

MEDIFOOT

STUDY REPORT

2022-6590/ 22 23 00231

Nail Protection Lotion

SUSPENSION TEST
ACCORDING TO EN 1650:2019
(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 - Test method and
requirements (Phase 2, Step 1)

JULY 2022

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SUSPENSION TEST ACCORDING TO EN 1650:2019

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)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	: Nail Protection Lotion
ACTIVE SUBSTANCES	: Alcohol Denat. 37% w/w, Salicylic Acid 0.5% w/w
APPEARANCE OF THE PRODUCT	: Liquid
STORAGE CONDITIONS	: Room temperature
LOT	: 2295M
METHOD	: EN 1650:2019
COMPANY NAME	: MEDIFOOT
STUDY SPONSOR	: THE NUCLAB
PRODUCT MANUFACTURER	: MEDIFOOT
RECEIPT DATE	: 06/05/2022
STUDY PERIOD	: 14/07/2022-21/07/2022
LAB ID	: 2022-5394/22 23 00204

SCOPE

This document specifies a test method and the minimum requirements for yeasticidal or fungicidal 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) is added to a test suspension of fungi (yeast cells or mould spores) 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 yeasticidal and/or fungicidal 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 fungi in each sample are determined and the reduction is calculated.

The test is performed using the vegetative cells of *Candida albicans* and the spores of *Aspergillus brasiliensis* (fungicidal activity) or only the vegetative cells of *Candida albicans* (yeasticidal activity) as test organisms.

Where indicated, additional specific fungicidal or yeasticidal activity shall be determined applying other interfering substances and test organisms in order to take into account intended specific use conditions. In this test were used as additional test organisms the reference strains: *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

TEST CONDITIONS

1. Test temperature: 20 °C
2. Contact time: 60 seconds
3. Interfering substance: Bovine Albumin 3g/L final concentration (dirty conditions).
4. Dilution Method: Neutralization Method - Pour Plate Technique
5. Neutralizer used: LPT Dilution Broth containing 3% polysorbate 80.
6. Incubation temperature: $30 \pm 1^{\circ}\text{C}$
7. Appearance of product test solutions: No precipitate during the test procedure (homogeneous solution)
8. According to EN 1650, 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%, 0,1%.

TEST ORGANISMS (Obligatory Test Microorganisms)

<i>Candida albicans</i>	ATCC 10231
<i>Aspergillus brasiliensis</i>	ATCC 16404

TEST ORGANISMS (Additional Test Microorganisms)

<i>Trichophyton mentagrophytes</i>	ATCC 9533
<i>Trichophyton rubrum</i>	ATCC 28188

FUNGICIDAL ACTIVITY FOR GENERAL PURPOSES

The product shall be deemed to have passed the EN 1650 standard if it demonstrates in a valid test a reduction of at least a 4 lg under the adopted test conditions with the chosen interfering substance simulating clean or dirty conditions defined by this European Standard when the test organisms are *Candida albicans* and *Aspergillus brasiliensis*.

YEASTICIDAL ACTIVITY FOR GENERAL PURPOSES

The product shall be deemed to have passed the EN 1650 standard (yeastcidal activity) if it demonstrates in a valid test a reduction of at least a 4 lg within the adopted test conditions with the chosen interfering substance simulating clean or dirty conditions defined by this European Standard when the test organism is *Candida albicans*.

ASSAY ACCEPTANCE CRITERIA AND EXPLANATIONS

1. Test Suspension (N) is between 1.5 to 5.0×10^7 CFU per mL ($7.17 \leq \log N_0 \leq 7.70$).
2. N_0 ($N/10$) is between 1.5 to 5.0×10^6 CFU per mL ($6.17 \leq \log N_0 \leq 6.70$).
3. Validation Suspension= N_v is between 3.0×10^2 and 1.6×10^3 .
4. N_{v0} ($N_v/10$) is between 30 and 160.
5. N_a is the number of survivors (cells) per ml in the test mixture at the end of contact time.
6. R (log reduction) = $N_0 - N_a$
7. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (N_{v0})
8. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (N_{v0})
9. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (N_{v0})
10. Control of weighted mean counts. Quotient is not lower than 5 and not higher than 15
11. V_c values = Count per ml (one plate or more)
12. If the count on one plate for yeasts is higher than 330, the number is reported as "> 330".
13. If the count on one plate for fungi is higher than 165, the number is reported as "> 165".
14. If a V_c value is lower than 14, the number is reported "< 14"

Test Results for *Candida albicans*

Test suspension

Test - suspension			(N and No)	
N	Vc1	Vc2	x mean	
10 ⁻⁵	154	161	log N	1.58E+07
10 ⁻⁶	17	15	No (N/10)	7.20
			log No	1.58E+06
				6.20
			6,17 < = logNo < = 6,70	
			Yes	

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	37	x mean	Vc 1	30	x mean	Vc 1	30	x mean	Vc 1	27	x mean
Vc 2	31	34	Vc 2	34	32	Vc 2	36	33	Vc 2	25	26
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	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%)	1 min	10 ⁰	0	0	< 14	< 140	< 2.15	6.20	> 4.05	≥ 4	PASS TEST
		10 ⁻¹	0	0							
50%	1 min	10 ⁰	0	0	< 14	< 140	< 2.15	6.20	4.05	≥ 4	PASS TEST
		10 ⁻¹	0	0							
0.1%	1 min	10 ⁰	> 330	> 330	> 3300	> 33000	> 4.52	6.20	< 1.68	≥ 4	FAILS TEST
		10 ⁻¹	> 330	> 330							

Test Results for *Aspergillus brasiliensis*

Test suspension

Test - suspension			(N and No)	
N	Vc1	Vc2	x mean	
10 ⁻⁶	33	31	log N	3.23E+07
10 ⁻⁷	3	4	No (N/10)	7.51
			log No	3.23E+06
				6.51
			6,17 < = logNo < = 6,70	
			Yes	

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	59	x mean	Vc 1	60	x mean	Vc 1	57	x mean	Vc 1	50	x mean
Vc 2	64	61.5	Vc 2	63	61.5	Vc 2	61	59	Vc 2	57	53.5
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?			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%)	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.51	< 2.29	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							
50%	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.51	< 2.29	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							
0.1%	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.51	< 2.29	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							

Test Results for *Trichophyton rubrum*

Test suspension

Test - suspension			(N and No)	
N	Vc1	Vc2	x mean	
10 ⁻⁶	39	37	log N	3.77E+07
10 ⁻⁷	3	4	No (N/10)	7.58
			log No	3.77E+06
				6.58
			6,17 < = logNo < = 6,70	
			Yes	

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	82	x mean	Vc 1	84	x mean	Vc 1	81	x mean	Vc 1	80	x mean
Vc 2	78	80	Vc 2	80	82	Vc 2	76	78.5	Vc 2	77	78.5
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?			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 Vc 1 and Vc 2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10 ⁰	0	0	< 14	< 140	< 2.15	6.58	> 4.43	≥ 4	PASS TEST
		10 ⁻¹	0	0							
50%	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.58	< 2.36	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							
0.1%	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.58	< 2.36	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							

Test Results for *Trichophyton mentagrophytes*

Test suspension

Test - suspension			(N and No)	
N	Vc1	Vc2	x mean	
10 ⁻⁶	39	47	log N	4.41E+07
10 ⁻⁷	6	5	No (N/10)	7.64
			log No	4.41E+06
				6.64
			6,17 < = logNo < = 6,70	
			Yes	

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	86	x mean	Vc 1	82	x mean	Vc 1	81	x mean	Vc 1	90	x mean
Vc 2	82	84	Vc 2	87	84.5	Vc 2	89	85	Vc 2	86	88
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	Dilution step	Vc 1	Vc 2	Average of Vc 1 and Vc 2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10 ⁰	0	0	< 14	< 140	< 2.15	6.64	> 4.50	≥ 4	PASS TEST
		10 ⁻¹	0	0							
50%	1 min	10 ⁰	43	49	46.0	460	2.66	6.64	3.98	≥ 4	FAILS TEST
		10 ⁻¹	4	5							
0.1%	1 min	10 ⁰	> 165	> 165	> 1650	> 16500	> 4.22	6.64	< 2.43	≥ 4	FAILS TEST
		10 ⁻¹	> 165	> 165							

TEST PRODUCT IDENTIFICATION

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PRODUCT MANUFACTURER	: MEDIFOOT
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LAB ID	: 2022-5394/22 23 00204

METHODOLOGY ABSTRACT

A test suspension of fungi is tested against a product test solution with the presence of interfering substance. The mixture is maintained at 20 °C for 60 seconds contact time. 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.

CONCLUSION

The product under test: "Nail Protection Lotion" did not demonstrate fungicidal activity for general purpose according to EN 1650:2019 (≥ 4 log reduction) under dirty conditions, at 20 ± 1 °C, when tested at product concentration:

Undiluted (80%) for 60 seconds contact time using as test organisms the reference strain: *Aspergillus brasiliensis*.

The product under test: "Nail Protection Lotion" demonstrated yeasticidal activity for general purpose according to EN 1650:2019 (≥ 4 log reduction), under dirty conditions, at 20 ± 1 °C, when tested at product concentrations:

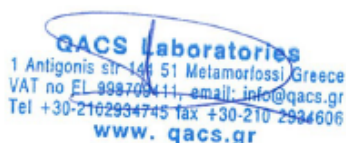
Undiluted (80%) for 60 seconds contact time using as test organisms the reference strain: *Candida albicans*

The product under test: "Nail Protection Lotion" demonstrated fungicidal activity for specific purpose according to EN 1650:2019 (≥ 4 log reduction) under dirty conditions, at 20 ± 1 °C, when tested at product concentration:

Undiluted (80%) for 60 seconds contact time using as test organisms the reference strains: *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

The method is accredited according to EN ISO/IEC 17025:2017 (Cert. No 195).

For the QACS Ltd Laboratory


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Date: 25/07/2022

Testing
Cert. No 195

STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1650:2019

Chemical disinfectants and antiseptics – evaluation of fungicidal and/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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<i>Aspergillus brasiliensis</i>	ATCC 16404

TEST ORGANISMS (Additional Test Microorganisms)

<i>Trichophyton mentagrophytes</i>	ATCC 9533
<i>Trichophyton rubrum</i>	ATCC 28188

CONCLUSION

The product under test: "Nail Protection Lotion" did not demonstrate fungicidal activity for general purpose according to EN 1650:2019 (≥ 4 log reduction) under dirty conditions, at 20 ± 1 °C, when tested at product concentration:

Undiluted (80%) for 60 seconds contact time using as test organisms the reference strain: *Aspergillus brasiliensis*.

The product under test: "Nail Protection Lotion" demonstrated yeasticidal activity for general purpose according to EN 1650:2019 (≥ 4 log reduction), under dirty conditions, at 20 ± 1 °C, when tested at product concentrations:

Undiluted (80%) for 60 seconds contact time using as test organisms the reference strain: *Candida albicans*

The product under test: "Nail Protection Lotion" demonstrated fungicidal activity for specific purpose according to EN 1650:2019 (≥ 4 log reduction) under dirty conditions, at 20 ± 1 °C, when tested at product concentration:

Undiluted (80%) for 60 seconds contact time using as test organisms the reference strains: *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

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.

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