

R2012FSFOR001

Antiviral activity of MARMOLEUM TOPSHIELD 2 surface against human coronavirus HCoV-229E for contact times of 2 and 5 hours according to ISO 21702 (2019) standard

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I. CONCLUSION

Antiviral activities of the MARMOLEUM TOPSHIELD 2 surface and non-active surface have been tested under conditions defined by the ISO 21702 (2019) protocol for contact times of 2 and 5 hours against human coronavirus HCoV-229E.

The stainless-steel surface is the control for this test.

- MARMOLEUM TOPSHIELD 2 surface, 2 hours

Under experimental conditions (25°C, 2 hours, 90% RH), the MARMOLEUM TOPSHIELD 2 surface shows an antiviral activity per cm² associated with a logarithmic reduction of 0.8log₁₀ (84.15%) efficiency under the ISO 21702 protocol.

PRODUCT	Contact time	Antiviral activity R (log ₁₀ cm ²)	Antiviral activity (%)
MARMOLEUM TOPSHIELD 2	2 hours	R=0.8	84.15

- MARMOLEUM TOPSHIELD 2 surface, 5 hours

Under experimental conditions (25°C, 5 hours, 90% RH), the MARMOLEUM TOPSHIELD 2 surface shows an antiviral activity per cm² associated with a logarithmic reduction of 2.0 log₁₀ (99.00%) efficiency under the ISO 21702 protocol.

PRODUCT	Contact time	Antiviral activity R (log ₁₀ cm ²)	Antiviral activity (%)
MARMOLEUM TOPSHIELD 2	5 hours	R = 1.9	98.74

III.2 Experimental conditions

Test surface: MARMOLEUM TOPSHIELD 2

Experimental Conditions	
Date	- 25/11/2020
Viral strain	Human coronavirus HCoV-229E
Inoculum volume	400 µL
Cover film	4 cm x 4 cm = 16 cm ²
Temperature	25°C ± 0.1
Humidity HR (%)	90% ± 5
Contact time	2 hours 5 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

IV. RESULTS

IV.1 Antiviral activity of the MARMOLEUM TOPSHIELD 2 surface against human coronavirus HCoV-229E for a contact time of 2 and 5 hours

a. Cell susceptibility

Surface	Log ₁₀ TCID ₅₀ /mL
SCDLP medium	6.1
MARMOLEUM TOPSHIELD 2	6.1
Non active surface	6.3
Active Surface: Difference < 0.5 log ₁₀ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	
Inactive Surface: Difference < 0.5 log ₁₀ <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 TCID₅₀ technique.

For viral recuperation on surface, the surfaces are submerging in 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 MARMOLEUM TOPSHIELD 2 and reference surfaces did not show cytopathic effects on MRC5 cells for a contact time of 2 and 5 hours, 90% RH.

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

c. Inactivation of antiviral activity

Product	Log ₁₀ TCID ₅₀ /mL
S_n = SCDLP medium	5.1
S_t = active surface	4.9
S_u = non Active surface (reference)	5.0
$S_n - S_u \leq 0,5$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no $S_n - S_t \leq 0,5$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> 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 MARMOLEUM TOPSHIELD 2 and reference surfaces against human coronavirus HCoV-229E under test conditions (25°C, 2 hours, 90% RH) are presented in appendices.

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID₅₀ technique on MRC5 cells.

Surface	Cytotoxicity (log ₁₀ TCID ₅₀)	Specimen	U ₀ (log ₁₀ TCID ₅₀ /cm ²)	U _{12h} (log ₁₀ TCID ₅₀ /cm ²)	U _{15h} (log ₁₀ TCID ₅₀ /cm ²)
Stainless steel	0.5	L1	4,9	4,6	4,7
		L2	4,7	4,3	4,6
		L3	5,1	4,7	4,3
		<i>Average</i>	4,9	4,5	4,5

Surface	Cytotoxicity (log ₁₀ TCID ₅₀)	Specimen	A ₀ (log ₁₀ TCID ₅₀ /cm ²)	A _{12h} (log ₁₀ TCID ₅₀ /cm ²)	A _{15h} (log ₁₀ TCID ₅₀ /cm ²)
MARMOLEUM TOPSHIELD 2	0.5	L1	5,3	3,6	2,6
		L2	4,8	3,8	2,7
		L3	4,8	3,7	2,4
		<i>Average</i>	5,0	3,7	2,6
		R (log ₁₀ TCID ₅₀ /cm ²)	/	0.8	1.9

R is the antiviral activity

U₀ is the average of the common logarithm of the number of TCID₅₀ recovered from three untreated test specimens immediately after inoculation

U_t is the average of the common logarithm of the number of TCID₅₀ recovered from three untreated test specimens

A₀ is the average of the common logarithm of the number of TCID₅₀ recovered from three treated test specimens immediately after inoculation

A_t is the average of the common logarithm of the number of TCID₅₀ recovered from three treated test specimens.

The logarithmic value of the number of TCID₅₀ recovered immediately after inoculation from untreated test specimen (U₀) satisfies the requirement below: $(L_{\max} - L_{\min}) / (L_{\text{mean}}) \leq 0.2$.



V. CONCLUSION

MARMOLEUM TOPSHIELD 2 surface shows antiviral activity of $0.8\log_{10}$ TCID₅₀/cm² (84.15%) against human coronavirus HCoV-229E after a contact time of 2 hours at 25°C, 90% RH.

MARMOLEUM TOPSHIELD 2 surface shows antiviral activity of $1.9\log_{10}$ TCID₅₀/cm² (98.74%) against human coronavirus HCoV-229E after a contact time of 5 hours at 25°C, 90% RH.

VI. ANNEXES

VI.1 Materials and reagents

Cell line

Name : MRC5 ATCC® CCL-171™

Number of passages: 22

Culture medium: EMEM (Lonza) with 10% of FCS (Dutscher), 1% of antibiotics (Gibco) et 1% of L-glutamine (Gibco)

Viral strain

Name: human coronavirus 229E ATCC® VR-740™

Viral test suspension: 4.22×10^7 (batch number: 102020229-9)

Quantification technique :

- Successive tenfold dilution in infection medium: EMEM (Lonza) with 2% of FCS (Dutscher), 1% of antibiotics (Gibco) et 1% of L-glutamine (Gibco)

- Add 100µL of every dilution on 8 wells on a 96 plate.

- Incubate 7 days at 34°C, 5% CO₂

VI.2 RAW DATA: TCID₅₀ quantification against human coronavirus HCoV-229E after 2 and 5 hours, visual reading of cytopathic effects (8 wells per dilutions)

• Table 1: Inactivation of antiviral activity

	Product	contact time (h)	dilutions (-log)							
			p	1	2	3	4	5	6	7
suppression of product's activity	SCDLP	/	44444444	44444444	44444444	44444444	11020333	0	0	0
		/	44444444	44444444	44444444	44444444	11424444	0	0	0
		/	44444444	44444444	44444444	44444444	11202222	0	0	0
	Stainless steel	/	44444444	44444444	44444444	44444444	00020222	0	0	0
		/	44444444	44444444	44444444	44444444	11423220	0	0	0
		/	44444444	44444444	44444444	44444444	00212111	0	0	0
	MARMOLEUM TOPSHIELD 2	/	44444444	44444444	44444444	44444444	10102200	0	0	0
		/	44444444	44444444	44444444	44444444	21212000	0	0	0
		/	44444444	44444444	44444444	44444444	11423200	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

• Table 2 : A0/U0

	Product	contact time (h)	dilutions (-log)							
			p	1	2	3	4	5	6	7
A0/U0	Stainless steel	0	44444444	44444444	44444444	44444444	11002301	0	0	0
		0	44444444	44444444	44444444	44444444	12100000	0	0	0
		0	44444444	44444444	44444444	44444444	14230021	0	0	0
	MARMOLEUM TOPSHIELD 2	0	44444444	44444444	44444444	44444444	0	0	0	0
		0	44444444	44444444	44444444	44444444	11203000	0	0	0
		0	44444444	44444444	44444444	44444444	11020300	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

• Table 3 : cytotoxicity

	Product	contact time (h)	dilutions (-log)							
			p	1	2	3	4	5	6	7
Cytotoxicity	Stainless steel	2	0	0	0	0	0	0	0	0
	MARMOLEUM TOPSHIELD 2	2	0	0	0	0	0	0	0	0
Cytotoxicity	Stainless steel	5	0	0	0	0	0	0	0	0
	MARMOLEUM TOPSHIELD 2	5	0	0	0	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

• Table 4 : cell susceptibility

	Product	contact time (h)	dilutions (-log)							
			p	1	2	3	4	5	6	7
cell susceptibility	SCDLP	/	44444444	44444444	44444444	44444444	00111102	0	0	0
	Stainless steel	/	44444444	44444444	44444444	44444444	00333212	0	0	0
	MARMOLEUM TOPSHIELD 2	/	44444444	44444444	44444444	44444444	01002222	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

• Table 5 : test

	Product	contact time (h)	dilutions (-log)							
			p	1	2	3	4	5	6	7
test	Stainless steel	2	44444444	44444444	44444444	44444444	10000020	0	0	0
		2	44444444	44444444	44444444	44444444	0	0	0	0
		2	44444444	44444444	44444444	44444444	03302000	0	0	0
	MARMOLEUM TOPSHIELD 2	2	44444444	44444444	44444444	100000020	0	0	0	0
		2	44444444	44444444	44444444	11020000	02000000	0	0	0
		2	44444444	44444444	44444444	20300001	0	0	0	0
test	Stainless steel	5	44444444	44444444	44444444	21111111	02011000	0	0	0
		5	44444444	44444444	44444444	44444444	03300000	0	0	0
		5	44444444	44444444	44444444	44444444	0	0	0	0
	MARMOLEUM TOPSHIELD 2	5	44444444	44444444	10010000	0	0	0	0	
		5	44444444	44444444	11030000	0	0	0	0	
		5	44444444	44444444	02000000	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