Final Report 2010/968i

ANTIMICROBIAL ACTIVITY AGAINST Staphylococcus aureus MRSA ON DRY DISINFECTANT

Study program: 2010/968 SAM

Contract n: PARA2010002003

Customer: EUROFINS PRODUCT TESTING A/S DK
SMEDESKOVVEJ 38
DK 8464 – GALTEN (DENMARK)

Test substance: DRY DISINFECTANT (G046-0001)

Study Director: Laura Brambilla ..... Released on: Oct 25th 2010
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SUMMARY

An assay was conducted on test substance DRY DISINFECTANT (G046-0001) in order to determine its antimicrobial effectiveness against *Staphylococcus aureus MRSA*, according to the protocol provided by the Sponsor.

For this purpose the microbial strain *Staphylococcus aureus MRSA* ATCC 33592 was exposed to the test substance at the following conditions:
- final concentrations: neat (0.310 g/carrier)
- contact times: 30 minutes - 8 hours - 24 hours
- temperature: room temperature (20 – 25°C)

The test was performed on paper carriers.

After sterilization, 2×2 cm paper squares were contaminated with an inoculum of *Staphylococcus aureus MRSA* and were then placed in sterile petri plates (three carriers for each plate). One set of squares were treated with the test substance and one set were left untreated as a control.
At different exposure times (30 minutes, 8 hours, 24 hours), three paper squares exposed to the test substance were removed from each petri plate and assayed for the microbial viable count.
The same procedure was performed for the untreated control.
The test was performed in moist conditions to simulate the probable environmental use conditions.

See Experimental Report 2010/968 for more details.

After the incubation time of 37°C ±1°C for 48 hours, the results are expressed as *Percent Kill (% Kill)* and *Percent Inhibition (%Inhibition)*; they are listed in the following table.

Exposure time	Average % Kill	Average % Inhibition
30 minutes	99.006	98.991
8 hours	>99.999	>99.993
24 hours	>99.999	>99.994

On the basis of obtained results, in compliance with the assay validity criteria, the test substance DRY DISINFECTANT (G046-0001) **causes a reduction >99%** calculated versus the time zero, against *Staphylococcus aureus MRSA* ATCC33592, after 30 minutes of contact and **causes a reduction >99.99%** calculated versus the time zero, against *Staphylococcus aureus MRSA* ATCC33592, after 8 hours of contact, in compliance with the provisions of the Sponsor method.
The test substance DRY DISINFECTANT (G046-0001) **causes an inhibition >90%** calculated versus each exposure time, against *Staphylococcus aureus MRSA* ATCC33592, after 30 minutes of contact, and **causes an inhibition >99.99%** calculated versus each exposure time, against *Staphylococcus aureus MRSA* ATCC33592, after 8 hours of contatct, in compliance with the provisions of the Sponsor method.

INTRODUCTION

A study was conducted on behalf of EUROFINS PRODUCT TESTING A/S DK in order to evaluate the antimicrobial effectiveness, in compliance with the protocol provided by the Sponsor.

The study was performed at the Test Facility Eurofins Biolab S.r.l. of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Antimicrobial activity	October 21st 2010	October 23rd 2010	C. Meroni

In this report:
- doses are expressed as milliliter (ml) of the test substance for 100 milliliter (ml) of water (%)
- the number of micro organisms, counted in colony-forming units per ml of test solution, are expressed as colony-forming units per ml (cfu/ml).

REFERENCES

1. Jennifer Dunham, B.S. Microchem Laboratory, Inc. – *The antimicrobial Activity of Stalosan-F and Various Competitive Products in Moist Conditions Using S. aureus Test 2* (Project ID Numbers 050405-1, 050412-2) – April 21, 2005.
2. EN 13697 August 2001 – Chemical disinfectants and antiseptics – Quantitative test for not porous surfaces for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2/step2).

FILING

The study program, all raw data are filed in the archives of Eurofins Biolab S.r.L for ten years after the issuing of the final report.
No retained sample will be kept.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the Eurofins Biolab S.r.L Procedures Manual.

TEST SUBSTANCE

The test substance consisted of a product used to sanitize and deodorize the animal quarters.

Name	DRY DISINFECTANT (G046-0001)
Stability	Not provided
Composition	Not provided

TESTED SAMPLE

The analysed sample, representative of the test substance, consisted of a brown powder contained in a plastic bag.

Batch	Not provided
Manufacture date	Not provided
Expiry date	Not provided
CoA – UDN#: M1318-003v1.0	Not provided
Receiving n.	EUITVI-11886
Receiving date	October 11th 2010
Id number	10.1403-S

Characterization of the test substance is under Sponsor's responsibility.

Experimentation Report 2010/968 – EVALUATION OF ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS MRSA (Sponsor method)

EXPERIMENTAL PROCEDURE

1 ASSAY SYSTEM

Microorganisms
The following test strain was used:
Staphylococcus aureus MRSA ATCC 33592

Conservation
The bacterial strain was kept frozen; before use, it was transplanted on TSA with sheep blood and kept in a refrigerator at 5°C ±3°C.

Preparation of the bacterial suspension
The bacterial strain was transplanted on TSA with sheep blood twice consecutively and incubated at 37°C ±1°C for 48 hours in microaerophilic conditions.
Within two hours from the beginning of the test, the final culture was suspended in the diluent using glass beads, and the suspension was diluted to a concentration of $1.5x10^8$-$5.0x10^8$ cfu/ml.
The colony number was determined performing the counting.

2 CULTURE MEDIA AND REAGENTS

Tryptone Soya Agar (TSA) MERCK

Diluent

Tryptone, pancreatic digestion of casein	1.0 g	MERCK
NaCl	8.5 g	MERCK
Distilled water q.s. to	1000 ml	

Sterile water for injection (WFI) EUROSPITAL

Neutraliser
The following neutraliser was selected:

Lecithin	3 g	MERCK
Polysorbate 80	30 g	MERCK
Sodium Thiosulfate	5 g	MERCK
L-histidine	1 g	MERCK
Saponin	30 g	SIGMA

Tryptone-treated water (q. s.) to 1000 ml

3 EQUIPMENT

Standard microbiology laboratory equipment.

Dry sterilization oven	MEMMERT
Steam autoclave	FEDEGARI
Incubator	MEMMERT
pHmeter	BECKMAN
Vortex stirrer	VELP
Chronometer	ARBORE
Micropipettes	GILSON
Petri plates 90 mm	GHIARONI
Paper squares (2x2 cm)	GHIARONI

4 EXPERIMENTAL DESIGN

Test temperature
The test was performed at room temperature (20 – 25°C).

Test conditions
The test substance was tested at the following test conditions:
- concentration: neat (0.310 g/carrier)
- contact times: 30 minutes – 8 hours – 24 hours

Carriers
Paper squares (2x2 cm) had been used to the performing the assay.

5 EXECUTION OF THE ASSAY

Assay
After sterilization, 2x2 cm paper squares were contaminated with 0.05 ml of the suspension 1.5 $x10^8$-5.0$x10^8$ cfu/ml and were then placed in sterile petri plates (three carriers for each plate).
One set of squares (3 plates, 9 carriers) were treated with the test substance that was sprinkled uniformly on the surface of the plate with a final dosage of 0.310 g/petri plate.
One set (4 plates, 12 carriers) were left untreated as a control.
At various exposure times (30 minutes, 8 hours, 24 hours), three paper squares exposed to the test substance were removed from each petri plate and tranferred to a test tube containing 10 ml of neutraliser and 5 g of glass beads. The test tubes had been strongly stirred on a vortex mixer in order to take off the bacteria from the carrier.
After a neutralization time of 5 minutes, the mixture had been diluted with serial ten-fold dilutions and 0.5 ml from each dilution had been transferred onto the surface of Tryptone Soya Agar (TSA) plate.
The plates were incubated at 37±1°C for 48 hours.
The same procedure was performed for the untreated control, including a zero time point, to evaluate the number of surviving cfu at different exposure times.
At the end of the incubation time, the number of cfu/plate was determined and multiplied by the appropriate dilution factor to determine the number of surviving cfu for each carrier.
All the test was performed in moist conditions to simulate the probable environmental use conditions.

Validation of neutralization and viability

Two 2x2 cm sterile paper squares were soaked in sterile deionized water for about 60 seconds. The carriers were then placed in a sterile plate and quickly sprinkled with the test substance.
Each square was transferred to a test tube containing 10 ml of neutraliser and 5 g of glass beads. The test tubes had been strongly stirred on a vortex mixer in order to take off the bacteria from the carrier.
After a neutralization time of 5 minutes, the mixture had been diluted with serial ten-fold dilutions and each tube had been inoculated with approximatly 1000 cfu/ml of the test strain; 0.5 ml from each dilution had been transferred onto the surface of Tryptone Soya Agar (TSA) plate.
The plates were incubated at 37±1°C for 48 hours.
At the same time two tubes of 10 ml of neutralizer were inoculated with approximatly 1000 cfu/ml of the test strain; 0.5 ml of this suspension had been transferred onto the surface of Tryptone Soya Agar (TSA) plate. The plates were incubated at 37±1°C for 48 hours.

6 CALCULATION AND EXPRESSION OF THE RESULTS

After the incubation time, the results are expressed as *Percent Kill (% Kill)* and *Percent Inhibition (%Inhibition)*:

$$\% \; Kill = \left(\frac{S_0 - S}{S_0} \right) \times 100$$

S = number of surviving cfu after exposure to the test substance
S_0 = original number of cfu at the time zero (control)

$$\% \; Inhibition = \left(\frac{S_T - S}{S_T} \right) \times 100$$

S = number of surviving cfu after exposure to the test substance
S_T = number of cfu at each exposure time (control)

ASSAY VALIDITY CRITERIA

In the validation of neutralization and viability test, similar numbers of colonies on all plates must occur for a valid assay.

RESULTS

Validation of neutralization and viability
The validation test complies with the assay validity criteria.

Assay
The average Percent Kill (% Kill) and average Percent Inhibition (%Inhibition) at the different contact times are listed in the following table:

Exposure time	Average % Kill	Average % Inhibition
30 minutes	99.006	98.991
8 hours	>99.999	>99.993
24 hours	>99.999	>99.994

DEVIATIONS

No deviations occured during the study.

CONCLUSIONS

On the basis of obtained results, in compliance with the assay validity criteria, the test substance DRY DISINFECTANT (G046-0001) **causes a reduction >99%** calculated versus the time zero, against *Staphylococcus aureus MRSA* ATCC33592, after 30 minutes of contact and **causes a reduction >99.99%** calculated versus the time zero, against *Staphylococcus aureus MRSA* ATCC33592, after 8 hours of contact, in compliance with the provisions of the Sponsor method.
The test substance DRY DISINFECTANT (G046-0001) **causes an inhibition >90%** calculated versus each exposure time, against *Staphylococcus aureus MRSA* ATCC33592, after 30 minutes of contact, and **causes an inhibition >99.99%** calculated versus each exposure time, against *Staphylococcus aureus MRSA* ATCC33592, after 8 hours of contatct, in compliance with the provisions of the Sponsor method.

ATTACHMENTS

ATTACHMENT	TITLE	NUMBER OF PAGES
N.1	RAW DATA EXPERIMENTATION 2010/968	1

eurofins | biolab	*1. Jennifer Dunham, B.S. Microchem Laboratory, Inc. – The antimicrobial Activity of Stalosan-F and Various Competitive Products in Moist Conditions Using S. aureus Test 2*
Project ID Numbers 050405-1, 050412-2	

ID. studio *(ID. Study)*: 2010/968 SAM

Data inizio *(Started on)*: 21/10/10 ID. campione *(ID. sample)*: 10.1403-S

Counting of the test strain

Microrganisms test	-7	-7	ufc/ml
Staphylococcus aureus MRSA ATCC33592	49	46	4.8E+08

Table n. 1: COUNTING FOR THE VALIDATION TEST (ufc/plate)

Microrganisms test	-1	-1	-1	AVERAGE
	NEUTRALIZATION (Counting in the presence of the product)			
Staphylococcus aureus MRSA ATCC33592	40	30	36	35
	VIABILITY (Counting in the absence of the product)			
	39	42	30	37

Table n. 2: MICROBIAL COUNTING VALUES (ufc/carrier)

Staphylococcus aureus MRSA ATCC33592				2.38E+07		
Contact time	**CONTROL**			**TEST**		
	CARRIER 1	*CARRIER 2*	*CARRIER 3*	*CARRIER 1*	*CARRIER 2*	*CARRIER 3*
T0	9.9E+05	1.2E+06	1.2E+06	n.d.	n.d.	n.d.
30 minutes	7.4E+05	1.4E+06	1.2E+06	2.6E+03	2.1E+03	2.9E+04
8 hours	3.0E+05	1.7E+05	2.1E+05	< 1.5E+01	< 1.5E+01	< 1.5E+01
24 hours	2.4E+05	2.8E+05	2.2E+05	< 1.5E+01	< 1.5E+01	< 1.5E+01

n.d.: non determinato

Table n. 3: AVERAGE COUNT FOR THE ASSAY (cfu/carrier)

Contact time	CONTROL	TEST
T0	1.1E+06	n.d.
30 minutes	1.1E+06	1.1E+04
8 hours	2.3E+05	> 1.5E+01
24 hours	2.5E+05	> 1.5E+01

n.d.: non determinato

Table n. 4: THE AVERAGE % KILL AND THE AVERAGE % INHIBITION AT THE DIFFERENT CONTACT TIME

Contact time	AVERAGE % KILL	AVERAGE % INHIBITION
30 minutes	99.006	98.991
8 hours	> 99.999	> 99.993
24 hours	> 99.999	> 99.994

Sigla tecnico *(Technician signature)*: Data fine *(Finished on)*: 23/10/10

Sigla Approvazione *(Approval signature)*: Data *(Date)*: 23/10/10