HAMILTON

ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 399165/20/POZ

A) IDENTYFICATION OF THE SAMPLE:	
Name of the product	**PP 5% - 6 pieces of the test sample and 12 pieces of the control sample (without anti-bacterial properties)** **Lot: 1** **Production date: 07-08-2020** **Expiration date: 11-08-2021**
Composition of the antibacterial coat	-
B) TEST METHOD :	
Method	**ISO 22196:2011 – Plastics- Measurement of antibacterial activity on plastics surfaces**
Neutralizer	SCDLP (Soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate)
C) EXPERIMENTAL CONDITIONS:	
Surface	50 mm x 50 mm, covered with a film size of 40 mm x 40 mm. Test and control samples were placed in sterile petri dishes.
Assay period	22/09/2020 – 25/09/2020
Temperature of incubation	35 ± 1 ° C/ 24h
Identyfication of the bacterial strains used	*Escherichia coli* ATCC 8739 *Staphylococcus aureus* ATCC 6538
Special remarks	The test was performed in several repetitions. Test samples were used to determine amount of live cells of bacteria.
Conclusion	The sample shows antibacterial activity against the used reference strains.

Date: 14.06.2021

Authorized by: Daria Depa, Analyst Specialist, Cosmetics Microbiology Laboratory
Approved by: Hanna Wachowska, Laboratory Director *(Approved with qualified electronic signature)*

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RESULTS

Inoculum:
E.coli 5,5 x10^5 cfu/ml
Staphylococcus aureus 9,4 x 10^5 cfu/ml

The number of vaiable bacterial on the control sample immediately after inoculation:
E.coli (U_0 E.coli)
1,5 x 10^5 cfu/ cm^2 = 5,18 log
1,1 x 10^5 cfu/ cm^2 = 5,05 log
1,6 x 10^5 cfu/ cm^2 = 5,19 log

Staphylococcus aureus (U_0 S.aureus)
4,8 x 10^5 cfu/ cm^2 = 5,68 log
3,3 x 10^5 cfu/ cm^2 = 5,51 log
3,3 x 10^5 cfu/ cm^2 = 5,52 log

The number of vaiable bacterial on the control sample after incubation for 24h:
E.coli (Ut E.coli)
4,6 x 10^6 cfu/ cm^2 = 6,66 log
1,1 x 10^7 cfu/ cm^2 = 7,04 log
3,4 x 10^6 cfu/ cm^2 = 6,53 log

Staphylococcus aureus (Ut S.aureus)
9,3 x 10^6 cfu/ cm^2 = 6,97 log
1,1 x 10^7 cfu/ cm^2 = 7,02 log
8,6 x 10^6 cfu/ cm^2 = 6,93 log

The number of vaiable bacterial after inoculation (24h 35 ± 1° C) (test sample):
E.coli (At E.coli)
1,0 x 10^1 cfu/cm^2 = 1,00 log
1,0 x 10^1 cfu/cm^2 = 1,00 log
1,0 x 10^1 cfu/cm^2 = 1,00 log

Staphylococcus aureus (At S.aureus)
1,0 x 10^1 cfu/cm^2 = 1,00 log
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CALCULATIONS

Antimicrobial efficacy (R) is determined by reducing the number of vaiable bacterial cells in the control sample (Ut) and the tested sample (At).

$R= (Ut – U_0) – (At - U_0) = Ut - At$

E.coli

R= (6,66-5,18)-(1,00-5,18)=1,48-(-4,18)=5,66

R=(7,04-5,05)-(1,00-5,05)=1,99-(-4,05)=6,04

R= (6,53-5,19)-(1,00-5,19)=1,34-(-4,19)=5,53

S.aureus

R= (6,97-5,68)-(1,00-5,68)=1,29-(-4,68)=5,97

R= (7,02-5,51)-(1,00-5,51)= 1,51 -(-2,94)= 4,60

R= (6,93-5,52)-(1,00-5,52)= 1,41-(-4,52)=5,93

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