



UNIVERSITY OF TURIN  
**DIPARTIMENTO DI SCIENZE DELLA SANITA' PUBBLICA E PEDIATRICHE**  
**DEPARTMENT OF PUBLIC HEALTH AND PAEDIATRICS**

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Tax Code 80088230018 – VAT n. IT02099550010

Turin, 11<sup>th</sup> December 2014

RE: results of the effectiveness test conducted on the disinfectant Peroxy Ag+ (H<sub>2</sub>O<sub>2</sub> 3%) used in the dental unit inlet water continuous disinfection simulator (Cefla Dental Group)

Following earlier agreements between the Company CEFLA and the Hygiene Applied Serology and Microbiology Laboratory of the University of Turin, tests were carried out on water samples taken from the continuous disinfection simulator, experimentally contaminated with *Legionella pneumophila* sg 1, in order to evaluate the dental unit inlet water disinfection system.

The tests were performed using as a reference the standard EN 13623 (2010).

**Test conditions:**

Inoculum:	<i>Legionella pneumophila</i> sg 1 (ATCC 33152)
Diluent:	hard water (HW)
Ambient temperature	approximately 22°C
Test product:	540 ±10 ppm of Peroxy Ag+ (H <sub>2</sub> O <sub>2</sub> 3%)
Neutraliser:	0.25 g/l catalase
Effluent sampling times:	after 9 minutes of contact in the tanks after 16 minutes of contact in the tanks
No. of repetitions:	3 for each contact time.
Test performance time:	28 <sup>th</sup> November - 9 <sup>th</sup> December



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**Procedure:**

From a 3 days' culture of *Legionella*, a test suspension was prepared at the concentration of  $4,6 \times 10^7$  /ml. 1.8 ml of suspension were used to contaminate the tank from which water was delivered to the tanks for mixing with Peroxy Ag+, containing 1.8 litres of hard water. After mixing, a 10 ml sample of final suspension was taken from the tank, and base 10 dilutions and selective medium seeding were performed, to grow *Legionella* and test the actual *Legionella* concentration in the tank.

The tank was connected to the simulator and water delivery was started. The effluent samples to submit to testing to measure bactericidal activity were taken after contact times of 9 minutes and of 16 minutes (*legionella* –peroxy). For each contact time, three effluent sampling repetitions were performed.

Immediately after harvesting, the samples were submitted to neutralisation and base 10 dilutions were prepared and selective medium seeding was performed to obtain *Legionella* growth.

The obtained results are shown in the tables here below.

**Test results**

	N	Vc <sub>1</sub>	Vc <sub>2</sub>	
Test suspension (N <sub>0</sub> )	10 <sup>-6</sup>	64	50	N <sub>0</sub> = 5.70 x 10 <sup>3</sup> lgN <sub>0</sub> = 3.75
	10 <sup>-7</sup>	< 15	< 15	
Validation suspension (N <sub>v</sub> )	10 <sup>-6</sup>	50	42	N <sub>v</sub> = 4.60 x 10 <sup>2</sup> lgN <sub>v</sub> = 2.66
	10 <sup>-7</sup>	< 15	< 15	



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Contact Time	Effluent sampling repetitions	Vc <sub>1</sub>	Vc <sub>2</sub>	$\bar{X}$ surviving microorganisms (N <sub>a</sub> )	lgN <sub>a</sub>	lgR
9 minutes	1	29	41	$\bar{x} = 6.33 \times 10^2$	2.80	0.95
	2	65	55			
	3	98	92			
16 minutes	1	78	82	$\bar{x} = 1.02 \times 10^3$	3.00	0.75
	2	106	112			
	3	110	128			

Average lgR= (0.95+0.75) : 2 = 0.85

**Dilution-neutralisation method test with 600 mg/l of product in hard water**

Experimental condition check (A)			Neutraliser check (B)			Validation method check (C)		
Vc <sub>1</sub>	41	$\bar{x} = 43$	Vc <sub>1</sub>	49	$\bar{x} = 55$	Vc <sub>1</sub>	43	$\bar{x} = 43$
Vc <sub>2</sub>	45		Vc <sub>2</sub>	61		Vc <sub>2</sub>	44	

Neutralisation is validated (A, B, C greater than  $0.5 \times N_{v0}$ ) with the tested neutraliser, at the product test concentration of 600 mg/l and for the test microorganism.

N<sub>0</sub> = number of microorganisms present in 1 ml of test mix from the beginning of the contact time.

N<sub>a</sub> = number of microorganisms surviving in 1 ml of test mix at the end of the contact time before neutralisation

N<sub>v</sub> = validation suspension

N<sub>v0</sub> = (N<sub>v</sub>/10) number of microorganisms present in the validation mix (A-B-C) at the beginning of the contact time  
V<sub>c</sub> = microorganisms per ml count

$\bar{x}$  = average of Vc<sub>1</sub> and Vc<sub>2</sub>;

R = reduction (lgR = lgN<sub>0</sub> - lgN<sub>a</sub>)



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**Conclusion:**

On the basis of the obtained results, the use of Peroxy Ag+ at the concentration of  $540 \pm 10$  ppm does not allow for a reduction of 99.99% (4 log) to be achieved to declare a bactericidal efficacy against *Legionella pneumophila* sg 1 according to the applicable EN standards.

However, the performed tests showed a reduction of the infective load ranging between a maximum value of 88.77% and a minimum value of 82.21% (logR average = 0.85; average reduction 85.87%).

The Lab Manager

Prof. Carla Zotti

The Test Manager

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