

**DETERMINATION OF BACTERICIDAL ACTIVITY FOR SURFACE DISINFECTION
ACCORDING TO UNE EN 13727:2012+A2:2015
TEST REPORT Nº 230008786***

a) Identification of the test laboratory	"Control Microbiológico Bilacon, S.L.U."
b) Identification of 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
- Product appearance	WITHISH (PLEASE SHAKE BEFORE USE)
- Batch number	AMS230120
- Expiration date	2024.01
- Manufacturer	SUCCESS GADGET, NANOTECNOLOGIA E NOVOS MATERIAIS, LDA.
- Storage conditions	AMBIENT TEMPERATURE
- Manufacturer's recommended product diluent	Not indicated
- Active substances and their concentration	HYDROGEN PEROXIDE (1.4% (w/w)); ETHANOL (50% (w/w))
d) Description of the sample by the laboratory	
- Delivery date	26/01/2023
- Product appearance	Transparent liquid
- Active substances and their concentration	Not requested
e) Test method and its validation	
- Method	Dilution – neutralization
- Technique	Pour plate
- Neutralizer	Lecitine (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 21/02/2023 to 23/02/2023
- Diluent of the product used during the test	Sterile distilled water
- Product test concentration	80%; 40%; 20%
- Aspect of product dilutions	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	5 minutes ± 10 seconds
- Temperature of assay	20°C ± 1°C
- Temperature of incubation	37± 1°C
- Identification of test strains	- <i>Pseudomonas aeruginosa</i> CECT 116 - <i>Escherichia coli</i> K12 CECT 433 - <i>Staphylococcus aureus</i> CECT 239 - <i>Enterococcus hirae</i> CECT 4081
g) Test results (See table A.1)	
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 5 lg.	
- For hand washing, at least one concentration of the product demonstrated a log reduction of less than 3 lg.	
- No precipitate was formed during the test procedure (test mixtures were homogeneous).	
i) Conclusion	
The tested sample of the product " CARE US ", batch AMS230120 , UNDILUTED (80%) shows bactericidal activity for surface disinfection, based on UNE EN 13727:2012+A2:2015, under clean conditions (aqueous bovine albumin solution 0.3g/l) at 20°C, for 5 minutes of contact.	

Technical manager

Laboratory technician

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Table A.1
Test results

Test organisms	Validation test suspension				Assay validation						Assay suspension			Test procedure at the concentration %			
	N _v and N _{v0}		N _{vB}		Control of the experimental conditions (A)		Control of neutralizer (B)		Validación de the method (C)					80	40	20	
	V _{c1}	V _{c2}	V _{c1}	V _{c2}	V _{c1}	V _{c2}	V _{c1}	V _{c2}	V _{c1}	V _{c2}	V _{c1}	V _{c2}					
<i>Pseudomonas aeruginosa</i> CECT 116	67	69	65	65	66	63	61	62	45	48	10 ⁻⁶	>330	>330	N = 3.4x10 ⁸ Log N = 8.52	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0 Na = <1.4x10 ² Log Na = <2.15 Log R = >5.37	10 ⁰ : >330; >330 10 ⁻¹ : 55; 57 Na = 5.6x10 ³ Log Na = 3.75 Log R = 3.77	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.00
	N _v = 6.8x10 ² N _{v0} = 68		N _{vB} = 6.5x10 ⁴		A = 64		B = 62		C = 46			10 ⁻⁷	33	34	N ₀ = 3.4x10 ⁷ Log N ₀ = 7.52		
<i>Escherichia coli</i> K12 CECT 433	72	68	69	64	66	65	64	66	58	59	10 ⁻⁶	>330	>330	N = 3.6x10 ⁸ Log N = 8.56	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0 Na = <1.4x10 ² Log Na = <2.15 Log R = >5.41	10 ⁰ : 19; 18 10 ⁻¹ : 1; 1 Na = 1.8x10 ² Log Na = 2.27 Log R = 5.29	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.04
	N _v = 7x10 ² N _{v0} = 70		N _{vB} = 6.6x10 ⁴		A = 66		B = 65		C = 58			10 ⁻⁷	38	34	N ₀ = 3.6x10 ⁷ Log N ₀ = 7.56		
<i>Staphylococcus aureus</i> CECT 239	95	97	94	91	93	90	89	87	78	79	10 ⁻⁶	>330	>330	N = 4.1x10 ⁸ Log N = 8.61	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0 Na = <1.4x10 ² Log Na = <2.15 Log R = >5.46	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.09	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.09
	N _v = 9.6x10 ² N _{v0} = 96		N _{vB} = 9.2x10 ⁴		A = 92		B = 88		C = 78			10 ⁻⁷	40	42	N ₀ = 4.1x10 ⁷ Log N ₀ = 7.61		
<i>Enterococcus hirae</i> CECT 4081	92	89	87	87	90	86	85	83	80	79	10 ⁻⁶	>330	>330	N = 3.6x10 ⁸ Log N = 8.56	10 ⁰ : 0; 0 10 ⁻¹ : 0; 0 Na = <1.4x10 ² Log Na = <2.15 Log R = >5.41	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.04	10 ⁰ : >330; >330 10 ⁻¹ : >330; >330 Na = >3.3x10 ⁴ Log Na = >4.52 Log R = <3.04
	N _v = 9x10 ² N _{v0} = 90		N _{vB} = 8.7x10 ⁴		A = 88		B = 84		C = 80			10 ⁻⁷	35	38	N ₀ = 3.6x10 ⁷ Log N ₀ = 7.56		

V_c: counts obtained in plate.

N: number of cells per ml in the test suspension.

N₀: number of cells per ml at the beginning of the contact time.

N_v: number of cells per ml in the validation suspension.

N_{v0}: number of cells per ml in mixtures A, B and C at the beginning of the contact time.

N_{vB}: (dilution method) number of cells per ml after 1: 100 dilution

Na: number of survivors per ml in the test mixture at the end of the contact time.

A: number of survivors in the control conditions of experiment A.

B: number of survivors in the neutralizing or filtrate control B.

C: number of survivors in the validation of method C.

Log R: Logarithmic reduction.

VERIFICATION OF THE METHODOLOGY:

a) N is comprised between 1.5 and 5x10⁸ (8.17 g, log N 8. 8.70).

N (modified method) is in the range of 1.5 to 5x10⁹ (9.17 g, log N 9.70).

N₀ is between 1.5 and 5x10⁷ (7.17 g, log N, 7.70).

b) N_{v0} is between 30 and 160.

N_v is between 3x10² and 1.6x10³.

N_v (modified method) is between 3x10³ and 1.6x10⁴

N_{vB} is between 3x10⁴ and 1.6x10⁵.

c) A, B and C are equal to or greater than 0.5 x N_{v0}

B (dilution-neutralization) is equal to or greater than 0.0005x N_{vB}

d) For the results calculated by weighted averages of two subsequent dilutions, the quotient of the mean of the 2 results is between 5 and 15