

R2012FSOGP001

Antiviral activity of POSTsilver/React Sheet surface against human coronavirus
HCoV-229E for contact times of 30 and 60 minutes
Protocol from ISO 21702 (2019) standard

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Report includes 12 pages



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I. CONCLUSION

Antiviral activities of the **POSTsilver/React Sheet surface** and non-active surface have been tested under conditions defined by the ISO 21702 (2019) protocol with contact times of 30 and 60 minutes against human coronavirus HCoV-229E.

The General PVC Sheet surface is the control for this test.

- **POSTsilver/React Sheet surface, 30 minutes**

Under experimental conditions (25°C, 30 minutes, 90% RH), the **POSTsilver/React Sheet surface** shows an antiviral activity per cm² associated with a logarithmic reduction of 0.2 log₁₀ (39.90%) efficiency under the ISO 21702 protocol.

PRODUCT	Contact time	Antiviral activity R (log ₁₀ /cm ²)	Antiviral activity (%)
POSTsilver/React Sheet surface	30 minutes	R = 0.2	36.90

- **POSTsilver/React Sheet surface, 60 minutes**

Under experimental conditions (25°C, 60 minutes, 90% RH), the **POSTsilver/React Sheet surface** shows an antiviral activity per cm² associated with a logarithmic reduction of 0.4 log₁₀ (60.19%) efficiency under the ISO 21702 protocol.

PRODUCT	Contact time	Antiviral activity R (log ₁₀ /cm ²)	Antiviral activity (%)
POSTsilver/React Sheet surface	60 minutes	R = 0.4	60.19

II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

. Quotation	DEV0248
. Order	12/11/2020

III. TEST CONDITIONS AND SAMPLES DATA

III.1 Samples identification

Surface	POSTsilver/React Sheet surface	General PVC Sheet
Appearance	Transparent	Transparent
Size (cm)	5 cm x 5 cm	5 cm x 5 cm
Thickness (mm)	1	1
Porous / non porous	Non porous	Non porous

Storage conditions: room temperature

Evaluation period: 12/2020

III.2 Experimental conditions

Test surface: POSTsilver/React Sheet surface

Experimental Conditions	
Date	- 04/12/2020
Viral strain	Human coronavirus HCoV-229E
Inoculum volume	400 µL
Cover film	4 cm x 4 cm = 16 cm ²
Temperature	25.3°C ± 0.1
Humidity HR (%)	90% ± 5
Contact time	30 minutes 60 minutes
Interfering substance	n.a.
Neutralisation	Recuperation 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 POSTsilver/React Sheet surface surface against human coronavirus HCoV-229E for contact times of 30 and 60 minutes

a. Cell susceptibility

Surface	Log ₁₀ TCID ₅₀ /mL
SCDLP medium	7.5
POSTsilver/React Sheet surface	7.1
Non active surface	7.4
Active Surface: Difference $\leq 0.5 \log_{10}$ <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	
Inactive Surface: Difference $\leq 0.5 \log_{10}$ <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 immersed in 10mL of SCDLP medium (recuperation buffer). The recuperation buffer cytotoxicity is determined by reading of cytopathic effect (CPE).

Under test conditions, the recuperation buffer from POSTsilver/React Sheet surface and the General PVC Sheet surface did not show cytopathic effects on MRC5 cells for a contact time of 30 and 60 minutes

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	4.8
S_t = active surface	4.8
S_u = non Active surface (reference)	4.9
$S_n - S_u \leq 0,5$ <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 POSTsilver/React Sheet surface and reference surfaces against human coronavirus HCoV-229E under test conditions (25°C, 30 and 60 minutes, 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 ₃₀ (log ₁₀ TCID ₅₀ /cm ²)	U ₁₆₀ (log ₁₀ TCID ₅₀ /cm ²)
General PVC Sheet surface	0.5	L1	4,9	4,3	4,1
		L2	5,1	4,3	4,3
		L3	5,1	3,9	4,1
		<i>Average</i>	5,0	4,2	4,2

Surface	Cytotoxicity (log ₁₀ TCID ₅₀)	Specimen	A ₀ (log ₁₀ TCID ₅₀ /cm ²)	A ₃₀ (log ₁₀ TCID ₅₀ /cm ²)	U ₁₆₀ (log ₁₀ TCID ₅₀ /cm ²)
POSTsilver/React Sheet surface	0.5	L1	4,8	3,8	3,7
		L2	4,9	4,1	3,8
		L3	4,9	4,1	3,9
		<i>Average</i>	4,9	4,0	3,8
		R (log ₁₀ TCID ₅₀ /cm ²)	/	0,2	0,4

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: $(L_{max} - L_{min}) / (L_{mean}) \leq 0.2$.



V. CONCLUSION

POSTsilver/React Sheet surface shows antiviral activity of 0.2 log₁₀ TCID₅₀/cm² (36.90%) and 0.4 log₁₀ TCID₅₀/cm² (60.19%) against human coronavirus HCo-229E after contact times of 30 and 60 minutes respectively at 25°C, 90% RH.



VI. ANNEXES

VI.1 Materials and reagents

- Cell line

Name : MRC5 ATCC® CCL-171™

Number of passages: 15

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 30 and 60 minutes, visual reading of cytopathic effects (8 wells per dilutions)

• Table 1: Inactivation of antiviral activity

	Product	contact time (min)	dilutions (-log)							
			p	1	2	3	4	5	6	7
supression of product's activity	SCDLP	/	44444444	44444444	44444444	44444444	10000020	0	0	0
		/	44444444	44444444	44444444	44444444	22142000	0	0	0
		/	44444444	44444444	44444444	44444444	22330000	0	0	0
	General PVC Sheet	/	44444444	44444444	44444444	44444444	24002142	0	0	0
		/	44444444	44444444	44444444	44444444	00124222	0	0	0
		/	44444444	44444444	44444444	44444444	00241000	0	0	0
	POSTsilver/React Sheet	/	44444444	44444444	44444444	44444444	22320000	0	0	0
		/	44444444	44444444	44444444	44444444	11420000	0	0	0
		/	44444444	44444444	44444444	44444444	00223000	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 (min)	dilutions (-log)							
			p	1	2	3	4	5	6	7
A0/U0	General PVC Sheet	0	44444444	44444444	44444444	44444444	10102230	0	0	0
		0	44444444	44444444	44444444	44444444	22330102	0	0	0
		0	44444444	44444444	44444444	44444444	11423200	0	0	0
	POSTsilver/React Sheet	0	44444444	44444444	44444444	44444444	10000111	0	0	0
		0	44444444	44444444	44444444	44444444	22324000	0	0	0
		0	44444444	44444444	44444444	44444444	22324000	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 (min)	dilutions (-log)							
			p	1	2	3	4	5	6	7
Cytotoxicity	General PVC Sheet	30	0	0	0	0	0	0	0	0
	POSTsilver/React Sheet	30	0	0	0	0	0	0	0	0
	General PVC Sheet	60	0	0	0	0	0	0	0	0
	POSTsilver/React Sheet	60	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 (min)	dilutions (-log)							
			p	1	2	3	4	5	6	7
Sensibility	SCDLP	/	44444444	44444444	44444444	44444444	44444444	11123222	0	0
	General PVC Sheet	/	44444444	44444444	44444444	44444444	44444444	11402222	0	0
	POSTsilver/React Sheet	/	44444444	44444444	44444444	44444444	44444444	22324000	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 (min)	dilutions (-log)							
			p	1	2	3	4	5	6	7
test	POSTsilver/React Sheet	30	44444444	44444444	44444444	01110200	0	0	0	0
		30	44444444	44444444	44444444	23002222	0	0	0	0
		30	44444444	44444444	44444444	10112033	0	0	0	0
	General PVC Sheet	30	44444444	44444444	44444444	11111111	0	0	0	0
		30	44444444	44444444	44444444	11142322	0	0	0	0
		30	44444444	44444444	44444444	02203330	0	0	0	0
test	POSTsilver/React Sheet	60	44444444	44444444	44444444	10110002	0	0	0	0
		60	44444444	44444444	44444444	22300000	0	0	0	0
		60	44444444	44444444	44444444	11101111	0	0	0	0
	General PVC Sheet	60	44444444	44444444	44444444	11232420	0	0	0	0
		60	44444444	44444444	44444444	11412222	0	0	0	0
		60	44444444	44444444	44444444	11203333	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