

## Bacteriological Safety of Water Filters for Dental Units: Evaluation of the Filtration Action against *S. Aureus* and *E. Coli*

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### Abstract:

**Introduction:** The safety and control of water for clinical use are of critical importance in dental practice, in fact it is needed to cool and irrigate instruments, burs and oral tissues during treatment. Cross - contamination linked to operative procedures is a threat to the health of clinician and patients with considerable risk of infection. The aim of this study was to evaluate the bacterial filtration of the water of a medical device applied to a dental unit.

**Methods:** A total of 6 new nano - reticular filters were analysed at different operating times to observe their filtration capability and performances in comparison to 6 used filters.

The sterilizing efficacy of the filter under test was analyzed towards bacterial loads *S. Aureus* and *E.coli*, with known titer using two categories of filters: new and used with different operating times verifying the bacterial load present in the outgoing water, since a contaminated 2 litres solution was previously injected. Statistical analysis was performed using the Shapiro - Wilk test and Stat view software from SAS Institute.

**Results:** The outcome of the evaluation shows that both the used and new filters had highlighted a highly capability of bacterial filtration that attested 99.9999 % in the volume analysed. A statistical difference was found in the bacterial water contamination before and after filtration. (P = 0.000000). No statistical difference were observed between new and used filters. (P = 0.96239).

**Conclusions:** The durability and the efficiency of the filters at different times were positive in controlling cross-contamination and risk of infection during dental procedures.

**Keywords:** Biological safety; Water safety Dental unit; Microbiological tests; *Aeruginosa*; *E.coli*.

### Introduction

The microbiological quality of water is a serious ecological problem for public health<sup>[1]</sup> and for medical devices<sup>[2]</sup>. Dental chair units (DCUs) contain integrated systems providing water to cool hard and soft oral tissues and also the surfaces of the instruments during dental treatment. In fact the microbiological quality of water used in dental units is crucial for the safety of dental personnel and the requirements of dental patients and may relate to cross - infection during the treatment<sup>[3]</sup> particularly in immune suppressed patients<sup>[4]</sup>. The fungi, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Legionella* species, *Staphylococcus*, *Streptococcus* are considered opportunistic pathogens in dentistry<sup>[5,6]</sup>. The presence of bacterial and water contamination for DCUs has been shown to be an important cause of cross - infection and cross - contamination<sup>[7]</sup>.

The aspects related to the biological safety of the water used during the dental chair work phases have been brought to the attention of the public health control authorities, in accordance with the new regulations, with particular reference to the new guidelines on the prevention of *Legionella* issued by the Italian Ministry of Health as early as 2015. In fact, ac-

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According to the new guidelines, the dentist is responsible not only for the working environment of his surgery but also for the biological safety of the water circulating in the dental unit that feeds the spray syringe, the glass and the rinsing basin and the flushing sprays for the cooling of the rotating or ultrasonic instruments (ultrasonic scaler, air polishing and air abrasion turbines). This is often carried out with water from the mains without bacteriological control or treated with inadequate drainage systems, with the consequent risk of biological contamination of the environment. So if there is legionella, virus or other pathogens in the water supply of the study, the environmental risk of bacterial and / or viral infection occurs when these contaminated water particles are aerosolized by introducing these pathogens into the oral cavity of the patient or into the environment surrounding the work area<sup>[8]</sup>. To not mention the further risk of direct blood contagion during working with direct water flushing instruments on a patient undergoing surgical treatments that expose the oral mucosa to bleeding. This risk of contamination can significantly increase during long interventions, due to the continued exposure of the patient and operators to the dispersion of contaminated aerosols in the work place<sup>[9]</sup>. In order to find a suitable solution, capable of sanitizing the incoming water to the dental unit, it was decided to test a medical filtration device specifically designed for the dental unit and currently available on the market. The purpose of this study was to measure the capacity of filtering *Aureus* and *E.coli* of a medical device of ultra filtration.

## Materials and Methods

A total of 6 new filters and 6 used filters of a medical device of ultra filtration were used in this study. The water filter has a nano - reticular structure 0.05  $\mu$  with activated vegetable carbon added as a purifying and anti-odour agent for incoming water upstream of the dental unit, called Koala® filter (ODON-TOKOALA Rome, Italy). This dental unit water filter system contains the finest coconut shell based granular activated carbon.

To this end, laboratory tests were performed at two different experimental sites with new and used filters and *S. aureus* and *E.coli* were taken as indicators for evaluating their efficiency in up to 2 years of operation.

At the Department of Medical, Oral and Biotechnological Sciences of the University of Chieti - Pescara the tests were performed on the bacterial colonies. The sterilizing efficacy of the filter under test was analyzed towards bacterial loads *S.Aureus* and *E.coli*, with known titer using two categories of filters: new (table 1) and used with different operating times (table 2) verifying the bacterial load present in the outgoing water, since a contaminated 2 litres solution was previously injected. Two litres of sterile distilled water were filtered to check for any initial contamination of the filtering system (white), then with the same filter the contaminated sample was filtered (continuous physiological solution). The determination of the microbial load at 37°C on Plate Count Agar (PCA) was performed: on the white filtrate on the contaminated physiological sample (verification of the initial titer), on the corresponding filter (determined residual microbial). Six trials were performed with, *S.Aureus* at initial titer of  $10^4$  and  $10^5$  ufc / ml, and six series *E.coli* at initial titer of respectively  $10^5$  and  $10^6$  ufc / ml. Each series included 6 filtrates of similar contamination samples carried out respectively with

a new filter and one already used, for a total of 12 experimental tests. The water for dental use is considered contaminated when waterlines are between 10,000 and 10,000,000cfu / ml.

**Table 1:** The average concentration of bacteria isolated before and after 10500 litres filtration.

New filters				
Bacterial strain	Bacterial suspension titer (ufc/ml)	Initial sample titer (ufc/ml)	White (ufc/ml)	Residual titer filtered (ufc/ml)
<i>S.Aureus</i>	$2,58 \times 10^8$	$4,1 \times 10^4$	0	0
<i>S.Aureus</i>	$2,53 \times 10^7$	$3,7 \times 10^5$	0	0
<i>S.Aureus</i>	$2,53 \times 10^7$	$3,7 \times 10^5$	0	0
<i>E.Coli</i>	$1,3 \times 10^9$	$1,4 \times 10^5$	0	0
<i>E.Coli</i>	$1,3 \times 10^9$	$1,3 \times 10^6$	0	<1
<i>E.Coli</i>	$1,3 \times 10^9$	$1,3 \times 10^6$	0	<1

**Table 2:** The average concentration of bacteria isolated in the water before and after filtration with used filter.

Used filters					
Filter*	Bacterial strain	Bacterial suspension titer (ufc/ml)	Initial sample titer (ufc/ml)	White (ufc/ml)	Residual titer filtered (ufc/ml)
Cartridge A	<i>S.Aureus</i>	$2,58 \times 10^8$	$6,6 \times 10^4$	0	0
Cartridge B	<i>S.Aureus</i>	$2,53 \times 10^7$	$4,0 \times 10^5$	0	0
Cartridge C	<i>S.Aureus</i>	$2,53 \times 10^7$	$4,0 \times 10^5$	0	0
Cartridge D	<i>E.Coli</i>	$1,3 \times 10^9$	$1,5 \times 10^5$	0	0
Cartridge E	<i>E.Coli</i>	$1,3 \times 10^9$	$1,5 \times 10^6$	0	< 1
Cartridge F	<i>E.Coli</i>	$1,3 \times 10^9$	$1,5 \times 10^6$	0	< 1

**Statistical evaluation:** A power analysis was performed using clinical software, freely available on the site <http://clincalc.com/stats/samplesize.aspx>, for determining the number of filters needed to achieve statistical significance for quantitative analyses of quantization of bacteria. A calculation model was adopted for dichotomous variables (yes / no effect) by putting the effect incidence designed to caution the reasons 10% for controls and 95% for treated. Alpha was set at 0.6, Beta at 0.1 and powers a 0.9.

The optimal number of filters for analysis is 6 tests and 6 for control.

The differences quantitative bacterial before and after water filtration were analyzed by Shapiro - Wilk test. The differences quantitative bacterial filtration between new and used filters was analyzed by Shapiro - Wilk test. A p -value  $\leq 0.05$  was considered statistically significant. Statistical analysis was performed using the Statview software from SAS Institute.

## Results

The new and the already used filters after 10500 liters filtration showed an effective retention capacity of the bacterial loads under examination, always recording a residual zero load, even in the presence of high initial contaminant loads ( $10^6$  ufc / ml) with both the bacterial strains used and in any case, demonstrating a reduction of 99.9999% in the volume analyzed. A sta-

tistical difference was found in the bacterial water contamination before and after filtration. ( $P = 0.000000$ ). No statistical different were observed between new and used filters. ( $P = 0.96239$ ) (Tables)

## Discussion

In the present study a statistical difference was found in the bacterial water contamination before and after filtration. No statistical different were observed between new and used filters. This result show the efficacy of the filter used towards bacterial loads *S. Aureus* and *E. coli*. The importance of the data on the safety of the water sanitization systems circulating in the dental unit, is of considerable importance, since it involves both medical and legal aspects of professional responsibility in the workplace. On the basis of the experiments carried out, we can state that the filtering system of the tested Medical Device is an effective barrier, impermeable to the passage of bacteria, including legionella. It has also shown a remarkable seal of its membranes, demonstrating the elimination of bacterial charges, even after some time on used filters.

Therefore, the validation of the system for biological sterilization of water entering the dental chair is the necessary prerequisite to guarantee the safety of its use, in compliance with current regulations.

The device under examination is a  $0.05 \mu$  ultra filtration system that places a nonporous physical barrier, impermeable to viruses and bacteria, with a feature demonstrating efficiency and duration equal to a year of work (8000 litres) in safety. With this premise, a series of tests were carried out to measure the effectiveness of the filtering barrier against common bacterial contaminants having equal and inferior dimensions to legionella, in order to exclude the possibility of contamination of the water in transit and exiting the filtering system in question.

In order not to alter the results of the microbial counts, the filters in question were preliminarily disinfected and rinsed and then tested with sterile saline water in order to evaluate their perfect initial sterility, including those already used.

A further observation was made regarding the presence inside the filtering system of activated vegetable carbon. This material is generally used to eliminate odours and chlorine from water, as well as to prevent the formation of algae in water circuits, but at the same time collects the bacterial charge beyond the membranes, for which the efficiency test performed on the filters used represents a significant data on the biological safety of the filter.

In light of the results obtained in the two experiments, the total zeroing by the filtering device of the bacterial and viral charges present in the pre-contaminated water, both at medium and high concentration, is fully demonstrated, for which the sterilizing action of this filter against the bacterial and viral colonies, included legionella, is clearly demonstrated. Moreover, this filtering capacity was maintained even on the used filters, demonstrating a considerable resistance in time of the filtering system and is in accordance with the current guidelines from the Italian Center for Disease Control and Prevention<sup>[10]</sup>.

This longevity of the membranes represents a further significant data on its safety and guarantees its efficiency over time. The filter to purify water should be used in dental practic-

es, especially in surgical procedures<sup>[11]</sup>.

From the results obtained it can be concluded that both the new and used filter devices show an absolute filtering capacity towards the *S. Aureus* and *E. coli* microorganisms.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**Ethical Approval:** This article does not contain any study involving humans or animals.

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