Rationale for R2A Dental Water testing methods

- Methods such as SimPlate and R2A differ significantly. SimPlate incubates at 35 degrees C and R2A incubates at 20 degrees C.

Dental biofilms develop at 20C. Many of the organisms that are common in dental units are not detected at 20 C. Idexx Simplate was developed to look for E Coli and other organisms that thrive at 35C or body temperature. We have tested Simplate side by side with R2A and SimPlate misses too many organisms to be appropriate for testing dental water.

- The incubation temperature can really be considered just another way to select for a certain bacterial population. A dental unit in not 35 C which is why every university that has tested it has stated that it is not appropriate for dental unit waterline testing.
- The incubation temperature for a method at 35C will tend to favor the detection of those bacterial populations that grow at temperatures near human body temperature (that's why that temp was selected for e coli) which is not going to show up in dental unit waterline biofilms.

In every paper written about dental unit waterline heterotrophic plate counts, R2A is used as the gold standard. There is not one university study that found Idexx SimPlate to be appropriate for use in dental bacterial counts.

Below is a peer reviewed paper by Dr. Nuala Porteous, Department of Comprehensive Dentistry, University of Texas Health and Science Center at San Antonio (UTHSCSA) that states:
"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."

In a similar peer reviewed paper
Comparison of in-office dental unit waterline test kits.

Morris BF1, Vandewalle KS, Hensley DM, Bartoloni JA.

Abstract
The authors conducted a study to determine the validity of two commercially available in-office dental unit waterline test kits compared to the gold standard, R2A agar. Samples were collected from the air/water syringes of dental units and cultured on HPC Samplers, Petrifilm AC Plates, and R2A agar plates. HPC Samplers and R2A agar plates were incubated for 7 days and counted manually using magnification. Petrifilm AC Plates were counted after incubation time of 5 and 7 days using an electronic-plate reader. Validity measurements were calculated using a cutoff value < or = 500 colony-forming units per milliliter. The accuracy for the HPC Sampler compared to R2A agar was 71%. The accuracy for the Petrifilm AC Plates at 5 and 7 days was 79% and 87% compared to R2A agar. The Petrifilm AC Plate (7-day incubation) demonstrated higher sensitivity, specificity, predictive values, and accuracy than the HPC Sampler kit.

Current Microbiology
April 2004, Volume 48, Issue 4, pp 243–246 Discrepancies in Bacterial Recovery from Dental Unit Water Samples on R2A Medium and a Commercial Sampling Device Authors Authors and affiliations Richard S. Smith Silvia A. Pineiro Ruby Singh Elaine Romberg Mohamed E. Labib Henry N. Williams
Article
DOI: 10.1007/s00284-003-4130-5
Cite this article as:
Smith, ., Pineiro, ., Singh, . et al. Curr Microbiol (2004) 48: 243.
doi:10.1007/s00284-003-4130-5

Abstract
Monitoring the number of bacterial colony-forming units is an important step in assuring compliance with the recommendation that water from dental units contain <200 CFU mL−1. Media that have been used for this purpose include R2A, a standard plate counting medium for water samples, and the Millipore HPC Sampler device, designed to facilitate sampling in dental offices.
Discrepancies between the two media have been observed. This study tested the hypothesis that differences in counts on the two media were due to the failure of some bacteria to grow on the HPC sampler or to grow at less efficiency than on R2A. Of four different bacterial colony phenotypes tested in three independent experimental trials, one phenotype did not grow on the HPC device, and another grew inconsistently and at lower efficiency. These results confirmed the hypothesis. From these findings, users of the HPC sampler should be aware that microbial undercounts may occur.

Evaluation of in-office dental unit waterline testing

Article in General dentistry 60(3):e142-7 · May 2012
Source: PubMed

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Abstract
In-office dental unit waterline (DUWL) testing systems are commercially available for monitoring DUWL bacteria. The current study compared Aquasafe, Petrifilm, and Heterotrophic Plate Count Sampler (HPCS) with R2A plating methodology, considered the gold standard for

enumerating heterotrophic bacteria in potable water. Samples were collected from 20 dental units.
Heterotrophic bacterial counts of ≤500 CFUs/mL were used as the cut-off for assessing in-office testing compared to R2A laboratory plating. Validity was assessed using sensitivity and specificity, along with positive and negative predictive values. Results were also compared using concordance and kappa statistics. All in-office tests demonstrated 100% specificity and positive predictive values, while sensitivity and negative predictive values were low (Petrifilm, 57%/50%; HPCS, 50%/46%; Aquasafe, 21%/35%). Concordance and kappa values for agreement with R2A plating were as follows: Petrifilm 70% ($\varkappa = 0.44$), HPCS 65% ($\varkappa = 0.38$), and Aquasafe 45% ($\varkappa = 0.14$). In-office DUWL testing with Aquasafe, Petrifilm, and HPCS agreed poorly with R2A plating methodology and is not valid or reliable as a means of accurately monitoring bacterial density in DUWL. These in-office test systems should not be used for assessing compliance with the ADA and CDC standard for acceptable heterotrophic bacterial counts in DUWLs (≤500 CFUs/mL