

## FINAL REPORT

Grant Number 17858100 (BR01); Contract with Dentist Select

Action Date: 5/15/12

Budget Period: 6/15/12 to 9/15/12

**Objective:** Test the Dentist Select Mouthrinse for its ability to remove volatile sulfur compounds (VSCs) from cultures of periodontal pathogens. The species to be tested are: *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 49046), and *Treponema denticola* (ATCC 35405). *Streptococcus mitis* N551 was selected as a negative control.

[It will turn out that *T. denticola* did not grow in broth cultures to an extent necessary to complete testing. *Tannerella forsythia* (ATCC 43037) and *Treponema socranskii* (ATCC 35535) were substituted for *T. denticola*, though neither produced large amounts of VSCs.]

**Materials:** Dentist Select supplied their test Mouthrinse, the 0.12% chlorhexidine (CHX) solution, BreathRx, Crest Pro Health, CloSys, Oxyfresh Professional Zinc Rinse and the halimeter used to measure VSCs. Dr. Banas supplied Listerine.

### Experimental Design:

#### Protocol

In order to test the Dentist Select Mouthrinse under controlled conditions that would simulate actual use, we chose to measure the reduction in VSCs following a 30 second exposure. The test organism was grown in thioglycollate broth with hemin and menadione (Anaerobe Systems; Morgan Hill, CA) until turbid. The culture was mixed by vortexing and 2ml was used to saturate a 4.25cm glass fiber filter (G8; Fisher Scientific, Pittsburgh, PA) placed at the bottom of a 50ml Erlenmeyer flask. The mouth of the flask was covered with aluminum foil to retain VSCs. A baseline reading of VSCs was obtained by removing the foil and placing a two-hole rubber stopper into the mouth of the flask. One hole was connected via tubing to the halimeter (previously equilibrated to zero parts per billion) while the other hole was left open to facilitate continuous airflow. After the halimeter reached its peak reading, the rubber stopper was removed from the flask and the foil replaced. The peak reading was recorded as the baseline reading and the halimeter was left to re-equilibrate to a stable reading at or near zero. The filter-containing flask was then rinsed for 30 seconds with 20ml Dentist Select Mouthrinse, CHX, or water. The rinse was poured off and a post-rinse reading taken with the halimeter. Again, the peak reading was recorded as the post-rinse level of VSCs. Three independent trials were completed. Since it was noted that the baseline readings tended to decrease with each additional test, the order in which the rinses were tested (Dentist Select, CHX, water) was varied with each independent trial. This strategy worked well as the mean baselines did not differ statistically for any of the organisms tested.

### Results:

The raw data for each test species are shown in the series of tables below:

#### Testing *Porphyromonas gingivalis*

Trial	Dentist Select	Chlorhexidine	Water
1 Baseline (ppb)	220	255	133
1 Post 30 sec. rinse (ppb)	-4	41	24
Percent Reduction	>100	84	82
2 Baseline (ppb)	367	193	286
2 Post 30 sec. rinse (ppb)	-2	37	44
Percent Reduction	>100	81	85
3 Baseline	226	319	447
3 Post 30 sec. rinse	-7	59	78
Percent Reduction	>100	82	83
Average % Reduction	100	82	83

Testing *Prevotella intermedia*

Trial	Dentist Select	Chlorhexidine	Water
1 Baseline (ppb)	114	96	71
1 Post 30 sec. rinse (ppb)	0	13	10
Percent Reduction	100	86	86
2 Baseline (ppb)	77	44	63
2 Post 30 sec. rinse (ppb)	1	7	7
Percent Reduction	99	84	89
3 Baseline (ppb)	25	59	67
3 Post 30 sec. rinse (ppb)	-36	9	10
Percent Reduction	>100	85	85
Average % Reduction	100	85	87

Testing *Tannerella forsythia*

Trial	Dentist Select	Chlorhexidine	Water
1 Baseline (ppb)	10	10	5
1 Post 30 sec. rinse (ppb)	-70	2	1
Percent Reduction	>100	80	80
2 Baseline (ppb)	5	4	4
2 Post 30 sec. rinse (ppb)	-13	1	1
Percent Reduction	>100	75	75
3 Baseline (ppb)	3	4	6
3 Post 30 sec. rinse (ppb)	-9	1	1
Percent Reduction	>100	75	83
Average % Reduction	100	77	79

Testing *Treponema socranskii*

Trial	Dentist Select	Chlorhexidine	Water
1 Baseline (ppb)	8	10	4
1 Post 30 sec. rinse (ppb)	-21	1	0
Percent Reduction	>100	90	100
2 Baseline (ppb)	7	4	4
2 Post 30 sec. rinse (ppb)	-161	1	1
Percent Reduction	>100	75	75
3 Baseline (ppb)	3	4	7
3 Post 30 sec. rinse (ppb)	-43	1	0
Percent Reduction	>100	75	100
Average % Reduction	100	80	92

Testing *Streptococcus mitis*

Trial	Dentist Select	Chlorhexidine	Water
1 Baseline (ppb)	3	4	2
1 Post 30 sec. rinse (ppb)	-65	1	0
Percent Reduction	>100	75	100
2 Baseline	3	2	2
2 Post 30 sec. rinse	-74	1	1
Percent Reduction	>100	50	50
3 Baseline (ppb)	2	4	3

3 Post 30 sec. rinse (ppb)	-97	2	2
Percent Reduction	>100	50	33
Average % Reduction	100	58	61

The order of testing for trial 1 was CHX, Dentist Select, water; for trial 2 it was Dentist Select, water, CHX; and for trial 3 it was water, CHX, Dentist Select.

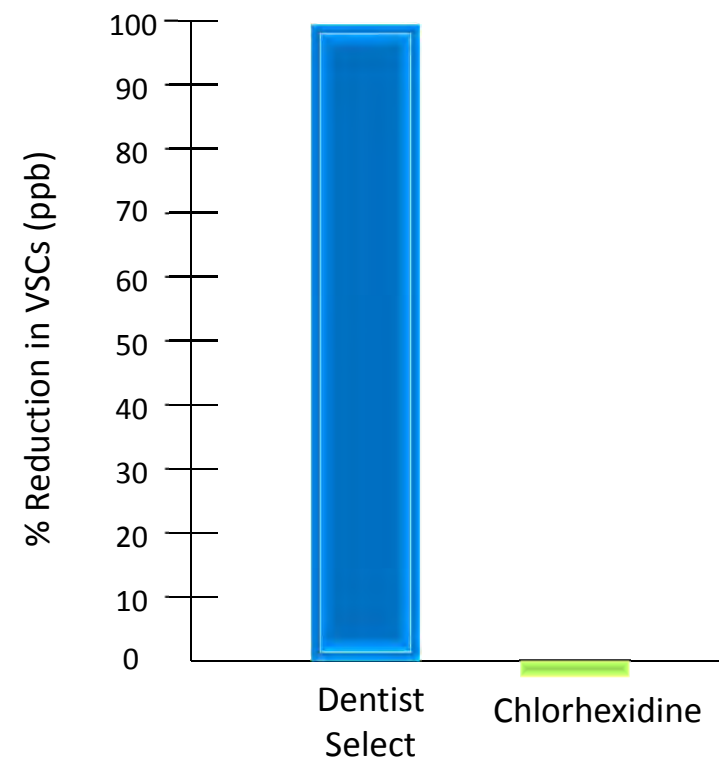
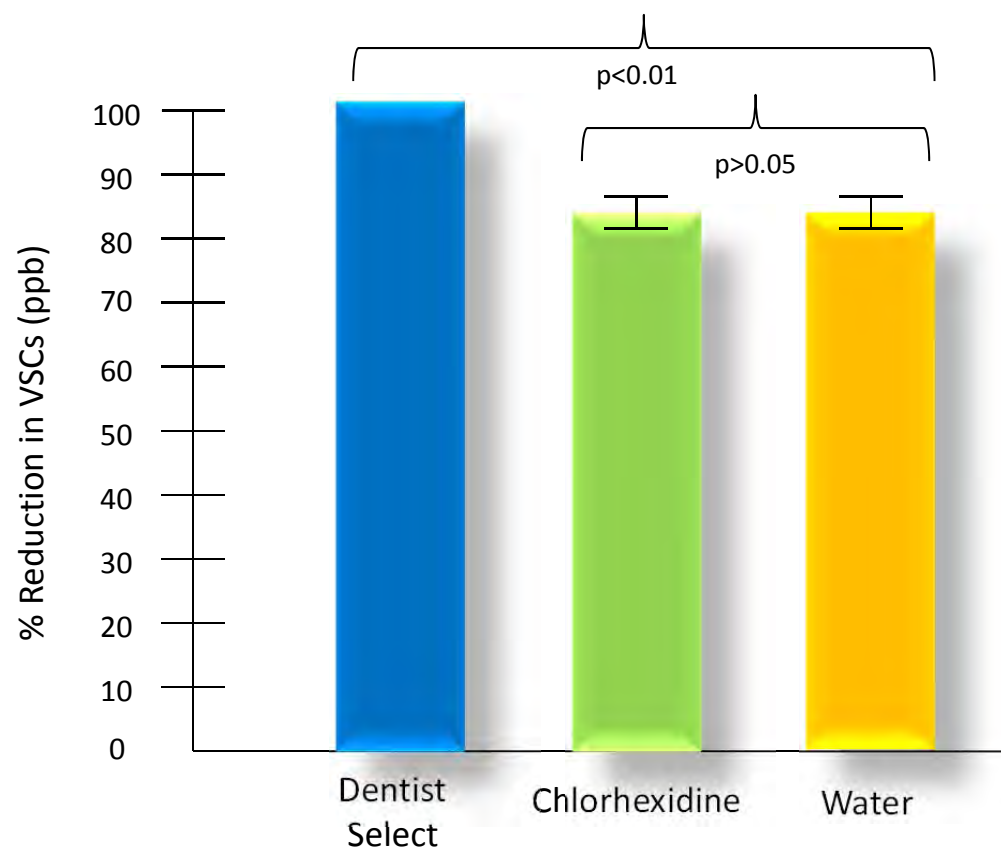
Pooling the data and applying statistics (ANOVA and Tukey HSD test) yields the following:

<i>Porphyromonas gingivalis</i>	Dentist Select	Chlorhexidine	Water
Mean Baseline VSCs (ppb)	271	256	289
Mean Post-Rinse VSCs (ppb)	0 <sup>#</sup> ± 3	46 ± 12	49 ± 27
Mean Percent Reduction	100 <sup>^</sup> ± 0	82 ± 2	83 ± 2

\*Significantly different than “Water” for the value in the horizontal category;  $p < 0.05$ .

<sup>#</sup>Values below 0 were rounded up to 0.

<sup>^</sup>Values above 100 were rounded down to 100.



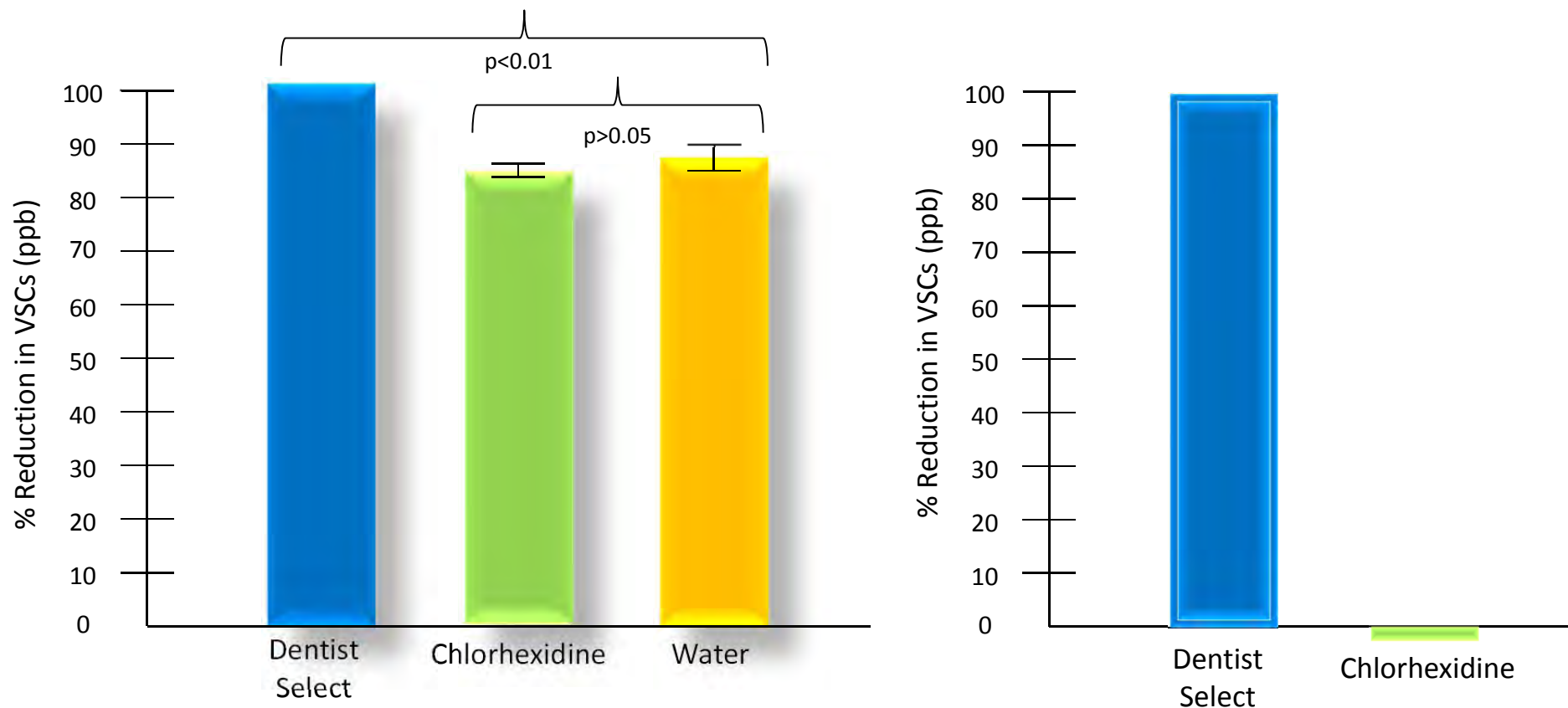
Dentist Select, but not chlorhexidine, removes significantly more VSCs produced by *P. gingivalis* than does water (left). When normalized to water (right), Dentist Select removes 100% of the remaining VSCs produced by *P. gingivalis* whereas chlorhexidine does not remove any of the remaining VSCs.

<i>Prevotella intermedia</i>	Dentist Select	Chlorhexidine	Water
Mean Baseline VSCs (ppb)	72	66	67
Mean Post-Rinse VSCs (ppb)	0 <sup>#</sup> ± 21	10 ± 9	9 ± 2
Mean Percent Reduction	100 <sup>^</sup> ± 0	85 ± 1	87 ± 2

\*Significantly different than “Water” for the value in the horizontal category based on ANOVA and Tukey HSD test;  $p < 0.05$ .

<sup>#</sup>Values below 0 were rounded up to 0.

<sup>^</sup>Values above 100 were rounded to 100.



Dentist Select, but not chlorhexidine, removes significantly more VSCs produced by *P. intermedia* than does water (left). When normalized to water (right), Dentist Select removes 100% of the remaining VSCs produced by *P. intermedia* whereas chlorhexidine does not remove any of the remaining VSCs.

Additional organisms tested included *Tannerella forsythia*, *Treponema socranskii*, and *Streptococcus mitis* which was originally chosen to be a negative control. Each of these species produced low amounts of VSCs. The baseline readings for *T. forsythia* ranged from 5-10ppb, 4-10ppb for *T. socranskii*, and 2-4ppb for *S. mitis*. Dentist Select removed all remaining VSCs whereas the low VSC values resulted in wide fluctuations for chlorhexidine (58-77% reductions) and for water (61-92% reductions).

During a telephone conference on August 2, 2012 it was decided that the Dentist Select Mouthrinse should be tested against other mouth rinses since chlorhexidine did not show an ability to reduce VSCs beyond the reduction obtained when rinsing with water. Dr. Downs sent several mouth rinses; we supplied Listerine. The protocol for testing was the same as before with the exception that only *P. gingivalis* was tested and that separate cultures of *P. gingivalis* were used for each group of three mouth rinses (the order of testing rotated within the group) and for the three trials using water as a control.

The raw data are shown in the table below:

Testing *P. gingivalis*

Trial	BreathRx	Crest Pro Health	CloSys	Oxyfresh Zinc	Dentist Select	Listerine	Water
1 Baseline (ppb)	433	286	173	414	329	187	412
1 Post Rinse	80	59	25	73	-25	35	54
% Reduction	82	79	86	82	>100	81	87
2 Baseline (ppb)	230	398	366	186	396	253	273
2 Post rinse	37	79	37	28	-72	46	50
% Reduction	84	80	90	85	>100	82	82
3 Baseline (ppb)	236	161	283	239	155	335	200
3 Post rinse	51	34	46	38	-95	53	32
% Reduction	78	79	84	84	>100	84	84
Average % Reduction	81	79	87	84	100	82	84

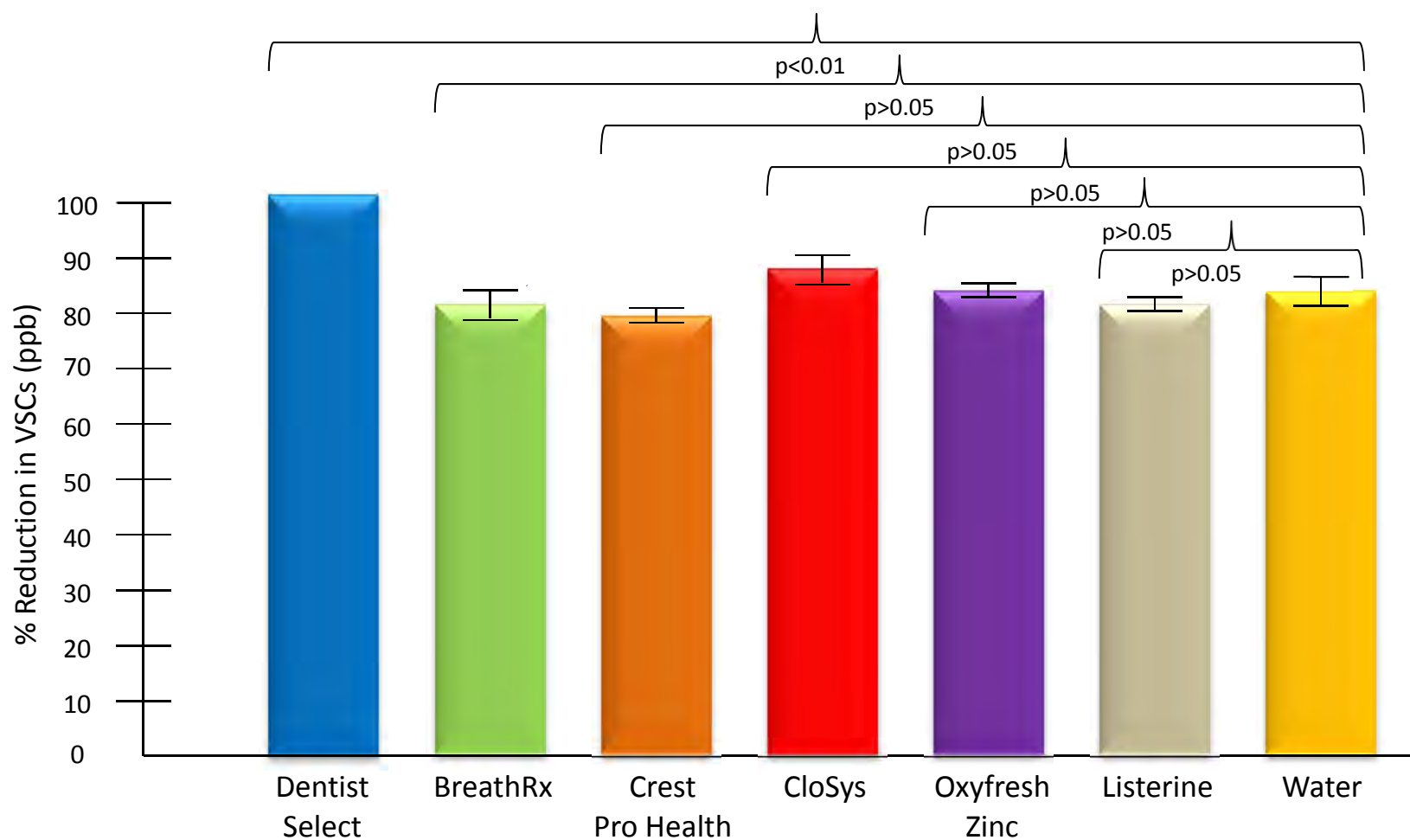
<i>Porphyromonas gingivalis</i>	Dentist Select	BreathRx	Crest Pro Health	CloSys	Oxyfresh Zinc	Listerine	Water
Mean Baseline VSCs (ppb)	293	300	282	274	280	258	295
Mean Post-Rinse VSCs (ppb)	0 <sup>#*</sup> ± 36	56 ± 22	57 ± 23	36 ± 11	46 ± 24	45 ± 9	45 ± 12
Mean Percent Reduction	100 <sup>^*+</sup> ± 0	81 ± 3	79 ± 1	87 ± 3	84 ± 2	82 ± 2	84 ± 3

\*Significantly different than “Water” for the value in the horizontal category based on ANOVA and Tukey HSD test;  $p < 0.01$ .

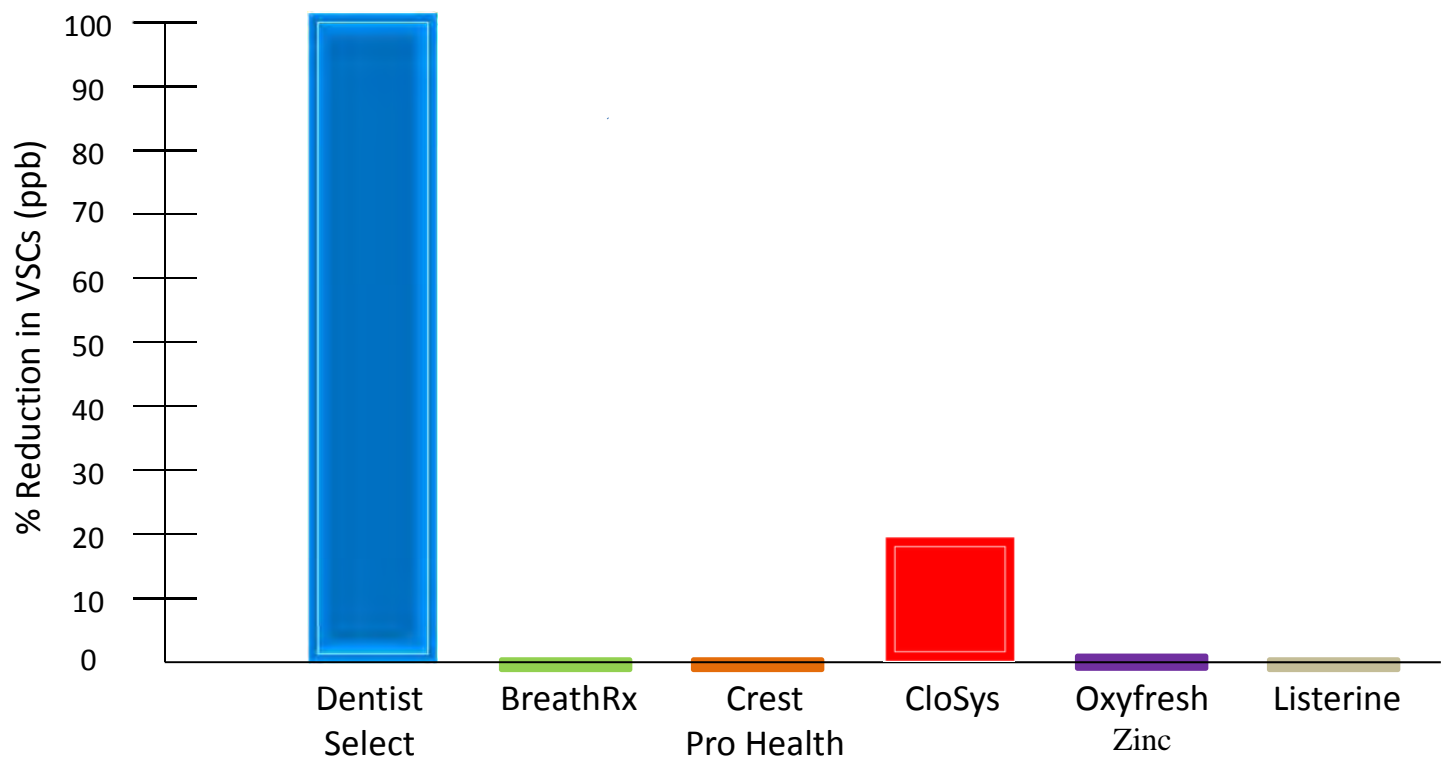
+Significantly different than all other values in the horizontal category based on ANOVA and Tukey HSD test;  $p < 0.01$ .

<sup>#</sup>Values below 0 were rounded up to 0.

<sup>^</sup>Values above 100 were rounded down to 100.



Dentist Select, but none of the other rinses, removes significantly more VSCs produced by *P. gingivalis* than does water (above). When normalized to water (below), Dentist Select removes 100% of the remaining VSCs produced by *P. gingivalis* whereas only CloSys among the other rinses removes any of the remaining VSCs.



In order to be sure that the apparent lack of VSC removal by the various mouth rinses was not obscured by the halimeter giving positive readings due to the detection of perfumes within the mouth rinses, the experiment was repeated using a blank filter (no *P. gingivalis*). The table below reveals that the rinses alone gave minimal positive readings on the halimeter.

Rinse	Trial 1	Trial 2	Average
BreathRx	1	1	1
Crest Pro Health	2	3	3
CloSys	-53	-31	-42
Oxyfresh Zinc	0	0	0
Listerine	3	3	3
Dentist Select	-1235	-1148	-1192

## Discussion:

The choice of experimental design was based on the objective of trying to simulate the effects of a 30 second rinse on removal of volatile sulfur compounds produced by species of bacteria that can colonize oral tissue. While the cosmetic benefit of VSC removal is the reduction in oral malodor, there is also an underlying hypothesis that VSCs are toxic to the host and can complicate treatment, maintenance of, and recovery from, oral diseases such as periodontal disease.

Bacterial species belonging to the red complex of periodontal pathogens were chosen as the source of VSCs. However, it proved difficult to obtain sufficient quantities of *T. denticola* in broth culture necessary to carry out the experiments. Therefore, another treponeme, *T. socranskii*, was substituted for *T. denticola*. In addition, *T. forsythia* was tested, as was the negative control (not expected to produce VSCs), *S. mitis*. *P. gingivalis* turned out to be the most prolific producer of VSCs and *P. intermedia* produced an intermediate amount. The other species produced negligible amounts.

In order to simulate, *in vitro*, oral tissues colonized with VSC-producing bacteria, thick glass fiber filters within Erlenmeyer flasks were saturated with broth containing the test species of bacteria. After rinsing for 30 seconds the filter retained more bacteria and VSCs than would the glass surfaces of the flask. Unlike some of the oral streptococci, the bacterial species tested in this report are not proficient at producing a strongly adherent biofilm on the surfaces of glass vessels, thus necessitating the use of the filter. Although rinsing with water alone removed over 80% of the VSCs from the filters, the amounts retained were sufficient to statistically distinguish the effects of different mouth rinses. It was noted that the baseline reading of VSCs tended to drop for sequential tests even though the bacterial culture was from the same source. To account for this variability, the different rinses were tested in different order each trial, and a given culture was used for a maximum of three test readings. Collectively, the means of the baseline readings were quite similar for each mouth rinse with no statistical variation detected. Accordingly, statistical differences in the post-rinse readings and percentages of VSCs removed cannot be attributed to variations in the baseline readings. Rather, they represent authentic differences in the capacities of the various mouth rinses to reduce detectable VSCs. The possibility that post-rinse readings for some mouth rinses were influenced by perfumes within the rinse was also tested using blank filters that contained no bacteria. BreathRx, Crest Pro Health and Listerine gave small positive readings in two independent trials. These readings were too small to mask an effect on VSC removal but may explain why the percentages of VSCs removed by these mouth rinses fell slightly below that of the water rinse control.

Of all the oral mouth rinses and antiseptics tested, only the Dentist Select Mouthrinse and CloSys appeared to remove VSCs over and above the water rinse control. However, CloSys appeared capable of only removing about 19% of the residual VSCs whereas Dentist Select consistently removed 100%. It should also be noted that the mean post-rinse VSCs and mean percent reduction in VSCs was not statistically different when comparing CloSys with water. Only Dentist Select was statistically different than the water rinse control. The main active ingredient of these two mouth rinses is chlorine dioxide, however, the form and concentration vary. There was only one instance in which Dentist Select did not remove 100% of the VSCs. In one of three trials with *P. intermedia*, 1 ppb was detected following a Dentist Select rinse. This is the smallest unit read by the halimeter and may have reflected the natural fluctuation that occurs around any given value. Since halimeter readings often entered negative territory following a rinse with Dentist Select, it is unlikely that the experimental design tested the full capacity of Dentist Select to remove VSCs. Consequently, while the chosen protocol was sufficient to demonstrate the efficacy of Dentist Select relative to other mouth rinses, a different model – or human volunteers – would be more appropriate to discern the upper limits of Dentist Select's capacity for removal or inactivation of VSCs.

## Overall Summary and Conclusion:

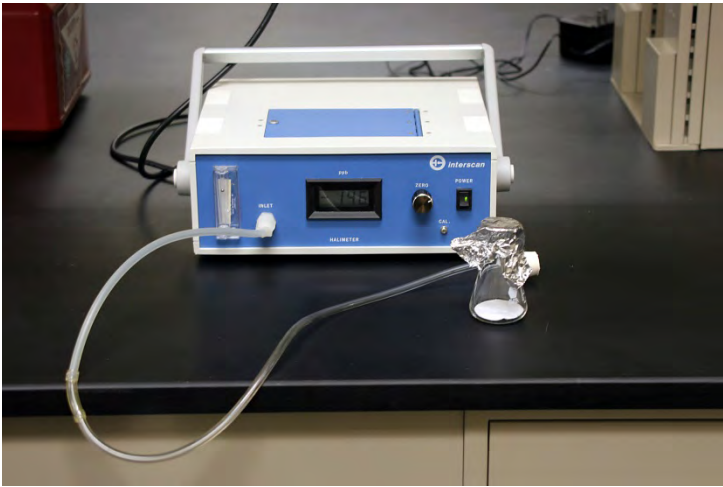
A 30 second rinse with Dentist Select was statistically more proficient at removing VSCs produced by *P. gingivalis* and *P. intermedia* than a 30 second rinse with chlorhexidine or water. A 30 second rinse with Dentist Select was also statistically more proficient at removing VSCs produced by *P. gingivalis* than a 30 second rinse with BreathRx, Crest Pro Health, CloSys, Listerine, and Oxyfresh Zinc.



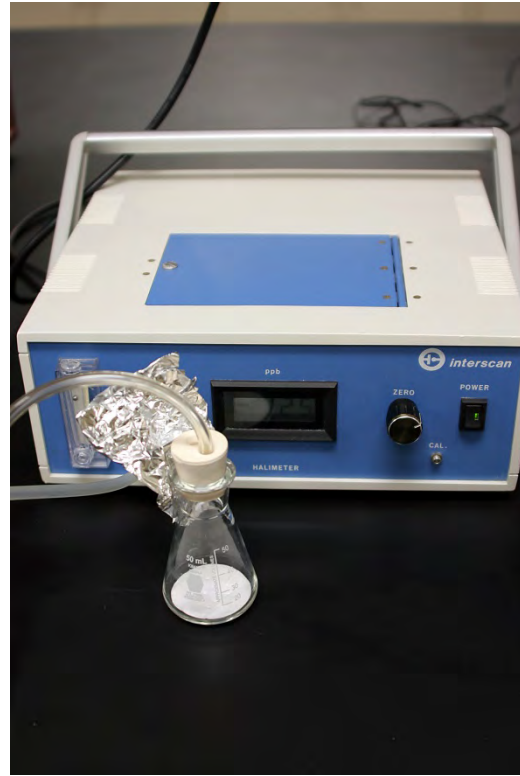
Submitted August 17<sup>th</sup>, 2012

## ADDENDUM

### Images of Experimental Set-Up:



Halimeter and flask with filter paper. The filter paper was wetted with 2ml of *P. gingivalis* culture. VSCs were measured by inserting the rubber stopper into the flask and recording the maximal reading (occurred within 5 seconds). The filter was rinsed for 30 seconds with 20ml of a test rinse, the rinse was poured out and the post-rinse reading recorded.



To take a reading, the rubber stopper was inserted into the flask; the second hole in the rubber stopper facilitated the proper air flow to the halimeter.



The filter after 2ml of *P. gingivalis* was added.



The filter after 20ml of a test rinse (Listerine) was added.

Raw data from four trials.

Testing *Porphyromonas gingivalis*

Trial	Tom's of Maine	TheraBreath	Smart Mouth	Dentist Select	Water
1 Baseline (ppb)	442	289	305	154	118
1 Post 30 sec. rinse (ppb)	56	35	46	-134	32
Percent Reduction	87.3	87.9	84.9	>100	72.9
2 Baseline (ppb)	120	218	157	145	177
2 Post 30 sec. rinse (ppb)	30	29	20	-34	33
Percent Reduction	75.0	86.7	87.3	>100	81.4
3 Baseline	145	170	256	333	349
3 Post 30 sec. rinse	34	14	31	-86	41
Percent Reduction	76.6	91.8	87.9	>100	88.3
4 Baseline (ppb)	203	281	223	335	244
4 Post 30 sec. rinse (ppb)	48	43	36	-60	54
Percent Reduction	76.4	84.7	83.9	>100	77.9
Average % Reduction	78.8	87.8	86.0	>100	80.1

As a control, each rinse was tested with the halimeter in the absence of *P. gingivalis* to determine if perfumes or other components within the rinse would elevate the halimeter reading and obscure a reduction in VSCs.

	Halimeter Reading*
Tom's of Maine	2
TheraBreath	1
Smart Mouth	1

\*Reading taken after rinsing a filter (without the usual 2ml of *P. gingivalis*) for 30 seconds with 20ml of the rinse and then pouring it off.

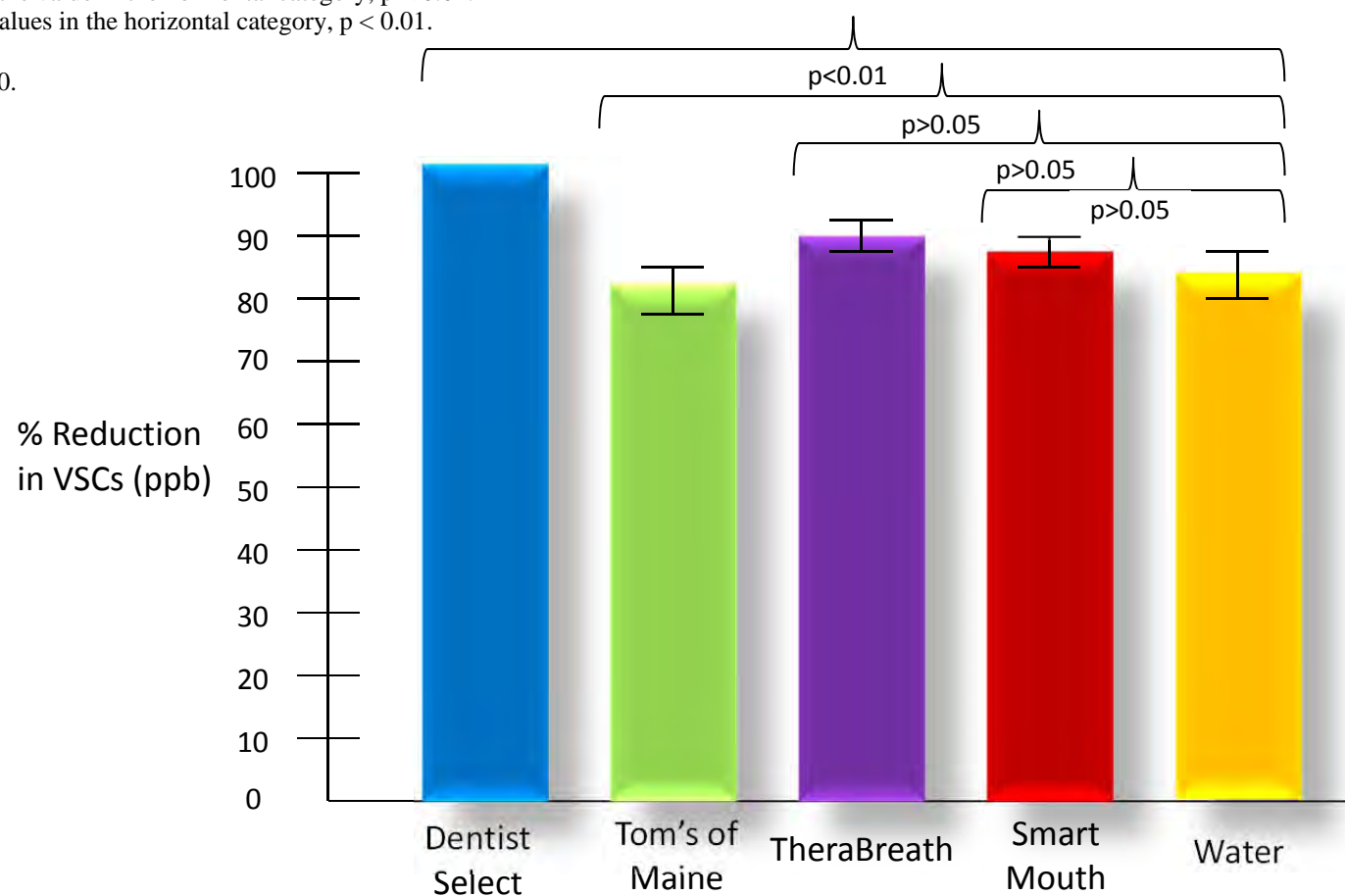
<i>Porphyromonas gingivalis</i>	Tom's of Maine	TheraBreath	Smart Mouth	Dentist Select	Water
Mean Baseline VSCs (ppb)	228	240	235	242	222
Mean Post-Rinse VSCs (ppb)	42	30	33	0 <sup>#*</sup>	40
Mean Percent Reduction	79 ± 6	88 ± 3	86 ± 2	100 <sup>^*+</sup>	80 ± 6

\*Significantly different than Water for the value in the horizontal category,  $p < 0.01$ .

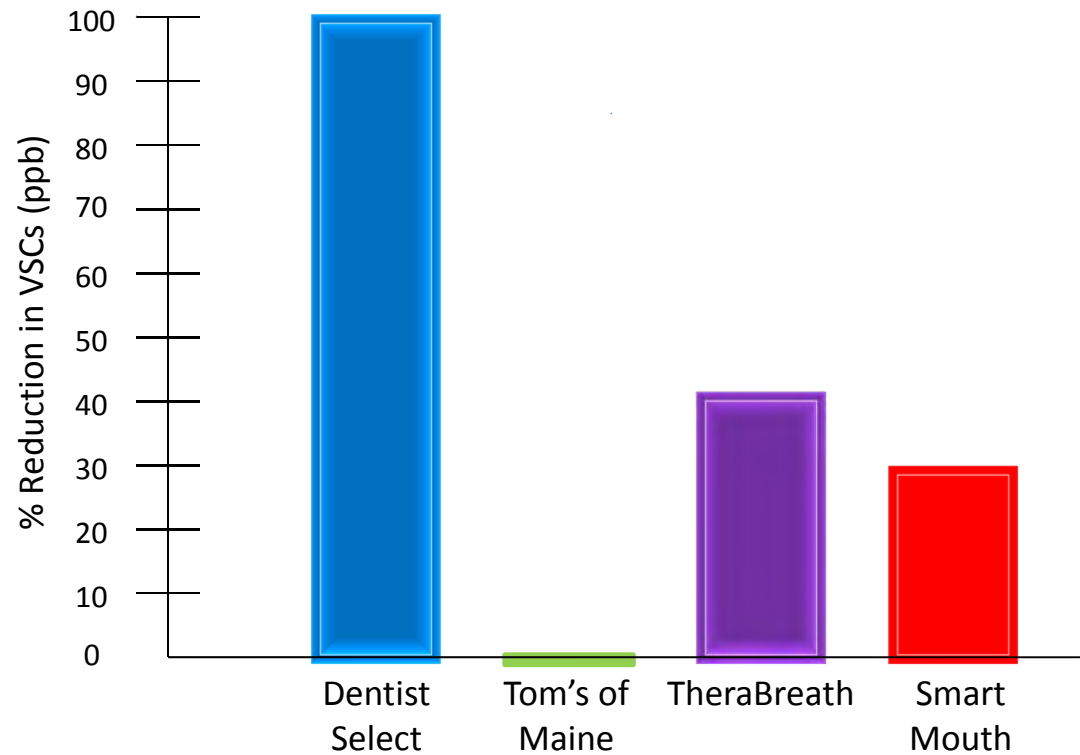
+Significantly different than all other values in the horizontal category,  $p < 0.01$ .

#Values below 0 were rounded to 0.

^Values above 100 were rounded to 100.



When normalized to water, Dentist Select removed 100% of the remaining VSCs. TheraBreath and Smart Mouth removed approximately 42% and 32% of the remaining VSCs, respectively. Tom's of Maine removed none of the remaining VSCs.



#### Discussion:

Tom's of Maine behaved like most of the other mouth rinses in that there was no indication that it was better than water at removing VSCs. TheraBreath and Smart Mouth joined CloSys as appearing modestly better than a water rinse when data were normalized to water. However, the average percent reductions in measured VSCs for TheraBreath and Smart Mouth did not differ statistically from water whereas the percent reduction for Dentist Select was statistically different from water. Therefore, in combination with earlier tests, it can be concluded that Dentist Select was the only rinse to remove statistically higher percentages of VSCs than did a water control. CloSys, TheraBreath, and Smart Mouth showed non-statistically significant trends towards removing more VSCs than a water rinse, and when normalized to water the data indicated an approximate 20-40% reduction in remaining VSCs. It would require several additional trials to determine whether these differences represent an authentic ability of the product or whether the apparent proficiencies in reducing VSCs were due to random experimental fluctuations. However, even if these reductions reflect a genuine property of the rinses, when the percent reductions in measurable VSCs were compared between the Dentist Select Mouthrinse and other mouthrinses, the Dentist Select Mouthrinse yielded statistically significantly greater reductions in VSCs than all other rinses ( $p < 0.01$  in all instances).

**Conclusion:**

Within the limits of the testing model, Dentist Select was the only rinse tested that resulted in a statistically significant reduction in VSCs beyond the background level of a negative control. In addition, the percent reductions in VSCs following a 30 second rinse with Dentist Select were statistically greater than the reductions observed with any other rinse tested.

A handwritten signature in black ink, appearing to read "J. B. B. B." with a stylized flourish at the end.

October 11, 2012