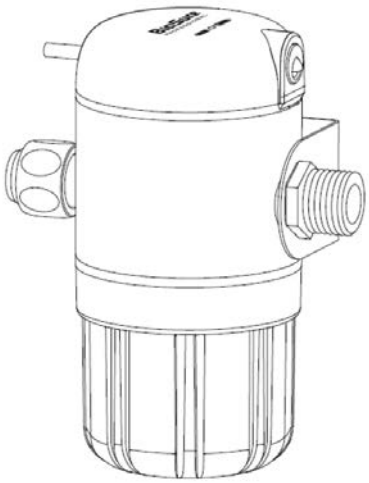
CHARACTERISATION OF THE COMPOSITION OF OZONIZED WATER

EXECUTION OF UNE-EN TESTS TO CERTIFY THE VIRUCIDE 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 virucidal activity of ozonized water** generated "in situ" with the equipment to be tested.

The standard that has been carried out is UNE-EN 14476:2019 + A2 - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics in the medical area. (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

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VIRUCIDAL ACTIVITY TEST
UNE-EN 14476:2019 + A2:2020 Standard

Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics in the medical area. (phase 2, step 1).

Test execution: 26/02/2024 – 09/03/2024
Analytical report: 5052/24

Methodology

The stipulated method in this Standard to evaluate the virucidal activity of ozonized water is based on the calculation of the infectivity reduction of different viruses that have been subjected to the effect of the ozonized water.

For the execution of the test, a solution of different types of viruses together with interfering substances is prepared to apply to the ozonized water sample, pure and/or diluted with hard water (300mg/Kg CaCo3).

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 viruses 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 mixture is kept at a specified temperature and for a defined period of time. After this contact time, an aliquot of the mixture is taken and the virucidal effect of the ozonized water immediately neutralized using a validated method (dilution of the sample in a cellular maintenance medium at ice temperature).

The different dilutions are transferred to cellular cultures (petri dishes, tubes or microtiter plate wells) in monolayer or in cell suspension. The infectivity tests are performed either through plate tests or quantal tests.

After incubation, and using Spearman-Kärber method or plate recounts, the infectivity titers are calculated and evaluated.

The virus infectivity reduction is calculated through the differences of the logarithms of viral titers before (virus control) and after the treatment with ozonized water.

The virus strains used have been:

- Poliovirus type 1. LSc-2ab (Piconavirus) passe 3
- Murine norovirus, strain S99 Berlin passe 2
- Adenovirus type 5, strain Adenoid 75, ATCC VR-5 passe 1
- Virus vaccina ATCC VR-1508 passe 2

The cell lines used have been:

- Vero, ref FTVE, rate 2, passes 7, 9, 11
- Raw 264.7, rate 3, passes 4, 6, 8

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

The test has been executed at a temperature of 20ºC ± lºC.

The contact period of time has been 1 minute ± 10 seconds.

The incubation temperatures have been 36ºC ± 1ºC and 37ºC ± 1ºC..

Results and conclusion

Results of product activity with **Poliovirus type 1** under **dirty conditions.**

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				Reduction of the 95% confidence interval after 1 min
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3g/L	0,5		0,5			≥ 6,64 ± 0,34
	90%		0,5		0,5			≥ 5,50 ± 0,32
	30%		0,5		6,74			0,43 ± 0,45
Control virus	NA	0,3 g/L	NA	7,07	7,02			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	5	3,07	NA
Formaldehyde virus control	0,70%	NA	NA	7,08	ND	ND	6,91	NA
NA: NON APPLICABLE; ND: NOT DONE								

Poliovirus type 1 titration test under **dirty conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)^3							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	4444	3202	0010	0000
				4444	4444	4444	4444	4444	3223	0201	0000
				4444	4444	4444	4444	4444	0332	0012	0000
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	4444	1020	0000
				4444	4444	4444	4444	4444	4444	2200	0120
				4444	4444	4444	4444	4444	4444	1000	0000
			1 min	4444	4444	4444	4444	4444	3343	1020	0000
				4444	4444	4444	4444	4444	2332	3020	0000
				4444	4444	4444	4444	4444	3312	1010	0000
Formaldehyde	0,7	NA	30 min	4444	4444	4444	3332	0220	0000	0000	ND
				4444	4444	4444	4333	1003	0000	0000	
				4444	4444	4444	3443	0101	0000	0000	
			60 min	4444	4444	0201	0001	0000	0000	0000	ND
				4444	4444	0020	1000	0000	0000	0000	
				4444	4444	2001	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test											

Results of product activity with **Adenovirus type 5** under **dirty conditions**.

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				*
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3g/L	0,5		0,5			≥ 5,67 ± 0,31
	90%		0,5		0,5			≥ 5,24 ± 0,32
	30%		0,5		5,66			0,33 ± 0,41
Control virus	NA	0,3 g/L	NA	6,08	6,18			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	2,58	1,33	NA
Formaldehyde virus control	0,70%	NA	NA	6,07	ND	ND	5,91	NA

NA: NON APPLICABLE; ND: NOT DONE

* Reduction of the 95% confidence interval after 1 min

Adenovirus type 5 titration test under **dirty conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)²							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	3023	0120	0000	ND
				4444	4444	4444	4444	3302	0011	0000	
				4444	4444	4444	4444	3323	0020	0000	
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	0322	0000	ND
				4444	4444	4444	4444	4444	0102	0100	
				4444	4444	4444	4444	4444	2020	0000	
			1 min	4444	4444	4444	4444	4213	3001	0000	ND
				4444	4444	4444	4444	4332	2002	1000	
				4444	4444	4444	4444	4211	1002	0000	
Formaldehyde	0,7	NA	30 min	4444	3323	2001	0000	0000	0000	0000	ND
				4444	2333	0010	0000	0000	0000	0000	
				4444	2033	2000	0000	0000	0000	0000	
			60 min	0302	2100	0000	0000	0000	0000	0000	ND
				2030	0001	0000	0000	0000	0000	0000	
				2020	0200	0000	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	

NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test

Results of product activity with **murine Norovirus** under **dirty conditions**.

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				*
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3g/L	0,5		0,5			≥ 5,57 ± 0,31
	95%		0,5		0,5			≥ 5,28 ± 0,30
	30%		0,5		5,57			0,41 ± 0,37
Control virus	NA	0,3 g/L	NA	5,99	6,03			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	3,16	1,74	NA
Formaldehyde virus control	0,70%	NA	NA	6	ND	ND	5,82	NA
NA: NON APPLICABLE; ND: NOT DONE			* Reduction of the 95% confidence interval after 1 min					

Murine Norovirus titration test under **dirty conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)²							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	2113	0011	0000	ND
				4444	4444	4444	4444	2322	2000	0000	
				4444	4444	4444	4444	2321	0000	0000	
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	0210	0000	ND
				4444	4444	4444	4444	4444	2012	2000	
				4444	4444	4444	4444	4444	1000	0010	
			1 min	4444	4444	4444	4444	3324	3011	0000	ND
				4444	4444	4444	4444	3333	2002	0000	
				4444	4444	4444	4444	2334	3022	0000	
Formaldehyde	0,7	NA	30 min	4444	4444	2001	0000	0000	0000	0000	ND
				4444	4444	0110	1000	0000	0000	0000	
				4444	4444	3002	2000	0000	0000	0000	
			60 min	4444	1000	0000	0000	0000	0000	0000	ND
				4444	0200	0000	0000	0000	0000	0000	
				4444	2001	0000	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test											

Results of product activity with **vaccinia virus** under **dirty conditions.**

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				Reduction of the 95% confidence interval after 1 min
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3g/L	0,5		0,5			≥ 6,70 ± 0,35
	90%		0,5		0,5			≥ 6,25 ± 0,29
	30%		0,5		6,73			0,36 ± 0,41
Control virus	NA	0,3 g/L	NA	7,07	7,02			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	5	3,07	NA
Formaldehyde virus control	0,70%	NA	NA	7,08	ND	ND	6,91	NA
NA: NON APPLICABLE; ND: NOT DONE								

Vaccinia virus titration test under **dirty conditions.**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)[a]							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	4444	3202	0010	0000
				4444	4444	4444	4444	4444	3220	0202	0000
				4444	4444	4444	4444	4444	0313	1012	0000
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	4444	1020	0000
				4444	4444	4444	4444	4444	4444	2201	0120
				4444	4444	4444	4444	4444	4444	1000	0000
			1 min	4444	4444	4444	4444	4444	3343	1020	0000
				4444	4444	4444	4444	4444	2332	3020	0000
				4444	4444	4444	4444	4444	3312	1010	0000
Formaldehyde	0,7	NA	30 min	4444	4444	4444	3332	0220	0000	0000	ND
				4444	4444	4444	4333	1003	0000	0000	
				4444	4444	4444	3443	0101	0000	0000	
			60 min	4444	4444	0201	0001	0000	0000	0000	ND
				4444	4444	0020	1000	0000	0000	0000	
				4444	4444	2001	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test											

Validation of test results under dirty conditions

Poliovirus type 1

Viral suspension titre for control virus (60 seconds): $\log 10^{-7,19}$
Cytotoxicity level: $\log 10^{-0,55}$
Maximum detectable virus inactivation: $\log 10^{-6,64}$

Adenovirus type 5

Viral suspension titre for control virus (60 seconds): $\log 10^{-6,19}$
Cytotoxicity level: $\log 10^{-0,52}$
Maximum detectable virus inactivation: $\log 10^{-5,67}$

Murine Norovirus

Viral suspension titre for control virus (60 seconds): $\log 10^{-6,15}$
Cytotoxicity level: $\log 10^{-0,58}$
Maximum detectable virus inactivation: $\log 10^{-5,57}$

Vaccinia virus

Viral suspension titre for control virus (60 seconds): $\log 10^{-7,25}$
Cytotoxicity level: $\log 10^{-0,55}$
Maximum detectable virus inactivation: $\log 10^{-6,70}$

Reference test (formaldehyde 1.4%)

Cytotoxicity level of 0.7% formaldehyde: $\log 10^{-0,5}$
Viral quantification in the reference test (formaldehyde) after 60 min and with

- **Poliovirus type 1:** $\log 10^{-3,16}$
- **Adenovirus type 5:** $\log 10^{-1,25}$
- **Murine Norovirus:** $\log 10^{-1,73}$
- **Vaccinia virus:** $\log 10^{-3,18}$

Confidence Interval

Virus titre with 95% confidence interval (60 seconds)

- **Poliovirus type 1:** $\log 10^{-6,72\pm0,34}$
- **Adenovirus type 5:** $\log 10^{-6,14\pm0,31}$
- **Murine Norovirus:** $\log 10^{-6,44\pm0,29}$
- **Vaccinia virus:** $\log 10^{-7,06\pm0,30}$

Cell susceptibility interference monitoring:

Titre of the viral suspension with untreated cells by the disinfectant test solution

- **Poliovirus type 1:** $\log 10^{-6,88}$
- **Adenovirus type 5:** $\log 10^{-6,21}$
- **Murine Norovirus:** $\log 10^{-6,18}$
- **Vaccinia virus:** $\log 10^{-6,91}$

Titre of the viral suspension with treated cells by the disinfectant test solution

- **Poliovirus type 1:** $\log 10^{-6,47}$
- **Adenovirus type 5:** $\log 10^{-5,55}$
- **Murine Norovirus:** $\log 10^{-5,56}$
- **Vaccinia virus:** $\log 10^{-6,54}$

Effectiveness control of product activity suppression:

Titre of the viral suspension after 30 minutes of incubation in an ice bath, without contact of the virus with disinfectant

- **Poliovirus type 1**: $\log 10^{-7,22}$
- **Adenovirus type 5**: $\log 10^{-5,72}$
- **Murine Norovirus**: $\log 10^{-6,13}$
- **Vaccinia virus:** $\log 10^{-7,14}$

Titre of the viral suspension by exposing the virus to disinfectant and incubation for 30 minutes in an ice bath

- **Poliovirus type 1**: $\log 10^{-6,61}$
- **Adenovirus type 5**: $\log 10^{-5,74}$
- **Murine Norovirus**: $\log 10^{-5,65}$
- **Vaccinia virus:** $\log 10^{-6,65}$

In **conclusion**, the ozonised water generated with the **BES Group Biosure Professional EOS7211-BX / WS1200X equipment** (Serial No.: 7211BXNA1101 - Production: 2023) **complies with the UNE-EN-14476: 2019+A2 standard** in dirty conditions at **concentrations of 100% (1.62ppm) and 90% (1.46ppm)** at a **contact time of 1 minute**, demonstrating at least a decimal logarithmic reduction of 4 of the infectivity of the following viruses:

- Poliovirus type 1, with a reduction of ≥ 6,64 ± 0,34 TCID50
- Adenovirus type 5, with a reduction of ≥ 5,67 ± 0,31 TCID50
- Murine Norovirus, with a reduction of de ≥ 5,57 ± 0,31 TCID50
- Vaccinia virus, with a reduction of ≥ 6,70 ± 0,35 TCID50

All checks and validations are within accepted limits.

Oviedo, 16th of March 2024

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Results of product activity with **Poliovirus type 1** under **clean conditions.**

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				Reduction of the 95% confidence interval after 1 min
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3g/L	0,5		0,5			≥ 6,65 ± 0,31
	90%		0,5		0,5			≥ 5,31 ± 0,27
	30%		0,5		6,72			0,36 ± 0,32
Control virus	NA	0,3 g/L	NA	7,07	7,02			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	5	3,07	NA
Formaldehyde virus control	0,70%	NA	NA	7,08	ND	ND	6,91	NA
NA: NON APPLICABLE; ND: NOT DONE								

Poliovirus type 1 titration test under **clean conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)[3]							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	4444	3211	0010	0000
				4444	4444	4444	4444	4444	3221	0202	0000
				4444	4444	4444	4444	4444	0332	0012	0000
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	4444	1021	0000
				4444	4444	4444	4444	4444	4444	2201	0120
				4444	4444	4444	4444	4444	4444	1000	0000
			1 min	4444	4444	4444	4444	4444	3343	1020	0000
				4444	4444	4444	4444	4444	2332	3020	0000
				4444	4444	4444	4444	4444	3312	1012	0000
Formaldehyde	0,7	NA	30 min	4444	4444	4444	3332	0220	0000	0000	ND
				4444	4444	4444	4333	1003	0000	0000	
				4444	4444	4444	3443	0101	0000	0000	
			60 min	4444	4444	0201	0001	0000	0000	0000	ND
				4444	4444	0020	1000	0000	0000	0000	
				4444	4444	2001	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test											

Results of product activity with **Adenovirus type 5** under **clean conditions**.

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				*
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3g/L	0,5		0,5			≥ 5,71 ± 0,32
	90%		0,5		0,5			≥ 5,49± 0,32
	30%		0,5		5,66			0,40 ,± 0,37
Control virus	NA	0,3 g/L	NA	6,08	6,18			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	2,58	1,33	NA
Formaldehyde virus control	0,70%	NA	NA	6,07	ND	ND	5,91	NA

NA: NON APPLICABLE; ND: NOT DONE * Reduction of the 95% confidence interval after 1 min

Adenovirus type 5 titration test under **clean conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)[a]							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3 g/L	1 min	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
	90%		1 min	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
	30%		1 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3023 3302 3323	0120 2011 3220	0000 0000 0000	ND
Cytotoxicity	1%	0,3 g/L	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
Virus Control	NA	0,3 g/L	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0322 0103 2020	0000 0100 0000	ND
			1 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4213 4332 4211	3001 2032 1002	0000 1000 0000	ND
Formaldehyde	0,7	NA	30 min	4444 4444 4444	3323 2333 2033	2001 0010 2000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
			60 min	0302 2030 2020	2100 0001 0200	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
Formaldehyde virus control	0,7	0,3 g/L	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND

NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test

Results of product activity with **Murine Norovirus** under **clean conditions**.

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				*
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3g/L	0,5		0,5			≥ 5,60 ± 0,32
	90%		0,5		0,5			≥ 5,32 ± 0,21
	30%		0,5		5,56			0,43 ± 0,39
Control virus	NA	0,3 g/L	NA	5,99	6,04			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	3,15	1,74	NA
Formaldehyde virus control	0,70%	NA	NA	6	ND	ND	5,82	NA

NA: NON APPLICABLE; ND: NOT DONE * Reduction of the 95% confidence interval after 1 min

Murine Norovirus titration test under **clean conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)[a]							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3 g/L	1 min	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
	90%		1 min	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
	30%		1 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	2213 2322 2321	0021 2001 0000	0000 0000 0000	ND
Cytotoxicity	1%	0,3 g/L	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
Virus Control	NA	0,3 g/L	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0210 2212 1000	0000 2000 0010	ND
			1 min	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	3324 3332 2334	3011 2302 3022	0000 0000 0000	ND
Formaldehyde	0,7	NA	30 min	4444 4444 4444	4444 4444 4444	2001 0110 3002	0000 1000 2000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
			60 min	4444 4444 4444	1000 0200 2001	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND
Formaldehyde virus control	0,7	0,3 g/L	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	ND

NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test

Results of product activity with **Vaccinia virus** under **clean conditions.**

Sample	Concentration	Interfering substance	Cytotoxicity level	Log 10 TCIDSO after...				Reduction of the 95% confidence interval after 1 min
				0 min	1 min	30 min	60 min	
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3g/L	0,5		0,5			≥ 6,65 ± 0,30
	90%		0,5		0,5			≥ 6,40± 0,32
	30%		0,5		6,74			0,36 ± 0,32
Control virus	NA	0,3 g/L	NA	7,07	7,02			NA
Formaldehyde	0,70%	NA	0,5	ND	ND	5	3,07	NA
Formaldehyde virus control	0,70%	NA	NA	7,08	ND	ND	6,91	NA
NA: NON APPLICABLE; ND: NOT DONE								

Vaccinia virus titration test under **clean conditions**

Sample	Concentration	Interfering substance	Contact time	Dilutions (log10)[a]							
				1	2	3	4	5	6	7	8
Device AVET AG - Model EOS 7211-BX / WDS1200X	100%	0,3 g/L	1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	90%		1 min	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
	30%		1 min	4444	4444	4444	4444	4444	3202	0010	0000
				4444	4444	4444	4444	4444	3003	0201	0000
				4444	4444	4444	4444	4444	0432	0012	0000
Cytotoxicity	1%	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
Virus Control	NA	0,3 g/L	0	4444	4444	4444	4444	4444	4444	1020	0000
				4444	4444	4444	4444	4444	4444	2202	0120
				4444	4444	4444	4444	4444	4444	1000	0000
			1 min	4444	4444	4444	4444	4444	3343	1020	0000
				4444	4444	4444	4444	4444	2332	3020	0000
				4444	4444	4444	4444	4444	3312	1010	0000
Formaldehyde	0,7	NA	30 min	4444	4444	4444	3332	0220	0000	0000	ND
				4444	4444	4444	4333	1003	0000	0000	
				4444	4444	4444	3443	0101	0000	0000	
			60 min	4444	4444	0201	0001	0000	0000	0000	ND
				4444	4444	0020	1000	0000	0000	0000	
				4444	4444	2001	0000	0000	0000	0000	
Formaldehyde virus control	0,7	0,3 g/L	NA	0000	0000	0000	0000	0000	0000	0000	ND
				0000	0000	0000	0000	0000	0000	0000	
				0000	0000	0000	0000	0000	0000	0000	
NA: NON APPLICABLE; ND: NOT DONE; 1 to 4, virus present and degree of cytopathic effect in 12 units of cell culture, or degree of cell injury in cytotoxicity test; O = virus not present, or absence of cellular lesion in cytotoxicity test											

Validation of test results under clean conditions

Poliovirus type 1

Viral suspension titre for control virus (60 seconds): $\log 10^{-7,21}$
Cytotoxicity level: $\log 10^{-0,56}$
Maximum detectable virus inactivation: $\log 10^{-6,65}$

Adenovirus type 5

Viral suspension titre for control virus (60 seconds): $\log 10^{-6,23}$
Cytotoxicity level: $\log 10^{-0,52}$
Maximum detectable virus inactivation: $\log 10^{-5,71}$

Murine Norovirus

Viral suspension titre for control virus (60 seconds): $\log 10^{-6,12}$
Cytotoxicity level: $\log 10^{-0,52}$
Maximum detectable virus inactivation: $\log 10^{-5,60}$

Vaccinia virus

Viral suspension titre for control virus (60 seconds): $\log 10^{-7,18}$
Cytotoxicity level: $\log 10^{-0,53}$
Maximum detectable virus inactivation: $\log 10^{-6,65}$

Reference test (formaldehyde 1.4%)

Cytotoxicity level of 0.7% formaldehyde: $\log 10^{-0,5}$
Viral quantification in the reference test (formaldehyde) after 60 min and with

- **Poliovirus type 1:** $\log 10^{-3,14}$
- **Adenovirus type 5:** $\log 10^{-1,50}$
- **Murine Norovirus:** $\log 10^{-1,53}$
- **Vaccinia virus:** $\log 10^{-3,16}$

Confidence Interval

Virus titre with 95% confidence interval (60 seconds)

- **Poliovirus type 1:** $\log 10^{-7,11\pm0,34}$
- **Adenovirus type 5:** $\log 10^{-6,22\pm0,28}$
- **Murine Norovirus:** $\log 10^{-6,16\pm0,26}$
- **Vaccinia virus:** $\log 10^{-7,14\pm0,33}$

Cell susceptibility interference monitoring:

Titre of the viral suspension with untreated cells by the disinfectant test solution

- **Poliovirus type 1:** $\log 10^{-6,94}$
- **Adenovirus type 5:** $\log 10^{-6,21}$
- **Murine Norovirus:** $\log 10^{-6,12}$
- **Vaccinia virus:** $\log 10^{-6,93}$

Titre of the viral suspension with treated cells by the disinfectant test solution

- **Poliovirus type 1:** $\log 10^{-6,43}$
- **Adenovirus type 5:** $\log 10^{-5,52}$
- **Murine Norovirus:** $\log 10^{-5,61}$
- **Vaccinia virus:** $\log 10^{-6,42}$

Effectiveness control of product activity suppression:

Titre of the viral suspension after 30 minutes of incubation in an ice bath, without contact of the virus with disinfectant

- **Poliovirus type 1**: $\log 10^{-7,13}$
- **Adenovirus type 5**: $\log 10^{-5,78}$
- **Murine Norovirus**: $\log 10^{-6,11}$
- **Vaccinia virus:** $\log 10^{7,14}$

Titre of the viral suspension by exposing the virus to disinfectant and incubation for 30 minutes in an ice bath

- **Poliovirus type 1**: $\log 10^{-6,69}$
- **Adenovirus type 5**: $\log 10^{-5,74}$
- **Murine Norovirus**: $\log 10^{-5,59}$
- **Vaccinia virus:** $\log 10^{-6,89}$

In **conclusion**, the ozonised water generated with the **BES Group Biosure Professional EOS7211-BX / WS1200X equipment** (Serial No.: 7211BXNA1101 - Production: 2023) **complies with the UNE-EN-14476: 2019+A2 standard** in clean conditions at **concentrations of 100% (1.62ppm) and 90% (1.46ppm)** at a **contact time of 1 minute**, demonstrating at least a decimal logarithmic reduction of 4 of the infectivity of the following viruses:

- Poliovirus type 1, with a reduction of ≥ 6,65 ± 0,31 TCID50
- Adenovirus type 5, with a reduction of ≥ 5,71 ± 0,32 TCID50
- Murine Norovirus, with a reduction of ≥ 5,60 ± 0,32 TCID50
- Vaccinia virus, with a reduction of ≥ 6,65 ± 0,30 TCID50

All checks and validations are within accepted limits.

Oviedo, 16th of March 2024

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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.
- The UNE-EN 14476 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).