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FROM THE ORIGINAL DOCUMENT

UNIVERSITY OF TURIN

DIPARTIMENTO DI SCIENZE DELLA SANITA' PUBBLICA E PEDIATRICHE

DEPARTMENT OF PUBLIC HEALTH AND PAEDIATRICS

DIRECTOR: PROF. LUCA CORDERO DI MONTEZEMOLO

Piazza Polonia, 94 – 10126 Turin (Italy)

Tax Code 80088230018 – VAT n. IT02099550010

TECHNICAL REPORT

on the disinfection systems in dental unit water lines.

Part I. Microbiological field tests

Purpose: checking the hygienic quality of the water delivered by a **Cefla Dental Group dental unit,** equipped with both **continuous disinfection (WHE)** and **non-continuous disinfection** (**BIOSTER**) systems.

In the period February-May 2013, we tested the effectiveness of the continuous sanitisation system with 550 ppm of H_2O_2 (Peroxy Ag+) installed on a dental unit of the Cefla Dental Group supplied to the Dental School of Turin.
The test dental unit, like all the other units at the Dental School, at the end of each work day was submitted to a disinfection cycle with H_2O_2 (Peroxy Ag+) at 3%. The treatment was performed in the morning in case of downtime due to holidays or weekend breaks.
During work on patients, the dental unit would deliver tap water mixed with hydrogen peroxide at the final concentration of 550 ppm H2O2.

The tests on the effectiveness of the sanitising system were not carried out by testing for Legionella, as the microbial loads detected at the Dental School (and systematically measured by this Department) were not such as to allow for anti-microbial effectiveness measurements; therefore, tests were performed on other microbial indicators (*Pseudomonas* spp, *P. aeruginosa* and total bacterial count at 22° and 37°C). All samplings were carried out in the morning and submitted to monitoring for three months, with sampling every 15 days.

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The test results, listed in the report dated 10th June 2013, show that in none of the test runs bacterial contamination was detected - except for the first (basal) test performed after installation and in the absence of sanitisation treatments.

Turin, 3rd August 2014

The Lab Manager Prof. Carla Zotti

The Test Manager Dr. Savina Ditommaso

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Turin, 10th June 2013

RE: scientific validation results regarding the continuous disinfecting system for inlet water to the Cefla Dental Group dental unit.

Following our earlier agreements and contract entered into by the Cefla Company, the Dental School and the Hygiene Applied Serology and Microbiology Laboratory of the University of Turin, tests were carried out on water samples taken from the dental unit, in order to evaluate the continuous disinfecting system (600 ppm of H2O2) for inlet water to the dental unit.

The tested parameters were:

a) Total Bacterial Count (TBC) at 22 and 37 °C
c) *Pseudomonas spp*
d) *Pseudomonas aeruginosa*

The tests were performed according to the reference standards: UNI EN ISO 6222 and UNI EN 12780

Monitoring was performed for 3 months, and samplings were repeated every fifteen days using outlet water from the syringe and turbine of the tested dental unit.
The results of the microbiological tests (expressed in Colony Forming Units, CFUs) are shown in the table below:

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Sampling date:	**TBC at 22°C**	**TBC at 37°C**	***Pseudomonas spp***	***P. aeruginosa***
Basal (2/2/ 2013)	>300 CFU/ml	>300 CFU/ml	>300 CFU/250 ml	>300 CFU/250 ml
26/02/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
12/03/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
26/03/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
08/04/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
22/04/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
07/05/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml
21/05/2013	not detected in 1 ml	not detected in 1 ml	not detected in 250 ml	not detected in 250 ml

Except for the first (basal) sampling, none of the tested samples during testing showed any microbiological contamination (no detected bacteria in the tested volume) and *Pseudomonas aeruginosa* was equally undetected.

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TECHNICAL REPORT on the disinfection systems in dental unit water lines.

Part II. Microbiological tests on prototype

Purpose: Validate continuous disinfection of inlet water to the dental unit on the device simulator (CEFLA Dental Group).

On the test bench prototype developed by CEFLA and installed at DSSPP tests were performed on the pathogen *Legionella pneumophila* sg. 1 (ATCC 33152) to determine the effectiveness of the Peroxy Ag^+ disinfectant at the concentration of 540 ± 10 ppm of H_2O_2.

The tests on water samples taken from the continuous disinfection device simulator were carried out by experimentally contaminating the device with *Legionella* in order to evaluate the dental unit inlet water sanitising system.

The test results are shown in the report dated 13th November 2013, and suggest that, in the samples taken from effluents after contact times of 7 minutes and 10 minutes, the 99.99% = 4 log reduction prescribed by the EN standards to be able to claim the bactericidal activity of the solution used had not been achieved.
In the sample taken after 60 minutes of hold time, a decrease >= 4 log was observed (reduction >= 99.99% of the inoculum).

Therefore, the obtained field results (report dated 10th June 2013) are possibly attributable not to the continuous system exclusively, but to the synergic action resulting from the protocol applied at the Dental School (treatment at 3% and continuous treatment).

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It is possible, but yet to be demonstrated, that the continuous system ensures the persistent presence of disinfectant, but the only way of evaluating the actual efficacy of the concentration used (540 +- 10 ppm of H_2O_2) in the absence of 3% treatment would be testing the dental unit in the absence of such treatment.

The continuous system alone at the concentration of 540 ± 10 ppm of H_2O_2 can help maintain, during one-hour activity pauses, a decrease >= 99.99% of any present *Legionella* loads.

Turin, 3rd August 2014

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Hygiene Applied Serology and Microbiology Laboratory

Turin, 13th November 2013

RE: results of the scientific validation of the continuous disinfecting system Simulator for inlet water to the Cefla Dental Group dental unit

Following our earlier agreements and contract entered into by the Cefla Company, the Dental School 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 device Simulator, experimentally contaminated with *Legionella pneumophila* sg 1, in order to evaluate the sanitising system (540 ±10 ppm of H_2O_2) for dental unit inlet water.

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

Test conditions:

Inoculum: *Legionella pneumophila* sg 1 (ATCC 33152)
Diluent: hard water (HW)
Ambient temperature (approximately 22°C)
Test product: 540 ±10 ppm of Peroxy Ag+ (H_2O_2 3%)
Neutraliser: 0.25 g/l catalase
Effluent sampling times: 7 min., 10 min., 60 min.
Test performance time: 10th September - 4th October
Calculation of results: $\Delta \log_{10} = \log_{10}$ inoculum $- \log_{10}$ sample.

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Before performing the test on the Simulator, neutralisation method validation tests were carried out (by filtration, mixed neutraliser, neutraliser with catalase). The test results showed that the most suitable method for Peroxy neutralisation is neutralisation with 0.25 g/l catalase.

Test results:

Flow sampling after 7 min contact time: $\Delta \log_{10} < 1$
Flow Sampling after 10 min contact time: $\Delta \log_{10} < 1$
Sampling after 60 min hold time: $\Delta \log_{10} \geq 4$

Conclusion:

On the basis of the obtained results the test product Peroxy Ag+, at a concentration of 540 ±10 ppm, showed bactericidal activity against *Legionella pneumophila* sg 1 only after a contact time of 60 minutes.

The Test Manager
Dr. Savina Ditommaso