



The impact of LightAir IonFlow technology on viruses

In particular the viruses underlying COVID-19, SARS, influenza, calici and rotaviruses

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Draft

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, calici 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-, calici- and rotaviruses 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.

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 battle against COVID-19, by offering an effective device that neutralizes 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, calici 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 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.

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 proves that the LightAir IonFlow ultrahigh density ionizer neutralizes viruses while they are still in the air.

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

3. The study at IrsiCaixa



IrsiCaixa
Institut de Recerca de la Sida

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 drugs and are participating in numerous clinical trials to reduce transmission and treat the progression of the disease.

In March 2021, IrsiCaixa tested whether the LightAir IonFlow ultrahigh-density ionizer neutralizes SARS-CoV-1 and SARS-CoV-2 viral viruses, as it had earlier been proven to do on influenza, calici 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. The specific aims were to assess the inhibition of SARS-CoV-1 and SARS-CoV-2 pseudoviral entry on HEK-293 cells expressing ACE2.

Different concentrations of the viruses were placed in droplets and added to human cells on a surface. The samples were then subjected to ionization for 30 respective 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

The IrsiCaixa report 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 when pseudoviruses are treated in drops. There is a clear inhibition of the pseudoviruses, they are destroyed and can't infect human cells.

This new IrsiCaixa study involving viruses underlying COVID-19 must be interpreted against the background of the longer, more elaborate study that was performed over a period of seven years at the Karolinska Institute in Stockholm, Sweden.

While the IrsiCaixa study is of course scientifically sound, its depth and methodology were necessarily simplified when compared to the much larger Karolinska study. Time was important factor to show a result quick and connect it to earlier studies. Thus it would be an oversimplification to interpret viral inhibition percentages in the same manner done in the longer study.

It must be emphasized that these tests were carried out to provide further proof of concept of the original findings from the Karolinska Institute study (see above), and in particular how applicable they would be to the new coronavirus underlying COVID-19.

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,
the principal investigator
of the IrsiCaixa study

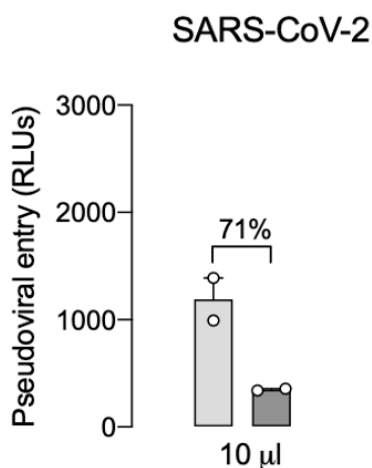
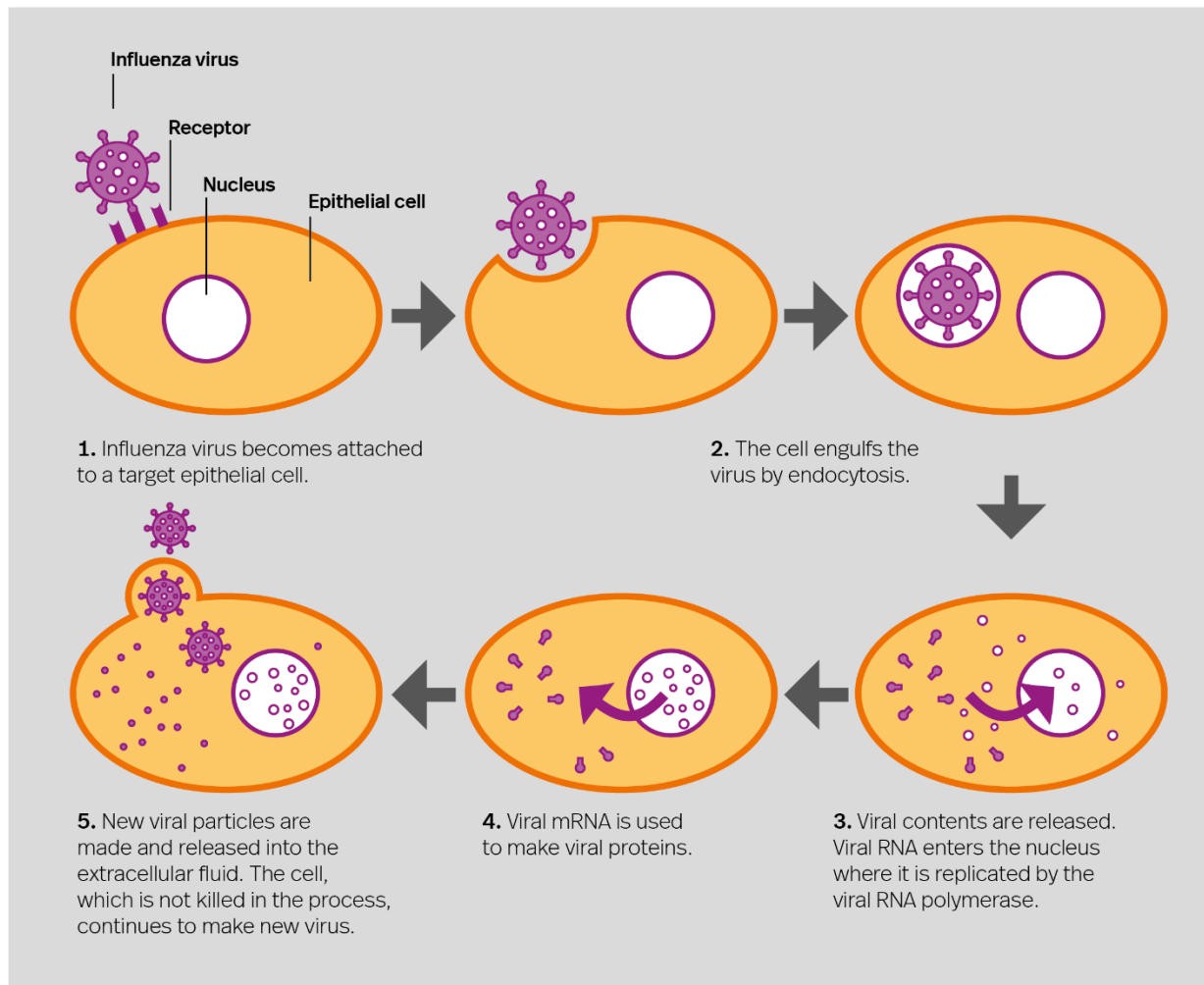


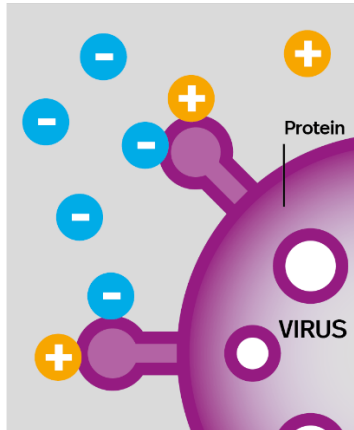
Figure 1. Effect of LightAir IonFlow ultrahigh-density ionization technology on SARS-CoV-2. SARS-CoV-2 pseudoviruses were placed in a drop of 10 µL, and added to the cells after treatment. Percentage indicates mean reduction of pseudoviral entry upon LightAir treatment.

4. Background on viruses and ionization

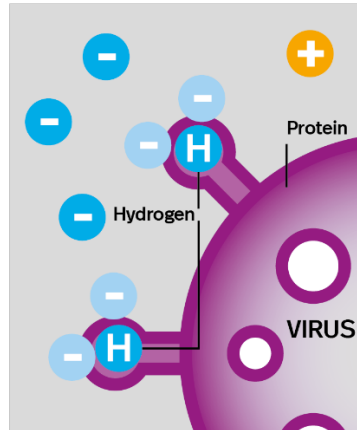
How cells are infected by a virus



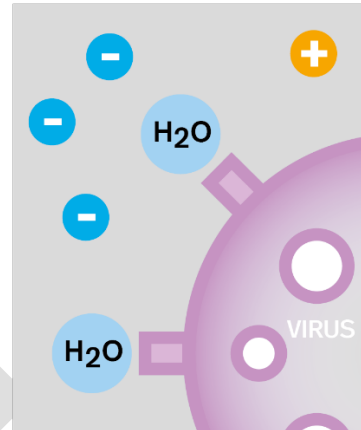
How LightAir IonFlow technology neutralizes a virus



Negative ions reach the virus protein spike. Negative ions attach to the virus.



By extracting hydrogen (H) from the protein on the surface, the spike transforms into water. Eliminating the spikes means the virus can't function.



The water returns to the air and the spike is gone, which neutralizes the virus. The virus can no longer infect human cells.

5. Conclusion

Challenge

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. This does however not need to cause such a huge negative effect on neither the individual nor society as seen with SARS-CoV-2 that causes COVID-19. By preventing the spread of viruses, we will be able to control them better and avoid the negative impact.

Obviously, providing pharmaceutical treatments and creating vaccines are of great importance for us as a society. It is a part of the prevent and protect individuals to be infected by that virus. Technology should be added to that mix and to take an active role in inhibiting the infections. While a vaccine is developed and designed to work on a specific virus, technology can take a broader spectrum and work to protect against several viruses. LightAir IonFlow ultrahigh density ionization has been tested and proven to destroy several influenza viruses and coronaviruses. By destroying the virus both in the air and on surfaces, it prevents the spread of viruses and by that we will be able to control them better and avoid the negative impact. It is important to understand that we aren't saying that technology is the solution but a part of the solution to protect and prevent future pandemics.

Research conclusions, 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, calici 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 present on surfaces.

Scientists from The Karolinska Institute found that IonFlow causes N1H1 (influenza/swine flu), rota and calici virus to lose 97% of its infectivity. Scientists at the IrsiCaixa research institute conclude that the IonFlow has similar effect on purpose-engineered viruses mimicking 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.

Implications

Health authorities provide directions and guidelines on how to best prevent the spread of virus infections in society. We 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 it will not spread infection, thus preventing the spread in situations where people meet.

Back to the New Normal

Restrictions and recommendations have been the case for more than a year now. As individuals, it affects us in various ways and for some more than others. Workplaces have been closed with the effect that we haven't met co-workers for a long time, been part of the buzz around the coffee machine, and other activities that normally feed us with positive energy and build the corporate culture. At home, we have been restricted/recommended to meet a few people at a time. We have stopped having birthday celebrations, kid playdays, larger dinner parties and other social indoor activities. Now we start to see the end of this and with fewer restrictions and recommendations, we are getting back to a more normal life. We all know it and talk about it as the New Normal and, LightAir is a good friend in the new normal.

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 to travel to a new host. The ones that remain are then 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 – a new normal where we will be aware and cautious, but not in lockdown or restrained from staying close to the people we love, and not being able to go 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 -80°C 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 pseudoviral 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).

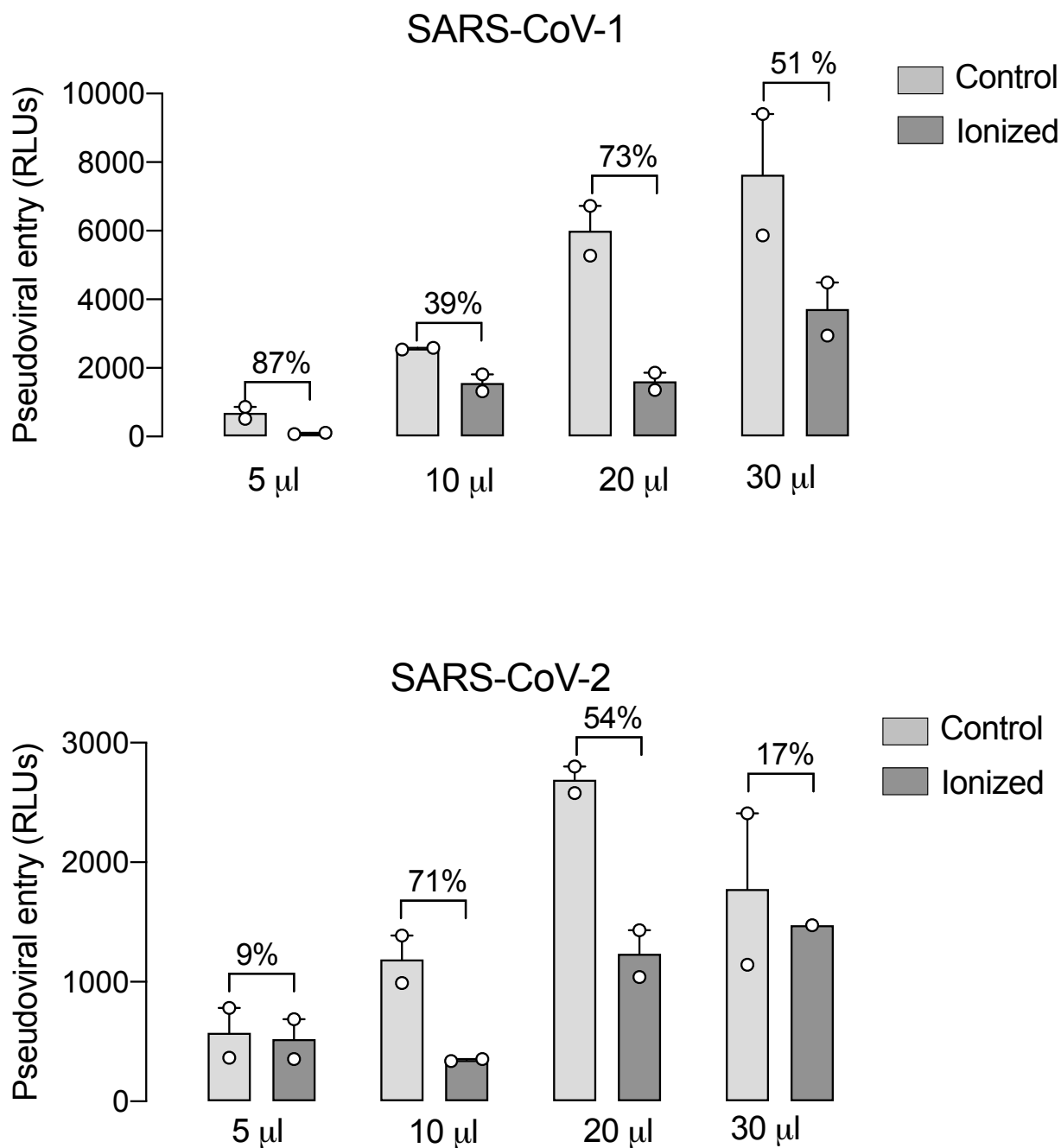


Figure 1. Effect of LightAir IonFlow high-density ionization technology on luciferase expression of reporter lentiviruses pseudotyped with SARS-CoV-1 and 2 Spike in ACE2 expressing HEK-293T cells. Different different amounts (5, 10, 20, 30 µL) of SARS-CoV-1 or -2 pseudoviruses were placed in a drop of 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 μ L (**Figure 2**, top panels). For SARS-CoV-2 pseudovirus, this reduction took place at 10 and 20 μ L (**Figure 2**, bottom panels). This effect was both after 30 to 60 minutes.

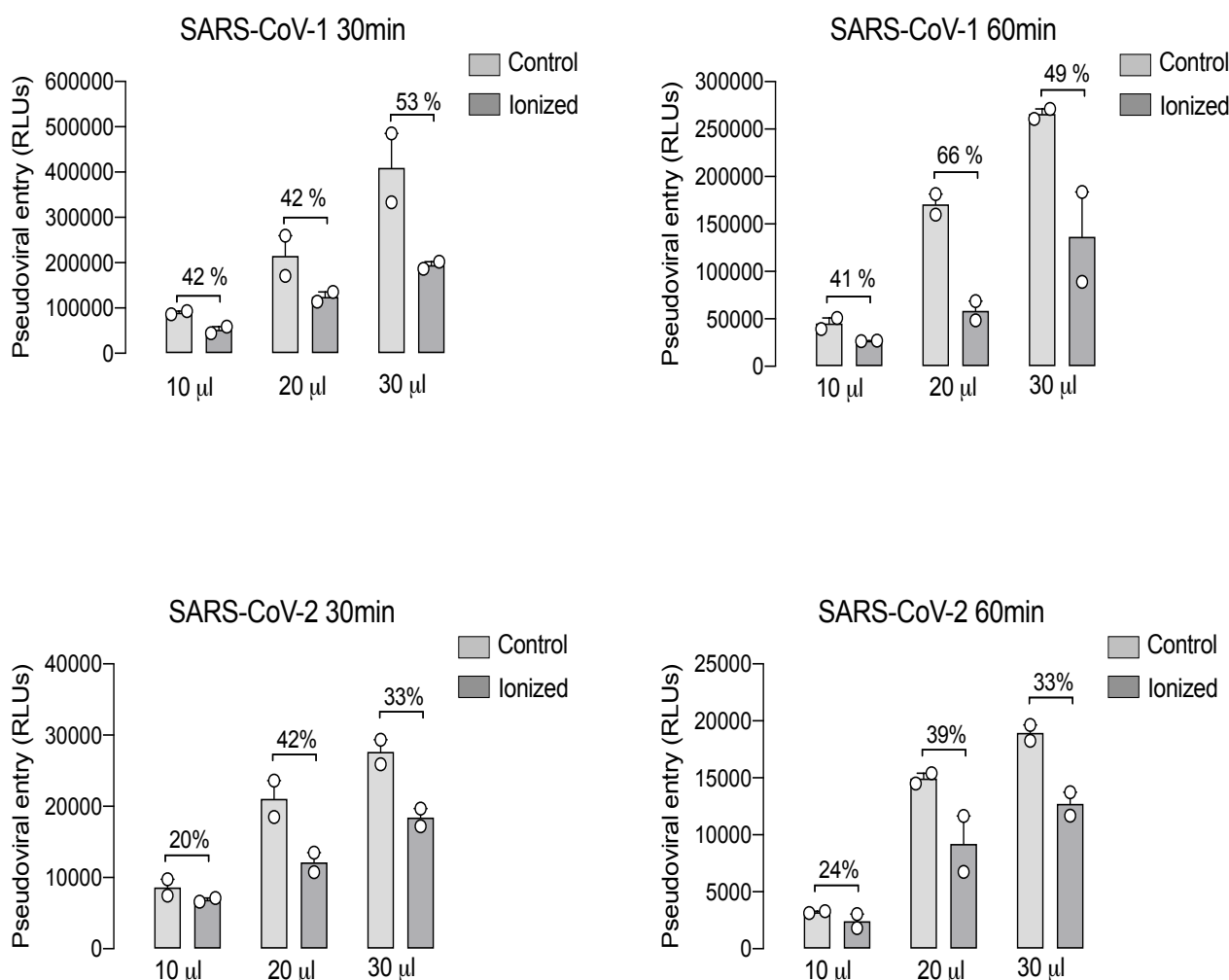


Figure 2. Effect of LightAir IonFlow high density ionization technology on luciferase expression of reporter lentiviruses pseudotyped with SARS-CoV-1 and 2 Spike in ACE2 expressing HEK-293T cells. Different amounts (10, 20, 30 μ L) of SARS-CoV-1 or -2 pseudoviruses were placed in a drop of 30 μ L of 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 pseudoviruses are treated in drops of 30 μ L.

Bibliography

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., et al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. Commun.* 11, 1620. doi:10.1038/s41467-020-15562-9.