


R2004FSAVA002

Antiviral activity of SUPERSUM 3C surface against human coronavirus HCoV-229E for a contact time of 1 and 24 hours.  
Adapted protocol from ISO 21702 (2019) standard

CLIENT	AVANZARE Monsieur Javier Pérez Avenida Lentiscales 4-6 26370 Navarrete SPAIN
TEST LABORATORY	SAS VirHealth Centre d’Innovation, Bâtiment Domilyon 321 avenue Jean Jaurès 69007 Lyon France
TECHNICAL CONTRIBUTION	Léa Szpiro, technical manager Loranne Durimel, laboratory technician

Quality validation
Name : Dr Vincent MOULES, CEO
Date : Lyon, 15/04/2021
Signature :


Report includes 10 pages



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## I. CONCLUSION

Antiviral activities of the SUPERSUM 3C surface and non-active surface have been tested under conditions defined by the ISO 21702 (2019) adapted protocol for a contact time of 1 and 24 hours against Human coronavirus HCOV-229E

The raw PE FILM surface is the control for this test.

- Human coronavirus HCOV-229E

Under experimental conditions (25°C, 1 hour), the SUPERSUM 3C surface shows an antiviral activity per cm<sup>2</sup> associated with a logarithmic reduction of 0.2log<sub>10</sub> (36.90%) efficiency under the ISO 21702 adapted protocol.

PRODUCT	Contact time	Antiviral activity R (log <sub>10</sub> cm <sup>2</sup> )	Antiviral activity (%)
SUPERSUM 3C	1 hours	<b>R= 0.2</b>	36.90

- Human coronavirus HCOV-229E

Under experimental conditions (25°C, 24 hours), the SUPERSUM 3C surface shows an antiviral activity per cm<sup>2</sup> associated with a logarithmic reduction of 0.8log<sub>10</sub> (84.15%) efficiency under the ISO 21702 adapted protocol.

PRODUCT	Contact time	Antiviral activity R (log <sub>10</sub> cm <sup>2</sup> )	Antiviral activity (%)
SUPERSUM 3C	24 hours	<b>R = 0.8</b>	84.15

## II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

. Quotation	DEV0425
. Order	Good for agreement date: 11/03/2021

## III. TEST CONDITIONS AND SAMPLES DATA

### III.1 Samples identification

Surface	SUPERSUM 3C	raw PE FILM
Appearance	Transparent	grey
Size (cm)	5 x 5	5 x 5
Thickness (mm)	50µm	50µm
Porous / non porous	Non porous	Non porous

Manufacturer: AVANZARE

Supplier: AVANZARE

Storage conditions: room temperature

Evaluation period: 04/2020

### III.2 Experimental conditions

Experimental Conditions	
Date	- 01/04/2020
Viral strain	Human coronavirus HCOV-229E
Inoculum volume	400 µL
Cover film *	4 cm x 4 cm = 16 cm <sup>2</sup>
Temperature	25°C ± 0.1
Humidity HR (%)	50% ± 5
Contact time	1 and 24 hours
Interfering substance	n.a.
Neutralisation	submerging in 10mL of SCDLP medium
Quantification	endpoint titration on permissive cells
Number of wells per dilution	8
Incubation temperature	34 ± 1 °C

\*: Test surfaces are very hydrophobic. A cover film was used in order to ensure the distribution of the inoculum over a total area of 16 cm<sup>2</sup>.

## IV. RESULTS

### IV.1 Antiviral activity of the SUPERSUM 3C surface against Human coronavirus HCOV-229E for a contact time of 1 and 24 hours

#### a. Cell susceptibility

Surface	Log <sub>10</sub> TCID <sub>50</sub> /mL
SCDLP medium	6.1
SUPERSUM 3C	6.3

Raw PE FILM	6.0
Active Surface: Difference < 0.5 log <sub>10</sub> <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	
Inactive Surface: Difference < 0.5 log <sub>10</sub> <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	

b. Cytotoxicity

The test surface cytotoxicity is determined by reading of cytopathic effect (CPE) on MRC5 permissive cells and quantified by TCID<sub>50</sub> technique.

For viral recuperation on surface, the surfaces are submerging in 10mL of SCDLP medium (recuperation buffer). The recuperation buffer cytotoxicity is determined by reading of cytopathic effect (CPE).

Under test conditions, the recuperations buffers from SUPERSUM 3C and reference surfaces did not show cytopathic effects on MRC5 cells for a contact time of 1 and 24 hours.

The test results are dependent on and take into account the cytotoxicity results.

c. Inactivation of antiviral activity

Product	Log <sub>10</sub> TCID <sub>50</sub> /mL
$S_n$ = SCDLP medium	5.1
$S_t$ = SUPERSUM 3C	5.0
$S_u$ = raw PE FILM (reference)	5.2
$S_n - S_u \leq 0.5$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	



$S_n - S_t \leq 0.5$   yes  no

*Explanations:*

$S_n$ : the average of the common logarithm of the infectivity titer of virus from three specimens of the SCDLP broth for negative control.

$S_u$ : the average of the common logarithm of the infectivity titer of virus recovered from three untreated test specimens;

$S_t$ : the average of the common logarithm of the infectivity titer of virus recovered from three test specimens.

d. Test

Raw data for antiviral activity of SUPERSUM 3C and reference surfaces against Human coronavirus HCOV-229E under test conditions (25°C, 1 and 24 hours) are presented in appendices.

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID<sub>50</sub> technique on MRC5 cells.

Surface	Cytotoxicity (log <sub>10</sub> TCID <sub>50</sub> )	Specimen	U <sub>0</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )	U <sub>t1h</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )	U <sub>t24h</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )
raw PE FILM	0.5	L1	5.1	4.2	3.8
		L2	5.2	4.3	4.1
		L3	5.2	4.2	4.1
		<i>Average</i>	5.2	4.2	4.0

Surface	Cytotoxicity (log <sub>10</sub> TCID <sub>50</sub> )	Specimen	A <sub>0</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )	A <sub>t1h</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )	A <sub>t24h</sub> (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )
SUPERSUM 3C	0.5	L1	4.8	4.3	3.2
		L2	4.9	3.9	3.3
		L3	5.0	3.9	3.2
		<i>Average</i>	4.9	4.0	3.2
		R (log <sub>10</sub> TCID <sub>50</sub> /cm <sup>2</sup> )	/	<b>0.2</b>	<b>0.8</b>

*R is the antiviral activity*

*U<sub>0</sub> is the average of the common logarithm of the number of TCID<sub>50</sub> recovered from three untreated test specimens immediately after inoculation*

*U<sub>t</sub> is the average of the common logarithm of the number of TCID<sub>50</sub> recovered from three untreated test specimens*

*A<sub>0</sub> is the average of the common logarithm of the number of TCID<sub>50</sub> recovered from three treated test specimens immediately after inoculation*

*A<sub>t</sub> is the average of the common logarithm of the number of TCID<sub>50</sub> recovered from three treated test specimens.*

The logarithmic value of the number of TCID<sub>50</sub> recovered immediately after inoculation from untreated test specimen (U<sub>0</sub>) satisfies the requirement below:  $(L_{max} - L_{min}) / (L_{mean}) \leq 0.2$ .

The number of virus recovered from each untreated test specimen after contact time of 24 hours should not be less than  $6.2 \times 10^2$  TCID<sub>50</sub>/cm<sup>2</sup>.



## VI. ANNEXES

### VI.1 Materials and reagents

- Cell line and viral strain

	Name	Number of passages	Batch number	Quantification
Cell line	MRC5 (ATCC CCL-171)	18	ATCC CCL-171-2	n.a.
Viral strain	HCoV-229E (ATCC VR-740)	n.a.	032021HCoV-3	1.00.10 <sup>8</sup> TCID <sub>50</sub> /mL

- Reagents

	Name	Batch number	Expiration Date	Preparation
Medium	EMEM	0000939603	14/10/2022	n.a.
Antibiotics	Penicillin and streptomycin	2240838	30/07/2021	1%
L-Glutamine	L-GLU	2248755	03/2022	1%
SVF	SVF	S73136	04/09/2024	10 % (culture) 2% (infection)

VI.2 RAW DATA: human coronavirus HCoV-229E

Product	Contact time (h)	Dilutions (-log)							
		P	1	2	3	4	5	6	7
Cytotoxicity									
raw PE FILM	1	0	0	0	0	0	0	0	0
SUPERSUM 3C	1	0	0	0	0	0	0	0	0
raw PE FILM	24	0	0	0	0	0	0	0	0
SUPERSUM 3C	24	0	0	0	0	0	0	0	0
Cell susceptibility									
SCDLP	/	44444444	44444444	44444444	44444444	44444444	10002324	0	0
raw PE FILM	/	44444444	44444444	44444444	44444444	44444444	10102200	0	0
SUPERSUM	/	44444444	44444444	44444444	44444444	44444444	02302222	0	0
A0/U0									
raw PE FILM	0	44444444	44444444	44444444	44444444	10102324	0	0	0
	0	44444444	44444444	44444444	44444444	11022222	0	0	0
	0	44444444	44444444	44444444	44444444	1402324	0	0	0
SUPERSUM 3C	0	44444444	44444444	44444444	44444444	01002340	0	0	0
	0	44444444	44444444	44444444	44444444	10232002	0	0	0
	0	44444444	44444444	44444444	44444444	10023241	0	0	0
Suppression of product's activity									
SCDLP	/	44444444	44444444	44444444	44444444	10023022	0	0	0
	/	44444444	44444444	44444444	44444444	14102222	0	0	0
	/	44444444	44444444	44444444	44444444	11111111	0	0	0
raw PE FILM	/	44444444	44444444	44444444	44444444	10232402	0	0	0
	/	44444444	44444444	44444444	44444444	22022222	0	0	0
	/	44444444	44444444	44444444	44444444	14123214	0	0	0
SUPERSUM 3C	/	44444444	44444444	44444444	44444444	10014200	0	0	0
	/	44444444	44444444	44444444	44444444	20320222	0	0	0
	/	44444444	44444444	44444444	44444444	11202232	0	0	0
TEST									
raw PE FILM	1	44444444	44444444	44444444	11102324	0	0	0	0
	1	44444444	44444444	44444444	11111111	0	0	0	0
	1	44444444	44444444	44444444	12120222	0	0	0	0
SUPERSUM 3C	1	44444444	44444444	44444444	11111111	0	0	0	0
	1	44444444	44444444	44444444	11001201	0	0	0	0
raw PE FILM	24	44444444	44444444	44444444	01102030	0	0	0	0
	24	44444444	44444444	44444444	10223240	0	0	0	0
	24	44444444	44444444	44444444	00121111	0	0	0	0
SUPERSUM 3C	24	44444444	44444444	10112032	00010000	0	0	0	0
	24	44444444	44444444	11111111	0	0	0	0	0
	24	44444444	44444444	11110232	0	0	0	0	0

Explanations:

- 1-4: degrees of CPE in 8 cell culture unit (microtiter plate)
- 0: no virus present
- n.a: not applicable
- n.d: not done