Evaluation of 3 dental unit waterline contamination testing methods

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Abstract

Previous studies have found inconsistent results from testing methods used to measure heterotrophic plate count (HPC) bacteria in dental unit waterline (DUWL) samples. This study used 63 samples to compare the results obtained from an in-office chairside method and 2 currently used commercial laboratory HPC methods (Standard Methods 9215C and 9215E). The results suggest that the Standard Method 9215E is not suitable for application to DUWL quality monitoring, due to the detection of limited numbers of heterotrophic organisms at the required 35°C incubation temperature. The results also confirm that while the in-office chairside method is useful for DUWL quality monitoring, the Standard Method 9215C provided the most accurate results.

The water that is supplied via dental unit waterline (DUWL) tubing to air/water syringes, handpieces, and ultrasonic scalers in a typical dental unit is fed directly from the main water supply or via a self-contained reservoir on the dental unit itself. DUWL tubing typically is 2 mm in diameter and made of either polyvinyl chloride or polyurethane. This tubing forms a complex network inside a dental unit, resulting in a high ratio of tubing surface area to water volume.[1] These factors, along with the periodic pooling of stagnant water inside the tubing, facilitate an ideal environment for bacterial growth and biofilm formation (up to 50μ thick) comprised of a heterogeneous population of organisms.[2,3] Microorganisms from the biofilm are continuously shed as the water flows through the DUWL tubing, resulting in microbial contamination of the patient treatment water.[4]

The *Centers for Disease Control and Prevention* (CDC) recommends that the water used in dental offices should meet the drinking water standard established by the *US Environmental Protection Agency* (EPA) of <500 colony forming units per milliliter (CFU/ml) for routine

dental treatment output water.[5,6] In order for dental practitioners to comply with these guidelines, DUWL monitoring should be performed as recommended by the dental unit manufacturers.[5] Waterline monitoring can be done in-office with chairside kits or via commercial laboratories. The purpose of monitoring is to measure the heterotrophic (organisms that use a carbon source for survival) plate count (HPC) of DUWL samples.

Currently, there is only 1 in-office, chairside kit available: the HPC Sampler (EMD Millipore), consisting of a removable dip paddle contained in a plastic sampler. The dip paddle contains a 0.45μ filter and an absorbent pad with dehydrated agar medium which absorbs 1 ml of the liquid sample, facilitating the recovery of stressed (that is, partially sanitized or nutritionally starved) aerobic bacteria in 7 days. According to the manufacturer, this method can produce accurate readings up to 300 CFU/ml; all counts >300 CFU/ml are considered too numerous to count (TNTC).[7] There is evidence from previous studies to show that, although the HPC Sampler underestimates bacterial counts compared with other methods, it is useful as a screening tool for regular DUWL quality monitoring in dental offices to ensure the water used in the treatment of patients meets the CDC/EPA recommendation of <500 CFU/ml.[8-10]

Dental offices can also utilize services offered by commercial laboratories for a more accurate assessment of water quality. Waterline samples are collected and mailed using kits that are supplied to offices by the commercial entities. Standard laboratory methods, as published in the "Standard Methods for the Examination of Water and Wastewater" (hereafter referred to as *Standard Methods*), are recommended by the American Public Health Association, American Water Works Association, and *Water Environment Federation.*[11] The list includes 4 standard methods and 5 types of media for use in different combinations as appropriate for testing purposes.[11]

Standard Method 9215C (a spread plate method on R2A medium) has long been considered the gold standard for analysis of DUWL quality.[12] This procedure, using a low nutrient R2A formulation (Becton, Dickson & Company) and room temperature incubation for 7 days, was designed for the detection of common water organisms. The disadvantages of this method are that it is time-consuming to prepare the R2A and it relies on a small volume of liquid (0.1 ml), which can become quickly absorbed if the agar dries out.[11]

Standard Method 9215E (SimPlate for HPC, IDEXX Laboratories, Inc.) is a user-friendly method that has been included in the list of Standard Methods in recent years.[11] A proprietary enzyme substrate is mixed with the water sample, and as bacteria metabolize the substrate they fluoresce after 48 hours of incubation at 35°C. The number of fluorescent wells are counted and converted to the most probable number (MPN), using a table provided by the manufacturers. The maximum MPN/ml recorded from an undiluted sample is 73.8; for more highly contaminated water samples, 10-fold serial dilutions can be used.[13] Since its introduction as a Standard Method, SimPlate for HPC has become widely used in commercial laboratories, and dental offices that use a mail-in laboratory service may be obtaining their results from this method.

A previous study by the authors found that bacterial counts were underestimated on the SimPlate for HPC compared to R2A agar.[14] The purpose of this experiment was to expand on those findings and to compare bacterial counts and genera from all 3 currently available monitoring methods: the spread plate R2A (Method 9215C), the SimPlate for HPC (Method 9215E), and the in-office HPC Sampler.

Materials and methods

The experiment was designed to collect an approximately uniform distribution of water sample contamination based on 3 source types and 7 exposure durations yielding a total of 63 waterline samples. Sterile collection bottles (100 ml), each containing sodium thiosulfate to neutralize residual chlorine (IDEXX Laboratories, Inc.) were used to collect samples from the handpiece lines, the air/water syringe lines, and the source tap water in 21 randomly selected dental operatories in a teaching institution.

Each sample was cultured on HPC Sampler, R2A agar (Method 9215C), and SimPlate for HPC (Method 9215E) according to manufacturer-recommended methods. The pH of the source tap water and the residual free chlorine level (mg/l) were tested before the experiment and were found to be 7.2 and 0.5mg/l, respectively. These levels were assumed to remain constant as previous readings in the institution had shown this to be the norm.[15]

Sample cultures

All laboratory procedures were conducted by 1 laboratory technician. A 10-fold serial dilution of each sample was made with phosphate buffer solution.

For Method 9215C, 0.1 ml of each sample was spread on R2A plates in triplicate, incubated at room temperature, and the microbial CFU/ml was recorded after 7 days.[11]

For Method 9215E, 10 ml of each solution were placed in the center of the SimPlates and the manufacturer's instructions were followed. Plates were incubated for 48 hrs at 35°C, and the MPN/ml was calculated.[16] Following the calculation of MPN/ml, liquid was collected (using an inoculating loop) from randomly selected fluorescent wells, then spread on R2A plates and incubated at room temperature for 7 days to prepare isolates for molecular identification.

For HPC Sampler cultures, an undiluted 10 ml sample was placed in the outer sheath, and the dip paddle was replaced for 30 seconds until 1 ml was absorbed. The remainder of the DUWL sample was discarded and the Sampler was incubated at room temperature for 7 days, at which point CFU/ml were recorded using the comparison chart provided by the manufacturer.[7]

Molecular identification

A selection of HPC Samplers and R2A plates with the largest bacterial colonies was transported to the Department of Microbiology at the University of Texas Health Science Center at San Antonio (UTHSCSA) for molecular identification, and a sequence-based

approach using the 16s ribosomal DNA regions as targets for the molecular identification isolates was performed.[17]

DNA isolation

Isolates were suspended in 600 µl cell lysis buffer (blood Maxwell LEV kit, Promega Corporation) in a 0.5 ml microfuge tube. The suspension was bead-beaten for 45 seconds to 1 minute to aid in cell wall breakdown. The suspension was then pelleted for 3 minutes at maximum speed in a microfuge according to the manufacturer's instructions. The supernatant was transferred to the Maxwell LEV cartridge and then mounted on the automated Maxwell system, resulting in 150 ng/µl of purified bacterial DNA after a 45-minute run.

Polymerase chain reaction

Polymerase chain reactions (PCR) were performed directly on 3 µl of the DNA supernatant in a 50 µl reaction using a 5 prime PCR Extender system (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. 16s amplicons were obtained using primers 27F and 1525R. Amplifications were performed in a PTC-100 thermocycler (MJ Research, Inc.) using the preprogrammed, 3-step protocol as the standard program for all reactions, consisting of 35 cycles using an annealing temperature of 55°C and 1 minute extension time. A 5 µl aliquot of the PCR reaction was run on a 0.7% agarose gel and stained with ethidium bromide to confirm amplification. The remaining PCR reaction (45 µl) was run on a gel as described above, then purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation), eluted in 30 µl sterile water according to the manufacturer's instructions, and incubated with proteinase K at 56°C for 15 minutes.

Sequencing

DNA obtained from the PCR reaction was prepared for sequencing by cleaning with a Qiaprep Spin Miniprep Kit (Qiagen Sciences, Inc.) according to manufacturer's instructions. The purified DNA was sequenced at the UTHSCSA Advanced Nucleic Acids Core facility. Sequences were then used to perform individual nucleotide-nucleotide searches of the ribosomal 16s region using the BLASTn algorithm at the *National Center for Biotechnology Information* website.[18] Identifications were calculated based on a percentage made from the alignment matches obtained from the top 3 BLAST searches for the 16s region to yield a variety level identification. The 3 highest percent identities for each isolate were analyzed for bacterial identification.

Statistical analysis

For the 3 types of detection methods, all possible pairwise Pearson and/or Spearman correlation coefficients with corresponding 95% confidence intervals were performed to determine if any significant association was observed among the 3 measurement methods, with log transformations performed if appropriate. Correlations were performed for all waterline samples and, if appropriate, separately for each waterline sample source type. Statistical analyses and graphics were performed using Stata 13.0 (StataCorp LP).

Results

As expected, the R2A measures approximated an exponential distribution; however, the SimPlate for HPC values approximated a uniform distribution ranging from a minimum of 0.4 MPN/ml to the maximum 73.8 MPN/ml, followed by 12 (19%) samples with unspecified values >73.8 MPN/ml, as the corresponding 1^{10} dilution plates did not provide any results. There were also 4 samples based on a 1^{10} dilution that had values ranging from 112 to 440 MPN/ml and 1 handpiece sample that could not be assayed due to technical error. For the purposes of graphs and correlations, an arbitrary value of 80 MPN/ml was used to represent all SimPlate for HPC values >73.8 MPN/ml.

Results for each of the methods can be seen in Table 1. The HPC Sampler detection method showed that 46 (73%) of the dip paddle surfaces were entirely covered with TNTC small microbial colonies. For 2 handpiece samples, no HPC counts were detected; otherwise, all handpiece and air/water samples counts were TNTC. For the purposes of graphs and correlations, an arbitrary value of 1,000 CFU/ml was used to represent TNTC results, and the 2 handpiece samples for which the HPC Sampler failed to detect CFUs were excluded as having implausible results. Specific HPC counts were detected for 14 of 21 source water samples. Due to the characteristics of the distribution of HPC measures, correlations were performed for all samples and for source water samples only (Table 2).

For the R2A with SimPlate for HPC, the overall Pearson correlation coefficient of 0.607 was moderate, while the corresponding Spearman rank correlation coefficient of 0.475 was lower. Correlations for each source type showed similar results for source water samples and poorer results for handpiece and air/water samples. To depict the pairwise association, a scatterplot was generated displaying the paired results for each sample with symbols indicating the source type (Chart).

For the HPC Sampler with SimPlate for HPC, the overall Pearson correlation coefficient of 0.650 was significantly lower than the overall Pearson correlation coefficient of 0.871 for SimPlate for HPC with R2A. Similarly, the corresponding Spearman correlation for HPC Sampler with R2A was higher than that for SimPlate with R2A, but the 2 Spearman correlations were not significantly different.

When restricted to the source water samples, the HPC Sampler with SimPlate for HPC Pearson correlation coefficient of 0.573 was significantly lower than the HPC sampler with R2A coefficient of 0.795, while the corresponding Spearman correlations were not significantly different.

Recovered microorganisms

As seen in Table 3, 16 genera of bacteria were recovered. The most commonly occurring genus was Sphingomonas, and only 2 species—*Cupriavidus metallidurans* and *Sphingomonas parapaucimobilis*—were found on all 3 culture media. *Micrococcus luteus* was the only gram-positive species found. All other recovered bacteria were gram-negative.

Discussion

This article describes an evaluation of 3 currently available methods for monitoring HPC bacteria in DUWLs. The SimPlate for HPC (Method 9215E) recovered the lowest numbers of microorganisms and the highest readings were found on spread plate R2A (Method 9215C), although it must be noted that all HPC methods enumerate only a fraction of microorganisms in any water sample and no single method will recover all genera.[19] The overall results are not altogether unexpected, since the media composition and incubation parameters were specifically designed to recover different microbial populations.

Statistical analysis showed moderate correlations between Method 9215E and the other 2 methods, while Method 9215C and HPC Samplers had high correlations. Correlations based on source tap water samples involved fewer arbitrary approximation values, resulting in a decrease in Pearson correlations and an increase in Spearman correlations.

Unlike the other 2 laboratory methods, serial dilutions of samples were not done prior to culturing on HPC Samplers due to its purposeful design as an in-office, chairside monitoring device. As stated earlier, previous studies have shown that HPC Samplers underestimate bacterial counts when compared to the spread plate R2A agar method, and some have attributed this to its failure to grow certain phenotypes.[20,21] The results of this study concur with those findings. However, this study also confirms the high sensitivity of the HPC Samplers, as microbial counts on the majority of the paddles were TNTC and 5 different species of bacteria were detected. For the 2 handpiece samples in which zero bacterial growth was recovered on HPC Samplers, a plausible explanation may be variation among kits, as it was unlikely due to laboratory error (based on the reliability of the standard laboratory methods employed).

The spread plate R2A agar (Method 9215C) has long been considered the gold standard for application to DUWL monitoring with the advantage of producing a true assessment of HPC contamination levels. In this study, accurate counts were obtained using serial dilutions, and 14 different genera of bacteria were detected on R2A plates.

The inclusion of SimPlate for HPC (Method 9215E) in the list of the Standard Methods endorses its use for analysis of drinking water and source water sampes.[11] It is recommended as an alternative to the pour plate method (9215A), which uses high nutrient plate count agar to test for general EPA compliance monitoring; studies have demonstrated good correlation between the 2 methods.[11,13,16] Both 9215A and 9215E methods require incubation periods of 48 hours at mammalian physiological incubation temperature (35°C), favoring the growth of bacteria from human and animal wastes.[22] However, a previous study showed that Method 9215E showed lower microbial counts when compared to the membrane filter method (9215D), which utilizes low nutrient R2A agar and incubation periods of 48 hours at 22°C-28°C.[13] Lower incubation temperature (22°C-28°C), along with a longer incubation time favor the growth of indigenous aquatic bacteria.[22] SimPlate for HPC was a method designed for higher incubation temperatures, and the results of this study add to the body of existing scientific evidence showing that Method 9215E underestimates microbial contamination at 22°C-28°C.[13,14,16]

Significance of microorganisms recovered

Culture plates that were selected for organism identification were based on recovered colony size, so the number of recovered organisms represents a mere snapshot of the total bacterial population. Not surprisingly, due to the limited number of microorganisms isolated on SimPlate for HPC, only 2 bacterial species were identified. However, it must also be noted that this method is not designed for recovering particular organisms, as stated in the Standard Methods.[11]

One gram-positive organism was identified on R2A: *M. luteus,* which is ubiquitously found in soil, dust, air, and water. Cases of infective endocarditis due to *M. luteus* have been reported in the literature.[23] All other microorganisms were gram-negative, which are known to have lipopolysaccharide molecules (endotoxins) in their cell wall that can trigger inflammatory responses in humans. Several studies have reported a significant association between the presence and severity of asthma and a raised concentration of airborne gram-negative bacteria in the indoor environment.[24] A significant correlation between endotoxin levels and high bacterial load in DUWLs has also been reported.[25]

Two species of Pseudomonas isolated on the HPC Samplers in this study have previously been recovered from DUWLs and reported as the causative organisms of postoperative dental infections and respiratory infections in immunocompromised patients.[26,27]

Only 2 bacterial types were common to the 3 culture methods tested in the study: *Cupriavidus metallidurans* and *Sphingomonas parapaucimobilis;* these were the only bacterial species recovered on SimPlate for HPC, verifying the limitations of this culture method for detection of common water organisms. *C. metallidurans* belongs to the α-Proteobacteria group, known to be the predominant survivor in chlorinated water distribution systems.[28,29]

The most frequently isolated genera in this study were Sphingomonas, also closely aligned with the phylogenic group α-Proteobacteria, and previously found in DUWL samples and ultrapure water.[2,15,29,30] A review of nosocomial infections concluded that the species *S. parapaucimobilis* has emerged in recent years as an opportunistic pathogen as it has been associated with many cases of bacteremia and other systemic infections in immunocompromised patients.[31]

Conclusion

The variety of potentially pathogenic organisms recovered from waterlines in this study reinforces the need for monitoring DUWL quality to ensure the delivery of high quality dental patient treatment water.

The study confirmed that Millipore HPC Samplers are useful for routine in-office, chairside DUWL quality monitoring when the benchmark CDC recommended level of <500 CFU/ml is used. The study also confirmed that the spread plate R2A agar method (9215C) provides the most accurate analysis of DUWL quality.

The laboratory SimPlate for HPC method (9215E) failed to detect microbial contamination of DUWL samples to the same extent as Method 9215C, most likely due to the specific design of SimPlate for HPC for the recovery of fast-growing organisms at 35°C. While Method 9215E clearly has value for application in EPA compliance monitoring, this study found that it is not acceptable for application in DUWL quality monitoring, when quantification of slow-growing water organisms at 22°C-28°C and a correct assessment of dental patient treatment water quality are required.

Dental offices can reliably use in-office, chairside Millipore HPC Samplers to screen DUWL quality, ensuring that patient treatment water is compliant with the EPA/CDC recommendation of <500 CFU/ml. However, for offices that rely on commercial laboratories to provide an accurate assessment of their DUWL quality, it is recommended that the spread plate R2A method (9215C) be requested, rather than the SimPlate for HPC method (9215E), when DUWL samples are submitted for analysis.

Acknowledgments

References

1. Mills SE. The dental unit waterline controversy: defusing the myths, defining the solutions. J Am Dent Assoc. 2000; 131(10):1427–1441. [PubMed: 11042982]

2. Szymanska J. Bacterial contamination of water in dental unit reservoirs. Ann Agric Environ Med. 2007; 14(1):137–140. [PubMed: 17655191]

3. Cunningham, AB.; Lennox, JE.; Ross, RJ., editors. [Accessed November 7, 2014] Biofilm growth and development. Biofilms: The Hypertextbook. Available at: http://www.biofilmbook.com

4. Lenz AP, Williamson KS, Pitts B, Stewart PS, Franklin MJ. Localized gene expression in *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol. 2008; 74(14):4463–4471. [PubMed: 18487401]

5. Kohn WG, Collins AS, Cleveland JL, et al. Guidelines for infection control in dental health-care settings – 2003. MMWR Recomm Rep. 2003; 52(RR17):1–61. [PubMed: 14685139]

6. United States Environmental Protection Agency. [Accessed November 7, 2014] National Primary Drinking Water Regulations. Available at: http://www.epa.gov/safewater/contaminants/index.html

7. [Accessed November 7, 2014] EMD Millipore [product guide]. Available at: http://www.millipore.com/catalogue/module/C10712

8. Momeni SS, Tomline N, Ruby JD, Dasanayake AP. Evaluation of in-office dental unit waterline testing. Gen Dent. 2012; 60(3):142–147.

9. Morris BF, Vandewalle KS, Hensley DM, Bartoloni JA. Comparison of in-office dental unit waterline test kits. Mil Med. 2010; 175(11):901–906. [PubMed: 21121503]

10. Bartoloni JA, Porteous NB, Zarzabal LA. Measuring the validity of two in-office water test kits. J Am Dent Assoc. 2006; 137(3):363–371. [PubMed: 16570470]

11. American Water Works Association. American Public Health Association. Water Environment Federation. Microbiological examination. In: Rice, EW.; Baird, RB.; Eaton, AD.; Clesceri, LS., editors. Standard Methods for the Examination of Water and Wastewater. 22nd ed. Washington, DC: 2012. p. 9.49-9.52.

12. De Paola LG, Mangan D, Mills SE, et al. A review of the science regarding dental unit waterlines. J Am Dent Assoc. 2002; 133(9):1199–206. quiz 1260. [PubMed: 12356251]

13. Stillings, A.; Herzig, D.; Roll, B. Comparative Assessment of the Newly-Developed Simplate™ Method With the Existing EPA-Approved Pour Plate Method for the Detection of Heterotrophic Plate Count Bacteria in Ozone-Treated Drinking Water. International Ozone Association Conference; October 1998; Available at: https://www.idexx.com/resource-library/water/water-reg-article8B.pdf

14. Porteous N, Sun Y, Dang S, Schoolfield J. A comparison of 2 laboratory methods to test dental unit waterline water quality. Diag Microbiol Infect Dis. 2013; 77(3):206–208.

15. Porteous N, Luo J, Hererra M, Schoolfield J, Sun Y. Growth and identification of bacteria in N-halamine dental unit waterline tubing using an ultrapure water source. Int J Microbiol. 2011; 767314

16. Jackson RW, Osborne K, Barnes G, et al. Multiregional evaluation of the SimPlate heterotrophic plate count method compared to the standard plate count agar pour plate method in water. Appl Environ Microbiol. 2000; 66(1):453–454. [PubMed: 10618266]

17. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol. 2008; 74(8):2461–2470. [PubMed: 18296538]

18. National Center for Biotechnology Information. [Accessed November 10, 2014] Blast: Basic Local Alignment Search Tool. Available at: http://blast.ncbi.nlm.nih.gov/Blast.cgi

19. Allen MJ, Edberg SC, Reasoner DJ. Heterotrophic plate count bacteria—what is their significance in drinking water? Int J Food Microbiol. 2004; 92(3):265–274. [PubMed: 15145585]

20. Smith RS, Pineiro SA, Singh R, Romberg E, Labib ME, Williams HN. Discrepancies in bacterial recovery from dental unit water samples on R2A medium and a commercial sampling device. Current Microbiol. 2004; 48(4):243–246. [PubMed: 15057447]

21. Cohen ME, Harte JA, Stone ME, O'Connor KH, Coen ML, Cullum ME. Statistical modeling of dental unit water bacterial test kit performance. J Clin Dent. 2007; 18(2):39–44. [PubMed: 17508622]

22. Reasoner DJ. Heterotrophic plate count methodology in the United States. Int J Food Microbiol. 2004; 92(3):307–315. [PubMed: 15145589]

23. Miltiadous G, Elisaf M. Native valve endocarditis due to *Micrococcus luteus:* a case report and review of the literature. J Med Case Rep. 2011; 5:251. [PubMed: 21714882]

24. Rennie DC, Lawson JA, Kirychuk SP, et al. Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. Indoor Air. 2008; 18(6):447–453. [PubMed: 18681911]

25. Huntington MK, Williams JF, Mackenzie CD. Endotoxin contamination in the dental surgery. J Med Microbiol. 2007; 56(Pt 9):1230–1234. [PubMed: 17761488]

26. Martin MV. The significance of the bacterial contamination of dental unit water systems. Br Dent J. 1987; 163(5):152–154. [PubMed: 3117080]

27. Langton Hewer SC, Smyth AR. Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. Cochrane Database Syst Rev. 2009; 4 CD004197.

28. von Rozycki T, Nies DH. *Cupriavidus metallidurans:* evolution of a metal-resistant bacterium. Antonie van Leeuwenhoek. 2009; 96(2):115–139. [PubMed: 18830684]

29. Williams MM, Domingo JW, Meckes MC, Kelty A, Rochon HS. Phylogenic diversity of drinking water bacteria in a distribution system simulator. J Appl Microbiol. 2001; 96(5):954–964. [PubMed: 15078511]

30. Oie S, Oomaki M, Yorioka K, et al. Microbial contamination of 'sterile water' used in Japanese hospitals. J Hosp Infect. 1998; 38(1):61–65. [PubMed: 9513069]

31. Ryan MP, Adley CC. *Sphingomonas paucimobilis:* a persistent Gram-negative nosocomial infectious organism. J Hosp Infect. 2010; 75(3):153–157. [PubMed: 20434794]

Manufacturers

Becton, Dickson & Company, Franklin Lakes, NJ

888.237.2862, www.bd.com

EMD Millipore, Billerica MA

781.533.6000, www.emdmillipore.com

IDEXX Laboratories, Inc., Westbrook, ME

800.548.6733, www.idexx.com

MJ Research, Inc., St. Bruno, Quebec, Canada

450.461.6245, mj-research.com

Promega Corporation, Madison, WI

608.274.4330, www.promega.com

Qiagen Sciences, Inc., Germantown, MD

240.686.7700, www.qiagen.com

StataCorp LP, College Station, TX

800.782.8272, www.stata.com

Thermo Fisher Scientific, Inc., Waltham, MA

800.678.5599, www.thermofisher.com
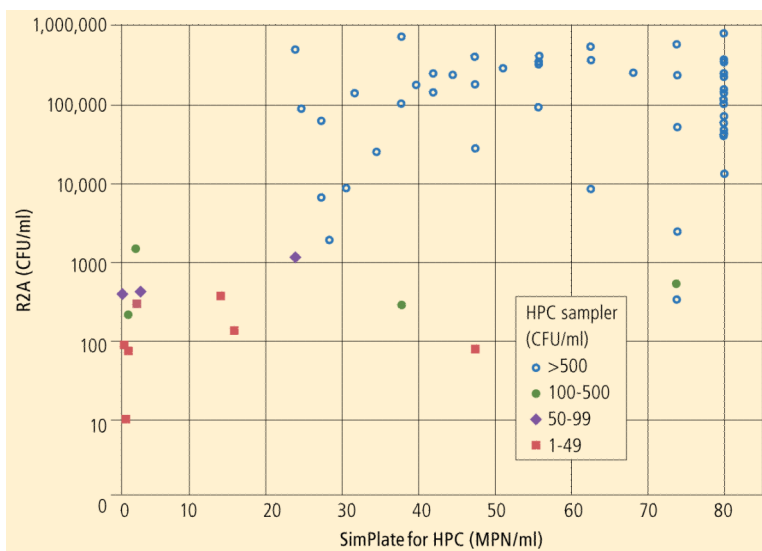
Chart.

R2A with SimPlate for HPC method scatterplot, displaying legend markers indicating the range of the corresponding HPC Sampler value for each sample.

Note that 2 handpiece samples for which no CFUs were detected by HPC Sampler were excluded. All samples with R2A >1500 CFU/ml had HPC Sampler values >500 CFU/ml, while only 1 sample with R2A <1500 CFU/ml had an HPC Sampler value >500 CFU/ml.

Table 1

HPC from DUWL samples

Dental unit	Sample source	R2A agar CFU/ml	SimPlate MPN/ml	HPC sampler CFU/ml
1	Handpiece	61,300	26.6	1000[a]
	Air/water	87,000	23.9	1000
	Source water	1,130	23.1	59
2	Handpiece	494,000	23.1	1000
	Air/water	102,000	37.2	1000
	Source water	293	2.1	12
3	Handpiece	142,000	41.4	1000
	Air/water	6,570	26.6	1000
	Source water	8,430	29.9	1000
4	Handpiece	138,000	31.1	1000
	Air/water	392,000	47.0	1000
	Source water	86.7	0.4	33
5	Handpiece	341,000	55.5	1000
	Air/water	235,000	44.0	1000
	Source water	1,470	1.9	360
6	Handpiece	252,000	68.0	1000
	Air/water	284,000	50.7	1000
	Source water	1,850	27.6	1000
7	Handpiece	642,000	62.3	0
	Air/water	573,000	73.8	1000
	Source water	327	73.8	1000
8	Handpiece	361,000	239.0	1000
	Air/water	535,000	62.3	1000
	Source water	73.3	1.0	20
9	Handpiece	178,000	47.0	1000
	Air/water	39,900	80.0[b]	1000
	Source water	10	0.6	1
10	Handpiece	151,000	80.0[b]	1000
	Air/water	323,000	55.5	1000
	Source water	24,600	33.9	1000
11	Handpiece	243,000	41.4	1000
	Air/water	176,000	39.2	1000

Dental unit	Sample source	R2A agar CFU/ml	SimPlate MPN/ml	HPC sampler CFU/ml
	Source water	27,700	47.0	1000
12	Handpiece	543,000	80.0[b]	0
	Air/water	47,300	80.0[b]	1000
	Source water	133	15.1	39
13	Handpiece	411,000	55.5	1000
	Air/water	244,000	80.0[b]	1000
	Source water	387	0.2	86
14	Handpiece	795,000	257.0	1000
	Air/water	722,000	37.2	1000
	Source water	213	1.0	151
15	Handpiece	233,000	73.8	1000
	Air/water	362,000	62.3	1000
	Source water	417	2.6	73
16	Handpiece	8,370	62.3	1000
	Air/water	139,000	80.0[b]	1000
	Source water	76.7	47.0	39
17	Handpiece	115,000	80.0[b]	1000
	Air/water	101,000	80.0[b]	1000
	Source water	2,390	73.8	600
18	Handpiece	33,400	NA	1000
	Air/water	70,700	112.0	1000
	Source water	283	37.2	216
19	Handpiece	42,300	80.0[b]	1000
	Air/water	13,100	80.0[b]	1000
	Source water	527	73.8	116
20	Handpiece	343,000	440.0	1000
	Air/water	226,000	80.0[b]	1000
	Source water	367	13.2	36
21	Handpiece	91,300	55.5	1000
	Air/water	57,700	80.0[b]	1000
	Source water	51,300	73.8	1000

[a]1000 CFU/ml

[b]MPN >73.8/ml

Abbreviations: CFU, colony forming units; DUWL, dental unit waterline; HPCs, heterotrophic plate counts; MPN, most probable number; NA, not available.

Table 2

Correlation coefficients (95% confidence interval).

Sample source	Pearson	Spearman
	R2A with SimPlate for HPC	
All	0.607 (0.421, 0.744)	0.475(0.256, 0.648)
Handpiece	0.103 (−0.356, 0.522)	0.174 (−0.291, 0.572)
Air/water	−0.068 (−0.486, 0.375)	−0.268 (−0.627, 0.185)
Source water	0.481 (0.062, 0.756)	0.521 (0.115, 0.778)
	Millipore with SimPlate for HPC	
All	0.650 (0.474, 0.776)	0.598 (0.406, 0.740)
Source water	0.573 (0.188, 0.805)	0.624 (0.263, 0.832)
	R2A with Millipore	
All	0.871 (0.793, 0.921)	0.734 (0.592, 0.832)
Source water	0.795 (0.554, 0.913)	0.797 (0.557, 0.914)

Table 3

Bacteria recovered from DUWL samples.

Acidovorax sp. [a]

Acidovoraxcitrulli

Acidovoraxtemperans

Afipia sp.

Blastomonas natatoria

Bradyrhizobium sp.

Bradyrhizobium yuanmingens

Caulobacter segnis

Cupriavidus basilensis

Cupriavidus metallidurans [b]

Methylobacterium extorquens

Methylobacterium oryzae

Methylobacterium populi

Methylobacterium radiotolerans

Methylobacterium rhodesianum

Methylobacterium thiocyanatum

Micrococcus luteus

Novosphingobium stygium

Pseudomonas koreen [c]

Pseudomonas libane [c]

Ralstonia sp.

Sphingobium sp.

Sphingobium amiense

Sphingomonas sp.

Sphingomonas adhaesiva

Sphingomonas parapaucimobilis [b]

Sphingomonas sanguinis

Sphingomonas trueperi

Sphingomonas yunnanensis

Sphingopyxis alaskensis

Sphingopyxis chilensis

Xenophilus aerolatus

Xulophilus ampelinus

[a]Grown on R2A and Millipore HPC Samplers

[b]Grown on all 3 media

[c]Grown on Millipore HPC Samplers only

No symbol: grown on R2A agar only