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Determination of the virucidal activity of a PVC floor covering with Evercare/Protecsol^2 surface treatment on the human coronavirus HCoV-229E for contact times of 2 and 5 hours. Protocol adapted from ISO 21702 (2019)

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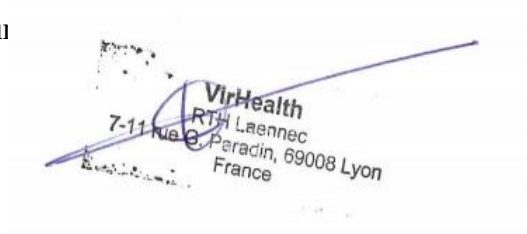
Name: Dr Vincent Moulès, General manager Date: 2020/6/25 Lyon Signatu 

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I. CONCLUSION

The virucidal activity of the PVC floor covering with Evercare / Protecsol^2 surface treatment was evaluated according to a protocol adaped from standard 21702(2019) for contact times of 2 and 5 hours on the human coronavirus HCov-229E in the presence of standardized interference (BSA 0.3g/L).

The 304L stainless steel surface is the reference surface for the test.

- PVC flooring with Evercare/Protecsol^2 surface treatment, 2h contact time

Under the experimental conditions, (20°C, 2h, BSA interference 0.3g/L), the Mipolam Evercare membrane shows a virucidal activity associated with a logarithmic mean reduction of 2.53 log_{10}/cm^2 corresponding to an efficiency of 99.70% according to the adapted protocol. of the ISO 21702 standard.

- PVC flooring with Evercare/Protecsol^2 surface treatment, 5h contact time

Under the experimental conditions, (20°C, 2h, BSA interference 0.3g/L), the Mipolam Evercare membrane shows a virucidal activity associated with a logarithmic mean reduction of 3.43 log_{10}/cm^2 corresponding to an efficiency of 99.97% according to the adapted protocol. of the ISO 21702 standard.

PRODUIT	Contact time (hour)	Interfering substance	Logarithmic reduction (log_{10}/ cm^2)	Virucidal efficacy (%)
PVC flooring with Evercare/ Protecsol^2 surface treatment	2	BSA 0.3g/L	2.53	99.70%
	5		3.43	99.97%

II. CONTRACT DOCUMENTS

This service is defined by the following documents:

. Quote	DEV0017
. Ordered	Good for agreement dated 19/05/2020

III. DATA CONCERNING THE SAMPLES AND TEST CONDITIONS

III.1 Identification of sampels

Test surface : PVC flooring with Evercare/Protecsol^2 surface treatment

Indicator surface : stainless steel surface

Appearance of the supplied product : blue, smooth, non-porous

Manufacturer : Gerflor

Supplier : Gerflor

Storage condition : room temperature

Evaluation period: 06/2020

III.2 Experimental conditions

Test surface: PVC flooring with Evercare/Protecsol^2 Surface treatment, surface considered as a porous surface: no

Experimental conditions	
Date	- 03/06/2020
Viral strain	- Human coronavirus HCoV-229E
Sample surface (cm²)	- 5 cm x 5 cm = 25 cm²
Inoculum surface (cm²)	- 4 cm x 4 cm = 16 cm²
Inoculum volume	- 400uL
Temperature	20°C
Interference substance	BSA 0.3g/L
Contact time	2 and 5 hours
Neutralization	- 10 mL of infection medium without SVF
Quantification	- Limit titration on permissice cells
Number of wells per dilution	6
Incubation temperature	34 ± 1 °C

IV. RESULTS

Virucidal activity of PVC flooring with Evercare/Protecsol^2 surface treatment on human coronavirus HCoV-229E for contact times of 2 and 5 hours.

a. Cellular sensitivity

Surface	LOG TCID50/mL
PVC with Evercare/Protecsol^2 surface treatment	6.5
Stainless steel surface	6.7
Difference < 1 log ☒ oui ☐ no	

The comparative assay of the human coronavirus HCoV-229E on MRC5 cells inoculated with the recovery buffers of the PVC floor covering with Evarcare / Protecsol2 surface treatment and the stainless steel surface results in a difference of less than 1 log10. The results show that the liquids recoverd from the test surfaces did not affect the sensitivity of MARC5 cells to the human coronavirus HCoV-229-E in the experimental procedure used.

b. Determination of cytotoxicity

The cytotoxicity of the test surface is determined by visual reading of the cytopathogenic effects (ECP) by microscopy and quantified by DICT50 on the permissive MRC5 cells.

For surface recovery of the virus, the membranes are immersed in a volume of 10mL of infection medium without SVF (recovery solution). The cytotoxicity of the recovery solutions is determined by visual reading of the ECP.

Under the test conditon, in the presence of the interfering substance (BSA 0.3g/L), the recover solutions of the PVC flooring with Evercare/Protecsol^2 surface treatment and of the stainless steel surface do not induce cytopathogenic effects. on MRC5 cells for contact times of 2 and 5 hours.

The results of the test are dependent and take into account the results of cytotoxicity

c. Trial

The raw data for the evaluation of the virucidal activity of PVC flooring with Evercare / Protecsol^2 surface treatment and of the Inox surface on the human coronavirus HCoV-229E under the test conditions (20°C, 2 and 5 hours, BSA interference 0.3g/L) are presented in the appendix

The results were determined by visual reading of cytopathogenic effects (ECP) by microscopy and quantified by DICT50 on the permissive MRC5 cells.

Surface	Interference condition	Cytotoxicity (log10 DICT50)	Support	T0 (log_{10} $DICT_{50}/cm^2$)	T2 (log_{10} $DICT_{50}/cm^2$)	T5 (log_{10} $DICT_{50}/cm^2$)
Stainless steel surface	BSA 0.3g/L	0.5	S1	5,3	5,2	5,2
			S2	5,3	5,3	5,2
			S3	5,30	5,50	5,30
			Average N1	**5,30**	**5,33**	**5,23**
			SD	0,00	0,11	0,04
PVC flooring with Evercare/ Protecsol^2 surface treatment	BSA 0.3g/L	0.5	S1	5,30	2,80	1,80
			S2	5,20	2,80	1,80
			S3	5,30	2,80	1,80
			Average N2	**5,27**	**2,80**	**1,80**
			SD	0,04	0,00	0,00
			Abatement D1 (log_{10} $DICT_{50}/cm^2$) *	**/**	**2,53**	**3,43**

N1: average quantity in log10 of virus (triplicate) stainless steel surface (BSA 0.3g/L)

N2: qaverage quantity in log10 of virus (triplicate) of PVC flooring with Evercare/Protecsol^2 surface treatment (BSA 0.3g/L)

** D1 : reduction in virucidal efficiency for each contact time (log10/cm²)*

D2=N1-N2'

V. CONCLUSION

PVC flooring with Evercare/Protecsol^2 surface treatment exhibits virucidal efficiencies of 99.70% (2.53 log10 $DICT_{50}/cm^2$) and 99.97% (3.43log10 $DICT_{50}/cm^2$) on the human coronavirus HCoV-229E respectively after a contact time of 2 and 5 hours in the presence of an interfering solution BSA 0.3g/L.

VI. APPENDIX

V.1 Materials and reagents

- Cell line

Name MRC5 ATCC® CCL-171™

Number of passes: 18

Culture medium: EMEM (Lonza, batch n°0000757679, 11/2020) with 10% of SVF (Dutscher, batch n°S16529S1810, 09/2022), 1% d'antibiotics (Gibco, batch n° 2145466, 12/2020) and 1% L-glutamine (Gibco, batch n° 2091579, 22/2021)

- Viral strain

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

Virale suspecsion: 2.37x10^7 (batch number:032020229-6)

Quantification technique:

- Serial dilutions in infection medium: EMEM (Lonza, batch n°0000757679, 11/2020) with 2% of SVF (Dutscher, batch n° S16529S1810, 09/2022), 1% of antibiotics (Gibco, batch n ° 2145466, 12/2020) and 1% L-glutamine (Gibco, batch n° 2091579, 22/2021)
- Add 100uL of each solution to a 96-well plate at the rate of 8 wells per dilution.
- Incubate 7 days at 34°C

V.2 Preparation of reagents

0.3g/L of BSA: Dissolve 0.3g of BSA (SIGMA ALDRICH : batch n° SLB26632) in 100 mL of sterile water, sterilization by filtration through a 0,2 µM membrane

V.3 Raw data : Quantification in DICT50/mL of coronavirus 229E with a contact time of 2 and 5 hours after visual microscopic reading of cytopathogenic effects (4 wells / dilution)

- Table 1 : Stop activity indicators

	Product	Interfering substance	Contact time (hour)	dilutions (-log)								TITER viral particles/mL	TITER LOG viral particles/ mL			
				p	1	2	3	4	5	6	7					
T0	PVC flooring with Evercare /Prot ecsol2 surface	BSA 0,3g/L	0	6	6	6	6	5	0	0	0	2,15E+05	5,3			0,7
			0	6	6	6	6	4	0	0	0	1,47E+05	5,2			0,8
			0	6	6	6	6	5	0	0	0	2,15E+05	5,3			0,7
	Stainless steel surface		0	6	6	6	6	4	1	0	0	2,15E+05	5,3			0,7
			0	6	6	6	6	5	0	0	0	2,15E+05	5,3			0,7
			0	6	6	6	6	4	1	0	0	2,15E+05	5,3			0,7

Explanations :
1-4 : degree of ECP in 4 cell culture units (microtiter plate)
0 : no ECP
C : observation of cytotoxicity on cells
n.d : not determined

- Table 2 : Cytotoxicity controls

	Product	Interfering substance	Contact time (hour)	dilutions (-log)								TITER viral particules /mL	TITER LOG viral particules/ mL
				p	1	2	3	4	5	6	7		
cytotoxicity	PVC flooring Evercare/ Prot ecsol2 surafce	BSA 0,3g/L	0	0	0	0	0	0	0	0	0	3,16E+00	0,5
			2	0	0	0	0	0	0	0	0	3,16E+00	0,5
			5	0	0	0	0	0	0	0	0	3,16E+00	0,5

Explanation:
1-4 : degree of ECP in 4 cell culture units (microtiter plate)
0 : no ECP
C : observation of cytotoxicity on cells
n.d : not determined

- Table 3 : Trial

	Product	Interfering substance	Contact time (hour)	dilutions (-log)							
				p	1	2	3	4	5	6	7
Testing	PVC flooring with Evercare / Protecsol 2	BSA 0,3g/L	2	6	6	0	0	0	0	0	0
				6	6	0	0	0	0	0	0
				6	6	0	0	0	0	0	0
	PVC flooring with Evercare / Protecsol 2		5	6	0	0	0	0	0	0	0
				6	0	0	0	0	0	0	0
				6	3	0	0	0	0	0	0
	Stainless steel surface	BSA 0,3g/L	**2**	6	6	6	6	3	1	0	0
				6	6	6	6	3	0	0	0
				6	6	6	6	4	1	0	0
	Stainless steel surface		5	6	6	6	6	4	0	0	0
				6	6	6	6	4	0	0	0
				6	6	6	6	3	2	0	0

Explanation :
1-4 : degree of ECP in 4 cell culture units (microtiter plate)
0 : no ECP
C : observation of cytotoxicity on cells
n.d : not determined