses*, are useful and important virological tools because of their safety and versatility, especially for emerging and re-emerging viruses. Due to its high pathogenicity and infectivity and the lack of effective vaccines and therapeutics, live SARS-CoV-2 must be handled under biosafety level 3 conditions, which has hindered the development of vaccines and therapeutics.

Pseudoviruses are manipulated in the laboratory to mimic a specific virus and its viral entrance into cells. They are ideal for testing the efficacy of antivirals, antibodies or strategies aimed at inhibiting viral entry.

https://www.the-scientist.com/news-opinion/what-pseudoviruses-bring-to-the-study-of-sars-cov-2-68457

Results

IrsiCaixa concludes that SARS-CoV-2 and SARS-CoV-1 pseudoviral entry are diminished after 30 or 60 minutes of treatment with LightAir IonFlow ultrahigh density ionization technology. There is a clear inhibition of the pseudoviruses, which means that they are destroyed and therefore can't infect humans or reproduce themselves.

The IrsiCaixa study involving viruses underlying COVID-19 extends the findings of the groundbreaking study that was performed over a period of seven years at the Karolinska Institute in Stockholm, Sweden.

These tests were carried out to provide further proof of concept of the original findings from the Karolinska Institute study (see previous section), and in particular how applicable they would be to the new coronavirus underlying COVID-19. Its depth and methodology were necessarily structured differently than the much larger Karolinska study.

Below is an extract from one of the trials using a 60-minute ionization treatment. Specific percentages shown in these tests are indicative of a clear virus inhibition capacity, and thus additional proof of concept. In order to publish single percentages tests must be multiplied during a longer test period.

Voice of the scientist

"We measured out various versions of pseudoviruses that mimic the entry of different coronaviruses into cells. The conclusion is that in the form of droplets on exposed surfaces, IonFlow technology has a clear inhibition on viral entry already within 30 minutes."

"Personally, I find this extremely interesting, and the potential of this technology to combat virus spreading should be further investigated. Not least in these times, and especially given that this technology attacks the viral entry process so viruses can no longer infect human cells."

Nuria Izquierdo Useros, PhD,
principal investigator
of the IrsiCaixa study

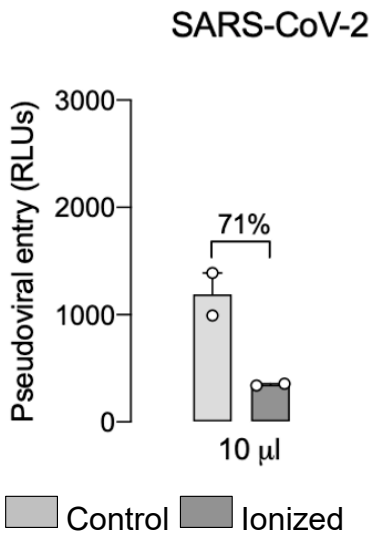
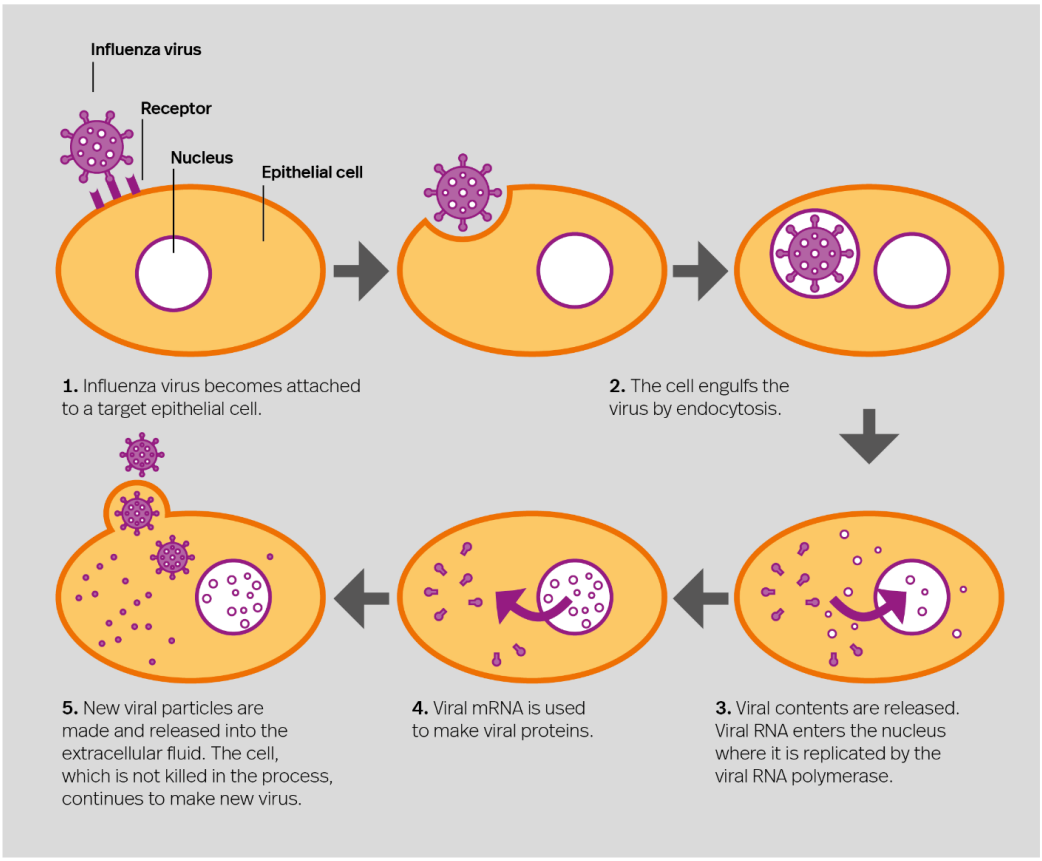


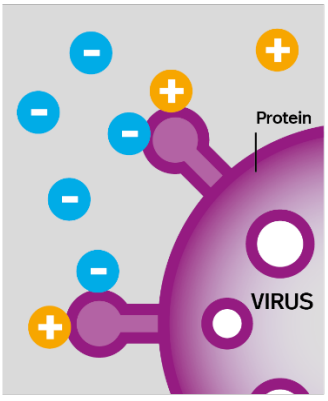
Figure 1. Effect of LightAir IonFlow ultrahigh-density ionization treatment (60 minutes) on SARS-CoV-2. This graph shows the results of placing 10 µL SARS-CoV-2 pseudovirus in a drop, and adding it to the cells after treatment. Percentage indicates the mean reduction of pseudoviral entry upon LightAir treatment.

4. Background on viruses and ionization

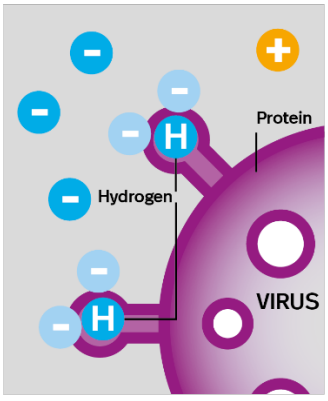
Viral entry – How cells are infected by a virus



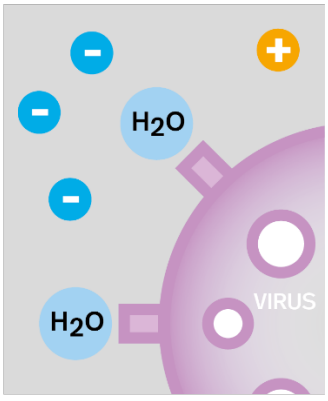
How LightAir IonFlow technology neutralizes a virus



Negative ions attach themselves to the virus protein spike.



The ions extract hydrogen (H) from the protein on the spike surface, creating water molecules.



The water is released to the air and the spike is destroyed, meaning that the virus can no longer infect human cells.

5. Conclusions

Main finding: IonFlow destroys virus in the air and on surfaces

LightAir IonFlow technology has been proven in two separate and independent scientific studies from IrsiCaixa and the Karolinska Institute to effectively neutralize the infectivity of pseudoviruses for COVID-19 (SARS-CoV-2) and SARS (SARS-CoV-1) as well as influenza virus, calicivirus and rotavirus, thereby making them harmless to human cells. This effect has been demonstrated both on viruses in aerosol form – still lingering in the air – and when the virus is present on surfaces.

Scientists from the Karolinska Institute found that IonFlow causes N1H1 (influenza/swine flu), rotavirus and calicivirus to lose 97% of its infectivity. Scientists at the IrsiCaixa research institute conclude that IonFlow has a similar measurable effect on purpose-engineered viruses that mimic the SARS-CoV-2.

Thus the more recent tests provide a proof of concept that the SARS-CoV-2 virus is structured in a similar way and can be counteracted, using the same principles established by the Karolinska Institute findings.

Challenges today and tomorrow

PREVENT is the magic word. Viruses are a fact that we need to accept now and in the future. It is also a fact that some viruses are, and will be, worse than others. But we envision a scenario where new viruses, such as the SARS-CoV-2 that causes COVID-19, will not have such devastating effects on health and society. By preventing the spread of viruses, we will be able to control them better and mitigate negative impacts.

Creating preventive vaccines and providing pharmaceutical treatments are of great importance for us as a society. It's now time for technology to be added to that mix and to take an active role in reducing infections.

Technology has a broader scope

While a vaccine is developed and designed to work on a specific virus, technology can take on a broader spectrum and work to protect against several viruses. LightAir IonFlow ultrahigh-density ionization has been tested and shows a proven virus inhibition capacity on every single virus examined. By destroying the virus both in the air and on surfaces, it prevents the spread of viruses, enabling us to control them better and mitigating negative impacts. We are not stating that technology alone is THE answer, but it is clearly a part of a broader solution to protect and prevent future pandemics.

Implications

Health authorities provide directions and guidelines on how to best prevent the spread of virus infections in society. LightAir can add another layer of safety, while adding active measures rather than strictly focus on reactive efforts. With the powerful and proven virus-inhibiting effect of the LightAir IonFlow ultrahigh-density ionization technology, an additional layer of safety can be added as society slowly opens. It is done by destroying viruses as they search for a new host. In an ionized, charged state they will not be able to enter human cells, thus preventing spreading in situations where people meet.

Creating a New Normal

As we publish this document, restrictions and recommendations have already been with us for a long period. This has affected us in various ways as individuals, and for some much more than others. Many people have died or become seriously ill. Workplaces have been closed, preventing us from meeting our co-workers for a long time, missing the buzz around the coffee machine, and other activities that normally give us positive energy and contribute to a positive corporate culture.

At home, we have been restricted in how many people we can meet. We have stopped having birthday celebrations, playdates for our kids, dinner parties with our friends, and other social indoor activities. Sooner or later, we will see the end of this. We will get back to a more normal life with fewer restrictions. We all know this and talk about it as a New Normal, and LightAir can be a trusted companion in this "reopening of life".

In commercial settings such as offices and schools the virus-inhibiting IonFlow is often combined with medical-grade air purification provided by the LightAir CellFlow air purifiers. We call this LightAir Health⁺. The CellFlow reduces the concentration of virus in the air with a 99.99% degree of filtration, thus stopping a virus from traveling to a new host. Viruses are immediately and continuously attacked and destroyed by the IonFlow virus inhibitor. Together with recommended measures from health authorities, the LightAir Health⁺ solution is part of a responsible plan to welcome students and employees back from lockdown.

We believe that by preventing the spread of viruses – and thereby Infections – we can get back to a new normal. We will be aware and cautious, but not in lockdown or restrained from being close to the people we love, and not prevented from going to school or work.

About LightAir

LightAir AB (publ) improves health and well-being by developing and selling efficient air purification. Solutions are based on two unique and patented technologies: IonFlow and CellFlow. IonFlow uses ultrahigh-density ionization to neutralize harmful viruses, while they're still in the air and on surfaces. CellFlow uses the medical-grade air filtration of the EcoPrecision™ filter to lower the concentration of common pollutants, including viruses, pollen, traffic pollution and ultra-fine dust. The two main segments targeted in this international market are Home Solutions and Professional Solutions. The company's share is listed on Nordic SME Sweden (Nordic Growth Market NGM AB) under LAIR. Read more at www.lightair.com

Impact of LightAir IonFlow high-density ionization technology on SARS-CoV-1 and SARS-CoV-2 pseudoviral infection *in vitro*

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1. AIMS and SCOPE of the research project:

The objective of this project was to evaluate the capacity of LightAir IonFlow high-density ionization technology to inhibit SARS-CoV-1 and SARS-CoV-2 pseudoviral entry into human target cells *in vitro.*

The **specific aim** was the following:

Assess the inhibition of SARS-CoV-1 and SARS-CoV-2 pseudoviral entry on HEK-293 cells expressing ACE2.

2. Material & Methods:

Cell Cultures. HEK-293T (ATCC repository) were maintained in DMEM with 10% fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin (all from Invitrogen). HEK-293T overexpressing the human ACE2 (Integral Molecular Company) were cultivated and maintained in DMEM (Invitrogen) with 10% fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin, and 1 µg/mL of puromycin (all from Invitrogen).

Pseudovirus production. HIV-1 reporter pseudoviruses expressing SARS-CoV-2 or SARS-CoV-1 Spike protein and luciferase were generated co-transfecting two plasmids. pNL4-3.Luc.R-.E- was obtained from the NIH AIDS repository. SARS-CoV-2.SctΔ19 or SARS-CoV-1.SctΔ19 were generated (Geneart) from the full protein sequence of SARS-CoV-2 spike with a deletion of the last

19 amino acids in C-terminal, human-codon optimized and inserted into pcDNA3.4-TOPO(Ou et al., 2020). Spike plasmid was transfected with X-tremeGENE HP Transfection Reagent (Merck) into HEK-293T cells, and 24 hours post-transfection, cells were transfected with pNL4-3.Luc.R-.E-. Supernatants were harvested 48 hours later, filtered with 0.45 µM (Millex Millipore) and stored at -80ºC until use. Pseudoviruses were titrated in HEK-293T overexpressing the human ACE2.

Pseudovirus assay. HEK-293T overexpressing the human ACE2 and TMPRSS2 were used to test the pseudorviral entry capacity of SARS-CoV-1 or SARS-CoV-2 in a final volume of 30 µL using 4 concentrations of viral particles in duplicates after 30 and 60 minutes of ionization performed with LightAir IonFlow high-density ionization technology in a biosafety cabin hood. The IonFlows collectors were cleaned before ionization. As positive controls, the same pseudoviral inputs not exposed to the treatment of LightAir technology but kept in a similar hood were used. 48h post-inoculation, cells were lysed with the Glo Luciferase system (Promega). Luminescence was measured with an EnSight Multimode Plate Reader (Perkin Elmer).

3. Deliverables

1. Capacity to inhibit SARS-CoV-1 pseudoviral entry into HEK-293 cells after 30 to 60 minutes of treatment with LightAir IonFlow high-density ionization technology.
2. Capacity to inhibit SARS-CoV-2 pseudoviral entry into HEK-293 cells after 30 to 60 minutes of treatment with LightAir IonFlow high-density ionization technology.

4. Results

4.1 Results 1st experiment (16/02/2021):

LightAir IonFlow high-density ionization technology was tested for 60 minutes against 4 concentrations (5, 10, 20 and 30 µL) of SARS-CoV-1 and SARS-CoV-2 pseudoviruses and were compared to untreated controls.

Compared to untreated controls, LightAir IonFlow high-density ionization technology reduced SARS-CoV-1 pseudoviral entry at 10, 20 and 30 µL (**Figure 1**, top panel). For SARS-CoV-2 pseudovirus, this reduction took place at 10 and 20 µL (**Figure 1**, bottom panel).

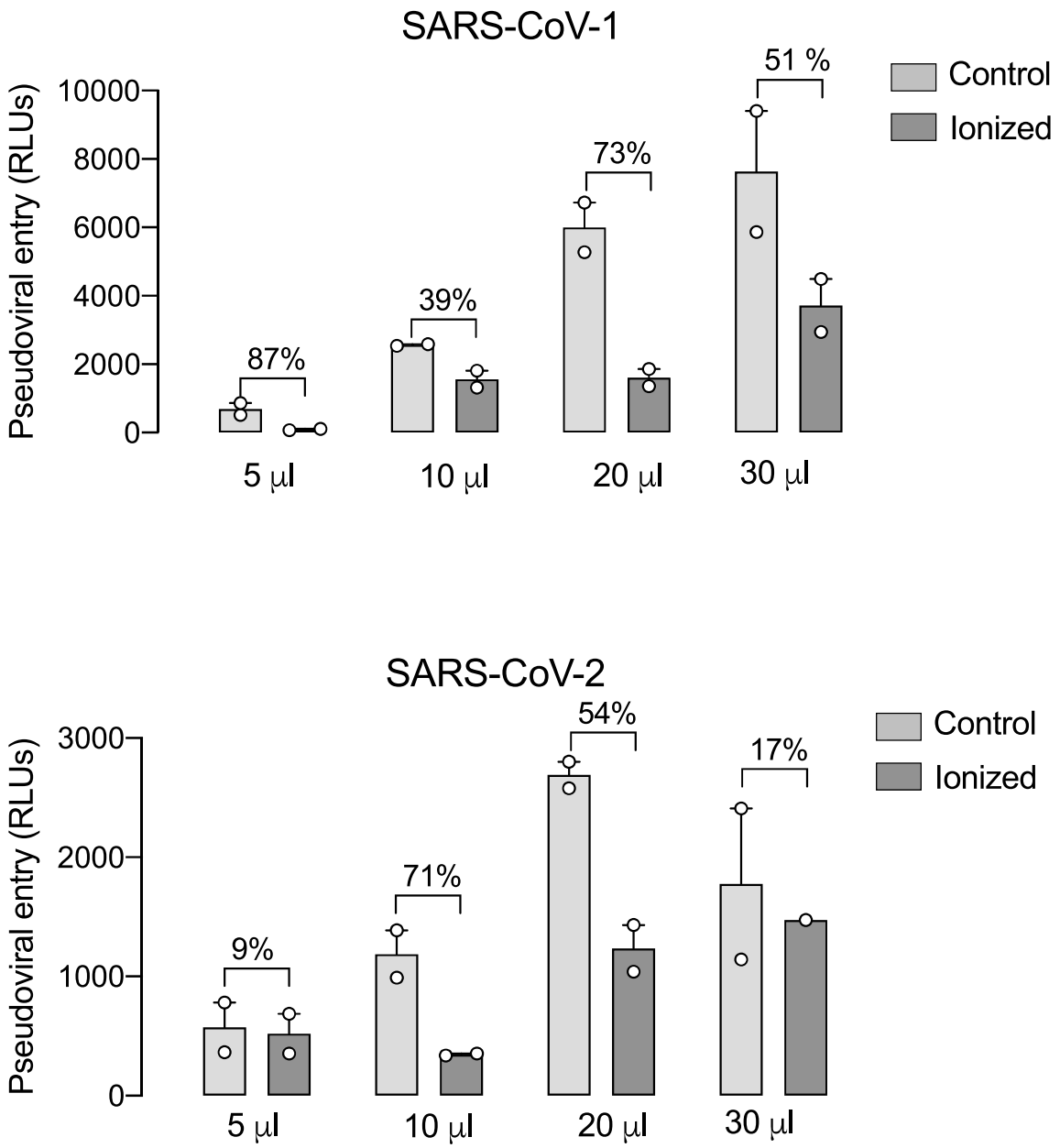

Figure 1. Effect of LightAir IonFlow high-density ionization technology on luciferase expression of reporter lentiviruses pseudotyped with SARS-CoV-1 and 2 Spike in ACE2 expressing HEK-293T cells. Diferent different amounts (5, 10, 20, 30 µL) of SARS-CoV-1 or -2 pseudoviruses were placed in a drop of 30 µL of final volume, and added to the cells after treatment. Percentage indicates mean reduction of pseudoviral entry upon LightAir treatment. Mean of two replicates is shown. RLUs: relative light units.

4.2 Results 2nd experiment (17/03/2021):

LightAir IonFlow high-density ionization technology was tested for 30 and 60 minutes against 3 concentrations (10, 20 and 30 µL) of SARS-CoV-1 and SARS-CoV-2 pseudoviruses and were compared to untreated controls.

Compared to untreated controls, LightAir IonFlow high-density ionization technology reduced SARS-CoV-1 pseudoviral entry at 10, 20 and 30 µL (**Figure 2**, top panels). For SARS-CoV-2 pseudovirus, this reduction took place at 10 and 20 µL (**Figure 2**, bottom panels). This effect was both after 30 to 60 minutes.

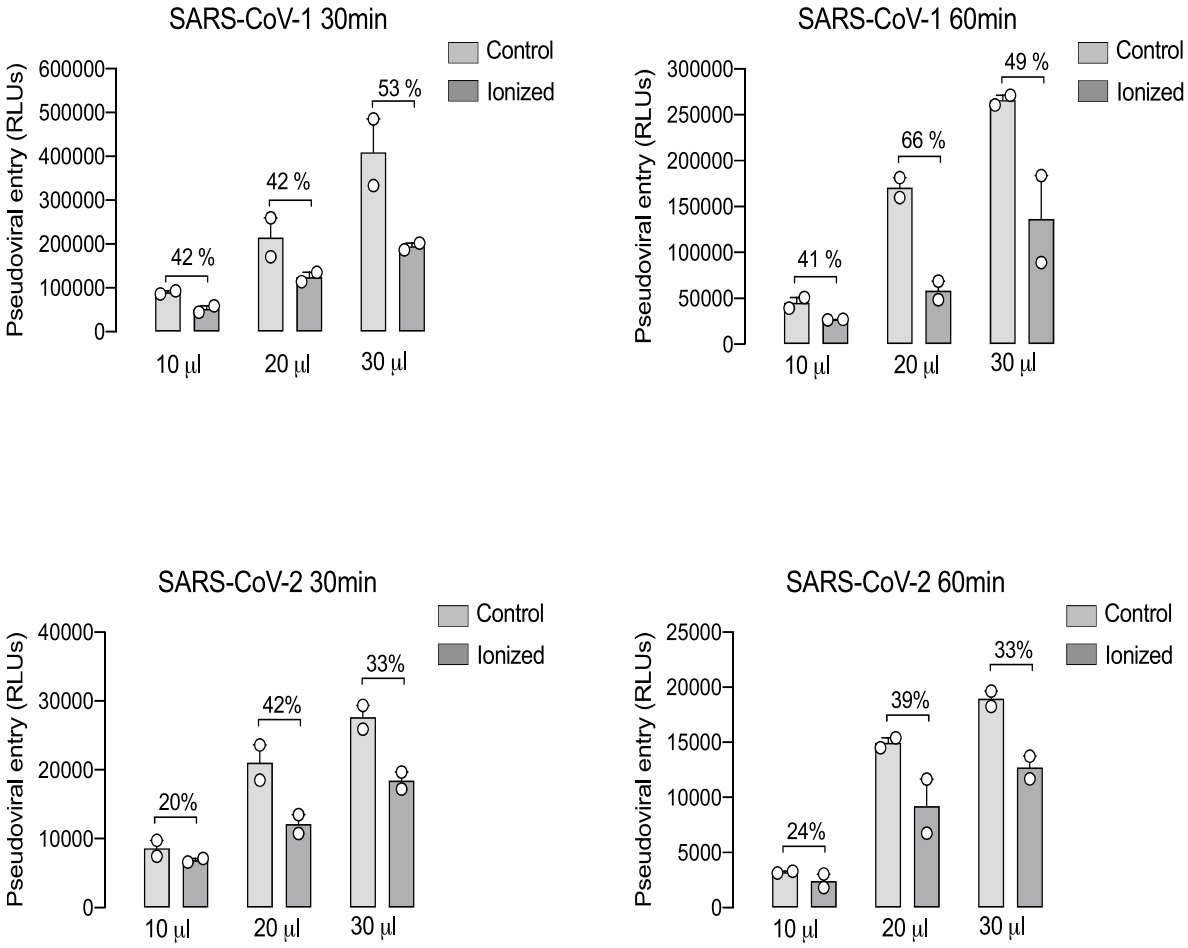

Figure 2. Effect of LightAir IonFlow high density ionization technology on luciferase expression of reporter lentiviruses pseudotyped with SARS-CoV-1 and 2 Spike in ACE2 expressing HEK-293T cells. Diferent different amounts (10, 20, 30 µL) of SARS-CoV-1 or -2 pseudoviruses were placed in a drop of 30 µL of final volume, ionized or not (control) for 30 or 60 min, and added to the cells after treatment. Percentage indicates mean reduction of pseudoviral entry upon LightAir treatment. Mean of two replicates is shown. RLUs: relative light units.

Conclusion

SARS-CoV-2 and SARS-CoV-1 pseudoviral entry are diminished after 30 or 60 minutes of treatment with LightAir IonFlow high density ionization technology when pseudviruses are treated in drops of 30 µL.

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