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Research Article

Comparison of the ActiDes-Blue and CARELA HYDRO-DES technology for the sanitation of contaminated cooling water systems in dental units

Vergleichende Untersuchung des ActiDes-Blue- und des CARELA HYDRO-DES Verfahrens zur Sanierung kontaminierter Kühlwassersysteme von Dentaleinheiten

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Abstract

Background: The hygienic-microbiological control of 6 dental units being in use for the past 16 years revealed a significantly increased microbial contamination of their cooling water system. In order to comply with the requirements of the drinking water directive ("Trinkwasserverordnung"), the commercially available production system ActiDes, producing on-site ActiDes-Blue which is based on hypochlorous acid (HOCl) and generated by anodic oxidation, was investigated.

Method: Water samples from the 6 contaminated dental units were examined for the total number of colony forming units (cfu), contamination with molds, *L. pneumophila* and *P. aeruginosa*. The control period for the total colony count was 4 weeks (8 samples/unit). The subsequent application phase of the ActiDes-Blue procedure was 6 months (31 samples/unit). Additionally, the redox potential and the pH value were measured.

Futhermore, the decontamination agent CARELA HYDRO-DES, a two component agent based on H_2O_2 with the addition of a mixture of sodium hydrogen sulphate and sulphuric acid in an aqueous solution effective at 0.1% and higher, was applied in a unit that had been put out of service for a month before. Before application, the system was first filled with a 5% solution of the alkaline pre-cleaning agent CARELA Solvent for bacterial slime; the system was left with this solution for 1 h. The pre-cleaning agent was then completely displaced from the system with tap water and a decontaminating solution of 5% CARELA HYDRO-DES and left in place for 1 h.

Results: Drinking water quality level was reached only twice during the control phase. The average values of the dental units ranged between 3,633 CFU/ml and 29,417 c/ml. During the application phase, drinking water level could be achieved in 11 water samples. In another 6 water samples a total colony count of <150 cfu/ml was reached. The average values for the dental units' total colony count ranged between 529 cfu/ml and 87,450 cfu/ml. No significant differences between the control phase and the action phase could be demonstrated.

During the control phase, contamination of the water samples with a mold was noticed so that examinations for molds were carried out beyond the scope of the drinking water directive. For this parameter as well, no significant differences between the phases of the study could be shown.

The *Legionella* load of the dental units was low. *L. pneumophila* were yielded in only 4 out of 130 water samples. During the control phase, twice colony counts at 50 cfu/1,000 ml and 110 cfu/1,000 ml were measured. During the action phase, counts with *Legionella spp*. could be measured at 5 cfu/1,000 ml for one unit only.

Also, with 1–10 cfu/100 ml, the *P. aeruginosa* contamination was low. During the application phase, it ranged between 0–7 cfu/100 ml.
Redox potential and pH value showed a slight decrease during the application phase.
Before treatment with CARELA Solvent and CARELA HYDRO-DES, the initial contamination of the total count of bacterial colonies was 1,432 cfu/ml at 22°C and 846 cfu/ml at 36°C as well as >1,000 cfu/100 ml for molds. 1 h after the decontamination, no bacteria and molds could be detected in 1,000 ml of tap water. Despite the fact that the unit was not used any longer, after 7 d the bacterial colony count was 3 cfu/ml at 22°C and 2 cfu/ml at 36°C while molds could not be detected. Even after a rest time of 14 d only 167 cfu/ml or 42 cfu/ml could be yielded. Molds were further not cultivable. A material damage could not be observed.
Discussion: Pertaining to the ActiDes technology's effectiveness, it has to be pointed out that the dental units investigated were those used for dental students' teaching and therefore were clearly less frequently used than clinically used units in a dental practice. This resulted in distinctly longer stagnation periods which favored formation of biofilms.
Conclusions: In summary, the ActiDes technology and ActiDes-Blue showed not to be sufficiently effective for the sanitation of contaminated water reservoirs in dental units under aggravated conditions of repeated and longer periods of non-use in connection with longer water stagnation periods. In comparison, the biofilm was sustainably eliminated through the combined application of CARELA® Solvent for Bacterial Slime with subsequent decontamination using CARELA® HYDRO-DES.

Keywords: microbial contamination, dental unit, decontamination, anodic oxidation of NaCl, CARELA® Solvent for Bacterial Slime, CARELA® HYDRO-DES, ActiDes, ActiDes-Blue

Zusammenfassung

Hintergrund: Bei der hygienisch-mikrobiologischen Überprüfung von 6 zahnärztlichen Dentaleinheiten, die seit 16 Jahren im Einsatz waren, wurde eine stark erhöhte mikrobielle Belastung des Kühlwassers auffällig. Um die Anforderungen der Trinkwasserverordnung zu erfüllen, wurde als ein handelsübliches Sanierungsverfahren das ActiDes Verfahren installiert, das auf der potentialkontrollierten anodischen Oxidation beruht und als aktives Wirkprodukt ActiDes-Blue erzeugt.
Methode: Wasserproben der 6 kontaminierten Dentaleinheiten wurden auf die Gesamtzahl koloniebildender Einheiten (KbE), Schimmelpilzkontamination, *L. pneumophila* und *P. aeruginosa* untersucht. Die Kontrollphase erstreckte sich für die Gesamtkoloniezahl über 4 Wochen (8 Proben/Einheit). Die anschließende Wirkphase für das ActiDes-Blue-Verfahren erstreckte sich auf knapp 6 Monate (31 Proben/Einheit). Zusätzlich wurden das Redoxpotential und der pH-Wert gemessen.
Zusätzlich wurde das Dekontaminationssystem CARELA HYDRO-DES, ein zwei-Komponenten Mittel auf Basis von H_2O_2 und einer Mischung aus Natrium-Hydrogensulfat und schwefeliger Säure in wässriger Lösung, welche bei Konzentrationen über 0,1% effektiv ist, in einer weiteren Zahnbehandlungseinheit eingesetzt, welche seit 1 Monat außer Betrieb gesetzt wurde. Vor Anwendung von CARELA HYDRO-DES wurden die wasserführenden Leitungen mit 5% CARELA Lösungsmittel für bakteriellen Schleim, einem alkalischen Vorreiniger, befüllt und über 1 Stunde belassen. Der alkalische Reiniger wurde vollständig entfernt und das System mit 5% CARELA HYDRO-DES über 1 Stunde behandelt.
Ergebnisse: In der Kontrollphase wurde nur zweimal Trinkwasserniveau erreicht. Die Mittelwerte der Dentaleinheiten lagen zwischen 3.633 KbE/ml und 29.417 KbE/ml. In der Wirkphase konnte bei 11 Wasser-

proben Trinkwasserniveau nachgewiesen werden. Bei weiteren 6 Wasserproben wurde eine Gesamtkoloniezahl <150 KbE/ml erreicht. Die Mittelwerte der Gesamtkoloniezahl für die Dentaleinheiten lagen zwischen 529 KbE/ml und 87.450 KbE/ml. In der statistischen Auswertung konnten keine signifikanten Unterschiede zwischen Kontroll- und Wirkphase aufgezeigt werden.
Während der Kontrollphase fiel eine Schimmelpilzbelastung der Wasserproben auf, so dass über den Rahmen der Trinkwasserverordnung hinaus auf Schimmelpilze untersucht wurde. Auch bei diesem Parameter war kein signifikanter Unterschied zwischen den Phasen der Studie nachweisbar.
Die *Legionella*-Belastung der Behandlungseinheiten war gering. Nur in 4 von 130 Wasserproben wurden *L. pneumophila* nachgewiesen. Zweimal trat eine Belastung während der Kontrollphase mit 50 KbE/1.000 ml bzw. 110 KbE/1.000 ml auf. Während der Wirkphase konnte nur bei einer Einheit eine *Legionella spp*.-Belastung mit jeweils 5 KbE/1.000 ml nachgewiesen werden.
Auch die *P. aeruginosa*-Belastung war mit 1–10 KbE/100 ml gering. Während der Wirkphase schwankte sie zwischen 0–7 KbE/100 ml.
Beim Redoxpotential und pH-Wert ergab sich ein leichter Abfall während der Wirkphase.
Vor Anwendung mit CARELA Solvent und CARELA HYDRO-DES betrugen die initialen KbE 1.432 KbE/ml bei 22°C und 846 KbE/ml bei 36°C sowie >1.000 KbE/100ml für Schimmelpilze. 1 Stunde nach Anwendung wurden keine Bakterien und Schimmelpilze in 1.000 ml Leitungswasser nachgewiesen. Obwohl die Behandlungseinheit nicht mehr in Verwendung stand, betrugen 7 d danach die Werte für Bakterien 3 KbE/ml bei 22°C und 2 KbE/ml bei 36°C, ohne Nachweis von Schimmelpilzen. Selbst nach 14 Tagen wurden nur 167 KbE/ml Bakterien bzw. 42 KbE/ml nachgewiesen werden. Schimmelpilze konnten weiterhin nicht nachgewiesen werden. Ein Materialschaden trat nicht ein.
Diskussion: Bei der Bewertung der Verfahrenswirksamkeit ist die Einschränkung zu machen, dass es sich um Behandlungseinheiten eines Studentenkurses handelte, die deutlich weniger frequentiert waren als das in der Zahnarztpraxis der Fall ist. Daraus ergaben sich deutlich längere Stagnationszeiten, die eine Biofilmbildung begünstigen.
Schlussfolgerungen: Insgesamt erwies sich das ActiDes Verfahren zur Sanierung des kontaminierten Wasserreservoirs zahnärztlicher Behandlungseinheiten unter den erschwerten Bedingungen wiederholter und längerer Phasen der Nichtbenutzung mit der damit verbundenen Wasserstagnation als nicht ausreichend wirksam.

Schlüsselwörter: mikrobielle Kontamination, Dentaleinheit, Dekontamination, anodische Oxidation von NaCl, CARELA® Bakterienschleimlöser, CARELA® HYDRO-DES, ActiDes, ActiDes-Blue

Introduction

On the occasion of a hygienic-microbial control of dental units being in service since 16 years in an old building of a center for dental and oral medicine (which meanwhile moved into a new building), a highly increased microbial contamination of the cooling water became obvious. In order to comply with the requirements of the drinking water directive (TrinkwV), a practicable and efficient method of restoration had to be established. It is known from literature that simple flushing of the water line is not sufficient to comply with the limits required by the drinking water directive [1], [2]. As a commercially available procedure, classified as environmentally friendly and cost efficient, the ActiDes procedure was therefore chosen and evaluated on this occasion. Its action principle is based on the hypochloric acid production through the potential-controlled anodic oxidation of NaCL, sodium hypochlorite and further radicals that are permanently fed into the pipe network of the installation [3]. The active product ActiDes-Blue is then released into the water line system of dental units. This is important because, even following a successful restoration of the dental unit, new biofilms possibly build up as microorganisms from unrestored pipe sections as well as – in minor quantities – from the public network and from the backlash of the

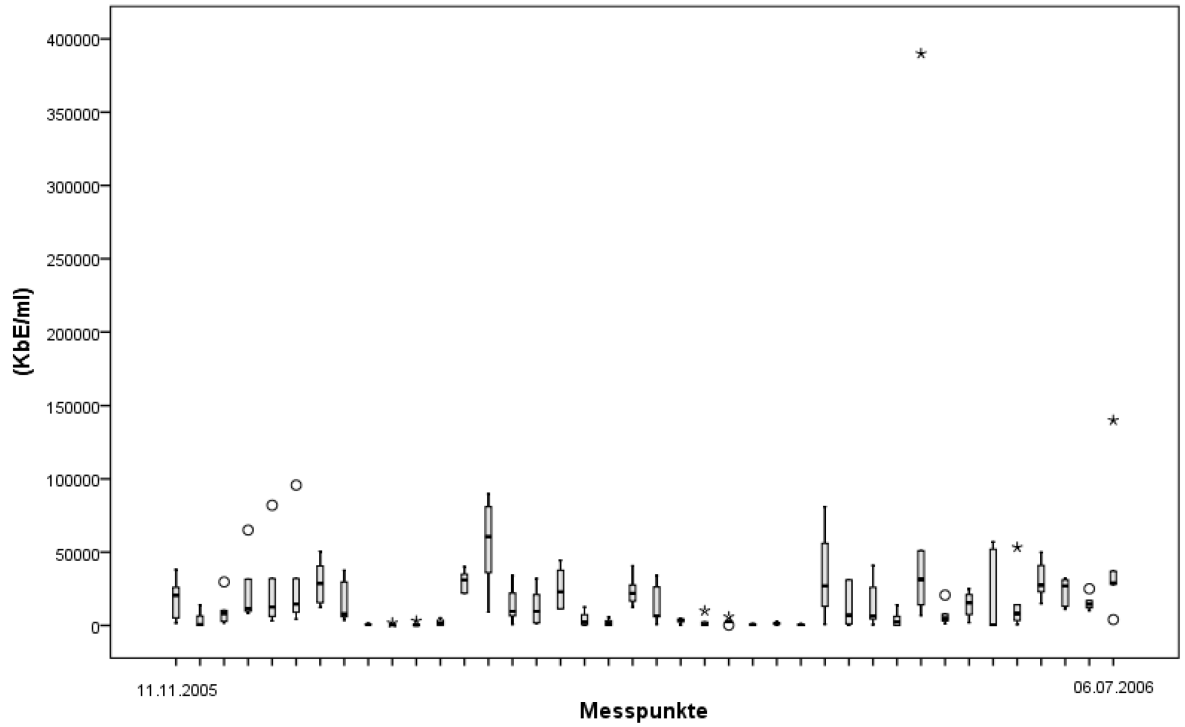

Figure 1: Boxplots (with outliers and extreme values) of the total colony count (cfu/ml) per sampling day during the assessment period

vacuum recovery valve technique can be introduced into the water system of the dental unit. Additionally, an introduction during maintenance and repair works is possible. For the purpose of evaluation of the ActiDes procedure, the decontamination performance in real operating mode was examined over a representative time period.

Methods

The analysis was carried out of 6 dental units that were only used for teaching of students. In these units, about 6 patients were treated over the day on four days of the week, Wednesdays excluded. The control phase was 4 weeks (starting 11/11/2005). During the application phase a dilution of the reactive solution ActiDes-Blue of 1:200 (so-called anolyte) was adjusted in the system for 27 weeks. A first high chlorination was carried out on 02/14/2006 and a second on 05/31/2006 (with a dilution of anolyte of 1:100). During the semester holidays (February), the lines were flushed once a week for 20 minutes using anolyte diluted by 1:100.

The water was drawn from the hose couplings of the two micro motors upon removal of the elbows, from the multifunctional syringe on the dentist side and the assistant side as well as from the mouth glass filling. Mixed samples of 250 and 1,000 ml were produced from each of the 3 samples. Sampling was carried out according to the recommendation of Pitten et al. [4]. The initial contamination was determined on 11/11/2005 (Friday) and afterwards in intervals of 3, 4, 6 and 7 d. After a free interval of 17 d, the contamination was determined again on the following day (Monday) as well as on Tuesday and Thursday of the same week. 7 d later, the ActiDes-Blue procedure was installed (start of the action phase). Further sampling was done after 7, 11, 12, 14, 21, 24, 27, 28, 31, 32, 34, 38, 39, 42 and 45 d. During the following semester holidays, sampling was done every Wednesday over the first 3 weeks, on Thursdays and Tuesdays during the 2 subsequent weeks. 2 samples each were taken in March and April (03/10 and 15, 04/07 and 20), 1 sample in May (05/19) and one sample every Thursday until the last sampling on 07/06.

The evaluation concerned the total colony count (in 250 ml), *P. aeruginosa* (in 1,000 ml), *L. pneumophila* (in 1,000 ml) and molds (in 250 ml) according to the method described in [5]. Furthermore, the redox potential and the pH value were measured.

Results

Total colony count

The total colony count ranged within the normal scope for drinking water in only 13 out the 186 water samples (Figure 1). After stagnation phases such as weekends, holidays or semester holidays, the values increased significantly (individual values/unit and detailed development in [6].

In order to compare the measured values, the Friedmann test for variance by ranks for dependent sample checks was deployed. During this test checks were carried out whether the central tendency of multiple subsequent measures differs significantly [7]. The test has the advantage that the measured values need not be distributed

Table 1: Subdivision of the test periods into 4 phases with the corresponding number of measures during these periods

summarized test period	Period
Pre	11/11/2005–12/08/2005 (prior to ActiDes-Blue application)
Post1	12/15/2005–02/07/2006 (following ActiDes-Blue application)
Post2	02/14/2006–05/19/2006 (following ActiDes-Blue application and 1st high-level chlorination)
Post3	06/08/2006–07/06/2006 (following ActiDes-Blue application and 2nd high-level chlorination)

normally and require ordinal scale level only. In Table 1 the individual test times have been summarized.
As the differences between the dental units were not significant, test were carried out only as to whether the measured values of the units were differing throughout the test times. Therefore, the measured values were categorized (Table 2).

Table 2: Categorizing of the measured values in 10 categories

Category	Total colony count CFU/ml
1	0–100
2	100.01–500
3	500.01–1000
4	1000.01–5000
5	5000.01–10000
6	10000.01–20000
7	20000.01–40000
8	40000.01–60000
9	60000.01–100000
10	100000.1– highest value

Every unit was assigned a ranking for all the test times. If the measured values did not differ, the assignment of each ranking at every measure time was equally likely for every unit, i.e. the ranking of the units would be distributed randomly over the test times [7]. The zero hypothesis of the Friedmann test indicates that the central tendencies do not differ from each other. The alternative hypothesis, however, indicates that at least two central tendencies differ from each other. The descriptive statistics are presented in Table 3, the medium rankings in Table 4 and the statistics for the Friedmann test is given in Table 5. Medium values and standard deviations were determined for the individual measure periods. N indicates the number of the chairs. Minimum and maximum designate the minimum and maximum measure values that were considered. The result of the Friedmann test is shown in Table 5, line “Exact significance”. No significant differences in the total colony count could be found for the different test times, i.e. the rankings were distributed randomly over the test times. The zero hypothesis is therefore to be maintained.

Table 3: Descriptive statistics

	N	Medium value	Standard deviation	Minimum	Maximum
Pre	6	6.00	1.27	5.00	8.00
Post1	6	5.83	0.41	5.00	6.00
Post2	6	6.17	1.17	5.00	8.00
Post3	6	6.83	0.75	6.00	8.00

Table 4: Ranks

	Medium rank
Pre	2.25
Post1	2.00
Post2	2.42
Post3	3.33

Table 5: Statistics for Friedmann test

N	6
Chi-square	4.380
df	3
Asymptotic significance	0.223
Exact significance	0.232
Point probability	0.012

The mold charge differed a lot during the tests (Figure 2). The threshold value of ≤100 cfu/ml [8] recommended by literature was exceeded for 64 out of the 156 measures. The highest individual values were documented prior to the application of ActiDes-Blue. Afterwards as well, values of >500 cfu/ml were reached at several treatment units. As to the mold charge, a wave-like sequence could be observed in analogy to the total colony count of bacteria [9]. The day of commissioning of the ActiDes-Blue procedure a medium value of 4.1 cfu/ml was measured. This increased to 94.2 cfu/ml within one week. After the water stagnation due to the Christmas holidays, the medium value had further increased to 560 cfu/ml, then decreased to <100 cfu/ml and only increased again to 206.3 cfu/ml following the first high chlorination. Following intercurrent variations, the medium values were only found to be below 100 cfu/ml after the second high chlorination.
The evaluation of the mold measures was carried out in analogy to the evaluation of the total colony count with the exception that 3 categories (0–100, >100–500 and >500–1000) were created instead of 10. For the mold contamination, too, no statistical differences could be found between the test times (Table 6, Table 7, Table 8).

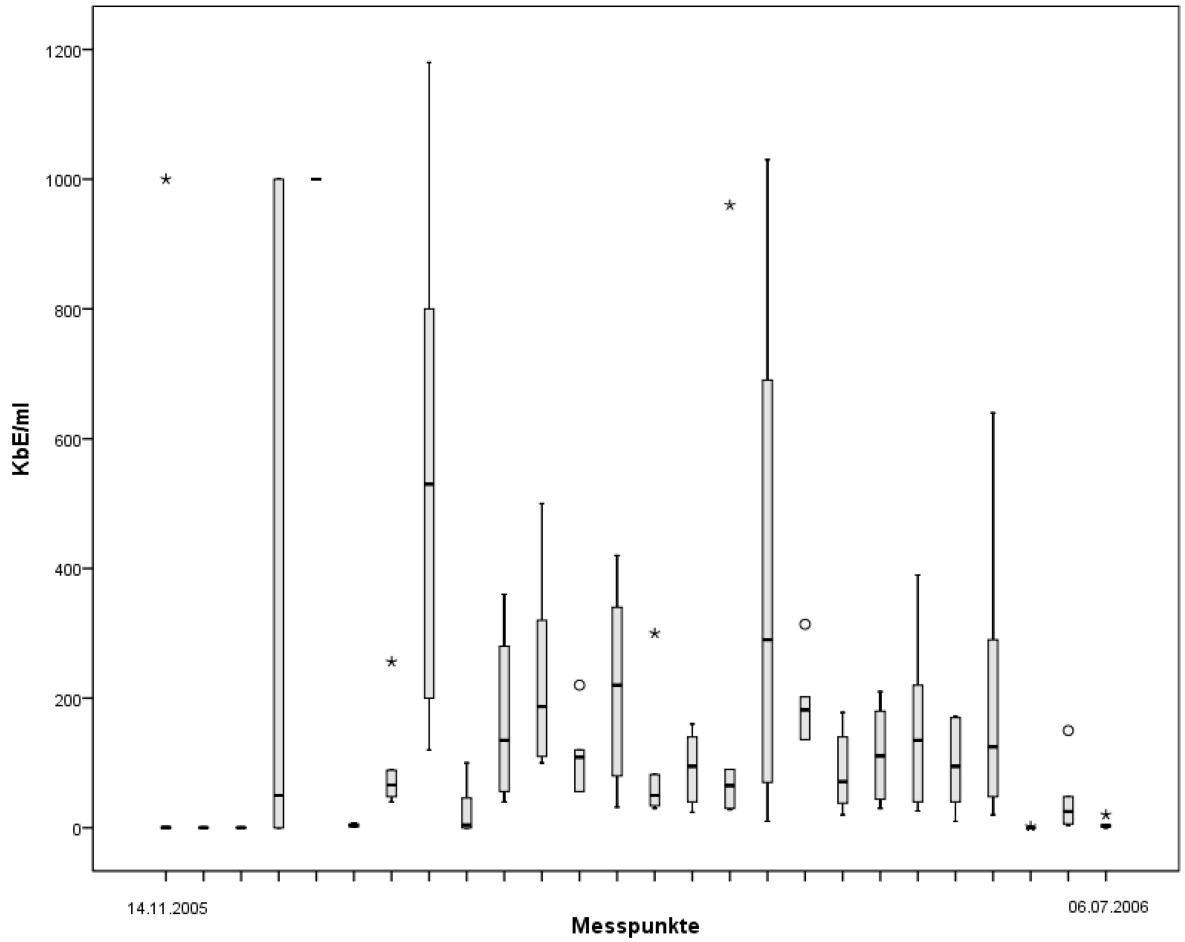

Figure 2: Boxplots (with outliers and extreme values) of the mold colonies for all treatment units (cfu/ml) per sampling day during the assessment period

Table 6: Descriptive statistics

	N	Medium value	Standard deviation	Minimum	Maximum
Pre	6	1.67	0.82	1.00	3.00
Post1	6	1.67	0.52	1.00	2.00
Post2	6	1.67	0.52	1.00	2.00
Post3	6	1.00	0.00	1.00	1.00

Table 7: Ranks

	Medium rank
Pre	2.75
Post1	2.83
Post2	2.83
Post3	1.58

Table 8: Statistics for Friedmann test

N	6
Chi-square	6.568
df	3
Asymptotic significance	0.087
Exact significance	0.090
Point probability	0.017

Legionella pneumophila

During the control phase, 50 and 110 cfu/1,000 ml resp. of *L. pneumophila* were proven at 2 of the 6 units only. After the application of ActiDes-blue one unit became negative, for the other 5 cfu/1,000 ml were proven after 1 and 3 weeks each. Afterwards, this unit was without positive results.

Pseudomonas aeruginosa

For three units, *P. aeruginosa* were proven neither during the control phase nor during the action phase. During the control phase, 5 CFU/100 ml were proven in one unit only. After the application of ActiDes-Blue, *Pseudomonas* spp. were yielded at different points of time, 11 times in total for this unit as well as for 2 further units (1 CFU/100 ml each, only once 2 CFU/100 ml).

Redox potentials and pH values

The medium value of the redox potential was 469.8 mVH during the control phase and 453.5 mVH during the application phase. The medium value of the pH value was 7.32 during the control phase and 7.24 during the application phase

Discussion

Despite the fact that a correlation with water stagnation became obvious for the colony count of the bacteria as well as of the molds the statistical assessment showed no significant differences between the individual measure times. That means that the values have not changed statistically in comparison to the values of the application phase with two high chlorinations. The decrease of redox potential and pH value indicate that biofilm parts were mobilized by ActiDes-Blue so that more active substances were used. The *L. pneumophila* detected in two units were eliminated after the application of the procedure or after the 3rd week of application. This could not be achieved completely for *P. aeruginosa*.

As the sanitation was not successful, the decontamination agent CARELA® HYDRO-DES (CARELA Wassertechnologie GmbH Petershagen, Germany) was applied on 04/26/2007 in a unit that had been put out of service for a month already due to the planned move into a new building. This is a two component agent based on H_2O_2 with the addition of a mixture of sodium hydrogen sulphate and sulphuric acid in an aqueous solution that is effective in concentrations of 0.1% and more [10]. As a precondition to the decontamination, the system was first filled with a 5% solution of the alkaline pre-cleaning agent CARELA® Solvent for bacterial slime that is used to break up slimes (contains sodium hydroxide in an aqueous solution); the system was left with this solution for 1 h. The pre-cleaning agent was then completely displaced from the system with tap water and a decontaminating solution of 5% CARELA® HYDRO-DES with a pH value of 2.0 was introduced and also left in the system for 1 h. Through activation of the dental unit, tap water was afterwards used for flushing until the indicator showed no more rests of active substances. The unit was then left with the remaining water in the system while not being used.

The initial contamination of the total count of bacterial colonies was 1,432 cfu/ml at 22°C and 846 cfu/ml at 36°C as well as >1,000 cfu/100 ml for molds. 1 h after the decontamination no bacteria and molds could be detected in 1,000 ml of tap water. Despite the fact that the unit was not used any more subsequently, after 7 d the bacterial colony count was 3 cfu/ml at 22°C and 2 cfu/ml at 36°C while molds could not be detected. Even after a rest time of 14 d only 167 cfu/ml or 42 cfu/ml could be yielded. Molds were further not cultivable. A material damage could not be observed.

Conclusion

Under the tested conditions, the ActiDes-Blue procedure showed not to be sufficiently effective. Compared to the practice, the application conditions were significantly less favorable though because the dental units reached only 1/5–1/6 of the utilization of a comparable practice. Biofilm formation was further favored by the semester holidays and non-utilization. In opposite, the biofilm was completely eliminated by the combined application of CARELA® Solvent for bacterial slime and subsequent decontamination with CARELA® HYDRO-DES.

Notes

Competing interests

The authors declare that they have no competing interests.

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