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JAMES W. McDOWELL, DARYL S. PAULSON and JOHN A. MITCHELL

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A simulated-use evaluation of a strategy for preventing biofilm formation in dental unit waterlines

JAMES W. McDOWELL, B.S.; DARYL S. PAULSON, Ph.D.; JOHN A. MITCHELL, Ph.D.

ABSTRACT

Background. Prevention of biofilm formation is important in the maintenance of dental unit waterline systems. Without effective control measures, the waterlines will become contaminated with routine use.

Methods. The authors used a simulated-use dental unit waterline system to evaluate the ability of a test product, A-dec ICX (A-dec, Newburg, Ore.), to prevent biofilm formation. They evaluated buffered distilled water and hard water models versus mixed-challenge suspensions of *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Results. The authors documented development of significant biofilm in untreated test units, while treated test units showed no indication of biofilm formation throughout the 16-week study. Student *t* tests and 95 percent confidence intervals performed on the plate count data confirmed that untreated test units had significantly greater bacterial populations than did treated test units ($P < .05$). Qualitative images by scanning electron microscopy verified these findings.

Conclusion. In this simulated clinical-use study, the test product effectively reduced bacterial counts in incoming water and produced water quality exceeding stated recommendations of the American Dental Association.

Clinical Implications. The test product provides an approach to dental unit waterline maintenance that is simple to use and that, by continuously preventing biofilm formation, reduces concerns about bacterial contamination in dental unit water.

Dental unit waterline contamination can pose a serious threat of infection to patients. Such contamination is the result of biofilms that adhere to the inner surfaces of the lines. Biofilms consist of bacterial cells immobilized in an organic polymer matrix that often is highly resistant to removal. The biofilm protects the bacteria both from being washed away by the water flow and from many types of antimicrobial water treatment.[1,2]

A new product provides an approach to dental unit waterline maintenance that may reduce concerns about bacterial contamination in dental unit water.

In 1995, the American Dental Association Board of Trustees adopted a statement on dental unit waterlines directing industry and the research community to take an "ambitious and aggressive course" to ensure the delivery of quality treatment water to patients.[3] Before the statement's publication, research findings revealing unacceptable bacterial contamination in dental unit water were becoming common.[4-7] In subsequent years, the dental profession developed a common understanding that dental unit waterlines will become contaminated if no control measures are applied[8]; as a consequence, during that time numerous products were introduced that addressed the dental unit waterline biofilm issue with some measure of success. Beyond the concern for efficacy, however, most of these products have issues of material incompatibility with dental equipment and are difficult or costly for the practitioner to use.[9]

Methods intended to improve the microbiological quality of dental unit water can be classified as preventive or remedial. For instance, microfiltration of the outflow of a dental unit water system is a remedial method. With disregard to the contamination upstream, these filters are designed to "catch" con-

DISCLOSURE
This study was supported by A-dec, Newburg, Ore., manufacturer of the biofilm prevention product described in this article.

tamination before exposing the patient. On the contrary, however, the ADA statement indicated that bacteria should be controlled in the "unfiltered output" of the dental unit because the Association recognized the need for systemic control of bacteria throughout the dental unit water system, not just at one point such as that provided by a filter.[7]

Another remedial method commonly used for controlling the quality of dental unit water is periodic "shock treatment" of the waterlines with an aggressive chemical, such as sodium hypochlorite or certain commercial products distributed for this purpose. Because such shock treatments are intended to eliminate contamination once it has developed on the internal surfaces of waterlines, their "clean-up" approach is a remedial method. The success of these treatment regimens is highly dependent on the quality of the water that is supplied daily to the system, because there typically is no residual protection against deterioration of water quality between applications. In addition, they generally are not convenient to apply, and their effectiveness often depends on administrative compliance measures. Furthermore, the shocking process, by its nature, is chemically aggressive, thereby resulting in increased potential for damage to the dental unit water system.

Ideally, a treatment process would prevent the development of biofilm contamination of the water system, be easily performed and offer continuous protection.

Ideally, a treatment process would prevent the development of biofilm contamination of the water system, be easily performed and offer continuous protection, thereby eliminating the root cause of poor dental unit water quality. Also, the process should provide continued efficacy during periods of nonuse, such as overnight and weekends. A treatment process exhibiting these attributes would be much easier to explain to patients and easier for practitioners to manage than remedial treatment processes.

A proactive approach is taken by a new product, A-dec ICX waterline tablets (A-dec, Newburg, Ore.), an effervescent tablet that is added directly to the dental unit water bottle at each refill. The product contains multiple active ingredients, including sodium percarbonate, silver nitrate and cationic surfactants, to provide both immediate and sustained residual protection against biofilm formation. The ingredients in ICX are regarded as safe for human consumption based on the incidental ingestion model accepted by the U.S. Food and Drug Administration for premarket clearance, consistent with generally recognized as safe ingestion models.

In this article, we describe our evaluation of the ICX system for its ability to prevent biofilm formation. We addressed several research questions in this study:

- How effective is the test product in preventing biofilm/microbial growth in dental unit waterlines during an extended period of simulated use?
- Are there any significant differences in treatment effects between the handpiece water coolant, or HPWC, lines and the air-water syringe, or AWS, line?
- Does the test product maintain acceptable water quality throughout the week and after weekends?
- Is the test product's effectiveness affected by water hardness?

This study simulated conditions in dental unit waterlines over a period of approximately four months. Our basic study design involved creating conditions that promoted growth of biofilms in dental unit water systems, using bacteria common in dental unit water and the environment. The test product was used to treat the test units, and effluent bacterial counts were tracked in both test and control (untreated) test units. In addition, we used scanning electron microscopy, or SEM, to inspect internal tubing surfaces for biofilm formation.

An added objective of this study was the validation of a laboratory test method for reproducibly assessing the efficacy of proposed treatment processes. Other published studies attempting to replicate dental unit biofilm formation in the laboratory generally have described inadequate models that are not well-suited to evaluating preventive treatment approaches. The test apparatus and procedures used in this study accurately reproduced the dental unit water flow typically observed with use of a dental unit.

MATERIALS AND METHODS

To re-create dental unit waterline conditions accurately and precisely over time, we used a series of 10 automated dental unit water systems (test units). Each of the 10 independent test units

contained all of the components of a typical water delivery system that included a water bottle, polyurethane supply tubing, a control system, three high-speed HPWC lines and one AWS line. (We used A-dec 500 [A-dec] components in the test units.) An electronic controller operated the test unit waterlines intermittently to simulate daily dental unit usage, using approximately 60 milliliters per simulated patient, a volume consistent with published clinical data.[10] The test program consisted of 10 simulated patient treatment cycles per day and a flushing of all waterlines at the start of each day and after each patient, as recommended by the Centers for Disease Control and Prevention.[11] We added the test product, A-dec ICX, to the water reservoir bottle at each refill. The test product does not contain any chlorine compounds associated with the production of trihalomethanes, a class of compounds that includes a number of suspected carcinogens.[12]

We divided the 10 test units into two groups of five, treating three of the test units in each group with the test product and using the remaining two as the untreated controls. In one group of five units, we filled the water bottles with distilled water buffered with a 1:100 dilution of phosphate-buffered saline. In the other group, we filled the bottles with identically buffered distilled water in which water hardness was adjusted to at least 200 milligrams per liter as calcium carbonate, or $CaCO_3$. We selected the hardness level as being representative of typical U.S. hard municipal water, based on a citation that 94 percent of the 100 largest cities in the United States were found to have a water hardness of less than 200 mg/L as $CaCO_3$.[13] We will refer to these solutions as "buffered distilled water" and "hard water," respectively. We inoculated the solutions used to fill the water bottles of the test units with a pooled challenge suspension of *Klebsiella pneumoniae* (American Type Culture Collection, or ATCC, no. 4352), *Pseudomonas aeruginosa* (ATCC no. 15442) and *Staphylococcus aureus* (ATCC no. 6538) to a level of approximately 10^2 to 10^3 colony-forming units, or CFU, per milliliter. This inoculum level was shown to result reliably in the development of a biofilm (J.W. McDowell, B.S.; D.S. Paulson, Ph.D.; J.A. Mitchell, Ph.D., unpublished data, July 2003) and approximates the 500 CFU/mL maximum heterotrophic plate count allowable by the U.S. Environmental Protection Agency's National Primary Drinking Water Regulations. We then secured the inoculated bottles on the test units and pressurized the test units for the daily (five-days-per-week) controller cycle.

The test program consisted of 10 simulated patient treatment cycles per day and a flushing of all waterlines at the start of each day and after each patient, as recommended by the Centers for Disease Control and Prevention.

We performed bacterial sampling from the HPWC lines of each of the test units on the first working day of each week before we refilled the water bottles. We again sampled the test unit HPWC lines after daily refilling and operation of the controller cycle for at least 15 minutes. Finally, on the last working day of the week, we sampled the HPWC lines and the AWS line after four hours of controller cycle operation. Before doing any sampling, we disinfected the outer surfaces of the HPWC and AWS water outlets by swabbing them with 70 percent isopropyl alcohol, air-drying them for at least 30 seconds and flushing them for at least two seconds. For each test unit, we sampled each of the three HPWC lines, and we pooled equal volumes from each for a composite HPWC sample. We plated the single weekly sample from the AWS line separately. We implemented a reduced sampling sequence comprising only composite HPWC lines before preparation on the first day of each week and after four hours of operation on the last working day for weeks 13, 14 and 15 of the 16-week study.

We prepared all samples for analysis using a validated neutralization process that included sodium thiosulfate and sodium thioglycolate in the diluting and plating media, to eliminate any remaining antimicrobial activity. We performed serial 10-fold dilutions and spread-plated them on R2A agar with neutralizers. We inverted the plates and incubated them at 20 to 25 C. After incubation, we enumerated colonies on the plates and characterized the microorganisms on the basis of morphology either as an expected type of the challenge species or as exogenous to the system.

At the conclusion of weeks 10 and 16, we randomly selected samples of waterline tubing, two HPWC lines and one AWS line from each test group (that is, the untreated control group and treated group for each water type), and excised

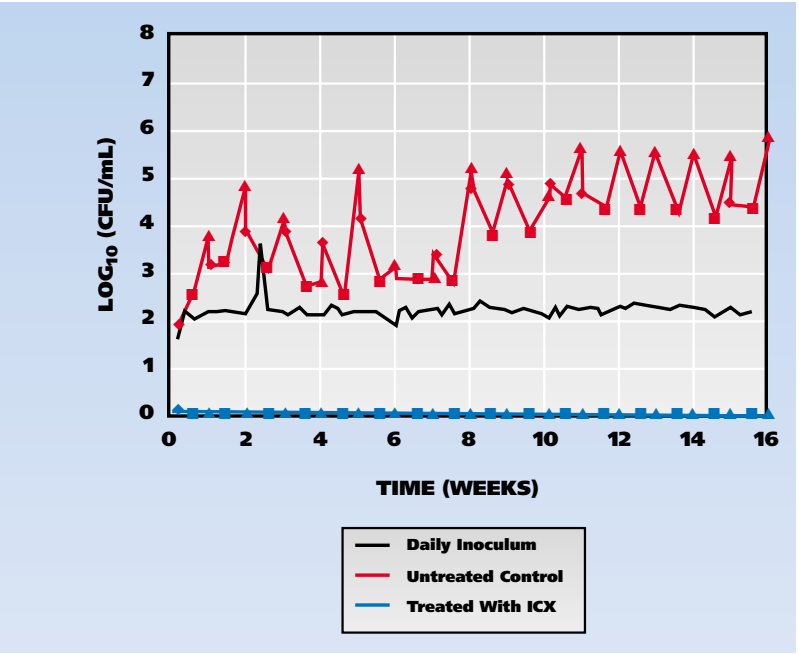

Figure 1. Bacteria levels in test units supplied with inoculated buffered distilled water over 16 weeks. For reference, the daily inoculation level also is shown. The untreated control group became contaminated rapidly, while the test product reduced the incoming bacteria levels to near zero for the duration of the study. CFU/mL: Colony-forming units per milliliter. ICX: A-dec ICX waterline tablets (A-dec, Newberg, Ore.).

them for SEM analysis. We purged the selected waterlines with air before aseptically removing a 2- to 3-centimeter section 50 cm from the outlet end of the HPWC or AWS line. We then spliced the lines together for continued operation. We fixed and stored the SEM samples in a 2.5 percent glutaraldehyde solution. In preparation for sputter coating, we removed the samples from the fixing solution, rinsed them in water, sliced them longitudinally and allowed them to air-dry for no more than two hours. We performed sputter coating with gold/palladium and SEM analysis at the Image and Chemical Analysis Laboratory at Montana State University in Bozeman on the samples from week 10 and week 16.

RESULTS

The populations of the daily inoculum—a mean population of 198 CFU/mL (2.30 $\log_{10}$/mL) for the buffered distilled water test group and a mean population of 165 CFU/mL (2.22 $\log_{10}$/mL) for the hard water test group—were consistent over the course of the study. The level of water hardness was maintained within the range of 200 to 252 mg/L as $CaCO_3$ during the course of the study.

Biofilm development in the untreated control test units, regardless of water type, produced effluent counts that rapidly exceeded those of the inoculum population. Three of the four untreated controls achieved greater than 4.0 $\log_{10}$/mL by the beginning of the third week, while the fourth untreated control did not reach greater than 4.0 $\log_{10}$/mL until the eighth week. However, the lagging untreated control had exogenous microorganisms in the effluent water samples that were excluded from the total plate count until the ninth week.

In contrast to the control group results, water samples from the six treated test units showed no appreciable bacterial counts—less than 3 CFU/mL—during the 16-week period of study. Figures 1 and 2 show the progression of mean plate counts in the effluent water from the test groups using buffered distilled water and hard water, respectively. For reference, the daily inoculum level also is shown. There is a one-day gap in the data plotted for the treated HPWC sample before daily use at day 14 in both Figures 1 and 2, because no zero-dilution plates were prepared on that day.

Water samples from the six treated test units showed no appreciable bacterial counts during the 16-week period of study.

We performed a series of two-sample Student *t* tests ($P = .05$) on data from samples taken during weeks 9 through 16, and we generated 95 percent confidence intervals. Because the control units supplied with hard water produced significantly higher bacterial populations than did those supplied with buffered distilled water, we evaluated the two groups of five test units separately. Regardless of group, however, comparative statistical analyses yielded the same outcomes. In both groups, the untreated control units produced significantly greater populations ($P < .05$) of bacteria than did treated units, which, in fact, were essentially free of viable bacteria. Among the treated units of both groups, there were no significant differences between bacterial populations attributable to variables such as the weekday on which or time of day at which samples were taken, or whether samples were from HPWC or AWS lines. Among samples from

the untreated control units of both groups, populations did not differ significantly on the basis of source (HPWC or AWS line), but did differ on the basis of time variables. That is, populations in HPWC samples taken before the refilling of the bottles on the first day of the week (Monday after a weekend) were significantly greater ($P < .05$) than populations in samples taken 15 minutes after recharging, which, in turn, were significantly greater ($P < .05$) than populations in the four-hour sample taken on the last day of the week (Friday).

SEM analysis corroborated the plate count results. In both the week 10 and week 16 SEM images, a proliferation of bacterial colonization was apparent in all of the untreated test unit samples, while we observed no bacterial colonization in any of the specimens from the treated test units. Figures 3 and 4 are representative SEM images of samples from the treated and untreated test units, respectively.

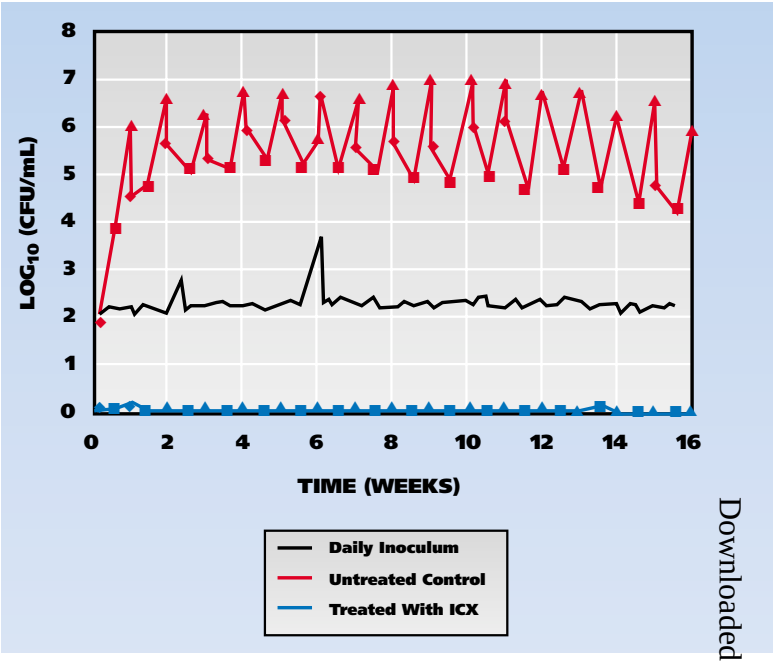

Figure 2. Plate count results for the test units supplied with hard water. For reference, the daily inoculation level also is shown. The results are similar to those for the buffered distilled water group, except that the level of contamination in the untreated control group was greater with hard water. CFU/mL: Colony-forming units per milliliter. ICX: A-dec ICX waterline tablets (A-dec, Newberg, Ore.).

DISCUSSION

Considerable attention was directed toward making the test system representative of actual dental units in clinical service. Each test unit accurately re-created a dental unit water system. We modeled the pattern of use after typical daily clinical use, and we selected clinically relevant bacterial challenge species. One simplification in this laboratory test design to be noted is that we introduced the challenge species exclusively in the water added to the supply bottle, while in the clinical setting there exists at least a theoretical possibility that contamination also could occur at the outlets of the waterlines. Sterilization of handpieces and syringe tips subsequent to each patient treatment, use of nonretracting dental units and compliance with CDC recommendations to flush after each patient[11] all are measures that substantially reduce this risk in the clinical setting.

Each test unit accurately re-created a dental unit water system. We modeled the pattern of use after typical daily clinical use, and we selected clinically relevant bacterial challenge species.

We chose the challenge species to represent gram-positive *(S. aureus)* and gram-negative *(K. pneumoniae* and *P. aeruginosa)* microorganisms common in the environment. *S. aureus* is associated with skin and mucous membranes and is isolated from food products, dust and water, including dental unit water.[14] *P. aeruginosa* and *K. pneumoniae* are microorganisms that produce glycocalices involved in biofilm formation and, reportedly, are common biofilm contaminants in dental unit water systems.[5,14,15] We added a dilute buffer to the challenge water to minimize the possibility of incidentally lysing the challenge bacteria owing to changes in osmotic pressure.

The water used to create the buffered distilled water and hard water was nonsterilized steam-distilled water. This resulted in the introduction of exogenous bacteria to all test units and a more diverse colonization of the untreated control test units in the buffered distilled water group. We identified *Brevundimonas vesicularis* and *Sphingomonas paucimobilis* on the basis of colony morphologies, but our analysis for exogenous species was not exhaustive.

We considered the study's first eight weeks a conditioning period during which test unit performance was stabilized. We conducted statistical analyses using the total plate counts from all

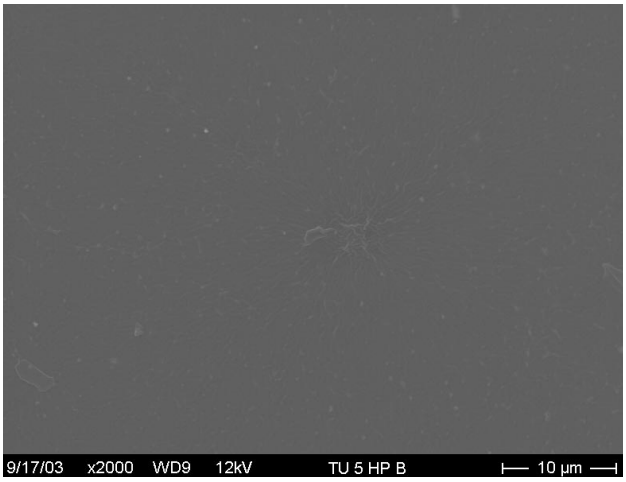

Figure 3. This scanning electron micrograph (×2,000 magnification) is typical of the surface of a handpiece water coolant line after 10 weeks of daily exposure to buffered distilled water inoculated with 10^2 to 10^3 colony-forming units per milliliter and treated with A-dec ICX waterline tablets (A-dec, Newberg, Ore.). The SEM analysis revealed no indication of microbial contamination in any of the test units treated with the test product.

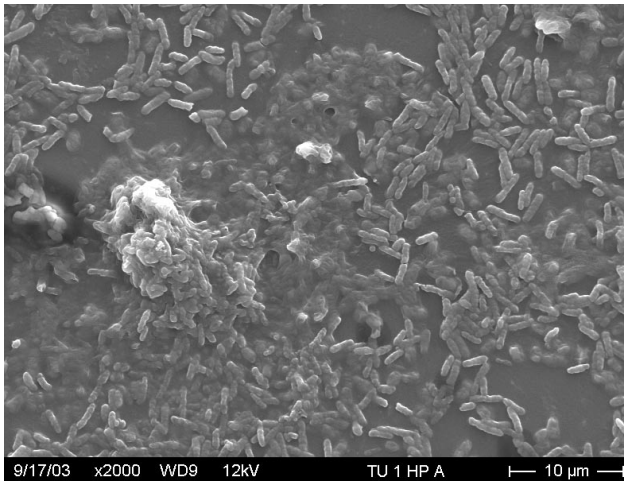

Figure 4. This scanning electron micrograph (×2,000 magnification) is typical of the surface of a handpiece water coolant line after 10 weeks of daily exposure to buffered distilled water inoculated with 10^2 to 10^3 colony-forming units per milliliter and not treated with the test product (that is, a waterline from the control group). Numerous microorganisms in an established biofilm matrix are evident.

units from the ninth through the 16th week. The data support consideration of a shorter conditioning period, but because we did not include exogenous organisms—which eventually composed a significant portion of the effluent counts from some of the test units—in plate count enumeration prior to week 9, we performed statistical analyses on data accumulated from that point. The spread-plate results from treated test units consistently were less than 3 CFU/mL, demonstrating that the test product was capable of reducing the level of contamination in the daily challenge water and maintaining high water quality throughout the dental unit at all times. After 16 weeks, there was still no indication that a "breakthrough" in or escalation of effluent plate counts might be forthcoming (Figures 1 and 2). This finding was reinforced by an SEM analysis that revealed no evidence of biofilm formation or colonization by any microorganisms in the treated test units (Figure 3). On the other hand, the effluent counts from the untreated test units clearly portrayed a developing biofilm, which was plainly revealed by the SEM analysis (Figure 4). Visual examinations and SEM analyses did not suggest any corrosion of or material damage to the treated test units.

Statistical analyses of the plate count data revealed significant differences between bacterial populations from the treated and untreated test units for all waterlines, times of day and weekdays sampled. Populations in samples from the untreated test units declined with increasing time of operation through the day and the week. At no time, however, did flushing alone reduce the effluent count to the level of less than 200 CFU/mL recommended by the ADA.[1] In fact, populations were often 100 to 1,000 times greater than this recommended level. The plate counts observed in the untreated control units were consistent with the range reported in many studies on the microbiological water quality of dental units in actual clinical service.[3,5,15-17]

CONCLUSION

In the presence of a bacterial challenge of 100 to 1,000 CFU/mL in the incoming water, A-dec ICX effectively prevented the development of biofilm and maintained water quality at a level consistently well below 200 CFU/mL at both high and low water-hardness levels under conditions that replicate clinical use. Persistence of the inhibition was observed during periods of inactivity typical of clinical practice. During the 16-week course of study, there was no "breakthrough" of microorganisms in the effluent samples from treated units, nor did we observe adherent microorganisms in SEM analyses of excised waterlines. On the other hand, the untreated controls developed extensive biofilm, resulting in contaminated water at levels consistent with clinical findings. This study did not seek to evaluate whether daily

use of A-dec ICX might reduce or eliminate the importance of flushing after each patient, as recommended by the CDC. Further study examining this question may be of interest.

This research demonstrates that A-dec ICX effectively controlled bacterial contamination in dental unit waterlines and prevented biofilm buildup during daily use and over weekend periods of inactivity, thereby meeting the ADA's recommended goal for dental unit water quality. Following CDC recommendations and dispensing one tablet into the supply water bottle before daily operation achieved proactive biofilm prevention in dental unit water supplied in this study. While more studies are warranted to continue investigation of the product's efficacy in a variety of end-user conditions, this study provides evidence that A-dec ICX offers dental practitioners promise for a convenient and effective way to maintain clean dental unit waterlines. ■

Mr. McDowell is the manager of clinical laboratories, Bioscience Laboratories, 300 N. Willson Ave., Suite 1, Bozeman, Mont. 59715, e-mail "jmcdowell@biosciencelabs.com". Address reprint requests to Mr. McDowell.

Dr. Paulson is president and chief executive officer, Bioscience Laboratories, Bozeman, Mont.

Dr. Mitchell is director of quality assurance, Bioscience Laboratories, Bozeman, Mont.

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