Quality Assurance & Control Systems Ltd.,1 Antigonis St., 144 51, Athens, Greece
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STUDY REPORT
SUSPENSION TEST
ACCORDING TO EN 1276

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas
Test method and requirements (Phase 2 step 1)

TEST SUBSTANCE IDENTIFICATION

CERTIFICATE ID	:	2017-3670 / 17 23 00048 / EN 1276
PRODUCT NAME	:	Germ Kill Alcohol 70°
PRODUCT TYPE	:	DISINFECTANT
ACTIVE SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol
APPEARENCE OF THE PRODUCT	:	LIQUID
STORAGE CONDITIONS	:	ROOM TEMPERATURE, DARKNESS
LOT	:	
METHOD	:	EN 1276
CONTACT TIME	:	5 minutes
DILUTION	:	AS IS
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM MEDICAL
PRODUCT MANUFACTURER	:	GERM KILL Alcohol 70°
RECEIPT DATE	:	25/05/2017
STUDY PERIOD	:	20/05/2016 - 23/05/2016
LAB ID	:	17 23 00048

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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 1276.

TEST SYSTEMS

Staphylococcus aureus	: ATCC 6538	-	LOT	4852821
Escherichia coli	: ATCC NCIMB 8879	-	LOT	4835111
Pseudomonas aeruginosa	: ATCC NCIMB 10421	-	LOT	4846231
Enterococcus hirae	: ATCC NCIMB 8191	-	LOT	678641

TEST METHOD

European Standard EN 1276 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.

D. A final concentration of 3g bovine albumin was used in testing for dirty conditions (EN 1276: 1997, Section 5.2.2.8.2, Bovine albumin solutions, Part b).

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RESULT REPORTING - CALCULATIONS

As specified in the European Standard EN 1 276 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

PRODUCT : Germ Kill Alcohol 70°
DILUTION : AS IS
METHOD : EN 1276
STUDY PERIOD : 20/05/2016 - 23/05/2016
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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	Average of Log_{10} Inoculum x 10^{-1}
S. aureus	1.8E+08	1.8E+07	8.26	7.26

Acceptable Average Inoculum Count= 1.5 to 5.0 x 10^8 CFU/ml

Escherichia coli

Test System	Average Inoculum CFU's per mL	Average Inoculum CFU's per mL x 10^{-1}	Average of Log_{10} Inoculum	Average of Log_{10} Inoculum x 10^{-1}
E. coli	4.2E+08	4.2E+07	8.62	7.62

Acceptable Average Inoculum Count= 1.5 to 5.0 x 10^8 CFU/ml

Pseudomonas aeruginosa

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

Acceptable Average Inoculum Count= 1.5 to 5.0 x 10^8 CFU/ml

Enterococcus hirae

Test System	Average Inoculum CFU's per mL	Average Inoculum CFU's per mL x 10^{-1}	Average of Log_{10} Inoculum	Average of Log_{10} Inoculum x 10^{-1}
E. hirae	4.7E+08	4.7E+07	8.67	7.67

Acceptable Average Inoculum Count= 1.5 to 5.0 x 10^8 CFU/ml

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TEST SUBSTANCE RECOVERY/SURVIVOR COUNTS

PRODUCT : Germ Kill Alcohol 70°
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Staphylococcus aureus

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

Escherichia coli

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

Pseudomonas aeruginosa

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

Enterococcus hirae

Test System	TOTAL COLONY AVERAGE	LOG_{10} OF TOTAL COLONIES
E. hirae	<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 : Germ Kill Alcohol 70°
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LAB ID : 17 23 00048

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

Escherichia coli

Test System	Average Inoculum CFU's per mL x 10^{-1}	TOTAL COLONY AVERAGE	LOG_{10} REDUCTION	SUMMARY
E. coli	7.62	<2.18	>5.44	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

Enterococcus hirae

Test System	Average Inoculum CFU's per mL x 10^{-1}	TOTAL COLONY AVERAGE	LOG_{10} REDUCTION	SUMMARY
E. hirae	7.67	<2.18	>5.49	PASS

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CONTROL ASSAYS

PRODUCT : Germ Kill Alcohol 70°
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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	120	132	126	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	144	130	137	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	74	70	72	15 - 300 CFU/mL	Acceptable

Escherichia coli

Test System	Testing Dilution	Replicate #1	Replicate #2	Average of Bacterial Colonies	Acceptable Colonies	Summary
E. coli	1:10 Diluted Bacterial Suspension Count	138	142	140	60 - 300 CFU/mL	Acceptable
	Validation of Expiremental conditions	117	129	123	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	136	127	132	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	81	69	75	15 - 300 CFU/mL	Acceptable

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CONTROL ASSAYS (continued)

PRODUCT : Germ Kill Alcohol 70°
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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	69	70	70	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	76	72	74	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	44	43	44	15 - 300 CFU/mL	Acceptable

Enterococcus hirae

Test System	Testing Dilution	Replicate #1	Replicate #2	Average of Bacterial Colonies	Acceptable Colonies	Summary
E. hirae	1:10 Diluted Bacterial Suspension Count	99	107	103	60 - 300 CFU/mL	Acceptable
	Validation of Expiremental conditions	101	103	102	30 - 300 CFU/mL	Acceptable
	Neutralization Toxicity Validation	157	116	137	30 - 300 CFU/mL	Acceptable
	Dilution Neutralization Validation	58	61	60	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, Escherichia coli, Pseudomonas aeruginosa, Enterococcus hirae.

Initial EN1276 test has been performed by QACS Ltd. - on identical sample declared by manufacturer, (Alcomedsept, batch: L153712, Expiry date: 12/2018, Study Report: 2016 -4035/16 23 00058). The raw data which have been used for this Report's issue are identical.

Signature Date: 23/05/2016

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STUDY SUMMARY / ABSTRACT

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Chemical disinfectants and antiseptics – Basic Bactericidal activity
Test methods and requirements (Phase 2 Step 1)

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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, Escherichia coli, Pseudomonas aeruginosa, Enterococcus hirae.

REMARKS : Initial EN1276 test has been performed by QACS Ltd. - on identical sample declared by manufacturer, (Alcomedsept, batch: L153712, Expiry date: 12/2018, Study Report: 2016 -4035/16 23 00058). The raw data which have been used for this Report's issue are identical.

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.