

Management of bacteriological quality of dental unit water systems at ACTA Amsterdam

Disinfection of DUW (pre-clinics) using peroxide, Alpron® and Bilpron®.

The Academic Center for Dentistry at Amsterdam (ACTA) is one of the largest Dental Schools in Europe. ACTA educates over 1000 students and treats 600 patients *per day* at more than 200 dental chairs. About 175 students *per year* start their practical dental training at the pre-clinical department. This large facility is situated on the first floor of the 8-story University of Amsterdam dental building at Louwesweg 1, Amsterdam. It consists of two halls A and B, housing 92 and 72 pre-clinical units respectively. Although the individual units are spatially organized in groups of 4, each unit is self supporting, provided with a phantom head, cooling water, rotary instruments, dental light and suction system. All units in A and B were manufactured and installed (in 1997) by KaVo. Municipal Amsterdam drinking water was used for feeding of all clinical and pre-clinical units.

The dental unit water systems (DUWS) of the *clinical* units installed in 1994 are treated with the built-in semi-automatic disinfection system of KaVo, using Oxygenal® fluid as the disinfectant. Oxygenal® contains 6% hydrogen peroxide and silver ions as the active principle. It is pumped at a 1:200 dilution into the incoming water resulting in nominal concentrations of 300 ppm of hydrogen peroxide in the DUW. At the end of each week an "Intensiv" disinfection protocol provides the DUWS with water of higher concentrations of Oxygenal® (i.e. nominal 2500 ppm hydrogen peroxide). According to KaVo, a properly managed KaVo dental unit could provide DUW of good bacteriological quality.

The concentration of hydrogen peroxide in the peripheral DUW can be checked by analytical test strips (Merckoquant® Peroxid-Test). We use test strips with a range of 0-1000 mg/l H₂O₂.

Legislation in the Netherlands asks for a risk analysis for buildings that supply water that may be aerosolized. If its occupants are exposed to the aerosol, measures are to be taken to control the bacteriological quality and minimize the risk of *Legionella* exposure.

Monitoring the total amount of cultivable aerobic bacteria per ml of peripheral DUW by a plate count technique (see appendix) and using the H₂O₂ test strips, we could show that very good correlations exist between the numbers of bacteria and the concentration of peroxide. Drinking water quality i.e. no more than 200 bacteria per ml (< 200 CFU/ml) was found when the peroxide concentration was consistently at 300 ppm.

Most DUW showing peroxide values of 0-100 ppm had bacteria in high numbers (5000 - 100.000 CFU/ml). This was found at ACTA as well as in several general dental practices in the Netherlands (unpublished results).

Untreated DUW or DUW from units with failing disinfection systems generally show bacteria in the range of 20.000 - >100.000 CFU/ml. This is not only the experience at ACTA or with KaVo units, but is a phenomenon encountered with most makes of units virtually worldwide.

DUWS at the pre-clinical department

Pre-clinical units (KaVo make, installed 1997) were fed by municipal water. The internal water system consists of relatively simple and short (2- 2.5 meters) narrow bore plastic tubes. In contrast to the clinical units no disinfection system was installed for these pre-clinical DUWS.

It was found that after a year of use, the numbers of bacteria in the DUW were high. In contrast, numbers of bacteria at the local tap water facilities in halls A en B were very low, generally <10 CFU/ml.

In June 1999 the median number of bacteria in 14 ad random sampled airrotor line waters of hall A was 12.500 CFU/ml (range 2200 - >100.000). Tap water in Hall A had < 100 CFU/ml.

Again, from samples taken in November 1999 several DUWS with >100.000 CFU/ml were found.

It was deduced that high numbers of bacteria in the DUW caused a high concentration of bacteria in the aerosol. The aerosol in fully occupied halls A & B emerging from some 90 and 70 units could, potentially, harm students and staff, given the frequent and substantial exposures. Especially staff and students presenting with respiratory illness or allergic symptoms could be vulnerable to these bacterial aerosols.

ACTA is a large and prominent Dental School. Proper sterilization, disinfection and hygiene are considered essential for the protection of patients, staff and students.

Also, in pre-clinical situations, students and staff should not be at risk.

It was decided to disinfect the pre-clinical DUWSs.

A central disinfection system for the feed water to the ACTA pre-clinical units could, theoretically, be one of the solutions. After some consideration, this was deemed to produce uncertain results, and was technically demanding or impossible indeed.

As there had been many successful (experimental, practical, and commercial) applications of disinfection of DUWSs by way of self contained water systems, it was decided to replace the municipal water feed system of the units with self contained DUWSs. All 164 units of halls A & B were fitted with a 1.5 liter transparent plastic bottle, pressurized with air from the unit, to deliver water of choice, containing any disinfectant. Installation and rebuilding of the DUWSs was completed in May 2000.

Disinfection of pre-clinical DUWS with Peroxide

From the 22nd of May 2000 onwards, students were asked to add Oxygenal® fluid 1:200 to fresh (tap) water in the bottles. This accounts for 0.03% (= 300 ppm) hydrogen peroxide, similar to the concentration in the KaVo DUWS of the clinical units. As an example, data from a single group of four units monitored for total counts in the effluent from the 3-in-1 syringe are shown below, before and after the disinfection regime.

unit	CFU/ml			
	May 19 (day -3)	May 31 (day 9)	June 7 (day 16)	July 6 (day 45)
501	>100.000	<300	400	2000
502	16.000	>100.000	>100.000	500
503	50.000	800	<100	100
504	50.000	11.000	<100	200

These and similar data (q.v.) account for the observation that units, also as parts within groups of four, now have independent water systems (i.e. self contained water systems by means of flask - or bottle feeding).

As we know that properly treated and Oxygenal® disinfected water normally contains <1000 CFU/ml, often <100 CFU/ml, we presumed that compliance with protocol was poor or erratic (see units 502 and 504). This was confirmed by staff members observing students and hearing complaints like "tiresome" or "cumbersome" protocols; "not my task"; "why bother"; "oh, forgotten" and so on. I have to admit that the facilities for filling bottles and dosing disinfectant were, indeed, not optimal and could have been improved. During busy weeks of practice, students use 4 or more liters of water per day (considerable more than the amount used in clinical units) adding to the task of refilling bottles and dosing disinfectant.

Urea Hydrogen peroxide

Because of logistic reasons, spilling, staining of floors, neglect, relatively poor compliance with protocol and also of costs for the 164 units with heavy use of unit water, we decided to replace the Oxygenal® fluid by hydrogen peroxide in the form of a solid : a urea hydrogen peroxide compound. This compound, known as Hydrogen Peroxide Urea Adduct, or Carbamide Peroxide (FLUKA product nr. 95307) comes in tablets of 1 gram that contain ca 30% of hydrogen peroxide, set free when dissolved in water.

Adding a single tablet to water in the bottle of the unit appeared simple and, presumably, could solve part of the spilling – and compliance problems. The urea peroxide disinfection period started end of the year 2000.

Although we had the impression that compliance with protocol was slightly better than during the Oxygenal® period, samples taken at random from the 3-in-1 syringe lines during the year 2001 showed that the results of the tablet protocol were disappointing. Several times, but in only 5-10% of samples, CFU values <200 were found while >100.000 occurred in another 5-10% of the samples. Median values on several widely scattered sampling occasions in 2001 and 2002 were generally 10.000 – 25.000 .

So, although low CFU values were scarce, the fact that low values were consistently found could be interpreted as circumstantial evidence that the disinfection regime, given adherence to protocol, *could* be appropriate. *From data using Urea Peroxide at general practitioner's units we know that it is – in fact - an effective DUW disinfectant.*

From consideration of factors jeopardizing effective disinfection in our preclinics it appears that: 1) Students are not consistently allocated to a given unit, but use several units, depending on time-tables, type of schooling, etcetera. This could mean that no strict responsibility is felt for maintenance and protocol. 2) Some units, erratically positioned in the hall, are used only infrequently, are in repair, etcetera. Standing idle means that the waterlines grow biofilm easily. 3) Long periods of inactivity during holidays, and periods of low activity due to time-tables frustrate regular flows of disinfected water. 4) Students seem to forget or neglect addition of tablets to the water 5) Bottles that happen to contain plain water and are left at the unit for some time grow bacteria in high numbers that colonize the waterlines easily and probably persistently.

Alpron® and Bilpron®

Alpron® and Bilpron® are disinfectants for dental unit water systems marketed by Alpro Dental-Produkte GMBH, St.Georgen, Germany.

Alpron® is a fluid concentrate on the basis of tosylchloramide (p-toluene sulfonchloramide) for addition to dental unit water. The concentrate may be pumped automatically 1:100 into the incoming water or dosed by hand to bottles for self contained DUWSs. There is ample evidence for successful application of Alpron® at general practitioner's DUWSs.

Bilpron® is a relatively new DUWS disinfectant to be used undiluted in a so called Weekend System. At the end of a week, the DUW is replaced by the (blue) Bilpron® fluid that remains in the DUWS to disinfect its contents. The active anti-microbial compounds are polyhexamethylene biguanide and p-hydroxybenzoic acid esters; EDTA serves as a calcium chelating agent. After the weekend period, the Bilpron® disinfectant is flushed out and replaced by fresh municipal water or water from an alternative source. An advantage of this principle is that plain water is used during the whole working-week.

Once (or a few times) a year the dentist should dissolve or remove the biofilm in the DUWS by means of a "biofilm removing set" This BRS is based on the principle of dissolving or removing the biofilm by application and flushing of highly alkaline hypochlorite followed by citric acid and a detergent as a pH-shock treatment. The manufacturer (i.e. Alpro) claims that, after use of their BRS and subsequent use of

Alpron® or the Bilpron® Weekend System, the DUW will be of excellent microbiological quality.

After demonstration of the BRS protocol at the pre-clinical units by staff of Alpro, we tested units subsequently treated with Alpron®. After two weeks of use, 11 out of 12 Alpron® units had CFU's/ml <100 ; one had CFU/ml = 300. We concluded that the BRS protocol could be effective for previously contaminated DUWSs.

In addition to the presumed advantage of plain water (opposed to water containing a disinfectant) as DUW for every day use, we reasoned that the Weekend System had potential for better compliance with protocol. By mutual arrangement with Alpro (Mr Helmes and Mr Gnatowski) we started a test with the aim of comparing Alpron® with Bilpron® and with Urea peroxide disinfection of pre-clinical units at ACTA. The trial started September 2003. In an effort to reduce non-compliance with the respective disinfection protocols we asked staff members to closely supervise students assigned to the experimental units.

First the BRS procedure was done on 30 units. Then the Alpron disinfection, Bilpron weekend disinfection protocol, and Urea peroxide disinfection were instituted at 8, 8, and 14 units respectively. One, 3, and 6 months after BRS, CFU's/ml were determined:

Table 1 median CFU/ml at 1, 3 and 6 months*

	1	3	6
Alpron	5000	1800	3600
Bilpron	1400	3450	600
Urea peroxide	10.500	11.000	20.000

* full data and ranges available.

It appeared that the Bilpron Weekend System performed (slightly) better than Alpron. Urea peroxide CFU's figures were high. Factors that complicated interpretation of the results were several experimental units that were in repair, had broken down, or had stood idle for some reason during a unknown period. Although compliance with protocols seemed good, daily or weekly attendance seemed to vary during the experiment. Therefore, statistical treatment of the data was considered inappropriate.

The advantages of the Bilpron® system urged us to consider a large scale trial. From April 2003 onwards, the Bilpron Weekend System was instituted at all 164 pre-clinical units. Groups of students (the corvee groups, supervised by staff) had the duty and responsibility for the weekend protocol. CFU's were determined in June, July and August 2003: Table 2.

Table 2 CFU/ml from units treated with Bilpron® protocol

2003	number	median	range
June 23	N=18	8500	10 - 130.000
July 9	N=25	40.000	<100 - >100.000
August 6	N= 8	100.000	$2 \cdot 10^4$ - $>10^6$

These puzzling and disappointing high numbers of CFU's were then explained by the phenomenon that not the DUWS proper, but the water within most of the *source bottles* was highly contaminated. It turned out that the students had found a more convenient and economical way of filling the DUWS with Bilpron. They used just a few bottles filled with Bilpron as a vehicle to replace the water in all units. Most bottles, containing amounts of (old) plain water were left for use next week. After several weeks, the contents of these bottles - that had never contained disinfectant - were (and remained), as a matter of course, contaminated.

From then on, all bottles were disinfected each week by rinsing with 0.1 % sodium hypochlorite.

Biofilms were then removed on August 20; monitoring of CFU's started August 22

Table 3 ----- CFU/ml -----

date	N	median	range	%<1000*	%>10.000**
22-08-2003	9	<100	<100 – 1700	89	0
01-10-2003	11	<100	<100 – 10.000	73	0
08-10-2003	15	1300	<100 – $1.4 \cdot 10^4$	33	13
07-11-2003	22	2900	<100 - $>10^5$	32	27
02-12-2003	18	1950	<100 - $>10^5$	45	33
18-12-2003	24	7000	1400 - $>10^5$	0	38

* Percentage of units that has DUW of less than CFU/ml = 1000. (CFU <1000 is arbitrarily considered of good microbiological quality)

** Percentage of units with DUW that contains $>10^4$ CFU/ml.

From the above data it is obvious that the median values and the % DUW $>10^4$ increased with time. DUWs of good quality decreased in number. As before, it is not clear whether this was due to fading compliance, free Fridays that frustrate the carrying out of protocol, or other factors. It appears that the situation at the ACTA pre-clinics calls for biofilm removal at least 3 or 4 times a year.

The next biofilm removing treatment of all units was done at February 12, 2004. Weekly Bilpron treatment was continued. Samples for determination of CFU/ml were taken as seen in table 4 :

Table 4 ----- CFU/ml -----

date	N	median	range	%<1000	%>10.000
18-02-2004	24	<150	<100 - >10 ⁵	75	13
10-03-2004	22	1650	<100 - 7.10 ⁴	36	21
18-05-2004	12	1700	100 - >10 ⁵	33	25
28-05-2004	biofilm treatment				
22-06-2004	24	650	<100 – 2.10 ⁴	58	8

We conclude that :

- 1) Untreated DUWSs contain large numbers of bacteria. This applies to clinical as well as pre-clinical units.
- 2) It is feasible to disinfect dental unit water *via* self contained water systems by adding peroxide preparations (e.g. Oxygenal® ; Urea peroxide); chloramide compounds (e.g. Alpron®) or biguanide/hydroxybenzoic acid esters (e.g. Bilpron®).
- 3) Adherence to daily and/or weekly protocol appears essential for maintenance of the anticipated and proper disinfection effects.
- 4) The source of DUW bacteria, i.e. the microbial biofilm adhering to the internal surfaces of the DUW lines, can be removed or diminished by treatment with appropriate agents. This facilitates effective action of daily or weekly applied disinfectants.
- 5) Adherence to protocol seems best with the Bilpron® Weekend System.
- 6) At the pre-clinical department of ACTA the better disinfectant effect was found for Bilpron®. Superior performance over Oxygenal®, Urea peroxide and Alpron® was observed.
- 7) Applying the Bilpron® system, the dental unit water and its aerosols are free of disinfectant compounds during the working week.
- 8) The Bilpron® Weekend system, supported by regular appliance of a biofilm removing set, is perceived as one of the best systems for dental unit water disinfection up to now.

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September 2004

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Appendix

Samples of DUW, drinking water or process water are surface plated onto HALF strength Plate Count Agar (e.g. Oxoid CM325 or Difco Bacto Plate Count Agar). Formula: Tryptone 2.5 gram; Yeast Extract 1.2 g; Glucose 0.5 g and Agar 12 g per liter TAP water. Sterilization at 121 °C, 15 min.

Optimal incubation 7 days at 30 °C.

Total heterotrophic aerobic counts are expressed as Colony Forming Units per ml (CFU/ml)

According to most Western Countries, drinking-water should have a total heterotrophic count of < 200 CFU/ml or < 100 CFU/ml. The American Dental Association as well as the CDC (Centers for Disease Control and Prevention) advocate use of dental unit water that contain < 100 CFU/ml.

Water (potable water) for human use in general should contain << 10.000 CFU/ml, preferably < 1000 CFU/ml.

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