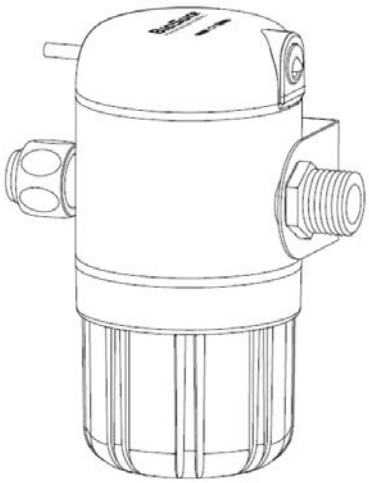
CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

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 UNE-EN 13727:2012+A2:2015 - 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 / WS1200X (N° de serie: 7211BXNA1101 – Production: 2023)	**Dissolved ozone concentration**	**1,62 mg/L**
	REDOX Potential	**921 mv**

Oviedo, 23rd of Februay 2024

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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**
Cepas bacterianas	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae* *Escherichia coli K12* *Listeria monocytogenes* *Salmonella enterica subsp. enterica*	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae* *Escherichia coli K12* *Listeria monocytogenes* *Salmonella enterica subsp. enterica*	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae* *Enterococcus faecium* *Listeria monocytogenes* *Salmonella enterica subsp. enterica*	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus hirae* *Listeria monocytogenes* *Salmonella enterica subsp. enterica*
Test temperature	20°C	20°C	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); L-histidine (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

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL (A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	97	96	91	96	94	94	91	78	74	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,85x10^3		NVB=9,35x10^4		A=95		B=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=9,85x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	39	36	41	38	41	39	38	36	39	34	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,75x10^3		NVB=3,95x10^4		A=40		B=37		C=36,5		10^{-7}	37	35	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,75x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	101	98	94	98	96	91	89	75	71	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,2x10^3		NVB=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=10,2x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Escherichia coli K12 CECT 433	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	41	38	41	36	41	37	38	35	38	33	10^{-6}	>330	>330	N=3,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,52	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,95x10^2		NVB=3,85x10^4		A=39		B=36,5		C=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													Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	95	96	91	96	91	93	91	77	73	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,75x10^3		NVB=9,35x10^4		A=93,5		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=9,75x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	100	96	92	96	94	93	90	78	73	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,2x10^3		NVB=9,4x10^4		A=95		B=91,5		C=75,5		10^{-7}	36	36	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,2x10^2													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.
Nv0: 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.
Log R: log reduction.

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

Clean conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL (A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	99	96	94	96	94	94	92	74	72	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,1x10^3		NVB=9,5x10^4		A=95		B=93		C=73		10^{-7}	44	42	N0=4,3X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,1x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	41	39	42	40	42	38	41	37	41	39	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=4x10^2		NVB=4,1x10^4		A=40		B=39		C=40		10^{-7}	34	34	N0=3,4X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=4x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	102	98	93	98	93	91	86	77	72	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,3x10^3		NVB=9,55x10^4		A=95,5		B=88,5		C=74,5		10^{-7}	43	46	N0=4,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,3x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Escherichia coli K12 CECT 433	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	38	34	39	35	39	37	42	39	40	38	10^{-6}	>330	>330	N=3,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,52	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,6x10^2		NVB=3,7x10^4		A=38		B=40,5		C=39		10^{-7}	36	36	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,6x10													Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	100	96	93	96	94	91	86	73	69	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,1x10^3		NVB=9,45x10^4		A=95		B=88,5		C=71		10^{-7}	44	46	N0=4,5X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,1x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	101	96	98	93	98	96	92	87	74	72	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,85x10^3		NVB=9,55x10^4		A=97		B=89,5		C=73		10^{-7}	36	34	N0=3,5X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,85x10^2													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.
Nv0: 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.
Log R: log reduction.

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

Dirty conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL (A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	98	96	92	96	91	93	88	74	72	10^{-6}	>330	>330	$N=4,2X10^8$	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$10,05x10^3$		NVB=$9,4x10^4$		A=93,5		B=90,5		C=73		10^{-7}	44	46	$N0=4,5X10^7$	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=$10,05x10^2$													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	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^{-1}: >300; >300
														Log N = 8,50	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$3,8x10^2$		NVB=$4x10^4$		A=40		B=38,5		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													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	96	99	94	99	94	91	89	76	71	10^{-6}	>330	>330	$N=4,1X10^8$	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$9,8x10^3$		NVB=$9,65x10^4$		A=96,5		B=90		C=73,5		10^{-7}	42	46	$N0=4,4X10^7$	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=$9,8x10^2$													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Escherichia coli K12 CECT 433	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	41	36	41	39	41	39	38	36	42	39	10^{-6}	>330	>330	$N=3,1X10^8$	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,52	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$3,85x10^2$		NVB=$4x10^4$		A=40		B=37		C=40,5		10^{-7}	35	34	$N0=3,45X10^7$	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,85x10													Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	99	99	96	99	96	92	88	77	73	10^{-6}	>330	>330	$N=4,7X10^8$	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$10,1x10^3$		NVB=$9,75x10^4$		A=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=$10,1x10^2$													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	99	96	91	96	93	92	87	74	71	10^{-6}	>330	>330	$N=3,2X10^8$	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = $<1,4x10^2$	Na = $<1,4x10^2$	Na = $>3,3x10^4$
	NV=$10,05x10^3$		NVB=$9,35x10^4$		A=94,5		B=89,5		C=72,5		10^{-7}	36	37	$N0=3,65X10^7$	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=$10,05x10^2$													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.
Nv0: 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.
Log R: log reduction.

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

Clean conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL(A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	101	97	98	95	98	93	93	89	75	71	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,9x10^3		NVB=9,65x10^4		A=95,5		B=91		C=73		10^{-7}	45	46	N0=4,55X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,9x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	39	36	42	38	42	37	39	35	38	34	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,75x10^2		NVB=4x10^4		A=39,5		B=37		C=36		10^{-7}	36	36	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,75x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	100	96	94	96	92	92	90	75	72	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,1x10^3		NVB=9,5x10^4		A=94		B=91		C=73,5		10^{-7}	46	43	N0=4,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,1x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Escherichia coli K12 CECT 433	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	42	37	39	35	39	37	40	35	40	36	10^{-6}	>330	>330	N=3,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,52	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,95x10^2		NVB=3,7x10^4		A=38		B=37,5		C=38		10^{-7}	37	35	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,95x10													Log N0 = 7,52	Log R = >5,41	Log R = >5,41	Log R = < 2,99
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	96	96	92	96	94	93	89	78	76	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,8x10^3		NVB=9,4x10^4		A=95		B=91		C=77		10^{-7}	42	43	N0=4,25X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,8x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	98	99	94	99	95	93	89	78	75	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10x10^3		NVB=9,65x10^4		A=97		B=91		C=76,5		10^{-7}	35	35	N0=3,5X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10x10^2													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.
Nv0: 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.
Log R: log reduction..

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

Dirty conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL (A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	98	99	95	99	97	94	92	78	74	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,05x10^3		NVB=9,7x10^4		A=98		B=93		C=76		10^{-7}	44	43	N0=4,35X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,05x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	40	35	38	35	38	33	41	36	38	35	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,75x10^2		NVB=3,65x10^4		A=35,5		B=38,5		C=36,5		10^{-7}	34	35	N0=3,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,75x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	101	97	98	95	98	96	91	88	77	73	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,9x10^3		NVB=9,65x10^4		A=97		B=89,5		C=75		10^{-7}	42	42	N0=4,2X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,9x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	97	97	93	97	95	91	89	78	73	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,95x10^3		NVB=9,5x10^4		A=96		B=90		C=75,5		10^{-7}	43	46	N0=4,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,95x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	100	98	96	98	96	91	89	76	72	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,1x10^3		NVB=9,7x10^4		A=97		B=90		C=74		10^{-7}	37	37	N0=3,7X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,1x10^2													Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus faecium ATCC 700221	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	101	97	99	95	99	95	92	88	76	71	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,9x10^3		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=9,9x10^2													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.
N0: number of cells per ml at the start of contact time.
Nv: number of cells per ml in the validation suspension.
Nv0: 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.
Log R: log reduction..

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

Clean conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		Nvb		EXPERIMENTAL CONDITION CONTROL(A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	97	96	91	96	94	91	86	75	73	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,95x10^3		NVB=9,35x10^4		A=95		B=88,5		C=74		10^{-7}	45	43	N0=4,4X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,95x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	41	38	39	34	39	34	42	39	41	38	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,95x10^3		NVB=3,65x10^4		A=36,5		B=40,5		C=39,5		10^{-7}	34	35	N0=3,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,95x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	101	96	91	96	94	92	90	74	69	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,25x10^3		NVB=9,35x10^4		A=95		B=91		C=71,5		10^{-7}	46	42	N0=4,4X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,25x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	101	98	96	92	96	93	91	86	77	72	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,95x10^3		NVB=9,4x10^4		A=94,5		B=88,5		C=74,5		10^{-7}	44	45	N0=4,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,95x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	100	98	94	98	96	92	89	78	76	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,2x10^3		NVB=9,6x10^4		A=97		B=90,5		C=77		10^{-7}	37	37	N0=3,7X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,2x10^2													Log N0 = 7,51	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus faecium ATCC 700221	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	102	98	97	95	97	94	91	86	73	69	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10x10^3		NVB=9,6x10^4		A=95,5		B=88,5		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													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.
N0: number of cells per ml at the start of contact time.
Nv: number of cells per ml in the validation suspension.
Nv0: 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.
Log R: log reduction..

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

Dirty conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NVB		EXPERIMENTAL CONDITION CONTROL(A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
Enterococcus faecium ATCC 700221	101	97	99	95	99	95	92	88	76	71	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,9x10^3		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=9,9x10^2													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

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NVB		EXPERIMENTAL CONDITION CONTROL(A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
Enterococcus faecium ATCC 700221	102	98	97	95	97	94	91	86	73	69	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10x10^3		NVB=9,6x10^4		A=95,5		B=88,5		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													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.
N0: number of cells per ml at the start of contact time.
Nv: number of cells per ml in the validation suspension.
Nv0: 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.
Log R: log reduction.

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

Dirty conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		Nvb		EXPERIMENTAL CONDITION CONTROL (A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	98	97	95	97	93	93	90	77	74	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,9x10^3		NVB=9,6x10^4		A=95		B=91,5		C=75,5		10^{-7}	46	46	N0=4,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,9x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	41	36	41	38	41	37	39	35	40	38	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,85x10^2		NVB=3,95x10^4		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=3,85x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	102	96	92	96	93	93	91	77	72	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,3x10^3		NVB=9,4x10^4		A=94,5		B=92		C=74,5		10^{-7}	45	44	N0=4,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,3x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	100	98	94	98	93	91	87	73	68	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,2x10^3		NVB=9,6x10^4		A=95,5		B=89		C=70,5		10^{-7}	45	45	N0=4,5X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,2x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	100	96	96	94	96	92	93	89	76	72	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=9,8x10^3		NVB=9,5x10^4		A=94		B=91		C=74		10^{-7}	35	36	N0=3,55X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,8x10^2													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.
Nv0: 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.
Log R: log reduction.

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

Clean conditions

Test organism	Validation suspension				Validation test						Test suspension				Concentration test procedure % (V/V)		
	Nv and Nv0		NvB		EXPERIMENTAL CONDITION CONTROL(A)		NEUTRALIZER CONTROL (B)		METHOD VALIDATION (C)						100%	90%	30%
Pseudomonas aeruginosa ATCC 15 442	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	99	96	93	96	91	94	90	74	70	10^{-6}	>330	>330	N=4,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,1x10^3		NVB=9,45x10^4		A=93,5		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=10,1x10^2													Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,09
Staphylococcus aureus ATCC 6538	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	39	36	38	33	38	34	40	38	38	35	10^{-6}	>330	>330	N=3,3X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,50	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=3,75x10^2		NVB=3,55x10^4		A=36		B=39		C=36,5		10^{-7}	37	35	N0=3,6X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,75x10													Log N0 = 7,53	Log R = >5,36	Log R = >5,36	Log R = < 2,99
Enterococcus hirae ATCC 10 541	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	101	97	92	97	92	91	87	73	71	10^{-6}	>330	>330	N=4,1X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,62	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,25x10^3		NVB=9,45x10^4		A=94,5		B=89		C=72		10^{-7}	46	42	N0=4,4X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,25x10^2													Log N0 = 7,61	Log R = >5,46	Log R = >5,46	Log R = < 3,09
Salmonella enterica ATCC 35664	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	104	102	98	96	98	93	91	88	73	68	10^{-6}	>330	>330	N=4,7X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,63	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,3x10^3		NVB=9,7x10^4		A=95,5		B=89,5		C=70,5		10^{-7}	46	44	N0=4,5X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,3x10^2													Log N0 = 7,66	Log R = >5,50	Log R = >5,50	Log R = < 3,13
Listeria monocytogenes ATCC 35152	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10^0:0 ; 0	10^0:0 ; 0	10^0: >300 ; >300
	103	101	97	93	97	93	91	86	74	71	10^{-6}	>330	>330	N=3,2X10^8	10^{-1}: 0; 0	10^{-1}: 0; 0	10^{-1}: >300; >300
														Log N = 8,51	Na = <1,4x10^2	Na = <1,4x10^2	Na = >3,3x10^4
	NV=10,2x10^3		NVB=9,5x10^4		A=95		B=88,5		C=72,5		10^{-7}	35	34	N0=3,45X10^7	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,2x10^2													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.
Nv0: 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.
Log R: log reduction..

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 Nº: 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 20ºC – 60 seconds of contact)
- **Surgical handrub treatment and surgical handwash** (Temperature 20ºC – 1 minute of contact)
- **Instrument disinfection** (Temperature 20ºC (70ºC for Enterococcus faecium) - 15 minutes contact)
- **Surface disinfection** (Temperature 20ºC - 1 minute contact)

Oviedo, 16th of March 2024

Daniel Cepedal Macías
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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.