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# Comparison of Methods to Enumerate Bacteria in Dental Unit Water Lines

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**Abstract.** Millipore HPC samplers are simple, self-contained test devices that can be used by personnel in dental offices who do not have microbiologic training to easily and economically monitor dental unit water quality without laboratory support. This study evaluated the correlation of HPC samplers to R2A agar for enumerating planktonic bacteria in dental unit treatment water. Eight different dental units were sampled. Five replicates were performed for each media at each dilution. The Pearson correlation coefficient between the R2A agar and HPC sampler is 0.89. These data suggest HPC samplers correlate with conventional laboratory-based R2A culture techniques for determining dental unit water line contamination.

Virtually all untreated dental unit water systems (DUWS) are colonized with aquatic biofilms that contaminate the water used for coolants and irrigants to levels as high as 160 million colony-forming units per milliliter (CFU/ml) [5, 12]. Even newly installed units may exhibit greater than 200,000 CFU/ml within 5 days of installation [2]. The American Dental Association (ADA) has proposed that technology be developed which will improve the quality of dental treatment water (DTW) to less than 200 CFU/ml by the year 2000. The ADA statement on water lines also calls for the development of methods to monitor the quality of dental treatment water [1]. To this date, no practical and cost-effective in-office method to regularly assess dental treatment water quality has been widely accepted.

Over a period of more than 30 years, a wide variety of different media have been used in attempts to enumerate DTW organisms [7]. Reports in literature indicate a wide range of culture media, temperatures, and incubation times have been used for culturing heterotrophic mesophilic bacteria. These organisms thrive in an aerobic, nutrient-poor, room-temperature environment. This environment is inconsistent with nutrient-rich media such as blood agars, heart-brain infusion, or trypticase soy agar. Incubation time and temperature should reflect the normal environment of these predominantly slow-growing organisms. Generally, the nutrient-poor media, with longer incubation times and 20–28°C temperatures yield higher CFU/ml [4, 9]. A test frequently used to monitor water quality is the heterotrophic plate count (Method 9215) described in Eaton et al. [4]. The use of this method requires specialized equipment and training. Agar plates must be refrigerated and have a short shelf-life. These limitations make this method impractical in the dental office.

HPC samplers are disposable, self-contained testing devices consisting of an 18-ml plastic vial into which a plastic paddle with an inner layer of nutrient medium covered with a membrane filter is inserted. The entire assembly is individually wrapped to maintain sterility during storage. The media is designed to accurately absorb 1 ml of sample, so that the number of colonies enumerated equals the actual number of CFU/ml [6].

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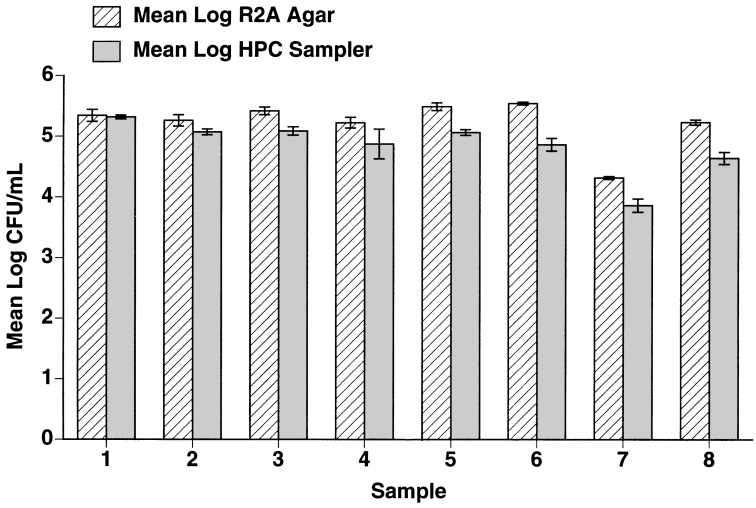


Fig. 1. Comparison of R2A agar versus HPC sampler.

These devices are simple to use, have their own built-in grid system, and require no laboratory support to obtain results. This makes them ideal for an in-office monitoring system for dental unit water.

The purpose of this study was to evaluate the correlation between HPC samplers and R2A agar plates in measuring planktonic bacteria from dental treatment water in dental units that are not undergoing treatment for controlling or removing biofilm.

### Materials and Methods

Eight water samples were taken from the air/water syringe (AWS) of 25-year-old A-dec (A-dec, Inc., Newberg, OR) dental units in daily clinical use. The dental units were attached to a chlorinated municipal water supply; none of the units had been previously treated for removal of biofilm. The dental units had been in use for varying lengths of time prior to the water being sampled. No attempt was made to flush the lines before sampling. Dental units currently being treated for water quality were not used due to the high frequency in which these units would deliver water with no recoverable bacteria. After removal of the AWS tip, a 100-ml water sample was aseptically collected in a sterile container with sodium thiosulfate to bind free chlorine and thereby neutralize its antimicrobial activity. Immediately after sampling it was brought to the laboratory area for processing. The sample was vortexed and serial dilutions were made to 1:10, 1:100, 1:1,000, and 1:10,000 with sterile buffered phosphate diluent.

Each dilution was then cultured as follows: five HPC samplers were each filled with sample to the manufacturer's mark, the media paddle placed inside for a timed period of 30 s, the water poured out of the container, the paddle shake-dried, and the paddle reinserted. The water sample was again vortexed and a 500-µl sample was spread-plated on each of five R2A agar plates. The agar samples were then inverted and incubated. The HPC samplers were incubated with the membrane facing down. Each of the eight water samples was processed in the manner described above within 1 h of collection. Negative controls were three of each of the following combinations: (1) unused R2A agar plates; (2) R2A agar plates with sterile phosphate buffered diluent; and (3) HPC samplers with sterile phosphate buffered diluent. All plates and HPC samplers were incubated at 24°C for 7 days. Plates and samplers were removed and counted manually with a Quebec Counter. This procedure was repeated for each of the eight samples. The number of counted colonies was recorded and converted to CFU/ml. The dilution of each sample that yielded the highest number of countable colonies was converted to $\log_{10}$. The Pearson correlation coefficient was calculated. The Pearson correlation coefficient can vary from 0 to 1.0, with 0 representing no correlation and 1.0 representing perfect correlation. Statistical texts consider a Pearson correlation coefficient of over 0.75 to be good to excellent [3].

## Results

For all negative controls, there were no recoverable colonies. In each sample, the number of colonies recovered from HPC samplers was less than R2A agar, but within 0.5 log (Fig. 1). The Pearson correlation coefficient of 0.89 was calculated for R2A agar and the HPC sampler.

## Discussion

Almost all studies of dental unit water systems have demonstrated unacceptably high levels of bacterial contamination, regardless of whether the units have independent reservoirs or are connected to municipal water [11]. Unless separate water systems are combined with other interventions such as periodic chemical treatment, there may be little to be gained by monitoring these units since the likelihood of untreated systems providing acceptable water is remote [8, 10, 12].

The success of dental water line treatment protocols has been shown to be technique-sensitive and dependent on conscientious compliance by dental health care workers [8]. Regardless of the methods used to treat DTW, it is imperative that some form of monitoring be used to ensure that protocols are followed. While more traditional microbiological methods of monitoring have been

demonstrated to be effective in recovering heterotrophic mesophilic organisms, their use as an in-office method is impractical. The HPC sampler devices are designed to produce readable biologic results from undiluted water samples when colony counts are below 200 CFU/ml [6]. This is ideally suited for monitoring DTW for compliance with the ADA goal of less than 200 CFU/ml.

Dental units are normally supplied with municipal water, sterile water, or freshly distilled/pasteurized water. The ADA goal of bacterial contamination not exceeding 200 CFU/mL of mesophilic heterotrophic bacteria is only a quantitative measure with no qualitative component. The dental practitioner should only need to monitor staff compliance of manufacturer-validated dental unit maintenance directions. The recovery of heterotrophic mesophilic bacteria, which make up the main population of aquatic biofilms, should provide adequate indication compliance. The use of in-office monitoring devices that are inexpensive and simple to use should encourage the adoption of a regular monitoring program for dental unit water.

Correlation coefficients from this study indicate a strong correlation between HPC samplers and traditional R2A culture techniques for quantitating heterotrophic mesophilic bacteria from DTW. Although the HPC samplers tend to underestimate true counts, it is a chairside test that does not require the services of a microbiological laboratory. There is no pipette measuring, no dilutions, no preparation of storage of media plates, and no special techniques to learn.

## Conclusion

These results suggest that dental water contamination as measured with HPC samplers correlate with R2A agar results. It may be possible to use the HPC sampler as a simple in-office method for clinical monitoring of dental unit water treatment protocols.

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