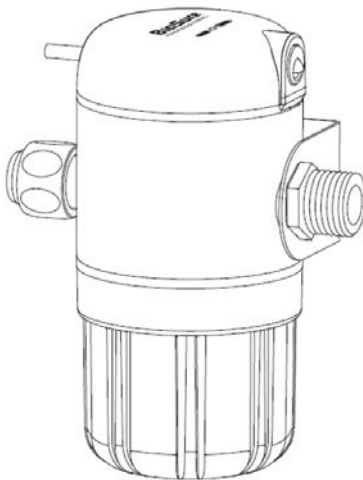


CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

EXECUTION OF UNE-EN TESTS TO CERTIFY THE BACTERICIDAL AND FUNGICIDE/LEVURICIDE 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 and fungicidal/yeasticidal activity of ozonized water** generated "in situ" with the equipment to be tested.

The standard that has been carried out is UNE-EN 13697:2015+A1:2020 - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas (phase 2, step 2).

Furthermore, the UNE-EN 13697:2015+A1:2020 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 February 2024



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BACTERICIDAL AND FUNGICIDAL ACTIVITY TEST

UNE-EN 13697:2015 + A1:2020 Standard

Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas (phase 2, step 2)

Test execution: 26/02/2024 – 06/02/2024

Analytical report: 5052/24

Metodología

The stipulated method in this Standard to evaluate the bactericidal and fungicidal activity of ozonized water on surfaces is based on the determination of the microorganisms surviving the process after the application of such water on a previously contaminated surface.

For the execution of the test, a solution of bacteria and fungi together with interfering substances is prepared to be applied on a stainless steel surface, creating a film that dries afterwards.

Next, the ozonized water object of study at different concentrations is applied so that it covers the dried film keeping a specific temperature and during a defined period of time.

Ozonated water is a transparent, colorless liquid, soluble in normalized hard water. The concentrations of ozonated water chosen to carry out the test have been 100%, 90% and 30%.

The following step is to transfer the surface to a previously validated neutralization medium in order for the disinfecting effect of the ozonized water to be immediately neutralized.

Finally, the number of surviving microorganisms that can be recovered from the surface is quantitatively determined.

The culture medium used for seeding is TSA (Tryptone Soy Agar) in the case of bacterial strains and MEA (Malt Extract Agar) in the case of fungal strains. The incubation temperatures were 36°C and 30°C, respectively.

Parallely, the number of bacteria and fungi on a surface treated with hard water (300mg/Kg CaCo3) instead of ozonized water is also determined and the reduction in viable recounts attributed to the product is calculated by difference.

The interfering substance used in dirty conditions is an aqueous solution of bovine albumin at a concentration of 3g/L. Under clean conditions, the aqueous solution is bovine albumin at a concentration of 0.3g/L.

The neutralizers used have been Lecithin (3g/l); Tween 80 (30ml/l); Sodium thiosulfate (5g/l); L-histidine (1 g/l); in phosphate buffer 0,0025N.

The bacterial and fungal strains used have been:

- *Pseudomonas aeruginosa* ATCC 15 442
- *Escherichia coli* ATCC 10 536
- *Staphylococcus aureus* ATCC 6 538
- *Enterococcus hirae* ATCC 10 541
- *Candida albicans* ATCC 10 231
- *Aspergillus niger* ATCC 16 404

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 test was carried out at a temperature between $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

The contact time in the case of bacterial strains has been 5 minutes $\pm$ 10 seconds and the incubation temperature $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

In the case of fungal strains, the contact time has been 15 minutes $\pm$ 10 seconds and the incubation temperature $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Results and conclusion

The following charts show the **results** of the test:

Dirty conditions

Microorganism	Bacterial suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100,00	90,00	30,00
<i>Pseudomonas aeruginosa</i> ATCC 15 442	(10-6) 227,225	(10-3) 105,108	(10-3) 121,117	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 24,24	(10-4) 11,7	(10-4) 12,15	10-4 135, 122	10-1 0, 0	10-1 0, 0	10-4 135, 122
	N:6,75	10-5 0, 0	10-5 0, 0	10-5 13, 13	10-2 0, 0	10-2 0, 0	10-5 13, 13
	NT:6,03	NT:6,08		Nc: 7,11 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,01	Nd: < 0,10 Nts: 0 R: > 7,01	Nd: < 0,10 Nts: 0 R: < 3,5
<i>Escherichia coli</i> ATCC 10 536	(10-6) 209,210	(10-3) 104,104	(10-3) 105,102	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 21,17	(10-4) 9,13	(10-4) 10,13	10-4 152, 157	10-1 0, 0	10-1 0, 0	10-4 163, 162
	N:6,72	10-5 0, 0	10-5 0, 0	10-5 15,14	10-2 0, 0	10-2 0, 0	10-5 15, 14
	NT:6,02	NT:6,01		Nc: 7,19 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,09	Nd: < 0,10 Nts: 0 R: > 7,09	Nd: < 0,10 Nts: 0 R: < 3,5
<i>Staphylococcus aureus</i> ATCC 6 538	(10-6) 262,265	(10-3) 124,126	(10-3) 119,122	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 23,26	(10-4) 12,13	(10-4) 12,13	10-4 163, 162	10-1 0, 0	10-1 0, 0	10-4 148, 145
	N:6,82	10-5 0, 0	10-5 0, 0	10-5 15, 14	10-2 0, 0	10-2 0, 0	10-5 13, 13
	NT:6,1	NT:6,08		Nc: 7,21 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,11	Nd: < 0,10 Nts: 0 R: > 7,11	Nd: < 0,10 Nts: 0 R: < 3,5
<i>Enterococcus hirae</i> ATCC 10 541	(10-6) 251,251	(10-3) 107,110	(10-3) 116,118	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 23,19	(10-4) 12,13	(10-4) 9,13	10-4 148, 145	10-1 0, 0	10-1 0, 0	10-4 135, 122
	N:6,79	10-5 0, 0	10-5 0, 0	10-5 13, 13	10-2 0, 0	10-2 0, 0	10-5 13, 13
	NT:6,04	NT:6,07		Nc: 7,16 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,06	Nd: < 0,10 Nts: 0 R: > 7,06	Nd: < 0,10 Nts: 0 R: < 3,5
Microorganism	Bacterial suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100,00	90,00	30,00
<i>Listeria monocytogenes</i> CECT 935	(10-6) 218,214	(10-3) 98,100	(10-3) 100,97	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 22,19	(10-4) 13,10	(10-4) 11,10	10-4 136, 124	10-1 0, 0	10-1 0, 0	10-4 133, 124
	N:6,73	10-5 0, 0	10-5 0, 0	10-5 13, 13	10-2 0, 0	10-2 0, 0	10-5 13, 12
	NT:6	NT:5,99		Nc: 7,11 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,01	Nd: < 0,10 Nts: 0 R: > 7,01	Nd: < 0,10 Nts: 0 R: < 3,5
<i>Salmonella enterica</i> ATCC 35664	(10-6) 217,213	(10-3) 117,116	(10-3) 98,98	10-3 >300, >300	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-7) 24,26	(10-4) 10,7	(10-4) 9,9	10-4 153, 154	10-1 0, 0	10-1 0, 0	10-4 160, 161
	N:6,74	10-5 0, 0	10-5 0, 0	10-5 15,14	10-2 0, 0	10-2 0, 0	10-5 15, 14
	NT:6,07	NT:5,99		Nc: 7,19 Nts: >100	Nd: < 0,10 Nts: 0 R: > 7,09	Nd: < 0,10 Nts: 0 R: > 7,09	Nd: < 0,10 Nts: 0 R: < 3,5

N: decimal logarithm of the cfu number per 0.05 ml of the test suspension.

NT: decimal logarithm of the cfu number per test surface for the neutralization test.

NC: decimal logarithm of the cfu number per test surface for neutralization control.

Nc: decimal logarithm of the cfu number per test surface for water control.

Nd: decimal logarithm of the cfu number per test surface for the disinfectant test.

R: Microbicidal effect.

Clean conditions

Microorganism	Bacterial suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100,00	90,00	30,00
<i>Pseudomonas aeruginosa</i> ATCC 15 442	(10-6) 253,257 (10-7) 23,21 N:6,8	(10-3) 121,117 (10-4) 9,5 10-5 0, 0 NT:6,08	(10-3) 101,104 (10-4) 13,9 10-5 0, 0 NT:6,01	10-3 >300, >300 10-4 135, 120 10-5 13, 12 Nc: 7,11 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,01	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,01	10-3 >300, >300 10-4 135, 122 10-5 13, 13 Nd: < 0,10 Nts: 0 R: < 3,5
<i>Escherichia coli</i> ATCC 10 536	(10-6) 239,237 (10-7) 20,18 N:6,77	(10-3) 113,109 (10-4) 9,9 10-5 0, 0 NT:6,05	(10-3) 120,116 (10-4) 12,13 10-5 0, 0 NT:6,07	10-3 >300, >300 10-4 152, 157 10-5 15,14 Nc: 7,19 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,09	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,09	10-3 >300, >300 10-4 163, 162 10-5 15, 14 Nd: < 0,10 Nts: 0 R: < 3,5
<i>Staphylococcus aureus</i> ATCC 6 538	(10-6) 217,219 (10-7) 20,17 N:6,73	(10-3) 107,103 (10-4) 12,12 10-5 0, 0 NT:6,02	(10-3) 121,124 (10-4) 11,13 10-5 0, 0 NT:6,09	10-3 >300, >300 10-4 163, 162 10-5 15, 14 Nc: 7,21 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,11	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,11	10-3 >300, >300 10-4 148, 145 10-5 13, 13 Nd: < 0,10 Nts: 0 R: < 3,5
<i>Enterococcus hirae</i> ATCC 10 541	(10-6) 223,227 (10-7) 20,23 N:6,75	(10-3) 112,110 (10-4) 12,12 10-5 0, 0 NT:6,05	(10-3) 117,119 (10-4) 13,17 10-5 0, 0 NT:6,07	10-3 >300, >300 10-4 144, 145 10-5 13, 13 Nc: 7,16 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,06	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,06	10-3 >300, >300 10-4 135, 122 10-5 13, 13 Nd: < 0,10 Nts: 0 R: < 3,5

Microorganism	Bacterial suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100,00	90,00	30,00
<i>Listeria monocytogenes</i> CECT 935	(10-6) 266,269 (10-7) 24,26 N:6,82	(10-3) 112,112 (10-4) 9,10 10-5 0, 0 NT:6,05	(10-3) 119,119 (10-4) 12,10 10-5 0, 0 NT:6,08	10-3 >300, >300 10-4 136, 124 10-5 13, 13 Nc: 7,11 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,01	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,01	10-3 >300, >300 10-4 133, 124 10-5 13, 12 Nd: < 0,10 Nts: 0 R: < 3,5
<i>Salmonella enterica</i> ATCC 35664	(10-6) 249,251 (10-7) 25,21 N:6,79	(10-3) 104,104 (10-4) 9,10 10-5 0, 0 NT:6,02	(10-3) 105,106 (10-4) 13,14 10-5 0, 0 NT:6,02	10-3 >300, >300 10-4 153, 154 10-5 15,14 Nc: 7,19 Nts: >100	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,09	10-0 0, 0 10-1 0, 0 10-2 0, 0 Nd: < 0,10 Nts: 0 R: > 7,09	10-3 >300, >300 10-4 160, 161 10-5 15, 14 Nd: < 0,10 Nts: 0 R: < 3,5

N: decimal logarithm of the cfu number per 0.05 ml of the test suspension.

NT: decimal logarithm of the cfu number per test surface for the neutralization test.

NC: decimal logarithm of the cfu number per test surface for neutralization control.

Nc: decimal logarithm of the cfu number per test surface for water control.

Nd: decimal logarithm of the cfu number per test surface for the disinfectant test.

R: Microbicidal effect.

Dirty conditions

Microorganism	Fungal suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100	90	30
Candida albicans ATCC 10 231	(10-5) 210,214	(10-3) 119,115	(10-3) 124,128	10-3 88,87	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-6) 24,23	(10-4) 10,9	(10-4) 13,11	10-4 7, 6	10-1 0, 0	10-1 0, 0	10-4 135, 133
	N:5,81			10-5 0, 0	10-2 0, 0	10-2 0, 0	10-5 13, 13
		NT:5,99	NT: 5,86	Nc: 5,93 Nts: >100	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: < 3,5
Aspergillus niger ATCC 16 404	(10-5) 210,214	(10-3) 119,115	(10-3) 124,128	10-3 86, 84	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-6) 26,24	(10-4) 10,9	(10-4) 9,13	10-4 8, 8	10-1 0, 0	10-1 0, 0	10-4 163, 159
	N:5,73			10-5 0, 0	10-2 0, 0	10-2 0, 0	10-5 15, 14
		NT:6,07	NT:6,1	Nc: 5,93 Nts: >100	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: < 3,5

Clean conditions

Microorganism	Fungal suspension	Validation test		Water control Nc	Test procedure at concentration %		
		NT	NC		100	90	30
Candida albicans ATCC 10 231	(10-5) 227,226	(10-3) 123,125	(10-3) 106,104	10-3 88,85	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-6) 26,28	(10-4) 11,13	(10-4) 11,11	10-4 7, 8	10-1 0, 0	10-1 0, 0	10-4 135, 129
	N:5,8			10-5 0, 0	10-2 0, 0	10-2 0, 0	10-5 13, 13
		NT:6,05	NT: 5,86	Nc: 5,93 Nts: >100	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: < 3,5
Aspergillus niger ATCC 16 404	(10-5) 227,226	(10-3) 123,125	(10-3) 106,104	10-3 83, 84	10-0 0, 0	10-0 0, 0	10-3 >300, >300
	(10-6) 25,23	(10-4) 11,13	(10-4) 9,9	10-4 8, 8	10-1 0, 0	10-1 0, 0	10-4 164, 161
	N:5,76			10-5 0, 0	10-2 0, 0	10-2 0, 0	10-5 15, 14
		NT:6,09	NT:6,02	Nc: 5,93 Nts: >100	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: > 5,83	Nd: < 0,10 Nts: 0 R: < 3,5

N: decimal logarithm of the cfu number per 0.05 ml of the test suspension.

NT: decimal logarithm of the cfu number per test surface for the neutralization test.

NC: decimal logarithm of the cfu number per test surface for neutralization control.

Nc: decimal logarithm of the cfu number per test surface for water control.

Nd: decimal logarithm of the cfu number per test surface for the disinfectant test.

R: Microbicidal effect.

In conclusion, indicate that the ozonated water generated with the device **BES Group Biosure Professional EOS7211-BX / WS1200X** (Serial N°: 7211BXNA1101 – Production: 2023):

- Complies with UNE-EN 13697:2015 + A1:2020 Standard (bactericidal) in dirty and clean conditions, at concentrations of **100% (1,62ppm) y 90% (1,42ppm)** with a **contact time of 5 minutes** against: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* y *Enterococcus hirae*, *Listeria monocytogenes* y *Salmonella entérica* **demonstrating at least a 4 lg reduction.**
- Complies with UNE-EN 13697:2015 + A1:2020 Standard (fungicidal) in dirty and clean conditions, at concentrations of **100% (1,62ppm) y 90% (1,42ppm)** with a **contact time of 15 minutes** against: *Candida albicans* y *Aspergillus niger* **demonstrating at least a 3 lg reduction.**

Oviedo, 16th of March 2024


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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.
- The UNE-EN 13697 standards is within the scope of the ISO 9001:2015 quality certification held by the company LABORATORIOS INNOAGRAL, S.L. owner of the laboratory where the samples were analyzed (Reference 9899-E).