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Contamination of Dental Waterlines: Efficacy of Seven Waterline Treatments and Three In-office Bacteria Test Kits

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CONTAMINATION OF DENTAL WATERLINES: EFFICACY OF SEVEN WATERLINE TREATMENTS AND THREE IN-OFFICE BACTERIA TEST KITS

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Table of Contents

Page

List of Tables

Page

List of Figures

Page

Abstract

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This study compared seven dental unit water line (DUWL) treatments and three in-office bacteria test kits. Sodium hypochlorite (NaOCl) 1:10 in tap water weekly; 3 drops of NaOCl in 1 liter of water; Dentapure® DP 40; ICX™ tablet; Sterilex® Ultra powder; Lines™; and Selective Micro® Dental-Clean. Traditional culture technique was compared to HPC Dental Sampler; Aquasafe™ Dental Unit Water Line Test Kit; and Bacteria in Water Test Kit. Eight dental units in the Virginia Commonwealth University Graduate Endodontic Clinic were randomly assigned treatment regimens. Samples were taken

weekly initially and after flushing for 1 minute. In conclusion NaOCl hypochlorite 1:10 in tap water once weekly, Sterilex® Ultra, Lines™, and Selective Micro® Dental-Clean were effective at all sample times while ICX™, 3 drops of NaOCl, and Dentapure® DP 40 were only effective after 1 minute flushing. There was no significant difference between the in-office test kits and traditional culture.

{CHAPTER 1 Introduction}

Contamination of dental unit water lines (DUWLs) by microbial biofilm is a well known Phenomenon. Numerous studies have tested levels of microorganisms in the DUWLs [1, 2, 3], the types of microorganisms found [4, 5, 6], and methods to reduce or eliminate the microorganisms [4, 7-17]. Tap water is treated to maintain <500 CFU/ml and therefore is not the cause of high numbers of microorganisms in dental waterlines. High numbers of microorganisms in dental unit waterlines are related to tubing design and materials, and ultimately laminar flow [18]. DUWLs have narrow diameters and low flow rates. This creates stagnation in the lines and provides an optimal environment for the establishment and maintenance of a bacterial biofilm [19]. The lumens of the small-bore hoses are colonized by a tenacious freshwater biofilm where the microorganisms are protected by a glycocalyx coating [21]. As a consequence, bacterial counts in water samples can reach $\leq$ 1,000,000 colony forming units per milliliter (CFU/ml) [20]. The Center for Disease Control (CDC) and the American Dental Association (ADA) recommend maintaining <500 CFU/ml in DUWLs but do not advocate any particular method to achieve this standard. As a result, self-contained units with plastic bottles that can be filled with any number of water treatments have largely replaced units directly plumbed to local water sources.

The first mention in the literature of microorganisms in DUWLs in the United States was by Abel in 1971 [22]. Since that time there has been considerable time and money spent exploring the role of DUWL contamination and the role that biofilms play in this contamination. Biofilm is a complex structure adhering to surfaces that are regularly in contact with water, consisting of colonies of bacteria and usually other microorganisms such as yeasts, fungi, and protozoa that secrete a mucilaginous protective coating in which they are encased. Biofilms can form on solid or liquid surfaces as well as on soft tissue in living organisms, and are typically resistant to conventional methods of disinfection. Dental plaque is a common example of biofilm [24]. Once the biofilm is formed, it gives the microorganisms within it a considerable advantage. It may require up to 1,000 times more antibiotic to reach and kill biofilm microorganisms compared to planktonic (free floating) [19]. Bacteria within biofilms have structural heterogeneity, genetic diversity, complex community interactions, and channels to distribute nutrients and communicate [24]. The microorganisms that are cultivable represent a very low percentage of the biofilm [19]. Some of the species isolated from DUWLs include Klebsiella, Legionella, Mycobacterium, Pseudomonas, Penicillium, and Acanthamoeba [5]. Pseudomonas aeruginosa and P. cepacia are reported to have increased resistance to antibiotics [5] and medically compromised patients have been reported to contract infections from DUWLs contaminated with P. aeruginosa [26].

Numerous methods have been studied and reported to be effective for meeting the CDC guidelines for DUWLs. These include sodium hypochlorite, glutaraldehyde, and isopropanol 15.3% [4]; distilled water and line cleaning [7]; ICX (sodium percarbonate, silver

nitrate and cationic surfactants) [8]; Lines (ethanol and Chlorhexidine) [9]; in-line bacteriological filters [10]; super-oxidized water [11]; electro-chemically activated water [13]; diluted sodium hypochlorite [7,14]; chlorine dioxide [5]; Listerine (ethanol, menthol, thymol, methyl salicylate, and eucalyptol), 0.5% sodium fluoride, Rembrandt (sodium fluoride), BioBlue (activated chlorine dioxide) and Dentosept (salvia, arnica, menthol, thyme, chamomile) [17]; and Sterilex® Ultra (alkaline peroxide with phase transfer catalyst) [15]. Choosing the right method is complicated by cost, time involved, compliance, efficacy, and possible clinical effects such as decreasing bond strength and allergic reactions. With so many factors and possible methods to obtain the same outcome, there may never be a consensus or directive handed down to practitioners to follow one protocol. This means practitioners must decide which product to use to meet the following objectives, 1) product protects patients and staff from DUWL contamination, 2) method is user-friendly and not labor intensive, and 3) product is cost effective.

The purpose of this study was to compare the effectiveness of 1:10 sodium hypochlorite in tap water once per week, 3 drops of sodium hypochlorite in 1 liter of tap water, Dentapure® DP-40 (elemental iodine), ICX™ tablet (sodium percarbonate, silver nitrate and cationic surfactants), Sterilex® Ultra Powder (alkaline peroxide with phase transfer catalyst), Lines™ (ethanol and Chlorhexidine), Selective Micro® Dental-Clean (chlorine dioxide) used as a waterline treatment. The study also compared traditional culture techniques to Millipore HPC Sampler (now called HPC Total Count), Pall-Aquasafe™ Dental Unit Water Line Test Kit, and Pro-Lab® Bacteria in Water Test Kit.

{CHAPTER 2 Materials and Methods}

Eight dental units in the Virginia Commonwealth University School of Dentistry Graduate Endodontic Clinic were selected for treatment. Dental treatment was performed in each unit on a regular basis. Treatments (including controls) were randomly assigned to a unit and then run for three weeks. Treatments were reassigned after the three week period to eliminate the unit as a variable in the treatments. A control was performed in the same manner as treatments.

The products chosen for the study were selected based on cost and ease of use. All treatments were performed according to manufacturer's directions. Treatments were performed weekly during the experimental period. Lines were flushed weekly for weekly treatment protocols. Continuous treatment protocols were not flushed weekly. Samples were taken each Tuesday morning at time zero (no flushing), and after 1 minute of flushing. Samples were taken for ten seconds to allow for measurement of bacterial count by more than one method.

Each unit was randomly assigned to one of the seven treatment protocols or to serve as a control as follows:

Treatment 1- 1:10 sodium hypochlorite (5.25%) in tap water once per week. The water bottle was filled with 550 ml of 1:10 sodium hypochlorite in tap water and connected to the dental unit. The solution was then flushed through the air water syringe until almost

empty and left overnight. The next morning the bottle was rinsed with tap water, filled with tap water, and then flushed through the air water syringe. The bottle was then filled with tap water and used for the week. The 1:10 sodium hypochlorite was run through the unit at the same time and day each week.

Treatment 2- 3 drops of sodium hypochlorite (5.25%) treatment continuously. The water bottle was filled with tap water and 3 drops sodium hypochlorite and used for the week.

Treatment 3- Dentapure® DP 40 dental water purifier (MRLB International, Inc, Fergus Falls, MN). The Dentapure® DP 40 was installed and the water bottle filled with tap water and used for the three week period.

Treatment 4- ICX™ tablet **(**A-dec, Newberg, Oregon) was placed in the water bottle and 1 liter of tap water was added. The bottle was used for the week.

Treatment 5- Sterilex® Ultra powder (Sterilex Corporation, Owings Mills, MD). The powder was mixed and used according to manufacturer's instructions. The water bottle was then filled with tap water and used for the week.

Treatment 6- Lines™ (Micrylium Laboratories Inc, Niagara Falls, NY). The solution was used according to manufacturer's instructions. The bottle was then filled with tap water and used for the day.

Treatment 7- Selective Micro® Dental-Clean (Selective Micro Technologies, Beverly, MA). The solution was prepared and used according to manufacturer's instructions. The bottle was then filled with tap water and used for the week.

Control- Tap water from the Graduate Endodontic Clinic.

Samples were taken for 10 seconds in 100 milliliter sterile sample containers. After samples were collected they were immediately transported to the laboratory. There samples were plated on the Simplate® for HPC (IDEXX Laboratories) and allowed to incubate according to manufacturer's instructions and read with a 365 nm UV light (Fisher Scientific).

The Millipore HPC Sampler, Pall-Aquasafe™ Dental Unit Water Line Test Kit, and Pro-Lab® Bacteria in Water Test Kit were then used (according to manufacturer's instructions) to test the samples. Due to the limited number of test kits, every sample was not run on them. When a sample was selected to be tested by the in-office kits, all three test kits were used to test the same sample. The bacterial count from the Simplate® for HPC was then compared to the bacterial count of the in-office test kits.

{CHAPTER 3 Results}

Each of the seven treatments and one control condition were randomly assigned to two different units, on different occasions. The treatment groups were compared using a repeated-measures mixed-model analysis of the log transformed bacteria counts, and a logistic regression analysis of the probability of an effective treatment. A repeated-measures mixed-model ANOVA was used to analyze the log-transformed counts. Units were considered a random effect in the model. Tukey's HSD was then used to identify which conditions were different.

The summary information obtained from each of the treatments on two occasions is shown in Table 1. The bacterial counts are presented in columns 3-6. The probability of an effective treatment is shown as determined by a logistic regression in columns 7 and 8. For instance, for the 1:10 NaOCl treatment in the first unit (63), the average count was 0.33 CFU/ml and 3 of 3 samples were below 500 CFU/ml. For the 1:10 NaOCl treatment in the first unit (68), the average count was 40 CFU/ml and 3 of 3 samples were below 500 CFU/ml.

Table 1: Summary Information

			HPC Initial				Count < 500	
Treatment	Unit	N	Mean	Min	Max	Median	No	Yes
1:10 NaOCl	63	3	0.33	0	1	0	0	3
	68	3	40.00	0	120	0	0	3
3 drops NaOCl	64	3	739.00	739	739	739	3	0
	66	3	175.67	77	299	151	0	3
Control	64	3	639.00	555	739	623	3	0
	66	3	533.67	355	739	507	2	1
Dentapure® DP 40	64	3	436.00	62	739	507	2	1
	67	3	739.00	739	739	739	3	0
ICX™	63	3	48.00	0	104	40	0	3
	66	3	492.67	0	739	739	2	1
Lines™	62	3	38.67	0	112	4	0	3
	63	3	104.67	1	287	26	0	3
Selective Micro® Dental-Clean	65	3	0.00	0	0	0	0	3
	67	3	0.33	0	1	0	0	3
Sterilex® Ultra	61	3	0.00	0	0	0	0	3
	62	3	0.00	0	0	0	0	3

In the first set of columns the heterotrophic plate counts (HPC) from the initial sample are summarized by giving the arithmetic mean, minimum, maximum, and median. In addition, the counts of the number of samples (out of n =3) whose counts were below 500.

The bacterial counts were skewed; therefore they were analyzed using a log transformation. Zero counts were analyzed as log (1/2). The following effects were included in the ANOVA model: Treatment group, week (3 occasions), and the week*treatment interaction. Neither the week nor the interaction effect was significant ($p > 0.6$). There was a significant difference between the treatment groups ($p < .0001$). The estimated bacteria counts under each treatment condition are shown in Table 2. Tukey's HSD indicated that all treatments except 3 drops NaOCl and Dentapure were superior to the Control (at alpha = 0.05).

Table 2: Estimated Bacteria Counts

	COUNT		
Treatment	**LS Mean**	**95% CI**	
1:10 NaOCl	1.15	0.42	3.19
3 drops NaOCl	378.43	123.51	1159.52
Control	643.71	210.09	1972.35
Dentapure® DP 40	548.67	240.62	1251.11
ICX™	20.50	9.07	46.34
Lines™	6.77	2.49	18.44
Selective Micro® Dental-Clean	0.62	0.20	1.95
Sterilex® Ultra	0.48	0.15	1.54

Table 3: Tukey's HSD

	Results		
Level			**Least Sq Mean**
Control	A		562.33
Dentapure® DP 40	A		538.36
3 drops NaOCl	A		419.35
ICX™	A	B	24.52
Lines™		B C	9.71
1:10 NaOCl		B C	1.03
Selective Micro® Dental-Clean		C	0.58
Sterilex® Ultra		C	0.49

Levels not connected by same letter are significantly different.

An inspection of the estimated odds-ratio of an effective treatment indicated that all treatments except 3 drops NaOCl, Dentapure® DP 40, and ICX™ are superior to control (Table 4). The large width of these score confidence intervals is due to the small sample size in each group (N = 6).

A second analysis considered whether the counts were below 500 (yes or no). See the right-hand columns (7 and 8) in Table 1. Logistic regression indicated that the groups were significantly different (LR chi-square = 32.9, df = 7, p-value < .0001).

Table 4: Estimated Probability of an Effective Treatment (Counts < 500) in Each Treatment Group (N = 6)

	Probability (Count < 500)		
Treatment	Estimate	95% CI	
1:10 NaOCl	1.00	0.61	1.00
3 drops NaOCl	0.50	0.19	0.81
Control	0.17	0.03	0.56
Dentapure	0.17	0.03	0.56
ICX	0.67	0.30	0.90
Lines	1.00	0.61	1.00
Selective Micro Dental-Clean	1.00	0.61	1.00
Sterilex Ultra	1.00	0.61	1.00

The counts from the initial sample were compared to the sample after one minute flushing. Since the CFU counts are skewed, the log-transformed values were analyzed using a repeated-measures mixed-model analysis. The analysis indicated that the occasion difference (initial vs. 1 minute) differed, depending upon the treatment (p-value = 0.0012). This can be seen in Table 5 with Dentapure® DP 40, the initial count had a geometric mean of 459 CFU/ml and this decreased to 51 CFU/ml. This ratio (initial : 1 minute) was 0.11 and indicates a change that was statistically significant (p-value = 0.0052). This may be seen in the 95% confidence interval (CI) on the ratio (0.08, 1.60), which includes 1.

Thus, it is plausible that the ratio of the initial to 1 minute CFU count is 1. On the other hand, in the case of 3 drops NaOCl, the ratio was 0.02 and the fact that the CI does not include 1 (0.005, 0.10). There was no significant change in 1:10 NaOCl, Control, Lines™, Selective Micro® Dental-Clean or Sterilex® Ultra. There was a significant decrease in 3 drops NaOCl, Dentapure® DP 40 and ICX™ and all three were <500 CFU/ml after 1 minute flushing. There were ten treatment occasions where the initial

sample was >500 CFU/ml and all 10 were <500 CFU/ml after 1 minute flushing. In addition, there were five occasions where Control was >500 CFU/ml and after 1 minute flushing 4/5 were <500 CFU/ml.

Table 5: Estimated CFU for Initial and after 1 Min. Flushing

Treatment	Occasion	Estimate	95% CI		
1:10 NaOCl	Initial	1.40	0.33	5.96	
	1 min.	0.50	0.12	2.09	
	Ratio	0.36	0.08	1.60	
3 drops NaOCl	Initial	334.59	78.58	1424.68	
	1 min.	7.18	1.72	30.02	
	Ratio	0.02	0.005	0.10	*
Control	Initial	569.12	133.66	2423.34	
	1 min.	170.92	40.89	714.37	
	Ratio	0.30	0.07	1.35	
Dentapure	Initial	459.21	107.85	1955.11	
	1 min.	51.00	12.20	213.17	
	Ratio	0.11	0.02	0.50	*
ICX	Initial	28.78	6.76	122.54	
	1 min.	1.22	0.26	5.70	
	Ratio	0.04	0.01	0.21	*
Lines	Initial	10.89	2.56	46.39	
	1 min.	19.36	4.63	80.91	
	Ratio	1.78	0.40	7.97	
Selective Micro Dental-Clean	Initial	0.56	0.13	2.39	
	1 min.	0.50	0.12	2.09	
	Ratio	0.89	0.20	4.00	
Sterilex Ultra	Initial	0.50	0.12	2.13	
	1 min.	0.50	0.12	2.09	
	Ratio	1.00	0.22	4.49	

* indicates significant differences

On the 14 occasions where both the Simplate® for HPC and the Pro-Lab® Bacteria in Water Test Kit were both used showed a mean for the Pro-lab of 1160 and a mean for the Simplate for HPC of 252. The differences between the paired measurements were

compared using a Wilcoxon sign-rank test and found to be not significantly different (p-value = 0.3594).

On the 14 occasions where both the Simplate® for HPC and the Pall-Aquasafe™ Dental Unit Water Line Test Kit were both used showed a mean for the Pall Medical of 59 and a mean for the HP initial of 252. The differences between the paired measurements were compared using a Wilcoxon sign-rank test and found to be not significantly different (p-value = 0.1094).

On the 14 occasions where both the Simplate® for HPC and the Millipore HPC Sampler were both used showed a mean for the Millipore of 3912 and a mean for the HP initial of 252. The differences between the paired measurements were compared using a Wilcoxon sign-rank test and found to be not significantly different (p-value = 0.0781).

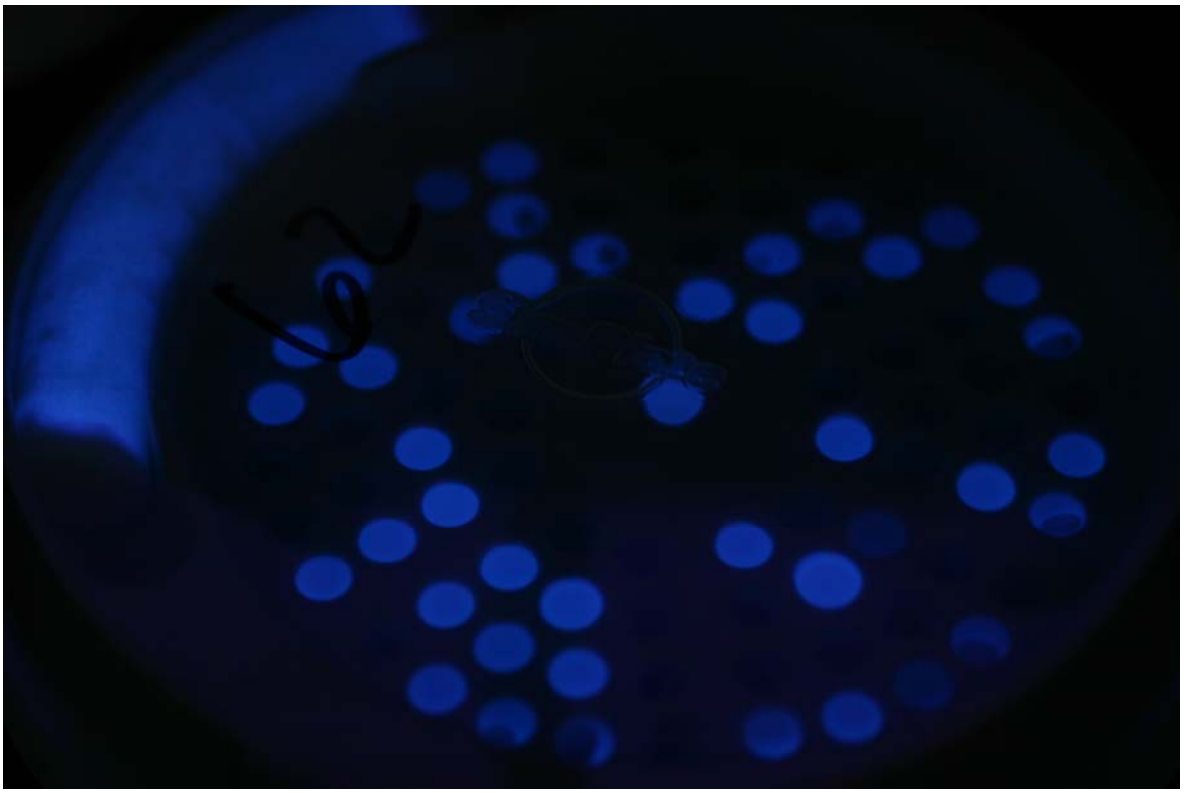

Figure 1: Simplate® for HPC showing positive wells illuminated by UV light

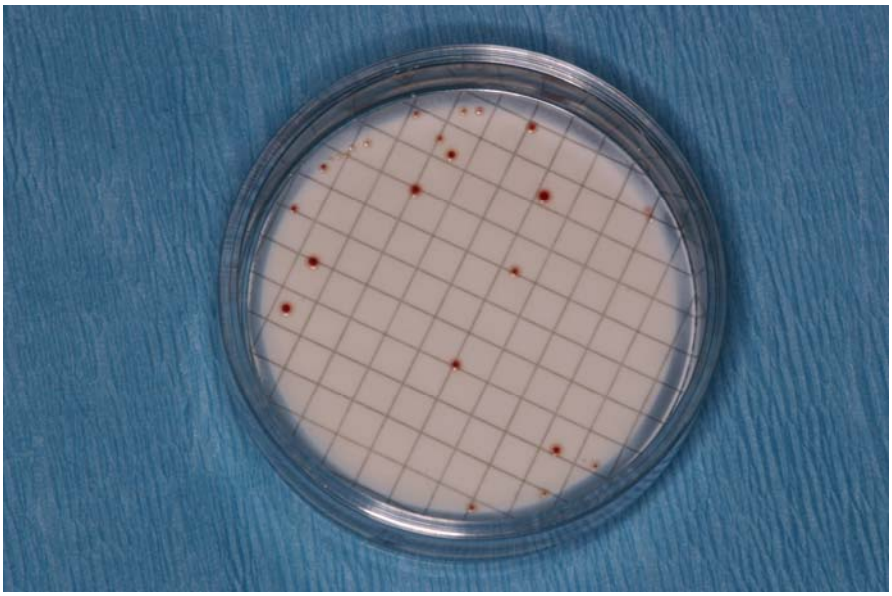

Figure 2: Pall-Aquasafe™ Dental Unit Water Line Test Kit

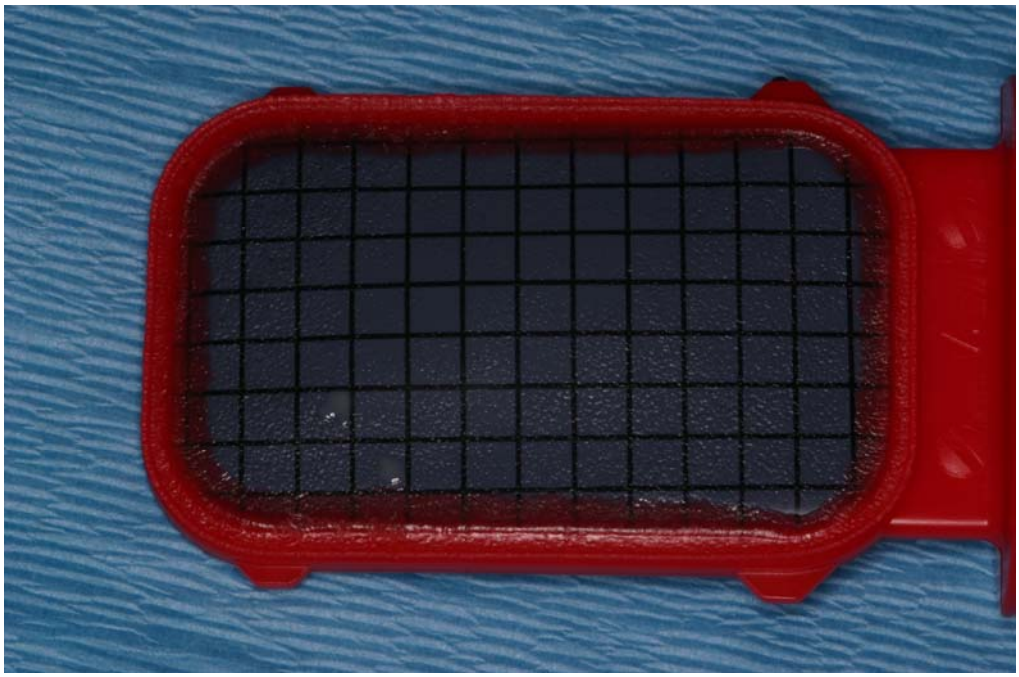

Figure 3: Millipore HPC Sampler

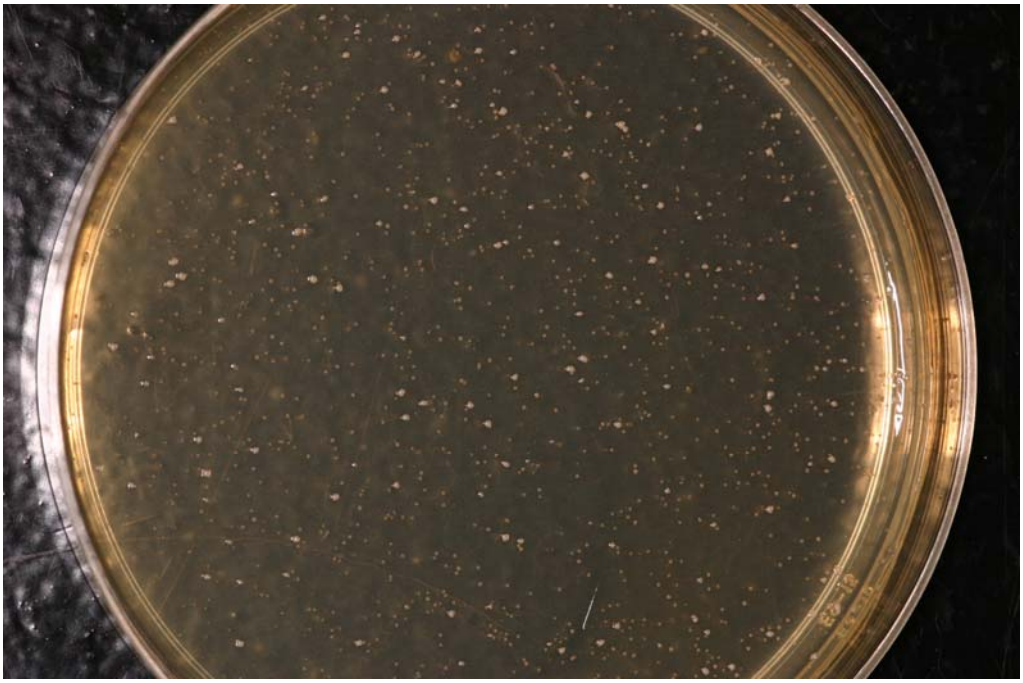

Figure 4: Pro-Lab® Bacteria in Water Test Kit

{CHAPTER 4 Discussion}

This study compared seven DUWL treatments on contaminated DUWLs. A 1:10 sodium hypochlorite in water dilution, Lines™, Selective Micro® Dental-Clean, and Sterilex® Ultra powder were all effective in maintaining counts <500 cfu/ml. Three drops of sodium hypochlorite in the self-contained bottle with tap water, Dentapure® DP 40, and ICX™ were not effective in maintaining counts <500 CFU/ml without 1 minute flushing. After 1 minute flushing 3 drops NaOCl, Dentapure® DP 40, and ICX™ had all >500 CFU/ml counts reduced to <500 CFU/ml. It is interesting to note that the effective treatments that did not require flushing are all weekly regimens, while the ineffective methods were all continuous regimens that are added directly to, or attached to the self-contained water bottle. Additionally, Sterilex® Ultra and Selective Micro® Dental-Clean had a substantive effect lasting nine weeks. This was discovered after reinoculating the units treated with these two products. The cultures continued to show counts < 2 CFU/ml for the rest of the observation period even with no treatment.

Flushing waterlines for 20-30 seconds between patients is still recommended by the CDC and untreated waterlines aren't likely to maintain a level of <500 CFU/ml [18]. This means a product such as the ones tested in this study must be used to meet this standard. Merely using supply water with <500 CFU/ml is not enough because this does not address the biofilm [18.] Self-contained systems in conjunction with a chemical treatment, filters

within the water line, as well as combinations of these have been shown to be effective [18].

ICX™, Dentapure® DP 40, and 3 drops NaOCl were only effective after 1 minute flushing. While most studies don't support flushing as a method to keep waterline levels <500 CFU/ml, it may still serve a valuable purpose by reducing cross-contamination and reducing the CFU count. The current study showed a difference in three of seven treatments with one minute flushing. This is an important point, given the manufacturer's directions require flushing for two minutes for the Dentapure® DP 40. Without this daily flush, this product could be ineffective.

Three in-office bacteria monitoring kits were also compared and were not statistically different in their ability to count heterotrophic bacteria from DUWL samples compared to culture methods. Bartoloni and associates found that both the HPC Dental Sampler (Millipore) and Clearline Water Test Kit (Kerr/Metrex) underestimated bacterial counts compared to culture [25]. The differences noted by this author were ease of use, cost, and ability to read. The Pro-Lab® Bacteria in Water Test Kit was the easiest to use while the Millipore HPC Sampler and Pall-Aquasafe™ Dental Unit Water Line Test Kit were similar in ease of use. The Pro-Lab® was the most expensive while the Millipore and Pall Medical were similar in price. The Pall Medical was similar to Millipore in ease of use, while the Pro-Lab was the most difficult to read due to its clear medium, off-white positive bacterial colonies, and thickness of the medium. The thickness meant you had to not only count across the plate horizontally, but also vertically. This was very visually distracting and difficult to read when the counts were high.

There are well over 20 commercially available products to treat DUWLs. The

products range from inexpensive, such as sodium hypochlorite, to expensive, such as the Dentapure® devices. Each product has advantages and disadvantages. This means each clinic that purchases these products must decide what works best. There is no perfect way to rid DUWLs of bacteria. The factors that come into play in this decision are compliance, cost, time, frequency, and efficacy of the given treatment regimen.

Reports have been published advancing the fact that there are bacteria in untreated DUWLs [27]. While Fotos [21] and Reinthaler [23] reported high antibody titers to Legionella in dental staff versus controls, there have not been any reported cases of legionellosis stemming from dental unit water in the literature. There are several methods available to monitor DUWL contamination. In-office systems were studied for their convenience. Unfortunately the small sample size did not allow discernment of their effectiveness as clearly as had been anticipated. The current CDC guidelines [18] recommend using a waterline treatment regimen, but do not recommend any monitoring. If an accepted method for DUWL decontamination is used, there is no requirement for monitoring DUWLs

{CHAPTER 5 Conclusion}

In conclusion, 1:10 Sodium Hypochlorite, Selective Micro® Dental-Clean, Lines™ and Sterilex® Ultra powder are effective treatments with no flushing required. ICX™, Dentapure® DP 40, and 3 drops NaOCl were not effective initially, but were effective after one minute flushing. The Pall-Aquasafe™ Dental Unit Water Line Test Kit and Millipore HPC Sampler were preferred over the Pro-Lab® for ease of use, however in-office testing does not appear to be necessary.

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Literature Cited

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VITA

Adam Davis is the fourth child of Herbert and Evelyn Davis. His father was a career navy officer and his mother was dental assistant. Adam graduated from Lebanon High School in 1989, received his B.S. from Bethel College in 1993, and received his D.D.S. from the University of Tennessee College of Dentistry in 2001. After graduation from dental school, he was commissioned in the US Navy Dental Corps where he completed a one year General Practice Residency. Following the residency he was assigned as the Dental Department Head of the USS Germantown, LSD 42, where he spent two years as the solo dentist caring for the sailors and marines onboard. His final tour of duty was at NAS Millington in Millington, Tennessee where he served for two years. During his final year in Millington he applied and was accepted in the Graduate Endodontic Residency at the Virginia Commonwealth University in Richmond, Virginia. Dr. Davis will receive his Master of Science in Dentistry and Certificate in Endodontics June 26, 2008.