

Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM

STUDY REPORT 18 23 00028

GERM KILL ALCOHOL

SUSPENSION TEST ACCORDING TO EN 1650:2008+A1:2013 (Phase 2 step 1)

Chemical disinfectants and antiseptics –
Quantitative suspension test for the evaluation of
fungicidal or yeasticidal activity of chemical
disinfectants and antiseptics used in food,
industrial, domestic and institutional areas

JANUARY 2018

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STUDY REPORT 18 23 00028

SUSPENSION TEST ACCORDING TO EN 1650:2008+A1:2013

Chemical disinfectants and antiseptics - evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (Phase 2 step 1)

PRODUCT NAME	:	GERM KILL ALCOHOL
SUBSTANCES AND THEIR CONCENTRATIONS	:	-
APPEARANCE OF THE PRODUCT	:	LIQUID
STORAGE CONDITIONS	:	ROOM TEMPERATURE, DARKNESS
LOT	:	-
METHOD	:	EN 1650:2008+A1:2013
CONTACT TIME	:	15 minutes
DILUTIONS	:	AS IS, 50%, 1%
DILUENT RECOMENDED BY THE MANUFACTURER	:	HARD 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	:	10/01/2018
STUDY PERIOD	:	17/01/2018-22/01/2018
LAB ID	:	2018-724/17 23 00028

OBJECTIVE

The objective of this study was to demonstrate the fungicidal activity of the test material under the requirements of European Standard EN 1650:2008+A1:2013 .

TEST SYSTEMS

Candida albicans	ATCC: 10231	Lot: 4435903
Aspergillus brasiliensis	ATCC: 16404	Lot: 3925233

TEST METHOD

European Standard EN 1650:2008+A1:2013 Chemical disinfectants and antiseptics. Evaluation of fungicidal activity. This EN test has two obligatory test micro-organisms the fungus: Aspergillus brasiliensis and the yeast: Candida albicans. The product under test has to achieve a log 4 kill against each test micro-organisms in 15 minutes at 20°C.

EXPERIMENTAL DESIGN/TEST CONDITIONS

Test temperature

The test has been conducted at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Concentrations

The test substance has been tested at dilutions: **AS IS(80%), 50%, 1%**

Contact times

A contact time of **15 minutes** has been used.

Interfering substance

A bovine albumin solution with final concentration **3g/L** has been used as interfering substance (dirty conditions)

CALCULATION AND EXPRESSION OF THE RESULTS

Calculation of the mycotic count (cfu/ml)

For the final assay (Na) and for the preliminary assay (A, B, C e Nv) and for the test suspension (N), the calculation of mycotic count is performed in the following way:

$$\text{cfu/ml} = \frac{C}{n \times V \times d}$$

C= total of colonies counted on both dishes number of counted dishes

n=number of counted dishes

V= volume used

d= dilution factor corresponding to the relevant dilution

The counting has been performed using the number of colonies counted on both dishes.

Only the dishes containing from 15 to 150 colonies have been used for the calculation of the results. In the assay, where the number of cfu on every dish counted was <15, the number of cfu/ml should be recorded as $<1.5 \times 10^2$.

Where the number of cfu on every dish counted was >150, the number of cfu/ml should be recorded as $>1.5 \times 10^3$.

Calculation of Viability reduction

Viability reduction has been calculated for each organism and test concentration using the following formula:

$$R = N \times 10^{-1} / N_a$$

where:

R = Viability reduction

N = bacterial count of test suspension

N_a = bacterial count of test mixture at the end of contact time

ASSAY VALIDITY CRITERIA

Verify the following:

N: must be included between 1.5×10^7 and 5.0×10^7

Nv: must be included between 3.0×10^2 and 1.6×10^3

B: must be equal to, or higher than 0.05 times Nv

C: must be equal to, or higher than 0.5 times B

A: must be equal to, or higher than 0.05 times Nv

where:

N= number of cfu/ml of the mycotic suspension

Nv= number of cfu/ml of the mycotic suspension in the preliminary assay

A= number of cfu/ml of the solution in the control of experimental conditions

B= number of cfu/ml of the validation of neutralizer non toxicity

C= number of cfu/ml of the validation of dilution-neutralization

TEST RESULTS FOR *Candida albicans* (YEASTICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)			
N	Vc1	Vc2	x mean
10 ⁻⁶	30	25	2.77E+07
10 ⁻⁷	3	3	log N
			7.44
			No (N/10)
			2.77E+06
			log No
			6.44
			6,17 < = logNo < = 6,70
			Yes

Validation and controls

Validation suspension (Nvo)				Experimental conditions (A)			Neutralizer control (B)				Method validation (C) Product conc.: AS IS(80%)				
Vc1	sum	134	x mean	Vc1	sum	112	x mean	Vc1	sum	129	x mean	Vc1	sum	92	x mean
	value1	value2			value1	value2			value1	value2			value1	value2	
	69	65		67	54	58		56	62	67		64,5	46	46	
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				Yes			Yes				Yes				

Test Results

Product concentration (%)	Contact time (min)	Vc1	Vc2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
as is	15 min	0	0	140	2.15	6.44	4.30	>4	PASS TEST
50%	15 min	0	0	140	2.15	6.44	4.30	>4	PASS TEST
1%	15 min	54545	54545	54545	4.74	6.44	1.71	>4	FAILS TEST

TEST RESULTS FOR *Aspergillus brasiliensis* (FUNGICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and Nvo)			
N	Vc1	Vc2	x mean
10 ⁻⁶	23	28	2.50E+07
10 ⁻⁷	2	2	log N
			7.40
			No (N/10)
			2.50E+06
			log No
			6.40
			6,17 < = logNo < = 6,70
			Yes

Validation and controls

Validation suspension (Nvo)				Experimental conditions (A)				Neutralizer control (B)				Method validation (C) Product conc.: AS IS (80%)			
Vc1	sum	98		Vc1	sum	110		Vc1	sum	120		Vc1	sum	94	
	value1	value2	x mean		value1	value2	x mean		value1	value2	x mean		value1	value2	x mean
	47	51	49		53	57	55		58	62	60		52	42	47
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				Yes				Yes				Yes			

Test Results

Product concentration (%)	Contact time (min)	Vc1	Vc2	Average	log Na	log N	log Reduction (N-Na)	Criteria	Result
as is	15 min	0	0	140	2.15	6.40	4.25	>4	PASS TEST
50%	15 min	0	0	140	2.15	6.40	4.25	>4	PASS TEST
1%	15 min	27273	27273	27273	4.44	6.40	1.96	>4	FAILS TEST

CONCLUSION

TEST SUBSTANCE IDENTIFICATION

PRODUCT NAME	: GERM KILL ALCOHOL
SUBSTANCES AND THEIR CONCENTRATIONS	: -
APPEARANCE OF THE PRODUCT	: LIQUID
STORAGE CONDITIONS	: ROOM TEMPERATURE, DARKNESS
LOT	: -
METHOD	: EN 1650:2008+A1:2013
CONTACT TIME	: 15 minutes
DILUTIONS	: AS IS, 50%, 1%
DILUENT RECOMENTED BY THE MANUFACTURER	: HARD WATER
STUDY SPONSOR	: Σ. ΚΟΥΖΟΥΝΑΣ ΚΑΙ ΣΙΑ ΕΕ ALCOFARM
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RECEIPT DATE	: 10/01/2018
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METHODOLOGY ABSTRACT

A test suspension of fungi (*C. albicans* & *Asp. brasiliensis*) is tested against a product test solution (at three concentrations) with the presence of interfering substance (bovine albumin -dirty conditions). The mixture is maintained at **(20 ± 1) °C for 15 minutes**. At the end of this contact time, an aliquot is taken, and the fungicidal and/or yeasticidal activity in this portion is immediately neutralized or suppressed. The numbers of surviving fungi in each sample are determined and the reduction is calculated.

RESULT

The test substance **GERM KILL ALCOHOL** demonstrated fungicidal and yeasticidal activity (>1.0 x 10⁴ Reduction in Viability or > 4 log reduction) under **dirty conditions, within contact time 15 minutes at 20±1 °C**, when tested at concentrations:

AS IS using as test organism the reference strain: *Candida albicans*.

50% using as test organism the reference strain: *Candida albicans*.

Signature date: 30/01/2018



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

STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1650:2008+A1:2013

Chemical disinfectants and antiseptics - evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (Phase 2 step 1)

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TEST SYSTEMS

Candida albicans ATCC: 10231 Lot: 4435903
Aspergillus brasiliensis ATCC: 16404 Lot: 3925233

RESULT

The test substance **GERM KILL ALCOHOL** demonstrated yeasticidal activity ($>1.0 \times 10^4$ Reduction in Viability or > 4 log reduction) under **dirty conditions**, within contact time **15 minutes** at **20±1 °C**, when tested at concentrations:

AS IS using as test organism the reference strain: *Candida albicans*.

50% using as test organism the reference strain: *Candida albicans*.

The test substance **GERM KILL ALCOHOL** demonstrated fungicidal activity ($>1.0 \times 10^4$ Reduction in Viability or > 4 log reduction) under **dirty conditions**, within contact time **15 minutes** at **20±1 °C**, when tested at concentrations:

AS IS using as test organism the reference strain: *Aspergillus brasiliensis*.

50% using as test organism the reference strain: *Aspergillus brasiliensis*

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