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$_2$O$_2$ (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$_2$O$_2$ (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 H$_2$O$_2$.

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
tpp, *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.
The test results, listed in the report dated 10\textsuperscript{th} 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, 3\textsuperscript{rd} August 2014

The Lab Manager               Prof. Carla Zotti

The Test Manager               Dr. Savina Ditommaso
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:
### Sampling Data

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>TBC at 22°C</th>
<th>TBC at 37°C</th>
<th>Pseudomonas spp</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (2/2/2013)</td>
<td>&gt;300 CFU/ml</td>
<td>&gt;300 CFU/ml</td>
<td>&gt;300 CFU/250 ml</td>
<td>&gt;300 CFU/250 ml</td>
</tr>
<tr>
<td>26/02/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>12/03/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>26/03/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>08/04/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>22/04/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>07/05/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
<tr>
<td>21/05/2013</td>
<td>not detected in 1 ml</td>
<td>not detected in 1 ml</td>
<td>not detected in 250 ml</td>
<td>not detected in 250 ml</td>
</tr>
</tbody>
</table>

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.

The Lab Manager  
Prof. Carla Zotti

The Test Manager  
Dr. Savina Ditommaso
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 H₂O₂.

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$_2$O$_2$) 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$_2$O$_2$ can help maintain, during one-hour activity pauses, a decrease $\geq$ 99.99% of any present Legionella loads.

Turin, 3$^{rd}$ August 2014

The Lab Manager          Prof. Carla Zotti
The Test Manager          Dr. Savina Ditommaso
Turin, 13\textsuperscript{th} 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 \textit{Legionella pneumophila} sg 1, in order to evaluate the sanitising system (540 ±10 ppm of H\textsubscript{2}O\textsubscript{2}) for dental unit inlet water.

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

\textbf{Test conditions:}

- Inoculum: \textit{Legionella pneumophila} sg 1 (ATCC 33152)
- Diluent: hard water (HW)
- Ambient temperature (approximately 22\textdegree C)
- Test product: 540 ±10 ppm of Peroxy Ag+ (H\textsubscript{2}O\textsubscript{2} 3%)
- Neutraliser: 0.25 g/l catalase
- Effluent sampling times: 7 min., 10 min., 60 min.
- Test performance time: 10\textsuperscript{th} September - 4\textsuperscript{th} October
- Calculation of results: $\Delta \log_{10} = \log_{10} \text{inoculum} - \log_{10} \text{sample}$.
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.