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Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL

STUDY REPORT

2020-7622/20 23 00658

Alco X Quat

SUSPENSION TEST
ACCORDING TO EN 1276:2019
(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)

SEPTEMBER 2020

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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 PRODUCT IDENTIFICATION

PRODUCT NAME	:	Alco X Quat
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethyl Alcohol Denat. 59% w/v (73,75% v/v) Benzalkonium Chloride (50%): 0.8%
APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	Not Listed
METHOD	:	EN 1276:2019
CONTACT TIME	:	5 minutes
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT MANUFACTURER	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
RECEIPT DATE	:	10/07/2020
STUDY PERIOD	:	07/08/2020-10/08/2020
LAB ID	:	2020-7622/20 23 00658

SCOPE

This document specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or - in the case of ready-to-use products - with water. Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.
This document applies to products that are used in food, industrial, domestic and institutional areas excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues except those for hand hygiene in the above considered areas

PRINCIPLE

A sample of the product as delivered and/or diluted with hard water (or water for ready-to-use products with the exception of handwash products whose first dilution is done in hard water is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at the chosen test temperature for the adopted contact time. At the end of this contact time, an aliquot is taken, and the bactericidal and/or the bacteriostatic activity in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving bacteria in each sample are determined and the reduction is calculated.
The test is performed using Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Enterococcus hirae as test organisms. For temperatures ≥ 40 °C only Enterococcus faecium shall be used. For testing of hand hygiene products, Pseudomonas aeruginosa, Escherichia coli K12, Staphylococcus aureus and Enterococcus hirae are used as test organisms.

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TEST CONDITIONS

1. The following procedure was performed in water bath at 20 °C
2. The test product was tested at 5 minutes contact time
3. A final concentration of 0.3g/L bovine albumin was used for testing (clean conditions)
4. Neutralization Method used: Dilution neutralization.
5. Neutralizer used: LPT Dilution Broth containing polysorbate 80.
6. According to EN 1276, products shall be tested at a minimum of three different concentrations to include one concentration in the active range and one concentration in the non-active range. In this case the product was tested: Undiluted (80%), 50%, 1%.

TEST ORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli	NCIMB 8879
Enterococcus hirae	NCIMB 8192

BACTERICIDAL ACTIVITY FOR GENERAL PURPOSES

The product shall be deemed to have passed the EN 1276 standard if it demonstrates in a valid test at least a 5 lg reduction, under the suitable test conditions for general purpose defined by this standard when the test organisms are Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Enterococcus hirae (E. faecium when the test temperature is ≥ 40 °C).

ASSAY ACCEPTANCE CRITERIA

1. Test Suspension (N) is between 1.5 to 5.0 X 10^8 CFU per mL ($8.17 \leq \log N \leq 8.70$)
2. No (N/10) is between 1.5 to 5.0 X 10^7 CFU per mL ($7.17 \leq \log No \leq 7.70$)
3. Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
4. Nvo (Nv/10) is between 30 and 160
5. Na is the number of survivors (cells) per ml in the test mixture at the end of contact time.
6. R (log reduction) = No - Na
7. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
8. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
9. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
10. 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 for 5 years.

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

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	1.89E+08
10^{-6}	192	187		
10^{-7}	20	17	log N	8.28
			No (N/10)	1.89E+07
			log No	7.28
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	32	x mean	VC 1	31	x mean	VC 1	34	x mean	VC 1	37	x mean
VC 2	37	34.5	VC 2	36	33.5	VC 2	39	36.5	VC 2	39	38
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 or Nva/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			Yes			Yes			Yes		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.28	> 5.13	≥ 5	PASS TEST
		10^{-1}	0	0							
50%	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.28	> 5.13	≥ 5	PASS TEST
		10^{-1}	0	0							
1%	5 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.28	< 2.76	≥ 5	FAILS TEST
		10^{-1}	> 330	> 330							

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

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.09E+08
10^{-6}	196	221		
10^{-7}	18	24	log N	8.32
			No (N/10)	2.09E+07
			log No	7.32
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	49	**x mean**	VC 1	37	**x mean**	VC 1	41	**x mean**	VC 1	47	**x mean**
VC 2	48	**48.5**	VC 2	30	**33.5**	VC 2	46	**43.5**	VC 2	52	**49.5**
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 or Nvo/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			**Yes**			**Yes**			**Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.32	> 5.17	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.32	> 5.17	≥ 5	**PASS TEST**
		10^{-1}	0	0							
1%	5 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.32	< 2.80	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.05E+08
10^{-7}	30	32		
10^{-8}	3	2	log N	8.48
			No (N/10)	3.05E+07
			log No	7.48
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	60	x mean	VC 1	61	x mean	VC 1	66	x mean	VC 1	54	x mean
VC 2	69	64.5	VC 2	67	64	VC 2	60	63	VC 2	62	58
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 or Nvo/1000?			x mean of C is >0,5*x mean of Nvo?		
Yes			Yes			Yes			Yes		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.48	> 5.34	≥ 5	PASS TEST
		10^{-1}	0	0							
50%	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.48	> 5.34	≥ 5	PASS TEST
		10^{-1}	0	0							
1%	5 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.48	< 2.97	≥ 5	FAILS TEST
		10^{-1}	> 330	> 330							

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

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.60E+08
10^{-6}	249	276		
10^{-7}	20	28	log N	8.42
			No (N/10)	2.60E+07
			log No	7.42
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	42	**x mean**	VC 1	55	**x mean**	VC 1	52	**x mean**	VC 1	51	**x mean**
VC 2	44	**43**	VC 2	46	**50.5**	VC 2	57	**54.5**	VC 2	46	**48.5**
30<x mean of Nvo < 160? **Yes**			x mean of A is > 0,5*x mean of Nvo? **Yes**			x mean of B is > 0,5*x mean of Nvo or Nv8/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.42	> 5.27	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	5 min	10^{0}	0	0	< 14	< 140	< 2.15	7.42	> 5.27	≥ 5	**PASS TEST**
		10^{-1}	0	0							
1%	5 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.42	< 2.90	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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CONCLUSION

TEST SUBSTANCE IDENTIFICATION

PRODUCT NAME	:	Alco X Quat
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethyl Alcohol Denat. 59% w/v (73,75% v/v) Benzalkonium Chloride (50%): 0.8%
APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	Not Listed
METHOD	:	EN 1276:2019
CONTACT TIME	:	5 minutes
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT MANUFACTURER	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
RECEIPT DATE	:	10/07/2020
STUDY PERIOD	:	07/08/2020-10/08/2020
LAB ID	:	2020-7622/20 23 00658

METHODOLOGY ABSTRACT

A test suspension of bacteria is tested against a product test solution at three different concentrations with the presence of interfering substance. The mixture is maintained at 20°C ±1°C for 5 minutes. 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 log reduction is calculated.

RESULT

The product under test: "Alco X Quat" demonstrated bactericidal activity according to EN 1276:2019 (≥ 5 log reduction), under clean conditions for 5 minutes contact time, at 20 ± 1 °C, when tested at product concentrations:

Undiluted (80%) using as test organisms the reference strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus hirae*.

For the QACS Ltd Laboratory,

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STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1276:2019

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)

PRODUCT NAME	:	Alco X Quat
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APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	Not Listed
METHOD	:	EN 1276:2019
CONTACT TIME	:	5 minutes
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TEST MICROORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli	NCIMB 8879
Enterococcus hirae	NCIMB 8192

RESULT

The product under test: “Alco X Quat” demonstrated bactericidal activity according to EN 1276:2019 (≥ 5 log reduction), under clean conditions for 5 minutes contact time, at 20 ± 1 °C, when tested at product concentrations:

Undiluted (80%) using as test organisms the reference strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli* and *Enterococcus hirae*.

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

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 for 5 years.

End of Test Report