

## **Test Facility**

Report No.: Version: Page: Print date: 2010/968 SAMi English 1 of 9 Oct 25<sup>th</sup> 2010

Final Report 2010/968i

# ANTIMICROBIAL ACTIVITY AGAINST Staphylococcus aureus MRSA ON DRY DISINFECTANT

Study program:	2010/968 SAM
Contract n:	PARA2010002003
<u>Customer</u> :	EUROFINS PRODUCT TESTING A/S DK SMEDESKOVVEJ 38 DK 8464 – GALTEN (DENMARK)
<u>Test substance</u> :	DRY DISINFECTANT (G046-0001)
Study Director: Laws Fonawh (Dr. L. Brambilla)	rllen Released on: Oct 25th 2000

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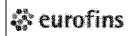
Eurofins Biolab S.r.l. Società con Socio unico sottoposta

a direzione e coordinamento della società
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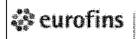
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#### **SUMMARY**

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  $\pm$ 1°C for 48 hours, the results are expressed as *Percent Kill (% Kill)* and *Percent Inhibition (%Inhibition)*; they are listed in the following table.

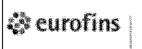
Exposure time	Average % Kill	Average % Inhibition
30 minutes	99.006	98.991
8 hours	>99.999	>99.993
24 hours	>99.999	>99.994

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 contact, in compliance with the provisions of the Sponsor method.

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#### 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 21 <sup>st</sup> 2010	October 23 <sup>rd</sup> 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.
- 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.

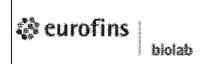
No retained sample will be kept.

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.

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#### **TEST SUBSTANCE**

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

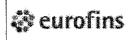
Name	DRY DISINFECTANT (G046-0001)  Not provided	
Stability		
Composition	Not provided	

#### **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 11 <sup>th</sup> 2010	
ld number	10.1403-S	

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



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## 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<sup>8</sup>-5.0x10<sup>8</sup> cfu/ml.

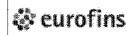
The colony number was determined performing the counting.

#### 2 CULTURE MEDIA AND REAGENTS

Tryptone Soya Agar (TSA)		MERCK
Diluent Tryptone, pancreatic digestion of casein NaCl Distilled water q.s. to	1.0 g 8.5 g 1000 ml	MERCK MERCK
Sterile water for injection (WFI)		EUROSPITAL
Neutraliser The following neutraliser was selected: Lecithin Polysorbate 80 Sodium Thiosulfate L-histidine Saponin Tryptone-treated water (q. s.) to 1000 in	3 g 30 g 5 g 1 g 30 g ml	MERCK MERCK MERCK MERCK SIGMA

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#### 3 **EQUIPMENT**

Standard microbiology laboratory equipment.

Dry sterilization oven
Steam autoclave
Incubator
pHmeter
Vortex stirrer
Chronometer
Micropipettes
Petri plates 90 mm
Paper squares (2x2 cm)

MEMMERT FEDEGARI MEMMERT BECKMAN VELP ARBORE GILSON GHIARONI GHIARONI

#### 4 EXPERIMENTAL DESIGN

#### Test temperature

The test was performed at room temperature  $(20 - 25^{\circ}C)$ .

#### 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<sup>8</sup>-5.0x10<sup>8</sup> 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.

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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.

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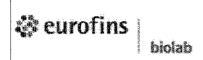
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#### **RESULTS**

#### 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 >99.993	
24 hours	>99.999 >99.9		

#### **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

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

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)

	-1	-1	-1	AVERAGE
Microrganisms test —	NEUTRALIZATION (Counting in the presence of the product)			
	40	30	36	35
Staphylococcus aureus MRSA ATCC33592	VIABILITY (Counting in the absence of the product)			oduct)
WINGA ATOCOSOS2	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):

Sigla Approvazione (Approval signature):

Data fine (Finished on): 23/10/10

Data (Date): 23/10/10