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Virucidal activity of the Agivir surface + 5000 cycles martindale on Human coronavirus HCoV-229E for a contact time of 5, 15 and 60min.
Adapted protocol from ISO 21702 (201) standard

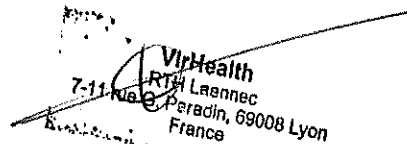
CLIENT	Mme Valérie Courault SERGE FERRARI BP54, 38352 La Tour-Du-Pin cedex, France
TEST LABORATORY	S.A.S VIRHEALTH Site Laennec-La Buire, 2ème étage, Bat B 7-11 rue Guillaume Paradin, 69372 Lyon Cedex 08
TECHNICAL CONTRIBUTION	Léa Szpiro, responsable laboratoire Dounia Bouchami, technicienne de laboratoire

Quality approval

Name : Dr Vincent Moulès, CEO

At Lyon, 2020/06/08

Signature :



VirHealth
RTM Laennec
7-11 rue Guillaume Paradin, 69008 Lyon
France

This report includes 10 pages



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I. CONCLUSION

Virucidal activity of the Agivir surface + 5000 cycles martindale and non-active surface have been tested under conditions defined by the ISO 21702 (2019) adapted protocol for contact times of 5, 15 and 60 minutes on the human coronavirus HCoV-229E with the chosen interfering substance (mucus/saliva mix)

The non-active surface is the control for this test

- Agivir surface + 5000 cycles martindale, 5 minutes of contact time

Under experimental conditions, (20°C, 5 minutes, Mucus/saliva interfering substance), the Agivir surface + 5000 cycles martindale shows a virucidal activity associated with a logarithmic reduction of 2.17 log₁₀ which is equivalent to a 99.32% efficiency under the ISO 21702 adapted protocol.

- Agivir surface + 5000 cycles martindale, 15 minutes of contact time

Under experimental conditions, (20°C, 15 minutes, Mucus/saliva interfering substance), the Agivir surface + 5000 cycles martindale shows a virucidal activity associated with a logarithmic reduction of 3.37 log₁₀ which is equivalent to a 99.96% efficiency under the ISO 21702 adapted protocol.

- Agivir surface + 5000 cycles martindale, 60 minutes of contact time

Under experimental conditions, (20°C, 60 minutes, Mucus/saliva interfering substance), the Agivir surface + 5000 cycles martindale shows a virucidal activity associated with a logarithmic reduction of 3.87 log₁₀ which is equivalent to a 99.99% efficiency under the ISO 21702 adapted protocol.

PRODUCT	Contact time (min)	Interfering substance	Logarithmic reduction (Log10)	Virucidal efficiency (%)
Agivir surface + 5000 cycles martindale	5	Mucus/saliva	2.17	99.32%
	15		3.37	99.96%
	60		3.87	99.99%



II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

- . Quotation: DEV0010
- . Order: Good for agreement date: 15/05/20

III. TEST CONDITIONS AND SAMPLES DATA

III.1 Samples identification

Test surface : Agivir surface + 5000 cycles martindale

Control surface : non-active surface

Product appearance: white, smooth and non-porous

Manufacturer : SERGE FERRARI

Supplier : SERGE FERRARI

Storage conditions : room temperature

Evaluation period : 05/2020

II.1 Experimental conditions

Test surface : Agivir surface + 5000 cycles martindale

Conditions expérimentales	
Date	- 27/05/2020
Viral strain	- Human coronavirus HCoV-229E
Sample size (cm ²)	- 1.5 cm x 1.5 cm = 2.25 cm ²
Inoculum size (cm ²)	- 1 cm x 1 cm = 1cm ²
Inoculum volume	- 50uL
Temperature	20°C
Interfering substance	Mucus/saliva
Contact time	5, 15 et 60 minutes
Neutralisation	- 2 mL of infection medium without FCS
Quantification	-endpoint titration on permissives cells
Number of wells per dilution	4
Incubation temperature	34 ± 1 °C



II. RESULTS

Virucidal activity of the Agivir surface + 5000 cycles martindale on human coronavirus HCoV-229E for a contact time of 5, 15 and 60 minutes

a. Cell susceptibility

Surface	LOG TCID50/mL
Agivir surface + 5000 cycles martindale	5.7
Non-active surface	5.7
Différence < 1 log <input checked="" type="checkbox"/> oui <input type="checkbox"/> no	

Comparative titre of human coronavirus HCoV-229E on MRC5 cells inoculated with Agivir surface + 5000 cycles martindale surface and non-active surface recuperation buffers show a difference less than 1log.

Results showed that recuperation buffers of the test surfaces didn't affect the MRC5 susceptibility to human coronavirus HCoV-229E under test conditions.

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 2mL of infection medium without FCS (recuperation buffer). The recuperation buffer cytotoxicity is determined by reading of cytopathic effect (CPE)

Under test conditions, with artificial mucus / saliva mix interference, the recuperations buffers from Agivir surface + 5000 cycles martindale and non-active surface didn't show cytopathic effects on MRC5 cells for a contact time of 5, 15 and 60 minutes.

The test results are dependant and take into account the cytotoxicity results.

c. test

Raw data for virucidal activity of Agivir surface + 5000 cycles martindale and non-active surface on human coronavirus HCoV-229E under test conditions (20°C, 5, 15 and 60 minutes, interference Mucus/Saliva) are presented in appendices

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

Surface	interfering substance	Cytotoxicity (log10 TCID50)	Support	T0 (log10 TCID50)	T5 (log10 TCID50)	T15 (log10 TCID50)	T60 (log10 TCID50)
Non-active surface	Mucus/saliva	0.5	S1	4,7	5	4,5	4,3
			S2	4,5	4,7	4,7	4,3
			S3	5	4,7	4,5	4,5
			Average N1'	4,73	4,80	4,57	4,37
			SD	0,18	0,13	0,09	0,09
Agivir surface + 5000 cycles martindale	Mucus/saliva	0.5	S1	5	2,7	1,3	0,5
			S2	4,7	2,5	1,3	0,5
			S3	4,5	2,7	1	0,5
			Average N2'	4,73	2,63	1,20	0,50
			SD	0,18	0,09	0,13	0,00
			Reduction D2 (log TCID50)*	/	2,17	3,37	3,87

N1' = viral quantity in log10 (average of triplicate) control membrane (mu: cus/saliva)

N2' = viral quantity in log 10 (average of triplicate) Agivir surface + 5000 cycles martindale (Mucus/ saliva)

*D2 : virucidal activity for every contact time (logarithmic reduction in log10)

$D2 = N1' - N2'$

V. CONCLUSION

The Agivir surface + 5000 cycles martindale shows virucidal efficiency of 99.32% (2.17 Log 10 TCID50), 99.96% (3.37 log 10 TCID50) and 99.99% (3.87 Log 10 TCID50) on human coronavirus HCoV-229E respectively after a contact time of 5, 15 and 60 minutes with mucus/saliva mixture interference.



VI. ANNEXES

V.1 Matériels et réactifs

- Cell line

Name: MRC5 ATCC® CCL-171™

Number of passages: 15

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

- Viral strain

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

Viral test suspension: 2.37×10^7 (batch number: 032020229-4)

Quantification technique:

- Successive tenfold dilution in infection medium: EMEM (Lonza, batch n°0000757679, 11/2020) with 2% of FCS (Dutscher, batch n° S16529S1810, 09/2022), 1% of antibiotics (Gibco, batch n° 2145466, 12/2020) and 1% of L-glutamine (Gibco, batch n° 2091579, 22/2021)
- Add 100µL of every dilution on 8 wells on a 96 plate.
- Incubate 7 days at 34°C, 5% CO₂

V.2 Reagent preparation

- mix 1mL of artificial saliva (ASTM2721) with 1mL of nasal epithelium mucus (EPI I18, Epithelix)

V.3 RAW DATA: TCID50 quantification of human coronavirus 229^E after 5, 15 and 60 minutes after Visual reading of cytopathic effects (4 wells per dilutions)

- Table 1 : stopping activity control

	Product	interfering substance	contact time (min)	dilutions (-log)							
				p	1	2	3	4	5	6	7
TO	Non-active surface	mucus / saliva	0	4	4	4	4	1	0	0	0
			0	4	4	4	3	1	0	0	0
			0	4	4	4	4	2	0	0	0
	Agivir + 5000 martindale		0	4	4	4	4	2	0	0	0
			0	4	4	4	3	2	0	0	0
			0	4	4	4	3	1	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 : cytotoxicity control

	Product	interfering substance	contact time (min)	dilutions (-log)							
				p	1	2	3	4	5	6	7
cytotoxicity	Non-active surface	mucus / saliva	5	0	0	0	0	0	0	0	0
			15	0	0	0	0	0	0	0	0
			60	0	0	0	0	0	0	0	0
	Agivir + 5000 martindale		5	0	0	0	0	0	0	0	0
			15	0	0	0	0	0	0	0	0
			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 3 : test

	Product	interfering substance	contact time (min)	dilutions (-log)							
				p	1	2	3	4	5	6	7
test	Non-active surface	mucus / saliva	5	4	4	4	4	2	0	0	0
			5	4	4	4	4	1	0	0	0
			5	4	4	4	4	1	0	0	0
	15		4	4	4	4	0	0	0	0	
	15		4	4	4	4	1	0	0	0	
	15		4	4	4	4	0	0	0	0	
	60		4	4	4	3	0	0	0	0	
	60		4	4	4	3	0	0	0	0	
	60		4	4	4	4	0	0	0	0	
	Non-active surface	mucus / saliva	5	4	4	2	0	0	0	0	
			5	4	4	0	0	0	0	0	
			5	4	4	1	0	0	0	0	
	15		3	0	0	0	0	0	0		
	15		2	1	0	0	0	0	0		
	15		2	0	0	0	0	0	0		
	60		0	0	0	0	0	0	0		
	60		0	0	0	0	0	0	0		
	60		0	0	0	0	0	0	0		
	Agivr + 5000 martindale	mucus / saliva	5	4	4	0	0	0	0	0	
			5	4	4	0	0	0	0	0	
5			4	4	1	0	0	0	0		
15	3		0	0	0	0	0	0			
15	2		1	0	0	0	0	0			
15	2		0	0	0	0	0	0			
60	0		0	0	0	0	0	0			
60	0		0	0	0	0	0	0			
60	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