R2007HEX001-1

Antiviral activity of PUREZONE060/PRZ150/PURECOVER surface on human coronavirus HCoV-229E for a contact time of 15 and 60min. Adapted protocol from ISO 21702 (201) standard

CLIENT	HEXIS Monsieur Christophe BAUDRION Z. I. Horizons Sud 34110 FRONTIGNAN FRANCE
PRESTATION EFFECTUÉE PAR	S.A.S VIRHEALTH Site Laennec-La Buire, 2éme étage, Bat B 7-11 rue Guillaume Paradin, 69372 Lyon Cedex 08
CONTRIBUTION TECHNIQUE	Léa Szpiro, responsable laboratoire Dounia Bouchami, technicienne de laboratoire

Approbateur/Validation Qualité
Nom : Dr Vincent Moulès, CEO Date : A Signatu 

This report includes 10 pages

SUMMARY

I. CONCLUSION

Antiviral activity of the PUREZONE060/PRZ150/PURECOVER surface and non-active surface have been tested under conditions defined by the ISO 21702 (2019) adapted protocol for contact times of 15 and 60 minutes on the human coronavirus HCoV-229E

Stainless steel surface is the control for this test.

- PUREZONE060/PRZ150/PURECOVER surface, 15 minutes of contact time

Under experimental conditions, (20°C, 15 minutes), the PUREZONE060/PRZ150/PURECOVER surface shows an antiviral activity associated with a logarithmic reduction of 1.30 $\log_{10}$ which is equivalent to a 94.99% efficiency under the ISO 21702 adapted protocol.

- PUREZONE060/PRZ150/PURECOVER surface, 60 minutes of contact time

Under experimental conditions, (20°C, 15 minutes), the PUREZONE060/PRZ150/PURECOVER surface shows an antiviral activity associated with a logarithmic reduction of 2.90 $\log_{10}$ which is equivalent to a 99.87% efficiency under the ISO 21702 adapted protocol.

PRODUCT	Contact time (min)	Logarithmic reduction ($\log_{10}$)	Antiviral efficiency (%)
PUREZONE060/PRZ150/PURECOVER	15	1.3	94,99%
	60	2.9	99.87%

II. CONTRACTUAL DOCUMENTS

The present service is defined by the following contractual documents:

. Quotation	DEV0033
. Order	Good for agreement date: 03/06/2020

III. TEST CONDITIONS AND SAMPLES DATA

III.1 Samples identification

Test surface: PUREZONE060/PRZ150/PURECOVER (batch N° C014023C)

The test surface (film) is marketed under the following names:

PUREZONE060, PRZ150 et PURECOVER

HEXIS has provided VirHealth with technical data sheets and a certificate attesting that the test surface evaluated in this test is identical for the 3 references.

Control surface: stainless steel

Product appearance: white, self-adhesive film, smooth and non-porous

Manufacturer: HEXIS

Supplier: HEXIS

Storage conditions: room temperature

Evaluation period: 06/2020

II.1 Experimental conditions

Test surface: PUREZONE060/PRZ150/PURECOVER

Experimental Conditions	
Date	- 19/06/2020
Viral strain	- Human coronavirus HCoV-229E
Sample size (cm²)	- 1.5 cm x 1.5 cm = 2.25 cm²
Inoculum size (cm²)	- 1 cm x 1 cm = 1cm²
Inoculum volume	- 50uL
Temperature	20°C
Contact time	15 and 60 minutes
Interfering substance	n.a.
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

III. RÉSULTS

Antiviral activity of the PUREZONE060/PRZ150/PURECOVER surface on human coronavirus HCoV-229E for a contact time of 15 and 60 minutes

a. Cell susceptibility

Surface	lOG TCID50/mL
PUREZONE060/PRZ150/PURECOVER	5.1
Stainless steel	5.0
Difference < 1 log_{10} ☒ yes ☐ no	

Comparative titre of human coronavirus HCoV-229E on MRC5 cells inoculated with PUREZONE060/PRZ150/PURECOVER surface and stainless steel surface recuperation buffer show a difference less than $1log_{10}$. Results showed that recuperation buffers of the test surfaces didn't affect the MRC5 susceptibility to human coronavirus HCoV-229E under test conditions.

a. 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, the recuperations buffers from PUREZONE060/PRZ150/PURECOVER and stainless steel surfaces didn't show cytopathic effects on MRC5 cells for a contact time of 15 and 60 minutes.

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

a. Essai

Raw data for antiviral activity of PUREZONE060/PRZ150/PURECOVER and stainless steel surfaces on human coronavirus HCoV-229E under test conditions (20°C, 5, 15 and 60 minutes) 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 (log10 TCID50)	Support	T0 (log10 TCID50)	T15 (log10 TCID50)	T60 (log10 TCID50)
PUREZONE060/ PRZ150/PUREC OVER	0.5	S1	5	3,5	2,1
		S2	5,1	4	2,4
		S3	5,1	4,1	2,4
		Average N1	**5,1**	**3,9**	**2,3**
		SD	0,1	0,3	0,1
stainless steel	0.5	S1	5	5	5,1
		S2	5,3	5,3	5,3
		S3	5	5,3	5,1
		Average N2	**5,1**	**5,2**	**5,2**
		SD	0,1	0,1	0,1
		Reduction D1 (log10 DICT50) *	**/**	**1,3**	**2,9**

N1: viral quantity in log10 (average of triplicate) stainless steel surface
N2: viral quantity in log 10 (average of triplicate) PUREZONE060/PRZ150/PURECOVER surface
** D: antiviral activity for every contact time (logarithmic reduction in log10) for1 cm^2 of test surface*
D1=N1-N2

V. CONCLUSION

PUREZONE060/PRZ150/PURECOVER surface shows antiviral activity of 94.99% (1.3 log_{10} TCID50) and 99.87% (2.90 log_{10} TCID50) on human coronavirus HCoV-229E after a contact time of 15 and 60 minutes respectively at 20°C.

VI. ANNEXES

- 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: 7.50x10^7 (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% $C0_2$

- V.3 RAW DATA : TCID50 quantification of human coronavirus 229E after 15 and 60 minutes, visual reading of cytophatic effetcs (4 wells per dilutions)

Tableau 1 : stopping activity control

	Product	Contact time	dilutions (-log)							
			P	1	2	3	4	5	6	7
T0	PUREZONE060/PRZ 150/PURECOVER	0	44444444	44444444	44444444	44444444	10011010	0	0	0
		0	44444444	44444444	44444444	44444444	10110110	0	0	0
		0	44444444	44444444	44444444	44444444	10111010	0	0	0
	stainless steel	0	44444444	44444444	44444444	44444444	00011110	0	0	0
		0	44444444	44444444	44444444	44444444	10111101	0	0	0
		0	44444444	44444444	44444444	44444444	01011001	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*

- Tableau 2 : cytotoxcicity

	Product	Contact time	dilutions (-log)							
			P	1	2	3	4	5	6	7
cytotoxicity	stainless steel	/	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
	PUREZONE060/PRZ 150/PURECOVER		0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			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*

- Tableau 3 : test

	Product	Contact time	dilutions (-log)							
			P	1	2	3	4	5	6	7
Essais	PUREZONE060/PRZ 150/PURECOVER	15	44444444	44444444	44444444	0	0	0	0	0
		15	44444444	44444444	44444444	10010110	0	0	0	0
		15	44444444	44444444	44444444	00011111	0	0	0	0
		60	44444444	00101111	0	0	0	0	0	0
		60	44444444	11011111	0	0	0	0	0	0
		60	44444444	11111110	0	0	0	0	0	0
	stainless steel	15	44444444	44444444	44444444	44444444	10011010	0	0	0
		15	44444444	44444444	44444444	44444444	10110111	0	0	0
		15	44444444	44444444	44444444	44444444	10111110	0	0	0
		60	44444444	44444444	44444444	44444444	00011111	0	0	0
		60	44444444	44444444	44444444	44444444	10111101	0	0	0
		60	44444444	44444444	44444444	44444444	01011001	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*