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Short report edition

STULV20AA3429-1	**Measurement of antiviral activity of REair Original Plus**		
SPONSOR	REair s.r.l		
	Via Montenapoleone 10		
	20121 Milano		
	Italy		
REFERENCE TEST METHOD	ISO 21702:2019 - Measurement of antiviral activity on plastics and other non-porous surfaces		
TEST ITEM			
PRODUCT NAME	REair Original Plus		
MATRIX OF THE PRODUCT	Fine ceramics		
BATCH	NA	CODE	NA
MANUFACTURING DATE	NA	EXPIRY DATE	For the life of the finished product
MANUFACTURER	REair s.r.l		
ACTIVE INGREDIENT	Confidential		
PARCEL REGISTRATION N.	IP-LV-2020135-AJD	RECEIVING DATE	July 07th 2020
STORAGE CONDITIONS	Room temperature		
Note: test performed at Eurovir Hygiene Institut qualified test site in Luckenwalde (Berlin), Germany, under the responsibility of Dr. Christian Jursch.			
ANALYSIS STARTING DATE	September 09th 2020	**ANALYSIS ENDING DATE**	September 17th 2020
EXPERIMENTAL CONDITIONS			
TEST TEMPERATURE	Room temperature (25±1°C) at ≥90%RH		
SPECIMEN DESCRIPTION	5x5 cm specimen (ceramics treated with antiviral).		
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.		
CONTACT TIME	24 hours (±5 minutes)		
INACTIVATION OF PRODUCT RESIDUES	Immediate 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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TEST SUMMARY	The Sponsor's test item – after the specimen sterilization procedure – was inoculated with the test viral suspension. Each inoculum was evenly distributed onto the surface area of each treated and untreated specimen and left adsorbing onto the surface. After that, all specimens were placed into a dedicated climatic chamber maintaining the test conditions of 25°C and ≥90% RH for the exposure time of 24 hours requested by the ISO 21702:2019 standard. The test was performed in triplicate (3 treated specimens and 3 untreated specimens). After the exposure time, all specimens were recovered and the residual viral inoculum was eluted from the surface and plated into 96 wells microplates containing the HRT-18 host cells susceptible to the Bovine coronavirus test virus. All microplates were incubated for virus infection propagation in a dedicated CO2 incubator. At the end of the incubation period the microplates were observed under inverted microscope to check the virus cytopathogenicity (CPE) and perform both the end-point titration (Spearman-Karber) and Large Volume Plating techniques, in order to bypass possible product residual cytotoxicity issue or virus titre reduction over time. In parallel, all needed method validation control were performed (here below reported). The reduction factor of the virus infectivity was calculated in Log values as per norm, by subtracting the average virus infectious titre of treated specimen (At) from the average virus infectious titre of untreated specimen (U_t) at the chosen contact time of 24 hours: $R= U_{t\,24}– A_{t24}$. Additionally, it was calculated by subtracting the average virus infectious titre of treated specimen (A_t) from the average virus infectious titre of untreated specimen (U_0) at t0.

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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 $LogTCID_{50}$, i.e. 5.00±0.24$LogTCID_{50}$/ml, both using the Spearman-Karber technique and the Large Volume Plating (LVP) technique.

Check of viral recovery (untreated surface)
The dose of infectious particles recovered immediately after inoculation from the untreated test specimens was 4.45±0.26$LogTCID_{50}$/ml. The dose of infectious particles recovered from each untreated test specimen after contact of 24 h was less than 3$LogTCID_{50}$. It was 0.35±0.10 LogTCID50/ml (average value from three control specimen). Only the LVP could be used to detect antiviral activity after 24 hours according to ISO 21702:2019.

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:
$R= U_t – A_t$.
Additionally, it was calculated by subtracting the average $LogTCID_{50}$ of treated specimen (A_t) from the average $LogTCID_{50}$ of untreated specimen (U_0) at t0.

The $LogTCID_{50}$ reduction was calculated by the standard Spearman-Karber method.

Table 1: antiviral efficacy rate of treated articles as per Annex F of ISO 18184:2019.

Antiviral efficacy value, *Mv*	Standard
$3,0 > Mv \geq 2,0$	Good effect

Bovine coronavirus (BCoV) is used as a surrogate virus for SARS-related viruses (eg. SARS-CoV or SARS-CoV-2) as it is closely related to SARS viruses (including SARS-CoV-2) and it is low pathogenic to humans whilst SARS viruses are highly pathogenic BSL-3 high containment viruses. BCoV belongs to the same genus of Betacoronavirus as SARS viruses and showed similar susceptibility to WHO formulations in published studies. In fact, its resistance to chemical disinfection proved to be at least comparable to the one of SARS virus, if not slightly higher.

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Cytotoxicity		
RESULTS	HRT-18 cell destruction	≤0.30Log$_{10}$
	Log reductions at the different contact times	
	Bovine coronavirus (Betacoronavirus 1)	**24 hours**
		Average (U_t – A_t) – according to standard
		2.00±0.10Log$_{10}$
		99%
		Average (U_0 – A_t)
		6.10±0.26Log$_{10}$
		//
	See Annex N.1 for the detail of the test results	
CONCLUSIONS	**The antiviral treatment causes an EFFECTIVE viral titre reduction of the model Coronavirus tested (BCoV) in the adopted test conditions (see Annex F of ISO 18184:2019). The treated surface does not have any cytotoxic effect on the host cell line, which means that there is no leaching of cytotoxic substances from the tested specimen after elution into a water-based medium.**	
Note: virus infectivity after 24 hours was not sufficient to calculate virus reduction according to S-K technique (limiting dilution). Virus Log reduction after 24 hours was therefore calculated via the LVP technique. The virus Log reduction here above reported in italics is calculated vs untreated surface at t0.		

- End of Report -

Reviewed and electronically signed for Study Technical Supervisor Approval by
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