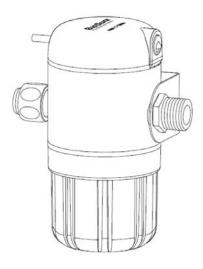


CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

EXECUTION OF UNE-EN TESTS TO CERTIFY THE BACTERICIDAL CAPACITY OF THE OZONIZED WATER GENERATED WITH THE DEVICE EOS7211-BX/WS1200X

Device: EOS7211-BX /WS1200X





Objective

The purpose of these analyses and tests is **to evaluate and demonstrate the bactericidal activity of ozonized water** generated "in situ" with the equipment to be tested.

The standard that has been carried out is <u>UNE-EN 13727:2012+A2:2015</u> - Quantitative suspension test for the evaluation of bactericidal activity in the medical area (phase 2, step 1).

Furthermore, the UNE-EN 13727:2012+A2:2015 standard include, in addition to the bacterial strains indicated therein, Salmonella and Listeria monocytogenes of special interest in the field of food industry.

The device responsible for generating the ozonated water provided by the company **BES Group Biosure Professional** has been the model **EOS7211-BX / WS1200X** (Serial number: 7211BXNA1101 – Production: 2023).



CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

Test execution: 15/02/2024 Analytical report: I-3937/24

Methodology

A sample of ozonized water, generated in situ, is collected with the equipment provided by [BES Group Biosure Professional model EOS7211-BX / WS1200X (Serial N° : 7211BXNA1101 – Production: 2023)] from cold tap water in the same laboratory where it will be analyzed.

The measurement of ozone concentration and REDOX potential is carried out immediately after generating the ozonated water.

The method used to determine each parameter is the following:

- Ozone concentration: UV-VIS spectrophotometry (potassium indigo trisulfonate wavelength 600nm).
- REDOX potential: potentiometry (ozonated water in circulation).

Results

Device	Parametres	Results
BES Group Biosure Professional EOS7211-BX /	Dissolved ozone concentration	1,62 mg/L
WS1200X (N° de serie: 7211BXNA1101 – Production: 2023)	REDOX Potential	921 mv

Oviedo, 23rd of Februay 2024

Daniel Cepedal Macías Technical Director inoQua | Food Health Institute



BACTERICIDAL ACTIVITY TEST IN THE MEDICAL AREA Standard UNE-EN 13727:2012+A2:2015

Quantitative suspension test for the evaluation of bactericidal activity in the medical area (phase 2, step 1)

Test execution: 26/02/2024 - 09/03/2024

Analytical report: 5052/24

Methodology

The test method established in this standard -to evaluate and demonstrate the bactericidal activity of ozonized water in the 4 activities of the medical area mentioned- is based on the determination of the microorganisms surviving the action of ozonized water through a dilution-neutralization procedure.

For this purpose, the ozonized water sample is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at the temperature and contact durations specified in the table depending on the activity concerned.

Ozonated water is a transparent, colorless liquid, soluble in normalized hard water. The concentrations of ozonated water chosen to carry out the test were 100%, 90% and 30% and will be the ones we refer to in the results tables. However, it must be taken into account that due to the dilution processes when incorporating the solution with the bacteria and interfering substances during the experimental development, the maximum possible concentration of the product to be tested according to the standard procedure is 80%.

The interfering substance used under dirty conditions is an aqueous solution of bovine albumin and sheep erythrocytes at a concentration of 3g/L and 3ml/L, respectively. Under clean conditions, the aqueous solution is bovine albumin at a concentration of 0.3 g/L.

The bacterial strains composing the suspension were those stipulated in the standard. Namely:

- Pseudomonas aeruginosa ATCC 15 442
- Escherichia coli K12 CECT 433
- Staphylococcus aureus ATCC 6538
- Enterococcus hirae ATCC 10 541
- Enterococcus faecium ATCC 700221

In addition, 2 bacterial strains of interest in the field of food safety are included in the tests evaluating surface disinfection:

- Listeria monocytogenes ATCC 35152
- Salmonella enterica subsp. enterica ATCC 35664



The following table lists the strains used and the temperatures at which the tests were carried out according to application activity and contact times:

APPLICATION ACTIVITIES	Hygienic handrub treatment and hygienic handwash	Surgical handrub treatment and surgical handwash	Instrument disinfection	Surface disinfection
	Pseudomonas aeruginosa Staphylococcus aureus	Pseudomonas aeruginosa Staphylococcus aureus	Pseudomonas aeruginosa Staphylococcus aureus	Pseudomonas aeruginosa Staphylococcus aureus
Cepas bacterianas	Enterococcus hirae Escherichia coli K12 Listeria monocytogenes	Enterococcus hirae Escherichia coli K12 Listeria monocytogenes	Enterococcus hirae Enterococcus faecium	Enterococcus hirae Listeria monocytogenes
	Salmonella enterica subsp. enterica	Salmonella enterica subsp. enterica	Listeria monocytogenes Salmonella enterica subsp. enterica	Salmonella enterica subsp. enterica
Test temperature	20°C	20℃	20°C (70°C Enterococcus faecium)	20°C
Contact time	60 seconds	1 minute	15 minutes	1 minute

After contact time has elapsed, the bactericidal and/or bacteriostatic action is immediately neutralized or suppressed with a previously validated neutralizing solution. In this case, the neutralizers used were Lecithin (3g/l); Tween 80 (30ml/l); Sodium thiosulphate (5g/l); Lhistidine (1 g/l); In phosphate buffer 0,0025N.

At the same time, the number of bacteria in a suspension treated with hard water (300 mg/Kg CaCo3) instead of ozonized water is also determined and the reduction in viable counts attributed to the product is calculated by difference.

Finally, the number of surviving microorganisms that can be recovered from the suspension is quantitatively determined. The culture medium used for sowing was TSA (Tryptone Soya Agar) and the incubation temperature was 36°C.



Results and conclusion

Results of the test are shown in the following table:

> Hygienic handrub treatment and hygienic handwash (Temperature 20°C - 60 seconds of contact).

Dirty conditions

	Val	idation s	uspens		EXPERIN		/alidatio	on test							Concentrat	ion test proced	ure % (V/V)
Test organism	N∨a	nd N V0	N	VB	COND		CON	ITROL (B)		IDATION (C)		Te	st suspe	ension	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Pseudomonas	100	97	96	91	96	94	94	91	78	74	10-6		>330	N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
aeruginosa											10 ,	>330	>550	Log N = 8,63	$Na = <1,4x10^2$	Na = <1,4x10 ²	Na =>3,3x10 ⁴
ATCC 15 442	NV=9	,85×10 ^a	NVB=9	,35x10 ⁴	A=	95	B=9	92,5	C=	76	10-7	45	46	N0=4,55X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=9	9,85x10 ²									10	45	46	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Staphylococcus	39	36	41	38	41	39	38	36	39	34	10-6	>330	>330	N=3,3X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
aureus ATCC 6538											10	>550	>330	Log N = 8,50	$Na = <1,4x10^2$	Na = <1,4x10 ²	Na =>3,3x10 ⁴
aureus Arcc 0036	NV=3	,75x10 ²	NVB=3	,95x10 ⁴	A=	40	B=	37	C=3	36,5	10-7	37	35	N0=3,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=	3,75×10									10	3/	33	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Enterococcus	103	101	98	94	98	96	91	89	75	71	10-6	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
hirae											10	>550	>330	Log N = 8,62	$Na = <1,4x10^2$	Na = <1,4x10 ²	Na =>3,3x10 ⁴
ATCC 10 541	NV=1	0,2x10 ³	NVB=9	9,6x10 ⁴	A=	97	B=	90	C=	73	10-7	45	45	N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=1	10,2x10 ²									10	45	45	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Escherichia coli	41	38	41	36	41	37	38	35	38	33	10-6	>330	>330	N=3,1X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
K12 CECT 433											10	>550	>330	Log N = 8,52	Na = <1,4x10 ²	Na = <1,4x10 ²	Na =>3,3x10 ⁴
K12 CECT 455	NV=3	,95×10²	NVB=3	,85x10 ⁴	A=	39	B=3	36,5	C=3	35,5	10-7	35	35	N0=3,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=	3,95x10									10	35	35	Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	100:>300;>300
Salmonella	100	95	96	91	96	91	93	91	77	73	10-6	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
enterica ATCC											10	>550	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na =>3,3x10 ⁴
35664	NV=9	,75x10³	NVB=9	,35x10 ⁴	A=9	3,5	B=	92	C	75	10-7	43	45	N0=4,4X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=9	9,75x10 ²									10	45	45	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	100:0;0	100:>300;>300
Listeria	104	100	96	92	96	94	93	90	78	73	10-6	>330	>330	N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10":>300;>300
monocytogenes											10 .	×330	×330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na =>3,3x10 ⁴
ATCC 35152	NV=1	0,2x10 ^a	NVB=9	9,4x10 ⁴	A=	95	B=9	91,5	C=7	75,5	10-7	36	36	N0=3,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na =>4,52
	NV0=1	10,2x10 ²									10 .	36	36	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time.

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

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



> Hygienic handrub treatment and hygienic handwash (Temperature 20°C - 60 seconds of contact).

Clean conditions

	Validation suspension		sion		\	/alidatio	on test							Canaan	tration test proc	adura 9/ ()/A/)	
Test organism	N V ar	nd N V0	N	VB	EXPERII COND CON		CON	RALIZER ITROL (B)	VALID	THOD DATION C)		Test	suspen	sion	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0; 0	10°: >300 ; >300
Pseudomonas	103	99	96	94	96	94	94	92	74	72		ACI	VCZ	N=4.2X10 ⁸	10-:0;0	10":0; 0	10": >300; >300
aeruginosa	103	99	96	94	96	94	94	92	/4	12	10 ⁻⁶	>330	>330	Log N = 8.63	Na = <1.4x10 ²	Na = <1.4x10 ²	Na = >3.3x10 ⁴
ATCC 15 442	NV=1	0,1x10 ³	NVB=9	5v10 ⁴	Δ-	95	B=	-03	C=	72				N0=4,3X10 ⁷	Log Na = <2,15	-,	Log Na =>4,52
7100 15 412		0.1x10 ²		,,,,,,,		55		55	- ŭ	,,,	10-7	44	42	Log NO = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2	LOG 140 - 7,02	10°:0:0	10°:0:0	10°: >300; >300
	41	39	42	40	42	38	41	37	41	39				N=3.3X10 ⁸	10 :0; 0	10 :0,0	10-1: >300; >300
Staphylococcus	41	33	42	40	42	36	41	37	41	33	10 ⁻⁶	>330	>330	Log N = 8,50	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus ATCC 6538	NV=	4x 10 ²	NVB=4	1.1x10 ⁴	A=	40	B=	:39	C=	40	_			N0=3,4X10 ⁷	Log Na = <2,15		Log Na = >4,52
	NVO	=4×10		,		-				-	10-7	34	34		Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2	Legite 1,cc	10°:0:0	10°:0:0	10°: >300; >300
Enterococcus	104	102	98	93	98	93	91	86	77	72				N=4,1X10 ⁸	10": 0: 0	10": 0: 0	10": >300; >300
hirae											10 ⁻⁶	>330	>330	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 10 541	NV=1	0,3x10 ³	NVB=9	,55x10 ⁴	A=9	95,5	B=8	38,5	C=7	4,5				N0=4,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,3x10 ²									10-7	43	46	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0:0	10°:0:0	10°: >300; >300
	38	34	39	35	39	37	42	39	40	38	40-6			N=3,1X10 ⁸	10-1: 0: 0	10-1: 0: 0	10-1: >300; >300
Escherichia coli											10 ⁻⁶	>330	>330	Log N = 8,52	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
K12 CECT 433	NV=	,6x10 ²	NVB=3	3,7x10 ⁴	A=	38	B=4	40,5	C=	39				N0=3,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	3,6x10									10-7	36	36	Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	102	100	96	93	96	94	91	86	73	69	10-6	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10.0	>330	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=1	0,1x10³	NVB=9	,45x10 ⁴	A=	95	B=8	38,5	C=	71	10-7		46	N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,1x10 ²									10-7	44	46	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300 ; >300
Listeria	101	96	98	93	98	96	92	87	74	72	10-6	>330	>330	N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10 -	>330	>330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=9	,85x10 ³	NVB=9	,55x10 ⁴	A=	97	B=8	39,5	C=	73	10-7	26	34	N0=3,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9	9,85x10 ²									10.,	36	34	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time.

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

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



> Surgical handrub treatment and surgical handwash (Temperature 20°C – 1 minute of contact)

Dirty conditions

	V	alidation	suspen	sion			'alidatio								Concentr	ation test proces	dure % (V/V)
Test organism	N ∨ ar	nd N V0	N [,]	VB	COND CONT		CON	ALIZER TROL B)	VALID	HOD ATION C)		Test	suspens	ion	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Pseudomonas	103	98	96	92	96	91	93	88	74	72	40-6			N=4,2X10 ⁸	10": 0: 0	10": 0: 0	10": >300; >300
aeruginosa											10-6	>330	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442	NV=10	0,05x10 ³	NVB=9	,4x10 ⁴	A=9	3,5	B=9	90,5	C=	73	10-7			N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,05×10 ²									10-7	44	46	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Chambadaaaaaaa	39	37	42	38	42	38	40	37	41	39	10-6	>330	>330	N=3,3X10 ⁸	10-1: 0; 0	10-1: 0; 0	10": >300; >300
Staphylococcus aureus ATCC 6538											10 °	>330	>330	Log N = 8,50	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus ATCC 6538	NV=3	3,8x10 ²	NVB=	4x10 ⁴	A=	40	B=3	88,5	C=	40	10-7	35	34	N0=3,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	3,8x10									10 .	35	34	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	100: >300; >300
Enterococcus	100	96	99	94	99	94	91	89	76	71	10 ⁻⁶	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
hirae											10	>550	2550	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 10 541	NV=9	9,8x10³	NVB=9	,65x10 ⁴	A=9	96,5	B=	90	C=7	73,5	10-7	42	46	N0=4,4X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	9,8x10²									10 .	42	46	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Escherichia coli	41	36	41	39	41	39	38	36	42	39	10-6	>330	>330	N=3,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
K12 CECT 433											10	>330	> 330	Log N = 8,52	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
K12 CECT 433	NV=3,	,85x10²	NVB=	4x10 ⁴	A=	:40	B=	37	C=4	10,5	10-7	35	34	N0=3,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3	3,85x10									10	33	54	Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	103	99	99	96	99	96	92	88	77	73	10-6	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10	/330	/330	Log N = 8,63	$Na = <1,4x10^2$	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=1	0,1x10³	NVB=9	,75x10 ⁴	A=9	97,5	B=	90	C=	:75	10-7	43	44	N0=4,35X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	10,1x10²									10 .	43	44	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Listeria	102	99	96	91	96	93	92	87	74	71	10-6	>330	>330	N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10 -	/550	/550	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=10	0,05x10 ³	NVB=9	,35x10 ⁴	A=9	94,5	B=8	39,5	C=7	72,5	10-7	36	37	N0=3,65X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,05×10 ²									10,	36	3/	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time. Nv: number of cells per ml in the validation suspension.

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



> Surgical handrub treatment and surgical handwash (Temperature 20°C – 1 minute of contact)

Clean conditions

	Validation suspension			sion		\	/alidatio	on test							Concor	ntration test pro	codure % (V/A/)
Test organism	N V an	d Nvo	N	VB	COND COND		CON	RALIZER ITROL (B)	VALI	THOD DATION (C)		Test	suspen	sion	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0:0	10°: >300 : >300
Pseudomonas	101	97	98	95	98	93	93	89	75	71		ACI	VCZ	N=4,2X10 ⁸	10":0;0	10":0;0	10": >300; >300
aeruginosa	101	97	90	95	90	95	95	69	/5	/1	10 ⁻⁶	>330	>330	Log N = 8.63		Na = <1.4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442	NIV-C	.9x10 ³	NVB=9	6Ev104	Λ-0	95.5	D-	:91	C-	73					Na = <1,4x10 ²		
ATCC 15 442		9.9x10 ²	INV D-9	,03810	A-3	15,5	Б-	91	C-	./3	10-7	45	46	N0=4,55X10 ⁷ Log N0 = 7,62	Log Na = <2,15 Log R = >5,46	Log Na = <2,15 Log R = >5,50	Log Na = >4,52
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2	LOG NU = 7,62	10°:0:0	10°:0:0	Log R = < 3,09 10°: >300 : >300
	39	36	42	38	42	37	39	35	38	34		ACI	VCZ	N=3.3X10 ⁸	10":0;0	10":0; 0	10": >300; >300
Staphylococcus	39	36	42	38	42	3/	39	35	38	34	10 ⁻⁶	>330	>330	N=3,3X10° Log N = 8,50	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus ATCC 6538	NV=3	75x10 ²	NVB=	4×10 ⁴	Δ=3	39,5	R-	:37	C-	36				N0=3,6X10 ⁷	Log Na = <2,15		Log Na = >4,52
		3.75×10	1440-	4710		,,,,	-	57	-	30	10-7	36	36	Log NO = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2	208 110 - 7,55	10°:0;0	10°:0:0	10°: >300; >300
Enterococcus	102	100	96	94	96	92	92	90	75	72		142		N=4,1X10 ⁸	10 :0; 0	10 :0; 0	10":>300;>300
hirae	102	100	50	34	30	32	32	30	/5	12	10-6	>330	>330	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 10 541	NV=10	0.1x10 ³	NVB=9	9.5x10 ⁴	A=	94	R=	91	C=7	73.5					Log Na = <2,15		Log Na = >4,52
A100 10 541		0.1x10 ²		JORZO	- "					5,5	10-7	46	43	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2	208 110 - 7,01	10°:0:0	10°:0:0	10°: >300; >300
	42	37	39	35	39	37	40	35	40	36				N=3.1X10 ⁸	10": 0: 0	10": 0: 0	10": >300; >300
Escherichia coli											10 ⁻⁶	>330	>330	Log N = 8,52	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
K12 CECT 433	NV=3.	95x10 ²	NVB=3	3.7×10 ⁴	A=	38	B=3	37.5	C=	38						Log Na = <2,15	Log Na = >4,52
	NV0=3	3.95x10		,							10-7	37	35		Log R = >5,41	Log R = >5,41	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	100	96	96	92	96	94	93	89	78	76	40-6			N=4,7X108	10": 0; 0	10": 0; 0	10":>300;>300
enterica ATCC											10 ⁻⁶	>330	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=9	,8x10³	NVB=9	,4x10 ⁴	A=	95	B=	91	C=	77	40.7			N0=4,25X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	9,8x10 ²									10-7	42	43	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Listeria	102	98	99	94	99	95	93	89	78	75				N=3,2X10 ⁸	10": 0: 0	10": 0: 0	10": >300; >300
monocytogenes											10 ⁻⁶	>330	>330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=	10x10 ³	NVB=9	,65x10 ⁴	A=	97	B=	91	C=7	76,5				N0=3,5X10 ⁷	Log Na = <2,15		Log Na = >4,52
	NV0=	10x10 ²									10-7	35	35	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time.

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

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



➤ Instrument disinfection (Temperature 20°C – 15 minutes of contact)

Dirty conditions

	V	alidation	suspen	sion		V	alidatio	n test							Concent	ration test proce	dura % (MAA)
Test organism	N V ar	d Nvo	N		EXPERIN COND CONT		CON	ALIZER TROL B)	VALID	HOD ATION (C)		Test	suspens	ion	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0:0	10°:0:0	10°: >300 : >30
Pseudomonas	103	98	99	95	99	97	94	92	78	74				N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >30
aeruginosa											10-6	>330	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442	NV=10	,05x10 ³	NVB=9	,7x10 ⁴	A=	98	B=	93	C=	76	10-7		42	N0=4,35X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,05x10 ²									10-7	44	43	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >30
	40	35	38	35	38	33	41	36	38	35	10-6			N=3,3X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >30
Staphylococcus											10-0	>330	>330	Log N = 8,50	$Na = <1,4 \times 10^{2}$	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus ATCC 6538	NV=3,	75x10 ²	NVB=3	,65x10 ⁴	A=3	35,5	B=3	38,5	C=3	36,5	10-7	24	25	N0=3,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	3,75×10									10	34	35	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	100: >300; >300
Enterococcus	101	97	98	95	98	96	91	88	77	73	10-6	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
hirae											10-4	>330	>330	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x104
ATCC 10 541	NV=9	,9x10 ³	NVB=9	,65x10 ⁴	A=	97	B=8	39,5	C=	75	10-7			N0=4,2X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	9,9x10²									10-7	42	42	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	102	97	97	93	97	95	91	89	78	73				N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10-6	>330	>330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=9	,95x10 ³	NVB=	9,5x10 ⁴	A=	96	B=	90	C=	75,5				N0=4,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9	,95x10 ²									10-7	43	46	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300 ; >300
Listeria	102	100	98	96	98	96	91	89	76	72	40-6			N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10-6	>330	>330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=1	0,1x10 ³	NVB=	9,7x10 ⁴	A=	97	B=	90	C=	74				N0=3,7X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,1x10 ²									10-7	37	37	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0:0	10°:0:0	10°: >300; >300
Enterococcus	101	97	99	95	99	95	92	88	76	71				N=4.2X10 ⁸	10": 0: 0	10": 0: 0	10": >300; >300
faecium ATCC			-			-			-	-	10-6	>330	>330	Log N = 8,63	Na = <1,4×10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
700221	NV=9	9,9x10 ³	NVB=	9,7x10 ⁴	A=	97	B=	:90	C=	73,5				N0=4.35X10 ⁷		Log Na = <2,15	Log Na = >4,54
		9.9x10 ²		,	-					-,-	10-7	42	45	Log N0 = 7,62	Log R = >5,46	Log R =>5,50	Log R = < 3,08

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time. Nv: number of cells per ml in the validation suspension.

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



➤ Instrument disinfection (Temperature 20°C – 15 minutes of contact)

Clean conditions

	Va	alidation	suspen	sion			/alidatio								Concer	tration test prod	redure % (V/V)
Test organism	Nv ar	d Nvo	N	VB	COND		CON	RALIZER TROL (B)	VALID	THOD DATION (C)		Test	suspen	sion			
															100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°:>300;>300
Pseudomonas	102	97	96	91	96	94	91	86	75	73	10-6	>330	>330	N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aeruginosa														Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442			NVB=9	,35×10 ⁴	A=	95	B=8	38,5	C=	74	10-7	45	43			Log Na = <2,15	Log Na = >4,52
		9,95×10 ²										- 1.0		Log N0 = 7,62		Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°:>300;>300
Staphylococcus	41	38	39	34	39	34	42	39	41	38	10-6	>330	>330	N=3,3X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aureus ATCC 6538														Log N = 8,50	Na = <1,4x10 ²	$Na = <1,4x10^2$	Na = >3,3x10 ⁴
uureus Arree 0000			NVB=3	,65x10 ⁴	A=3	36,5	B=4	10,5	C=3	39,5	10-7	34	35	N0=3,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	3,95×10											- 55	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	100:>300;>300
Enterococcus	104	101	96	91	96	94	92	90	74	69	10-6	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
hirae											10	>330	-330	Log N = 8,62	$Na = <1,4x10^2$	$Na = <1,4x10^2$	Na = >3,3x10 ⁴
ATCC 10 541),25x10 ³	NVB=9	,35x10 ⁴	A=	95	B=	91	C=7	71,5	10-7	46	42	N0=4,4X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	0,25x10 ²									10	40	42	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	101	98	96	92	96	93	91	86	77	72	10-6	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10	>550	2550	Log N = 8,63	$Na = <1,4x10^2$	$Na = <1,4x10^2$	Na = >3,3x104
35664	NV=9	,95x10³	NVB=	9,4x10 ⁴	A=9	94,5	B=8	38,5	C=7	74,5	10-7		45	N0=4,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9	9,95x10 ²									10 .	44	45	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Listeria	104	100	98	94	98	96	92	89	78	76	40-6			N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10-6	>330	>330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=1	0,2x10 ³	NVB=	9,6x10 ⁴	A=	97	B=9	90,5	C=	77				N0=3,7X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	10,2x10 ²									10-7	37	37	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Enterococcus	102	98	97	95	97	94	91	86	73	69				N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
faecium ATCC											10-6	>330	>330	Log N = 8,63	$Na = <1,4x10^2$	Na = <1,4x10 ²	Na = >3,3x10 ⁴
700221	NV=	10×10³	NVB=	9,6x10 ⁴	A=9	95,5	B=8	38,5	C=	71				N0=4,3X10 ⁷	Log Na = <2,15		Log Na = >4,52
	NV0=	10x10 ²									10-7	44	42	Log N0 = 7,62		Log R = >5,50	Log R = < 3,09

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time. Nv: number of cells per ml in the validation suspension.

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



➤ Instrument disinfection (Temperature 70°C for E. faecium – 15 minutes of contact)

Dirty conditions

	٧	alidation	susper	sion			/alidati	on test							Concer	tration test prod	edure % (V/V)
Test organism	Nva	nd Nvo		VΒ	EXPERI	MENTAL		RALIZER ITROL		THOD	1	Tes	t susper	nsion	Concer	iti ation test prot	eduic 70 (V/V)
			l N	VB		TROL ^(A)		(B)		DATION (C)					100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Enterococcus	101	97	99	95	99	95	92	88	76	71	10-6	>330	>330	N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
faecium ATCC											10	/550	/330	Log N = 8,63	Na = <1,4x10 ²	$Na = <1,4x10^{2}$	Na = >3,3x10 ⁴
700221	NV=9	9,9x10³	NVB=	9,7x10 ⁴	A=	97	B=	90	C=	73,5	10-7	42	45	N0=4,35X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,54
	NV0=	9,9x10²									10 .	42	45	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,08

➤ Instrument disinfection (Temperature 70°C for E. faecium – 15 minutes of contact)

Clean conditions

	V	alidation	suspen	sion		V	alidatio	n test							Concentr	ation test proced	9/ (\/\A/\
Test organism	Nv a	nd Nvo	NI	VB	EXPERIM		NEUTR CON	ALIZER		HOD		Test s	uspensi	on	Concentra	ation test proced	ure % (v/v)
rest organism	14V al	1440	IN.	VB		ROL ^(A)		B)	VALID.						100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1						10°:0;0	10°:0;0	10°: >300; >300				
Enterococcus	102	98	97	95	97	94	91	86	73	69	10-6	>330	>330	N=4,2X108	10": 0; 0	10-1: 0; 0	10-1: >300; >300
faecium ATCC											10	/550	/330	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
700221	NV=	10×10³	NVB=9	9,6x10 ⁴	A=9	5,5	B=8	38,5	C=	71	10-7	44	42	N0=4,3X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	10x10 ²									10	444	42	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time. Nv: number of cells per ml in the validation suspension.

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



➤ Surface disinfection (Temperature 20°C – 1 minute of contact)

Dirty conditions

	V	alidation	suspen	sion		V	alidatio	n test							_		
Test organism	Nv a	nd N V0	N	VR	COND	ITION	CON		VALID	HOD ATION		Test	suspens	ion		ration test proced	
				••	CONT	ROL ^(A)	(В)	()	C)					100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Pseudomonas	100	98	97	95	97	93	93	90	77	74	10-6	>330	>330	N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aeruginosa											10	7550	7550	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442	NV=9	,9x10³	NVB=9	,6x10 ⁴	A=	95	B=9	91,5	C=7	75,5	10-7	46	46	N0=4,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	9,9x10²									10	40	40	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Staphylococcus	41	36	41	38	41	37	39	35	40	38	10 ⁻⁶	>330	>330	N=3,3X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aureus ATCC 6538											10	1 330	- 550	Log N = 8,50	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus Arcc 0550	NV=3,		NVB=3	,95x10⁴	A=	39	B=	37	C=	39	10-7	35	35	N0=3,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3	3,85x10									10	33	33	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Enterococcus	104	102	96	92	96	93	93	91	77	72	10 ⁻⁶	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
hirae											10	-550	-550	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 10 541	NV=1	0,3x10³	NVB=9	,4x10 ⁴	A=9	4,5	B=	92	C=7	74,5	10-7	45	44	N0=4,45X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	.0,3x10 ²									10			Log N0 = 7,61	Log R = >5,46		Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Salmonella	104	100	98	94	98	93	91	87	73	68	10-6	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10	7550	-550	Log N = 8,63	$Na = <1,4x10^{2}$	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=1	0,2x10 ³	NVB=9	9,6x10 ⁴	A=9	5,5	B=	89	C=7	70,5	10-7	45	45	N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	.0,2x10 ²									10	45	42	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	100:0;0	100:>300;>300
Listeria	100	96	96	94	96	92	93	89	76	72	10-6	>330	>330	N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10 -	∕ 530	>530	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=9	,8x10³	NVB=9	,5x10 ⁴	A=	94	B=	91	C=	74	40-7			N0=3,55X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=	9,8x10²									10-7	35	36	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

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

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

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



➤ Surface disinfection (Temperature 20°C – 1 minute of contact)

Clean conditions

	Vä	alidation	susper				Validat	ion tes	t						Concentrat	ion test procedu	ure % (V/V)
Test organism	N V an	d N VO	N		CONDI CONT		CON	ALIZER TROL B)	VALI	THOD DATION (C)		Т	est susp	pension	100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Pseudomonas	103	99	96	93	96	91	94	90	74	70	10-6	>330	>330	N=4,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aeruginosa											10	>550	>550	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 15 442	NV=10	0,1x10³	NVB=9	,45×10 ⁴	A=9	3,5	B=	92	C=	72	10-7	42	43	N0=4,25X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	.0,1x10 ²									10	42	43	Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Staphylococcus	39	36	38	33	38	34	40	38	38	35	10-6	>330	>330	N=3,3X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
aureus ATCC 6538											10	>550	>550	Log N = 8,50	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
aureus ATCC 0556	NV=3,	75x10 ²	NVB=3,	,55x10 ⁴	A=	36	B=	39	C=3	36,5	10-7	37	35	N0=3,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3	3,75x10									10	3/	33	Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Enterococcus	104	101	97	92	97	92	91	87	73	71	10 ⁻⁶	>330	>330	N=4,1X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
hirae											10 °	>330	>330	Log N = 8,62	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 10 541	NV=10	,25x10 ³	NVB=9,	,45x10 ⁴	A=9	4,5	B=	89	C=	72	10-7	46	42	N0=4,4X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
1	NV0=10	0,25x10 ²									10 .	46	42	Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°:>300;>300
Salmonella	104	102	98	96	98	93	91	88	73	68	10 ⁻⁶	>330	>330	N=4,7X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
enterica ATCC											10	/330	/550	Log N = 8,63	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
35664	NV=10	0,3x10³	NVB=9	9,7x10 ⁴	A=9	5,5	B=8	9,5	C=7	70,5	10-7	46	44	N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=1	.0,3x10 ²									10 .	46	44	Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10°:0;0	10°:0;0	10°: >300; >300
Listeria	103	101	97	93	97	93	91	86	74	71	10-6	. 226	. 220	N=3,2X10 ⁸	10": 0; 0	10": 0; 0	10": >300; >300
monocytogenes											10-3	>330	>330	Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
ATCC 35152	NV=10	0,2x10 ³	NVB=9	,5x10 ⁴	A=	95	B=8	8,5	C=7	72,5				N0=3,45X10 ⁷		Log Na = <2,15	Log Na = >4,52
	NV0=1	0,2x10 ²									10-7	35	34	Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99

Vc: plate counts.

N: number of cells per ml in test suspension.

NO: number of cells per ml at the start of contact time. Nv: number of cells per ml in the validation suspension.

NvO: number of cells per ml in mixtures A, B and C at the beginning of contact time.

NvB: (dilution method) number of cells per ml after dilution 1:100

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

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

B: number of survivors in the neutralizer/filtrate control B.

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



In conclusion, it should be noted that the ozonized water used in the test-generated in situ with the device BES Group Biosure Professional EOS7211-BX / WS1200X (Serial N°: 7211BXNA1101 – Production: 2023), complies with the Standard UNE-EN 13727:2012+A2:2015, in clean and dirty conditions, for concentrations 100% (1,62ppm) and 90% (1,46ppm) against the reference strains Pseudomonas aeruginosa ATCC 15 442, Escherichia coli K12 CECT 433, Staphylococcus aureus ATCC 6538, Enterococcus hirae ATCC 10541, Enterococcus faecium ATCC 700221, Listeria monocytogenes ATCC 35152 and Salmonella enterica subsp. enterica ATCC 35664, demonstrating at least a 5 lg reduction in the following medical area application activities:

- Hygienic handrub treatment and hygienic handwash (Temperature $20^{\circ}\text{C} 60$ seconds of contact)
- Surgical handrub treatment and surgical handwash (Temperature 20°C 1 minute of contact)
- **Instrument disinfection** (Temperature 20°C (70°C for Enterococcus faecium) 15 minutes contact)

• **Surface disinfection** (Temperature 20°C - 1 minute contact)

Oviedo, 16th of March 2024

Daniel Cepedal Macías Technical Director inoQua | Food Health Institute



Notes:

- The results of this Study only attest to the samples analyzed.
- This report may not be reproduced in whole or in part without the prior written permission of the author
- The samples have been analyzed in a laboratory authorized by the Ministry of Health and Sanitary Services of the Principality of Asturias, an independent private laboratory for analysis and sanitary control of food, water and beverages, with registration number 05/O, since February 1997. It has been accredited by ENAC, according to standard UNE-EN ISO/IEC 17025, for carrying out tests in the environmental sector, as indicated in accreditation number 780/LE1514, since March 2010 and collaborating entity of the Administration Hydraulics in matters of control and surveillance of water quality and management of discharges into the public hydraulic domain under Order MAM/985/2006.