Direction de la Recherche Fondamentale
Département Médicaments et technologies pour la Santé
Service de Pharmacocinétique et d'Immunoanalyse
Laboratoire Innovations Technologiques pour la Détection et le Diagnostic

24/02/2021

STUDY REPORT
ASSESSMENT OF THE INACTIVATION OF THE SARS-CoV2 BY THE BIOVITAE® LAMP

For the attention of:
NEXTSENSE S.r.l.
Via della Rotonda, 36
00186 Roma, Italy

1 - PURPOSE

The objective of the present study is to evaluate the impact of the Biovitae® lamp on the survival of infectious SARS-CoV2 virus dried on a plastic surface.

2 - IMPLEMENTATION

The study was carried out by CEA/Li2D in its biosafety level 3 laboratory (BSL3 lab) using a purified batch of infectious virus, previously prepared and quantified by plaque-based assays (see § 5.1).

Fig 1: Biovitae® lamp

The Biovitae® device supplied by Nextsense S.r.l. has been installed in a type II biosafety cabinet (BSC II, HERASAFE® KS12), without disturbing its running, at a height of 53 cm from the work surface.

Fig 2: Biovitae® lamp installed in a BSC II of the BSL3 laboratory with a height of 53 cm from the work surface.

Preliminary tests were carried out (08/01/2021) to define the optimal parameters for carrying out the tests, in particular on the following points:

- How to prepare the virus
- Viral suspension dilution medium
- Drying time of virus droplets in the plates
- Range of processing times to be used

3 - VIRUS BATCH USES

The SARS-CoV2 virus strain used is **2019-nCov/Italy-INMI1**.
The infectious virus is produced by infection of VERO-E6 cells (ATCC CRL-1586) in culture in T150 flasks (NUNC™) in DMEM + 5 % fetal calf serum (FCS) medium, with a multiplicity of infection of 0.001. After a 72 hour incubation at 37°C under 9 % CO_2, the culture supernatant is harvested and clarified by centrifugation.
The virus is then purified by ultracentrifugation on a sucrose gradient, concentrated by ultracentrifugation and resuspended in TNE saline buffer (Tris 10 mM, NaCl 100 mM, EDTA 1 mM, pH 7.4) with the addition of 0.3 g/L bovine serum albumin (BSA), according to the standard NF EN 14476+A2 2019.

The batch of virus used in the study was titrated at **2.15 10^7 PFU/mL** *(infectious particles per mL)*.

4 - VIRUS DEPOSITION AND DRYING

The viral suspension is diluted to 10^5 PFU/mL in TNE + BSA 0.03 % saline buffer and dispatched in sterile 6-well polystyrene plates (COSTAR™) under a total volume of 100 µL per well (i.e. 10^4 PFU/well before drying) in 20 droplets of 5 µL. Two wells (on the middle column) of each plate used receive the viral suspension and will be simultaneously illuminated by the Biovitae® lamp thus constituting duplicates. After dispatching, the droplets are dried for 1 hour in the BSC airflow. Irradiation tests are carried out immediately after this drying phase after putting back the lid on the boxes in order to protect the virus from the airflow and to have only the effect of the lamp.

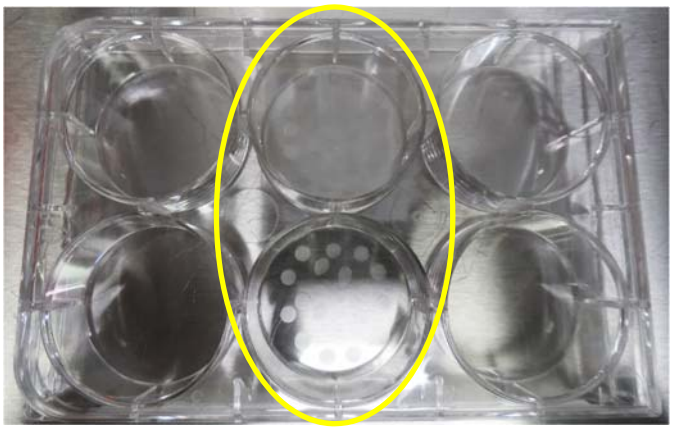

Fig 3: 5 µL droplets of viral suspension dried in 6-well plate.

5 - EVALUATION OF THE BIOVITAE® LAMP

5.1 - Conditions of Test 1

Date:	01/02/2021
Temperature:	21°C
Hygrometry:	26.1 %
Lamp/sample distance:	53 cm
Medium used for the virus:	TNE buffer (Tris 10 mM, NaCl 100 mM, EDTA 1mM, pH 7.4) + 0.3 g/L BSA

"Control" plates containing dried virus are tested in parallel under a control lighting constitute by the fluorescent lighting originally fitted to the Biological Safety Cabinet, consisting of two OSRAM L 30W/840 tubes, 895 mm long, delivering a light with a temperature of 4000K.

The light distribution of this control lighting is as follows:

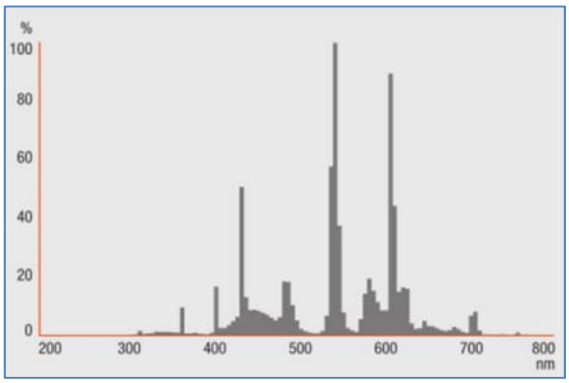

Fig 4: Light distribution of the control lighting used *(Ref OSRAM)*

All **"Test"** plates are irradiated only with the Biovitae® lamp placed 53 cm above the dried virus samples (fluorescent light of the BSC turned off).

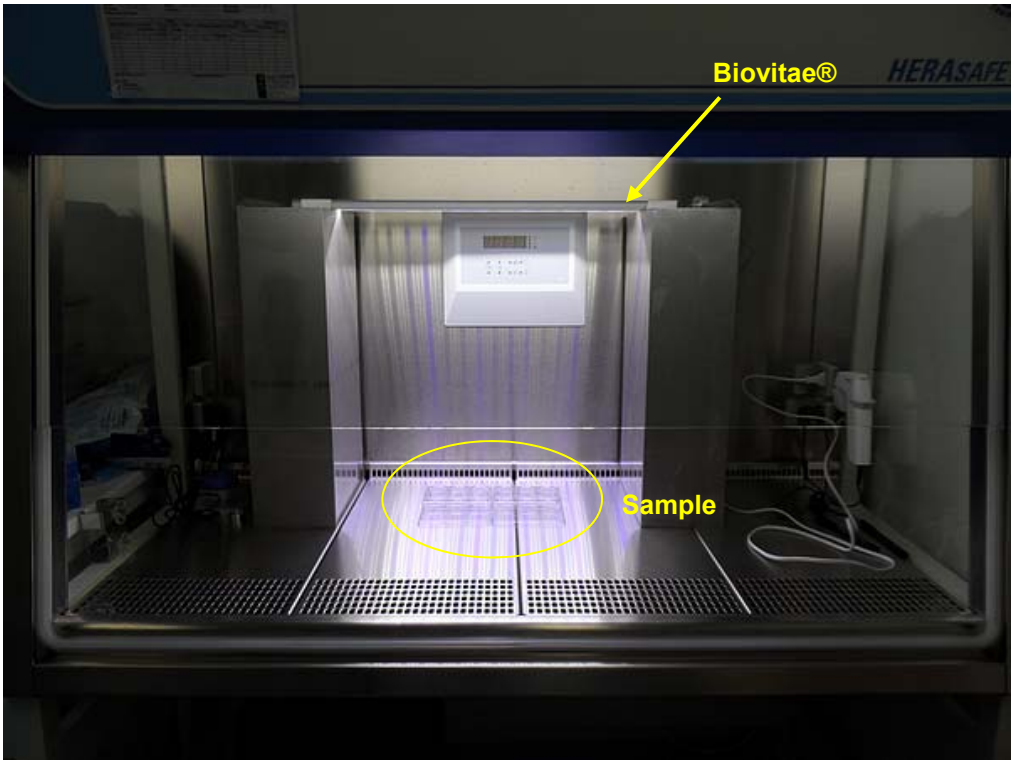

Fig 5: Dried viral samples placed under the Biovitae® lamp in a biosafety cabinet.

Summary table of conditions used:

Plate	Lamp	Qty virus before drying	Virus dilution medium	Irradiation time
Control 0		10^4 PFU	TNE + 0.03% BSA	0
Control 1	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	30’
Control 2	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	60’
Control 3	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	90’
Test 1	Biovitae®	10^4 PFU	TNE + 0.03% BSA	30’
Test 2	Biovitae®	10^4 PFU	TNE + 0.03% BSA	60’
Test 3	Biovitae®	10^4 PFU	TNE + 0.03% BSA	90’

After treatment, 1.2 mL of DMEM culture medium + 2.5% fetal calf serum (FCS) is added to each well containing dried virus (control and test plates) to resuspend the virus particles.

Each viral suspension is then titrated according to the following protocol:
The viral suspension is titrated in a 12-well plate on doublet VERO-E6 cells and the revelation is performed in crystal violet after 3 days of incubation.
Twenty-four hours prior to titration, 12-well plates are inoculated with 3.10^6 VERO-E6 cells per plate in DMEM + 5% FCS culture medium. The plates are incubated overnight at 37°C under 9 % CO_2.
- Serial dilutions of the virus are made from 10^{-1} to 10^{-8} (or other suitable dilutions) in DMEM + 2.5 % FCS culture medium.
- Two "negative control" wells containing only culture medium are also made.

- The culture medium is removed and then 500 µL of each dilution is distributed to each well (in duplicate).
- Plates are incubated for this phase of infection for 45 minutes at 37°C under 9 % CO_2.
- After 45 min incubation, 2 mL of a volume/volume mixture of carboxymethyl cellulose with DMEM + 10% FCS culture medium is dispatched in each well.
- Plates are incubated for 3 days at 37°C under 9 % CO_2.
- The medium is removed from each well.
- 500 µL of crystal violet is distributed in each well and incubated for 20 minutes at room temperature.
- The crystal violet is removed and a wash is carried out with phosphate saline buffer (PBS) pH 7.4.
- PBS is eliminated and plaques are counted.

The viral titer is calculated and expressed in plaque-forming units (PFU).

5.2 Results of Test 1

The results obtained after treatment at different irradiation times with the Biovitae® lamp of the SARS-CoV2 virus dried on the surface of the sterile 6-well boxes are as follows:

Time (min)	Control fluorescent lighting (pfu)	Biovitae (pfu)
0	2490	2490.0
30	771	119.0
60	172	6.3
90	100	1.5

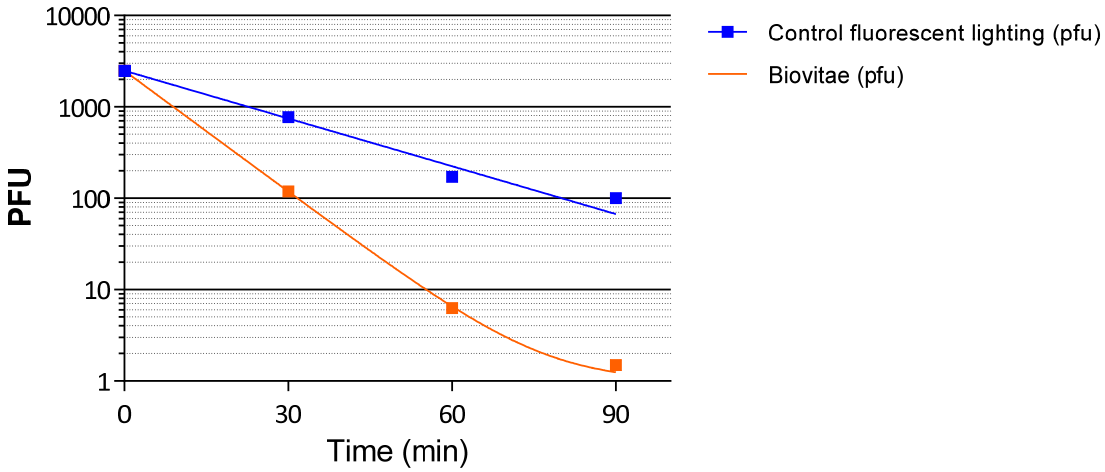

Fig 6: Evolution of the number of residual infectious particles (PFU) after irradiation at different times with the Biovitae® lamp and the control fluorescent lighting.

Time (min)	Control fluorescent lighting (pfu)	Biovitae (pfu)
0	0.00	0.00
30	0.51	1.32
60	1.16	2.60
90	1.40	3.22

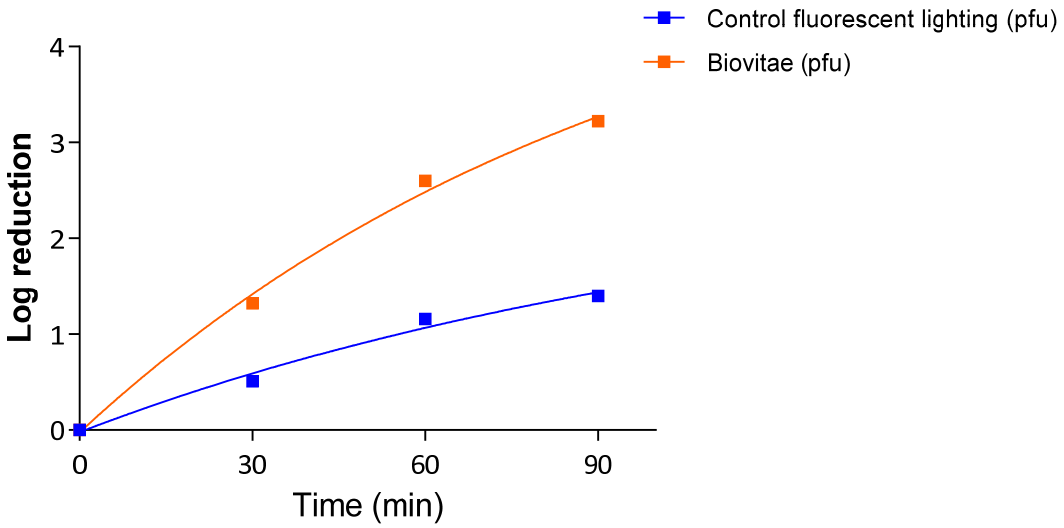

Fig 7: Logarithmic reduction in the number of residual infectious particles (PFU) after irradiation with the Biovitae® lamp and the control lighting at different times.

5.3 Conditions of Test 2

Date:	09/02/2021
Temperature:	21°C
Hygrometry:	51 %
Lamp/sample distance:	53 cm
Medium used for the virus:	TNE buffer (Tris 10 mM, NaCl 100 mM, EDTA 1mM, pH 7.4) + 0.3 g/L BSA

Control plates containing dried virus are tested in parallel under the control fluorescent lighting in the Biological Safety Cabinet consisting of 2 OSRAM L 30W/840 tubes (see details § 5.1).

All **Test** plates are irradiated only with the Biovitae® lamp placed 53 cm above the dried virus samples.

Illumination conditions were **identical** to those of Test 1.

Summary table of conditions used:

Plate	Lamp	Qty virus before drying	Virus dilution medium	Irradiation time
Control 0		10^4 PFU	TNE + 0.03% BSA	0
Control 1	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	30’
Control 2	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	60’
Control 3	2 fluorescent tubes	10^4 PFU	TNE + 0.03% BSA	90’
Test 1	Biovitae®	10^4 PFU	TNE + 0.03% BSA	30’
Test 2	Biovitae®	10^4 PFU	TNE + 0.03% BSA	60’
Test 3	Biovitae®	10^4 PFU	TNE + 0.03% BSA	90’

Sample processing and viral titration were identical to those of Test 1 (see 5.1).

5.4 Results of Test 2

The results obtained for the Test 2 after treatment at different irradiation times with the Biovitae® lamp are as follows:

Time (min)	Control fluorescent lighting (pfu)	Biovitae (pfu)
0	1950.0	1950.0
30	266.0	111.0
60	71.1	1.2
90	22.5	0.3

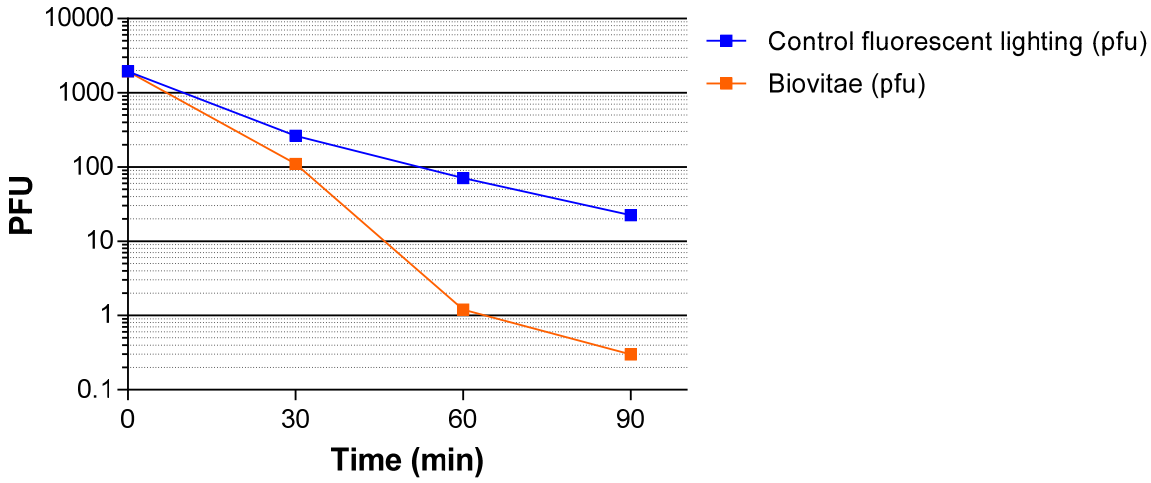

Fig 8: Evolution of the number of residual infectious particles (PFU) after irradiation at different times with the Biovitae® lamp and the control fluorescent lighting

Time (min)	Control fluorescent lighting (pfu)	Biovitae (pfu)
0	0.00	0.00
30	0.87	1.24
60	1.44	3.21
90	1.94	3.81

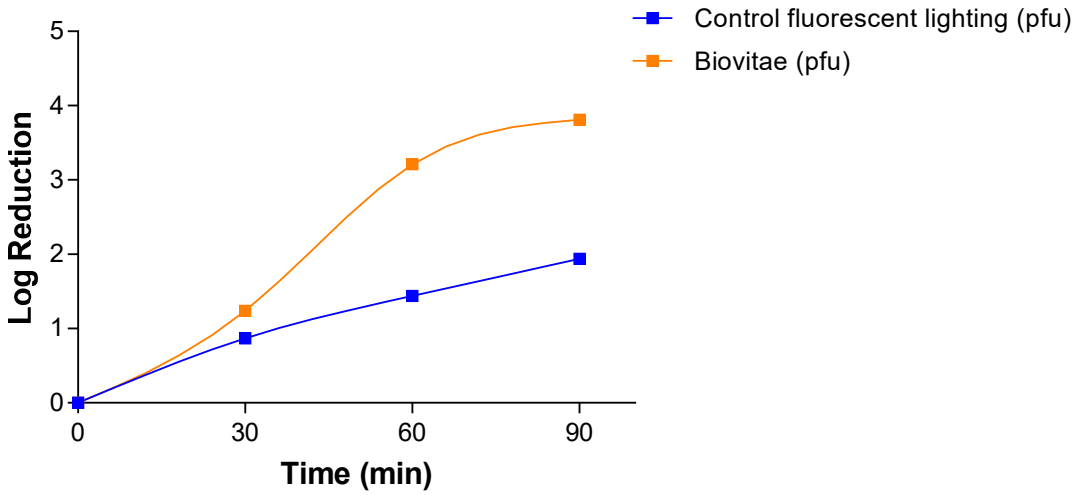

Fig 9: Logarithmic reduction of the number of residual infectious particles (PFU) after illumination with the Biovitae® lamp and the control fluorescent lighting at different times.

7 - CONCLUSION OF THE STUDY

The evaluation was carried out in our BSL3 laboratory under a biosafety cabinet. It was done by comparing the effect of a "control" lighting (fluorescent tubes originally fitted to the biosafety cabinet) with the effect of the Biovitae® lamp placed 53 cm from the viral samples. On average and under the conditions of the test, an average log reduction of 2.21 ± 0.40 times greater with Biovitae® lighting than with control lighting is noted. For the different times tested, 30, 60 and 90 minutes, this factor is 2.1 ± 0.82, 2.24 ± 0.01 and 2.13 ± 0.24 respectively.

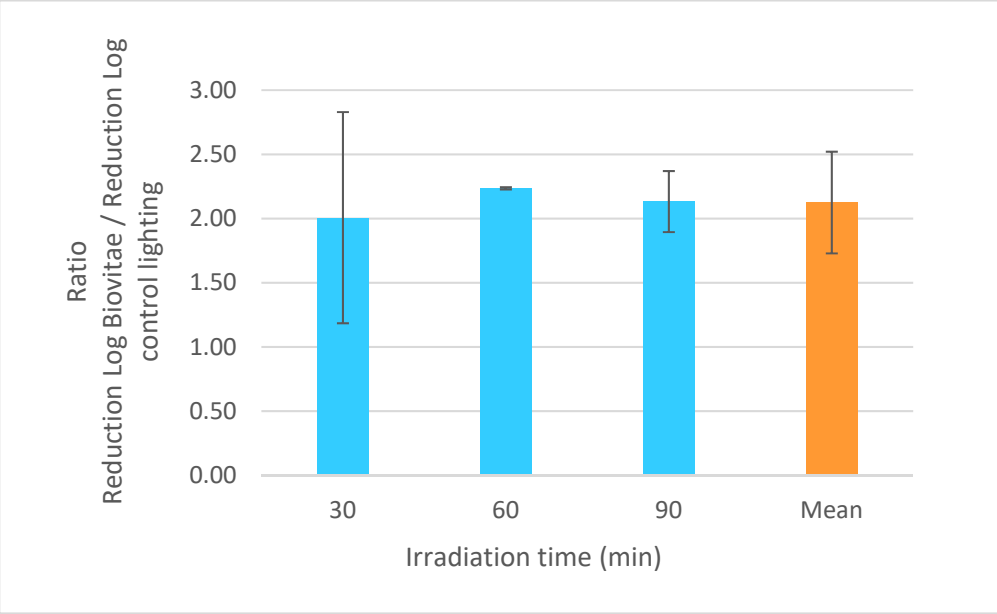

Fig 10: Ratio of the logarithmic reduction measured with Biovitae® on that measured with the control lighting for the different times tested (blue bars) and averaged over all times (orange bar).

The decrease observed under the "control" conditions should be considered as the "natural" decrease in the infectivity of the dried virus. This study shows that light irradiation of dried SARS-CoV2 on a plastic surface (polystyrene) with the Biovitae® lamp accelerates the loss of infectivity compared to the same viral preparation placed under control conditions. The logarithmic reduction in the number of infectious viral particles is on average increased by a factor of about two with the Biovitae® lamp under the conditions of our tests.

Report writer :
Laurent BELLANGER

Signature numérique de
BELLANGER Laurent
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