



ZAPRAY®

S U S T A I N A B L E D I S I N F E C T I O N

Micro-biological test results

VIRAL TEST

>99,99999%
REDUCTION

8,4 mJ/cm²
UVC LED dose
radiated

Onderwerp: Re: field tests UVC LED disinfectant RAY-ONE

Datum: vrijdag 29 januari 2021 om 13:11:34 Midden-Europese standaardtijd

Van: Hans Nauwynck

Aan: Duncan Verstraeten - ZAPARAY

1° Material and methods

Virus: porcine respiratory coronavirus - virus titer: 10exp8.0TCID50/ml (surrogate for SARS-CoV-2)
Aliquots of 250µl of virus were brought into one well of the special 6-well plates (FEP models), delivered by the company. One 6-well plate was not exposed to UV-C light (control). The other 6-well plates were exposed to UV-C light of the device ZAPARAY RAY-ONE. Positions in the device and exposure times changed depending on the experiment (see results).
Afterwards, the fluids were collected and titrated on ST-cells.

2° Results

Control

Virus titer in non-exposed 6-well plate: 10exp8.0TCID50/ml (reference control value)

Experiment 1 (ZAPARAY RAY-ONE with reflectors)

Three 6-well plates were placed at three different positions in the device (Center, Lateral, Front) as mentioned in the proposal. Two exposure times were used: 30 seconds and 60 seconds

- Center UV-C exposed for 30": ≤10exp0.8TCID50/ml (negative)
- ~~Lateral UV-C exposed for 30": ≤10exp0.8TCID50/ml (negative)~~
- Front UV-C exposed for 30": ≤10exp0.8TCID50/ml (negative)
- Center UV-C exposed for 60": ≤10exp0.8TCID50/ml (negative)
- Lateral UV-C exposed for 60": **10exp2.8TCID50/ml**
- Front UV-C exposed for 60": ≤10exp0.8TCID50/ml (negative)

Experiment 2 (ZAPARAY RAY-ONE with reflectors)

Four 6-well plates were used. They were enclosed in a plastic bag. The plate was positioned in the center of the device as mentioned in the proposal. Four exposure times were used: 30, 60, 90 and 120 seconds.

- Center UV-C exposed for 30": ≤10exp0.8TCID50/ml (negative)
- ~~Center UV-C exposed for 60": ≤10exp0.8TCID50/ml (negative)~~
- Center UV-C exposed for 90": ≤10exp0.8TCID50/ml (negative)
- Center UV-C exposed for 120": ≤10exp0.8TCID50/ml (negative)

Experiment 4 (ZAPARAY RAY-ONE without reflectors)

Four 6-well plates were used. The plate was positioned in the front of the device as mentioned in the proposal. Four exposure times were used: 10, 20, 40 and 60 seconds.

- Front UV-C exposed for 10": **10exp5.6TCID50/ml**
- ~~Front UV-C exposed for 20": 10exp2.0TCID50/ml~~
- Front UV-C exposed for 40": ≤10exp0.8TCID50/ml (negative)
- Front UV-C exposed for 60": ≤10exp0.8TCID50/ml (negative)

3° Conclusions

The device ZAPARAY RAY-ONE with and without reflectors is powerful to inactivate coronaviruses. Within 1 minute a virus reduction of >5log10 was obtained (mostly >7.2log10). A plastic bag did not affect the inactivating power of the device. Without reflectors, the ZAPARAY RAY-ONE was still very performant.

Experiment 3 will be performed next Monday (results on Friday).

Onderwerp: Experiment 3

Datum: zondag 7 februari 2021 om 12:27:19 Midden-Europese standaardtijd

Van: Hans Nauwynck

Aan: Duncan Verstraeten - ZAPARAY

1° Material and methods

Virus: porcine respiratory coronavirus - virus titer: 10exp8.0TCID50/ml (surrogate for SARS-CoV-2)
Aliquots of 250µl of virus were brought into one well of the special 6-well plates (FEP models), delivered by the company. One 6-well plate was not exposed to UV-C light (control). The other 6-well plates were exposed to UV-C light of the device ZAPARAY RAY-ONE. Positions in the device and exposure times changed depending on the experiment (see results).
Afterwards, the fluids were collected and titrated on ST-cells.

2° Results

Control

Virus titer in non-exposed 6-well plate: 10exp8.0TCID50/ml (reference control value)

Experiment 3 (ZAPARAY RAY-ONE with reflectors)

Seven 6-well plates were prepared and placed one by one at the front left side of the device as mentioned in the proposal. Increasing exposure times were used: 5, 10, 15, 20, 30, 40 and 60 seconds.

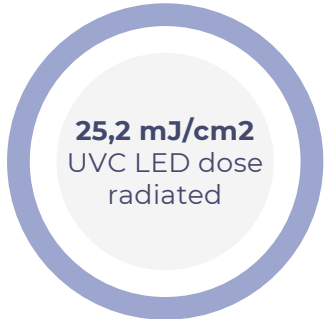
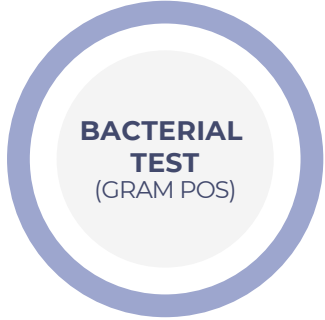
- Front UV-C exposed for 5": **10exp5.6TCID50/ml**
- Front UV-C exposed for 10": **10exp2.8TCID50/ml**
- Front UV-C exposed for 15": ≤10exp0.8TCID50/ml (negative)
- Front UV-C exposed for 20": ≤10exp0.8TCID50/ml (negative)
- Front UV-C exposed for 30": ≤10exp0.8TCID50/ml (negative)
- Front UV-C exposed for 40": ≤10exp0.8TCID50/ml (negative)
- Front UV-C exposed for 60": ≤10exp0.8TCID50/ml (negative)

3° Conclusions

The device ZAPARAY RAY-ONE with reflectors is powerful to inactivate coronaviruses. Within 15 seconds a virus reduction of >7.2log10 was obtained.

Prof. dr. Hans Nauwynck

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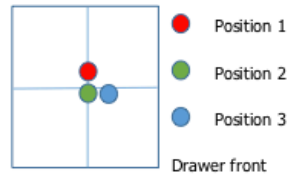
Test Report

Evaluation of the Efficacy of a UV Light Disinfection System.

Client Details: **Zaparay**
 Client Contact Name: Mieke Flour
 Client Email: mieke@zaparay.com
 PO Number: -
 Date Of Report: 11/08/21
 Melbec Reference Number: **30054**

Method Overview:

Mix test organism and Bovine Serum Albumin (low level soiling as per EN14561). Add 50µl of the test mixture onto one face of a 2cm stainless steel cube (as per EN13697). Test each face orientation individually. Dry onto the surface. Place triplicate test cubes in the drawer (see diagram below). Set the machine for 30 minutes. At 3 min open the drawer to stop the machine and remove the cubes. Prepare control cubes in the same way as the test cubes but without exposure to UV. After exposure recover the organisms from the cubes by placing the cube face down onto glass beads in 10ml of saline. Tenfold serially dilute to obtain countable numbers and carry out pour plates using TSA (35 - 38°C, 48h±6h). Log reduction after exposure calculated by comparison of test recovery and control recovery.



Layout of Test Discs in Drawer (5cm between centre points)

Test Organism:
Staphylococcus aureus ATCC 6538



Test Results:

Recovery from Test Cubes.

Test Replicate & position	Orientation of Inoculated Face Cfu/cube face					
	Facing the Back of the Drawer	Facing the Front of the Drawer	Facing the Right of the Drawer	Facing the Left of the Drawer	Facing Upwards	Facing Downwards
1	1.32 x 10 ⁵	1.10 x 10 ³	4.40 x 10 ⁴	4.20 x 10 ⁵	2.50 x 10 ⁵	6.80 x 10 ⁴
2	3.10 x 10 ⁴	2.00 x 10 ³	1.70 x 10 ⁴	1.37 x 10 ⁴	2.70 x 10 ⁵	4.10 x 10 ³
3	1.60 x 10 ⁴	3.20 x 10 ³	8.00 x 10 ³	8.80 x 10 ⁴	4.20 x 10 ⁴	2.60 x 10 ⁴
Mean Log	4.76	3.32	4.36	5.24	5.27	4.51

Recovery from Control Cubes:

Test Replicate	Cfu/cube face
1	1.90 x 10 ⁸
2	1.70 x 10 ⁸
3	2.30 x 10 ⁸
Mean Log	8.29

Log Reduction of Test Organism on Test Cube Compared to Control Cube:

Facing the Back of the Drawer	Orientation of Inoculated Face Mean Log Reduction					
	Facing the Front of the Drawer	Facing the Right of the Drawer	Facing the Left of the Drawer	Facing Upwards	Facing Downwards	
3.53	4.97	3.93	3.05	3.02	3.78	

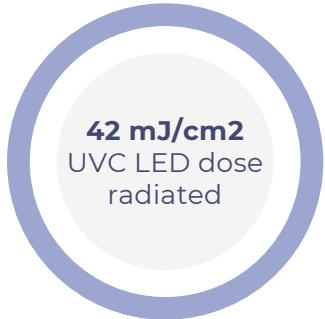
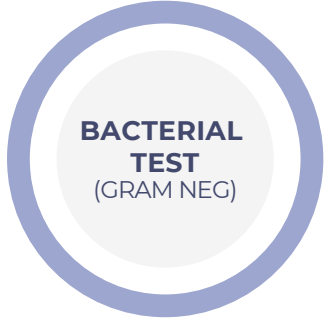
Ambient Temperature (°C) and Relative Humidity Values (%):

	Orientation of Inoculated Face					
	Facing the Back of the Drawer	Facing the Front of the Drawer	Facing the Right of the Drawer	Facing the Left of the Drawer	Facing Upwards	Facing Downwards
Ambient Temperature	20.4°C	20.0°C	20.0°C	20.3°C	20.4°C	20.0°C
Relative Humidity	56.5	52.8	53.9	52.7	52.0	51.2

Control	
Ambient Temperature	19.7°C
Relative Humidity	52.5

Conclusion:

Exposure to the UV light in the prototype device gave a reduction of the test organism on each face of the cube. There was some variability depending on the orientation of the cube.



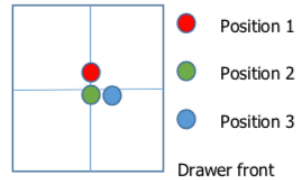
Test Report

Evaluation of the Efficacy of a UV Light Disinfection System.

Client Details: **Zaparay**
 Client Contact Name: Mieke Flour
 Client Email: mieke@zaparay.com
 PO Number: -
 Date Of Report: 19/09/21
 Melbec Reference Number: **31680**

Method Overview:

Mix test organism and Bovine Serum Albumin (low level soiling as per EN14561). Add 50µl of the test mixture onto the surface of a 2cm stainless steel discs (as per EN13697) Dry onto the surface. Place triplicate test discs in the drawer (see diagram below). Set the machine for 30 minutes. At 5 min open the drawer to stop the machine and remove the discs. Prepare control discs in the same way as the test discs but without exposure to UV. After exposure recover the organisms from the discs by placing the disc face down onto glass beads in 10ml of saline. Tenfold serially dilute to obtain countable numbers and carry out pour plates using Malt Extract Agar (29 - 31°C, 48h±6h). Log reduction after exposure calculated by comparison of test recovery and control recovery.



Layout of Test Discs in Drawer



Test Organism:
Pseudomonas aeruginosa ATCC 15442

Test Results

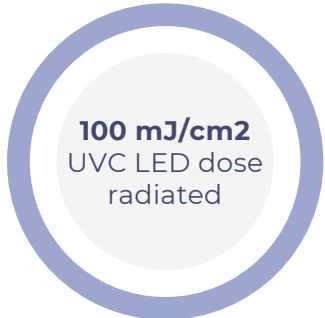
Test Organism	Test Replicate & position	Test	cfu/disc		Log Reduction (Mean Control Log - Test Log)	Mean Log Reduction (Mean Control Log - Mean Test Log)
			Control Replicate	Control		
<i>Pseudomonas aeruginosa</i>	1	3.2×10^2	1	4.80×10^6	4.05	>5.06
	2	$<1.0 \times 10^1$	2	4.00×10^6	>5.56	
	3	$<1.0 \times 10^1$	3	2.10×10^6	>5.56	
	Mean Log	<1.50	Mean Log	6.56	-	

Relative Humidity and Temperature

Test Organism	RH %	Temp °C
<i>Pseudomonas aeruginosa</i>	56.7	23

Conclusion:

Exposure to the UV light in the prototype device gave a mean log reduction of >5.06 for the *Pseudomonas aeruginosa* with a contact time of 5 minutes.



Test Report

Evaluation of the Efficacy of a UV Light Disinfection System.

Client Details: **Zaparay**
 Client Contact Name: Mieke Flour
 Client Email: mieke@zaparay.com
 PO Number: -
 Date Of Report: 16/01/22
Melbec Reference Number: 35994 – Mycobacterium terrae

Method Overview:

Mix test organism and Bovine Serum Albumin (low level soiling as per EN14561). Add 50µl of the test mixture onto the surface of a 2cm stainless steel discs (as per EN13697) and spread over the entire surface. Dry onto the surface. Place two test discs in the drawer, one disc directly on the weak spot and one disc directly on the powerful spot. Set the machine for 30 minutes. At 5 min open the drawer to stop the machine and remove the discs. Prepare control discs in the same way as the test discs but without exposure to UV. After exposure recover the organisms from the discs by placing the disc face down onto glass beads in diluent. Tenfold serially dilute to obtain countable numbers and carry out pour plates using Middlebrook and Cohn 7 H 10 medium with 10 % OADC enrichment (36°C±1°C, 21d). Log reduction after exposure calculated by comparison of test recovery and control recovery. Carry out two runs.

Test Organism:
Mycobacterium terrae NC 10856

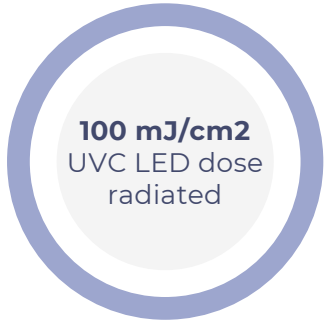


Test Results:

Test Organism	Test Run	cfu/disc log/disc			Log Reduction (Control Log – Test Log)	
		Test		Control	Weak	Strong
		Weak	Strong			
<i>Mycobacterium terrae</i>	1	1.0 x 10 ¹ 1.0	<1.0 x 10 ¹ <1.0	1.85 x 10 ⁷ 7.27	6.27	>6.27
	2	1.0 x 10 ¹ 1.0	<1.0 x 10 ¹ <1.0	1.19 x 10 ⁷ 7.08	6.08	>6.08

Conclusion:

Exposure to the UV light in the prototype device gave log reductions of >6.0 for the *Mycobacterium terrae*. There did not appear to be a significant difference in the reductions achieved on the strong and weak spots in the machine.



Test Report

Evaluation of the Efficacy of a UV Light Disinfection System.

Client Details: **Zaparay**
 Client Contact Name: Mieke Flour
 Client Email: mieke@zaparay.com
 PO Number: -
 Date Of Report: 23/12/21
Melbec Reference Number: 35994 – *Aspergillus brasiliensis*

Method Overview:

Mix test organism and Bovine Serum Albumin (low level soiling as per EN14561). Add 50µl of the test mixture onto the surface of a 2cm stainless steel discs (as per EN13697) and spread over the entire surface. Dry onto the surface. Place two test discs in the drawer, one disc directly on the weak spot and one disc directly on the powerful spot. Set the machine for 30 minutes. At 5 min open the drawer to stop the machine and remove the discs. Prepare control discs in the same way as the test discs but without exposure to UV. After exposure recover the organisms from the discs by placing the disc face down onto glass beads in diluent. Tenfold serially dilute to obtain countable numbers and carry out pour plates using Malt Extract Agar (25°C±2.5°C, 3-5d). Log reduction after exposure calculated by comparison of test recovery and control recovery. Carry out two runs.

Test Organism:
Aspergillus brasiliensis ATCC 16404



Test Results:

Test Organism	Test Run	cfu/ disc log/ disc			Log Reduction (Control Log – Test Log)	
		Test		Control	Weak	Strong
		Weak	Strong			
<i>Aspergillus brasiliensis</i>	1	3.75 x 10 ⁵ 5.57	4.90 x 10 ⁵ 5.69	2.5 x 10 ⁶ 6.40	0.83	0.71
	2	4.25 x 10 ⁵ 5.63	4.75 x 10 ⁵ 5.68	3.3 x 10 ⁶ 6.52	0.89	0.84

Conclusion:

Exposure to the UV light in the prototype device gave log reductions of between 0.71 and 0.89 with the *Aspergillus brasiliensis*. There did not appear to be a significant difference in the reductions achieved on the strong and weak spots in the machine.

External studies have documented the UVC radiation doses required to reduce pathogens with a certain percentage, also referred to as a 'LOG value'.

Columns 1-6 in the right hand tables reveal the UVC dose in mJ/cm² needed for a LOG1-6 reduction.

A typical dose required for a 99,99% (LOG4) bacterial reduction is +10mJ/cm².

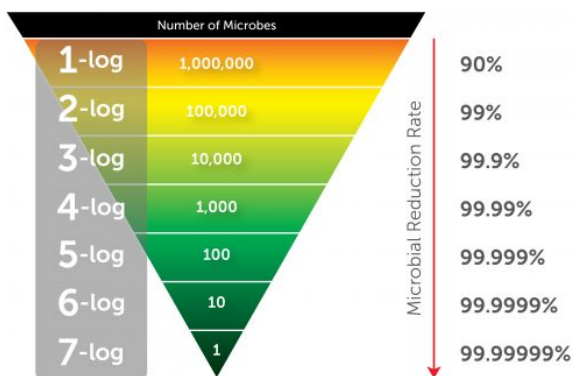
Viruses and bacteria are most susceptible to UVC (typ LOG1=90% @ 1,3mJ/cm²) The Spore *Aspergillus b.* is known to be one the most resistant organisms. (LOG1=90% @ 122mJ/cm²)

ZAPARAY's RAY-ONE and RAY-TWO provide 100mJ/cm² after a single radiation cycle.

Online reference literature:

[NIST](#) - [IUVA](#)

<https://www.zaparay.com/science>



LOG 1 to 6 : measured doses

Bacterium	Lamp Type	1	2	3	4	5	6
<i>Staphylococcus aureus</i>							
	LP	2.1	3.2				
(hem)	LP	2.6					
ATCC 25923	LP	3.9	5.4	6.5	10		
ATCC 25923	LP	4.4	5.8	6.4	7.3	9	

		Fluence (UV Dose) (mJ cm ⁻²) for a Given Log Reduction Without Photoreactivation							
Bacterium	Lamp Type	1	2	3	4	5	6	Notes	Reference
<i>Pseudomonas aeruginosa</i>									
ATCC 10145	LP	2.8	5.5	7	9.3				[27]
ATCC 15442	LP	1.6	3	4.8	8				[28]
NCTC 13437 – Antibiotic resistant	LP	0.7	1.5	2.3	6				[31]
<i>Mycobacterium terrae</i>									
ATCC 15755	LP	3.8 ± 1.3	9.3	16					[35, 36]
Spore									
	Lamp Type	1	2	3	4	5	Protocol?	Notes	Reference
<i>Aspergillus brasiliensis</i> (previously known as <i>Aspergillus niger</i>) ATCC 16404	LP	122	226	293			yes		[13]

LOG1 dose for PRCV: 1,3mJ/cm²

Porcine Respiratory Coronavirus (PRCV)	254	1.3*
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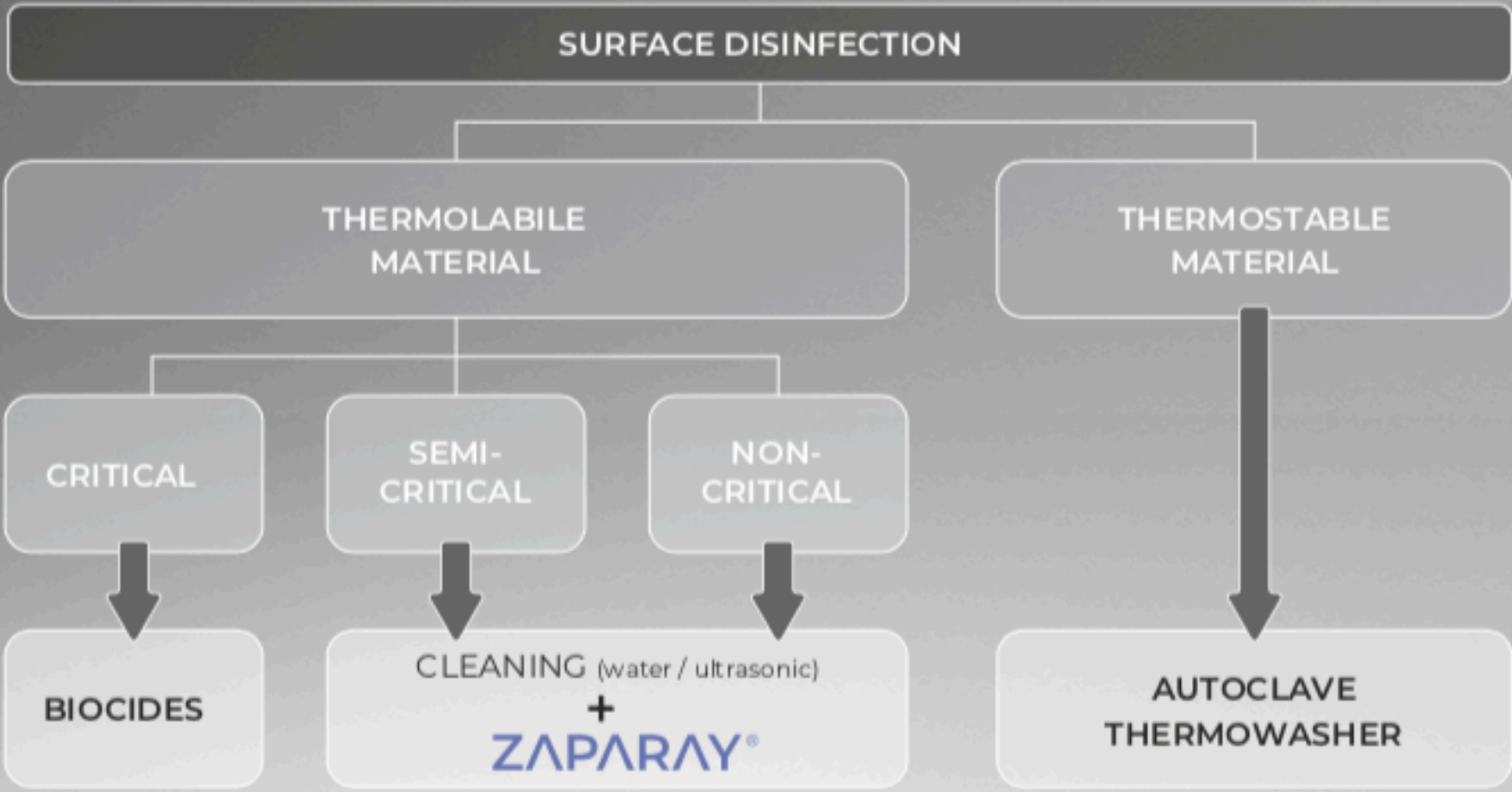
Heßling et al.: Ultraviolet irradiation doses for coronavirus inactivation ...



ZAPRAY®

S U S T A I N A B L E D I S I N F E C T I O N

Micro-biological test results



100 mJ/cm²
UVC LED dose
per cycle

ZAPARAY®
RAY-ONE



100 mJ/cm²
UVC LED dose
per cycle

REAL TIME
UVC DOSE
LOGGING

ZAPARAY®
RAY-TWO

