UNIVERSITÀ
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Department of Life Science

Report (version 1.0)

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EVALUATION OF VIRUCIDE ACTIVITY OF A STETHOSCOPE DISINFECTION SYSTEM: STET CUBE

INDEX

TARGET

It is recognized that UV-C light has the ability to stop microbial and viral growth, its physical approach is considered a good compromise between cost and effectiveness, which is why it is progressively becoming more and more widespread in health care and home.

The correct use of UV-C technology takes the following parameters into account: distance from the light source (m), spatial light distribution, radiant power (W), irradiance (W/m^2), inversely proportional to the square of the distance, and radiation times (min). It allows a deep disinfection of objects that are exposed to an adequate dose of UV-C rays, where the dose (J/m^2) is the product of irradiation time and irradiance. Predictive models, that take into account the parameters described above, make it possible to predict the disinfection capacity of systems based on UV-C technology. In particular, once the dose corresponding to a specific reduction in microbial load has been established, they enable the relative UV-C irradiation times to be predicted for each distance, and viceversa.

The aim of this study is to determine the virucide activity, against the SARS-CoV-2, of the UV-C radiation which occurs in the stethoscope disinfection system "Stet Cube".

STETHOSCOPE DISINFECTION SYSTEM

The device (Stet Cube) is equipped with a UV-C led, installed on the bottom of the box in order to radiate the stethoscope diaphragm to be disinfected. The device has an automatic double level of treatment: 3 minutes for standard disinfection followed by 2 minutes more for deeper disinfection. When the box lid is closed, a status blue light indicator (flashing for 3 minutes and steady the following 2 minutes) shows that the treatment is ongoing or complete, while an yellow light indicates whether the battery is low or there is a malfunction (Figure 1).

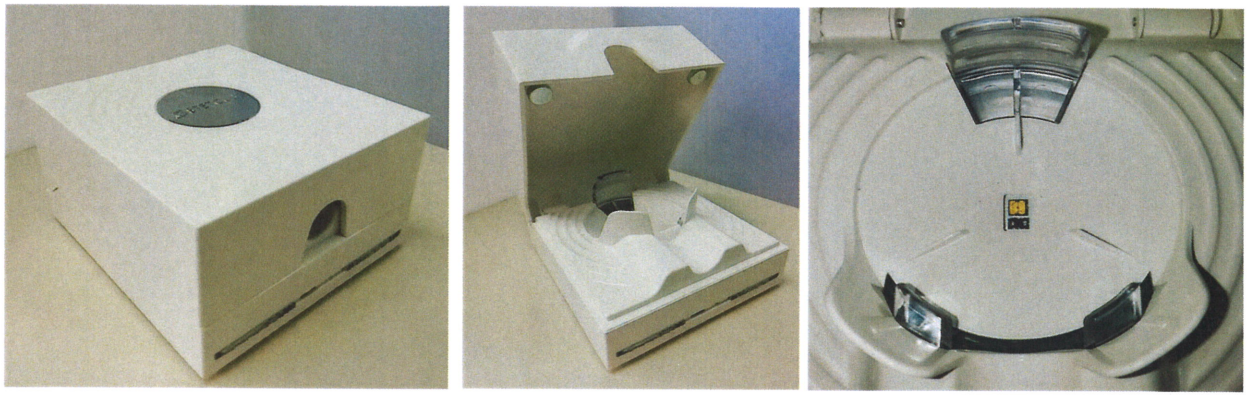


Figure 1 Stethoscope disinfection system: Stet Cube

On the left when it is closed, in the centre when it is open, on the right placement for the stethoscope head

PARAMETERS ESTABLISHED FOR THE TESTS

Name of product tested: Stet Cube (PCB ver. 1.3)

Period of analysis: 10/06/20 – 13/06/20

Identification of Viral strain: SARS-CoV-2 (Lot: VMR –SARSCPV2 VERO E6_28042020)

Contact time protocol: 3 min (flashing blue light); 5 min (flashing +steady blue light)

Repetition of tests: 3 times

Temperature of incubation: 37°C

Incubation period: 3 days

OPERATIVE TECHNIQUE

All repetitions were tested for SARS-CoV-2 concentration by TCID50% using VERO E6 C1008 (ATCC CRL-1586) cell line.

Set-Up

The UV light was activated by closing the lid.

Experiment method

A crystal (UV permeable) was positioned on the box support (Figure 2 and 3), then 100 µL of viral suspension were inoculated on the centre of the crystal at concentration of $10^{7.2}$ TCID50/mL.

After the exposure time, It was tested the residual virus activity by evaluating the Tissue Culture Infective Dose 50% (TCID50%).

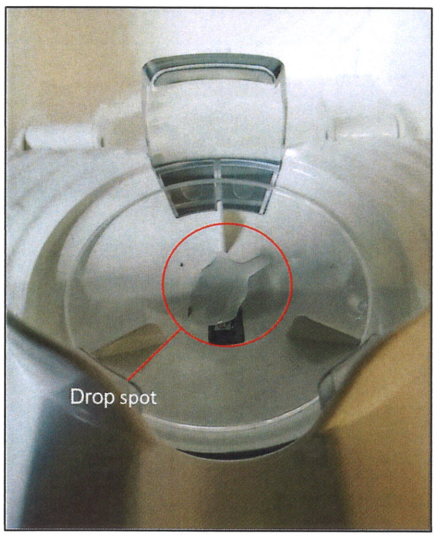


Figure 3 Drop spost position on the crystal support

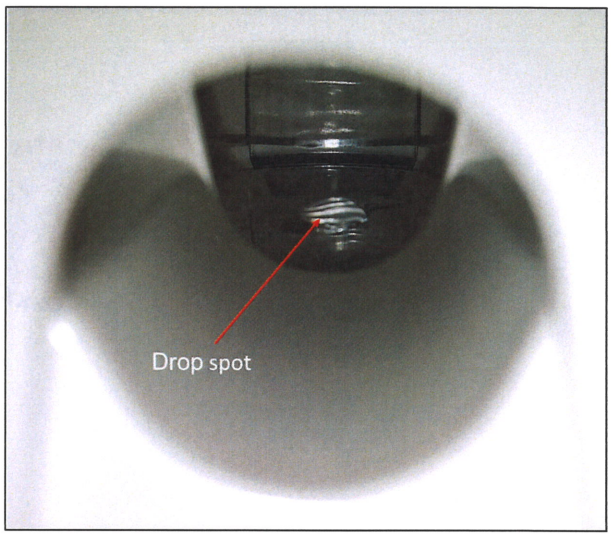


Figure 2 Drop spot position (box-closed lid)

The surfaces were irradiated as by protocol.

Examined samples:

- 3 samples inoculated with viruses and subjected to the action of UV-C as per protocol;
- 3 samples inoculated but not treated with UV-C to determine viral titer after recovery and examined immediately after inoculation.

The collected suspensions were used to inoculate a 48-wells plate into which the VERO E6 cell cultures were fixed.

Subsequent decimal dilutions were inoculated for a total of 10 dilutions. Each dilution was inoculated in 4 wells.

The plates were incubated for 3 days at 37°C ±2°C at 5% CO_2 in a humidified atmosphere.

After the exposure time, it was tested the residual virus activity by evaluating the Tissue Culture Infective Dose 50% (TCID50%).

Viral titers were determined according to the method developed by Spearman-Karber where percent reduction of virus is determined according to the following formula:

$$[1-(T/C)]*100$$

where:

T = Log_{10} of Virus Test Carrier

C = Log_{10} of Virus Control Carrier

RESULTS

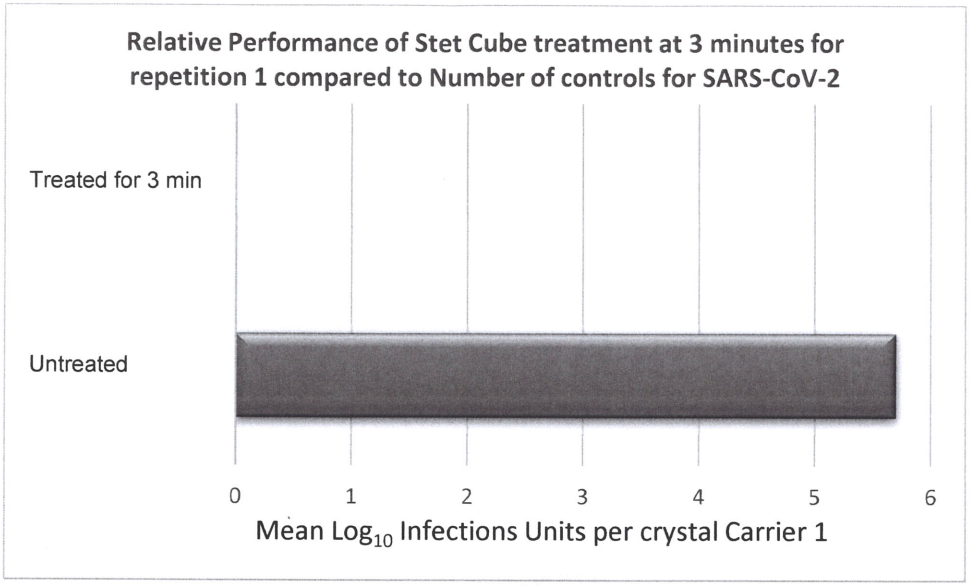
The results are presented in the following tables 1 and 2 and graphs 1-6

Table 1: Tests of Stet Cube with 3 minutes of UV-C exposition

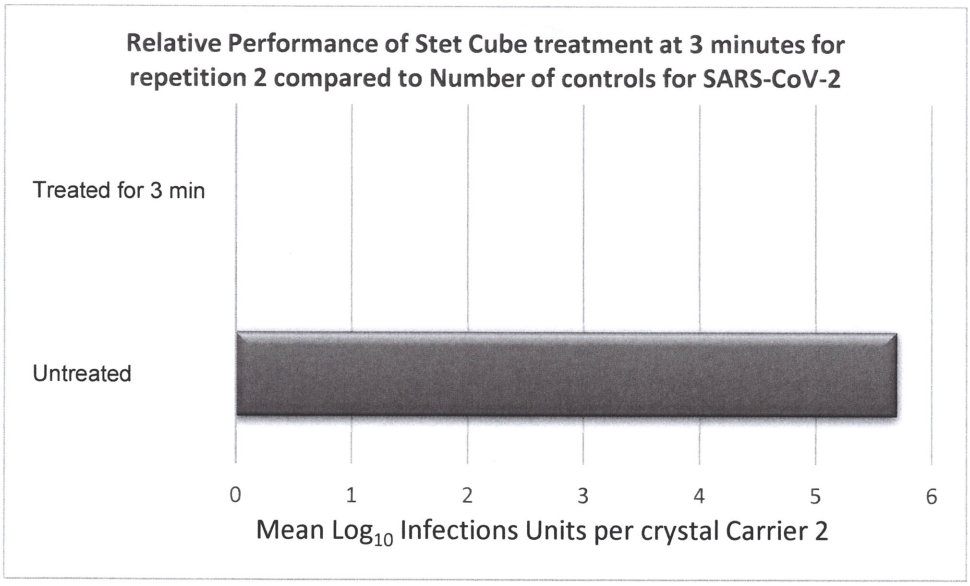
Repetition	Time	Log suspension virus TCID50%	Log TCID 50% after treatment	Log reduction TICID50%
1	3 min	7.2	1.5*	5.7
2	3 min	7.2	1.5*	5.7
3	3 min	7.2	1.5*	5.7

* The value of Log TCID50% = 1.50 means total viral inactivation

Graph 1



Graph 2



Graph 3

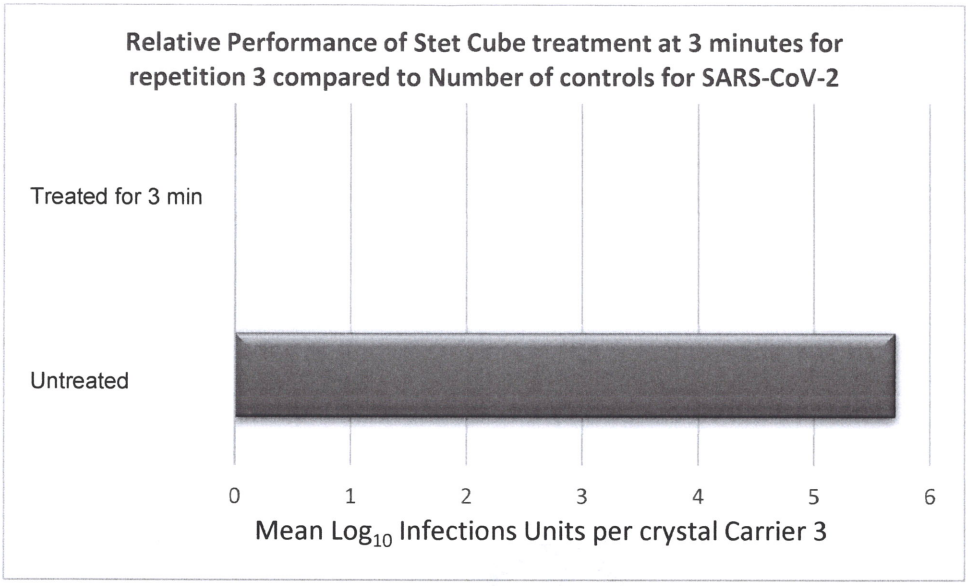
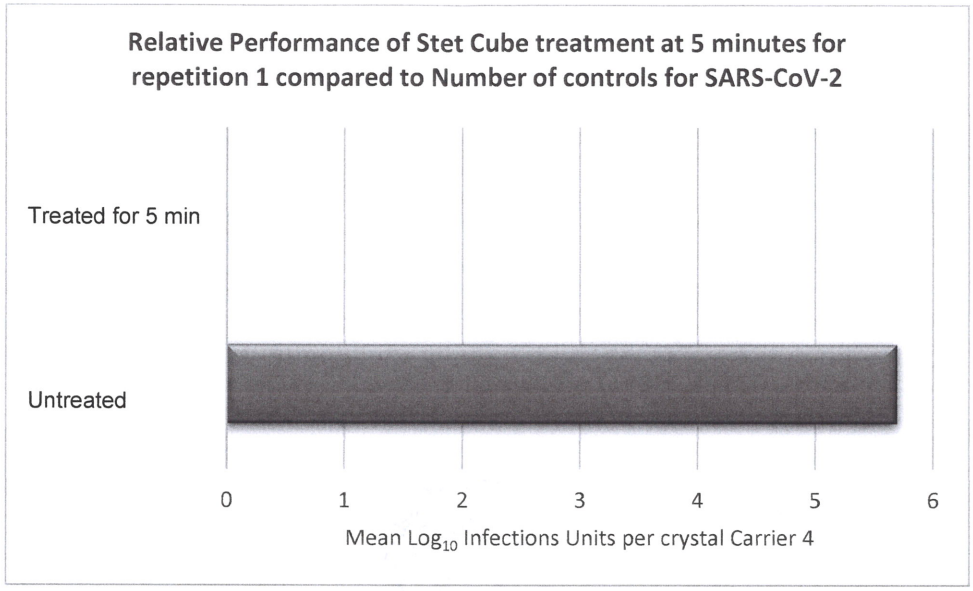


Table 2: Tests of Stet Cube with 5 minutes of UV-C exposition

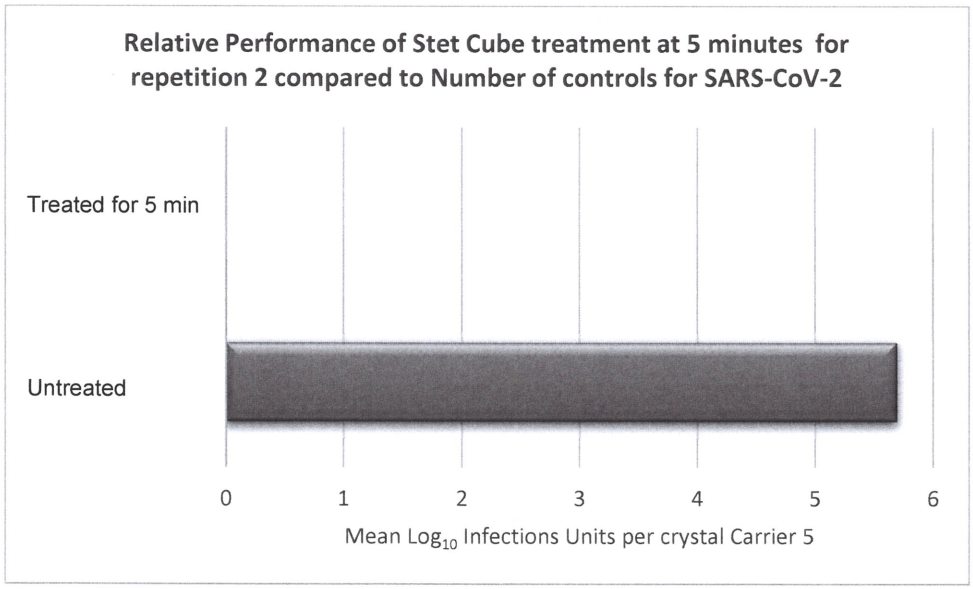
Repetition	Time	Log suspension virus TCID50%	Log TCID 50% after treatment	Log reduction TICID50%
1	5 min	7.2	1.5*	5.7
2	5 min	7.2	1.5*	5.7
3	5 min	7.2	1.5*	5.7

* The value of Log TCID50% = 1.50 means total viral inactivation

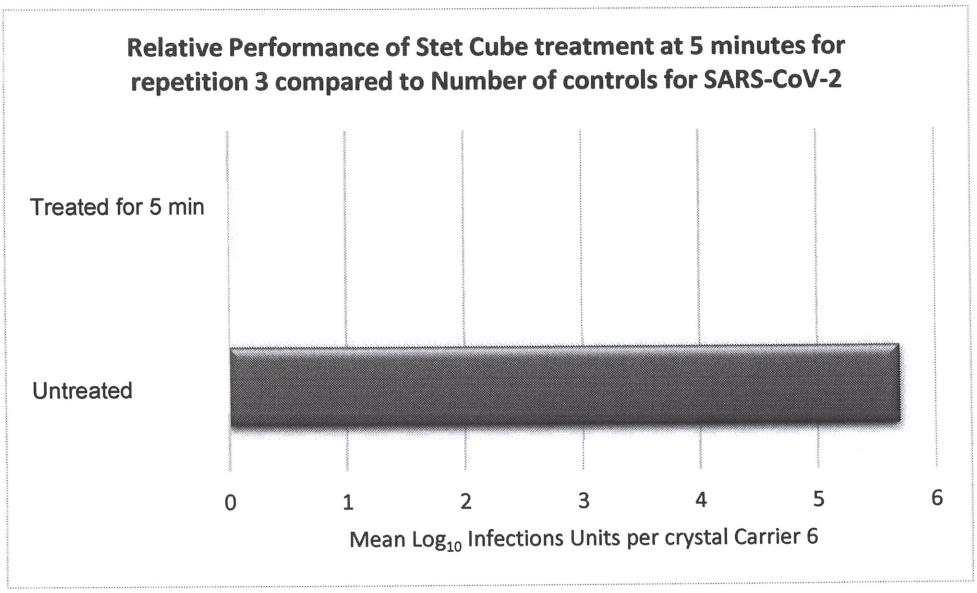
Graph 4



Graph 5



Graph 6



DISCUSSION AND CONCLUSIONS

The test showed that 5.7 Log_{10} reduction was reached, when tested against SARS–CoV-2 with both the contact time of 3 and 5 minutes, for all the 3 repetitions.

REFERENCES

ZIMBRO, M.J. ET AL., 2009*: DIFCO & BBL MANUAL –MANUAL OF MICROBIOLOGICAL CULTURE MEDIA*- SECOND EDITION

RAMAKRISHNAN, M.A., Determination of 50% endpoint titer using a simple formula, World J Virol. 2016 May 12; 5(2): 85–86.

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