Bacterial Counts in Dental Unit Waterlines Treated with BioBlue™(*Lines ™*)

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OSAP Symposium, June 2002

Abstract

The objective was to test the efficacy of BioBlue (New Trademark "*Lines*") (Micrylium Laboratories), containing chlorhexidine as the active ingredient, to reduce bacterial levels in functioning dental units. Baseline water samples were first taken from three treatment units and three similar controls with self-contained reservoir systems, according to the ADA guidelines: reservoir bottles were filled with tap water; lines were flushed for 20-30 seconds before 100 ml pooled samples from four individual waterlines on each unit were collected in bottles containing one-tenth of a percent of sodium thiosulfate to neutralize residual chlorine. Control units did not have any chemical treatment during the study period. They were flushed daily according to the ADA/CDC recommendations and chlorine level of the source water was carefully monitored. Test units were treated according to the manufacturer's instructions: two ounces of undiluted BioBlue(*Lines*) was run though lines until visible, left in lines overnight, and flushed out next morning. This initial treatment was repeated for six nights during the winter holiday period while the units were not in use. After winter break, overnight treatment was continued once a week for twelve weeks. Weekly water samples were collected in bottles containing Sodium Thiosulphate on the afternoon before overnight treatment, and immediately taken to the laboratory. Triplicate ten-fold serial dilutions in phosphate buffer solution were made and agitated vigorously for 15 seconds. Samples were plated on R2A agar using the spread-plate technique and incubated at 22-28º C for 7 days. Mean CFU/ml in treatment units declined from 23, 389 ($\pm$20,085) at baseline to 6($\pm$10) in week 4; to 5 ($\pm$2) in week 12. Statistical analysis, using the one-tail t-test, showed a significant decrease (p<.0005, df=52) between treatment and control units. Intermittent treatment of dental unit waterlines with BioBlue(*Lines*) resulted in significantly reduced bacterial counts to levels that were consistently below the ADA goal of 200 CFU/ml for eight weeks.

Purpose of Study

The purpose of this study was to evaluate the ability of BioBlue(*Lines*) to reduce bacterial levels in dental unit waterlines.

Materials and Methods

No chemicals were added to treatment and control units for one week before the study began. All lines were flushed daily according to ADA/CDC recommendations. Bacterial and chlorine levels of source tap water were monitored.

3 Treatment Units
- 100 ml baseline water samples were collected
- Lines were treated overnight for 6 nights during winter break
- Water samples were taken after initial treatment
- BioBlue(*Lines*) was added as overnight treatment once a week for twelve weeks
- BioBlue(*Lines*) was flushed out next morning
- Weekly water samples were collected on afternoon before overnight treatment

3 Control Units
- 100 ml baseline water samples were taken
- No chemicals were added
- Lines were flushed daily

Laboratory Procedures
- Sodium thiosulfate was added to water samples to neutralize residual chlorine
- Ten-fold serial dilutions in phosphate buffer were made
- 100 µl of water samples were spiral-plated on R2A
- Samples were incubated for 7-10 days at 22-28ºC
- Bacterial colonies were counted after 7 days
- Mean number of colony forming units per milliliter (mean CFU/ml) were recorded

Results

	Baseline	*AIT	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Test units														
Mean cfu/ml	23388.89	5791.11	14.44	330.00	258.89	5.56	11.11	11.11	11.11	17.78	58.89	15.56	13.33	4.44
SD	20084.72	2364.16	13.88	401.34	255.09	9.62	19.25	11.71	8.39	25.02	99.13	13.47	15.28	1.92
Control units														
Mean cfu/ml	14122.22	13594.44	3244.44	2383.33	855.56	387.78	482.22	444.44	284.44	447.78	417.78	1743.33	603.33	918.89
SD	6393.60	9395.38	3950.29	2155.68	435.04	379.10	573.27	339.48	164.80	251.76	261.82	1792.13	372.87	1225.27

*after initial treatment,
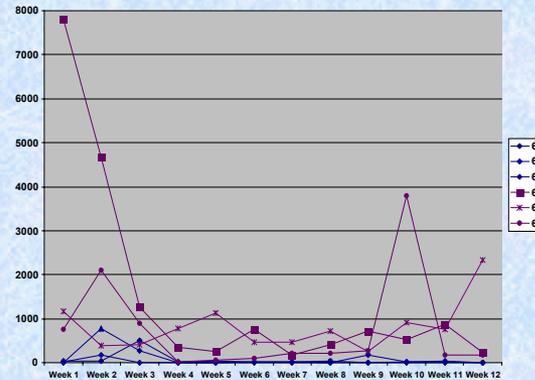1-tail t-test, P=0.0003

Figure I

Figure 1 shows the trends observed in individual units over the twelve week treatment period.
A steady decline in bacterial counts was seen in all three treatment units (63, 64, 65) until Week 4, after which low counts were steadily maintained for the remainder of the study.
A decrease in counts was also noted in control units (61, 62, 66) after the study began. Although fluctuations were observed in individual units, bacterial levels were reduced through strict adherence to ADA/CDC recommendations and close monitoring of bacterial and chlorine levels of source tap water.
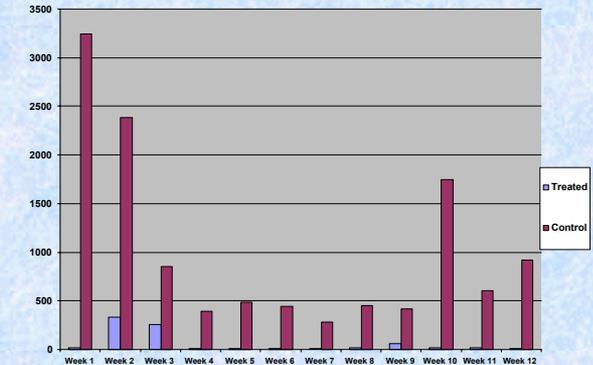
Figure 2

Figure 2 shows the mean number of CFU/ml found in Treatment and Control Units for the duration of the study

Conclusions

- Intermittent treatment of dental unit waterlines with BioBlue(*Lines*) resulted in significantly lower counts of heterotrophic, mesophilic bacteria than in untreated control units.
- Bacterial counts were maintained at sufficiently low levels to meet the ADA recommendation of 200 CFU/ml for eight consecutive weeks