*FINAL REPORT*

# Analysis of the germicidal effect of the SOLFIX AIR SF0P001 machine against feline coronavirus as a surrogate of SARS-CoV-2

Cod. Document: ENT - 01 / 07-000131 / R 00

**CONFIDENTIAL**

Donostia-San Sebastián

| ELABORATED |
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| Eva Pérez<br>Oihane Mitxelena |
| Date: 15/07/2021 |

**Cod. Doc:**
FINAL REPORT CEIT-UNAV-CUN - SIGNED

Ceit
Pº Manuel Lardizábal, 15
20018 San Sebastián - Spain
Tel +34 943 212 800 – Fax +34 943 213 076 – www.ceit.es

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# 1. Introduction

Exposure to air pollutants and respiratory diseases is a severe problem for public health systems around the world. The most common sources of unhealthy air are tobacco smoke, indoor and outdoor air pollution, and the presence of toxic particles, fumes or pathogens in the air. According to the World Health Organisation (WHO), influenza, together with the various viruses that cause it, is responsible for around 290,000 and 650,000 premature deaths worldwide each year, and the measles virus for more than 140,000 deaths each year (Rowe et al. 2021).

According to data published by the WHO, the US Centre of Disease Control (CDC) or Johns Hopkins University (JHU) and collected in Rome's work, the various pandemics that have occurred in the XX and XXI centuries, including the current SARS-CoV-2 pandemic, have had a significant impact on global mortality, causing numerous losses in the international population.

*Table 1. Incidence of pandemics in the XX and XXI centuries. Adapted from (Rowe et al. 2021)*

| Virus | Year | Deaths in world population |
|---|---|---|
| SARS-CoV-2 | 2019 | 0.02% |
| H1N1 pdm | 2009 | 0.001-0.007% |
| H3N2 | 1968 | 0.03% |
| H1N1 | 1918 | 1-3% |

Since the SARS-CoV-2 pandemic broke out globally between February and March 2020, there has been much controversy in identifying the routes of transmission and, therefore, which methods should be considered to control and slow the spread of those infected. According to information compiled by the World Health Organization (WHO) (World Health Organization 2020), the virus causing COVID-19 is mainly transmitted from person to person, so understanding the origin and how and where such transmissions take place is key to determine effective infection prevention measures.

In terms of transmission mechanisms, current evidence suggests that transmission of the virus occurs both in situations of direct contact and in close or even indirect contact with an infected person. Saliva or respiratory secretions, as well as droplets (with diameters of 5-10 µm) exhaled in breathing, coughing, sneezing or talking and singing are the primary means of transmission. A significant number of cases of aerosol transmission have also been observed. These are very small droplets, with diameters less than 5 µm, which are expelled during breathing and can remain suspended in the air and move with it for several hours (Castaño et al. 2020). A study by researchers in Australia showed that the viral load in aerosols is higher than in droplets or particles (Gralton et al. 2013), highlighting the transmission potential of this route. In addition, aerosols have a number of drawbacks, including the fact that they may remain in suspension longer and travel greater distances before settling on a surface.

On the other hand, over the months, studies have been published on the transmission of the virus in indoor and outdoor environments. Analysing the results of these studies, it is clear that

the degree of contamination in indoor environments is much higher than outdoor infections. Proof of this is the study published by the University of California in November 2020 (Bulfone et al. 2021). The following table shows some of the conclusions of the study.

*Table 2. Comparative study of the incidence of different virus types, including SARS-CoV-2, outdoors and indoors (adapted from Bulfone et al., 2021)*

| Virus studied | Estimate of effect outdoor | Estimate of effect indoor | Relative Estimate of effect | Number of participants in the study |
|---|---|---|---|---|
| SARS-CoV-2 | 2/7,324 cases | 7,322/7,324 cases | <1% of transmission happened outdoors | 7,324 cases totalling 318 outbreaks |
| SARS-CoV-2 | 4/103 cases | 99/103 cases | 5% of work-related cases occurred outdoors | 103 possible work-related cases among a total of 690 local transmissions |
| SARS-CoV-2 | Raw data not available | Raw data not available | Odds of transmission in closed environments 18,7 times greater than in open air | 110 cases: 27 primary cases and 83 secondary cases |
| SARS-CoV-2 | 1/7 super-spreading events | 6/7 super-spreading events | Odds ratio of super-spreading in closed environment 32,6 | 110 cases: 27 primary cases and 83 secondary cases |
| SARS-CoV-2 | 95/10,926 cases | 10,831/10,926 cases | <1% of transmission happened outdoors | 10,926 cases, cases totalling 201 events of transmission |
| H1N1 2009 influenza | 0/3 cases | 24/29 cases | Out of 32 total people in a holiday camp, 29 travelled together in a train wagon | 32 people at a holiday camp |
| H1N1 1918 influenza | 28/820 deaths sleeping in hammocks outside | 39/267 deaths sleeping in cabins inside | Risk ratio of 4,28 | Total 1,217 people on the ship |

* superspreading defined as events where the number of secondary cases generated by a single primary case is greater than the 95th percentile of the distribution (i.e. transmission to three or more persons)

Therefore, taking into account the data above, it could be argued that most respiratory diseases could be prevented by improving air quality and controlling unhealthy air. Furthermore, the importance of ventilation and aeration and the benefits of having an effective ventilation system to reduce the risk of infection indoors seems obvious.

This work arose from the interest of the company SolfixAir in characterising its photocatalytic purifier SFP0P001 (Figure 1). Specifically, their interest lies in knowing the degree of reduction

of pollutants, specifically coronavirus, in a closed passenger compartment using their catalytic purifier.


*Figure 1: Image of the SFP0P001 photocatalytic purifier used in the study.*

Feline coronavirus (FcoV) has been used as a surrogate of SARS-CoV-2 for the study. FCoV is a safe virus that is used in *in vitro* research to study coronavirus properties, including physicochemical resistance and therapeutic strategies (Centres for Disease Control and Prevention CDC, 2009). Indeed, some drugs currently introduced for the treatment of SARS-CoV-2 have also been successfully tested *in vivo* in cats for the treatment of FCoV-infected cats with severe clinical signs of peritonitis (Pedersen et al., 2018, 2019).

This study has been conducted at Ceit's facilities in San Sebastian, with the collaboration of professionals from the Clínica Universidad de Navarra (CUN) and the Universidad de Navarra in Pamplona.

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## 2. Objectives

The main objective of this study is to analyse the effectiveness of the photocatalytic purifier SFP0P001, testing its capacity to disinfect the air against feline coronavirus (FCoV) as a surrogate of SARS-CoV-2 that may be present in the space where the purifier is placed.

In order to develop this main objective, it will be necessary to meet the following partial objectives:

1. To develop a test bench adjusted to the measurements of the equipment to ensure a transmission of the airflow through the equipment, without leakages to the outside.
2. To analyse the disinfectant effect of the equipment with feline coronavirus (FCoV) as a surrogate for SARS-CoV-2.

# 3. Description of the project

The following section contains a description of the task plan that has been carried out to complete each of the objectives of the study.

## 3.1. Task 1: Development of a specific test bench for the SOLFIX AIR SF0P001 equipment

In order to be able to carry out the air purification tests without any risk for the technicians in charge of these tasks, it is necessary to build a specific test bench to carry out the effectiveness study of SOLFIX AIR SF0P001.

For the test bench, the interior of a Telstar BIO2A biological hood has been used. This space consists of 0.37 $m^3$, an air volume that has been evaluated to determine the effectiveness of the machine. The set-up for the experiments has been assembled inside the hood. This consisted of the SOLFIX AIR SF0P001 machine, the virus nebuliser and nine Petri dishes in which the samples were collected (triplicate) after the three operating times of the machine, as shown in the following diagram (Figure 2). In this case, the biological hood has a custom-made door so that the plate lids can be opened and closed to collect samples according to the analysed times.

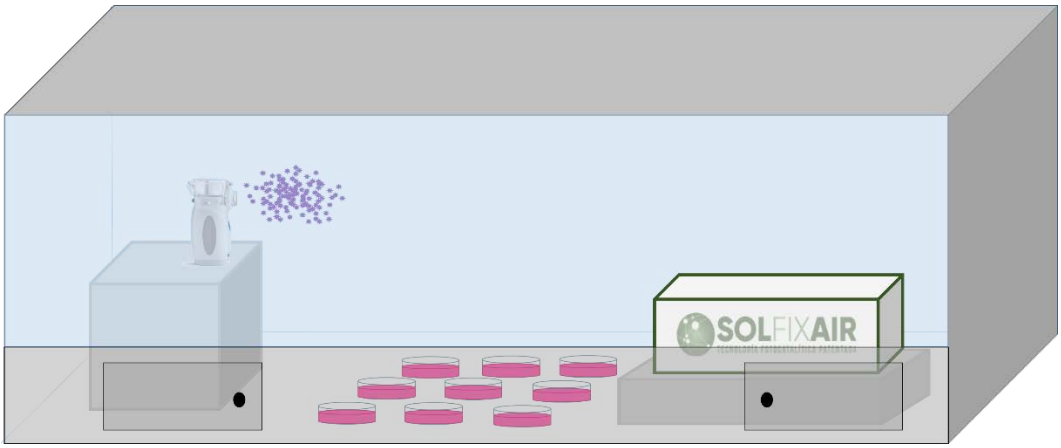


*Figure 2: Diagram of the experimental set-up.*

The SOLFIX AIR SF0P001 machine provided by the company to Ceit has been modified and has a flow rate of 40 $m^3$/h. In agreement with the company and in order to adapt the suction process of the machine, it was decided to place it in a horizontal position (instead of vertical), with the lower part of the machine facing the nebuliser. In the same way, the nebuliser was placed at a horizontal distance of 35 cm, but in a higher position than the SOLFIX AIR SFP0P001 machine, in order to prevent the nebulised virus from being deposited too quickly. Finally, the set-up of the experiment was the one shown in Figure 3.


*Figure 3: Experimental set-up before starting the assays.*

## 3.2. Task 2: Analysis of the germicidal effect of equipment against feline coronavirus (FCoV) as a surrogate for SARS-CoV-2

Once the test bench was established, the effectiveness of the SOLFIX AIR SF0P001 machine against FCoV samples as a surrogate of SARS-CoV-2 was analysed. In this case, the aim is to check the germicidal capacity of the equipment and to find out the air recirculation rounds needed to reduce FCoV population as a surrogate of SARS-CoV-2.

For this purpose, it is necessary to initially carry out a control test, using the machine without its purifying interior (identified as empty machine), and then the actual test with the complete SOLFIX AIR SF0P001 machine.

In the control test, three main elements were placed inside the biological hood: the empty SOLFIX AIR SF0P001 machine, nine closed Petri dishes containing 1 mL of virus medium and the nebuliser with a known concentration of FCoV as a surrogate of SARS-CoV-2, 5 x $10^5$ PFU/mL. The steps followed to perform the control assay were as follows:

1) The sample was nebulised so that it was suspended in the air. This air was then recirculated by the machine.

*Figure 4: Nebulization of virus in the hood.*

2) To collect the T0 samples, three of the nine Petri dishes inside the hood were opened, in order to obtain the value of the initial nebulised concentration. This way, after nebulising the entire sample, the virus was allowed to settle for 1 minute and then the lids of the Petri dishes were closed.

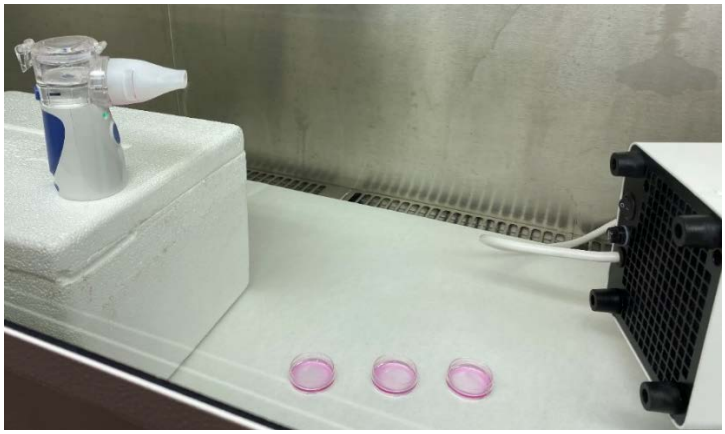

*Figure 5: Collection of the three samples in Petri dishes at time T0, after complete nebulization of FCoV as a surrogate for SARS-CoV-2.*

3) Once the T0 sample was acquired, the SOLFIX AIR SF0P001 equipment was turned on for one minute. In this case, being the control test, the machine was used empty, so no UV light was observed in the machine.
4) After one minute functioning, the machine was switched off and the next 3 Petri dishes, located between the nebuliser and the SOLFIX AIR SF0P001 machine, were uncovered and kept open for one minute. This way the samples called T1 were collected.
5) After covering the three T1 plates, the SOLFIX AIR SF0P001 machine was put into operation once again, but in this case, it was kept on for 5 minutes.

6) After this time, the last three Petri dishes were uncovered and, after waiting one minute to collect the samples, they were closed.

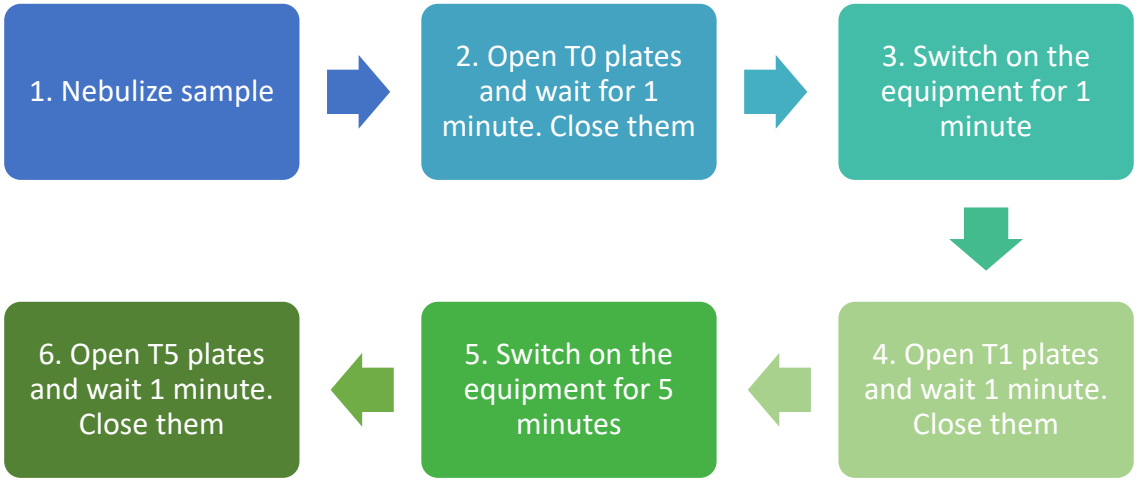


*Figure 6: Flow chart of the test procedure performed.*

Once the control test has been carried out, the entire hood was disinfected so that the test could be carried out with the real machine. It is worth mentioning that instead of being the SOLFIX AIR SF0P001 equipment, this was modified and the one provided to the Ceit had a flow rate of 40 $m^3/h$. The steps to follow during the assay were exactly the same as with the empty machine (as described in the diagram Figure 6).

All the virus quantification was carried out with a 6-well plate assay process, in which the virus titration was accounted. This process, for which different dilutions were made for each of the samples obtained, is a standardised protocol that takes a week to obtain the counted virus plates.

# 4. Results

This section explains the results obtained for each of the tests carried out by Ceit for the company SolfixAir.

## 4.1. Nebulization of the virus

After nebulization of the virus and before determining the effectiveness of the machine, two parameters were analysed to determine if the functionality of the virus was damaged by the nebulization process. Firstly, it was determined whether the nebulized virus was still active enough to infect the cells. Without virus activity, the test would be inconclusive. Secondly, it was measured how much of the starting concentration of virus was nebulized, using the process of counting the titration of virus on a plate mentioned in section 3.

As mentioned above, in order to check the effectiveness of the virus after fogging, it is necessary to check whether the virus is still active. Therefore, once the sample was nebulised, the samples obtained at T0 were incubated on cells grown in T25 flasks for 3 days. At the same time, as a control, the same cell line was incubated with uninfected medium. This way, it was found that the virus did not lose its infectious capacity after nebulization, as it continued infecting the same cells as before nebulization (Figure 7).

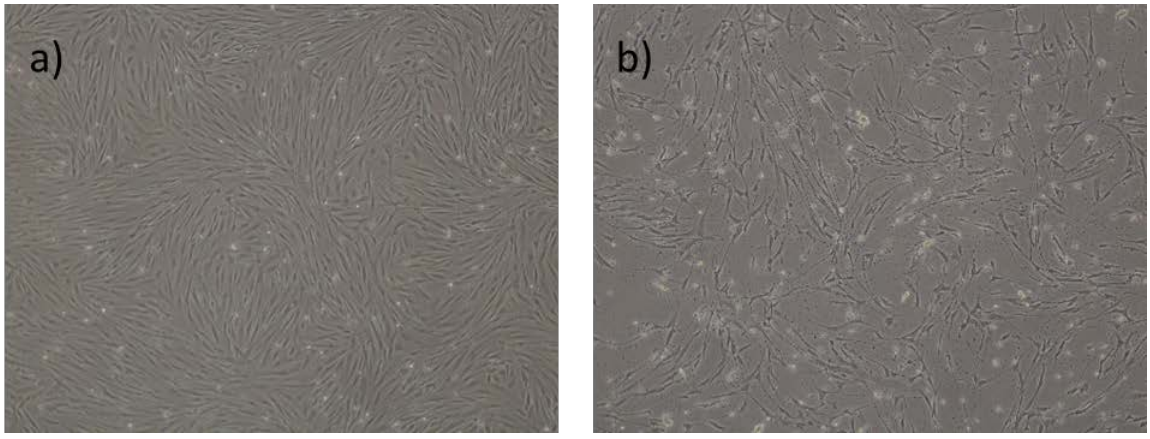


*Figure 7: a) Control cell culture, no virus infection; b) cell culture infected with the virus sample obtained after nebulization (T0). It can be seen that the cells in image b) are infected.*

After checking that the nebulized virus was still active, it was possible to count the amount of virus that was nebulized, i.e. it was possible to determine the titration of the virus obtained as a starting point T0. In this process, it was found that almost half of the amount of virus was lost during the nebulization process (Figure 8).

The initial nebulized concentration (referred to as T0) from the starting point in the air purification test with the empty machine was 2.5 x $10^5$ PFU/mL, while the one counted for the full machine test was 1.7 x $10^5$ PFU/mL.

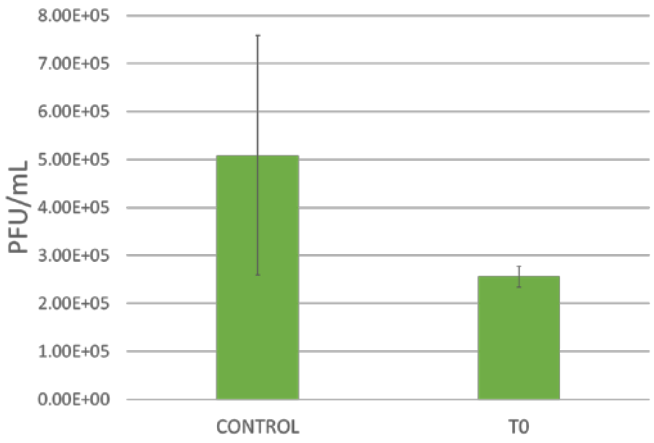


*Figure 8: FCoV concentration as a surrogate of baseline SARS-CoV-2 (control) before ebulization and virus concentration obtained after fogging (T0) in the empty machine assay.*

## 4.2. Effect of air recirculation with empty machine

As mentioned above, the first test performed in this study was using the empty SOLFIX AIR SF0P001 machine. Following the procedure explained in section 3, an 85% reduction in the concentration of FCoV as a surrogate of SARS-CoV-2 was observed (Figure 9).

Since this reduction of virus is maintained over time, even after 5 minutes of operation of the equipment, it is estimated that some of the nebulized sample remains in suspension while some adheres to the walls. Therefore, only 15% of the nebulized virus remains suspended in the air of the hood. This means that only an amount of 4 x $10^4$ PFU/mL can be filtered out by the equipment, while the rest of the virus has remained deposited on the different surfaces of the test bench.

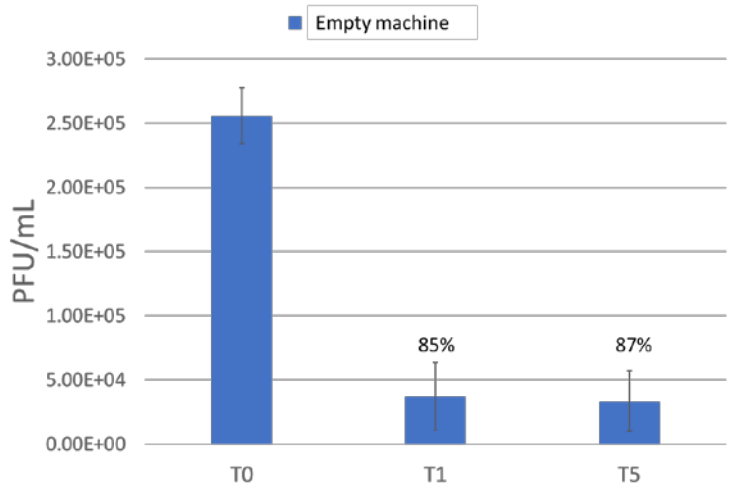


*Figure 9: FCoV nebulized concentration as a surrogate of SARS-CoV-2 (T0) and that obtained after one minute (T1) and five minutes (T5) of empty machine operation.*

## 4.3. Effectiveness of the SOLFIX AIR SF0P001 machine

Once the control test was carried out with the empty SOLFIX AIR SF0P001 machine, the efficiency of the entire machine was evaluated. Following the same procedure explained above, it was found that just by waiting one minute with the machine running, the virus remaining in the air was reduced by 100% (Figure 10). Moreover, this percentage was maintained after five minutes of machine operation.

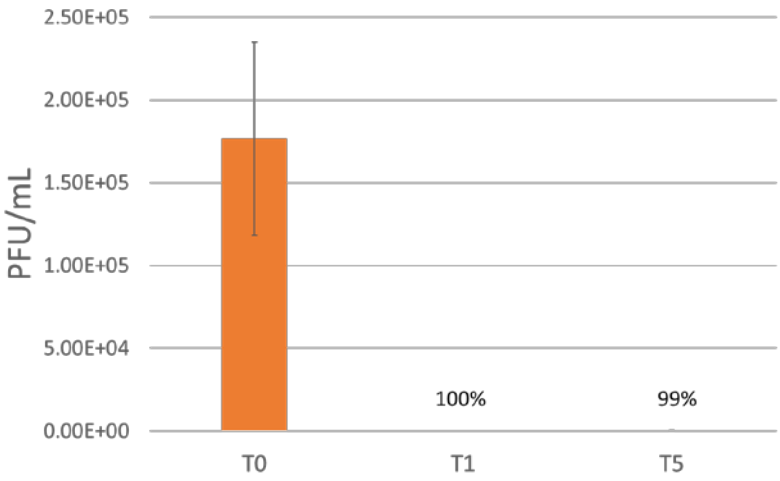


*Figure 10: FCoV nebulized concentration as a surrogate for SARS-CoV-2 (T0) and that obtained after one minute (T1) and five minutes (T5) of operation of the complete SOLFIX AIR SF0P001 machine.*

Based on the control test, it is estimated that only 15% of the nebulized sample is suspended in the air of the experimental set-up. Therefore, it can be assumed that the SOLFIX AIR SF0P001

machine reduces 100% of the FCoV as a surrogate of SARS-CoV-2 suspended in the air when it is at a maximum concentration of 4 x $10^4$ PFU/mL. In this case, it should be mentioned that taking into account the size of the work bench (0.37 $m^3$) and the flow rate of the machine provided by SolfixAir (40 $m^3$/h), 1.8 air rotations would be necessary to ensure the reduction of the coronavirus. In other words, 1.8 air rotations with the SOLFIX AIR SF0P001 machine are necessary to eliminate a maximum concentration of 4 x $10^4$ PFU/mL suspended in the air contained in the test bench.

On the other hand, as it can be seen in the comparative graph of the two tests (Figure 11), the initially nebulized virus concentration was higher in the test with the empty machine. This may also have caused the difference in the final virus reduction to be greater with the full machine, as the initial starting virus was lower.

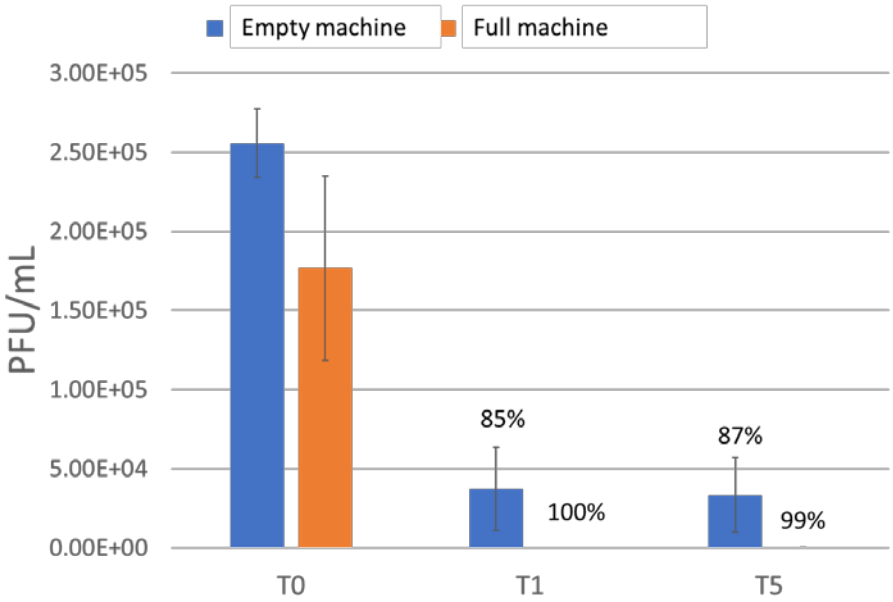


*Figure 11: FCoV nebulized concentration as a surrogate for SARS-CoV-2 (T0) and that obtained after one minute (T1) and five minutes (T5) of operation of the empty (blue) and full (orange) SOLFIX AIR SF0P001 machine.*

# 5. Conclusions

After the tests carried out in Ceit to evaluate the effectiveness of the SOLFIX AIR SF0P001 equipment of the SolFixAir company with a modified flow of 40 $m^3$/ h, the following conclusions have been reached:

- The virus has been nebulized, keeping it active, although 50% of the virus is lost in the process.
- It has been estimated that only 15% of the nebulized virus is suspended in the air of the test bench before being sucked into the machine. This reduces significantly the virus concentration to be evaluated.
- The SOLFIX AIR SF0P001 equipment completely reduces the concentration of FCoV as surrogate of SARS-CoV-2 in one minute, being the testing space of 0.37 $m^3$ and the maximum initial concentration of 4 x $10^4$ PFU / mL. This means that, in this environment and with this flow (40 $m^3$/ h), 1.8 air rotations are necessary to take place in 1 minute, to obtain a purified air.