

## ORIGINAL ARTICLE

## Comparison of different treatment modalities for oral halitosis

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**Abstract**

**Objectives.** To assess the effects on intra-oral halitosis by a mouth rinse containing zinc acetate (0.3%) and chlorhexidine diacetate (0.025%) with and without adjunct tongue scraping. **Materials and methods.** Twenty-one subjects without a diagnosis of periodontitis were randomized in a cross-over clinical trial. Organoleptic scores (OLS) were assessed to define intra-oral halitosis by total volatile sulfur compound (T-VSC) measurements and by gas chromatography. **Results.** Twenty-one subjects with a mean age of 45.7 years (SD: ±13.3, range: 21–66). The OLS were significantly lower following active rinse combined with tongue scraping ( $p < 0.001$ ) at all time points. Immediately after, at 30 min, and at day 14, the T-VSC values were lower in the active rinse sequence than in the negative rinse sequence ( $p < 0.001$ ,  $p < 0.001$  and  $p < 0.05$ , respectively). At 30 min and at day 14, the hydrogen sulfide (H<sub>2</sub>S) and methyl mercaptan (MM) values were lower in the active rinse sequence compared to the inactive rinse sequence ( $p < 0.001$ ). The inactive rinse sequence with tongue scraping reduced T-VSC at 30 min ( $p < 0.001$ ) but not at 14 days. Similar reductions in T-VSC, H<sub>2</sub>S and MM were found in the active rinse sequence with or without tongue scraping. **Conclusion.** The use of a tongue scraper did not provide additional benefits to the active mouth rinse, but reduced OLS and tongue coating index.

**Key Words:** halitosis, mouth rinse, tongue scraper

**Introduction**

Halitosis is considered as a social and a psychological problem. Available data suggest that the prevalence of halitosis with an oral etiology (intra-oral halitosis) is high [1–3]. Oral halitosis can be caused by several intra-oral factors such as tongue coating, periodontal diseases, tooth decay, unclean dentures, mucosal ulcerations and diseases, mouth breathing and poor oral hygiene [3]. Approximately 40% of individuals affected by halitosis have no underlying organic disease [4]. Extra-oral halitosis may be caused by respiratory tract conditions such as sinusitis, tonsillitis, bronchiectasis, lung or liver disease [5].

Intra-oral halitosis has been associated with bacterial production of hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (MM) and dimethyl sulfide (DMS) [6,7]. Anaerobic bacteria in periodontal pockets and on the dorsum of the tongue can degrade sulfur-containing amino acids, resulting in the formation of volatile sulfur compounds (VSC) [8–12]. Recent data also suggest that  $\beta$ -galactosidase activity in saliva is an important factor in intra-oral halitosis [13]. It is of interest that the activity of this enzyme was not related to the presence of bacteria associated with periodontitis, suggesting that intra-oral halitosis may in certain cases be present independent of such bacteria [13].

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Intra-oral halitosis has been studied with different methods including a subjective organoleptic scoring system (OLS) with a scale between 0–5 [14]. OLS is considered as the ‘gold standard’ to diagnose intra-oral halitosis. Objective assessments of intra-oral levels of VSCs can be performed with a device assessing total volatile sulfur compounds (T-VSC) or by gas chromatography [15,16]. Currently, gas chromatography is considered as the most accurate device to detect VSC in breath air [17].

Different treatment strategies including mechanical debridement of teeth, rinsing with antimicrobial agents and/or the use of metal salts have been proposed for the management of intra-oral halitosis [18]. The treatment of intra-oral halitosis in patients with periodontitis has focused on periodontal therapy, improvement of oral hygiene and the use of a tongue scraper by the patient [19,20]. Thus, data suggest that the use of a tongue scraper may reduce the level of intra-oral halitosis also in subjects who do not have periodontitis [21,22]. The data are, however, contradictory. Although a significant reduction in VSC may occur over 3 months the mean VSC scores at 3 months remained at much higher levels than suggested as the cut-off level of VSC by gas chromatography to define absence of VSC causing intra-oral halitosis [23].

In a recent systematic review, the authors found no evidence that diet modification, the use of a sugar-free chewing gum, tongue cleaning by brushing, scraping the tongue or the use of zinc containing toothpaste resulted in clinically important results in regards to the control for intra-oral halitosis [4].

The aim of the present randomized single blinded cross-over clinical trial was to compare the efficacy of four intervention modalities to control for intra-oral halitosis in subjects with a diagnosis of intra-oral halitosis but without a diagnosis of periodontitis.

## Materials and methods

The Ethics Committee at the University of Lund, Sweden, approved the study. All subjects signed an informed consent. Advertisements in the local newspaper, on message boards and on the web page at the University of Kristianstad, Sweden, were used to recruit subjects. The study was conducted between 2008 and 2009 and was performed at the dental clinic of the University of Kristianstad, Sweden.

- Inclusion criteria: (1) halitosis of intra-oral origin, (2) OLS  $\geq 2$  and (3) T-VSC  $\geq 160$  ppb, as determined with a Halimeter<sup>®</sup>.
- Exclusion criteria: (1) untreated periodontitis defined as the presence of more than one periodontal pocket with a probing pocket depth  $\geq 6$  mm, (2) open caries lesions, (3) pregnancy, (4) systemic medications known to cause hypo-salivation, (5) systemic antibiotic therapy within the preceding

3 months prior to the study, (6) current smoker or (7) a medical history with a disease known to be associated with extra-oral halitosis.

The subjects were given detailed verbal and written instructions regarding food intake to exclude a diet that may have an impact on oral malodor. They were given routine oral hygiene measures including the sequence assigned rinsing and as defined tongue scraping and what to do before each visit at the clinic. They were specifically asked; (I) not to consume food containing onions, garlic or hot spices within 48 h before assessments, (II) not to drink alcoholic beverages within 12 h before assessments, (III) not to eat or drink within 5 h before assessments (subjects were allowed to drink water until 3 h before assessments), (IV) not to perform oral hygiene measures, tongue cleaning or use any mouth-rinse in the morning of the examination day and (V) not to use scented cosmetics, perfume or after-shave lotions in the same morning as the study assessments were performed. Subjects were instructed not to change their oral hygiene habits during the study period. During each of the four study sequences, the subjects came to the clinic at the same time during the morning hours at baseline, day 1 and at day 14.

At study end-point, all subjects had participated in all four study intervention protocol sequences using: (I) the active test mouth rinse alone, (II) the active test mouth rinse with the use of a tongue scraper, (III) the inactive mouth-rinse alone and (IV) the inactive mouth-rinse with the use of a tongue scraper. The different test sequences were separated by a washout period of 1 week. Subjects were randomly assigned to protocol sequence order (Latin square) (Table I) using a computer-based randomization software program IBM<sup>®</sup>/SPSS<sup>®</sup> 18.0 (IBM<sup>®</sup>, Corporation Somers, NY). The two rinse solutions (active and inactive rinse) were distributed in coded bottles. The study subjects and the examiner (SEA) were unaware of sequence assignment. The subjects were instructed to rinse with 10 ml of the provided solution during 1 min twice daily and then to spit out the rinse solution. The subjects were instructed to rinse after breakfast and before bed-time.

The active mouth-rinse included water, glycerin, sorbitol, alcohol (1.8%), zinc acetate (0.3%), chlorhexidine diacetate (0.025%), sodium fluoride (0.05%),

Table I. Sequencing of cases to protocol order using the design of a Latin square.

Procedure	Sequence			
Active rinse alone	I	II	III	IV
Active rinse + tongue scraping	II	III	IV	I
Negative control rinse alone	III	IV	I	II
Negative control rinse + tongue scraping	IV	I	II	III

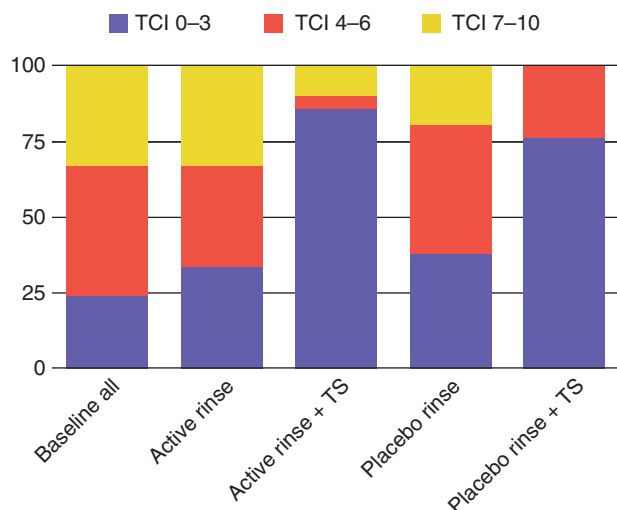


Figure 1. Frequency distribution of tongue coating index (baseline overall frequency distribution) and TCI scores for the four study groups, active rinse, active rinse plus tongue scraping (TS), control rinse and control rinse with tongue scraping (TS), at baseline and at day 14.

hydrogenated Castro oil, citric acid, acesulfame potassium, menthol and *Mentha piperita* (SB12<sup>®</sup>, Antula Healthcare AB, Stockholm, Sweden). The composition of the inactive mouth-rinse contained the same ingredients except that the inactive mouth-rinse did not include zinc acetate, chlorhexidine diacetate or sodium fluoride.

According to the study protocol, a tongue scraper (Halita<sup>®</sup>, DentAid, Barcelona, Spain) was used in two of the study sequences. For the adjunct use of the tongue scraper, the subjects were instructed and trained in how to use the tongue scraper. Briefly, they were shown to pull out the tongue, apply the tongue scraper to the dorsum of the tongue and perform five strokes. They were instructed to cover the dorsum of the tongue as far posterior as possible. This procedure was to be performed twice daily and before using the rinsing solution. Study subjects, but not the examiner (SEA), knew, of course, if they, during the specific study sequence, had used the tongue scraper or not. After the conclusion of the study all subjects responded to a questionnaire about the use of rinse and tongue scraper to control for compliance.

Study assessments were performed as follows: (I) Day 1: baseline values before intervention, (II) Day 1: immediately after intervention, (III) Day 1: 30 min after intervention and (IV) Day 14: 8–12 h after the last intervention the evening before. At baseline, the participants had not been eating during 5 h preceding the assessments. One and the same investigator (SEA) performed all registrations. The examiner was trained and calibrated in judging intra-oral halitosis at a clinic specialized in the treatment of intra-oral halitosis. Subjective assessments of intra-oral halitosis were performed using an arbitrary 0–5 scale (0 = no

halitosis to 5 = offensive halitosis) [19]. The tongue coating index (TCI) was used to assess the extent of tongue coating [24].

The Halimeter<sup>®</sup> (Interscan Corporation, Chatsworth, CA, USA) was used to assess total VSC in breath air. The OralChroma<sup>™</sup> (ABIMEDICAL Corporation, Kawasaki City, Japan) was used to assess H<sub>2</sub>S, MM and DMS in breath air from study subjects and consistent with the use of these devices in other studies of intra-oral halitosis [15,16,17].

### Statistics

Sample size was estimated based on the assumption that the negative control rinse would provide limited to no effects on VSCs, whereas the active rinse should reduce VSCs by 40%. Thus, a sample size of 20 subjects should provide statistical power (85%). The Kolmogorov-Smirnov test was used to identify that data for all variables failed to demonstrate a normally distribution pattern. The Kruskal-Wallis ANOVA and Univariate ANOVA with the Bonferroni post-hoc test were used to compare baseline sequence conditions. Further data analysis between and within study sequences for the study group sequences were studied by Wilcoxon signed rank test, by Kruskal-Wallis ANOVA and by repeated Mann Whitney U-tests. Data were also assessed by Spearman rank correlation. Significance was declared at  $p < 0.05$ .

## Results

### Subject characteristics

A total of 53 subjects were screened for intra-oral halitosis resulting in the inclusion of 21 adults (10 females) with confirmed intra-oral halitosis. All 21 subjects completed the study. The mean age of these subjects was 45.7 years (SD:  $\pm 13.3$ , range: 21–66).

At baseline in each study sequence, all study subjects had an OLS  $\geq 2$ . Reliability tests performed between the baseline organoleptic scorings of the four treatments sequences demonstrated a high level of reliability (Cronbach's  $\alpha$  varying between 0.63–0.87 ( $p < 0.01$  and  $p < 0.001$ , respectively)).

Comparisons by Kruskal-Wallis ANOVA failed to identify baseline sequence differences in the distribution of T-VSC scores ( $p = 0.83$ ), the levels of H<sub>2</sub>S scores ( $p = 0.62$ ), the levels of MM scores ( $p = 0.46$ ), the levels of DMS scores ( $p = 0.90$ ) and the levels of T-VSC scores ( $p = 0.27$ ). Univariate ANOVA with the Bonferroni post-hoc test confirmed these results ( $p$ -values varying between 0.27–1.0).

Baseline bleeding on probing at  $\geq 20\%$  of surfaces (four per tooth) was, on average, found in 23.8% (5/21) of the subjects and with the highest subject BOP score at 35% of surfaces. Statistical analysis failed to

Table II. Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, mean values and standard deviation (SD) for the active rinse alone at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Active rinse values expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	<i>p</i> -values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	2.0	0.0	0.0	2.0	0.001	0.001	0.01
	25th %	2.0	0.0	0.0	1.0			
	75th %	3.0	0.0	1.0	2.0			
	Mean	2.6	0.1	0.3	1.6			
	SD	0.8	0.3	0.6	0.6			
T-VSC	Median	164.0	64.0	64.0	113.0	0.001	0.001	0.01
	25th %	123.5	58.0	58.0	73.5			
	75th %	277.0	75.5	66.0	150.0			
	Mean	262.9	67.8	62.8	122.0			
	SD	264.6	13.9	7.5	55.3			
Hydrogen sulfide	Median	492.0	0.0	0.0	51.0	0.001	0.001	0.01
	25th %	113.0	0.0	0.0	6.5			
	75th %	1005.5	0.0	0.0	244.5			
	Mean	806.4	0.4	1.8	159.4			
	SD	973.6	2.0	5.2	235.4			
Methyl mercaptan	Median	67.0	30.0	11.0	14.0	0.01	0.001	NS
	25th %	12.5	0.0	0.0	0.0			
	75th %	250.5	50.0	39.0	48.0			
	Mean	165.7	55.2	26.5	50.5			
	SD	247.1	102.5	39.5	93.9			
Dimethyl sulfide	Median	28.0	10.0	8.0	12.0	0.01	0.01	NS
	25th %	13.0	0.0	0.0	0.0			
	75th %	68.0	30.5	23.5	35.5			
	Mean	51.4	24.8	13.3	71.9			
	SD	61.5	40.3	18.8	180.3			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

identify significant correlations between the percentage sites with bleeding and T-VSC, H<sub>2</sub>S, MM and DMS scores.

#### *Changes in organoleptic scoring (OLS) results*

In comparison to pre-treatment scores, significantly lower OLS were identified immediately after intervention, 30 min after intervention ( $p < 0.001$ ) in both the active rinse sequence alone and in the active rinse plus tongue scraping sequence. Significantly lower OLS scores were also obtained at day 14 for the active rinse alone ( $p < 0.01$ ) and for the active rinse plus tongue scraping sequence ( $p < 0.001$ ). In the negative control rinse group with tongue scraping, significantly lower OLS were found immediately after intervention and at 30 min after intervention ( $p < 0.001$ ). Statistical analysis failed to demonstrate differences in OLS between baseline and 14 days in the negative control rinse sequence alone ( $p = 0.32$ ), but demonstrated

significantly lower OLS in the negative control rinse with the tongue scraping sequence ( $p < 0.01$ ). Thus, at day 14 in the active rinse sequence 38.1% of the subjects had a negative OLS score while 66.7% of the subjects in the active rinse with tongue scraping sequence had a negative OLS score. Thus, at day 14 in the negative control rinse sequence 23.8% of the subjects had a negative OLS score while 33.3% of the subjects in the negative control rinse sequence with tongue scraping had a negative OLS score.

#### *Differences in tongue coating index (TCI change) at day 14*

The distributions of TCI at baseline and at day 14 are presented (Figure 1). Analysis by Mann-Whitney U-test identified that the change in TCI between baseline and day 14 was significantly lower in the active rinse sequence with tongue scraping than in the sequence with active rinsing alone ( $p < 0.001$ ). The



Table III. Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, mean values and standard deviation (SD) for the active rinse in combination with tongue scraping at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Active rinse and tongue scraping expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	<i>p</i> -values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	0.0	1.0	0.001	0.001	0.001
	25th %	2.0	0.0	0.0	1.0			
	75th %	3.0	0.0	0.5	2.0			
	Mean	2.5	0.0	0.3	1.3			
	SD	1	0	0.6	0.7			
T-VSC	Median	154.0	64.0	64.0	91.0	0.001	0.001	0.01
	25th %	110.0	61.5	58.0	71.5			
	75th %	275.0	68.0	71.5	118.0			
	Mean	220.9	65.1	64.1	130.9			
	SD	163.1	5.8	7.8	132.1			
Hydrogen sulfide	Median	311.0	0.0	0.0	38.0	0.001	0.001	0.05
	25th %	82.5	0.0	0.0	0.0			
	75th %	772.0	0.0	0.0	205.0			
	Mean	628.0	0.8	3.8	195.3			
	SD	960.5	3.7	10.6	362.6			
Methyl mercaptan	Median	56.0	10.0	8.0	9.0	0.001	0.001	0.05
	25th %	15.5	0.0	0.0	0.0			
	75th %	139.0	35.0	14.0	42.0			
	Mean	136.4	25.0	10.3	44.6			
	SD	236.9	36.0	15.3	96.7			
Dimethyl sulfide	Median	42.0	8.0	0.0	12.0	0.01	0.001	NS
	25th %	17.5	0.0	0.0	0.0			
	75th %	77.0	18.0	19.5	74.5			
	Mean	59.2	15.8	17.1	41.5			
	SD	64.0	27.9	33.2	55.4			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter.

TCI change was also lower in the negative control rinse with tongue scraping sequence than in the negative control rinse sequence alone ( $p < 0.001$ ). Statistical analysis failed to demonstrate a difference in the TCI change between the active and the inactive rinse sequences without tongue scraping ( $p = 0.09$ ) and between the active rinse sequence with tongue scraping vs the negative control rinse sequence with tongue scraping ( $p = 0.64$ ). Statistical analysis failed to demonstrate a correlation (Spearman rank correlation) between changes in TCI values between baseline and day 14 vs changes in T-VSC, H<sub>2</sub>S, MM or DMS.

#### *The intervention effects on VSC levels (within-subject analysis)*

Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, mean, standard deviation and the  $p$ -values in the four study sequences various test combinations are presented for T-VSC, H<sub>2</sub>S, MM and DMS at the different time points for

the active mouth rinse sequence alone (Table II) and in combination with tongue scraping (Table III), for the inactive control rinse sequence (Table IV) and for the inactive rinse with tongue scraping (Table V). The changes in H<sub>2</sub>S and MM levels in the four sequences between baseline and day 14 are presented in box-plot diagrams (Figures 2 and 3).

#### *Comparisons between sequences at the different time points for T-VSC, H<sub>2</sub>S, methyl mercaptan and dimethyl sulfide values*

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the T-VSC values immediately after ( $p < 0.001$ ), at 30 min ( $p < 0.001$ ) and at day 14 ( $p < 0.05$ ). Further analysis by repeat Mann-Whitney U-tests identified that immediately after, at 30 min and at day 14, the T-VSC values were significantly lower in the active rinse group compared to T-VSC values in the placebo

Table IV. Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, mean values and standard deviation (SD) for the negative control placebo rinse alone at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Negative control rinse values expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	<i>p</i> -values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	1.0	2.0	0.001	0.001	NS
	25th %	2.0	0.0	1.0	1.5			
	75th %	3.0	1.0	2.0	3.0			
	Mean	2.6	0.3	1.4	2.3			
	SD	1.0	0.5	0.9	1.0			
T-VSC	Median	180.0	111.0	130.0	183.0	NS	NS	NS
	25th %	99.0	86.0	87.0	85.5			
	75th %	287.0	183.0	259.5	297.5			
	Mean	242.1	227.0	225.0	221.7			
	SD	215.5	310.3	261.5	166.6			
Hydrogen sulfide	Median	239.0	187.0	226.0	272.0	NS	NS	NS
	25th %	89.0	52.5	95.5	92.0			
	75th %	912.0	519.5	514.0	816.5			
	Mean	625.9	754.1	491.3	599.2			
	SD	886.3	1742.4	693.1	690.7			
Methyl mercaptan	Median	51.0	35.0	31.0	115.0	0.05	NS	NS
	25th %	27.0	3.5	9.0	31.0			
	75th %	191.5	91.5	102.5	184.5			
	Mean	215.6	125.6	124.9	140.1			
	SD	420.8	314.0	331.3	164.7			
Dimethyl sulfide	Median	40.0	25.0	17.0	35.0	NS	NS	NS
	25th %	10.0	9.0	10.0	16.0			
	75th %	82.0	52.5	69.5	89.0			
	Mean	54.1	43.2	50.1	185.0			
	SD	56.7	53.3	65.6	600.9			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

rinse group ( $p < 0.001$ ,  $p < 0.001$  and  $p < 0.05$ , respectively). Statistical analysis failed to demonstrate differences for T-VSC values between the placebo rinse alone and the inactive rinse sequence with tongue scraping ( $p$ -values varying between 0.19–0.78). The T-VSC values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse sequence and tongue scraping sequence immediately after procedure ( $p < 0.001$ ), at 30 min after procedure ( $p < 0.001$ ) and at day 14 ( $p < 0.05$ ). Statistical analysis failed to demonstrate differences for T-VSC values between the active test rinse sequence and the active rinse sequence with tongue scraping ( $p$ -values varying between 0.33–0.98).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedures for the H<sub>2</sub>S values at 30 min and at day 14 ( $p < 0.001$ ). Further analysis by repeat Mann-Whitney U-tests identified that, at 30 min, and at day 14, the H<sub>2</sub>S values were significantly lower in

the active rinse sequence compared to H<sub>2</sub>S values in the inactive rinse sequence ( $p < 0.001$ ). Statistical analysis failed to demonstrate differences for H<sub>2</sub>S values between the inactive rinse sequence and the combined rinse and tongue scraping sequence ( $p$ -values = 0.90 and 0.91, respectively). The H<sub>2</sub>S values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse and tongue scraping sequence both at 30 min and at day 14 ( $p < 0.001$ ). Statistical analysis failed to demonstrate differences for H<sub>2</sub>S values between the active test rinse sequence and the active test rinse and tongue scraping sequence ( $p$ -values = 0.90 and 0.93, respectively).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the MM values immediately after ( $p < 0.05$ ) and at 30 min after procedure ( $p < 0.01$ ). Repeat Mann-Whitney U-tests identified that, at 30 min and at day 14, the MM values were significantly lower in the active rinse sequence compared to MM values in

Table V. Median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, mean values and standard deviation (SD) for the negative control placebo rinse in combination with tongue scraping at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Negative control rinse and tongue scraping expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	<i>p</i> -values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	1.0	2.0	0.001	0.001	0.01
	25th %	2.0	0.0	0.5	1.0			
	75th %	3.0	0.5	2.0	2.5			
	Mean	2.5	0.3	1.3	1.8			
	SD	0.9	0.7	0.9	1.0			
T-VSC	Median	186.0	100.0	90.0	142.0	0.01	0.001	NS
	25th %	108.5	84.0	69.5	87.0			
	75th %	286.0	204.0	176.0	293.0			
	Mean	246.1	172.5	172.9	193.5			
	SD	231.6	166.2	203.0	125.5			
Hydrogen sulfide	Median	367.0	380.0	216.0	271.0	NS	0.01	NS
	25th %	138.5	55.3	48.0	111.0			
	75th %	692.5	667.8	503.5	946.0			
	Mean	847.4	561.8	386.3	606.1			
	SD	1396.0	781.4	553.9	706.1			
Methyl mercaptan	Median	93.0	44.5	33.0	61.0	NS	0.01	NS
	25th %	22.5	2.5	8.0	18.0			
	75th %	265.0	102.3	167.5	173.0			
	Mean	180.1	98.3	85.2	123.7			
	SD	231.5	155.1	106.9	168.1			
Dimethyl sulfide	Median	48.0	27.5	26.0	41.0	NS	NS	NS
	25th %	7.0	2.3	0.0	0.0			
	75th %	101.0	58.3	84.0	117.5			
	Mean	64.6	40.8	42.0	99.5			
	SD	64.6	46.5	42.9	141.7			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

the inactive rinse sequence at 30 min ( $p < 0.05$ ) and at day 14 ( $p < 0.001$ ). Statistical analysis failed to demonstrate differences for MM values between the inactive rinse alone and the inactive rinse sequence with tongue scraping ( $p$ -values = 0.86, and 0.27, respectively). The MM values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse with tongue scraping sequence both at 30 min ( $p < 0.001$ ) and at day 14 ( $p < 0.01$ ). Statistical analysis failed to demonstrate differences for MM values between the active test rinse sequence and the active test rinse with tongue scraping sequence ( $p$ -values = 0.32 and 0.71, respectively).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the DMS values immediately after ( $p < 0.05$ ) and at 30 min after procedure ( $p < 0.01$ ). Repeat Mann-Whitney U-tests identified that, at 30 min after procedure, the DMS values were significantly

lower in the active rinse sequence compared to DMS values in the inactive rinse sequence ( $p < 0.01$ ). At the other study time points, statistical analysis failed to demonstrate DMS differences between these two procedures. Statistical analysis failed to demonstrate differences between the inactive rinse sequence and the inactive rinse sequence with tongue scraping, as well as between active rinse and active rinse with tongue scraping.

## Discussion

Baseline data assessments consistently confirmed that, at the beginning of each intervention sequence, baseline T-VSC, H<sub>2</sub>S, MM and DMS scores were comparable and that the 1 week wash-out period was sufficient to control for any effect that the preceding study sequence might have had on intra-oral halitosis. Thus, for this type of study of intra-oral halitosis in

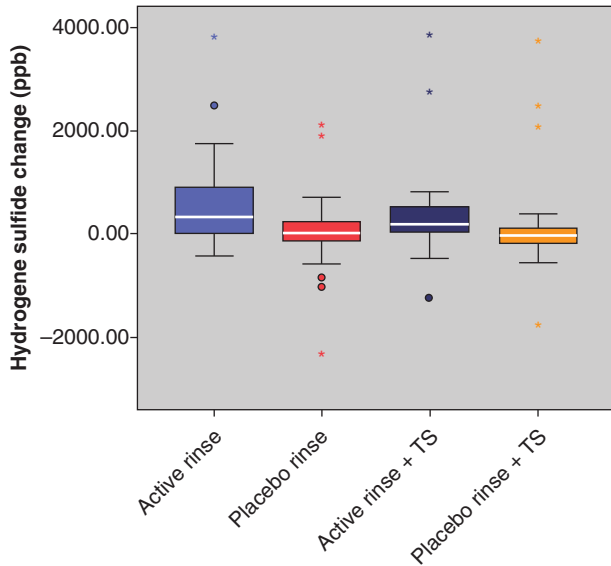


Figure 2. Change for Hydrogen sulfide between baseline and at day 14 for the four interventions. Notice that a positive value suggests a reduction in  $H_2S$  at day 14 ( $\lambda$  0 outlier value, \* extreme outlier value) (TS, tongue scraping).

subjects who did not have a diagnosis of periodontitis, the cross-over study design, including a 1-week washout period was appropriate.

The data failed to demonstrate baseline differences at each sequence for the four treatment modalities. Hence, the use of the cross-over design was appropriate. Due to the lack of normal distribution of the T-VSC,  $H_2S$ , MM and DMS scores the statistical analysis was performed with non-parametric tests which did not fully allow us to control for study sequence allocation. Nevertheless, the analysis clearly demonstrated statistical differences by sequence

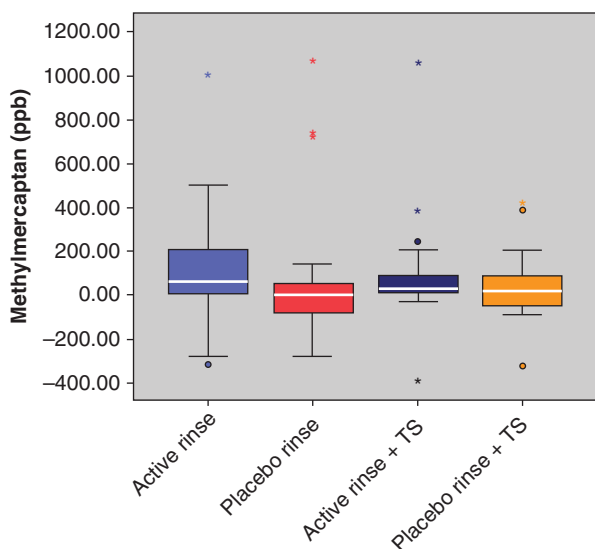


Figure 3. Change for Methyl mercaptan between baseline and at day 14 for the four interventions. Notice that a positive value suggests a reduction in MM at day 14 ( $\lambda$  0 outlier value, \* extreme outlier value) (TS, tongue scraping).

modality and that rinsing alone with the active rinse solution consistently demonstrated the highest ability to reduce the VSCs studied.

The present study design with the inclusion of a tongue scraper did not allow for a fully double-blind design. The rinse products were, however, bottled in the same type of bottles and labeled such that the subjects and the investigator were unaware if the subjects had been using the active or negative control rinse solutions during the dedicated study sequence. The flavoring agent may have affected the professional assessments using the organoleptic scoring system immediately after rinsing and at 30 min following interventions. Therefore, the OLS at these time points may be less accurate than the Halimeter<sup>®</sup> and OralChroma<sup>™</sup> readings.

Other studies assessing the effects of therapy in subjects with intra-oral halitosis and periodontitis have shown that periodontal intervention reduces intra-oral halitosis [20,25]. In the present study, we identified that intra-oral halitosis can occur in subjects who do not have periodontitis. The present study also suggested that the adjunct use of a tongue scraper provided limited impact on intra-oral halitosis.

Compared to 30 min after intervention, less reductions of VSC were observed at day 14. The explanation might be that the participants rinsed with or without tongue scraping the evening before and did not brush their teeth or used the rinsing solution or the tongue scraper 8–12 h before the registrations in the morning of day 14. This 8–12 h time frame may have allowed the accumulation of VSC before the assessments. Bacterial re-growth resulting in elevated production of VSC may have occurred. This could explain the trend of higher values of VSC at day 14. It should, however, be observed that the VSC values at day 14 were lower than at baseline in the active rinse sequences but not in the sequence with the placebo rinse.

Other studies have suggested that mechanical methods including tongue brushing or tongue scraping to clean the dorsum of the tongue reduce the levels of VSC in exhaled air [10,11,26–28]. The present study demonstrated that tongue scraping had limited effects in reducing levels of VSC in comparison to the effects of the active rinse solution. Mechanical cleaning of the tongue may have a short time effect on intra-oral halitosis [21].

The chemicals used in the active mouth rinse had effects on intra-oral halitosis. One possible explanation may be a chemical binding and inactivation of VSC by ingredients of the active mouth-rinse. Other data suggest that the use of chlorite anions and chlorine dioxide in a mouth rinse may have effects on intra-oral halitosis [28,29]. Metal ions, including zinc, have been used for several years in the treatment of intra-oral oral halitosis [30–33]. Dentifrices with either  $Zn^{++}$  or baking soda significantly reduce



VSC levels [6,34]. A reduction of intra-oral halitosis following chlorhexidine rinses has also been reported [12,35]. Furthermore, data suggest a synergistic effect between chlorhexidine and zinc, which may explain the efficacy in binding VSC thereby controlling for intra-oral halitosis [36]. Zinc salts are approved therapeutics by FDA (US Food and Drug Administration) with anti-inflammatory and anti-bacterial effects. Therefore, subjects with intra-oral halitosis could be recommended to use a mouth-rinse with the active ingredients studied (zinc and chlorhexidine) for the control of intra-oral halitosis.

The present study demonstrated that the active rinse alone without the tongue scraping provided the most reliable change (reduction) in intra-oral halitosis as defined by T-VSC, H<sub>2</sub>S and MM assessments. The report in a recent systematic review and our findings that the use of a tongue cleaner provides marginal or no effects on intra-oral halitosis are consistent [4].

In conclusion, rinsing with a zinc-acetate and chlorhexidine diacetate containing mouth rinse resulted in a clinically relevant reduction of intra-oral halitosis during a study period of 2 weeks. The use of a tongue scraper did not provide additional benefits to the active rinse. The removal of tongue coating debris with a tongue scraper does not seem to influence VSC levels in breath air in subjects who do not have periodontitis.

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