

Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM

STUDY REPORT 18 23 00068

ALCOMED SEPT

**SUSPENSION TEST
ACCORDING TO EN 1276:2009
(Phase 2 step 1)**

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)

APRIL 2018

QACS LTD, 1 Antigonis str, 144-51 Metamorfossi, Greece. Tel: +30-210-2934745,
fax: +30-210-2934606, email: info@qacs.gr website: www.qacs.gr

STUDY REPORT 18 23 00053

SUSPENSION TEST ACCORDING TO EN 1276:2009

Chemical disinfectants and antiseptics - Basic Bactericidal activity

Test methods and requirements (Phase 2 Step 1)

PRODUCT NAME : ALCOMED SEPT
SUBSTANCES AND THEIR CONCENTRATIONS : ETHYL ALCOHOL, 95% v/v
APPEARANCE OF THE PRODUCT : LIQUID
STORAGE CONDITIONS : ROOM TEMPERATURE, DARKNESS
LOT : L175811
METHOD : EN 1276:2009
CONTACT TIME : 30 seconds and 60 seconds
DILUTIONS : AS IS / 50% / 1%
DILUENT RECOMENDED BY THE MANUFACTURER : WATER
STUDY SPONSOR : Σ. KOYZOYNAΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT SUPPLIER : Σ. KOYZOYNAΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT MANUFACTURER : Σ. KOYZOYNAΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
RECEIPT DATE : 30/03/2018
STUDY PERIOD : 13/04/2018-16/04/2018
LAB ID : 2018-2571/18 23 00068

OBJECTIVE

The objective of this study was to demonstrate the bactericidal activity of the test material under the requirements of European Standard EN 1276:2009

TEST SYSTEMS

Pseudomonas aeruginosa	NCIMB: 10421	LOT : 02022005
Staphylococcus aureus	ATCC: 6538	LOT : 4854821
Escherichia coli	NCIMB: 8879	LOT : 07062005
Enterococcus hirae	NCIMB: 8192	LOT : 21121999

TEST METHOD

European Standard EN 1276:2009 - 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.

This protocol for bactericidal activity is performed with four test bacteria species, two Gram positive bacteria (*Staphylococcus aureus* and *Enterococcus hirae*) and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The disinfectant has to achieve a log 5 kill against each of the test bacteria within 1 minute.

TEST CONDITIONS

- Each test system was harvested off of the appropriate agar slants and diluted to the desired concentration using the appropriate diluent.
- The test substance was tested for **30 seconds (additional)** and **60 seconds (obligatory)** contact Time.
- The test plates were read and recorded after ~ **48hours incubation time**.
- A final concentration of **0.3g/L** bovine albumin/L was used for testing (**clean conditions**)
- The following procedure was performed in water bath at **20 °C**

SAMPLE PREPERATION

The sample was tested at three final concentrations: **AS IS (80%) / 50% / 1%**.

RESULT REPORTING - CALCULATIONS

As specified in the European Standard EN 1276:2009 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 and treatment tube recovery count were averaged, and the Reduction in Viability was calculated.

ASSAY ACCEPTANCE CRITERIA

1. Average inoculum's counts of 1.5 to 5.0×10^8 CFU per mL were achieved for all test systems.
2. Test Suspension (N) is between 1.5 to 5.0×10^7 CFU per mL ($7.17 \leq \log N \leq 7.70$)
3. No is between 1.5 to 5.0×10^6 CFU per mL ($6.17 \leq \log N \leq 6.70$)
4. Validation Suspension=Nv is between 3.0×10^2 and 1.6×10^3 (Nv0 is between 30 and 160)
5. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nv)
6. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nv)
7. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nv)
8. Control of weighted mean counts. Quotient is not lower than 5 and not higher than 15

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.

TEST RESULTS FOR *Pseudomonas aeruginosa* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)			
N	Vc1	Vc2	
10^{-7}	23	27	x mean 2.73E+08
10^{-8}	4	6	log N 8.44
			No (N/10) 2.73E+07
			log No 7.44
			7,17 <= logNo <= 7,70 Yes

Validation and controls

Validation suspension (Nvo)	Experimental conditions (A)				Neutralizer control (B)	Method validation (C) Product conc.: 80% (as is)							
	VC 1	45	30 sec	x mean		VC 1	39	30 sec	x mean				
	VC 2	48	30 sec	47		VC 2	46	30 sec	43				
VC 1	49		VC 1	41	60 sec	x mean	VC 1	48	60 sec	x mean			
VC 2	56	53	VC 2	45	60 sec	43	VC 2	40	42	VC 2	42	60 sec	45
30<x mean of Nvo < 160?		x mean of A is >0,5*x mean of Nvo?		x mean of B is >0,5*x mean of Nvo?		x mean of C is >0,5*x mean of Nvo?							
Yes		30 sec Yes 60 sec Yes		Yes		30 sec Yes 60 sec Yes							

Test Results

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	30 sec	0	0	140	2.15	7.44	5.29	>5	PASS TEST
50%	30 sec	0	0	140	2.15	7.44	5.29	>5	PASS TEST
1%	30 sec	6000	6000	6000	3.78	7.44	3.66	>5	FAILS TEST

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	60 sec	0	0	140	2.15	7.44	5.29	>5	PASS TEST
50%	60 sec	0	0	140	2.15	7.44	5.29	>5	PASS TEST
1%	60 sec	6000	6000	6000	3.78	7.44	3.66	>5	FAILS TEST

TEST RESULTS FOR *Staphylococcus aureus* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)				
N	Vc1	Vc2	x mean	
10 ⁻⁶	185	158		1.72E+08
10 ⁻⁷	18	17	log N	8.24
			No (N/10)	1.72E+07
			log No	7.24
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)	Experimental conditions (A)				Neutralizer control (B)	Method validation (C) Product conc.: 80% (as is)				
	VC 1	48	30sec	x mean		VC 1	38	30sec	x mean	
	VC 2	42	30sec	45		VC 2	43	30sec	41	
VC 1	36	x mean	VC 1	43	60sec	x mean	VC 1	42	60sec	x mean
VC 2	41	39	VC 2	40	60sec	42	VC 2	40	60sec	41
30-x mean of Nvo < 160?		x mean of A is >0,5*x mean of Nvo?		x mean of B is >0,5*x mean of Nvo?		x mean of C is >0,5*x mean of Nvo?				
Yes		30sec Yes 60sec Yes		Yes		30sec Yes 60sec Yes				

Test Results

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	30sec	0	0	140	2.15	7.24	5.09	>5	PASS TEST
50%	30sec	136	173	155	2.19	7.24	5.05	>5	PASS TEST
1%	30sec	6000	6000	6000	3.78	7.24	3.46	>5	FAILS TEST

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	60sec	0	0	140	2.15	7.24	5.09	>5	PASS TEST
50%	60sec	73	55	140	2.15	7.24	5.09	>5	PASS TEST
1%	60sec	6000	6000	6000	3.78	7.24	3.46	>5	FAILS TEST

TEST RESULTS FOR *Escherichia Coli* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)				
N	Vc1	Vc2	x mean	2.14E+08
10 ⁻⁶	204	226		
10 ⁻⁷	19	21	log N	8.33
			No (N/10)	2.14E+07
			log No	7.33
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)	Experimental conditions (A)				Neutralizer control (B)	Method validation (C) Product conc.: 80% (as is)				
	VC 1	39	30sec	x mean		VC 1	47	30sec	x mean	
	VC 2	46	30sec	43		VC 2	32	30sec	40	
VC 1	45	x mean	VC 1	42	60sec	x mean	VC 1	39	60sec	x mean
VC 2	47	46	VC 2	43	60sec	43	VC 2	41	60sec	40
30*x mean of Nvo < 160?		x mean of A is > 0,5*x mean of Nvo?		x mean of B is > 0,5*x mean of Nvo?		x mean of C is > 0,5*x mean of Nvo?				
Yes		30sec Yes 60sec Yes		Yes		30sec Yes 60sec Yes				

Test Results

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	30sec	0	0	140	2.15	7.33	5.18	>5	PASS TEST
50%	30sec	0	0	140	2.15	7.33	5.18	>5	PASS TEST
1%	30sec	6000	6000	6000	3.78	7.33	3.55	>5	FAILS TEST

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	60sec	0	0	140	2.15	7.33	5.18	>5	PASS TEST
50%	60sec	0	0	140	2.15	7.33	5.18	>5	PASS TEST
1%	60sec	6000	6000	6000	3.78	7.33	3.55	>5	FAILS TEST

TEST RESULTS FOR *Enterococcus hirae* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)				
N	Vc1	Vc2	x mean	2.77E+08
10 ⁻⁷	22	31		
10 ⁻⁸	3	5	log N	8.44
			No (N/10)	2.77E+07
			log No	7.44
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)	Experimental conditions (A)				Neutralizer control (B)	Method validation (C) Product conc.: 80% (as is)				
	VC 1	54	30sec	x mean		VC 1	62	30sec	x mean	
VC 2	60	30sec	57	VC 2	53	30sec	58			
VC 1	59	x mean	VC 1	48	60sec	x mean	VC 1	48	60sec	x mean
VC 2	62	61	VC 2	53	60sec	51	VC 2	66	60sec	57
30*x mean of Nvo < 160?		x mean of A is >0,5*x mean of Nvo?		x mean of B is >0,5*x mean of Nvo?		x mean of C is >0,5*x mean of Nvo?				
Yes		30sec Yes 60sec Yes		Yes		30sec Yes 60sec Yes				

Test Results

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	30sec	0	0	140	2.15	7.44	5.30	>5	PASS TEST
50%	30sec	364	182	273	2.44	7.44	5.01	>5	PASS TEST
1%	30sec	60000	60000	60000	4.78	7.44	2.66	>5	FAILS TEST

Product concentration (%)	Contact time	VC 1	VC 2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
80% (as is)	60sec	0	0	140	2.15	7.44	5.30	>5	PASS TEST
50%	60sec	0	0	140	2.15	7.44	5.30	>5	PASS TEST
1%	60sec	60000	60000	60000	4.78	7.44	2.66	>5	FAILS TEST

CONCLUSION

TEST SUBSTANCE IDENTIFICATION

PRODUCT NAME	:	ALCOMED SEPT
SUBSTANCES AND THEIR CONCENTRATIONS	:	ETHYL ALCOHOL, 95% v/v
APPEARANCE OF THE PRODUCT	:	LIQUID
STORAGE CONDITIONS	:	ROOM TEMPERATURE, DARKNESS
LOT	:	L175811
METHOD	:	EN 1276:2009
CONTACT TIME	:	30 seconds and 60 seconds
DILUTIONS	:	AS IS / 50% / 1%
DILUENT RECOMENDED BY THE MANUFACTURER	:	WATER
STUDY SPONSOR	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT MANUFACTURER	:	Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
RECEIPT DATE	:	30/03/2018
STUDY PERIOD	:	13/04/2018-16/04/2018
LAB ID	:	2018-2571/18 23 00068

METHODOLOGY ABSTRACT

A test suspension of bacteria (*P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*) is tested against a product test solution (at three concentrations AS IS, 50%, 1%) with the presence of interfering substance (bovine albumin -clean conditions). The mixture is maintained at 20°C ±1°C for 30 seconds and 60 seconds. At the end of this contact time, an aliquot is taken, and the bactericidal activity in this portion is immediately neutralized or suppressed. The numbers of surviving flora are determined and the reduction is calculated.

RESULT

The test substance: **ALCOMED SEPT demonstrated** bactericidal activity according to EN1276 (> 1.0 x 10⁵ Reduction in Viability or > 5 log reduction) under **clean conditions, within contact time 30 seconds at 20±1 °C**, when tested at concentrations:

AS IS(80%) using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

50% using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

The test substance: **ALCOMED SEPT demonstrated** bactericidal activity according to EN1276 (> 1.0 x 10⁵ Reduction in Viability or > 5 log reduction) under **clean conditions, within contact time 60 seconds at 20±1 °C**, when tested at concentrations:

AS IS(80%) using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

50% using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

Signature date: 20/04/2018



Lagiopoulos Giorgos
Agronomist-Food Technologist M.Sc.
Study Manager

STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1276:2009
Chemical disinfectants and antiseptics - Basic Bactericidal activity
Test methods and requirements (Phase 2 Step 1)

PRODUCT NAME : ALCOMED SEPT
SUBSTANCES AND THEIR CONCENTRATIONS : ETHYL ALCOHOL, 95% v/v
APPEARANCE OF THE PRODUCT : LIQUID
STORAGE CONDITIONS : ROOM TEMPERATURE, DARKNESS
LOT : L175811
METHOD : EN 1276:2009
CONTACT TIME : 30 seconds and 60 seconds
DILUTIONS : AS IS / 50% / 1%
DILUENT RECOMENDED BY THE MANUFACTURER : WATER
STUDY SPONSOR : Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT SUPPLIER : Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
PRODUCT MANUFACTURER : Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
RECEIPT DATE : 30/03/2018
STUDY PERIOD : 13/04/2018-16/04/2018
LAB ID : 2018-2571/18 23 00068

TEST SYSTEMS:

Pseudomonas aeruginosa	NCIMB: 10421	LOT : 02022005
Staphylococcus aureus	ATCC: 6538	LOT : 4854821
Escherichia coli	NCIMB: 8879	LOT : 07062005
Enterococcus hirae	NCIMB: 8192	LOT : 21121999

RESULT

The test substance: **ALCOMED SEPT demonstrated** bactericidal activity according to EN1276 (> 1.0 x 10⁵ Reduction in Viability or > 5 log reduction) under **clean conditions, within contact time 30 seconds at 20±1 °C**, when tested at concentrations:

AS IS(80%) using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

50% using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

The test substance: **ALCOMED SEPT demonstrated** bactericidal activity according to EN1276 (> 1.0 x 10⁵ Reduction in Viability or > 5 log reduction) under **clean conditions, within contact time 60 seconds at 20±1 °C**, when tested at concentrations:

AS IS(80%) using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

50% using as test organisms the reference strains: *P. aeruginosa*, *S. aureus*, *E. coli* & *E. hirae*.

Results refer to the sample as received and analyzed on the period specified above.

The test report shall not be reproduced except in full, without written approval of the laboratory.

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.

End of Test Report