Quality Assurance & Control Systems Ltd.,1 Antigonis St., 144 51, Metamorfosi, Greece
Tel: +30 210 2934745 • Fax: +30 210 2934606 • email: info@qacs.gr • website: www.qacs.gr

STUDY REPORT
SUSPENSION TEST
ACCORDING TO EN 1040

Chemical disinfectants and antiseptics – Basic Bactericidal activity
Test methods and requirements (Phase 1)

TEST SUBSTANCE IDENTIFICATION

CERTIFICATE ID	:	2016-4035 / 16 23 00057 / EN 1040
PRODUCT NAME	:	ALCOMEDSEPT
PRODUCT TYPE	:	DISINFECTANT
ACTIVE SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol
APPEARENCE OF THE PRODUCT	:	LIQUID
STORAGE CONDITIONS	:	ROOM TEMPERATURE, DARKNESS
LOT	:	L153712
METHOD	:	EN 1040
CONTACT TIME	:	5 minutes
DILUTION	:	AS IS
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM MEDICAL
PRODUCT MANUFACTURER	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM MEDICAL
RECEIPT DATE	:	6/5/2016
STUDY PERIOD	:	20/5/2016 - 23/5/2016
LAB ID	:	16 23 00057

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OBJECTIVE

The objective of this study was to demonstrate the bactericidal activity of the test material under the requirements of European Standard EN 1040 (at least 5 log reduction).

TEST SYSTEMS

Staphylococcus aureus	: ATCC	6538	-	LOT 4852821
Pseudomonas aeruginosa	: ATCC	15442	-	LOT 4846231

TEST METHOD

European Standard EN 1040 Chemical disinfectants and antiseptics. Basic bactericidal activity.

NOTES

A. Each test system was harvested off of the appropriate agar slants, coarse filtered through sterile glass wool, and diluted to the desired concentration using the appropriate diluent.
Test suspensions prepared without glass beads and a mechanical shaker were previously used in the laboratory and have provided the desired level of inoculum.

B. The test substance was tested at 5 minutes Contact Time

C. The test plates were read and recorded after ~ 2 nights incubation.

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RESULT REPORTING - CALCULATIONS

As specified in the European Standard EN 1040 methodology, a Reduction in Viability (R) was calculated for each test system. Each of the 2 replicate counts for each test system's inoculum count (x 10^{-1}) and treatment tube recovery count were averaged, and the Reduction in Viability was calculated.

Recoveries on the test plates represent a 10^{-1} dilution. A recovery of 67 CFU would result in a recovery of 6.7 x 10^2 CFU per mL. A recovery of 12 colonies results in a recovery reported as < 1.5 x 10^2 CFU per mL, countable plates are those containing between 15 and 300 colonies as stated in the protocol. Test plates with greater than 300 colonies are reported as > 3.0 x 10^3 CFU per mL.

Examples of a calculation has been shown below:

To determine the Reduction in Viability (R), the following calculation was performed:

$$R = \frac{\text{Inoculum Count x } 10^{-1}}{\text{Treatment Tube Recovery}}$$

An inoculum count of 4.87 x 10^8 CFU per mL and a recovery count of 1.5 x 10^2 CFU per mL would result in the following equation:

$$\frac{4.87 \text{ X } 10^8 \text{ x } 10^{-1}}{1.5 \text{ X } 10^2}$$

Resulting in a Reduction in Viability (R) of 3.24 x 10^5.

ASSAY ACCEPTANCE CRITERIA

1. Average inoculum's counts of 1.5 to 5.0 X 10^8 CFU per mL were achieved for all test systems.

2. Average Bacterial Suspensions control counts of 6.0 x 10^2 to 3.0 x 10^3 CFU per mL were achieved for all test systems.

3. Average recovery values for the Neutralization Toxicity Validation control assays were equal to or greater than 0.05 times the bacterial suspension control count (i.e. 30 to 300 CFU per mL).

4. Average recovery values for the Experimental Conditions Validation control equal to or greater than 0.05 times the bacterial suspension control count (i.e. 30 - 300 CFU per mL).

5. Average recovery values for the Dilution Neutralization Validation control assay were equal to or greater than 0.5 times the recovery values obtained in the Neutralizer Toxicity Validation control assay (Le. 15 to 300 CFU per mL).

ARCHIVING

The laboratory book which contains all the information (raw data and results) regarding the study and the study reports are kept in the laboratory archives during 2 years.

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TEST RESULTS INOCULUM COUNTS / INOCULUM COUNTS LOG10

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Staphylococcus aureus

Test System	Average Inoculum CFU's per mL	Average Inoculum CFU's per mL x 10^{-1}	Average of Log_{10} Inoculum (N)	Average of Log_{10} Inoculum x 10^{-1} (N_0)
S. aureus	1.8E+08	1.8E+07	8.26	7.26

Pseudomonas aeruginosa

Test System	Average Inoculum CFU's per mL	Average Inoculum CFU's per mL x 10^{-1}	Average of Log_{10} Inoculum (N)	Average of Log_{10} Inoculum x 10^{-1} (N_0)
P. aeruginosa	5.0E+08	5.0E+07	8.70	7.70

- Acceptable Average Count= 1.5 to 5.0 x 10^8 CFU/ml
- $8.17 \leq \log N \leq 8.70$
- $7.17 \leq \log N_0 \leq 7.70$

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TEST SUBSTANCE RECOVERY/SURVIVOR COUNTS

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Staphylococcus aureus

Test System	TOTAL COLONY AVERAGE	LOG_{10} OF TOTAL COLONIES
S. aureus	<1.5E+02	<2.18

Pseudomonas aeruginosa

Test System	TOTAL COLONY AVERAGE	LOG_{10} OF TOTAL COLONIES
P. aeruginosa	<1.5E+02	<2.18

Where the number of cfu on all plates counted is <15 the viable count is recorded as <1.5E+02 cfu/ml

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LOG REDUCTION

PRODUCT : ALCOMEDSEPT
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Staphylococcus aureus

Test System	Average Inoculum CFU's per mL x 10^{-1}	TOTAL COLONY AVERAGE	LOG_{10} REDUCTION	SUMMARY
S. aureus	7.26	<2.18	>5.08	PASS

Pseudomonas aeruginosa

Test System	Average Inoculum CFU's per mL x 10^{-1}	TOTAL COLONY AVERAGE	LOG_{10} REDUCTION	SUMMARY
P. aeruginosa	7.70	<2.18	>5.52	PASS

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CONTROL ASSAYS

PRODUCT : ALCOMEDSEPT
DILUTION : AS IS
METHOD : EN 1040
STUDY PERIOD : 20/5/2016 - 23/5/2016
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Staphylococcus aureus

Test System	Testing Dilution	Replicate #1	Replicate #2	Average of Bacterial Colonies	Acceptable Colonies	Summary
S. aureus	1:10 Diluted Bacterial Suspension Count	118	123	121	60 - 300 CFU/mL	Acceptable
	Validation of Expiremental conditions	112	119	116	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	144	130	137	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	65	70	68	15 - 300 CFU/mL	Acceptable

Pseudomonas aeruginosa

Test System	Testing Dilution	Replicate #1	Replicate #2	Average of Bacterial Colonies	Acceptable Colonies	Summary
P. aeruginosa	1:10 Diluted Bacterial Suspension Count	84	75	80	60 - 300 CFU/mL	Acceptable
	Validation of Expiremental conditions	77	79	78	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	76	72	74	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	53	43	48	15 - 300 CFU/mL	Acceptable

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CONCLUSION

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Bactericidal activity demonstrated (> 1.0 x 10^5 Reduction in Viability or > 5 log reduction) in **5 minutes** at 20±1 °C for referenced strains Staphylococcus aureus, Pseudomonas aeruginosa.

Signature Date: 23/5/2016

D. Melissos
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METHODOLOGY ABSTRACT : Each test system was harvested off of the appropriate agar slants, coarse filtered through sterile glass wool, and diluted to the desired concentration using the appropriate diluents. Test suspensions prepared without glass beads and a mechanical shaker were previously used in the laboratory and have provided the desired level of inoculum. Reduction in Viability (R) was calculated for each test system.

RESULT : Bactericidal activity demonstrated (> 1.0×10^5 Reduction in Viability or > 5 log reduction) in **5 minutes** at 20±1 °C for referenced strains Staphylococcus aureus, Pseudomonas aeruginosa.

CONCLUSION : PASS TEST

The samples will be stored by the laboratory during 1 month from the end test date.
The study report and raw data will be stored by the laboratory during 2 years.