MEASURING THE VALIDITY OF SEVERAL DENTAL UNIT WATERLINE TEST METHODS AND THE EFFECTIVENESS OF A CONTINUOUS AND INTERMITTENT IODINE-BASED CLEANER

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LIST OF ABBREVIATIONS

ADA	American Dental Association
ANOVA	Analysis of variance
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming unit
DHCP	Dental health care personnel
DUWLs	Dental unit waterlines
EPA	Environmental Protection Agency
HPC	Heterotrophic plate count
ML	Milliliter

A. ABSTRACT

MEASURING THE VALIDITY OF SEVERAL DENTAL UNIT WATERLINE TEST METHODS AND THE EFFECTIVENESS OF A CONTINUOUS AND INTERMITTENT *IODINE-BASED CLEANER*

Objective: To determine the validity of two in-office water test kits compared to R2A agar (gold standard), and to measure the effectiveness of two iodine dental unit waterline cleaners.

Methods: Over a twelve-week period, nine dental units from a dental school were monitored. Three dental units were randomly assigned to three treatment groups: controls, use of an intermittent iodine-based cleaner, and use of a continuous iodine-based cleaner. Each unit was equipped with an independent water reservoir. A total of 351 split samples were collected, cultured via the three test methods, manually counted, and validity was assessed using two different cut-off values: ≤ 200 colony-forming units per milliliter [American Dental Association (ADA) goal], and ≤ 500 colony-forming units per milliliter [Centers for Disease Control and Prevention (CDC) recommendation, and Environmental Protection Agency (EPA) mandate].

Results: Both in-office test kits showed marginal validity compared to R2A agar. Also, results failed to demonstrate the effectiveness of two iodine products to consistently meet recommended water sanitation standards.

Conclusions: The in-office test kits underestimated bacteria levels, producing inaccurate measures of bacterial levels compared to the gold standard. Also, both the continuous and intermittent iodine chemical agents displayed ineffectiveness in reducing microbial contamination to both the ADA recommendations or CDC/EPA guidelines. The data suggests this could result in a lack of compliance with either recommendation for water quality in dental unit waterlines.

B. NAME OF PROJECT

MEASURING THE VALIDITY OF SEVERAL DENTAL UNIT WATERLINE TEST METHODS AND THE EFFECTIVENESS OF A CONTINUOUS AND INTERMITTENT IODINE-BASED CLEANER

C. APPLICANT'S ROLE IN PROJECT

This project was originated by the applicant Dr. Joseph A. Bartoloni. The applicant conducted the study, analyzed the data, and prepared the final report. Study design and statistical consultation was provided by Dr. Nuala Porteous and Ms. Lea Ann Zarzabal.

D. STATEMENT OF PURPOSE

The purpose of this project was threefold: (1) to determine the validity of two in-office water testing kits compared to a spread plate bacterial culturing technique using R2A agar (gold standard), (2) to evaluate the effectiveness of a continuous and an intermittent iodine-based dental unit waterline cleaner to reduce heterotrophic bacteria in dental treatment water to less than or equal to 200 colony-forming units per milliliter, and less than or equal to 500 colony-forming units per milliliter, and (3) to improve the methodology of test methods for dental unit waterline contamination.

E. BACKGROUND AND REVIEW OF THE LITERATURE

During the past two decades infection control practices have become a significant part of dentistry. The basis of dental infection control is to create and maintain a safe clinical environment to eliminate or reduce disease transmission between patients and dental health care personnel (DHCP). Recently, an emerging issue in dental infection control has surfaced; reducing the exposure of patients and dental staff to microbes present in dental treatment water (i.e., non-sterile water used for dental therapeutic purposes).

It has long been recognized that dental treatment water delivered by dental unit

waterlines (DUWLs) can be contaminated by microorganisms originating from the water supply (1-6). Studies have shown that dental treatment water can be contaminated with levels up to one million microorganisms per milliliter of water. DUWLs provide a large quantity of water via a network of small-bore tubing to the dental handpieces, air-water syringe, and the ultrasonic scaler. The water is used for irrigation, cooling of dental burs, and for oral rinsing. The microorganisms found in dental treatment water vary with the geographic location, and include fungi, amoebae, protozoa, nematodes, as well as saprophytic and opportunistic gram-negative bacteria (7). A majority of the detected microbes are of very low pathogenicity or are opportunistic pathogens that cause harmful infections under special conditions or in immuncompromised patients (8). In particular, studies have shown that DUWLs harbor small numbers of opportunistic pathogens responsible for respiratory disease, namely *Pseudomonas aeruginosa* (9, 10), *Legionella spp.* (11-15), and non-tuberculous *Mycobacterium spp.* (16), which can infect both patients and DHCP. Recently, two researchers (17, 18) have found significant levels of endotoxin derived from the cell walls of gram-negative bacteria in DUWLs. These endotoxins have been shown to cause localized inflammation and fever.

Patients undergoing dental treatment may be infected by DUWL contamination via one of four ways: hematogenous spread during surgical procedures, local mucosal contact (oral or conjunctival), ingestion or inhalation. DHCP are mainly exposed through inhalation. Several studies have addressed the issue of DHCPs chronic exposure to DUWL contamination (19-21).

Dental units are either connected to municipal distribution systems for potable water or are fitted with an independent water reservoir (i.e., separate container used to hold and supply water). Both systems are subject to contamination. Depending upon infection control practices, waterborne, free-floating (i.e., planktonic) microorganisms flow through the dental tubing, and

can settle on the inner tubing surface, initiating a chain of events resulting in colonization, microcolony formation, and eventually biofilm development (22). Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces (23). The bacterial biofilms consist of microcolonies on a surface, and within these microcolonies the bacteria have developed into organized communities with functional heterogenicity. Biofilms constitute a protected mode of growth that allows survival in a hostile environment. These structures have been shown to be 500 times more resistant to antibacterial agents then isolated colonies, and are the causes of many persistent and chronic bacterial infections in patients undergoing a range of medical procedures (24). Singh (25) found that bioflim in DUWLs harbor a vast diversity of viable organisms including 55 cultivated biofilm isolates. Biofilm has been shown to be the primary source of contamination in dental treatment water (7, 26, 27)

Although the majority of biofilm microbes originate from the public water supply and, in general, do not pose a risk of disease for healthy dental patients, individuals with weakened immune systems may be prone to infection from these same organisms (28). To date no disease transmission arising from DUWL microbial contamination has been conclusively documented, but there is irrefutable scientific evidence that dental treatment water is of poor microbiological quality, and often would fail to meet United States drinking water standards (29-31).

The Environmental Protection Agency (EPA) is responsible for establishing national drinking water standards. This organization has mandated a standard for potable water in community water systems of 500 colony-forming units per milliliter (CFU/mL) or less for noncoliform bacteria (32). Also, advisory organizations and professional associations have issued various recommendations for dental treatment water. In 1993, the Centers for Disease Control and Prevention (CDC) recommended to dental offices the installation and maintenance

of antiretraction valves on dental units to limit retraction of contaminated fluids from the operating environment, and the flushing of units at the beginning of the day and between patients (33). This group also mentioned that only sterile irrigants should be used for surgical procedures, and published new infection control guidelines in 2003 recommending that coolant/irrigant water used for nonsurgical dental procedures should be as low as reasonably achievable, at a minimum $\leq$ 500 CFU/mL (34). In 1995, the American Dental Association (ADA) developed the ADA Statement on Dental Unit Waterlines. This statement urged increased efforts by researchers and dental manufacturers to improve the design of dental equipment to reliably deliver dental treatment water of 200 CFU/mL or less of heterotrophic, mesophilic bacteria in unfiltered output water (30). This ceiling level was derived from the standard set by the Association for the Advancement of Medical Instrumentation for water quality in hemodialysis units (28). Also, the Organization for Safety and Asepsis Procedures has issued a Position Paper regarding DUWLs which identifies practical recommendations for clinicians (35). Pankhurst (36) has developed a risk assessment protocol to analyze the hazards from biofilm microbes in DUWLs on the respiratory health of the dental team and patients.

A number of methods to control or eliminate DUWL contamination have been evaluated. Currently available technologies include: independent reservoirs, chemical treatment, sterile water delivery systems, filtration and a combination of these methods. An independent reservoir, also called a separate bottle system or clean water system, isolates the dental unit from municipal water, and allows the use of water whose initial microbiological quality is known. It does not, however, produce sterile water at the port of exit. It also permits the delivery of chemical agents to clean the DUWLs. Chemical treatment is a standard procedure for treating both drinking and recreational water in this country. Several dental unit manufacturers of independent reservoirs

have recommended specific chemical treatment regimens for use with their equipment. Treatment can be continuous or intermittent. To be effective, chemical treatment must be performed on a regular schedule to control or eliminate biofilms (37). Many chemical agents have been studied including chlorine compounds (38-42), glutaraldehyde (43, 44), alcohol (45), chlorhexidine (46, 47), hydrogen peroxide (48, 49), commercial mouthrinses (50, 51), and iodine compounds (52, 53). Sterile water delivery systems utilize single-use disposable or autoclavable tubing to bypass the dental unit providing sterile irrigating solutions directly to dental handpieces. Filtration uses membranes to trap microorganisms near the point of use. Presently a national standard for products intended to improve the quality of dental treatment water is being developed. This standard will address efficacy, biosafety and compatibility of various chemical agents with dental equipment and materials (54).

When choosing one of the above technologies to address DUWL contamination, it is imperative to monitor the results periodically via established test methods (34). Monitoring is a process used to evaluate the effectiveness of products or protocols designed to improve the quality of dental treatment water. It is used by the clinical staff to monitor compliance with the manufacturer instructions, and to assess the in-use performance of a manufacturer-validated device or protocol. Noncompliance and technique errors are the most common reasons for clinical failure that can be identified by a monitoring procedure. The ADA (55) has suggested that test methods for monitoring should be consistent with Method 9215 in the *Standard Methods for the Examination of Water and Wastewater* (56). Monitoring can be accomplished using a microbiological laboratory or by using an in-office test kit designed to measure the quantity of heterotrophic bacteria.

Method 9215, also called the heterotrophic plate count (HPC), can be determined by pour plate, spread plate, or membrane filter methods. The HPC provides an approximation of the number of viable bacteria and so yields useful information about water quality (56). The most common culture media used to grow and measure heterotrophic bacteria from water samples include R2A agar, HPC agar, and plate count agar. The R2A agar is not as nutrient rich as HPC or plate count agar, but is well suited for enumeration of bacteria that grow in low-nutrient environments such as drinking water (57). Reasoner (58) found that R2A medium yielded significantly higher bacterial counts than did plate count agar, and recommended incubating at 28 degrees C for 5 to 7 days. Williams (59) showed that a low-nutrient medium with reduced incubation temperatures (25 or 30 degrees C) recovered greater numbers of bacterial colony forming units than on enriched media of blood agar or trypticase soy agar. Linde (60) showed that microbiologic surveillance of hemodialysis fluids could be performed more precisely with R2A media combined with room temperature incubation for 10 days.

Comparing different DUWL studies has indicated validity problems of the various test methods in terms of bacterial CFU counts. This can be due to different water systems, different dental units (61), and different kinds of culture media and incubation conditions to determine bacterial load (62). Noce (63) found that time and temperature selected for plate incubation could dramatically affect study interpretations and conclusions concerning the clinical acceptability of water exiting DUWLs. Most heterotrophic bacteria thrive in an aerobic, nutrient-poor, room-temperature environment, and incubation time and temperature should reflect the normal environment of these predominantly slow-growing organisms (64). Most microbiologists believe using a low-nutrient medium like R2A with incubation temperatures of 20 to 25 degrees C, and an incubation period of at least 7 days yields the highest total bacterial

numbers when evaluating waterborne bacteria (63). Today, R2A agar is considered the gold standard for measuring heterotrophic bacteria in water.

Karpay (64, 65) has found that an in office test kit (Millipore HPC Samplr) was accurate, and correlated well with the R2A agar, while Smith (66) determined that this device resulted in an underestimation of bacteria in DUWLs compared to R2A agar.

F. DESCRIPTION OF THE PROJECT

This project was designed to determine if two chairside water testing kits measured bacterial levels as accurately as the gold standard. Secondly, the cleaning ability of two iodine products was investigated to evaluate the ability to reduce bacterial contamination to recommended levels.

G. PROCEDURES AND METHODS

Over a twelve-week period, nine dental units located in a dental school were monitored. All dental units were equipped with independent reservoirs (A-dec). Tap water from the dental operatory sink was used as the source water to fill the independent reservoirs. The following steps were utilized in collecting the water samples. The sink faucet was flushed for one minute prior to dispensing water into the independent reservoir, and attaching to the dental unit. All dental treatment water outlets were flushed for 30 seconds followed by cleaning with an alcohol pad. From each individual unit, pooled twenty (20) milliliter water samples were collected from five outlets (high-speed handpiece, low-speed handpiece, two air-water syringes, and ultrasonic scaler), and placed into a 100 milliliter sterile collection bottle containing sodium thiosulfate (Idexx Corporation) to neutralize any residual halogen present. Water samples were taken at baseline and once per week for 12 weeks. All samples were collected on Monday afternoons before initiation of chemical treatment on Wednesday mornings for the intermittent use product.

All the dental units were in use during Monday mornings. Three dental units were randomly assigned to three treatment groups:

Group 1: Independent reservoirs were filled with tap water only (controls). No further treatment was performed on these units.

Group 2: Same treatment as Group 1, with the addition that on Wednesday mornings, a solution of Dentacide (intermittent iodine-based DUWL cleaner) was placed in the independent reservoir and flushed through five outlets until the lines were filled with the solution. The solution was left in the lines for four hours and flushed with tap water for one minute prior to patient treatment on Wednesday afternoons.

Group 3: A Dentapure DP-40 cartridge (continuous iodine-based DUWL cleaner) was installed after baseline water sampling, and replaced every thirty days as per manufacturer instructions. No further treatment was performed on these units.

Enumeration of bacteria was performed by three techniques: two in-office test kits (Millipore HPC Samplers, Clearline Water Test Kit) that are currently available on the market, and a spread plate technique using R2A agar. Part of the water samples were processed directly without dilution using the in-office devices according to the methods described by each manufacturer. The configuration of the in-office devices permits the draw-through of 1 milliliter of sample to affix microorganisms to the filter surface for subsequent culturing, allowing for direct counts of bacteria after incubation.

The remainder of the water samples was immediately taken to the laboratory. Ten fold serial dilutions in phosphate buffer were made (10^{-1}, 10^{-2}, 10^{-3}), and each vortexed for 15 seconds. One-tenth of a milliliter of each dilution was plated on the R2A agar in triplicate using the spread plate method. All samples were incubated at 22 to 28 degrees C for 7 days.

Also, tap water from the dental operatory sinks was tested weekly using R2A agar. The water faucets were flushed for one minute and the water samples were collected and processed as discussed above. Any bacterial counts exceeding the EPA mandate for potable water was immediately retested to ensure levels were below 500 CFU/mL throughout the study period.

After incubation, the colonies were counted manually using magnification. The bacterial counts of each in-office kit, and the triplicate platings including correction for dilution made for the R2A agar, were calculated weekly. The exact counts were converted to $\log_{10}$ equivalents to achieve a symmetrical distribution resembling the normal distribution curve. A geometric mean was determined for each of the three treatment methods. Validity was assessed by measuring the sensitivity, specificity, predictive values, and accuracy of the two in-office test kits using two different cut-off values ($\leq$ 200 CFU/mL and $\leq$ 500 CFU/mL). Also, an analysis of variance (ANOVA) was conducted on the three treatment methods using the mean of the $\log_{10}$ values for each week to determine if bacterial counts differed significantly between the groups. Significant differences between individual treatments were investigated using the Tukey's test. Significance of differences was assumed at $p < 0.05$.

H. FINDINGS

Bacterial counts for the operatory sink tap water ranged from 0 CFU/mL to 1080 CFU/mL for the study period. Ninety-three percent of these water samples met EPA standards for potable water. The remaining 7 percent of samples were immediately rechecked, and found to be within the EPA guidelines. A total of 351 split samples for dental treatment water were collected, and cultured via the three test methods. Validity measurements are displayed in Tables 1 and 2 for both the ADA goal and the CDC recommendation/EPA mandate, respectively.

Results indicate that both in-office test kits had low sensitivity, high specificity, variable positive predictive value, low negative predictive value, and widely ranging accuracy for both cut-off values. This indicates that both tests did poorly in identifying water samples with high bacterial levels, but did relatively well in identifying water samples with low bacterial levels. Also, when both tests indicated high bacterial levels, a majority of the water sample results for the Millipore HPC Samplers were confirmed by the R2A agar, but only half of the Clearline samples agreed with the gold standard at $\leq$ 500 CFU/mL. However, when both tests indicated low bacterial levels, results were contradictory with the R2A agar a majority of the time. Overall, both devices underestimated the microbial levels throughout the study period leading to inaccurate measures of bacterial levels compared to R2A agar producing marginal validity.

Tables 3, 4, 5 show the CFU/mL for the three treatment methods utilizing the three test methods. Values highlighted in red indicate bacterial levels that meet the ADA goal of $\leq$ 200 CFU/mL, while values in yellow indicate the remainder of samples that met the CDC recommendation/EPA mandate of $\leq$ 500 CFU/mL.

The gold standard R2A agar was used to determine the effectiveness of the three treatment methods. Using Dentacide as the treatment method, only 25 percent and 44 percent of the water samples met the ADA goal and CDC recommendation/EPA mandate respectively. Using the Dentapure DP-40 only 11 percent and 28 percent met the ADA goal and CDC recommendation/EPA mandate. For the controls, the values were 3 percent and 8 percent accordingly. Overall, the geometric mean values were: 9,478 for the controls, 1,262 for the Dentapure DP-40, and 709 for Dentacide. Using an ANOVA (Appendix 1), significant differences were found between the three treatment methods (P=0.0037). Using the Tukey's Test, no differences were found between the Dentapure DP-40 and the Dentacide, but both were

significantly different from the control units (Appendix 2). Although both treatment methods reduced bacterial levels compared to the controls, both treatment methods failed to consistently meet the ADA or CDC/EPA recommended levels.

I. DISCUSSION

With increased emphasis on water quality standards as part of improved infection control in dentistry, monitoring should become part of an established, effective quality assurance program. The monitoring of DUWLs should be practical, cost-effective, easy to implement/interpret, and be fully integrated into quality assurance office practices. When properly administered, monitoring should yield high-quality data. An effective program must control all factors from sample collection, to processing, culturing, and data reporting.

The methods for recovering microbes from DUWLs have changed over time. Methods have evolved from nutrient-rich media with short, and high-temperature incubation periods, to the use of nutrient-poor media with long, and room-temperature incubation periods. Dental practices have two means of monitoring DUWLs; shipping samples to a microbiology laboratory or using an in-office test kit. The in-office test kits are designed to measure bacterial colonies from undiluted water samples, with incubation at room temperature for 7 days. These devices seek to eliminate the need for an incubator, and do not require special packaging, handling or shipment of samples to a laboratory, potentially simplifying procedures for dental offices.

Only two studies have been conducted comparing the Millipore HPC Samplers to R2A agar, and to date there are no validity studies regarding the Clearline Water Test Kit. Karpay (65) showed that the Millipore HPC Samplers agreed with R2A agar 92.6 percent of the time. However, he stated that the Millipore HPC Samplers generally underestimated colony counts when compared to R2A agar, and the results of his study confirmed the overall superiority of

R2A agar spread plating techniques. Also, Smith (66) showed that some bacteria failed to grow on the HPC Sampler compared to R2A agar resulting in reduced microbial counts.

The validity results of this study showed that test measurement methods have a dramatic effect on the HPC values. Both the Millipore HPC Samplers and the Clearline Water Test Kits consistently underestimated bacterial levels compared to the gold standard R2A agar, leading to inaccurate counts. Both manufacturers withheld proprietary information on the ingredients of their respective media. However, our results suggest the ingredients do not mimic those found in R2A agar resulting in the discrepancies between test measurement methods. No specific medium, temperature or incubation time will allow for ideal conditions to measure all microbes at all times. Karpay (64) has concluded, each combination of microbes yields an estimate that varies as a result of the proportion of different species present and their characteristics.

Dentistry needs a standard protocol for the handling of water samples. This data supports the notion for continual development of accurate in-office test kits that can provide valid measures of bacterial counts. It would be prudent for dental manufacturers to develop a medium that is consistent with the R2A formulation. Without accurate in-office test kits, many dentists will be reluctant to monitor DUWLs using microbiologic laboratory testing.

Also, this study demonstrated the problems encountered with cleaning DUWLs. The intricate design of dental units with an extensive network of narrow bore plastic tubing encourages biofilm formation which makes cleaning by chemical agents challenging.

It is difficult to compare previously published studies regarding the effectiveness of DUWL iodine cleaners due to the myriad of different environmental conditions under which the evaluations were conducted (i.e., type of iodine preparation, concentration, contact time, testing methods, incubation conditions). Growth conditions can dramatically affect susceptibility to

chemical agents, and must be considered when evaluating the effectiveness of a DUWL cleaning product. Again the need for standardization of test conditions is apparent.

Mills (53) showed that 10 percent povidine-iodine with subsequent use of sterile water effectively reduced microbial contamination in clinical use DUWLs. He allowed the agent to remain in contact for 12 hours; did not neutralize the samples with sodium thiosulfate; used dextrose agar plates for culturing, and incubated from 48-120 hours at room temperature. Walker (52) found that 10 percent betadine resulted in a 100 percent reduction in biofilm total viable counts in an established biofilm laboratory model. Source water was sterile water; samples were neutralized with sodium thiosulfate; plated on R2A agar, and incubated at 37 degrees C for 7 days.

The results of this study indicated that the two iodine treatment methods did reduce bacterial counts compared to no treatment, but did not sufficiently reduce microbial counts to levels recommended by the ADA or CDC/EPA. Since a majority of operatory sink tap water samples met EPA standards, the high level of bacteria recovered is probably due to biofilm formation, followed by the shedding of planktonic microbes into the treatment water. The effectiveness results from this study may be due to the source water. Tap water contains a small number of viable bacteria, which can multiply quickly inside the DUWLS, initiating biofilm formation. This could potentially overwhelm the use of the intermittent or continuous iodine products tested. Also, the minimal and erratic usage pattern of the dental units may have contributed to the biofilm formation.

Kettering (46) demonstrated that water source selection plays an important role in achieving and maintaining consistent cleaning of DUWLs. Both Eleazer (45) and Williams (67)

recommend that tap water not be used with independent water reservoirs. Results of this study may have been different if initially sterile was used as the source water.

One other potential explanation could be that the microbes present may be resistant to iodine. This study site has been using Dentacide for an extended period of time at the minimal contact time of 4 hours. This could potentially result in iodine resistance of DUWL microbes. No studies to date have addressed this particular issue.

An effective DUWL cleaner should reduce planktonic contamination to recommended levels, and ideally remove/control biofilm formation. In the future, dental manufacturers may need to redesign dental units to reduce biofilm and microbial contamination. In the meantime, the DHCP needs to address the problems directly and use available technologies consistently.

J. CONCLUSIONS

To date, there is minimal epidemiological evidence that microbial contamination from DUWLs is a significant infection risk for patients and dental staff. However, the potential for infection does exist, and the effects of DUWL contamination requires further evaluation. Therefore, every effort must be made to improve the microbiological quality of dental treatment water to meet recommended levels established by the ADA and CDC/EPA.

Following the methods of this study, both in-office test devices (Millipore HPC Samplers, and Clearline Water Test Kit) demonstrated marginal validity, underestimating bacteria levels compared to the R2A agar. Also, both iodine cleaning agents appeared to have limited benefits in reducing microbial contamination to recommended levels.

No single medium or incubation time/temperature will recover all viable bacteria in a water sample. It seems prudent to use a medium that will provide the highest estimate possible, when documenting bacterial levels related to water treatment protocols. Monitoring is becoming

more widely accepted as a means to assess the effectiveness/compliance of treatment methods. Regardless of the treatment methods, monitoring should be used to ensure that protocols are followed. The development of an accurate chairside test kit that is inexpensive, and simple to use would further encourage the adoption of a regular monitoring program for DUWLs

Future research is needed to develop standard ways to monitor bacteria levels accurately, and to generate DUWL cleaners that can consistently decrease microbial contamination to meet recommended guidelines. The dental community must share the common goal of improving the quality of dental treatment water. Definitive guidance on the management and monitoring of bacterial contamination is then possible from professional, regulatory, and advisory bodies. This will ensure that both patients and staff are appropriately protected.

Dental professionals should be wary of products that do not include evidence of independent testing of cleaning effectiveness, and valid monitoring procedures. The general public is becoming more aware of the water quality issues in dentistry. As more studies are conducted and published, it is expected that dental practices will have improved procedures to address this public health issue.

K. SUGGESTED CHANGES IF THE PROJECT WAS REPEATED

If I had the opportunity to repeat this project, I would have used sterile water as the source water to better determine the effectiveness of the two iodine products. As to the measurement of validity, I feel the study design appropriately tested the two in-office test kits. A limitation of this study is the number of dental units which could be tested. This testing and culturing is very labor intensive. Nevertheless, it is desirable to increase the statistical power in such studies.

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M. Tables

TABLE 1

VALIDITY MEASURES FOR TWO TEST PRODUCTS AT $\leq$ 200 CFU/mL (ADA)

Product	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
Millipore	66%	93%	99%	27%	69%
Clearline	18%	93%	95%	14%	28%

TABLE 2

VALIDITY MEASURES FOR TWO TEST PRODUCTS AT $\leq$ 500 CFU/mL (CDC/EPA)

Product	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
Millipore	30%	97%	96%	31%	47%
Clearline	1%	97%	50%	24%	25%

TABLE 3

DENTAPURE DP-40 (CFU/mL)

Unit	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	
A	82	35	82	148	187	39	**464**	**379**	**322**	**255**	**308**	**258**	**412**	Millipore
B	**475**	**338**	572	**491**	**447**	159	628	575	658	**356**	537	571	**374**	Millipore
C	163	0	20	53	**273**	6	175	25	100	**205**	**417**	65	**416**	Millipore
A	**312**	33	35	63	69	1	102	59	20	18	17	48	**214**	Clearline
B	**351**	51	97	51	150	58	70	70	185	40	105	130	25	Clearline
C	31	0	1	29	156	1	81	1	51	82	113	21	145	Clearline
A	2293	1037	**367**	**483**	1220	133	1087	780	1000	1083	154333	26866	23733	R2A
B	17300	1943	1673	1363	1510	**330**	1837	1863	2920	977	1557	1600	1060	R2A
C	1466	**263**	50	132	710	40	**327**	557	1500	1120	**433**	597	1130	R2A

TABLE 4

DENTACIDE (CFU/mL)

Unit	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	
D	1	1	98	17	**307**	592	24	562	725	78	143	76	523	Millipore
E	26	136	16	92	**355**	**307**	22	**412**	389	1	5	1	59	Millipore
F	5	137	**275**	70	552	**415**	174	188	92	**358**	58	108	**372**	Millipore
D	2	1	28	1	41	177	4	213	1	23	51	12	58	Clearline
E	8	96	1	85	79	63	4	25	67	3	1	1	10	Clearline
F	1	79	59	61	278	87	69	74	28	31	19	20	180	Clearline
D	527	120	2500	58	4966	27200	163	2396	6333	1127	47	**417**	3567	R2A
E	720	19366	**250**	**453**	1093	187333	200	1580	7167	1253	27	12	**440**	R2A
F	510	2443	1487	120	4566	43333	**407**	**457**	**280**	1233	10	1263	1123	R2A

TABLE 5

CONTROLS (CFU/mL)

Unit	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	
G	25	71	**378**	**382**	641	770	634	755	745	546	643	648	**380**	Millipore
H	**420**	58	**346**	**440**	654	1155	**481**	**466**	692	101	**318**	615	675	Millipore
I	70	**356**	571	**217**	**342**	544	512	**319**	545	**312**	**335**	135	**481**	Millipore
G	41	14	83	92	176	92	**431**	85	167	81	167	230	31	Clearline
H	**256**	35	**293**	**283**	**250**	**296**	134	**221**	120	38	66	183	144	Clearline
I	57	**330**	102	144	**383**	**266**	620	138	**210**	45	185	15	58	Clearline
G	957	1427	2953	1556	14266	20666	15200	22333	18000	11633	1966	17500	2733	R2A
H	2463	422660	1700	2096	16100	253666	26600	29166	221333	1237	**263**	2873	4666	R2A
I	637	288000	4867	**437**	26166	251000	18833	4933	15633	9700	43	1480	16433	R2A

In tables 3, 4, 5 values highlighted in red indicate bacterial levels that meet the ADA goal of ≤ 200 CFU/mL, while values in yellow indicate the remainder of samples that met the CDC recommendation/EPA mandate of ≤ 500 CFU/mL.

APPENDIX 1

The ANOVA Procedure

Class Level Information

Class	Levels	Values
type	3	1 2 3

Number of observations 27

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The ANOVA Procedure

Dependent Variable: count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	6.29208896	3.14604448	7.12	0.0037
Error	24	10.60275778	0.44178157		
Corrected Total	26	16.89484674			

****

Interpretation:

ANOVA is used to test for differences among three or more independent means.
Null hypothesis-all group means are equal.
Alternative hypothesis-at least one of the group means differs from the others.
The test statistic for ANOVA is a ratio F, of the between-group variance to the within-group variance.
Assumptions-variable is normally distributed, variance is the same in each group (same sample size), and observations are independent.

Conclusion:

We reject the null hypothesis and conclude that the means are not identical for the three treatment methods at the 0.05 level.

Due to the significant results, a specific pairwise post-hoc comparison was performed (Tukey's test) because we can not determine from the ANOVA which treatment methods are not equal.

APPENDIX 2

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for count

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.441782
Critical Value of Studentized Range	3.53170
Minimum Significant Difference	0.7825

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	type
Control	A	3.9767	9	2
Dentapure DP-40	B	3.1012	9	1
	B			
Dentacide	B	2.8506	9	3

Interpretation:

Tukey's test was used as a pairwise post-hoc comparison test after the ANOVA had resulted in a significant F test. The Tukey's HSD (honestly significant difference) test is applicable only for pairwise comparisons, but permits us to compare all pairs of means (controls the error rate for all comparisons simultaneously). It is the most accurate and powerful procedure to use in this situation, and is more conservative than other pairwise post-hoc comparison tests. Power is the ability to detect a difference if one actually exists, so high power means the null hypothesis is correctly rejected more often.

Conclusion:

Dentapure DP-40 and Dentacide are not significantly different from each other, but both are significantly different from the control group.