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A comparison of two laboratory methods to test dental unit waterline water quality

Nuala Porteous[*,1], **Yuyu Sun**[2], **Shichien Dang**[1], and **John Schoolfield**[1]

[1]Department of Comprehensive Dentistry, The University of Texas Health Science Center at San Antonio San Antonio, TX 78229, USA

[2]The University of Massachusetts, 1 University Ave, Lowell, MA 01854

Abstract

The performance of two APHA standard laboratory methods, the R2A spread plate and the SimPlate™ for heterotrophic plate count (HPC), for quantifying heterotrophic microorganisms in dental waterline samples was evaluated. Microbial counts were underestimated on SimPlate™ compared with R2A and the results indicated a poor correlation between the two methods.

Keywords

Dental unit waterlines; Dental unit waterline contamination; Dental unit waterline monitoring

Waterlines in functioning dental units have been shown to contain bacterial biofilms up to 50 microns thick, comprised of a heterogeneous population of microorganisms (Porteous et al., 2011; Szymanska 2007). Although bacterial biofilms remain fixed to the tubing wall, microbes are continuously sloughed off as the water flows through, causing contamination of the patient treatment water (Cunningham et al., 2011; Lenz et al., 2008).

The Centers for Disease Control and Prevention (CDC) recommends that dental offices should ensure that the level of non-coliform bacteria in patient treatment water meets the U.S. Environmental Protection Agency (EPA) drinking water standard of <500 colony forming units per milliliter (CFU/mL) (U.S. EPA 1999; CDC 2003). Dental practitioners are encouraged to monitor the level of dental unit waterline (DUWL) contamination regularly in order to comply with this recommendation. Monitoring DUWL quality can be done by using in-office chairside kits or by using a mail-in service provided by commercial laboratories.

Standard laboratory testing methods have been established by The American Public Health Association, American Water Works Association, and The Water Environment Federation (APHA et al., 2012). Four different methods (9215B-9215E) and five different types of media are recommended for use with specific applications. Each method is designed to provide the heterotrophic plate count (HPC), an estimate of the number of live heterotrophic bacteria in water samples. The use of low-nutrient media such as R2A agar (Beckton, Dickson and Company, Sparks, MD) is considered best suited to the cultivation of a variety

[*]**Corresponding Author:** Nuala Porteous, BDS, MPH, Associate Professor/Research, Department of Comprehensive Dentistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, MC 7914, San Antonio, TX 78229-3900, 210-567-6334, 210-567-6348 (fax), porteous@uthscsa.edu.

of slow-growing, indigenous water organisms (Reasoner 2004). The spread plate method (9215C), using R2A agar allows microbial colonies to grow on the agar surface at 20 to 28°C over a period of 7 days. The limitation of this method is that it relies on a small volume of water sample, which can be absorbed if the agar is dry (APHA et al., 2012). However, this is generally accepted as the most appropriate method for culturing organisms from DUWL samples (Bartoloni et al., 2006).

This most recent addition to the list of standard methods is 9215E, the SimPlate™ for HPC (IDEXX Laboratories, Westbrook, ME). It is a less tedious method than 9215C that merely involves mixing the water sample with a proprietary substrate and as microbial enzymes metabolize the substrate, they fluoresce after 48 hours of incubation at 35°C (APHA et al., 2012). The number of fluorescent wells are counted and converted to the most probable number (MPN), using a table provided by the manufacturers (Stillings et al., 1998). As this method becomes more widely used, dental offices may be obtaining results from commercial laboratories that use this method rather than Method 9215C. The purpose of this experiment was to compare Method 9215C and 9215E for culturing DUWL samples.

An a *priori* power analysis, performed using PASS 11 software (NCSS Inc., Kaysville, UT), was first conducted to determine sample size. Fifteen functioning dental units in a teaching clinic were randomly selected from 300 dental operatories and water samples were taken from the handpiece and air/water syringe lines on each unit, and from the source faucet water in each operatory. One-hundred mL sterile collection bottles that contained sodium thiosulfate to neutralize residual chlorine (IDEXX Labs, Westbrook, ME) were used to collect a total of 45 samples. Ten-fold serial dilutions of each sample were made with phosphate buffer solution.

For the R2A cultures, 0.1mL of each solution was spread on R2A plates in triplicate, incubated at room temperature, and the microbial CFU/mL was recorded after 7 days (APHA, Method 9215C). For the SimPlate™ cultures, 10 mL of each solution were placed in the center of the SimPlates™ and manufacturers' instructions were followed. Plates were incubated for 48 hrs at 35°C (Jackson et al., 2000) and the MPN/mL was calculated.

Statistical analyses and graphics were performed using Stata 12.0 (StataCorp LP, College Station, TX). Microbial counts for each of the methods are provided in Table 1. As expected, the R2A measures approximated an exponential distribution; however, the SimPlate™ for HPC values approximated a uniform distribution between 0 and an upper threshold value of >73.8 MPN/mL, so correlations were performed instead of paired Student's t-test using log transformed R2A measures and raw HPC values. The overall Pearson correlation coefficient of 0.423 with 95% c.i. of (0.148, 0.637) was weak, while the corresponding Spearman rank correlation coefficient of 0.216 with 95% c.i. of (−0.082, 0.480) was poorer, which suggested that the Pearson correlation was influenced by extreme values. Correlations for each source type were also performed (Figure 1) with similar results.

To depict the pairwise association, a scatterplot (Figure 2), displaying the paired results for each sample with symbols indicating the source type, illustrates the extreme values that inflated the Pearson coefficient relative to the Spearman coefficient were three source water samples with virtually undetectable contamination.

A previous study showed that the SimPlate™ for HPC method produced similar results to the pour plate 9215 B method that uses the less sensitive plate count agar and incubation at 35°C, but lower counts than the membrane filter R2A method (9215D) that uses room temperature incubation for 7 days (Stillings et al., 1998). Our study showed similar results. Furthermore, many of our undiluted samples resulted in the maximum number of fluorescent

wells, corresponding to a MPN of >73.8/mL; yet, ten-fold dilutions did not provide the expected results with the majority of those showing zero fluorescent wells.

In summary, the SimPlate™ for HPC method failed to detect microbial levels in DUWL samples to the same extent as the R2A spread plate method. Due to potential undesirable consequences of DUWL contamination for dental personnel and patients regular monitoring and accurate assessment of DUWL quality is essential (Atlas 1995; Ricci 2012; CDC 2003). As some dental offices rely on commercial laboratories to provide this service, it is recommended that the R2A spread plate 9215C method be used for analyzing DUWL samples. Other findings of note in this study, such as the high microbial levels found in the source water and DUWL samples should be further investigated.

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	Correlation Coeffs with 95% Confidence Bounds			
Sample Source	Pearson		Spearman	
All	0.423	(0.148, 0.637)	0.216	(-0.082, 0.480)
Handpiece	-0.210	(-0.652, 0.339)	-0.444	(-0.779, 0.089)
Air/Water	0.187	(-0.360, 0.638)	0.200	(-0.348, 0.646)
Source Water	0.639	(0.188, 0.867)	0.456	(-0.073, 0.785)

Figure 1.
Correlations between R2A spread plate and SimPlate™ for HPC values.

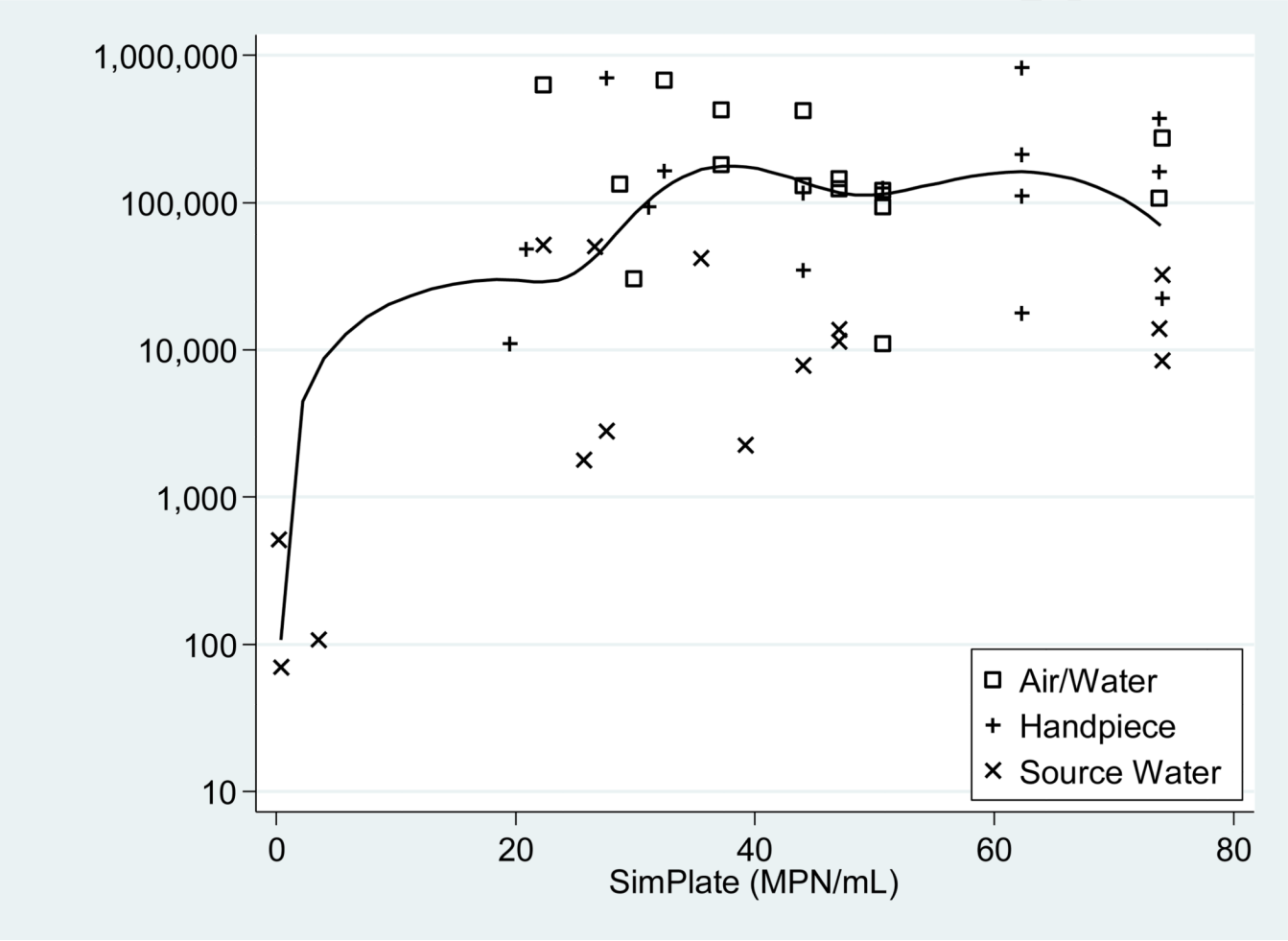

Figure 2.
Scatterplot of SimPlate™ (MPN/mL) values matched with R2A (CFU/mL) values. A log base 10 transformation was applied to the R2A (vertical) axis. A median spline curve was used to illustrate the correlation between the SimPlate™ and R2A values.

TABLE 1

Number of colony forming units/per milliliter (CFU/mL) and Most Probable Number/milliliter (MPN/mL) obtained from handpiece lines, air/water syringes and source water for each dental unit.

Dental Unit	Sample Source	CFU/mL (R2A)	MPN/mL(SimPlate™)
1	Handpiece	10,967	19.5
	Air/Water	275,333	*74.0
	SourceWater	11,500	47.0
2	Handpiece	17,733	62.3
	Air/Water	11,033	50.7
	SourceWater	2,800	27.6
3	Handpiece	48,333	20.9
	Air/Water	146,333	47.0
	SourceWater	7,867	44.0
4	Handpiece	35,000	44.0
	Air/Water	682,667	32.4
	SourceWater	13,967	*74.0
5	Handpiece	94,000	31.1
	Air/Water	424,667	37.2
	SourceWater	513	0.2
6	Handpiece	372,000	*74.0
	Air/Water	419,333	44.0
	SourceWater	2,240	39.2
7	Handpiece	126,000	50.7
	Air/Water	124,000	47.0
	SourceWater	8,400	*74.0
8	Handpiece	110,667	62.3
	Air/Water	94,000	50.7
	SourceWater	32,333	*74.0
9	Handpiece	22,500	*74.0
	Air/Water	130,667	44.0
	SourceWater	42,000	35.5
10	Handpiece	162,000	*74.0
	Air/Water	107,667	*74.0
	SourceWater	70	0.4
11	Handpiece	117,000	44.0
	Air/Water	30,333	29.9
	SourceWater	51,667	22.3
12	Handpiece	703,333	27.6
	Air/Water	633,333	22.3

Dental Unit	Sample Source	CFU/mL (R2A)	MPN/mL(SimPlate™)
	SourceWater	107	3.5
13	Handpiece	165,000	32.4
	Air/Water	121,333	50.7
	SourceWater	50,333	26.6
14	Handpiece	213,333	62.3
	Air/Water	134,333	28.7
	SourceWater	13,767	47.0
15	Handpiece	822,000	62.3
	Air/Water	182,333	37.2
	SourceWater	1,783	25.7

* MPN of >73.8/mL