QACS LAB
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# STUDY REPORT

# 2020-7622/20 23 00656

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

Alco X Quat

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

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

SEPTEMBER 2020

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Quantitative Non-Porous Surface Test According to EN 13697:2015+A1 2019 for the evaluation of fungicidal 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)<br>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 |
| PRODUCT MANUFACTURER | : | Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε.-ALCOFARM MEDICAL |
| RECEIPT DATE | : | 10/07/2020 |
| STUDY PERIOD | : | 12/08/2020-14/08/2020 |
| LAB ID | : | 2020-7622/20 23 00656 |

## Test Method Principle

A test suspension of fungi 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 fungi 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

- Fungicidal activity on surfaces for general purposes is characterized by the concentration of the tested product for which a 3 log or more reduction in viability is demonstrated under clean or dirty conditions, when the test organisms are *Candida albicans* and *Aspergillus brasiliensis*
- Yeasticidal activity on surfaces for general purposes is characterized by the concentration of the tested product for which a 3 log or more reduction in viability is demonstrated under clean or dirty conditions, when the test organisms are *Candida albicans*.

## 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: Fungi at 30 °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%.

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## Test Method

Dilution-Neutralization, plate count, pour plate method.

## Neutralizer

Neutralizer used: LPT Dilution Broth containing polysorbate 80

## Test Microorganisms

The yeasticidal-fungicidal activity is evaluated using the following two strains:

| | |
|---|---|
| *Candida albicans* | ATCC 10231 |
| *Aspergillus brasiliensis* | ATCC 16404 |

## Verification of Methodology

1. Test Suspension (N) for fungi is between 5,57 ≤ N ≤ 6,10 Logs
2. Test Suspension (N) for yeasts is between 5,57 ≤ N ≤ 6,10 Logs (6,57 ≤ N ≤ 7,10 lg for C. albicans under clean conditions)
3. Water control Nc ≥ 5,27 Logs for fungi
4. Neutralizer control "NC" (verification of the absence of toxicity of the neutralizer) NC - Nc is not greater than ± 0,3 lg
5. Method validation "NT" (dilution-neutralization validation) NT - Nc is not greater than ± 0,3 lg
6. Test "Nd" – determination of microbicidal concentrations
7. The log reduction (R) is expressed in logarithm. R = Nc – Nd
8. 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% | |
| *Candida albicans* | **≥3 Pass** | **≥3 Pass** | <3 Fail | 5 minutes |
| *Aspergillus brasiliensis* | <3 Fail | <3 Fail | <3 Fail | 5 minutes |

## Results Criteria

The log10 viability reduction should be:
3 log10 or more for fungi.

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## Conclusion

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

Undiluted using as test organisms the reference strain: *Candida albicans*.

## 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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# STUDY REPORT

# 2020-7622/20 23 00656

## Alco X Quat

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

STUDY SUMMARY
AND
APPENDICES

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## Study Summary / Abstract

Quantitative Non-Porous Surface Test According to EN 13697:2015 for the evaluation of fungicidal 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)<br>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 |
| PRODUCT MANUFACTURER | : | Σ. ΚΟΥΖΟΥΝΑΣ & ΣΙΑ Ε.Ε. - ALCOFARM MEDICAL |
| RECEIPT DATE | : | 10/07/2020 |
| STUDY PERIOD | : | 12/08/2020-14/08/2020 |
| LAB ID | : | 2020-7622/20 23 00656 |

## Test Microorganisms

The yeasticidal-fungicidal activity is evaluated using the following two strains:

| | |
|---|---|
| *Candida albicans* | ATCC 10231 |
| *Aspergillus brasiliensis* | ATCC 16404 |

## Result

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

Undiluted using as test organisms the reference strains: *Candida albicans*.

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## Appendix No 1: Compiled Raw

| Test organisms | Fungal 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 | 50.00% | | 5min | 1.00% | | 5min |
| ***Candida albicans*** | | | | | | | | | | 10-2 | >300 | >300 | $10^{-0}$ | 0 | 0 | $10^{-0}$ | 32 | 45 | $10^{-0}$ | >300 | >300 |
| | 10-6 | >300 | >300 | 10-2 | >300 | >300 | 10-2 | >300 | >300 | 10-3 | 92 | 86 | 10-1 | 0 | 0 | 10-1 | 4 | 5 | 10-1 | >300 | >300 |
| | 10-7 | 28 | 34 | 10-3 | 44 | 52 | 10-3 | 54 | 61 | 10-4 | 9 | 7 | 10-2 | 0 | 0 | 10-2 | 0 | 0 | 10-2 | 300 | 300 |
| ATCC 10231 | 10-8 | 3 | 4 | 10-4 | 6 | 4 | 10-4 | 6 | 6 | 10-5 | | | Nd | <0.1 | | Nd | 2.59 | | Nd | 5.48 | |
| | N | 6.89 | | NT | 5.68 | | NC | 5.76 | | Nc | 5.95 | | Nts | 0 | | Nts | 16 | | Nts | >100 | |
| | | | | | | | | | | Nts | >100 | | **R =** | **5.85** | | **R =** | **3.36** | | **R =** | **0.47** | |
| | **6,57 ≤ N ≤ 7,10** | | VALID | **NT-Nc<0.3lg** | | VALID | **NC-Nc<0.3lg** | | VALID | Nc ≥ 5,27 | | VALID | PASS = R ≥ 3 log | | PASS | PASS = R ≥ 3 log | | PASS | PASS = R ≥ 3 log | | FAIL |
| ***Aspergillus brasiliensis*** | | | | | | | | | | 10-2 | >165 | >165 | $10^{-0}$ | >165 | >165 | $10^{-0}$ | >165 | >165 | $10^{-0}$ | >165 | >165 |
| | 10-5 | >165 | >165 | 10-2 | >165 | >165 | 10-2 | >165 | >165 | 10-3 | 39 | 25 | 10-1 | >165 | >165 | 10-1 | >165 | >165 | 10-1 | >165 | >165 |
| | 10-6 | 23 | 25 | 10-3 | 29 | 30 | 10-3 | 19 | 24 | 10-4 | 2 | 3 | 10-2 | 165 | 165 | 10-2 | 165 | 165 | 10-2 | 165 | 165 |
| ATCC 16404 | 10-7 | 2 | 3 | 10-4 | 2 | 2 | 10-4 | 3 | 2 | 10-5 | | | Nd | 5.22 | | Nd | 5.22 | | Nd | 5.22 | |
| | N | 5.78 | | NT | 5.47 | | NC | 5.33 | | Nc | 5.51 | | Nts | >100 | | Nts | >100 | | Nts | >100 | |
| | | | | | | | | | | Nts | >100 | | **R =** | **0.29** | | **R =** | **0.29** | | **R =** | **0.29** | |
| | **5.57 ≤ N ≤ 6.10** | | VALID | **NT-Nc<0.3lg** | | VALID | **NC-Nc<0.3lg** | | VALID | Nc ≥ 5,27 | | VALID | PASS = R ≥ 3 log | | FAIL | PASS = R ≥ 3 log | | FAIL | PASS = R ≥ 3 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. |
| | R = Nc – Nd, PASS ≥ 4 log for bacteria & ≥ 3 log for fungi | | For non-active concentrations, Nts may be not countable |

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## Appendix No 2: Tables

**Table no 1: Microbial Suspensions**

| **Test microorganisms** | **Microbial test suspension** |
|---|---|
| | N in log 10 values |
| *Candida albicans* ATCC 10231 | 6.89 |
| *Aspergillus brasiliensis* ATCC 16404 | 5.78 |

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

| **Test microorganisms** | **Validation test:** log 10 values | |
|---|---|---|
| | NT | NC |
| *Candida albicans* ATCC 10231 | 5.68 | 5.76 |
| *Aspergillus brasiliensis* ATCC 16404 | 5.47 | 5.33 |

**Table no 3: Assays**

| **Test microorganisms** | N | Nc | Log reduction (R) | | |
|---|---|---|---|---|---|
| | Inoculum | Water control | Product dilution Undiluted | Product dilution 50% | Product dilution 1% |
| *Candida albicans* ATCC 10231 | 6.89 | 5.95 | 5.85 | 3.36 | 0.47 |
| *Aspergillus brasiliensis* ATCC 16404 | 5.78 | 5.51 | 0.29 | 0.29 | 0.29 |

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