

**STUDY REPORT**  
**2020-7622/20 23 00655**

**Σ. KOYZOYNAS & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL**

**Alco X Quat**

**QUANTITATIVE NON-POROUS SURFACE TEST**  
**ACCORDING TO EN 13697:2015+A1 2019**  
**(phase 2/step2)**

**Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas.**  
**(phase 2, step 2)**

**SEPTEMBER 2020**

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## STUDY REPORT 2020-7622/20 23 00655

Quantitative Non-Porous Surface Test According to EN 13697:2015+A1 2019 for the evaluation of bactericidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. (phase 2, step 2)

PRODUCT NAME	: Alco X Quat
SUBSTANCES AND THEIR CONCENTRATIONS	: Ethyl Alcohol Denat. 59% w/v (73,75% v/v) Benzalkonium Chloride (50%): 0.8% w/v
APPEARANCE OF THE PRODUCT	: Liquid
STORAGE CONDITIONS	: Room Temperature, Darkness
LOT	: Not provided
METHOD	: EN 13697:2015+A1 2019
CONTACT TIME	: 5 minutes
DILUTIONS	: Undiluted, 50%, 1%
STUDY SPONSOR	: Σ. KOYZOYNAΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT SUPPLIER	: Σ. KOYZOYNAΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT MANUFACTURER	: Σ. KOYZOYNAΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
RECEIPT DATE	: 10/07/2020
STUDY PERIOD	: 12/08/2020-14/08/2020
LAB ID	: 2020-7622/20 23 00655

### Test Method Principle

A test suspension of bacteria in a solution of interfering substances is inoculated onto a test stainless steel surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The surface is maintained at a specified temperature for a defined period of time. The surface is transferred to a previously validated neutralization medium so that the action of the disinfectant is immediately neutralized. The number of surviving organisms which can be recovered from the surface is determined quantitatively. The number of bacteria on the surface treated with hard water in place of the disinfectant is also determined and the reduction in viable counts attributed to the product is calculated by difference.

### Activity on Non-Porous Surfaces for General Purposes

Bactericidal activity on surfaces for general purposes is characterized by the concentration of the tested product for which a 4 log or more reduction in viability is demonstrated under clean or dirty conditions when the test organisms are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae* and *Escherichia coli*.

### Test Conditions

1. The test plates were read and recorded after ~ 48 hours incubation time.
2. The following procedure was performed between 18 °C ± 1 °C and 25 °C ± 1 °C.
3. Interfering substance: A final concentration of 0.3g/L bovine albumin was used (clean conditions according to EN 13697 2015+A1 2019) for all micro-organisms under test.
4. Contact time: 5 minutes for all micro-organisms under test.
5. Incubation temperatures: Bacteria at 37 °C ± 1 °C.
6. Appearance of product test solution: Homogeneous solution.
7. According to EN 13697 2015+A1 2019, 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, 50%, 1%.

## Test Method

Dilution-Neutralization, plate count, pour plate method.

## Neutralizer

Neutralizer used: LPT Dilution Broth containing polysorbate 80.

## Test Microorganisms

The bactericidal activity is evaluated using the following four strains:

<i>Pseudomonas aeruginosa</i>	NCIMB 10421
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Enterococcus hirae</i>	NCIMB 8192
<i>Escherichia coli</i>	NCIMB 8879

## Verification of Methodology

1. Test Suspension (N) for bacteria is between  $6,57 \leq N \leq 7,10$  Log  
( $7,57 \leq N \leq 8,10$  lg for *P. aeruginosa* under clean conditions)
2. Water control  $N_c \geq 6,27$  Logs for bacteria
3. Neutralizer control "NC" (verification of the absence of toxicity of the neutralizer) NC -  $N_c$  is not greater than  $\pm 0,3$  lg
4. Method validation "NT" (dilution-neutralization validation) NT -  $N_c$  is not greater than  $\pm 0,3$  lg
5. Test "Nd" - determination of microbicidal concentrations
6. The log reduction (R) is expressed in logarithm.  $R = N_c - N_d$
7. Nts (number of cfu remaining on test surface) is less than 100 cfu/ml for active concentrations. If not, the recovery of microorganisms has not been sufficient. For non-active concentrations, Nts may be not countable.

## Test Surfaces

Stainless steel discs (2 cm diameter discs) 304 with grade 2b finish on both sides (EN 10088-1). The surfaces are flat made. The surfaces are only used once and subsequently discarded.

## Results

Quantitative non-porous surface test, EN 13697 for product under test.  
The antimicrobial activity values are shown on the table below:

Microorganisms	Product dilutions			Contact time
	Undiluted	50%	1%	
<i>Pseudomonas aeruginosa</i>	≥ 4 Pass	≥ 4 Pass	< 4 Fail	5 minutes
<i>Escherichia coli</i>	≥ 4 Pass	≥ 4 Pass	< 4 Fail	5 minutes
<i>Staphylococcus aureus</i>	≥ 4 Pass	≥ 4 Pass	< 4 Fail	5 minutes
<i>Enterococcus hirae</i>	≥ 4 Pass	≥ 4 Pass	< 4 Fail	5 minutes

## Results Criteria

The log<sub>10</sub> viability reduction should be:  
4 log<sub>10</sub> or more for bacteria.

## Conclusion

In accordance with EN 13697:2015+A1 2019, the product under test: “Alco X Quat”, possesses bactericidal activity on surfaces in 5 minutes contact time at 20°C under clean conditions at concentration:

Undiluted for referenced strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*.

## Results authenticity

The study concerned by this report was carried out according to the experimental protocol, under responsibility and quality plan of the QACS Ltd laboratory. All the observations and data recorded during this trial are reported in this study report.

For the QACS Ltd Laboratory

  
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Signature date: 15/09/2020

Lagiopoulos Giorgos

Agronomist, Food Technologist M.Sc.

Study Manager

## 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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**Alco X Quat**

QUANTITATIVE NON-POROUS SURFACE TEST  
ACCORDING TO EN 13697:2015+A1 2019  
(phase 2/step2)

STUDY SUMMARY  
AND  
APPENDICES

## Study Summary / Abstract

Quantitative Non-Porous Surface Test According to EN 13697:2015+A1 2019 for the evaluation of bactericidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. (phase 2, step 2)

PRODUCT NAME	:	Alco X Quat
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethyl Alcohol Denat. 59% w/v (73,75% v/v) Benzalkonium Chloride (50%): 0.8% w/v
APPEARANCE OF THE PRODUCT	:	Liquid
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	Not provided
METHOD	:	EN 13697:2015+A1 2019
CONTACT TIME	:	5 minutes
DILUTIONS	:	Undiluted, 50%, 1%
STUDY SPONSOR	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
PRODUCT SUPPLIER	:	Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL
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## Test Microorganisms

The bactericidal activity is evaluated using the following four strains:

<i>Pseudomonas aeruginosa</i>	NCIMB 10421
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Enterococcus hirae</i>	NCIMB 8192
<i>Escherichia coli</i>	NCIMB 8879

## Result

In accordance with EN 13697:2015+A1 2019, the product under test: "Alco X Quat", possesses bactericidal activity on surfaces in 5 minutes contact time at 20°C under clean conditions at concentration:

Undiluted for referenced strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*.

### Appendix No 1: Compiled Raw

Test organisms	Bacterial Test Suspension			Validation Test						Water control : 20°C			Test procedure at concentrations % (V/V)			Test procedure at concentrations % (V/V)			Test procedure at concentrations % (V/V)										
				NT			NC			Nc			Undiluted			5min			50%			5min			1%			5min	
<i>Pseudomonas aeruginosa</i> NCIMB 10421	10-7	245	260	10-3			10-3			10-3	>300	>300	10 <sup>0</sup>	0	0	10 <sup>0</sup>	34	45	10 <sup>0</sup>	>300	>300								
	10-4	>300	>300	10-4	>300	>300	10-4	>300	>300	10-4	75	69	10-1	0	0	10-1	2	5	10-1	>300	>300								
	10-8	24	25	10-5	41	37	10-5	39	35	10-5	9	7	10-2	0	0	10-2	0	0	10-2	300	300								
	10-9	3	2	10-6	4	3	10-6	4	4	10-6			Nd	<0.1		Nd	2.60		Nd	5.48									
	N	7.80		NT	6.59		NC	6.57		Nc	6.86		Nts	0		Nts	49		Nts	>100									
7.57 ≤ N ≤ 8.10			VALID	NT-Nc<0.3lg			VALID	NC-Nc<0.3lg			VALID	Nc ≥ 6.27			VALID	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			FAIL		
<i>Staphylococcus aureus</i> ATCC 6538	10-6	232	244	10-3	>300	>300	10-3	>300	>300	10-3	>300	>300	10-0	0	0	10-0	12	14	10-0	>300	>300								
	10-7	21	25	10-4	31	34	10-4	35	44	10-4	78	85	10-1	0	0	10-1	1	2	10-1	>300	>300								
	10-8	3	3	10-5	3	4	10-5	3	5	10-5	7	9	10-2	0	0	10-2	0	0	10-2	300	300								
	10-9	3	3	10-6	3	4	10-6	3	5	10-6			Nd	<0.1		Nd	2.11		Nd	5.48									
	N	6.77		NT	6.51		NC	6.60		Nc	6.91		Nts	0		Nts	4		Nts	>100									
6.57 ≤ N ≤ 7.10			VALID	NT-Nc<0.3lg			VALID	NC-Nc<0.3lg			VALID	Nc ≥ 6.27			VALID	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			FAIL		
<i>Escherichia coli</i> NCIMB 8879	10-6	>300	>300	10-3	>300	>300	10-3	>300	>300	10-3	>300	>300	10 <sup>0</sup>	0	0	10 <sup>0</sup>	22	28	10 <sup>0</sup>	>300	>300								
	10-7	31	35	10-4	36	39	10-4	54	52	10-4	91	86	10-1	0	0	10-1	2	3	10-1	>300	>300								
	10-8	4	4	10-5	3	4	10-5	4	6	10-5	9	8	10-2	0	0	10-2	0	0	10-2	300	300								
	10-9	4	4	10-6	3	4	10-6	4	6	10-6			Nd	<0.1		Nd	2.40		Nd	5.48									
	N	6.92		NT	6.57		NC	6.72		Nc	6.95		Nts	0		Nts	31		Nts	>100									
6.57 ≤ N ≤ 7.10			VALID	NT-Nc<0.3lg			VALID	NC-Nc<0.3lg			VALID	Nc ≥ 6.27			VALID	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			FAIL		
<i>Enterococcus hirae</i> NCIMB 8192	10-6	>300	>300	10-3	>300	>300	10-3	>300	>300	10-3	>300	>300	10-0	0	0	10-0	24	16	10-0	>300	>300								
	10-7	34	37	10-4	49	42	10-4	61	52	10-4	42	49	10-1	0	0	10-1	2	2	10-1	>300	>300								
	10-8	3	4	10-5	4	5	10-5	4	7	10-5	4	5	10-2	0	0	10-2	0	0	10-2	300	300								
	10-9	3	4	10-6	4	5	10-6	4	7	10-6			Nd	<0.1		Nd	2.30		Nd	5.48									
	N	6.95		NT	6.66		NC	6.75		Nc	6.66		Nts	0		Nts	12		Nts	>100									
6.57 ≤ N ≤ 7.10			VALID	NT-Nc<0.3lg			VALID	NC-Nc<0.3lg			VALID	Nc ≥ 6.27			VALID	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			PASS	PASS = R ≥ 4 log			FAIL		
Verification of Methodology	6.57 ≤ N ≤ 7,10 Logs for bacteria (7,57 ≤ N ≤ 8,10 lg for P.aeruginosa under clean conditions)												NC - Nc is not greater than ± 0,3 lg						Nts is less than 100 cfu/ml for active concentrations										
	5,57 ≤ N ≤ 6,10 Logs for fungi (6,57 ≤ N ≤ 7,10 lg for C.albicans under clean conditions)												NT - Nc is not greater than ± 0,3 lg						If not, the recovery of microorganisms has not been sufficient. For non-active concentrations, Nts may be not countable										
R = Nc - Nd, PASS ≥ 4 log for bacteria & ≥ 3 log for fungi																													

## Appendix No 2: Tables

**Table no 1: Microbial Suspensions**

Test microorganisms	Microbial test suspension
	N in log 10 values
<i>Pseudomonas aeruginosa</i> NCIMB 10421	7.80
<i>Staphylococcus aureus</i> ATCC 6538	6.77
<i>Escherichia coli</i> NCIMB 8879	6.92
<i>Enterococcus hirae</i> NCIMB 8192	6.95

**Table no 2: Validation of the neutralization**

Test microorganisms	Validation test: log 10 values	
	NT	NC
<i>Pseudomonas aeruginosa</i> NCIMB 10421	6.59	6.57
<i>Staphylococcus aureus</i> ATCC 6538	6.51	6.60
<i>Escherichia coli</i> NCIMB 8879	6.57	6.72
<i>Enterococcus hirae</i> NCIMB 8192	6.66	6.75

**Table no 3: Assays**

Test microorganisms	N	Nc	Log reduction (R)		
	Inoculum	Water control	Product dilution Undiluted	Product dilution 50%	Product dilution 1%
<i>Pseudomonas aeruginosa</i> NCIMB 10421	7.80	6.86	6.76	4.26	1.38
<i>Staphylococcus aureus</i> ATCC 6538	6.77	6.91	6.81	4.80	1.43
<i>Escherichia coli</i> NCIMB 8879	6.92	6.95	6.85	4.55	1.47
<i>Enterococcus hirae</i> NCIMB 8192	6.95	6.66	6.56	4.36	1.18

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 for 5 years.

End of Test Report