

Test Facility Certification M.S. 85/2009

Report No.: Version: Page: Print date: 2010/882 SAMi English 1 of 11 Sept 22nd 2010

Final Report N. 2010/882 SAMi

SUSPENSION BACTERICIDAL EFFECTIVENESS FOR VETERINARY FIELD ON SAGEWASH SANITIZER 250ppm

Study Program No:

2010/882 SAM

Contract No:

PPR12010029702

Sponsor:

EUROFINS LABORATORIES LTD D3 Broadoak Business Park AshburT Road West, Trafford Park (M17 1RW Manchester, UK)

Test substance:

SAGEWASH SANITIZER 250ppm



Date of issue: Sept 12 Mad 200

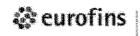
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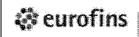


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SUMMARY

A series of assays were conducted on the test substance SAGEWASH SANITIZER 250ppm in order to determine its bactericidal effectiveness for the uses for which the product is specifically intended. The test substance, consists of white powder compressed in a cylindrical form that was put in a spray gun before the use. According to the Sponsor instructions, this device was connected to the tap water and the test solution was obtained after 10 minutes of supply.

The bactericidal effectiveness has been verified with the following experimentation:

- phase 2/step 1 bactericidal activity in suspension for veterinary field Method of dilutionneutralization in which 4 different bacterial strains, Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442, Proteus vulgaris ATCC 13315, Enterococcus hirae ATCC 10541, have been exposed to the following conditions test:
- final test concentrations: as such (80% maximum concentration testable) 50% 25%
- contact time: 5 minutes
- test temperature: 30°C ± 1°C

A solution of skim milk has been used as interfering substance with final concentration of 1%.

On the basis of obtained results, in compliance with the assay validity criteria, the test substance SAGEWASH SANITIZER 250ppm results **BACTERICIDAL** with the concentration of 80% after 5 minutes of contact, using a 1% final concentration of skim milk, in compliance with the provisions of EN1656:2000.

See Experimental Report 2010/882 for more details.

INTRODUCTION

A study has been conducted on behalf of EUROFINS LABORATORIES Ltd in order to prove the bactericidal and effectiveness in compliance with European regulations.

The study was performed at the Test Facility Biolab S.p.A. of Vimodrone (MI) - via B. Buozzi n. 2 (Italy).

In this report:

- Doses are expressed as grams of the test substance for 100 milliliters of water (%)
- the number of microorganisms, counted in colony-forming units per milliliter of test solution, are expressed as colony-forming units per milliliter (cfu/ml).

EXPERIMENTATION	START	END	RESEARCHER
Bactericidal activity in suspension for veterinary field — Method of dilution-neutralization	Sept 17 th 2010	Sept 20 th 2010	C. Meroni

TERMS AND DEFINITIONS

Bactericidal:

a chemical agent or formulation, which kills vegetative bacterial forms under certain conditions.

Bactericidal activity:

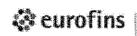
capability of a product to produce a reduction in the number of bacteria under certain conditions.

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BIBLIOGRAPHY

1. EN 1656 January 2000 - Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field. Test methods and requirements (Phase 2/Step1).

FILING

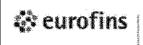
The study program, all raw data are filed in the archives of Eurofins Biolab S.r.L for ten years after the issuing of the final report.

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 consists of a disinfectant product in veterinary field.

Product	SAGEWASH SANITIZER 250ppm
Stability	Not provided
Composition	See Attachment #1

ANALYSED SAMPLE

The analysed sample, representative of the test substance, consists of white powder compressed in a cylindrical form, protected by transparent colourless plastic covering. Before the start of the experimentation, the tablet was put in a spray gun that was also provided by the Sponsor. This device was connected to the tap water and according to the Sponsor instructions, the test solution was obtained after 10 minutes of supply.

Batch	Not provided	
Manufacturing date	Not provided	
Expiry date	Not provided	
СоА	Not provided	
Receiving	EUITVI-11232	
Date	Sept 13 th 2010	
#ID	10.1286-S	

The characterization of the test substance is under Sponsor responsibility.

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Experimentation Report 2010/882 - EVALUATION OF BACTERICIDAL ACTIVITY IN SUSPENSION FOR VETERINARY FIELD - Method of dilution-neutralization (EN 1656:2000)

EXPERIMENTAL PROCEDURE

ASSAY SYSTEM

Microorganisms

The following test strains have been used:

Pseudomonas aeruginosa Staphylococcus aureus Enterococcus hirae Proteus vulgaris

ATCC 15442 ATCC 6538 ATCC 10541 ATCC 13315

Maintenance

Bacterial strains have been kept frozen; before their use they have been transplanted on TSA slants and kept in a refrigerator at 4°C ± 2°C.

Preparation of the bacterial suspensions

Bacterial strains have been transplanted on TSA slants two times in a row and incubated at 37°C ±1°C for 18 hours.

In two hours from the beginning of the test the final culture has been suspended in diluent using glass beads and the suspension has been diluted in order to reach a count between 1.5 x10⁸ and 5x10⁸ cfu/ml. The number of colonies has been determined doing the counting.

2. **CULTURAL MEDIA AND REAGENTS**

Tryptone Soya Agar (TSA)	MERCK
--------------------------	-------

Diluent

Tryptone, pancreatic

casein digestion 1.0 **MERCK** g NaCl **MERCK** 8.5 g

Distilled water q.s. to 1000 ml

EUROSPITAL Sterile water for injection (WFI)

Interfering substances

The interfering substance used was prepared a concentration 10 times higher than the final concentration.

Skim milk 10% solution

10 g Skim milk (free of antibiotics and additives) 100 ml Distilled water q.s. to

Sterilization at 105°C for 30 minutes

The final concentration test of skim milk solution was 1%.

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3. EQUIPMENT

Dry sterilization oven Steam autoclave Thermostat pHmeter Vortex stirrer Chronometer Micropipettes MEMMERT FEDEGARI MEMMERT BECKMAN VELP ARBORE GILSON

4. EXPERIMENTAL DESIGN

Test temperature

The test was conducted at 30°C ± 1°C.

Concentrations and contact time

The test substance has been tested at the following conditions:

- final test concentrations: 80% (maximum concentration testable) 50% 25%
- contact time: 30 minutes

Interfering substances

A skim milk solution having a final concentration of 1% has been used as interfering substance.

Neutralizer

The following neutralizer was selected: MERCK Lecithin 3 g **MERCK** Polysorbate 80 30 g **MERCK** Sodium Thiosulfate 5 g **MERCK** L-histidine 1 g **SIGMA** Saponin 30 g Triptone-treated water q.s. to 1000 ml

•

5. ASSAY EXECUTION

5.1 Preliminary assay

A preliminary assay was conducted prior to the execution of the assay.

The test sample, the bacterial suspension and the interfering substances have been previously stabilized at the test temperature, while the neutraliser and the water have been previously stabilized at the temperature of $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Count of bacterial suspensions

Bacterial suspensions have been diluted to a concentration between $6x10^2$ and $3x10^3$ cfu/ml. This suspension has been additionally diluted with a decimal dilution and then the number of colonies, by inclusion in Agar, after an incubation period of 48 hours at 37°C ±1°C has been determined; then the N_v value has been calculated.

Preparation of the test substance

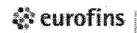
The test substance has been diluted to the maximum concentration tested in the assay.

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Validation of experimental conditions

1 ml of interfering substances and 1 ml of bacterial suspension containing between 6x102 and 3.0x103 cfu/ml, have been put in a test tube.

The components have been left in contact for 2 minutes and after 8 ml of hard water have been added, left in contact at the temperature planned by the test for the longest contact time among the ones to be tested. At the end of the contact time the mixture has been vortex-stirred and a count in double by inclusion in Agar has been made.

After an incubation period of 48 hours at 37°C ±1°C the number of colony-forming units/ml of the mixture has been determined and the A value has been calculated.

Validation of neutralizer toxicity

For each test strain in a test tube have been mixed 8 ml of neutralizer, 1 ml of distilled water and 1 ml of bacterial suspension containing between $6x10^2$ and $3x10^3$ cfu/ml, left in contact, at the temperature of 20°C ±1°C, for 5 minutes. At the end of the contact time the mixture has been vortex-stirred and a count in double by inclusion in Agar has been made.

After an incubation period of 48 hours at 37°C ±1°C the number of colony-forming units/ml of the mixture has been determined and the B value has been calculated.

Validation of the dilution-neutralisation

For each test strain in a test tube have been mixed 1 ml of interfering substances, 1 ml of diluent and 8 ml of the test substance at the highest concentration used in the test, left in contact, at the temperature planned by the assay for the longest contact period. . At the end of the contact time, 1 ml of the mixture has been transferred into a test tube containing 8 ml of neutralizer; left in contact for 5 minutes. Afterwards 1 ml of bacterial suspension containing between 6x10² and 3x10³ cfu/ml, has been added, left in contact, at the temperature of 20°C ± 1°C, for 30 minutes.

At the end of the contact time, the mixture has been vortex-stirred and a count in double by inclusion in Agar has been made.

After an incubation period of 48 hours at 37°C ±1°C the number of colony-forming units/ml has been determined and the C value has been calculated.

5.2 Assay

Count of the bacterial suspensions

Dilutions up to 10⁻⁶, 10⁻⁷ of bacterial suspensions with a concentration between 1.5 x 10⁸ and 5 x 10⁸ cfu/ml have been prepared. A count in double by inclusion in Agar has been made. The number of colony-forming units per ml of the suspension after an incubation period of 48 hours at 37°C ±1°C has been determined; then the N value has been calculated.

Assay execution

The assay sample, the bacterial suspensions and the interfering substance have been previously stabilized at the test temperature, while the neutraliser and the water have been stabilized at the temperature of 20°C ±1°C.

The test substance has been prepared with a concentration 1.25 times the requested test concentrations. For each bacterial strain and each concentration of the test substance a test tube has been prepared containing 1 ml of interfering substance and 1 ml of bacterial suspension with a concentration between 1.5 x108 and 5.0x108 cfu/ml, at the temperature planned by the test. After a contact period of 2 minutes 8 ml of test substance have been added and left in contact for the planned periods and at the test execution

After the contact time, 1 ml of the mixture has been put in a test tube containing 8 ml of neutralizer and 1 ml of distilled water. After 5 minutes ±10 sec. of neutralization the mixture has been stirred and a count in double by inclusion in Agar has been made.

After an incubation period of 48 hours at 37°C ±1°C the number of cfu/dish has been determined and the Na value has been calculated.

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6. CALCULATION AND EXPRESSION OF THE RESULTS

The count is made using the number of colonies counted on both dishes.

Only dishes containing between 15 and 300 colonies have been used to calculate the results.

In the assay, where the number of cfu on all counted dishes was <15, the number of cfu/ml has been registered as <1.5 x 10². Where the number of cfu on all counted dishes was >300 the number of cfu/ml has been registered as >3.0 x 10³.

Calculation of live units (cfu/ml)

Test bacterial suspension

For the test bacterial suspension the calculation of the bacterial count is made in the following way:

$$N(ufc/ml) = \frac{c}{(n_1 + 0.1n_2)d}$$

sum of colonies counted on both dishes C number of counted dishes of the first dilution n₁ number of counted dishes of the second dilution n_2 dilution factor correspondent to the first dilution

Assay and preliminary assay

For the assay (Na) and for the preliminary assay (A,B,C e N_v) the calculation of the bacterial count is done in the following way:

$$ufc / ml = \frac{c}{n \times V \times d}$$

sum of colonies counted on both dishes

number of counted dishes

volume used

dilution factor correspondent to the performed dilution

Calculation of vitality reduction

Vitality reduction was calculated for each organism and test concentration using the following formula:

$$R = \frac{N \times 10^{-1}}{Na}$$

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ASSAY VALIDITY CRITERIA

N: must be included between 1.5×10^8 and 5×10^8 cfu/ml Nv: must be included between 6×10^2 and 3×10^3 cfu/ml

B: is equal or higher than 0.05 times Nv C: is equal or higher than 0.5 times B A: is equal or higher than 0.05 times Nv

where:

N = count in cfu/ml of the bacterial suspension in the preliminary assay
 A = count in cfu/ml of the solution to verify the experimental conditions

B = count in cfu/ml for the control of neutralizer toxicity

c = count in cfu/ml of neutralizer effectiveness.

The test substance is considered bactericidal when for each bacterial strain 10°C (30°C in the case of disinfectants for breasts) after 5 minutes of contact, it causes a vitality reduction of at least 10⁵.

RESULTS

Preliminary assay

The N, A, B and C values for each bacterial strain are included in the validity criteria. The specific values are shown in Attachment #2.

Assay

The vitality reduction values at the different concentrations are shown below and in Attachment #2:

Γ	CONTACT TIME	AND TESTED CON	ICENTRATIONS
		5 minutes	, , , , , , , , , , , , , , , , , , , ,
TEST MICROORGANISMS	80%	50%	25%
Staphylococcus aureus ATCC 6538	>2.5×10 ⁵	<1.2×10 ⁴	<1.2×10 ⁴
Pseudomonas aeruginosa ATCC 15442	>3.3×10 ⁵	<1.7×10 ⁴	<1.7×10 ⁴
Proteus vulgaris ATCC 13315	>2.6×10 ⁵	<1.3×10 ⁴	<1.3×10 ⁴
Enterococcus hirae ATCC 10541	1.7×10 ⁵	<1.5×10 ⁴	<1.5×10 ⁴

DEVIATIONS

No deviations occurred during the study.

CONCLUSIONS

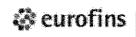
On the basis of obtained results, in compliance with the assay validity criteria, the test substance SAGEWASH SANITIZER 250ppm results **BACTERICIDAL** with the concentration of 80% after 5 minutes of contact, using a 1% final concentration of skim milk, in compliance with the provisions of EN1656:2000.

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ATTACHMENTS

ATTACHMENT	TITLE	NUMBER OF PAGES
N.1	COMPOSITION	1
N.2	RAW DATA EXPERIMENTATION 2010/882	2

D-U-N-S 429117112

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SAGEWASH MASTER PARTS LIST

SWS-01

Sagewash Sanitizer

SWS-02

Masterpack 6 x SWS-01

SWST-01

HTH Calcium Hypochlorite Tablets - One Six Pack

SWST-02

HTH Calcium Hypochlorite Tablets - Six Six Packs

SWST-COMBO

SWS-01 + SWST-01

SWST-COMBO-M

Masterpack – six x COMBO

SWS-104

Female Quick Connector

SWS-105

Male Quick Connector

SWS-106

Flow Control Handle

SWS-107

Pivot Connector

SWS-108

Hydro Body

bold items not available separately

SWS-109

Hydro Venturi Plate

SWS-110

Hydro Venturi Tube

SWS-111

Tablet Container

SWS-112

Adjustable Nozzle

SWS-113

Sweep Nozzle

SWS-114

Stainless Steel (SS) Fasteners - Set of Five

SWS-115

Hydro Seal

よる。ようによう。	Prova quantitativa in sospensione per la valutazione dell'attività battericida dei disinfettanti chimici e antisettici utilizzati nel
***************************************	campo veterinario
in the second se	(Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in
	veterinary areas)
Mod. PS/MIC/011.E	Norma (Standard): UNI EN 1656:2000 - phase2/step1
Rev.3	Pagina 1 di 2 (Page 1 of 2)

Data inizio (Started on):

17/09/10

ID. studio (ID. Study):

2010/882 SAM

ID. campione (ID. sample): 10.1286-S

Test Microrganisms		Z		^N	,	A		8	}	ပ	
	Dil.	cfu/plate	cfu/plate	cfu/plate	cfu/plate						
	မှ	>300	>300	2.6	88	82	67	7.4	ά	63	64
Staphylococcus	<u> </u>	31	43	0	9.	3	5	5		3	-
		3.7E	3.7E+08	7.3E+02	+02	7.5E+01	+01	5.6E+01	+01	6.4E+01	+01
Dselidomobas	9-	>300	>300	80	402	98	108	60	76	26	. 80
aeruginosa	-7	51	48	S S	7	3	2	20			
ATCC15442		5.0E	5.0E+08	9.9E+02	+02	9.7E+01	+01	9.3E+01	:+01	9.2E+01	+01
	စှ	>300	>300	98	60	υχ	76	74	72		78
Proteus vulgaris	2-	36	43	3	7	}		-			
		4.0E	4.0E+08	8.9E+02	+02	7.8E+01	+01	7.3E	7.3E+01	7.3E	7.3E+01
	9-	>300	>300	106	102	96	102	104	108	86	95
Enterococcus hirae	2-	46	43	2	1	8		- <u>!</u>			
)		4.5E	4.5E+08	1.0E+03	+03	9.9E+01	:+01	1.1	1.1E+02	9.7	9.7E+01

N: conteggio sospensione batterica ufc/ml (N: count of the bacterial suspension cfu/ml)

Nv: conteggio sospensione batterica per li saggio preliminare ufc/ml (Nv: count of the bacterial suspension in the preliminary assay cfu/ml)

A: conteggio nalla convalida delle condizioni sperimentali ufc/ml (A: count in the experimental conditions verification solution cfu/ml)

B: conteggio nel controllo di tossicità del neutralizzante ufc/ml (B: count in the neutraliser toxicity control cfu/ml)

C: conteggio nel controllo dell'efficacia del neutralizzante ufc/ml (C: count in the neutraliser effectiveness control cfu/ml)

	Prova quantitativa in sospensione per la valutazione dell'attività battericida dei disinfettanti chimici e antisettici utilizzati nel
CTOTIS	campo veterinario
	(Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in
	veterinary areas)
Mod, PS/MIC/011.E	Norma (Standard): UNI EN 1656:2000 - phase2/step1
Rev.3	Pagina 2 di 2 (Page 2 of 2)

17/09/10 Data inizio (Started on):

2010/882 SAM ID. studio (ID. Study):

ID. campione (ID. sample): 10.1286-S

		CONCENTRA	CONCENTRATIONS AND CONTACT TIMES (cfu/plate)	ONTACT TIME	S (cfu/plate)		
Test Microrganisms	%08	5 MIN	%09	5 MIN	25%	5 MIN	
Stanhvlococcus aureus	0	0	>300	>300	>300	>300	
ATCC6538	Na=	<1,5E+02	Na=	>3,0E+03	Na=	>3,0E+03	
	R= >	2.5E+05	R= <	1.2E+04	R= <	1.2E+04	
Pseudomonas aerudinosa	0 .	0	>300	>300	>300	>300	
ATCC15442	Na:	<1,5E+02	Na=	>3,0E+03	Na=	>3,0E+03	
	-K=	3.3E+05	R= <	1.7E+04	R= <	1.7E+04	
	0	0	>300	>300	>300	>300	
Proteus vulgaris ATCC13315	Nan	<1,5Ë+02	Na=	>3,0E+03	Na=	>3,0E+03	
	- ₩	2.6E+05	\#\ -\	1.3E+04	R= ^	1.3E+04	
Enterococcus hise	19	34	>300	>300	>300	>300	
ATCC10541	Na=	2.7E+02	Na=	>3,0E+03	Na≔	>3,0E+03	Na = count of the test mixture (cfu/ml)
	R=	1.7E+05	R= ^	1.5E+04	R≕ <	1.5E+04	R = vitality reduction

Sigla tecnico (Technician signature):

Sigla Approvazione (Approval signature):

Data fine (Finished on): 20/09/10

Data (Date): 20/09/10