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41520 EL VISO DEL ALCOR (Sevilla)
SPAIN

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TEST REPORT

06 July 2020

1 Sample Information

Sample name	PRIMER PLUS
Sample reception	11/05/2020
Sample no.	392-2020-00189101
Analysis period	01/05/2020 - 02/07/2020

2 Results

Please see enclosure with detailed results.

Eurofins Product Testing A/S

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SPONSOR	IEDISA, S.A.		
	POL. IND. POLIVISO. C/CARPINTEROS, 25		
	41520, EL VISO DEL ALCOR, SEVILLE		
	SPAIN		
MONITOR	Eurofins Product Testing A/S		
	Smedeskovvej 38		
	8464 Galten		
	Denmark		
TEST METHOD	EN 14476:2013+A2:2019 / UNI EN 14476:2019 - Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase 2/Step 1).		
TEST ITEM			
PRODUCT NAME (*)	*PRIMER PLUS*		
MATRIX OF THE PRODUCT (*)	Primer		
BATCH N. (*)	8103	EUROFINS PRODUCT TESTING A/S SAMPLE N. (*)	392-2020-00189101
MANUFACTURING DATE (*)	07-May-2020	EXPIRY DATE	Not provided
MANUFACTURER	Not provided		
ACTIVE INGREDIENT (*)	Calcium Hydroxide 10%		
MATERIAL ITEM ALIQUOT	LV-MAT-IJE2-20-141-0D86:a		
PARCEL REGISTRATION N.	IP-LV-2020133-AGP	RECEIVING DATE	12-May-2020
STORAGE CONDITIONS (*)	Room temperature (20°C ± 5°C)		
(*) INFORMATION PROVIDED BY THE SPONSOR			
ANALYSIS STARTING DATE	12-June-2020	**ANALYSIS ENDING DATE**	01-July-2020
EXPERIMENTAL CONDITIONS			
NOTE	Due to the nature of the product, a preliminary cytotoxicity has been evaluated. On the basis of the preliminary cytotoxicity results (See Addendum N.1), the determination of the residual virus titer has been performed by the Large-Volume-Plating (LVP) method at the test concentration of 50%, since the residual cytotoxicity was lower than the one detected with the test concentration of 80% and 66%. Using the LVP, the lowest apparently non-cytotoxic dilution of the test mixture was added to ice-cold medium after the required contact time and this dilution was added to a defined number of wells containing the host cell line in 100 µl cell culture medium. In parallel, the standard method (Spearman-Kärber) was performed for all the test item concentrations, in order to determine the residual virus in case the test item concentrations resulted as non-active.		

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TEST TEMPERATURE	20°C ± 1°C
CONCENTRATIONS	80% (Neat) - 66% - 50% The item dilutions of 66% and 50% have been prepared 1.25 times higher than the final tested concentrations, using water for injections. The dilutions were homogeneous. Test item dilution was stable throughout test (no precipitation in the test mixture).
PRODUCT APPEARANCE	White thick liquid (paint)
CONTACT TIME	30 minutes
INACTIVATION OF THE PRODUCT	Dilution in iced culture Medium. Due to the nature of the product, filtration with S400 HR columns MicroSpinTM (and iced culture Medium) proved to be not applicable.
INTERFERING SUBSTANCE	Bovine serum albumin (BSA) with a final concentration of 0.3 g/L (0.03% - simulating clean conditions for the medical area).
INCUBATION TEMPERATURE	37°C ± 1°C (with 5% CO_2)
TEST VIRUS	*Bovine Coronavirus (Betacoronavirus 1), strain S379 Riems* (RVB-0020)
CELL LINE	*PT* (CCLV-RIE 11)
VALIDITY AND EFFICACY CRITERIA	***Check of cytotoxicity of the test item*** The test item shall not be cytotoxic at the concentrations tested, i.e. its contribution in terms of CPE shouldn't be visible in the test; in any case, the cytotoxicity shall be low enough to at least enable a titre reduction of 4 Log to verify the method. ***Assay of viral activity (virus titration)*** The minimum titre of the virus suspensions is at least 10^8 $TCID_{50}$/ml; in any case, it shall be sufficiently high to at least enable a titre reduction of 4 Log to verify the method. ***Check of cellular sensitivity to virus*** The difference of the value of $TCID_{50}$ among the cellular cultures treated with the test item and the ones not treated with the test item (i.e. PBS only) must be < 1 Log. ***Check of suppression of disinfectant activity*** The difference of the value of $TCID_{50}$ among the cellular cultures treated with the inactivated test item and the ones treated only with the viral inoculum must be ≤ 0.5 Log.
	The test item is considered virucidal when, within 60 minutes of contact, it causes a reduction of viral assay of at least 4 Log compared to control virus when the test organisms are *Adenovirus Type 5, Poliovirus Type 1 and Murine norovirus (MNV, strain S99).* In case of specific use conditions for which other contact times, temperatures, test organisms and interfering substances are applied instead of or in addition to the standard ones, the test item shall demonstrate at least 4 Log reduction under the chosen test conditions.

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RESULTS	**Log reductions at concentrations and contact time**			
		30 minutes		
		80% (Neat)	**66%**	**50%**
	Bovine Coronavirus, strain S379 Riems	1.00±0.000	1.00±0.000	>4.17±0.346
	See Addendum N.1-2			
	Note: for 80% and 66% test concentrations, the reported log reduction values have been determined according to Spearman-Kärber method for virus titre calculation; meanwhile for 50% test concentration, the reported log reduction value has been determined according to LVP method for virus titre calculation.			
CONCLUSIONS	**CAUSES a reduction ≥ 4 Log** against *Bovine Coronavirus (Betacoronavirus 1)* at the test item concentration of 50%, after 30 minutes of contact time, in the adopted test conditions, using bovine serum albumin at the final concentration of 0.3 g/L (0.03% - simulating clean conditions for the medical area).			
	A residual cytotoxicity was shown on PT cell line with test item concentration of 80% (Neat) and 66%, and the virus suspension titre was not sufficient to at least enable a titre reduction of 4 Log, but no virus detections were observed at the set contact time. Meanwhile at test concentration of 50% a lower residual cytotoxicity was detected and a reduction of 4 Log was observed with LVP method; so the method was verified.			
ADDENDUM	N. 1: RAW DATA ELABORATION – PRELIMINARY CYTOTOXICITY (*1 PAGE*) N. 2: RAW DATA ELABORATION – TEST (*16 PAGES*)			

Reviewed and electronically signed for Study Technical Supervisor Approval by
Elisa Anna Maccagni, Employee
for Eurofins Biolab Srl, on 02-Jul-2020 12:59:29 UTC+02:00