

## Daylong Effect of Rinsing With Water Diluted Antiplaque® Toothpaste Combined With Tooth Brushing on Volatile Sulphur Compound Levels

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### Abstract

Halitosis, complained by most adult population, is a common oral condition. About 85 % of the cases happened because of the microbial activity in the oral cavity that produces Volatile Sulphur Compound (VSC) which has unpleasant odour. Daily oral prophylaxis can reduce the oral VSC levels. **Objective:** to determine the daylong effect of Antiplaque® toothpaste in daily oral prophylaxis: as dentifrice for tooth brushing and, in modified way, as oral rinse; on the VSC level. **Methods:** a double blind, clinical experimental study, involving 120 subjects with or without halitosis complain, divided in 2 treatment groups (oral rinse group and the combination of tooth brushing and oral rinse group, using Antiplaque® toothpaste) and 2 control groups (same procedure using placebo toothpaste). The daylong VSC levels measured with halimeter were taken twice, in the afternoon of day I and day II. **Results:** there are significant reduction of daylong VSC levels (Wilcoxon signed rank test,  $P < 0.05$ ), but not in the treatment groups using the placebo toothpaste. The result on combination of tooth brushing and oral rinse using Antiplaque® toothpaste are significant compared to the oral rinse treatment using Antiplaque® toothpaste alone (Mann Whitney,  $P < 0.05$ ). **Conclusion:** tooth brushing with Antiplaque® toothpaste combined with oral rinse using the modified Antiplaque® toothpaste can significantly reduce daylong VSC levels.

**Keywords:** VSC levels, daylong reduction, tooth brushing, oral rinse, Antiplaque® toothpaste

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### Introduction

Halitosis or oral malodour, is a common oral condition that becomes a reason for a patient to see a dentist.<sup>1</sup> Complained by most adult population,<sup>2</sup> halitosis become a wide world

concern since this condition can cause social and psychological problems to those who suffer from it. A study in United States found that 24% of 60 years old people or more have been told that they suffer from halitosis.<sup>3</sup> While a Japanese

research found that up to 25% of the population has a Volatile Sulphur Compound (VSC) concentration, which is an indicator of halitosis, above the socially accepted score.<sup>4</sup> Based on the patient data at Cipto Mangunkusumo Hospital in Jakarta, Indonesia during 2001, approximately 3.5% of the patient that visited the Oral Medicine clinic complained about halitosis.<sup>5</sup>

Many things can be linked as the cause of halitosis, but about 85% of the cases happened because the microbial activity in the oral cavity.<sup>2,6</sup> Inside the oral cavity of a person with a clean, good oral hygiene, and full dentition with healthy periodontal tissue, the cause of halitosis usually comes from the back of the tongue. It is suspected because of the postnasal drip that is putrefied by microorganisms on the dorsum of the tongue.<sup>2</sup> This activity produces the VSC that causes the awful smell.

Halitosis complains can be very annoying, especially for the sufferers and people around them, so that so many ways are performed to reduce it. One of oral hygiene procedures that can be done daily to reduce halitosis is tooth brushing,<sup>7,8,9</sup> which was proven to reduce the VSC level up to 3 hours.<sup>10</sup> Another way to reduce halitosis is the use of oral rinse or

mouthwash. Several literature stated that the use of oral rinse containing chlorhexidine, cetylridinium chloride,<sup>11</sup> and hydrogen peroxide,<sup>8</sup> as some oral rinses that effectively reduce halitosis. A study in North America reported that millions of dollars were expenses annually to buy a variety of breath refresher such as oral rinses, mouth spray, candies, and gums.<sup>12</sup> At this moment, many products of oral rinse and toothpastes are offered in the market as solutions to reduce halitosis. Since that, this is a study that trying to explore the possibility of halitosis treatment by modifying one of the toothpaste in the market to be used as oral rinse water diluted in the water and combined it with tooth brushing using the same toothpaste.

#### **Aim and Objective**

This study's objectives are to determine the daylong effect of rinsing with water diluted toothpaste and combined with tooth brushing using the same toothpaste on the VSC levels, the Plaque Index (PI) scores, the Oral Hygiene Index Simplified (OHI-S) scores, Debris Index (DI) scores, the organoleptic scores and the Tongue Index (TI) scores.

## **Materials and Methods**

This is a double blind, clinical experimental study, held between May-August 2003 and performed at the Oral Medicine Clinic on Faculty of Dentistry University of Indonesia. The subject's inclusion criteria were male or female, age 17-30 years old. The subject was excluded if a smoker, routine alcohol consumer, with systemic disease (such as diabetes mellitus, pulmonary diseases, upper respiratory tract diseases, hepatic diseases, or metabolic disorder), long range antibiotic consumer who still eat antibiotics 2 weeks prior the study, routine user of oral rinse or mouthwash, routine consumer of odorous spices (such as garlic, onion, and other odorous food), and a woman on menstrual period.

The subjects were divided into 4 groups; 2 groups performed the oral rinse treatment with Antiplaque® toothpaste and placebo, and 2 groups performed the tooth brushing and oral rinse using Antiplaque® toothpaste and placebo. Either the subjects nor the examiner knew which one was the Antiplaque® toothpaste or the placebo, since all the toothpastes were placed in white tubes marked only as 'X' and 'Y', given by the manufacture. Oral Rinse is the action of rinsing the mouth using 20 ml mineral water

diluted 1 cm of given toothpaste. Tooth brushing and Oral Rinse is the combination of the procedure to brush the teeth with Bass method for 2 minutes and continued with using the 20 ml water diluted 1 cm of given toothpaste as oral rinse.

The subjects were given the informed consent and the questionnaire in order to separate the subjects that fulfil the inclusion criteria. They should not wear any scented cosmetics and perfume before the measurement, not eat, drink, chewing gums or candies, flush or gargle at least 2 hours prior the measurement. The subjects should not eat odoriferous food at least 48 hours prior the measurement and should not consume antibiotics at least 72 hours prior the measurement. At the same time, the examiner should not use any scented cosmetics and perfume when taking the measurement and should not smoke, eat, drink, chew gum or candy, rinse with mouthwash, at least 5 minutes prior the measurement.

The daylong measurement consisted of 2 times measurement, before and after the treatment. The before measurements are taken at 2-4 pm day I and the treatments are performed 2 times: at 10 pm on day I and at 6 pm on day II.

The after measurements are taken at 2-4 pm on day II.

VSC level is the level of Volatile Sulphur Compounds in the breathing air of the subjects. The measurement was conducted using the Industrial Portable Sulphide Monitor Halimeter (Interscan Corp, model 1170), which was zeroed on ambient air prior to each measurement. A disposable plastic straw was inserted in the airway of the monitor. The Subject was refrained from talking and to close the mouth for 3 minutes, and breathe through the nose. Then, the subject was asked to insert the straw approximately 2.5-5 cm into the oral cavity and to breathe through the nose. Results were recorded as peak part per billion (ppb) sulphide equivalents. The VSC scores are taken for 3 times, and the average score were recorded. The straw was changed for each subject. The halimeter should be warmed up 30 minutes before the first measurement and placed on the site that away from the crowd. The halimeters should be calibrated interhalimeter to gain the same standard VSC level.

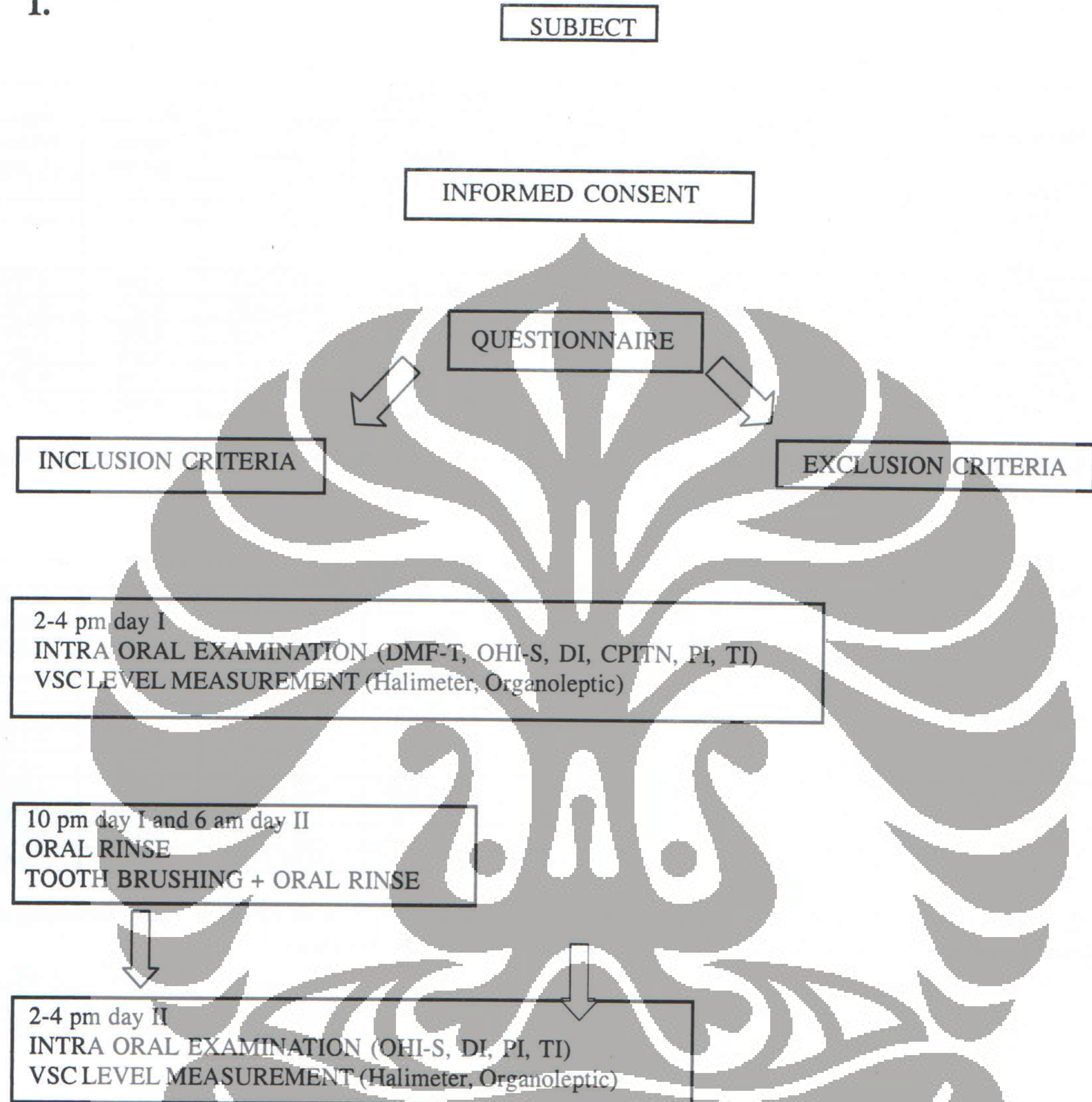
Organoleptic technique was done by using a separating board (50x70 cm) with a little hole in the middle, prepared from unscented material. This board was used to separate the subject and

the examiner, in order to prevent the subjective measurement of the examiner. A disposable plastic straw (diameter 5.9 mm, 18.4 mm long) placed in the board's hole. The straw inserted into the subject's mouth approximately 2.5-5 cm. The subject was asked to breathe through his/her nose slowly, while the examiner smelled the odour at the end of the straw and behind the board. The result was recorded based on the organoleptic scale (0: no detectable odour; 1: questionable odour; 2: slight malodour; 3: moderate malodour; 4: strong malodour; 5: severe halitosis).

Intra oral examination that were taken on the study were Decay Missing, Filling-Tooth (DMF-T) status according WHO standard, Debris Index (DI) and Oral Hygiene Index Simplified (OHI-S) score by Greene and Vermilion,<sup>13</sup> Plaque Index (PI) and Gingival Index (GI) by Loe and Silness,<sup>14,15</sup> and the Community of Periodontal Index and Treatment Needs (CPITN), and also Tongue Index (TI) that measure the coating on the dorsum of the tongue.<sup>16</sup>

The sample collection flow is described in picture 1.

1.



Picture 1. Sample collection flow

No	Measurements	Oral Rinse Mean						Wilcoxon signed rank test	Mann-Whitney U test
		Antiplaque*		Wilcoxon signed rank test	Placebo Treatment		Wilcoxon signed rank test		
		Before	After		Before	After			
1	DMF-T	3.93 ± 4.13	ND	-	3.67 ± 2.71	ND	-	-	
2	CPITN	0.83 ± 1.78	ND	-	0.83 ± 1.68	ND	-	-	
3	Calculus Index	0.62 ± 0.36	ND	-	0.61 ± 0.41	ND	-	-	
4	OHIS	0.78 ± 0.49	0.68 ± 0.39	0.005	0.81 ± 0.56	0.77 ± 0.49	0.206	0.775	
5	Debris Index	0.15 ± 0.24	0.05 ± 0.12	0.007	0.49 ± 0.31	0.14 ± 0.26	0.257	0.775	
6	Tongue Index	0.67 ± 0.46	0.53 ± 0.49	0.239	0.60 ± 0.61	0.27 ± 0.45	0.005	0.358	
7	Plaque Index	8.73 ± 5.73	5.03 ± 2.82	0.003	10.9 ± 4.67	8.37 ± 3.75	0.006	0.609	
8	Organoleptic	0.53 ± 0.57	0.53 ± 0.58	0.743	0.75 ± 0.75	0.55 ± 0.49	0.118	0.254	
9	VSC	161.7 ± 10.98	150.8 ± 10.98	0.000	102.73 ± 8.00	158.03 ± 13.5	0.666	0.000	

**Table 1.** VSC levels and Intra Oral Status Score Mean Before and After Oral Rinse Treatment Using the Water Diluted Antiplaque\* and Placebo Toothpaste.

No	Measurements	Tooth Brushing and Oral Rinse Mean						Wilcoxon signed rank test	Mann-Whitney U test
		Antiplaque*		Wilcoxon signed rank test	Placebo		Wilcoxon signed rank test		
		Before	After		Before	After			
1	DMF-T	2.20 ± 2.43	ND	-	4.07 ± 4.36	ND	-	-	
2	CPITN	0.80 ± 1.45	ND	-	1.10 ± 1.86	ND	-	-	
3	Calculus Index	0.62 ± 0.32	ND	-	0.72 ± 0.43	ND	-	-	
4	OHIS	0.74 ± 0.37	0.67 ± 0.35	0.018	0.95 ± 0.53	0.86 ± 0.49	0.022	0.316	
5	Debris Index	0.12 ± 0.17	0.05 ± 0.18	0.018	0.23 ± 0.19	0.14 ± 0.24	0.022	0.316	
6	Tongue Index	0.45 ± 0.49	0.27 ± 0.41	0.077	0.40 ± 0.48	0.22 ± 0.39	0.077	1.000	
7	Plaque Index	8.73 ± 3.43	4.67 ± 2.83	0.000	9.67 ± 4.12	7.37 ± 3.53	0.008	0.205	
8	Organoleptic	0.65 ± 0.59	0.23 ± 0.41	0.003	0.33 ± 0.46	0.28 ± 0.43	0.429	0.019	
9	VSC	172.1 ± 10.55	150.07 ± 11.5	0.000	84.17 ± 2.67	134.03 ± 8.95	0.171	0.000	

**Table 2.** VSC levels and Intra Oral Status Score Mean Before and After Combination of Tooth Brushing and Oral Rinse Treatment Using the Water Diluted Antiplaque\* and Placebo Toothpaste.

Before all the measurement were taken, the room for the experiment may not be cleaned with scented floor cleaner or equipped with automatic or manual air freshener. The room should be clear from dental material or alcohol scent, with room temperature and humidity maintained in the optimum state. There were only 2 persons allowed sitting near the halimeter while taking measurement and the room should not be contaminated by the food odour (restricted from eating).

This study use then non parametric statistical analysis. The Wilcoxon Signed Rank Test was used to determine pair wise comparisons in groups. The Mann-Whitney Test was used to determine pair wise comparisons between groups, at the  $P < 0.05$  significance level. Spearman's correlation coefficients were employed to analyze the correlation among the result of the different measurement techniques. Since there were several examiners in this study, to examine the validity of interexaminer agreement, we used the coefficient agreement of Kappa, which had resulted "very good agreement" for the scoring on tongue coating index and organoleptic test.

## Result

Total subjects who completed this study were 120 persons. The subjects include 84 females (70%) and 36 males (30%). The average age of all subjects is  $22.07 \pm 2.86$  years old, while the females were  $22.08 \pm 2.92$  years old and males were  $22.06 \pm 2.64$  years old. The subjects were divided into 4 groups that each consisted of 30 subjects. They were given the toothpaste signed X for Antiplaque® toothpaste and Y for placebo.

All the intraoral status and VSC levels were recorded before and after all treatments. On table 1, it is shown the score means for the first treatment groups, who performed the oral rinse with water diluted toothpaste with given toothpaste and did the daily tooth brushing with the regular toothpaste. Table 1 shows that after the oral rinse treatments; there were reductions on the mean of the intraoral status such as Debris Index, OHI-S, Tongue Index, Plaque Index, and Organoleptic scores, and also reduction on the VSC levels for Antiplaque® toothpaste. The result for placebo toothpaste was almost the same, but there was an increase on VSC level mean after the treatment was done. Since there was no professional dental treatment performed on the subjects, the DMF-T, CPITN, and Calculus

Index were not measured again after the treatment.

The Wilcoxon signed rank test for two paired samples were performed to analyze the significance of the reduction on VSC levels, OHI-S, Plaque Index, Debris Index, Tongue Index and Organoleptic scores. The Mann-Whitney U test for two independent samples was performed to analyze the significance of the reduction means of VSC levels and intraoral status score between two different toothpastes used in the study. The Spearman's correlation test was performed to analyze the correlation on the reduction of VSC levels, organoleptic and intra-oral status, for all the treatments.

The result of the Wilcoxon signed rank test showed significant result ( $P < 0.05$ ) when the treatment was performed with Antiplaque<sup>®</sup> toothpaste for VSC level, Debris Index, OHI-S and Plaque Index but not statistically significant for organoleptic and tongue index ( $P > 0.05$ ). On the other hand, for the treatment using placebo toothpaste, significant result ( $P < 0.05$ ) were shown only on Plaque Index and Tongue Index score means. The Mann Whitney U test showed significant result ( $P < 0.05$ ) only on VSC level. It means that there is significant difference between

Antiplaque<sup>®</sup> and placebo toothpaste in reducing the VSC level, when the toothpastes used as oral rinse. The Spearman's test of correlation showed that before and after the oral rinse with Antiplaque<sup>®</sup> toothpaste or the placebo toothpaste, there is no significant correlation ( $P > 0.05$ ) for all the measurement.

Table 2 showed the combination treatment of tooth brushing and oral rinse result using Antiplaque<sup>®</sup> toothpaste and placebo toothpaste. There were reduction on all measurement score mean taken in this study, for both toothpastes. The Wilcoxon signed rank test showed significant result ( $P < 0.05$ ) when the treatment was performed with Antiplaque<sup>®</sup> toothpaste on all measurements taken, except on Tongue Index ( $P > 0.05$ ). The treatment using the placebo toothpaste showed significant result ( $P < 0.05$ ) only for Debris Index, OHI-S, and Plaque Index. The Mann-Whitney U test showed significant result ( $P < 0.05$ ) on the VSC level and organoleptic score. It means that there is significant difference between Antiplaque<sup>®</sup> and placebo toothpaste in reducing the VSC level and organoleptic score for the combination treatment of tooth brushing and oral rinse. The Spearman's test of correlation showed that



before and after the tooth brushing and oral rinse treatment with Antiplaque® toothpaste or the placebo toothpaste, there is no significant correlation ( $P>0.05$ ) for all the measurement.

### Discussion

In this study, it has been assessed daylong reduction on halitosis associated variables following rinsing and the combination of tooth brushing and rinsing using water diluted Antiplaque® toothpaste, as opposed to a placebo toothpaste. Measurements carried out some 8-10 hours after the treatment were compared with the baseline measurements carried out at about the same time on the previous day. Measurement were carried out in a blind fashion using several techniques, which were measurement of oral sulphides using a portable monitor and the measurement of intraoral status such as DMF-T, Debris Index, OHI-S, Tongue coating Index, and Plaque Index. The measurements were corroborated by organoleptic rating of two odour judges in order to reduce the possibility of subjective results.

The treatments performed in this study were tooth brushing and rinsing, two of daily oral hygiene practice that most people do. In this

study, a brand of toothpaste easily found in the market, Antiplaque®, is used in usual and modified way, as regular toothpaste used for tooth brushing, and as an oral rinse after being diluted in 20 ml of water. Antiplaque® toothpaste is a brand of toothpaste that contains 4 active ingredients: cloxifenol 0.3%, sodium monofluorophosphate 0.8%, oleum caryophilly, and arnica tincture; that claimed to act as antiseptic, anti inflammation, anti caries, and tooth sensitivity reducer.<sup>17-19</sup> This study is trying to prove whether this toothpaste also has an effect to reduce VSC and also can be used in a modified way such as oral rinse. In this study, Antiplaque® toothpaste was compared to placebo toothpaste containing the same colour and taste, but without active ingredients, and both of the toothpaste types were placed in similar tubes.

The daylong time sequences, which is 8-10 hours after the treatments were performed, was chosen because some study revealed that cloxifenol, one of the active ingredients in Antiplaque® toothpaste, can be detected on oral mucosa and dental plaque on 8 hours after usage.<sup>20</sup> Another reason was that after dental plaque being deposited, it requires minimum 8

hours of maturation to produce VSC.<sup>21</sup> So, in order to prove the effectiveness of Antiplaque<sup>®</sup> toothpaste, a 8-10 hours sequences after the toothpaste used for tooth brushing or as water diluted toothpaste, was chosen.

The result showed that on the modified use of the Antiplaque<sup>®</sup> toothpaste, the VSC level was significantly reduced, as compared to the placebo toothpaste, in both treatment groups. In the oral rinse group and in the combination treatment group of tooth brushing and oral rinse, the use of Antiplaque<sup>®</sup> toothpaste significantly reduced the daylong VSC, compared to the placebo toothpaste. But, the reduction is more significant in the combination treatment group where Antiplaque<sup>®</sup> toothpaste was used for both tooth brushing and oral rinse, when compared to oral rinse group where Antiplaque<sup>®</sup> toothpaste was used only as water diluted oral rinse and use other toothpaste for tooth brushing. The reason of the VSC level reduction after the treatment with Antiplaque<sup>®</sup> toothpaste is that tooth brushing is considered quite adequate in reducing maintaining odour at non-objectionable level in 50% of the population,<sup>22</sup> although tooth brushing alone only resulted in less than 25% reduction of hydrogen sulphide and mercaptan,<sup>21</sup> the major oral VSC.

The use of water diluted Antiplaque<sup>®</sup> toothpaste as oral rinse for an adjunct of tooth brushing, probably increase the effect of the active agents in the toothpaste, since oral rinse is considered as a simple and effective means of distributing agent around the bacterial plaque and preventing gingivitis.<sup>23</sup>

The reduction of Plaque Index in all treatment groups, either in Antiplaque<sup>®</sup> toothpaste or the placebo groups, are significant. But the comparison between toothpastes and between treatments did not result in significant values. It means that all of the treatments in this study can reduce the number of plaque. Possibly, it is due to the mechanical effect of the same tooth brushing procedure that was performed in all groups although using the different toothpastes.

The most common personal oral hygiene procedure, tooth brushing, is the most common method for plaque control. There is substantial evidence that manual toothbrushes are effective in removing bacterial plaque and preventing gingivitis.<sup>24</sup> Although comparison of the reduction between the Antiplaque<sup>®</sup> and placebo toothpaste groups are not significant, still the reduction of Plaque Index are higher in the Antiplaque<sup>®</sup> toothpaste groups, even in the oral rinse group.

It is due to the fluoride and cloxifenol contains in Antiplaque® toothpaste. Fluoride in dentifrice and oral rinses have shown to prevent the accumulation of plaque and to reduce existing plaque.<sup>25</sup> Cloxifenol or triclosan, has otherwise a clear antibacterial and antiplaque effect in combination with sodium lauryl sulfate (SLS).<sup>20</sup> The use of Antiplaque® toothpaste as oral rinse might answer the reason of lower plaque index in the combination treatment group, since it is a fact, that most people experience difficulties in maintaining adequate levels of plaque control, particularly at interproximal sites. Hence, a simple and effective means of distributing agent around the mouth by oral rinses, provides the rationale for chemical control of plaque as a means of augmenting mechanical oral hygiene procedure.<sup>26</sup>

On Debris Index measurement, the result shows that there are reductions on all treatment groups. There is a significant reduction on the Antiplaque® toothpaste oral rinse group but not in the placebo one, though the comparison between the two groups is not significant. In both group on the combination of tooth brushing and oral rinse, there are significant reduction on Debris Index, though the comparison between the two groups is also not significant. The comparison

between the two different treatments is also not significant. Possibly, because all groups use oral rinses. With or without active agents, most oral rinse have limited effect to clean large size debris.<sup>27</sup> Another reason is the measurement took place in the afternoon about 2-4 hours after lunch time, when the subjects of the study could already had their meals prior the measurement. So, the presence of debris in the oral cavity is not impossible and the type of the meals also had influence on it.

On oral Hygiene Index Simplified measurements, the results are just the same as Debris Index. It happens because the OHI-S score comprises of Debris Index score and Calculus Index score. On this study, there was no dental professional treatment for the subjects, so there was no treatment for their calculus also. It means that the fluctuation of the OHI-S score only influenced by the fluctuation of Debris Index. In other words, the reduction of Debris Index on all treatment groups caused the reduction of OHI-S too.

The results show that Tongue Index score was reduced in both oral rinse groups, but the reduction only significant in Placebo group. While in the combination treatment groups, the reduction

on Tongue Index were the same and none of them is significant. On comparison analysis, none of them is significant. Possibly, it is due to the absence of tongue cleaning procedure in all treatment groups. Dorsum of the tongue often considered as the primary source of VSC production both in periodontally healthy and diseased population,<sup>28</sup> and selected bacterial group colonizing the dorsal surface of the tongue play an important role in oral malodour formation.<sup>29</sup> The presence of tongue coating, which has been shown to correlate with malodour, is related to the bacteria density or total number of bacteria the tongue sample.<sup>30</sup> A study revealed the possibility of using an anti microbial mouth rinse such as Chlorhexidine that eliminating the flora of the tongue, would reduce bad breath.<sup>14</sup> But the result in the present study did not show a significant result for tongue coating reduction, except in the placebo oral rinse group and the possible reason of this result might be due the of meals taken by the subjects prior the measurement.

For the organoleptic measurement, the results show that on the oral rinse treatment group, there is no significant reduction in both toothpaste, and the comparison between them

is not significant either. But in combination treatment groups, there is a significant reduction in the Antiplaque<sup>®</sup> toothpaste group but not in the placebo group and the comparison between is also significant. There is also a significant difference between the comparison of the combination treatment and the oral rinse groups. The significant result probably is caused by the multiple use of the toothpaste for tooth brushing and for oral rinse. The act of tooth brushing is effective in removing bacterial plaque<sup>24</sup> and in reducing the odour slightly above threshold concentration to acceptable levels.<sup>22</sup> The act or most oral rinse have a limited effect on cleansing the large size debris, temporary reducing the micro organisms, and give the senses of freshness.<sup>27</sup> The reduction of the microbial load in the oral cavity is important for the control of oral malodour and that, consequently, the growth of bacteria appears to be critical for oral malodour production.<sup>28</sup> These actions reduces the oral odour that resulted in the reduction of organoleptic scores.

### Conclusion

Compared to placebo toothpaste, the use of Antiplaque<sup>®</sup> toothpaste as water diluted

toothpaste for oral rinse, in combination with daily tooth brushing with or without Antiplaque® toothpaste can significantly reduce halitosis marked by the reduction of the daylong VSC levels, and some intra oral status. For suggestion, there is a need for further study on the effect of Antiplaque® toothpaste on larger number of subjects and more variable, such as on older age groups or subjects with halitosis complain.

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