

Quantitative assay of the virucide efficacy of the Surface and air purifier equipment BOXER 320 Olinco (bases on UNE-EN 14476)

Order: **C21159**

Report: **Report_OLINCO_210315**

Issued to:

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Sample reception:	02/23/2021
Assay start:	03/01/2021
Assay finish:	03/08/2021
Report issued:	13/17/2021

Introduction

Recent studies have shown that COVID19 is also airborne. In this line, Health Authorities have warned about the need to take measures that minimize the presence of the virus in closed environments and have offered recommendations to avoid contagion by this means.

Air purification systems have traditionally been used to remove potentially harmful particles, such as bacteria or allergens. The need to adapt its use to include viruses (10 to 100 times smaller than bacteria) among the elements to be purified has posed a technological challenge to which manufacturers have responded with solutions whose efficacy must be proven.

For obvious reasons, using SARS-CoV-2 viruses for experimental purposes is not possible. Therefore, laboratories must test the virucidal capacity of these machines with viruses that have similar characteristics. For this purpose, the use of non-pathogenic viruses that allow tests on their viability is recommended. Among these viruses there is the vMC0 strain of Mengo virus, which has been genetically modified to make it harmless, and the bacteriophage MS2, a virus that infects bacteria (*Escherichia coli*) and, therefore, harmless to humans and that allows infectivity tests to be carried out.

Goal

To obtain a quantitative estimate of the virucidal capacity of the air purifying machine with **BOXER 320** OLINCO technology on viruses spread in an air volume of 25 m³, at different treatment times through an adaptation of the UNE-EN 14476 standard.

Assay room

Closed room: 3 m × 3 m × 2,5 m

Test: Modified for quantitation

Summary of the test procedure

A total of 6×10^{11} viral particles of the bacteriophage MengovirusS2 (with RNA) are diluted in 10 ml of water and sprayed at constant intervals with a floor fan facing upwards at an angle of 45 ° until all the volume is spread.

Samples were taken with the Sartorius 16757 Air Sampler, MD8 Airport with gelatin collection plates using a volumetric flow rate of 50 l / min for 5 minutes to a total volume per sample of 250 l (0.25 m³).

A control experiment is carried out with air under turbulence and without the machine to obtain the baseline kinetics of virus decay. These kinetics are used as a reference to quantitatively evaluate the performance of the purifier equipment studied.

The plates with the samples are subjected to RNA extraction and then quantified using the qRT-PCR technique.

Table 1. Assay variables.

<i>Organisms tested</i>	Mengovirus VCM0
<i>Dilution medium used</i>	Phosphate buffer saline (PBS)
<i>Neutralizing medium used</i>	RNA later
<i>Tested system</i>	Purifier equipment technology ActivePure®
<i>Flow rate tested</i>	113,9 liters per second
<i>Disinfection method</i>	Air circulation through the equipment
<i>Description of samples</i>	Aquous viral suspension
<i>Sample quantity</i>	6x10 ¹¹ viral particles
<i>Treatment times</i>	10 min; 30 min; 60 min; 180 min.
<i>Air sampling system</i>	Sartorius 16757 Air Sampler
<i>Sample volume</i>	250 m ³
<i>Deviations from the standard method</i>	n.a.

The results expressed below correspond exclusively to the samples tested.

Results against Mengovirus vMCO

The recovery of virus at the different test times is shown in Table 1.

Table 1. Viral load in the sampling volume at the different tested times for both control and treatment conditions.

Time (min)	Control GU*/0,25 m ³	Treatment GU/0,25 m ³	% vs control	% vs init
0	1,87E+03	9,53E+02	49%	
10	1,86E+02	5,75E+01	69%	90%
30	5,72E+00	1,16E+00	80%	100%
60	2,86E+00	0,00E+00	100%	
180	0,00E+00	0,00E+00		

* (GU: Genomic units)

Viruses are no longer detectable after 60 min with the loads used.

These results indicate that after 30 minutes of operation the air treatment mediated by the action of the **BOXER 320** machine is 400% more effective than the basal reduction.

Comparison of virus elimination kinetics between basal (control) and machine-mediated conditions indicates negative exponential kinetics with different rates. The rate of disappearance of the virus in the air in the presence of the machine is higher (Figure 2; Table 2) than that of the baseline conditions (without the machine).

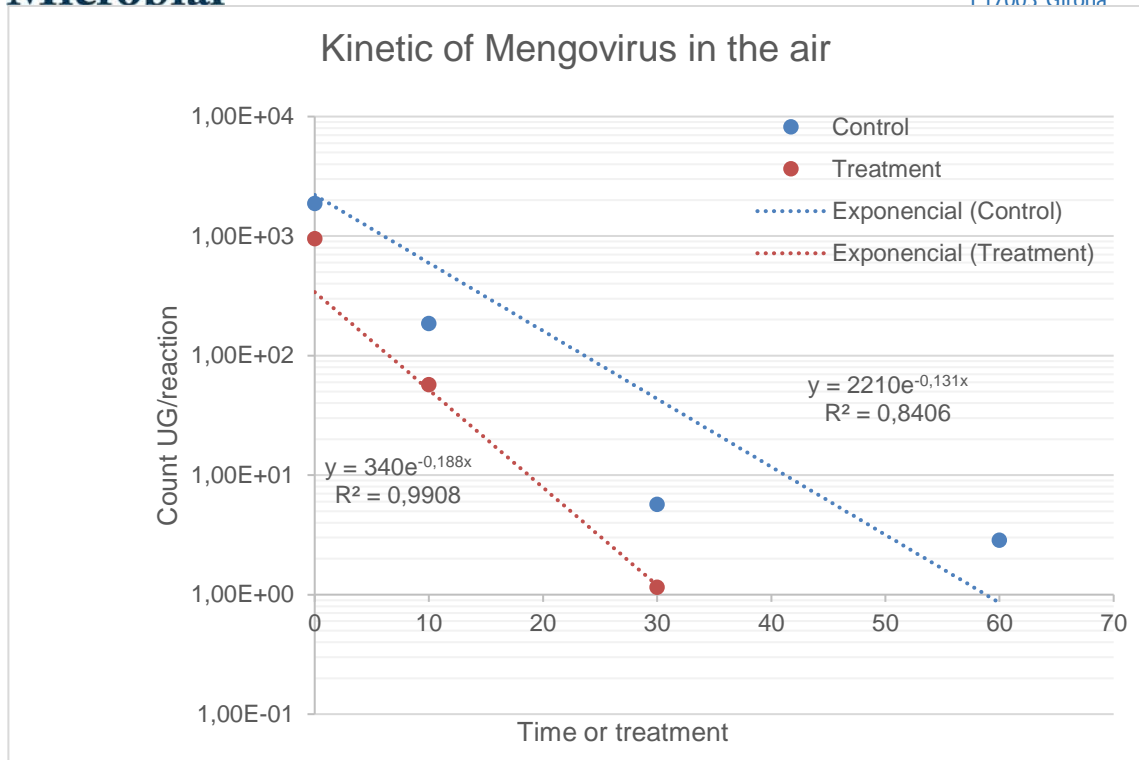


Figure 1. Comparative kinetics of MS2 virus disappearance.

The kinetics can be adjusted to a negative exponential, whose disappearance rate is expressed in table 2.

Table 2. Kinetic disappearance parameters of the suspended viruses.

Condition	Disappearing rate	R2	Rate differential
Control	-0.131	0.840	
Treatment	-0.188	0.991	0.057

Since they are exponential kinetics, the longer the treatment time, the greater the effect of the rate differential on the efficacy of the treatment. This is best expressed in short times, in which the measurement of viruses is possible, but it can be extrapolated by modeling the results under theoretical conditions (figure 2)

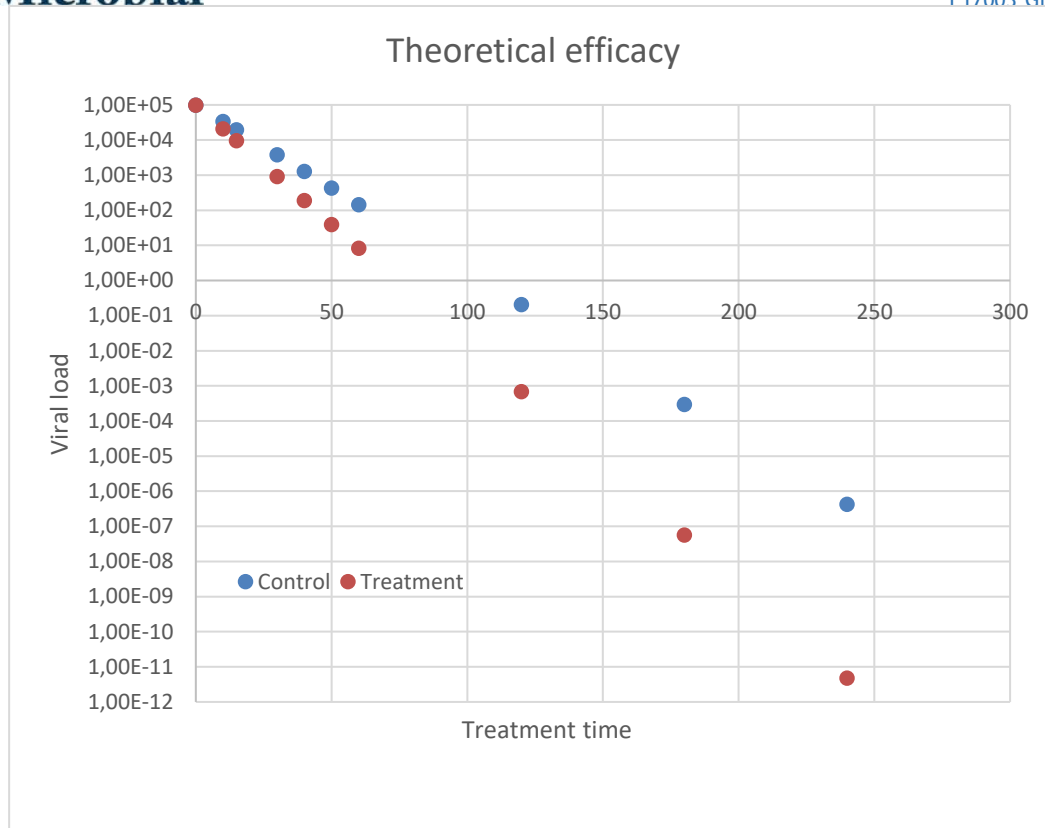


Figure 2. Theoretical kinetics of virus elimination under control and treatment conditions. The rate differential generates a response efficiency that increases with time

Table 3. Simulation of the virus disappearance kinetics in the air using the parameters measured in the test.

Time	Control	Treatment	x effic.	% effic.	% Control	% Treat.	Vol (m ³)
0	1,00E+05	1,00E+05				0,00%	
10	3,36E+04	2,09E+04	1,61	61%	38%	79,11%	4,15
30	3,79E+03	9,12E+02	4,15	415%	76%	99,09%	12,45
40	1,27E+03	1,90E+02	6,68	668%	85%	99,81%	16,60
50	4,27E+02	3,98E+01	10,74	1074%	91%	99,96%	20,75
60	1,43E+02	8,31E+00	17,26	1726%	94%	99,99%	24,90
120	2,06E-01	6,91E-04	298,00	29800%	100%	100,00%	49,80
180	2,95E-04	5,74E-08	5144,32	514432%	100%	100,00%	74,70
97			100,00				

Conclusions

The use of the purifying machine tested presents a higher rate of virus disappearance in air than the control conditions with an exponential rate differential of -0.057 min^{-1} .



The simulation from the kinetics obtained experimentally, indicates that the technology **Boxer 320** is effective for the purification of the air in terms of elimination of viral particles.

This efficacy increases exponentially with the treatment time, according to the following values:

- 30 minutes: 200%; treatment virus elimination is two fold (2x) higher than basal.
- 60 minutes: 415%; 4.15 times higher efficiency.
- 97 min: Efficacy is 100 x greater than the control.

Girona, March 15th, 2021

Signed:



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