



Eurochem Ricerche S.r.l.
Analisi, ricerca e sviluppo, consulenza
Viale del Lavoro, 6 – 35035 Mestrino (PD) – ITALY
Tel. +39 049 9002844 – Fax +39 049 6458057
info@eurochemricerche.it www.eurochemricerche.it

Study n°RAP01410

Mestrino, March 13th 2020

<p align="center">ASSESSMENT OF THE BACTERICIDAL ACTIVITY ON STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA according to EN 1040mod.</p>

Study N°: RAP01410

Manufacturer/Supplier: ONE MORE SRL
STRADA ACQUASALATA, 5A
00000 47899 SERRAVALLE SM
SAN MARINO

Sample: GEL IGIENIZZANTE 64% ALCOHOL

Batch: 021006 DEL 02.023.20

Eurochem Ricerche S.r.l. ref: ACC01246

Storage conditions: room temperature
away from light

Appearance of the product

Physical form Gel
Colour Colorless

Active substances and their
concentrations: Alcohol 64%

Periodo of testing:

Acceptance date: 03/05/2020
Analysis date : 03/11/2020
Performed by A. Agostini
Sector manager D.ssa Chiara Cuba



Eurochem Ricerche S.r.l.

Analisi, ricerca e sviluppo, consulenza

Viale del Lavoro, 6 – 35035 Mestrino (PD) – ITALY

Tel. +39 049 9002844 – Fax +39 049 6458057

info@eurochemricerche.it www.eurochemricerche.it

Study n°RAP01410

PURPOSE

The assessment of the bactericidal activity of the product analyzed in the present study was conducted in relation to its ability to reduce the viability of two bacterial strains in vitro, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The microbial strains indicated were put in contact with the product for 1 minute. At the end of the contact time, the titer of bacteria remained alive is compared to those present at the beginning of the test itself.

EXPERIMENTAL PART

MATERIALS

Strains used and growing conditions:

TEST MICROORGANISMS	GROWTH CONDITIONS
<i>Staphylococcus aureus</i> DSM799	Tryptone Soy Agar (37°C; 24-48 hours)
<i>Pseudomonas aeruginosa</i> DSM 939	Tryptone Soy Agar (37°C; 24-48 hours)

METHOD

An aliquot of 20g of product to be tested is prepared in a sterile container and inoculated with 0.2 ml of microbial suspension at a concentration equal to 10^7 - 10^8 CFU / ml. Product inoculated with the bacterial strain is kept at 25 ° C in the dark and analyzed after 1 minute. At the end of the contact time, the antibacterial activity of the product is stopped by neutralization and the titer of the bacteria left alive is determined, making sowings in scaled dilutions with a step of 1:10 on semi-solid culture medium. At the end of the incubation period, necessary for the growth of plate colonies, the number of colony forming Units per ml or per gram (Ufc / ml or Ufc / g) present at the end of the contact period between product and microbial strain is evaluated.

RESULTS

For the contact time considered, the bacterial count was assessed at the end of the incubation period. The results was compared with the bacteria count at the time of inoculation. Comparing the data it was possible to determine the vitality reduction factor (R), with logarithmic expression. The reduction of bacterial load was also expressed as a percentage (R%), corresponding to the percentage difference in the bacterial load between the moment of inoculation and the end of the contact period between product microbial strain.



Eurochem Ricerche S.r.l.

Analisi, ricerca e sviluppo, consulenza

Viale del Lavoro, 6 – 35035 Mestrino (PD) – ITALY

Tel. +39 049 9002844 – Fax +39 049 6458057

info@eurochemricerche.it www.eurochemricerche.it

Study n°RAP01410

Test organism: *Staphylococcus aureus*

Validation and controls

Validation suspension			Neutralizer control			Method validation		
(A)			(B)			(C)		
Vc1	87	A=96	Vc1	111	B =97	Vc1	89	C =95
Vc2	105		Vc2	83		Vc2	101	
			B ≥ 0.5 A ?			C ≥ 0.5 A ?		
			Yes:	X	No:	Yes:	X	No:

Test suspension and test

Test suspension:	N	Vc1	Vc2	Average = 46.5 x 10 ⁷ , lg N = 8.66
	10 ⁻⁶	>330	>330	No = N/100; lg No = 7.66
	10 ⁻⁷	48	45	

Test

GEL IGIENIZZANTE 64% ALCOHOL	Contact time	Na	lg Na	lg R	R%
	1 min	<10	<1.00	>6.66	>99,99998%

Test organism: *Pseudomonas aeruginosa*

Validation and controls

Validation suspension			Neutralizer control			Method validation		
(A)			(B)			(C)		
Vc1	122	A=118	Vc1	110	B =103	Vc1	93	C =93.5
Vc2	114		Vc2	96		Vc2	94	
			B ≥ 0.5 A ?			C ≥ 0.5 A ?		
			Yes:	X	No:	Yes:	X	No:

Test suspension and test

Test suspension:	N	Vc1	Vc2	Average = 61.0 x 10 ⁷ , lg N = 8.78
	10 ⁻⁶	>330	>330	No = N/100; lg No = 7.78
	10 ⁻⁷	52	70	

Test

GEL IGIENIZZANTE 64% ALCOHOL	Contact time	Na	lg Na	lg R	R%
	1 minuto	<10	<1.00	>6.78	>99,99998%

Explanations::

Vc = count per ml

Average = mean of Vc1 and Vc2

No = number of cells per ml in the mixtures at the beginning of the contact time

Na = number of survivors per ml in the test mixtures at the end of the contact time (1 min)

R = viability reduction (lg R = lg No – lg Na)



Eurochem Ricerche S.r.l.

Analisi, ricerca e sviluppo, consulenza

Viale del Lavoro, 6 – 35035 Mestrino (PD) – ITALY

Tel. +39 049 9002844 – Fax +39 049 6458057

info@eurochemricerche.it www.eurochemricerche.it

Study n°RAP01410

CONCLUSION

The study carried out has made it possible to verify the antibacterial efficacy of a product called **GEL IGIENIZZANTE 64% ALCOHOL** (Batch **021006 of 02.023.20**) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The product demonstrated a significant reduction in bacterial viability, under the conditions described above after 1 minute of contact (>99.99998%).

Laboratory Manager

Dr. Chiara Cuba

Laboratory Director

Stefano Pandolfo