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SPONSOR	UV SMART B.V.		
	OLOF PALMESTRAAT 16		
	2616LR DELFT		
	THE NETHERLANDS		
STUDY MONITOR	EUROFINS BACTIMM		
	MIDDENKAMPWEG 19		
	6545 CH, NIJMEGEN		
	THE NETHERLANDS		
TEST METHOD	EN 16777:2018 / UNI EN 16777:2019 - Chemical disinfectants and antiseptics — Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase 2/step 2)		
TEST ITEM			
PRODUCT NAME	*UV SMART D25*		
MATRIX OF THE PRODUCT	Biocide and Antimicrobials (Device for surface disinfection based on treatment with UV-C)		
BATCH N.	N/A	CODE	N/A
MANUFACTURING DATE	N/A	EXPIRY DATE	N/A
MANUFACTURER	UV SMART B.V.		
ACTIVE INGREDIENT	N/A		
MATERIAL ITEM ALIQUOT	LV-MAT-F5PH-20-076-0450:a		
PARCEL REGISTRATION N.	IP-LV-2020027-ABC	RECEIVING DATE	27-Jan-20
STORAGE CONDITIONS	Room Temperature		
ANALYSIS STARTING DATE	17-Mar-20	**ANALYSIS ENDING DATE**	25-Mar-20
EXPERIMENTAL CONDITIONS			
TEST TEMPERATURE	Room Temperature (18-25°C)		
CONCENTRATION	N/A The cycle was performed according to Manufacturer's instruction for use		
PRODUCT APPEARANCE	N/A		
CONTACT TIME	According to the standard disinfection cycle of the device		
INACTIVATION OF THE PRODUCT	N/A		

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INTERFERING SUBSTANCE	A 0.03% of final concentration of a bovine serum albumin was used as interfering substance (simulating clean conditions)
INCUBATION TEMPERATURE	37°C ± 1°C (with 5% CO_2)
TEST STRAIN	*Adenovirus Type 5* (ATCC VR-5)
CELL LINE	*HeLa* (ATCC CCL-2)
CULTURE MEDIUM	***Growth Medium*** for cell multiplication: Eagle's minimal essential medium (EMEM) supplemented with 10% Foetal Bovine Serum (EMEM+10% FBS) ***Maintenance Medium*** for virus propagation: Eagle's minimal essential medium (EMEM) supplemented with 2% Foetal Bovine Serum (EMEM+2% FBS)
CARRIERS	Stainless steel carriers with 2 cm diameter and frosted glass slides were used as test surfaces.
EXECUTION OF THE ASSAY	***Titration of the virus suspension*** The virus suspension showing concentration in about 10^8 $TCID_{50}$/ml (or sufficiently high to at least enable a titre reduction of 4 Log) was diluted by means serial dilutions 1:10 with maintenance Medium, starting from the virus stock suspension. Each dilution was placed six-fold, transferring 0.1 ml in 96 wells microplates containing the cellular confluent monolayer (>90%) without any culture Medium. After 1 hour of incubation at the indicated temperature, 0.1ml of maintenance Medium was added. The outline of the microplate did not receive the viral inoculum but only culture Medium and was used as control of cellular line. At the end of the required incubation period, the cellular culture was observed with inverted microscope to detect any cytopathic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Kärber method. ***Preparation of the inoculum suspension*** Under a laminar flow, nine volumes of the test virus suspension were added to one volume of interfering substance. Just before use, the inoculum suspension was mixed. ***Preparation of the test carrier*** The test surfaces were inoculated, under laminar air flow, with 10 µL of inoculum suspension that was left to dry in a horizontal position until visible dry but no longer than 60 minutes, at room temperature under the laminar air flow cabinet. The inoculum was applied over the carrier paying attention to deliver it in the centre of the carrier avoiding to touch the carrier edges. ***Test*** For each test surface, 2 inoculated carriers were transferred into the device and subjected to the UV-C disinfection cycle with the inoculum placed upwards and 2 inoculated carriers were transferred and treated upside down. At the end of the treatment time, each test surface was transferred in a 6-well plate and the inoculum was recovered with 0.9 ml of ice-cold culture Medium without FBS for each test surface by pipetting up and down all over the surface for 60 seconds to re-suspend the virus. Immediately after elution, eight serial dilutions 1:10 were prepared in ice-cold maintenance Medium and each dilution was placed six-fold, transferring 0.1 ml in 96 wells microplates containing the cellular confluent monolayer (>90%) without any culture Medium. After 1 hour of incubation at the indicated temperature, 0.1 ml of maintenance Medium was added. The outline of the microplate did not receive the viral inoculum but only culture Medium and was used as control of cellular line. At the end of the required incubation period, the cellular culture were observed with inverted microscope to detect any cytopathic effect (CPE) due to the residual virus

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	and the corresponding $TCID_{50}$ was calculated by means of Spearman – Kärber method. The same procedure was performed for two inoculated carriers, but without the UV-C treatment. The viral recovery from these carriers was performed at T0. The reduction of virus titre was calculated from titre differences between treated carriers and not-treated carriers.
EVALUATION OF THE VIRUCIDAL ACTIVITY	For the evaluation of virucidal efficacy the virus titre before and after exposure to the disinfection cycle of the device was determined and the reduction (R) calculated including its 95% confidence interval. The virus titrations were conducted in such a way that the virus titre exhibited a 95% confidence interval of ± 0.5 Log for the Spaerman-Kärber method.
VALIDITY AND EFFICACY CRITERIA	***Assay of viral activity (virus titration)*** The minimum titre of the virus suspensions is at least 10^8 $TCID_{50}$/ml; in any case, it shall be sufficiently high to at least enable a titre reduction of 4 Log to verify the method.
	The test item is considered virucidal on the test surface if it demonstrates a 4 log or more reduction in the titre for *Adenovirus* and *Murine Norovirus* at the specific contact time and at a temperature between 18±1°C and 25±1°C, with the chosen interfering substance.

RESULTS

Log reductions

TEST VIRUS	Stainless steel (upwards)	Stainless steel (upside down)
Adenovirus Type 5 (ATCC VR-5)	≥4.08 ± 0.000	≥4.08 ± 0.000
	Glass (upwards)	**Glass (upside down)**
Adenovirus Type 5 (ATCC VR-5)	≥4.00 ± 0.000	≥4.00 ± 0.000

See Addendum N.1

CONCLUSIONS	On the basis of the results obtained in compliance with the assay validity criteria of EN16777:2018 and Sponsor requirements, the cycle performed by *UV SMART D25* device **RESULTS EFFECTIVE** in reducing the titre of *Adenovirus Type 5* (ATCC VR-5) by at least 4 log, using a 0.03% final concentration of bovine serum albumin solution.
ADDENDA	N. 1: RAW DATA ELABORATION (6 PAGES)

Reviewed and electronically signed for Study Technical Supervisor Approval by
Camilla Carloni, employee
for Eurofins Biolab Srl, on 27-Mar-2020 08:21:32 UTC+01:00

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Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2)

Norma (Standard): EN16777:2018/ UNI EN16777:2019

EDR: 1-P-QM-TEM-9037744

Data inizio *(Started on)*: 17/03/20

Data fine test (*Test finished on*): 23/03/20

Rapporto No *(Report No)*: STULV20AA1358-1

ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Titolazione virus *(Virus Titration)*
Adenovirus Type 5 ATCC VR-5

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus *(Virus dilution)*								K-
			1	2	3	4	5	6	7	8	
Adenovirus Type 5 ATCC VR-5	B	0	4	4	4	4	4	3	2	0	0
	C	0	4	4	4	4	4	3	1	0	0
	D	0	4	4	4	4	4	3	0	0	0
	E	0	4	4	4	4	4	3	2	0	0
	F	0	4	4	4	4	4	3	0	0	0
	G	0	4	4	4	4	4	3	1	0	0
	Endpoint	0.0	100.0	100.0	100.0	100.0	100.0	100.0	66.7	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **7.17** ± **0.400**

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla tecnico (*Technician signature*): _____ Data (*Date*): 26/03/20

Sigla Approver (*Approver signature*): _____ Data (*Date*): 26/03/2020

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Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2)

Norma (Standard): EN16777:2018/ UNI EN16777:2019

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ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Recupero dell'inoculo prima dell'essiccamento

Adenovirus Type 5 ATCC VR-5

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus (Virus dilution)								K-
			3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
Recupero dell'inoculo prima dell'essiccamento Standard Steel BSA 0.03% final concentration 0 min	B	0	4	4	4	3	2	0	0	0	0
	C	0	4	4	4	3	2	0	0	0	0
	D	0	4	4	4	3	0	0	0	0	0
	E	0	4	4	4	3	2	0	0	0	0
	F	0	4	4	4	3	0	0	0	0	0
	G	0	4	4	4	3	0	0	0	0	0
	Endpoint	0.0	100.0	100.0	100.0	100.0	50.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **7.00** ± **0.447**

Carrier 1

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus (Virus dilution)								K-
			3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
Recupero dell'inoculo prima dell'essiccamento Glass BSA 0.03% final concentration 0 min	B	0	4	4	4	3	0	0	0	0	0
	C	0	4	4	4	2	1	0	0	0	0
	D	0	4	4	4	3	2	0	0	0	0
	E	0	4	4	4	3	0	0	0	0	0
	F	0	4	4	4	3	2	0	0	0	0
	G	0	4	4	4	2	0	0	0	0	0

Cell destruction: **VALID**

Log TCID50: **7.00** ± **0.447**

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla tecnico (*Technician signature*): _____ Data (*Date*): 26/03/20

Sigla Approver (*Approver signature*): _____ Data (*Date*): 26/03/2020

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Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2)

Norma (Standard): EN16777:2018/ UNI EN16777:2019

EDR: 1-P-QM-TEM-9037744

Data inizio *(Started on)*: 17/03/20

Data fine test (*Test finished on*): 23/03/20

Rapporto No *(Report No)*: STULV20AA1358-1

ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Virus Control *(Virus control)*

Adenovirus Type 5 ATCC VR-5

Carrier 1

Condizioni testate *(Test condition)*

Inoculum

Standard Steel

BSA 0.03% final concentration

0 min

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	4	4	3	2	0	0	0	0	0
C	0	4	4	3	1	0	0	0	0	0
D	0	4	4	2	2	0	0	0	0	0
E	0	4	4	2	2	0	0	0	0	0
F	0	4	4	3	0	0	0	0	0	0
G	0	4	4	3	2	0	0	0	0	0
Endpoint	0.0	100.0	100.0	100.0	83.3	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **6.33** ± **0.346**

Carrier 2

Condizioni testate *(Test condition)*

Inoculum

Standard Steel

BSA 0.03% final concentration

0 min

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	4	4	3	2	0	0	0	0	0
C	0	4	4	3	1	0	0	0	0	0
D	0	4	4	3	1	0	0	0	0	0
E	0	4	4	3	1	0	0	0	0	0
F	0	4	4	3	2	1	0	0	0	0
G	0	4	4	3	3	1	0	0	0	0
Endpoint	0.0	100.0	100.0	100.0	100.0	33.3	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **6.83** ± **0.400**

Log TCID50 (Average): **6.58** ± **0.265**

Carrier 1

Condizioni testate *(Test condition)*

Inoculum

Glass

BSA 0.03% final concentration

0 min

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	4	4	3	1	0	0	0	0	0
C	0	4	4	3	2	0	0	0	0	0
D	0	4	4	2	2	0	0	0	0	0
E	0	4	4	2	2	0	0	0	0	0
F	0	4	4	3	2	0	0	0	0	0
G	0	4	4	3	2	0	0	0	0	0
Endpoint	0.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **6.50** ± **0.000**

Carrier 2

Condizioni testate *(Test condition)*

Inoculum

Glass

BSA 0.03% final concentration

0 min

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	4	4	3	1	0	0	0	0	0
C	0	4	4	2	1	0	0	0	0	0
D	0	4	4	2	1	0	0	0	0	0
E	0	4	4	3	2	0	0	0	0	0
F	0	4	4	3	1	0	0	0	0	0
G	0	4	4	3	1	0	0	0	0	0
Endpoint	0.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: **6.50** ± **0.000**

Log TCID50 (Average): **6.50** ± **0.000**

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla tecnico (*Technician signature*): _____ Data (*Date*): 26/03/20

Sigla Approver (*Approver signature*): _____ Data (*Date*): 26/03/2020

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Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2)

Norma (Standard): EN16777:2018/ UNI EN16777:2019

EDR: 1-P-QM-TEM-9037744

Data inizio *(Started on)*: 17/03/20

Data fine test (*Test finished on*): 23/03/20

Rapporto No *(Report No)*: STULV20AA1358-1

ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Procedura test *(Test procedure)*

Adenovirus Type 5 ATCC VR-5

Carrier 1

Condizioni testate *(Test condition)*

UV Smart D25

Standard Steel

BSA 0.03% final concentration

25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Carrier 2

Condizioni testate *(Test condition)*

UV Smart D25

Standard Steel

BSA 0.03% final concentration

25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Log TCID50 (Average): ≤ **2.50** ± **0.000**

Reduction (Average): ≥ **4.08** ± **0.000**

Carrier 1

Condizioni testate *(Test condition)*

UV Smart D25

Glass

BSA 0.03% final concentration

25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Carrier 2

Condizioni testate *(Test condition)*

UV Smart D25

Glass

BSA 0.03% final concentration

25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Log TCID50 (Average): ≤ **2.50** ± **0.000**

Reduction (Average): ≥ **4.00** ± **0.000**

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla tecnico (*Technician signature*): _____ Data (*Date*): 26/03/20

Sigla Approver (*Approver signature*): _____ Data (*Date*): 26/03/2020

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Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2)

Norma (Standard): EN16777:2018/ UNI EN16777:2019

EDR: 1-P-QM-TEM-9037744

Data inizio *(Started on)*: 17/03/20

Data fine test (*Test finished on*):

Rapporto No *(Report No)*: STULV20AA1358-1

ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Procedura test *(Test procedure)*

Adenovirus Type 5 ATCC VR-5

Carrier 1
Condizioni testate *(Test condition)*

UV Smart D25
Standard Steel (Upside down)
BSA 0.03% final concentration
25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Carrier 2
Condizioni testate *(Test condition)*

UV Smart D25
Standard Steel (Upside down)
BSA 0.03% final concentration
25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Log TCID50 (Average): ≤ **2.50** ± **0.000**

Reduction (Average): ≥ **4.08** ± **0.000**

Carrier 1
Condizioni testate *(Test condition)*

UV Smart D25
Glass (Upside down)
BSA 0.03% final concentration
25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Carrier 2
Condizioni testate *(Test condition)*

UV Smart D25
Glass (Upside down)
BSA 0.03% final concentration
25 sec

Replica	K-	Diluizione virus (Virus dilution)								K-
		3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	
B	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
Endpoint	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cell destruction: **VALID**

Log TCID50: ≤ **2.50** ± **0.000**

Log TCID50 (Average): ≤ **2.50** ± **0.000**

Reduction (Average): ≥ **4.00** ± **0.000**

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla tecnico (*Technician signature*): _____ Data (*Date*): 26/03/20

Sigla Approver (*Approver signature*): _____ Data (*Date*): 26/03/2020

eurofins	Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area — Test method and requirements (phase2/step2) Norma (Standard): EN16777:2018/ UNI EN16777:2019	EDR: 1-P-QM-TEM-9037744 Pagina *(Page)* 6 / 6

Data inizio *(Started on)*: 17/03/20 Data fine test (*Test finished on*): 23/03/20

Rapporto No *(Report No)*: STULV20AA1358-1 ID Campione *(ID sample)*: LV-MAT-F5PH-20-076-0450:a

Result summary

Attività virucida *(Virucidal activity)*
Adenovirus Type 5 ATCC VR-5

Prodotto *(Product)*	**UV Smart D25**	
Sostanza interferente *(Interfering substance)*	**BSA 0.03% final concentration**	
Tempo di contatto *(Contact time)*	25 sec	
Concentrazione *(Concentration)*	**Riduzione Log *(Log Reduction)***	**Status**
Standard Steel	≥ 4.08 ± 0	PASS
Glass	≥ 4 ± 0	PASS
Standard Steel (Upside down)	≥ 4.08 ± 0	PASS
Glass (Upside down)	≥ 4 ± 0	PASS

Data verifica Approver (*Approver verification date*): 25/03/20

Sigla Approver *(Approver signature)*: _____ Data *(Date)*: 25/03/2020