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STULV20AA2264-1	**Measurement of antiviral activity of INTERCEPT CU22™ Film**		
SPONSOR	INTERCEPT Technology GmbH		
	Am Goldberg 2		
	99817 Eisenach		
	GERMANY		
REFERENCE TEST METHOD	ISO 21702:2019 - Measurement of antiviral activity on plastics and other non-porous surfaces		
TEST ITEM			
PRODUCT NAME	INTERCEPT CU22™ Film		
MATRIX OF THE PRODUCT	INTERCEPT CU22™ extruded PE Film with polymerized Copper		
BATCH	NA	CODE	NA
MANUFACTURING DATE	NA	EXPIRY DATE	NA
MANUFACTURER	INTERCEPT Technology GmbH		
ACTIVE INGREDIENT	Polymerized Copper		
PARCEL REGISTRATION N.	IP-LV-2020121-AFW	RECEIVING DATE	April 30th 2020
STORAGE CONDITIONS	Room temperature		
ANALYSIS STARTING DATE	May 27th 2020	**ANALYSIS ENDING DATE**	June 06th 2020
EXPERIMENTAL CONDITIONS			
TEST TEMPERATURE	Room temperature (25±1°C) at ≥90%RH		
SPECIMEN DESCRIPTION	5x5 cm specimen (PE film treated with antiviral). As control specimen 5x5 PE foil inert uncoated specimen were used.		
VIRAL INOCULUM	400 µl of viral inoculum with known viral titre were applied onto each specimen evenly distributed. The inoculum was left adsorbing and drying onto the specimen at room temperature and under biosafety hood.		
PRODUCT APPLICATION	NA		
VOLUME APPLIED	NA		
CONTACT TIME	30 minutes, 1 hour, 24 hours (±5 minutes)		
INACTIVATION OF PRODUCT RESIDUES	Dilution-neutralization in cell culture medium (no detoxification needed)		
INCUBATION TEMPERATURE	37°C ± 1°C (with 5% CO_2)		
TEST VIRUS	*Bovine Coronavirus (BCoV)* - strain S379 Riems		
CELL LINE	HRT-18 cells (human rectal carcinoma cells)		

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VALIDITY AND EFFICACY CRITERIA	***Check of cytotoxicity of the test item*** The test item was not cytotoxic, i.e. its contribution in terms of CPE was not visible in the test. ***Assay of viral infectivity (virus titration)*** The titre of the starting viral suspension was sufficiently high to at least enable a theoretical viral titre reduction of 4 $LogTCID_{50}$. ***Check of viral recovery (untreated surface)*** The dose of infectious particles recovered immediately after inoculation (as well as after 30 minutes and 1 hour) from the untreated test specimens was around $6LogTCID_{50}$. The dose of infectious particles recovered from each untreated test specimen after contact of 24 h was no less than $3LogTCID_{50}$. It was 4.86±0.35 LogTCID50/ml. ***Check of host cells susceptibility to virus and suppression of antiviral activity (neutralization)*** The difference of the average value of $TCID_{50}$ among the cellular cultures treated with the treated samples or untreated samples and then with the viral inoculum and the ones treated only with the viral inoculum (negative control) was ≤ 0.5 $LogTCID_{50}$. ***Accuracy of virus control among the three replicas*** The maximum difference of the value of $TCID_{50}$ among the cellular cultures treated with the viral inoculum recovered from the 3 different untreated specimen was ≤ 0.5 Log. ***Antiviral efficacy*** The $LogTCID_{50}$ reduction factor (R) is calculated as per ISO 21702 :2019 standard, i.e. subtracting the average $LogTCID_{50}$ of treated specimen (At) from the average $LogTCID_{50}$ of untreated specimen (U_t) at the chosen contact times: $R= U_t – At$. The $LogTCID_{50}$ is calculated by the standard Spearman-Karber method and by the Large Volume Plating method as confirmatory test.
	Bovine coronavirus is used as a surrogate virus for SARS-related viruses as it belongs to the same Betacoronavirus genus and showed similar susceptibility to WHO formulations in published studies.

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Cytotoxicity				
RESULTS	HRT-18 cell destruction	≤0.50 (Log)		
	Log reductions at the different contact times			
	Bovine coronavirus (Betacoronavirus 1)	**30 minutes**	**1 hour**	**24 hours**
		Average		
		ND	ND	2.36±0.43Log_{10}
		ND	ND	99.6%
	See Annex N.1 for the detail of the test results			
CONCLUSIONS	**The antiviral treatment causes a good viral titre reduction within 24 hours of contact time in the adopted test conditions. The treated surface does not have any cytotoxic effect on the host cell line.**			
ANNEX	N. 1: RAW DATA ELABORATION			

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Table 1: product description

Test item [Function]	Product designation	Description	Test surface inoculated and covered by PE-foil
Test squares 5 cm x 5 cm *(equipped with the active component(s)*	INTERCEPT CU22 Film	test squares with a semi-soft characteristic; flexible material with a slightly textured surface with a thickness of about 1 mm	4 cm x 4 cm = 16 cm^2
Test squares 5 cm x 5 cm *(non-coated control)*	PE-foil Control	test squares prepared from PE-foil; flexible material with a smooth and flat surface with a thickness of 0,1 mm	4 cm x 4 cm = 16 cm^2

Table 2: cytotoxicity control

Product(s)	Exposure	Sample ID	Dilution factor (lg) / VF = 4							Titer per 100 µL (lg TD_{50})	Titer per 1 mL (lg TD_{50})
			-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2		
INTERCEPT CU22 Film	24 h at 25 °C and 90% humidity	T-1	0/4 [1]							≤ 0,30	≤ 1,30
		T-2	0/4							≤ 0,30	≤ 1,30
		T-3	0/4							≤ 0,30	≤ 1,30
PE-foil Control		T-4	0/4							≤ 0,30	≤ 1,30
		T-5	0/4							≤ 0,30	≤ 1,30
		T-6	0/4							≤ 0,30	≤ 1,30

[1] = first number: number of cell cultures with a visible cytotoxic alteration; second number: total number of cell cultures

Table 3: verification of cells susceptibility

Test sample(s)	Sample ID	Dilution factor [1]	Dilution (lg) / VF = 4										Titer per 100 µL (lg ID_{50})
			-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6	
untreated cells	VK/E-1	n.a.	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	0/4		5,1 ± 0,37
	VK/E-2		4/4	4/4	4/4	4/4	4/4	4/4	1/4	1/4	1/4	0/4		4,35 ± 0,45
	VK/E-3		4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4				4,5 ± 0,0
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	9/12	4/12	2/12	0/12		4,65 ± 0,27
INTERCEPT CU22 Film (treated cells)	E-1	VF = 1	4/4	4/4	4/4	4/4	4/4	4/4	1/4	2/4	0/4			4,35 ± 0,4
	E-2		4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	0/4	1/4	0/4	4,8 ± 0,47
	E-3		4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4	1/4	0/4	4,8 ± 0,37
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	8/12	5/12	0/12	2/12	0/12	4,65 ± 0,28
PE-foil Control (treated cells)	E-4	VF = 1	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	0/4			4,65 ± 0,4
	E-5		4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4		4,95 ± 0,4
	E-6		4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4				4,35 ± 0,26
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	10/12	4/12	1/12	0/12		4,65 ± 0,24

[1] = dilution (or dilution factor) of the test sample(s) distributed to the detection cells when the *Large Volume Plating* (LVP) method was used
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[3] = virus titer A (virus titration on untreated cells) minus virus titer B (virus titration on treated cells)

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Table 4: verification of the suppression of the residual antiviral activity

Test sample(s)	Sample ID	Dilution (lg) / VF = 4											Titer per 100 µL (lg ID_{50})	Δ Titer [3] (lg ID_{50})	After-effect present [4]	Verification acc. clause 6.6 passed [5]
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6				
Cell culture medium (negative control)	VN-1	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4			4,95 ± 0,26	-	-	n.a.
	VN-2	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4		4,95 ± 0,4	-	-	
	VN-3	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4		4,95 ± 0,4	-	-	
Average virus titer (equivalent to S_n)		12/12	12/12	12/12	12/12	12/12	12/12	12/12	7/12	2/12	0/12		4,95 ± 0,22	-		
Resuspension medium derived from INTERCEPT CU22 Film (treated test specimens)	N-1	4/4	4/4	4/4	4/4	4/4	4/4	3/4	3/4	0/4			4,8 ± 0,37	0,15 ± 0,43	no	yes
	N-2	4/4	4/4	4/4	4/4	4/4	4/4	2/4	2/4	0/4			4,5 ± 0,42	0,45 ± 0,48	no	
	N-3	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4				4,5 ± 0,0	0,45 ± 0,22	no	
Average virus titer (equivalent to S_t)		12/12	12/12	12/12	12/12	12/12	12/12	9/12	5/12	0/12			4,6 ± 0,24	$S_n - S_t = 0,35 \pm 0,33$		
Resuspension medium derived from PE-foil Control (untreated test specimens)	N-4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	1/4	0/4		4,8 ± 0,37	0,15 ± 0,43	no	yes
	N-5	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4				4,5 ± 0,0	0,45 ± 0,22	no	
	N-6	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	0/4			4,65 ± 0,4	0,3 ± 0,46	no	
Average virus titer (equivalent to S_u)		12/12	12/12	12/12	12/12	12/12	12/12	11/12	3/12	1/12	0/12		4,65 ± 0,21	$S_n - S_u = 0,3 \pm 0,30$		

[1] = the test virus was added directly into the test sample as resuspended from the test item. Incubation was t = 30 min. at 25 °C.
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[3] = virus titer A (negative control [VN]) minus virus titer B (treated test specimen [N-1 to N-3]) or minus virus titer c (untreated test specimen [N-4 to N-6])
[4] = according to the EN 14476 an ongoing residual disinfecting activity (after effect) of the product(s) applies as not given when Δ Titer is ≤ lg 0,5
[5] = verification acc. ISO 21702, clause 6.6.3.3 is passed when Δ Titer is ≤ lg 0,5

Table 5a: titration of the virus suspension and the virus material recovered from the virus control

Test sample(s)	Sample ID	Dilution (lg) / VF = 4											Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})	Verification passed [2]
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6			
Virus suspension when added directly to the resuspension medium	Aus-1	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	1/4	5,7 ± 0,55	6,75 ± 0,25	-
	Aus-2	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4		5,55 ± 0,30		
	Aus-3	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	1/4	6,0 ± 0,42		
Average virus titer		12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	9/12	2/12	2/12	5,75 ± 0,25		
Virus material as recovered from the non-coated control test item after t = 0 min.	VK-1	4/4 1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	3/4	0/4	5,85 ± 0,46	6,60 ± 0,27 *(equivalent to 6,39/cm²)*	yes $(L_{max} - L_{min})/(L_{mean}) = 0,107$
	VK-2	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	0/4	5,7 ± 0,42		
	VK-3	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	1/4	0/4	5,25 ± 0,52		
Average virus titer		12/12	12/12	12/12	12/12	12/12	12/12	12/12	11/12	6/12	5/12	0/12	5,60 ± 0,27		
Virus material as recovered from the non-coated control test item after t = 24 hours	VK-4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	0/4			4,65 ± 0,46	4,86 ± 0,35 *(equivalent to 4,66/cm²)*	yes *(4,66/cm² > 2,8/cm²)*
	VK-5	4/4	4/4	4/4	3/4	0/4							2,55 ± 0,3		
	VK-6	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4				4,35 ± 0,3		
Average virus titer		12/12	12/12	12/12	11/12	8/12	8/12	6/12	2/12	0/12			3,86 ± 0,35		

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[2] = verification acc. ISO 21702, clause 8.2.2 is passed when $(L_{max} - L_{min})/(L_{mean})$ is ≤ lg 0,2

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Table 5b: titer of virus control at 30 minutes and 1 hour

Test sample(s)	Sample ID	Dilution (lg) / VF = 4											Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})	Average virus titer
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6			
Virus material as recovered from the non-coated control test item after t = 30 min.	VK-7	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4	5,55 ± 0,46	6,55 ± 0,34 *(equivalent to 6,34/cm²)*	6,59 ± 0,21 *(equivalent to 6,38/cm²)*
	VK-8	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	3/4	0/4	1/4	5,55 ± 0,52		
Average virus titer		8/8	8/8	8/8	8/8	8/8	8/8	8/8	7/8	5/8	1/8	1/8	5,55 ± 0,34		
Virus material as recovered from the non-coated control test item after t = 1 hour	VK-9	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4	0/4		5,55 ± 0,3	6,63 ± 0,25 *(equivalent to 6,42/cm²)*	
	VK-10	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4	1/4	0/4	5,7 ± 0,42		
Average virus titer		8/8	8/8	8/8	8/8	8/8	8/8	8/8	6/8	8/8	1/8	0/8	5,63 ± 0,25		

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures

Table 6: Maximum RF (reduction factors) at 30 minutes, 1 hour and 24 hours

Test virus	Dilution [1] factor	Incubation time (h)	Virus titer per 1 mL [1] *[lg ID_{50} ± $KI_{95\%}$]*	detection limit *[lg ID_{50} / mL]*	max. detectable virus reduction *(RF_{max})* [2]
Bovine Coronavirus (S379 Riems)	Virus titration using the limiting dilution method (Spearman & Kärber)				
	VF = 4	24	4,86 ± 0,35	lg ID_{50} = 1,30	3,56
		1	6,59 ± 0,21	lg ID_{50} = 1,30	5,29
		0,5			
	Virus titration by Large Volume Plating (LVP) inoculating 48 cell cultures [3]				
	VF = 1	24	4,86 ± 0,35	lg ID_{50} = -0,79	5,65
		1	6,59 ± 0,21	lg ID_{50} = -0,79	7,38
		0,5			

[1] = input virus (virus control), cf. Tab. 5
[2] = maximum detectable virus reduction (RF_{max}) when no residual virus was detectable. With LVP the detection limit was calculated with the modified Poisson-Formula (cf. Ref 7).
[3] = when 48 cell culture units were inoculated; V = 10,4 mL and v = 9,6 mL.

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Table 7: inactivation tests (3 test replicas) by Spearman-Karber method (S-K)

Test sample(s)	Sample ID	Incubation time	Dilution (lg) / VF = 4										Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})
			-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6	
INTERCEPT CU22 Film	In-1	24 h	4/4[1]	4/4	2/4	1/4	0/4							1,95 ± 0,4	2,5 ± 0,25
	In-2		4/4	1/4	1/4	0/4								1,2 ± 0,37	
	In-3		4/4	3/4	0/4									1,35 ± 0,26	
Average virus titer			12/12	8/12	3/12	1/12	0/12							1,5 ± 0,25	
INTERCEPT CU22 Film	In-4	0,5 h	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	2/4	1/4	5,85 ± 0,57	6,70 ± 0,25
	In-5		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4	5,55 ± 0,46	
	In-6		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4		5,7 ± 0,0	
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	8/12	3/12	1/12	5,7 ± 0,25	
INTERCEPT CU22 Film	In-7	1 h	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4		5,25 ± 0,3	6,45 ± 0,21
	In-8		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	0/4		5,4 ± 0,35	
	In-9		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	0/4	5,7 ± 0,42	
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	6/12	1/12	0/12	5,45 ± 0,21	

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures.

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Table 8: estimation of the reduction factors (RF)

INTERCEPT CU22 Film	In-1	24 h	4,86 ± 0,35	2,95 ± 0,4	1,91 ± 0,53	2,36 ± 0,43 *(equivalent to an average reduction of 99,6% within 24 h)*
	In-2			2,2 ± 0,37	2,66 ± 0,5	
	In-3			2,35 ± 0,26	2,51 ± 0,43	
	In-4	0,5 h	6,59 ± 0,21	6,85 ± 0,57	-0,26 ± 0,61	*Not detectable*
	In-5			6,55 ± 0,46	0,04 ± 0,51	
	In-6			6,7 ± 0,0	-0,11 ± 0,21	
	In-7	1 h	6,59 ± 0,21	6,25 ± 0,3	0,34 ± 0,37	*Not detectable*
	In-8			6,4 ± 0,35	0,19 ± 0,41	
	In-9			6,7 ± 0,42	-0,11 ± 0,47	

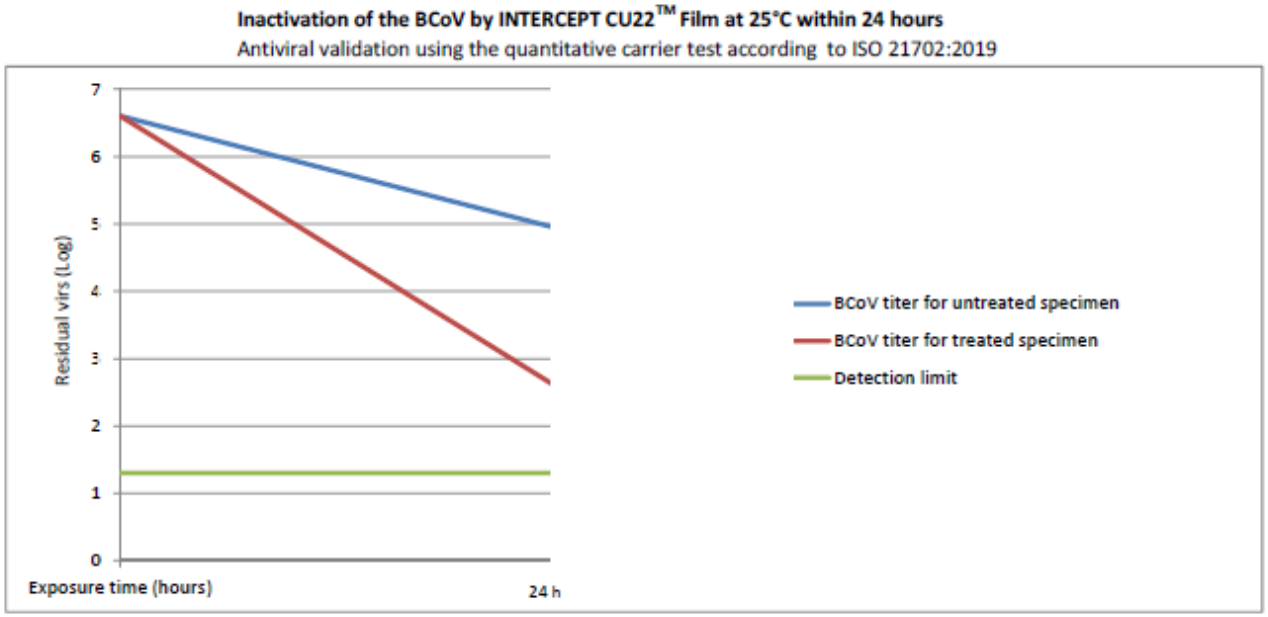

Fig.1

Reviewed and electronically signed for Study Technical Supervisor Approval by
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