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TEST REPORT

07 July 2021

Sample Information

Sample name	Purifica+ (Lot no. 190221)
Sample reception	03/06/2021
Sample no.	392-2021-00261503
Analysis period	27/05/2021 - 07/07/2021

Results

Please see enclosure with detailed results for the following test:

- ISO 22196; Measurement of antibacterial activity on plastics and other non-porous surfaces.

Conclusion

On the basis of the obtained results, in compliance with the assay validity criteria, can be stated that the test items "Purifica+ Batch: 190221" tested at 4 hours of contact time, **has antimicrobial activity** (Log reduction >2 Log) against S. aureus and E. coli, respectively in adopted experimental conditions.

Eurofins Product Testing A/S



Jeanette K. Pedersen
Analytical Service Manager

Version History

Report date	Report number	Modification
07/07/2021	392-2021-00261503_FP_EN	Current version

The results are only valid for the tested sample(s).

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392-2021-00261503_FP_EN

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SPONSOR	Colorificio MP srl Via G. Pastore, 2 47922 Rimini (RN) Italy		
TEST METHOD	ISO 22196 - Measurement of antibacterial activity on plastics and other non-porous surfaces		
TEST ITEM			
MATRIX OF THE PRODUCT	Biocide and Antimicrobials.		
PRODUCT NAME	Purifica +		
BATCH	190221	CODE	Sample 3
MANUFACTURING DATE	Not Provided	EXPIRY DATE	19/02/2023
ACTIVE INGREDIENT	Photocatalytic Titanium Dioxide		
MATERIAL ITEM ALIQUOT	LV-MAT-FOV7-21-0154-0J82:a		
UNTREATED			
PRODUCT NAME	SAMPLE CONTROL		
MATERIAL ITEM ALIQUOT	LV-MAT-FOV7-21-0154-0J83:a		
PARCEL REGISTRATION N.	IP-LV-2021148-ADT	RECEIVING DATE	28-May-2021
ANALYSIS STARTING DATE	10-Jun-2021	ANALYSIS ENDING DATE	12-Jun-2021

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EXPERIMENTAL CONDITIONS			
TEST STRAINS	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	ATCC 6538P ATCC 8739	
INOCULUM VOLUME	0,4 ml		
SPECIMENS SIZE	25 cm ²	COVER FILM SIZE	1600 mm ²
REAGENTS	<p>The validity of media and reagents have been verified according to Internal procedure.</p> <ul style="list-style-type: none"> - Suspension medium: 1/500 nutrient broth (1/500 NB) - Nutrient Broth (NB) - Water for Injection (WFI) - Plate count agar (PCA) - Neutralizer CEN (NEU CEN) - Phosphate-buffered physiological saline (PBSS) 		
EQUIPMENT	<p>The validity of instruments and equipment has been assured following internal procedures before starting the analyses. Standard microbiology laboratory equipment has been used, and in particular:</p> <ul style="list-style-type: none"> - Laminar flow filtered work area - Spectrophotometer - Micropipettes - Climate Chamber 35±1°C, RH>90% 		
MATERIALS	Cover film, that does not affect bacterial growth or absorb water, made of polyethylene low density, that is 0,05-0,10 mm thick is has been used.		
TREATMENT	Life lamp 2200lm has been tuned on 20 minutes before start of the test. Specimens have been illuminated for 1 h by Life lamp 2200lm lamp at the distance of about 30 cm from the light source before inoculum. The test was performed at a contact time of 4 hours with illumination of Life lamp 2200lm lamp during the test.		
ASSAY	<p>Three specimens of 5.0 × 5.0 cm square samples for each of the treated specimens provided by Sponsor and of Negative control have been prepared for each strain and t0 and time point tested.</p> <p>Separately for each test strain, 0,4 ml of standardized culture at 2,5-10×10⁵ cells/ml has been added to the specimen then the inoculum has been covered and gently press down with a 40x40 mm film so that the test inoculum spreads to, but does not leak beyond, the edges of the film.</p> <p>The specimens inoculated have been exposed to Life lamp 2200lm lamp during the test for 4h (contact time) at Laboratory Temperature condition.</p> <p>At t0 and after the specified contact time, each specimen has been recovered and neutralized with 16 ml of validated neutralizer; viable microorganisms have been enumerated by pour plate method on TSA at 35±1°C for 24-48 hours; then bacterial colonies from each dilution series have been counted and recorded and the Logarithmic reduction of bacteria from Treated versus Negative Control samples at specified contact time has been calculated.</p>		

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CALCULATION	<p>Number of colonies recorded in plates containing 30 to 300 colonies has been used for calculation. If the number of colonies in plates containing the 1 ml aliquots of undiluted recovered from specimen is <30, this number is used. When there are no colonies recovered in any plates the number of colonies is considered as “<1”.</p> <p>For each test specimen, the number of viable bacteria recovered has been calculated according with following equation:</p> $N = (100 \times C \times D \times V)/A$ <p>where <i>N</i> is the number of viable bacteria recovered per cm² per test specimen; <i>C</i> is the average plate count for the duplicate plates; <i>D</i> is the dilution factor for the plates counted; <i>V</i> is the volume, in ml, added to the specimen; <i>A</i> is the surface area, in mm², of the cover film. The geometric mean of the number of viable bacteria recovered for each set of test specimens has been calculated and this value expressed to two significant figures.</p>
ASSAY VALIDITY CRITERIA	<p>When the three conditions are satisfied, the test is deemed valid. If any of these conditions are not met, the test is not considered valid and the specimens shall be retested.</p> <p>1) The logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement:</p> $(L_{max} - L_{min})/(L_{mean}) \leq 0,2$ <p>where - <i>L</i>_{max} is the Log of the maximum number of viable bacteria found on a specimen; - <i>L</i>_{min} is the Log of the minimum number of viable bacteria found on a specimen; - <i>L</i>_{mean} is the Log of the mean number of viable bacteria found on the specimens.</p> <p>2) The average number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall be within the range 6,2×10³ cells/cm² to 2,5×10⁴ cells/cm².</p> <p>3) The number of viable bacteria recovered from each untreated test specimen at 4 h shall not be less than 6,2×10¹ cells/cm².</p>
Calculation of the Antibacterial Activity	<p>When the test is deemed valid, the antibacterial activity is calculated using following formula:</p> $R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$ <p>Where: <i>R</i> is the antibacterial activity; <i>U</i>₀: average of the Log cells/cm², recovered from untreated test specimens at t₀; <i>U</i>_t: average of the Log cells/cm², recovered from untreated test specimens after 4 h; <i>A</i>_t: average of the Log cells/cm², recovered from treated test specimens after 4 h.</p>
Antibacterial effectiveness	<p>The value of the antibacterial activity can be used to characterize the effectiveness of an antibacterial agent. According to ISO 22196:2011, the antibacterial-activity values used to define the effectiveness shall be agreed upon by all interested parties.</p>

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RESULTS	Assay Validity Criteria were satisfied. The number of viable bacteria in the test inoculum and average number of viable bacteria recovered from each specimen (expressed as cfu/cm ²) and the values of U_0 , U_t and A_t , and the antibacterial activity calculated are reported:																																
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