HAMILTON

ENCLOSURE No. 1 TO REPORT OF ANALYSIS NO. 28080/21/JSHS/Z1

A) IDENTYFICATION OF THE SAMPLE:	
Name of the product	**JUPOL Antimicrob - JUPOL Protect**
Expiration date	Not indicated
The active substance	Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides: CAS: 68424-85-1; 0.25%.
B) TEST METHOD :	
Method	**ISO 22196:2011**
C) EXPERIMENTAL CONDITIONS:	
Assay period	23/02/2021 – 28/02/2021
Contact time	24 hours
Assay temperature	35ºC ± 1ºC
Incubation temperature	35ºC ± 1ºC
Relative humidity during the incubation	>90%
Identification of the strains used	Staphylococcus aureus CECT-240 (ATCC-6538-P) Escherichia coli CECT-516 (ATCC-8739)
Inoculum coating with film	Yes

Results of the assay

- Controls and validation. ..... See tables 1, 2, 4 and 5 attached.
- Evaluation of bactericidal activity ..... See tables 3 and 6 attached.

Special remarks

Not required.

Date: 24.03.2021

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

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Conclusion

The product **JUPOL Antimicrob= JUPOL Protect t**, batch Lab. sample, shows an antibacterial activity index (R) of **> 3.31** when inoculated with a suspension of *Staphylococcus aureus* CECT-240 (ATCC 6538-P) and shows an antibacterial activity index (R) of **> 3.97** when it is inoculated with a suspension of *Escherichia coli* CECT-516 (ATCC 8739), on the test surface, when compared with non-additive containing pieces **JUB 0**, and tested in accordance with ISO 22196: 2011 standards.

Note: The results obtained correspond to the product received in the laboratory.

Comments.

The antibacterial activity index (R) represents the difference between the number of bacteria of each species on the surface treated with antibacterial material and the surface without antibacterial material, after incubation at 35°C ± 1°C for 24 hours. The higher it is the index value, the more antibacterial activity it has the antibacterial material. The standard ISO 22196: 2011 does not indicate ranges of values to consider a product that is very/ not very effective, depending on the values obtained for the antimicrobial activity index (R).

The standard JIS Z 2801: 2010+ A2012 states that the antimicrobial efficacy (R) obtained with the mentioned standard must not be lower than 2.0.

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Experimental results with *Staphylococcus aureus* CECT-240 (ATCC-6538P)

Table 1.- Validation and controls.

Test pieces without treatment after inoculation	Piece no. 1		Piece no.2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	106	107	99	105	112	100
10^{-3}	<30	<30	<30	<30	<30	<30
Value *N*	6.66×10^3		6.38×10^3		6.63×10^3	
Average *N*	6.56×10^3					
U_0	3.66		3.74		3.68	
Average U_0	3.69					
(Lmax-Lmin)/(Lmean) ≤ 0.2? Yes $6.2 \times 10^3 \leq$ Average $N \leq 2.5 \times 10^4$? Yes						

Table 2.- Suspension of assay.

Suspension of assay (*N*)	*N*	Vc_1	Vc_2	$X = 2.68 \times 10^5$ $2.5 \times 10^5 \leq X \leq 10 \times 10^5$? Yes
	10^{-3}	267	268	
	10^{-4}	28	26	

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Table 3.- Results of the activity test with the product.

Test pieces without treatment after incubation	Piece no. 1		Piece no.2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_1	Vc_2	Vc_1
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	145	168	140	128	123	131
10^{-3}	<30	<30	<30	<30	<30	<30
Value Ut	3.99		3.92		3.90	
Nº. bacteria	≥ 6.2 x 10^1? Yes		≥ 6.2 x 10^1? Yes		≥ 6.2 x 10^1? Yes	
Average Ut	3.94					
Test pieces with treatment after incubation	**Piece no. 1**		**Piece no.2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_1	Vc_2	Vc_1
10^{-1}	<1	<1	<1	<1	<1	<1
10^{-2}	<1	<1	<1	<1	<1	<1
10^{-3}	<1	<1	<1	<1	<1	<1
Value At	<0.63		<0.63		<0.63	
Average At	<0.63					
Antimicrobial activity (R)	$R = (Ut-U_0) - (At - U_0) = Ut - At = >3.31$					

Explanations:

R: antibacterial activity;

***U₀*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the untreated test samples immediately after inoculation;

***Ut*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the inoculated untreated test samples after 24 hours;

***At*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the inoculated test samples treated after 24 hours;

***Lmax*:** maximum logarithm (e.g. logarithm base 10) number of viable bacteria found in a test piece;

***Lmin*:** minimum logarithm (e.g. logarithm base 10) number of viable bacteria found in a test piece;

***Lmean*:** average of logarithm (e.g. logarithm base 10) numbers of viable bacteria found in the three test pieces;

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Test results with *Escherichia coli* CECT-516 (ATCC 8739).

Table 4.- Validation and controls.

Test pieces without treatment after inoculation	Piece no. 1		Piece no.2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	154	133	125	116	160	145
10^{-3}	<30	<30	<30	<30	<30	<30
Value *N*	8.95×10^3		7.53×10^3		9.52×10^3	
Average *N*	8.60×10^3					
U_0	3.95		3.88		3.98	
Average U_0	3.94					
(Lmax-Lmin)/(Lmean) ≤ 0.2? Yes $6.2 \times 10^3 \leq$ Average $N \leq 2.5 \times 10^4$? Yes						

Table 5.- Suspension of assay.

Suspension of assay (*N*)	*N*	Vc_1	Vc_2	$X = 4.35 \times 10^5$ $2.5 \times 10^5 \leq X \leq 10 \times 10^5$? Yes
	10^{-3}	>300	>300	
	10^{-4}	45	42	

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Table 6.- Results of the activity test with the product.

Test pieces without treatment after incubation	Piece no. 1		Piece no.2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_1	Vc_2	Vc_1
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	>300	>300	>300	>300	>300	>300
10^{-3}	47	61	56	68	71	79
Value Ut	4.53		4.59		4.67	
Nº. bacteria	$\geq 6.2 \times 10^1$? Yes		$\geq 6.2 \times 10^1$? Yes		$\geq 6.2 \times 10^1$? Yes	
Average Ut	4.60					
Test pieces with treatment after incubation	**Piece no. 1**		**Piece no.2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_1	Vc_2	Vc_1
10^{-1}	<1	<1	<1	<1	<1	<1
10^{-2}	<1	<1	<1	<1	<1	<1
10^{-3}	<1	<1	<1	<1	<1	<1
Value At	<0.63		<0.63		<0.63	
Average At	<0.63					
Antimicrobial activity (R)	$R = (Ut\text{-}U_0) - (At - U_0) = Ut - At = >3.97$					

Explanations:

R: antibacterial activity;

***U_0*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the untreated test samples immediately after inoculation;

***Ut*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the inoculated untreated test samples after 24 hours;

***At*:** average of logarithm numbers of viable bacteria in CFU/ cm^2, recovered from the inoculated test samples treated after 24 hours;

***Lmax*:** maximum logarithm (e.g. logarithm base 10) number of viable bacteria found in a test piece;

***Lmin*:** minimum logarithm (e.g. logarithm base 10) number of viable bacteria found in a test piece;

***Lmean*:** average of logarithm (e.g. logarithm base 10) numbers of viable bacteria found in the three test pieces;

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