SURFACE VIRUCIDAL ACTIVITY WITHOUT MECHANICAL ACTION AGAINST Bovine Coronavirus (BCoV) ON UV SMART D25 IN CLEAN CONDITIONS

Contract n: PO9YPH200158-01 / R2BUPH190310-04

Sponsor:
UV SMART B.V.
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THE NETHERLANDS

Study Monitor:
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Test item: UV SMART D25

Study Director: Camilla Carloni ..... Released on: June 10th, 2020
(Camilla Carloni)

Original copy 1 of 1

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COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development , Paris 1998.

- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE and 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).

- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.

- GLP Certification N. 2019/25 released by the Italian Ministry of Health on September 12th, 2019 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affect the quality or integrity of the study.

Camille Carloni

Study Director
(Camilla Carloni)

June 10th, 2020
Date

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved Study Plan and the Standard Operating Procedures of Eurofins Biolab S.r.l.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

QAU INSPECTIONS	
PHASE	**DATE**
Experimentation: -Audit process-based -Audit study-based	January, 30th-February 04th 2019 //
Documentation: - Study Plan - Raw data - Final report	 May 12th, 2020 May 29th, 2020 May 29th,June 03rd,11th, 2020

This report accurately reflects the raw data.

Giovanna Patrizia Bianco
QA GLP
(Giovanna Patrizia Bianco)

June 11th, 2020
Date

SUMMARY

This study was conducted on test item UV SMART D25 in order to determine its surface virucidal effectiveness in medical area against *Bovine Coronavirus* (BCoV) without mechanical action, in clean condition, for the uses for which the product is specifically intended, according to the Sponsor requirements.

For this purpose the following tests were performed:
- virucidal activity in suspension. Filtration method in which a viral suspension of against *Bovine Coronavirus* (BCoV) was inoculated on surface and then treated with the test item in the following test conditions:
- final test concentrations: the standard cycle set on the device has been run according to Instruction for Use provided by the Sponsor
- contact time: the standard contact time was 25 seconds
- temperature test: room temperature (between 18-25°C)
- interfering substance: a bovine serum albumin solution (BSA) with a final concentration of 0.03% (simulating clean conditions).

The selected viral test suspension was exposed to the treatment with the test item with the test conditions above described. After the contact time provided by the standard cycle set on the device, the viral suspension was inoculated in the appropriate cellular monolayer.
After the incubation period, cellular cultures were observed with the inverted microscope for the detection of cytopathic effects (CPE) produced by viral multiplication.
The verification of the methodology has been performed with different tests, in order to verify the compliance to the EN16777 requirements:
1. ASSAY OF VIRAL ACTIVITY (virus titration)
2. CHECK OF VIRUS DRYING ON THE TEST CARRIERS (Dry control, after the treatment time)

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 against *Bovine Coronavirus* (BCoV) by at least 4 log, using a 0.03% final concentration of bovine serum albumin solution, in compliance with EN16777:2018 and Sponsor requirements.

INTRODUCTION

This study was conducted in order to assess the suspension virucidal efficacy of the test item in conformity to EU regulatory requirements and according to the Sponsor requirements.

This study has been carried out at the Test Facility Eurofins Biolab S.r.l. on behalf of the Sponsor on the test item UV SMART D25.

In this report:
- doses are expressed as milliliter (ml) of the test item for 100 milliliter (ml) of water (%)
- the virus titres are expressed as TCID50: 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units.

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Virucidal activity in surface - Filtration method EN16777:2018	12-May-20	10-Jun-20	I. Perna - E. Valtorta

TERMS AND DEFINITIONS

Virucidal: a chemical agent or a formulation that inactivates viruses under certain conditions.

Virucidal activity: the capability of a product to produce a reduction in the number of viruses under certain conditions.

BIBLIOGRAPHY

EN16777:2018 - 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)

FILING

The Study Plan, the Final Report, Amendments (if present) and all raw data are filed in the archives of Eurofins Biolab S.r.l. for ten years after the issuing of the Final Report.
At the end of the study, the device has been returned to the Sponsor.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the GLP Test Facility Eurofins Biolab S.r.l.

TEST ITEM

The test item consists of Biocide and Antimicrobials (Device for surface disinfection based on treatment with UV-C). The device is represented by a white apparatus with a lid under which the area for arranging the instrument to be treated is located.

Name	UV SMART D25
Code	Not provided
Stability	N/A
Composition	N/A
Storage	Room Temperature
Batch	N/A
Manufacturing date	Not provided
Expiry date	N/A
CoA	N/A
Parcel registration number	IP-LV-2020027-ABC
Receiving date	27-Jan-20
Material aliquot number	LV-MAT-IJE2-20-097-0C18:a

The test item and the information concerning the test item were provided by the Sponsor.
All data related to the test item are under the responsibility of the Sponsor and have not been verified by the test facility.

Experimental Report

TEST METHOD

1 ASSAY SYSTEM

Virus
Bovine Coronavirus (BCoV) RVB-0020

The virus was kept at <–196°C in liquid nitrogen; before use it was multiplied in the appropriate cellular line as shown in the table below:

VIRUS STRAINS	HOST CELL LINES	INCUBATION TIME (Days)	INCUBATION TEMPERATURE
Bovine Coronavirus (BCoV)	*PT*	1-4	37±1°C (CO_2 5%)

2. CELLS, MEDIA AND REAGENTS

Cellular culture
PT (calf kidney cells) CCLV-RIE 0011

The cellular line was kept at <–196°C in liquid nitrogen; before viral inoculum, it appeared as confluent monolayer.

The cell debris was removed by centrifugation (400 g_N for 15 minutes), and the supernatant containing the virus was used for the test (test virus suspension).

Culture Medium and reagent

DMEM	Dulbecco's Modified Eagle Medium
EMEM	Eagle's minimal essential medium
FBS	Foetal Bovine Serum
PEN-STREP (1%)	Antibiotics
PBS	Phosphate Buffer Saline
TRYPSINE-EDTA	
TRYPAN BLUE	
BSA	Bovine Serum Albumin

Growth Medium for cell multiplication: DMEM supplemented with 10% Fetal Bovine Serum (FBS).
Maintenance Medium for virus propagation: EMEM supplemented with 2% FBS.

Interfering substance (clean conditions)
Bovine albumin solution (BSA) with a final concentration 10 times higher than the final concentration of 0.03% (simulating clean conditions):

bovine albumin	0.3 g
water for injection q.s. to	100 ml
(sterilized through a 0.45 μm. filter)	

3 EQUIPMENT

Ordinary microbiology laboratory equipment and in particular:

Incubator (37±1°C+5% CO_2)	THERMO SCIENTIFIC
Aspiration station	GILSON
Vortex stirrer	VELP

Chronometer	OREGON SCIENTIFIC
Micropipettes	GILSON - EPPENDORF
Water bath	JULABO
Centrifuge	NEYA - EPPENDORF
Inverted microscope	OPTIKA - NIKON
Ice Maker	EURFRIGOR
Microplates 96 wells	CORNING

4 EXPERIMENTAL DESIGN

Test temperature
The test was performed at room temperature (between 18-25°C).

Experimental conditions
The test item has been used with the following conditions:
- The standard cycle set on the device has been run according to Instruction for Use provided by the Sponsor* (see below)
- contact time: the standard contact time was 25 seconds
- viral inoculum: a volume of 10 µl has been placed on the test carriers and spread

*The device provided to Eurofins for evaluation of virucidal activity consisted in a prototype and the following instruction have to be followed before test execution for warming it up:

1. Put Euro plug in to the back of the device
2. Switch the device on in the back next to the plug
3. Read instruction on screen:
    - Close lid for system check
    - When lid is closed push the "Stop button"
    - System checks itself
    - Run the system with at least 2x disinfection runs to check lamp sets
4. Before testing, run at least 2x disinfection run to check lamp sets

Interfering substance
A 0.03% of final concentration of a bovine serum albumin solution was used as interfering substance (simulating clean conditions).

Carriers
Stainless steel carriers with 2 cm diameter were used as test surfaces.

5 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 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 surface was 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

2 carriers were inoculated with 10 µl of the inoculum suspension previously prepared. Immediately after drying, the inoculated carriers were exposed to the UV cycle set on the device at the test temperature with the inoculum placed upwards.

The result was the mean value obtained by two carriers.

At the end of the treatment cycle, each test surface was transferred in a 6-well plate and the inoculum was recovered with 1 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 and the corresponding $TCID_{50}$ was calculated by means of Spearman-Kärber method.

Dry control

The same procedure above described for the test has been performed for the dry control.

Two carriers have been inoculated and dried, but they were not subjected to the UV-C treatment. The viral recovery from these carriers was performed after the treatment time provided by the standard cycle on the device.

At the end of this time, each test surface was transferred in a 6-well plate and the inoculum was recovered with 1 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.

This control was performed on two carriers and the result was the mean value.

At the end of the required incubation period, the cellular culture were 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.

The reduction of virus titre was calculated from titre differences between treated carriers and not-treated carriers.

6 CALCULATION AND EXPRESSION OF RESULTS

The infecting activity was determined by means of Spearman-Kärber method that uses the following formula to calculate the value of $TCID_{50}$:

$$-\text{Log } TCID_{50} = -(-x_0) - \{[R/100] - 0.5\} \times \text{Log dilution factor}$$

where:

$TCID_{50}$ = 50% end point

x_0 = Log of the lowest dilution with 100% of positive reaction (CPE formation)

R = sum (%) of positive cultures

The standard error (S) was calculated as follow:

$$S = \sqrt{d^2 \sum\{[pi(1 - pi)]/(n - 1)\}}$$

Where:
S = standard error of Log titre
d = Log of dilution factor
pi = observed reaction rate
n = number of wells

95% confidence interval of the titre is approximately 2 S. When calculating the titre, the pre-dilution of the sample was taken into account and the titre was calculated to the same volume.

Evaluation of the virucidal activity
For the evaluation of virucidal efficacy the virus titre before and after exposure to the product 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 Spearman-Kärber method.

Calculating the reduction and its 95% confidence interval
The reduction (*R*) was calculated as the difference between the Log titre of drying control (a) and the Log titre of residual virus (rest virus) after exposure to the UV-C cycle of the device (b).
The reduction (*R*) was so calculated as follows:

$$R_T = a - b$$

Where:
R_T = reduction from the test run
a = Log $TCID_{50}$/ml of control titration of the test run (drying control)
b = Log $TCID_{50}$/ml of "rest virus" titration of the test run

(In case no virus multiplication was observed in the highest concentration, this value was preceded by the sign ≤; in case virus multiplication can be observed in all dilutions, this value was preceded by the sign ≥).
The 95% confidence interval of the R was calculated as follows:

$$K_{R(T)} = 2 \times \sqrt{Sa^2 + Sb^2}$$

Where:
$K_{R(T)}$ = 95% confidence interval of the R of the test run
S_a = standard error of control titration of the test run (drying control)
$2S_a$ = 95% confidence interval of control titration of the test run (drying control)
S_b = standard error of "rest virus" titration of the test run
$2S_b$ = 95% confidence interval of "rest virus" titration of the test run

If no virus can be detected any more in the test run with disinfectant ("rest virus"), the 95% confidence interval was calculated according to:

$$K_{R(Tkv)} = 2 \times \sqrt{Sa^2}$$

where:
$K_{R(Tkv^*)}$ = 95% confidence interval of the R in the case no virus can be detected in the test run with disinfectant ("rest virus")

S_a = standard error of control titration of the test run (drying control)

The reduction and the 95% confidence interval for each test run was calculated.

ASSAY VALIDITY CRITERIA

The test of virucidal activity is valid if the following criteria are fulfilled:

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.

INTERPRETATION OF RESULTS

The test item is considered virucidal if it demonstrates in a valid test a reduction in titre of at least 4 Log, with the chosen interfering substance, temperature condition and contact time, defined by the European Standard and according to the use condition of the product, when the test organism are *Adenovirus Type 5* and *Murine norovirus (MNV, strain S99)*.
In case of specific use conditions for which other contact times, temperatures, test organisms and interfering substances are applied instead of or in addition to the standard ones, the test item shall demonstrate at least 4 Log reduction under the chosen test conditions.

RESULTS

Validation of virucidal test
The validation tests comply with the validity criteria. The specific values are shown in Addendum N.1.

Virucidal activity
The vitality reduction expressed as logarithm values for each tested conditions are shown below and in the Addendum N.1:

TEST VIRUS	**Log Reductions after the standard cycle of the device UV SMART D25**
***Bovine Coronavirus* (BCoV) RVB-0020**	≥4.00 ± 0.000

DEVIATIONS

No deviation has been recorded from Study Plan.

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 against *Bovine Coronavirus* (BCoV) by at least 4 log, using a 0.03% final concentration of bovine serum albumin solution, in compliance with EN16777:2018 and Sponsor requirements.

ADDENDA

ADDENDUM	TITLE	NUMBER OF PAGES
N.1	RAW DATA ELABORATION	4

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Addendum N_1 CE 10/06/2020

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)* 1 / 4

Data inizio *(Started on)*: 13/05/20 Data fine test (*Test finished on*): 15/05/20

Rapporto No *(Report No)*: STULV20AA1779-1 GLP ID Campione *(ID sample)*: LV-MAT-IJE2-20-097-0C18:a

Titolazione virus *(Virus Titration)*
Betacoronavirus 1 (Bovine Corona Virus) strain S379 Riems RVB-0020

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus *(Virus dilution)*								K-
			1	2	3	4	5	6	7	8	
Betacoronavirus 1 (Bovine Corona Virus) strain S379 Riems RVB-0020	B	0	4	4	4	4	4	2	0	0	0
	C	0	4	4	4	4	2	1	0	0	0
	D	0	4	4	4	4	4	2	0	0	0
	E	0	4	4	4	4	2	2	1	0	0
	F	0	4	4	4	4	4	2	0	0	0
	G	0	4	4	4	4	4	1	0	0	0
	Endpoint	0.0	100.0	100.0	100.0	100.0	100.0	100.0	16.7	0.0	0.0

Cell destruction: **VALID**
Log TCID50: **6.67** ± **0.346**

Data verifica Approver (*Approver verification date*): 25/05/20

Sigla tecnico *(Technician signature)*: _____ Data *(Date)*: 10/06/20

Sigla Approver *(Approver signature)*: _____ Data *(Date)*: 10/06/2020

Data inizio *(Started on)*: 13/05/20 Data fine test (*Test finished on*): 15/05/20

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Controllo Essiccamento (Drying Control)

Betacoronavirus 1 (Bovine Corona Virus) strain S379 Riems RVB-0020

Carrier 1

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus (Virus dilution) 3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	K-
Inoculum	B	0	4	4	1	1	0	0	0	0	0
	C	0	4	4	3	2	0	0	0	0	0
	D	0	4	4	3	1	0	0	0	0	0
BSA 0.03% final concentration	E	0	4	4	1	1	0	0	0	0	0
	F	0	4	4	1	1	0	0	0	0	0
25 sec	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**

Carrier 2

Condizioni testate *(Test condition)*	Replica	K-	Diluizione virus (Virus dilution) 3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	K-
Inoculum	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
BSA 0.03% final concentration	E	0	4	4	3	2	0	0	0	0	0
	F	0	4	4	3	1	0	0	0	0	0
25 sec	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**

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

Data verifica Approver (*Approver verification date*): 25/05/20

Sigla tecnico *(Technician signature)*: _____ Data *(Date)*: 10/05/20

Sigla Approver *(Approver signature)*: _____ Data *(Date)*: 10/05/2020

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Procedura test *(Test procedure)*

Betacoronavirus 1 (Bovine Corona Virus) strain S379 Riems RVB-0020

Carrier 1
Condizioni testate *(Test condition)*

UV Smart D25
N.A.
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
N.A.
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*): 25/05/20

Sigla tecnico *(Technician signature)*: _____ Data *(Date)*: 200620

Sigla Approver *(Approver signature)*: _____ Data *(Date)* : 10/06/2020

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Result summary

Attività virucida *(Virucidal activity)*
Betacoronavirus 1 (Bovine Corona Virus) strain S379 Riems RVB-0020

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**
N.A.	≥ 4 ± 0	PASS

Data verifica Approver (*Approver verification date*): 25/05/20

Sigla Approver *(Approver signature)*: Data *(Date)* : 10/06/2020

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