

DETERMINATION OF BACTERICIDAL AND FUNGICIDAL ACTIVITY FOR SURFACE DISINFECTION

ACCORDING TO UNE EN 13697:2015 + A1:2019

TEST REPORT N° 02/2023

a) Identification of the test laboratory		Laboratório de Microbiologia Aplicado à Saúde (LMAS) Centro de Engenharia Biológica, Universidade do Minho	
b) Identification of the client			
- Name		Success Gadget, Nanotecnologia e Novos Materiais, Lda	
- Address		Rua Filipa Borges, 1245, 4750-823 Barcelos, Portugal	
c) Identification of the sample (data provided by the client, the laboratory is not responsible for the information provided by the client)			
- Product name		Care Us	
- Batch number		AMS220902	
- Expiration date		2023.09	
- Storage conditions		Ambient temperature	
- Manufacturer		Success Gadget, Nanotecnologia e Novos Materiais, Lda	
- Manufacturer recommended product diluent		Not disclosed	
- Active substances and their concentration		Hydrogen Peroxide (1.4% (w/w)); Ethanol (50% (w/w))	
- Product Appearance		Whitish (Please shake before use)	
d) Description of the sample by the laboratory			
- Delivery Date		05/09/2022	
- Product Appearance		Transparent Liquid	
- Active substances and their concentration		Not request	
e) Test method and its validation			
- Method		Dilution - neutralization	
- Technique		Pour plate	
- Neutralizer		Lecithin (3g/l); Tween 80 (30ml/l); Saponin (30 g/l); L-hystidine (1g/l); Sodium thiosulfate(5g/l); In phosphate buffer 0,0025N	
f) Experimental Conditions			
- Period of analysis		From 06/09/2022 to 23/09/2022	
- Diluent of the product used during the test		Sterile distilled water	
- Product test concentration		100%; 75%; 50%	
- Aspect of product dilution		Transparent solutions, soluble in sterile distilled water	
- Stability of the mixture during the procedure		Stable	
- Interfering substances		Aqueous bovine albumin solution 0.3 g/L	
- Contact times		60 seconds ± 5 seconds	
- Temperature of assay		(20 ± 1) °C	
- Temperature of incubation		(30 ± 1) °C	
- Identification of the test strains		- <i>Pseudomonas aeruginosa</i> ATCC10145 - <i>Escherichia coli</i> ATCC 25922 - <i>Staphylococcus aureus</i> ATCC 6538 - <i>Candida albicans</i> SC 5314 - <i>Enterococcus hirae</i> DSM 28619 - <i>Aspergillus niger</i> MUM 9235	
g) Test results (See table A)			
h) Special remarks regarding the results			
- All controls and validation were within their baseline limits. - At least one concentration of the product demonstrated a log reduction of less than 4 lg, for bacteria and a log reduction of less than 3 log, for fungal.			
i) Conclusion			
For the tested sample of the product “Care Us” batch AMS220707, DILUTED (75%) shows bactericidal and fungicidal activity, based on EN 13697:2015+A1:2019 under clean conditions (aqueous bovine albumin solution 0.3 g/L) at 20 °C, for 60 second of contact.			

Technical manager

Laboratory technician

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Test Organisms	N (Test and validation suspension)			NC (Neutralizer toxicity control)			NT (Method Validation)			Nc			Nd (Test procedure at the concentration, %)		
		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}	100	75	50
<i>Staphylococcus aureus</i> ATCC 6538	10 ⁻⁶	250	259	10 ⁻⁴	39	31	10 ⁻⁴	30	28	10 ⁻⁴	33	36	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : > 330; > 330 10 ⁻¹ : > 330; > 330 N _a = > 3.3 × 10 ⁵
	10 ⁻⁷	39	40	10 ⁻⁵	4	4	10 ⁻⁵	3	3	10 ⁻⁵	4	5			
	N = 6.9 × 10 ⁶ Log N = 6.82			NC = 3.5 × 10 ⁶ Log NC = 6.55			NT = 2.9 × 10 ⁶ Log NT = 6.46			Nc = 3.5 × 10 ⁶ Log Nc = 6.55			Log N _a = < 0.1 Log R = > 6.45	Log N _a = < 0.1 Log R = > 6.45	Log N _a = > 5.52 Log R = < 1.03
<i>Escherichia coli</i> ATCC 25922	10 ⁻⁶	164	195	10 ⁻⁴	33	38	10 ⁻⁴	44	37	10 ⁻⁴	32	37	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : > 330; > 330 10 ⁻¹ : > 330; > 330 N _a = > 3.3 × 10 ⁵
	10 ⁻⁷	17	22	10 ⁻⁵	4	5	10 ⁻⁵	5	5	10 ⁻⁵	4	4			
	N = 4.3 × 10 ⁶ Log N = 6.65			NC = 3.6 × 10 ⁶ Log NC = 6.56			NT = 4.1 × 10 ⁶ Log NT = 6.61			Nc = 3.5 × 10 ⁶ Log Nc = 6.54			Log N _a = < 0.1 Log R = > 6.44	Log N _a = < 0.1 Log R = > 6.44	Log N _a = > 5.52 Log R = < 1.02
<i>Pseudomonas aeruginosa</i> ATCC 10145	10 ⁻⁶	244	234	10 ⁻⁴	27	21	10 ⁻⁴	28	27	10 ⁻⁴	33	36	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : > 330; > 330 10 ⁻¹ : > 330; > 330 N _a = > 3.3 × 10 ⁵
	10 ⁻⁷	32	29	10 ⁻⁵	6	4	10 ⁻⁵	4	4	10 ⁻⁵	4	5			
	N = 6.1 × 10 ⁶ Log N = 6.79			NC = 2.6 × 10 ⁶ Log NC = 6.42			NT = 2.9 × 10 ⁶ Log NT = 6.46			Nc = 2.4 × 10 ⁶ Log Nc = 6.37			Log N _a = < 0.1 Log R = > 6.27	Log N _a = < 0.1 Log R = > 6.27	Log N _a = > 5.52 Log R = < 0.85
<i>Enterococcus hirae</i> DSM 28619	10 ⁻⁶	166	143	10 ⁻⁴	39	42	10 ⁻⁴	48	44	10 ⁻⁴	33	37	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : > 330; > 330 10 ⁻¹ : > 330; > 330 N _a = > 3.3 × 10 ⁵
	10 ⁻⁷	17	14	10 ⁻⁵	7	7	10 ⁻⁵	6	5	10 ⁻⁵	4	5			
	N = 3.9 × 10 ⁶ Log N = 6.59			NC = 4.3 × 10 ⁶ Log NC = 6.53			NT = 4.7 × 10 ⁶ Log NT = 6.67			Nc = 3.6 × 10 ⁶ Log Nc = 6.55			Log N _a = < 0.1 Log R = > 6.45	Log N _a = < 0.1 Log R = > 6.45	Log N _a = > 5.52 Log R = < 1.03

Test Organisms	N (Test and validation suspension)			NC (Neutralizer toxicity control)			NT (Method Validation)			Nc			Nd (Test procedure at the concentration, %)		
		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}	100	75	50
<i>Candida albicans</i> SC 5314	10 ⁻⁵	162	160	10 ⁻³	19	19	10 ⁻³	19	15	10 ⁻³	20	17	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 3; 2 10 ⁻¹ : 0; 0 N _a = < 1.4×10 ²	10 ⁰ : > 165; > 165 10 ⁻¹ : > 165; > 165 N _a = > 3.3 × 10 ⁵
	10 ⁻⁶	17	18	10 ⁻⁴	3	2	10 ⁻⁴	5	3	10 ⁻⁴	3	3			
	N = 4.1×10 ⁵ Log N = 5.61			NC = 2.0×10 ⁵ Log NC = 5.29			NT = 1.9×10 ⁵ Log NT = 5.28			Nc = 1.9×10 ⁵ Log Nc = 5.28					
<i>Aspergillus niger</i> MUM 9235		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}		V _{C1}	V _{C2}	100	75	50
	10 ⁻⁵	140	139	10 ⁻³	18	21	10 ⁻³	34	18	10 ⁻³	26	22	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0	10 ⁰ : 1; 2 10 ⁻¹ : 0; 0 N _a = < 1.4×10 ²	10 ⁰ : > 165; > 165 10 ⁻¹ : > 165; > 165 N _a = > 3.3 × 10 ⁵
	10 ⁻⁶	20	25	10 ⁻⁴	3	3	10 ⁻⁴	5	2	10 ⁻⁴	4	4			
N = 3.7×10 ⁵ Log N = 5.57			NC = 1.0×10 ⁵ Log NC = 5.31			NT = 2.7×10 ⁵ Log NT = 5.42			Nc = 2.6×10 ⁶ Log Nc = 5.41			Log N _a = < 0.1 Log R = > 5.31			

Vc: counts obtained in plate.

N: number of cells per 0.025 mL in the test suspension.

Nd: number of survivors per surface at the end of the contact time.

Nc: number of survivors per water control surface.

NC: number of survivors per neutraliser toxicity control surface.

NT: number of survivors per method validation control surface.

Log R: Logarithmic reduction ($Log R = Log Nc - Log Nd$)

Verification of the methodology:

- N** is comprised between 1.5 and 5×10⁸ ($6.57 < log N < 7.10$), for the bacterial test suspension.
N is comprised between 1.5 to 5×10⁷ ($5.57 < log N < 6.10$), for the fungal test suspension.
- Log NC – Log Nc is not** greater than ± 0.3.
- Log NT – Log Nc is not** greater than ± 0.3.
- The mean counts from duplicate plate used for calculation of N, NC, Nc, NT and Nd are between 14 and 330 for bacteria and yeast strains and 14 and 165 for mould strains;
- Nts is less than 100 cfu/ml for active concentrations. If not, the recovery of micro-organisms has not been sufficient. For non active concentrations, Nts may be not countable.