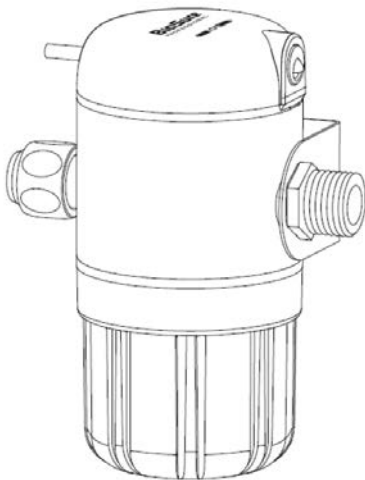


CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

EXECUTION OF UNE-EN TESTS TO CERTIFY THE 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 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 13624:2022 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in medicine (phase 2, step 1).

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




 Daniel Cepedal Macías
 Technical Director
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FUNGICIDAL ACTIVITY TEST IN THE MEDICAL AREA **Standard UNE-EN 13624:2014**

Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in medicine (Phase 2, step 1).

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

Analytical report: 5052/24

Metodología

The test method established in said standard to evaluate and demonstrate the fungicidal/yeasticidal activity of ozonized water in the 4 activities of the medical area mentioned is based on the determination of the fungi 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 fungi (yeast cells and mould spores) in a solution of an interfering substance. The mixture is maintained at the temperature and contact durations specified in Table 1 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 fungi, yeast 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 fungal strains used are:

- *Candida albicans* ATCC 10 231
- *Aspergillus brasiliensis* ATCC 16404

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
Fungal strains	<i>Candida albicans</i> (vegetative cells)	<i>Candida albicans</i> (vegetative cells)	<i>Candida albicans</i> (vegetative cells) <i>Aspergillus brasiliensis</i> (conidiospores)	<i>Candida albicans</i> (vegetative cells) <i>Aspergillus brasiliensis</i> (conidiospores)
Test temperature	20°C	20°C	20°C	20°C
Contact time	60 seconds	1 minute	15 minutes	1 minute

After the contact time has elapsed, the fungicidal and/or fungistatic 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.

In parallel, the number of fungi in a suspension treated with hard water (300mg/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 is MEA (malt extract Agar) and incubation temperature of the cultures was 30°C.

- **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%		
Candida albicans ATCC 10 231	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	95	99	97	91	89	74	72		>330	>330		N=4,2X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300; >300	
	NV=10,1x10 ³		NVB=9,7x10 ⁴		A=98		B=90		C=73						Log N = 8,64	Na = <1,4x10 ²	Na = <1,5x10 ²	Na = >3,7x10 ⁴
	NV0=10,1x10 ²										10 ⁻⁷	44	42		N0=4,3X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
Aspergillus brasiliensis ATCC 16404	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	36	38	35	38	36	42	37	41	36		>330	>330		N=3,3X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300; >300	
	NV=3,7x10 ³		NVB=3,65x10 ⁴		A=37		B=39,5		C=38,5						Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
	NV0=3,7x10										10 ⁻⁷	35	36		N0=3,55X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
														Log N0 = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,08	

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%		
Candida albicans ATCC 10 231	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	99	97	93	97	95	94	90	77	72		>330	>330		N=4,2X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300; >300	
	NV=10,15x10 ³		NVB=9,5x10 ⁴		A=96		B=92		C=74,5						Log N = 8,64	Na = <1,4x10 ²	Na = <1,5x10 ²	Na = >3,7x10 ⁴
	NV0=10,15x10 ²										10 ⁻⁷	44	46		N0=4,5X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
Aspergillus brasiliensis ATCC 16404	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	37	40	36	40	35	40	35	38	34		>330	>330		N=3,3X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300; >300	
	NV=3,85x10 ³		NVB=3,8x10 ⁴		A=37,5		B=37,5		C=36						Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
	NV0=3,85x10										10 ⁻⁷	37	36		N0=3,65X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
														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.

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%		
Candida albicans ATCC 10 231	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10 ⁰ : 0	10 ⁰ : 0	10 ⁰ : >300 ; >300	
	102	100	97	95	97	93	91	88	73	69		>330	>330		N=4,2X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300 ; >300	
																Log N = 8,64	Na = <1,4x10 ²	Na = >3,7x10 ⁴
																N0=4,5X10 ⁷	Log Na = <2,15	Log Na = >4,52
															Log N0 = 7,62	Log R = >5,46	Log R = < 3,08	
Aspergillus brasiliensis ATCC 16404	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10 ⁰ : 0	10 ⁰ : 0	10 ⁰ : >300 ; >300	
	38	35	39	35	39	36	39	34	42	39		>330	>330		N=3,3X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300 ; >300	
															Log N = 8,51	Na = <1,4x10 ²	Na = >3,3x10 ⁴	
															N0=3,65X10 ⁷	Log Na = <2,15	Log Na = >4,52	
														Log N0 = 7,53	Log R = >5,37	Log R = < 2,98		

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%	
Candida albicans ATCC 10 231	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10 ⁰ : 0	10 ⁰ : 0	10 ⁰ : >300 ; >300
	101	98	99	97	99	95	92	88	78	76		>330	>330		N=4,2X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300 ; >300
															Log N = 8,64	Na = <1,4x10 ²	Na = >3,7x10 ⁴
															N0=4,3X10 ⁷	Log Na = <2,15	Log Na = >4,52
														Log N0 = 7,62	Log R = >5,46	Log R = < 3,08	
Aspergillus brasiliensis ATCC 16404	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2		Vc1	Vc2		10 ⁰ : 0	10 ⁰ : 0	10 ⁰ : >300 ; >300
	39	35	39	35	39	36	42	40	41	39		>330	>330		N=3,3X10 ⁸	10 ⁻¹ : 0; 0	10 ⁻¹ : >300 ; >300
															Log N = 8,51	Na = <1,4x10 ²	Na = >3,3x10 ⁴
															N0=3,5X10 ⁷	Log Na = <2,15	Log Na = >4,52
														Log N0 = 7,53	Log R = >5,37	Log R = < 2,98	

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%	
Candida albicans ATCC 10 231	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	10 ⁻⁶	>330	>330	N=4,2X10 ⁸	10 ⁰ :0; 0	10 ⁰ :0; 0	10 ⁰ :>300; >300
	104	102	99	95	99	96	92	89	75	70				Log N = 8,64	Na = <1,4x10 ²	Na = <1,5x10 ²	Na = >3,7x10 ⁴
	NV=10,3x10 ³		NVB=9,7x10 ⁴		A=97,5		B=90,5		C=72,5		10 ⁻⁷	43	42	NO=4,25X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=10,3x10 ²													Log NO = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,08
Aspergillus brasiliensis ATCC 16404	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	10 ⁻⁶	>330	>330	N=3,3X10 ⁸	10 ⁰ :0; 0	10 ⁰ :0; 0	10 ⁰ :>300; >300
	38	33	42	37	42	40	40	37	42	40				Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
	NV=3,55x10 ²		NVB=3,95x10 ⁴		A=41		B=38,5		C=41		10 ⁻⁷	36	37	NO=3,65X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=3,55x10													Log NO = 7,53	Log R = >5,37	Log R = >5,34	Log R = < 2,98

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%	
Candida albicans ATCC 10 231	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	10 ⁻⁶	>330	>330	N=4,2X10 ⁸	10 ⁰ :0; 0	10 ⁰ :0; 0	10 ⁰ :>300; >300
	101	97	99	95	99	96	94	91	78	73				Log N = 8,64	Na = <1,4x10 ²	Na = <1,5x10 ²	Na = >3,7x10 ⁴
	NV=9,9x10 ³		NVB=9,7x10 ⁴		A=97,5		B=92,5		C=75,5		10 ⁻⁷	44	42	NO=4,3X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=9,9x10 ²													Log NO = 7,62	Log R = >5,46	Log R = >5,50	Log R = < 3,08
Aspergillus brasiliensis ATCC 16404	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	Vc1	Vc2	10 ⁻⁶	>330	>330	N=3,3X10 ⁸	10 ⁰ :0; 0	10 ⁰ :0; 0	10 ⁰ :>300; >300
	42	39	40	36	40	38	39	36	41	36				Log N = 8,51	Na = <1,4x10 ²	Na = <1,4x10 ²	Na = >3,3x10 ⁴
	NV=4,05x10 ²		NVB=3,8x10 ⁴		A=39		B=37,5		C=38,5		10 ⁻⁷	37	35	NO=3,6X10 ⁷	Log Na = <2,15	Log Na = <2,15	Log Na = >4,52
	NV0=4,05x10													Log NO = 7,53	Log R = >5,37	Log R = >5,34	Log R = < 2,98

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.

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**, 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 UNE-EN 13624:2014, in clean and dirty conditions, for the concentrations 100% (1,62ppm) y 90% (1,42ppm)**, to the reference strains *Candida albicans* ATCC 10 231, *Aspergillus brasiliensis* ATCC 16404, **demonstrating at least a 4 lg reduction** in the following application activities in the medical area:

- **Hygienic handrub treatment and hygienic handwash** (Temperature 20°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


inoQua Instituto
de SALUD ALIMENTARIA
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.