A Salivary Incubation Test for Evaluation of Oral Malodor: A Pilot Study

Marc Quirynen,*† Hong Zhao,*† Pieter Avontroodt,* Catherine Soers,* Martine Pauwels,† Wim Coucke,‡ and Daniel van Steenberghe*

Background: Breath odor is scored by different techniques, each with its own shortcomings. Organoleptic ratings are uncomfortable for the patient, subjective, influenced by external parameters including food and cosmetics, and especially lack international calibration. Portable sulphide monitors are relatively expensive and neglect several major malodorous molecules (e.g., butyric and propionic acids, putrescine, and cadaverine). Gas chromatography necessitates expensive devices and experienced technicians. This pilot study explored the applicability of a new technique (saliva incubation) by comparing its discrimination power, in a morning bad breath inhibition study of antiseptics, to those of hydrogen sulphide (H_2S) measurement devices and organoleptic ratings.

Methods: After a professional cleaning, 8 periodontally healthy students abstained from all means of mechanical plaque control for 5 experimental periods of 7 days, with intervening washout periods of at least 2 weeks. During each experimental period, the students rinsed only twice daily with different antiseptics. At day 7, morning breath was scored clinically (volatile sulphide compound [VSC] level and organoleptic ratings), and 1.5 ml of saliva was collected and divided between 3 glass tubes that were sealed and incubated (37°C, anaerobic chamber). Immediately after collection and after 3 and 6 hours of incubation, the headspace air in one of the tubes was examined for VSC production and organoleptic measurements.

Results: The investigations of the incubated saliva correlated well with the 7-day intraoral VSC recordings and organoleptic ratings ($P \leq 0.005$). Moreover, evaluations showed a similar interproduct ranking for their efficacy in malodor control. The power analyses indicated a higher discrimination power for the saliva incubation test than for the intraoral registrations.

Conclusions: The strong correlation between odor production of incubated saliva and clinical assessments suggests that the saliva incubation test may be used as an indirect method to measure oral malodor and can be employed to investigate the antimalodor effectiveness of oral hygiene products. *J Periodontol 2003;74:937-944.*

KEY WORDS

Chlorhexidine/therapeutic use; halitosis; mouthwashes/therapeutic use; saliva/microbiology; sulfur compounds.

* Department of Periodontology, School of Dentistry, Oral Pathology and Maxillo-Facial Surgery, Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium.
† Research Group for Microbial Adhesion, Catholic University of Leuven.
‡ Statistic consultant, Heverlee, Belgium.

Breath malodor, defined as a foul or offensive odor of expired air, may be caused by a number of factors, both intra- and extraoral (gingivitis/periodontitis, nasal inflammation, chronic sinusitis, diabetes mellitus, liver insufficiency, etc.), and can be linked to more serious underlying medical problems including primary biliary cirrhosis, uremia, lung carcinoma, decompensated liver cirrhosis, and trimethylaminuria.[1-5] In most cases, however, bad breath originates from the oral cavity itself.[5,6] Moreover, in some countries, the proportion of halitophobic patients reaches high levels.[7] The principal components of oral malodor are volatile sulphide compounds (VSC), especially hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulphide [$(CH_3)_2S$][1] or compounds such as butyric and propionic acid, putrescine, and cadaverine.[8] These compounds result from the proteolytic degradation by predominantly anaerobic Gram-negative oral microorganisms (e.g., *Porphyromonas gingivalis, Prevotella intermedia/nigrescens*) on various sulphur-containing substrates, e.g., food debris, saliva, blood, and epithelial cells.[1,9-11]

There are a variety of direct and indirect methods to assess oral malodor. The main direct approaches are organoleptic ratings, gas chromatography, and sulphide monitoring. Organoleptic ratings involve sniffing the mouth air (expired or not), smelling the tongue coating (on an odorless spoon), or smelling the wrist

after licking (often used for self-examination), followed by an arbitrary rating on a 4- or 5-number scale.[12] Since oral malodor is perceived via an olfactory stimulus, this direct method may be considered the most ideal technique.[13] However, this technique has many shortcomings including subjectivity,[14,15] low interexaminer reproducibility,[12] and lack of official standards. There also may be confounding factors such as the psychological/physiological state of the judge including hunger, menstrual cycle, head position, degree of attentiveness,[12] and need for precautions such as abstaining from eating garlic, onion, or spicy food; refraining from smoking and drinking coffee; and avoiding scented cosmetics before assessment.[7,16] Moreover, for most patients, an organoleptic examination gives an uncomfortable and/or embarrassing feeling.[7] Gas chromatography, initially used to measure volatile substances in fecal and urinary samples,[17] also offers a high specificity for volatile sulphur compounds. The main disadvantages of this technique are high costs for the equipment, the need for a skilled operator, lack of portable devices, and time required for detection and measurement. Therefore, this approach is not yet practical for quantitative measurement of large populations.[12] In the last 10 years or so, the use of a portable sulphide monitor for measuring halitosis has been introduced.[15,18] A sulphide monitor§ detects hydrogen sulphide and methyl mercaptan, some of the most common VSCs found in malodor. However, this device cannot distinguish different sulfides and does not measure all odorous compounds. Moreover, the sensitivity of the sulphide monitor for mercaptans, which are organoleptically more offensive than hydrogen sulphide,[19] is significantly lower,[20] which may explain a poor correlation between VSC scores and organoleptic ratings.[17] Furthermore, this instrument must be recalibrated periodically because of the loss of sensitivity to the sulphur compounds with time.[18]

Because of the variability and technical problems with the direct oral malodor measurements, research has been conducted on indirect methods. Saliva is believed to be one of the main sources of oral malodor because it contains a large reservoir of sulphur-containing substrates that can be hydrolyzed and further degraded to volatile sulphur compounds.[1,21] Therefore, salivary samples may be used for an indirect malodor examination. Some earlier data on the volatiles' production, measured from petri dishes with microbial cultures from patients' plaque samples, were encouraging.[22]

This study aimed to evaluate the reliability of this indirect test via a series of correlation analyses with intraoral organoleptic and sulphide ratings. Moreover, in order to test the discrimination power of the technique, it was introduced as an additional diagnostic tool in an antimalodor efficacy study of various antiseptics.

MATERIALS AND METHODS

Experimental Design

Eight medical students (3 males and 5 females; mean age, 20 years) volunteered for a double-blind, randomized, crossover clinical trial. They had at least 24 teeth, with no signs of ongoing periodontitis, no caries or extensive dental restorations, and they had not taken systemic antibiotics for the past 4 months. Three weeks prior to the study, all participants underwent a thorough professional cleaning and were instructed to exercise meticulous self-performed plaque control so that all subjects had clinically healthy gingiva. This preparatory phase was followed by 5 experimental periods (each starting with a professional cleaning) during which each participant abstained from all mechanical plaque control for 7 days, but instead rinsed twice daily for 1 minute. The last 10 seconds consisted of gargling with one of the following formulations: 10 ml of a 0.2% chlorhexidine (CHX) alcohol mouthrinse (CHX-Alc);‖ 10 ml of a 0.05% CHX plus 0.05% cetylpyridinium chloride plus 0.14% zinc lactate mouthrinse (CHX-CPC-Zn);¶ a freshly prepared slurry from a toothpaste (3 cm toothpaste in 10 ml water) containing amine fluoride (350 ppm F^-) and stannous fluoride (1,050 ppm F^-) (AmF-SnF_2^{Sl});# 10 ml of an amine fluoride (125 ppm F^-) stannous fluoride (125 ppm F^-) mouthrinse (AmF-SnF_2^{Mr});** or 10 ml of a placebo solution (placebo), comparable to the previous solution but without fluoride. Immediately after recording the 7-day breath parameters (see below), the participants received a comprehensive professional tooth cleaning and were instructed to take up again their meticulous home care. After a washout period of at least 14 days, the next experimental period followed with another mouthrinse. A total of 5 experimental periods were completed until all subjects had rinsed at random with each formulation.

Morning Breath Evaluation

On day 7 of each experimental period, the volunteers reported at 8 a.m. for both organoleptic ratings and VSC measurements. That morning, the volunteers refrained from drinking, eating, gargling, smoking, and using any scented cosmetic products.[16,23] The evening prior to the evaluations, alcohol consumption, smoking, and the use of deodorants, shampoo, etc. were forbidden. Participants rinsed once with the mouthrinse 30 minutes prior to the measurements. This delay was to avoid a direct influence of the rinse.[24]

The VSC levels were measured by a portable sulfide monitor§ as suggested by Rosenberg and coworkers.[18]

§ Model RH-17E, Halimeter, Interscan Corp., Chatsworth, CA.
‖ Corsodyl, SmithKline Beecham, Genval, Belgium.
¶ Halita, Dentaid S.A., Barcelona, Spain.
# Meridol toothpaste, GABA International AG, Münchenstein, Switzerland.
** Meridol mouthrinse, GABA International AG.

A disposable, flexible drinking straw was connected to the factory-supplied tubing and inserted in the subjects' mouth, with the top of the straw 3 cm behind the incisors, while the mouth was kept slightly opened. This procedure was performed twice, once after the mouth had been closed for 2 minutes, and once without a previous closure of the mouth.[25] The results, both the peak and plateau values, were recorded as parts per billion (ppb) sulphide equivalents.

The breath was organoleptically scored by the same periodontist, calibrated for this purpose, using a 0 to 4 rating.[23] This included ratings of the mouth air (smell in the mouth while the subject is counting) and of the tongue coating (small sample removed with an odorless spoon).[23] The examiner was blinded to the rinse used and the VSC scores. Scores ranged from 0 to 4: 0 represents no odor; score 1 was given for a doubtful to faint odor; 2 for a definite odor; 3 for a strong odor; and 4 for very strong bad breath.[26]

In Vitro Salivary Incubation Test

Immediately after breath assessments, 1.5 ml of unstimulated saliva was collected and equally divided into 3 sterile glass test tubes (15.5 cm in length, diameter 1.5 cm). These test tubes were flushed with CO_2 and sealed with a rubber cup and tape. One tube was immediately reopened to measure the VSC (baseline value). The 2 remaining tubes were incubated at 37°C in an anaerobic chamber under an atmosphere of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen, for 3 and 6 hours, respectively. Immediately after removal from the chamber, the odor of each tube was assessed both organoleptically and via the sulphide monitor. For the organoleptic ratings, the evaluator sniffed the headspace air of the tubes and rated the smell as described above. The VSC levels were measured with the same portable sulphide monitor as used in a clinic, zeroed on ambient air prior to each measurement. A disposable plastic straw was inserted 5 cm into the tube. The peak VSC score was determined in ppb sulphur equivalents.

Our previous pilot study indicated that the use of glass tubes, together with the above-mentioned sealing procedure, prevented false VSC registrations due to the inflow of gas from the incubation chamber and misjudgments of the organoleptic scores due to the smell of the hardware.

Microbiological Parameters

The bacterial load of the saliva incubated at both 0 and 6 hours was analyzed via aerobic and anaerobic culturing. From the corresponding test tubes, 500 µl was taken using a sterile plastic loop[††] and suspended in 3 ml reduced transport fluid (RTF).[27] Serial 10-fold dilutions were prepared in RTF. For all samples, dilutions 10^{-1} to 10^{-5} were plated in duplicate using a spiral plater[‡‡] onto non-selective blood agar plates[§§] supplemented with hemine (5 mg/l), menadione (1 mg/l), and 5% sterile horse blood. After 7 days of anaerobic (80% N_2, 10% CO_2, and 10% H_2) and aerobic incubation at 37°C, the total number of anaerobic and aerobic colony forming units (CFU)/ml was counted.

Statistical Analysis

The statistical analysis included an evaluation of the correlation between the indirect saliva test and different intraoral breath parameters (Pearson correlation coefficient) as well as a power analysis to estimate the most sensitive (discriminating) parameters. The interproduct comparison started with a repeated measures analysis with the respective parameter as the dependent variable, and period, product, time for VSC values after 3 and 6 hours' incubation, and the interaction product × time as independent variables. If the product parameter had a significant impact, differences between the 5 products were detected via a set of pair-wise comparisons, corrected for simultaneous hypothesis testing using the Tukey-Kramer method for multiple comparisons. Before each analysis, the residuals were tested for normality by a normal QQ-plot. In case of a deviation from normality, data were transformed by a log transformation. The Akaike's information criterion was used to choose the best fitting error correlation matrix. A carry-over effect could not be detected. The power analysis examined the Type II error (number of cases out of 1,000 data sets, randomly generated by Monte Carlo Sampling according to the fitted error correlation matrix, in which the null hypothesis, i.e., there are no differences between the products, is not rejected). All power was calculated for a fixed significance level of 0.95; the power is expressed as 1-β, with β representing the probability of making a Type II error. For the incubation VSC data, this analysis was performed for either the 6-hour observation or both the 3- and 6-hour data, taking the patient as a repeated factor into consideration. Based on the results obtained in this study, the number of patients needed for an intraoral VSC recording, to obtain the same power as for the VSC recording via the incubation test, has also been estimated.

RESULTS

VSC Scores of Incubated Saliva Versus Intraoral VSC Recording

The VSC data from the incubated saliva are depicted in Figure 1. The baseline values were extremely low for all products (overall mean 29.3 ± 13.3, ranging from 12 to 74 ppb; the mean value per product ranged

†† Biomérieux SA, Montalieu, France.
‡‡ Spiral Systems, Inc., Cincinnati, OH.
§§ Blood Agar Base II, Oxoid, Basingstoke, U.K.

from 25.9 to 33.4 ppb). After 3 and 6 hours, the VSC values increased significantly, but this increase was clearly (P <0.0001) product dependent (Table 1). The inter-product differences after 3 and 6 hours' incubation were similar (P = 0.76), in the following order: CHX-Alc < CHX-CPC-Zn < AmF-SnF_2^{Mr} < AmF-SnF_2^{Sl} < placebo. A statistically significant difference could be detected between the placebo solution and all other products (P <0.001) and between CHX-Alc and AmF-SnF_2^{Sl} (P = 0.05). The latter became obvious when the 2 time periods (3 and 6 hours) were both included.

The VSC values for the 2 direct intraoral recordings (with and without previous closure of the mouth for 2 minutes) are also included in Figure 1. The values were slightly higher after closure of the mouth, but the tendency over the products (CHX-CPC-Zn < CHX-Alc ≤ AmF-SnF_2^{Mr} < AmF-SnF_2^{Sl} < placebo) was comparable for both types of recording. The statistical analysis on this data showed fewer interproduct differences than for the indirect test (Table 1). The statistical analyses detected a significant difference only between the CHX-CPC-Zn solution on one hand, and the AmF-SnF_2^{Sl} (P≤0.005) and placebo solution (P≤0.001) on the other hand, respectively (Table 1).

The Pearson correlation coefficients between VSC values of the incubation test and the intraoral recordings were high (r, ± 0.60) and statistically significant (Table 2).

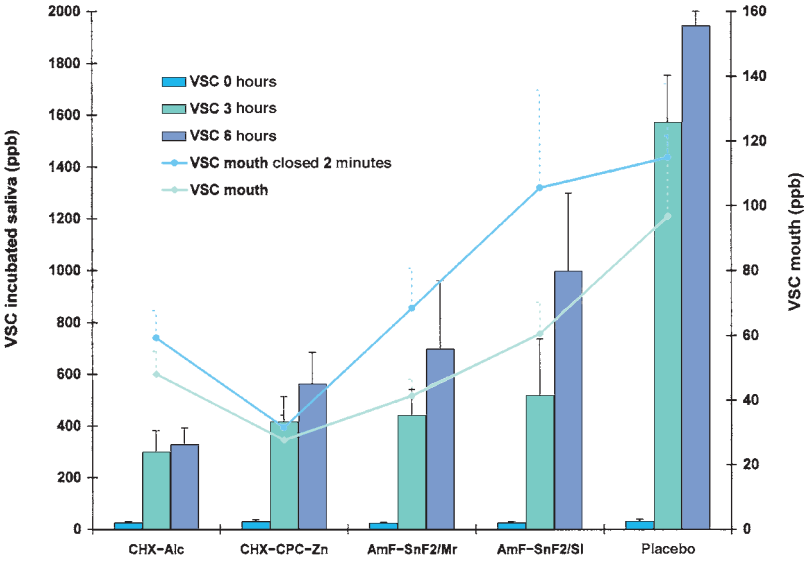

Figure 1.
Oral and saliva VSC values at different time points.

VSC Scores of Incubated Saliva Versus Organoleptic Mouth Odor Ratings

Figure 2 shows the 3- and 6-hour VSC measurements from incubated saliva and their relationship with the clinical organoleptic ratings. Both parameters resulted in a similar ranking of the different products, although the differentiation between the

Table 1.

Probability Scores for Interproduct Differences in VSC Values and Organoleptic Ratings (OR)

Interproduct Comparison		Intraoral VSC	Incubated Saliva			
			VSC		OR	
			6 Hours	3 and 6 Hours	3 Hours	6 Hours
CHX – Alc	CHX – CPC – Zn	0.194	0.861	0.633	0.962	0.956
CHX – Alc	AmF – SnF_2^{Mr}	0.988	0.723	0.395	0.685	0.711
CHX – Alc	AmF – SnF_2^{Sl}	0.432	0.178	**0.050**	0.685	0.091
CHX – Alc	Placebo	0.159	0.001	0.000	0.001	0.000
CHX – CPC – Zn	AmF – SnF_2^{Mr}	0.076	0.998	0.995	0.962	0.956
CHX – CPC – Zn	AmF – SnF_2^{Sl}	**0.005**	0.676	0.642	0.962	0.306
CHX – CPC – Zn	Placebo	**0.001**	**0.001**	**0.000**	**0.002**	**0.000**
AmF – SnF_2^{Mr}	AmF – SnF_2^{Sl}	0.719	0.824	0.857	1.000	0.711
AmF – SnF_2^{Mr}	Placebo	0.366	**0.001**	**0.000**	**0.022**	**0.000**
AmF – SnF_2^{Sl}	Placebo	0.971	**0.016**	**0.000**	**0.022**	**0.000**

Boldface type indicates a significant difference.

Table 2.

Pearson Correlation Coefficient Between VSC Values of Incubated Saliva (3 or 6 Hours) and Various Parameters

Compared parameters		Correlation Coefficient r	R^2	*P*
VSC saliva 3 hours	VSC mouth closed 2 minutes	0.600	0.360	**0.000**
VSC saliva 6 hours	VSC mouth closed 2 minutes	0.580	0.337	**0.000**
VSC saliva 3 hours	OR mouth air	0.538	0.289	**0.000**
VSC saliva 6 hours	OR mouth air	0.459	0.211	**0.003**
VSC saliva 3 hours	OR tongue coating	0.443	0.196	**0.004**
VSC saliva 6 hours	OR tongue coating	0.391	0.153	**0.013**
VSC saliva 6 hours	CFU/ml aerobic 6 hours	0.534	0.285	**0.000**
VSC saliva 6 hours	CFU/ml anaerobic 6 hours	0.505	0.255	**0.001**
VSC saliva 6 hours	OR saliva 6 hours	0.798	0.637	**0.000**
VSC saliva 3 hours	VSC saliva 6 hours	0.809	0.654	**0.000**

Boldface type indicates a significant difference.

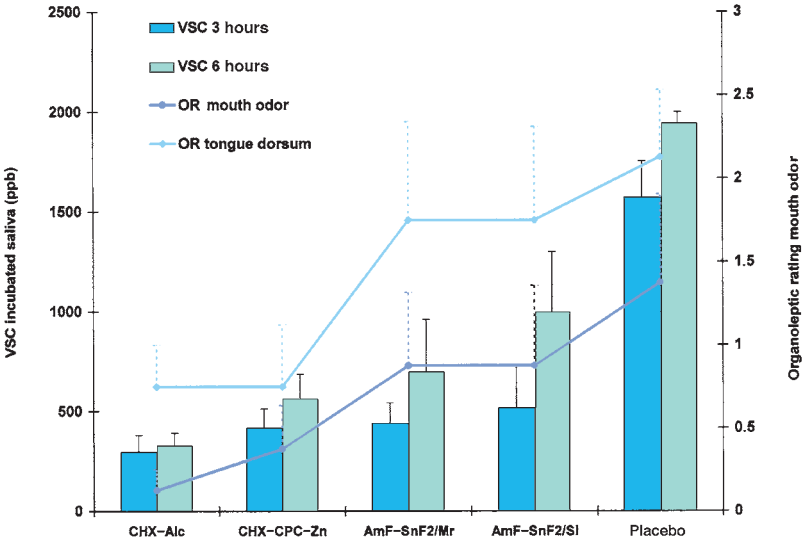

Figure 2.
VSC values in ppb (3 and 6 hours) of incubated saliva versus organoleptic ratings of mouth odor. For each observation, both the mean value and the standard error of the mean are shown.

2 AmF-SnF_2 solutions, and between CHX-CPC-Zn and CHX-Alc when the tongue coating was considered, was less obvious with the organoleptic ratings. The statistical analysis of the organoleptic ratings showed no significant interproduct differences ($P = 0.249$ for mouth air and $P = 0.099$ for tongue coating, respectively), probably due to the large standard deviations. The relationship between both parameters is, however, illustrated by significant correlation coefficients (Table 2).

VSC Analyses and Organoleptic Ratings of Incubated Saliva

Figure 3 represents the VSC values and the organoleptic ratings of the incubated saliva immediately after collection and after 3 and 6 hours' incubation. For both parameters, a clear increase after incubation could be detected. The ranking of the different test products is comparable for both parameters, with a better discrimination after 6 hours (Table 1). The strong correlation between both parameters is illustrated by the high correlation coefficient (r = 0.80, Table 2).

Relationship Between VSC Values and Bacterial Load of Incubated Saliva at 0 and 6 Hours

The changes in VSC values and number of bacteria in incubated saliva over 6 hours are shown in Figure 4. At saliva collection, some interproduct differences ($P = 0.001$) in microbial load could be detected (Table 3), both for the aerobically and anaerobically cultured samples. The order from lowest to highest was again CHX-Alc, CHX-CPC-Zn, AmF-SnF_2^{Mr}, AmF-SnF_2^{Sl}, and placebo, for both cultures. The sole exception was AmF-SnF_2^{Mr}, which was lower than CHX-CPC-Zn for the aerobic cultures at baseline. When the saliva was incubated for 6 hours, even more distinct interproduct differences appeared, with the same ranking as above. This ranking was similar to that found for the 6-hour VSC scores, resulting in a significant correlation coefficient between both parameters (r, 0.50).

Power Analysis for the Different VSC Recordings

Finally, the VSC measurements in the mouth and from the incubated saliva were compared to each other to find the most powerful parameter (Table 4). From the data, representing the percentage of 1,000 randomly Monte-Carlo generated data sets in which a significant interproduct difference could be detected, it can be concluded that the VSC analyses from the incubated saliva were more prone to find significant differences. With the intraoral registration, the CHX-CPC-Zn formulation, in particular, could be discriminated from the other products, whereas the VSC analyses made a distinction between all products and the placebo. Based on

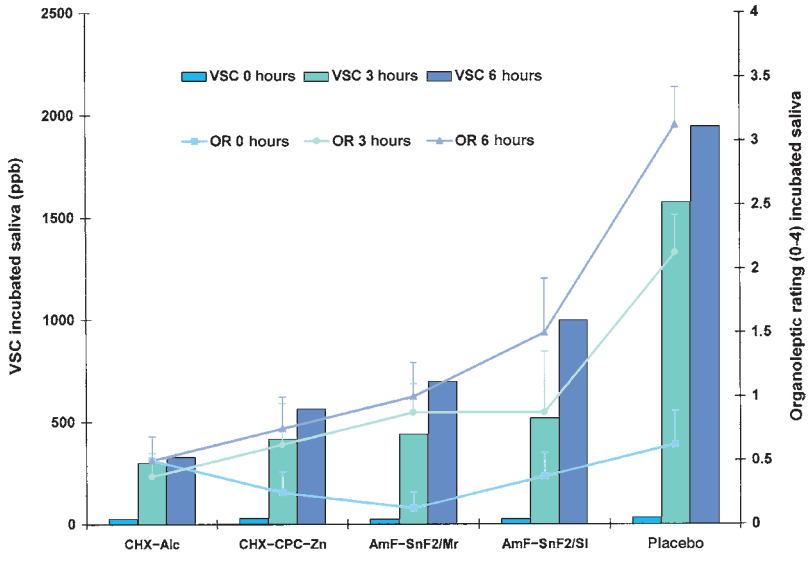

Figure 3.
VSC values (ppb) and organoleptic ratings of incubated saliva at 0, 3, and 6 hours.

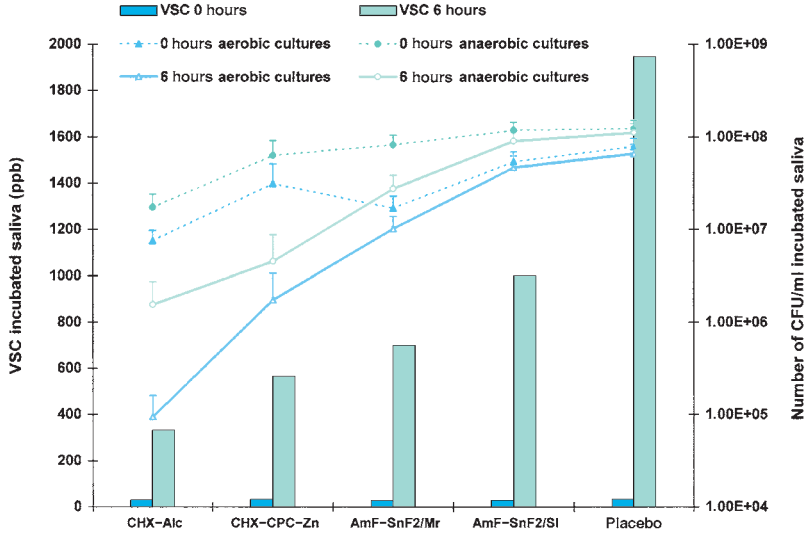

Figure 4.
Changes over time in VSC values in ppb (baseline and 6 hours) and bacterial load (baseline and 6 hours, aerobic and anaerobic CFU/ml) of incubated saliva.

all parameters of this data set, an additional statistical analysis indicated that, for the intraoral recordings to reach the same interproduct discrimination as the VSC measurement on the incubated saliva, 1.5 to 2× more patients had to be included in the study.

DISCUSSION

The data of this pilot study indicate that a saliva incubation test mimics the intraoral malodor-producing process. The degree of correlation between the clinical malodor data and those from the incubated saliva (VSC rating and organoleptic scores) in this study was high and comparable, or even superior to, the correlation coefficients reported between different clinical parameters for malodor.[15,25,28] Moreover, the discrimination power of the indirect saliva incubation test was found to be superior to intraoral registrations. The power could even be improved via the combined analysis of the 3- and 6-hour data (statistically corrected for repeated use of the subjects). In contrast, with intraoral registrations, one needs 1.5 to 2× more patients in order to find the same interproduct differences. This can be confirmed by a similar study from our group,[29] in which comparable interproduct differences mentioned in this paper were obtained for clinical recordings involving 16 subjects. The saliva incubation test thus might save time and costs and seems a valuable approach for oral malodor measurement, especially for comparing the antimalodor efficacy of different products. It is, however, clear that these observations have to be confirmed in real halitosis patients, although one should keep in mind that morning breath odor is often used as a clinical study model by most researchers dealing with halitosis. It may not be ideal, but because of the psychological impact of breath malodor on patients, one should be reluctant to involve them in clinical trials. Moreover, one can expect that in real halitosis patients, the power of a saliva test will increase even further. This incubation test does not have the shortcomings of several direct methods including subjectivity,[14,15] low interexaminer reproducibility,[12] or lack of official standards. It is also not influenced by confounding factors such as psychological/physiological state of the judge[12] and does not need precautions such as abstaining from eating garlic, onion, and spicy food; refraining from smoking and drinking coffee; and avoiding scented cosmetics before assessment.[7,16] In our study, participants abstained because of the clinical intraoral examination. It also avoids the uncomfortable and/or embarrassing feeling[7] to the patient during an intraoral evaluation.

Our data are somehow contradictory to a previous study that failed to correlate data from incubated saliva with clinical observations.[30] However, when repeated sampling was performed, correlation with oral malodor appeared.

While changes in bacterial load after incubation were negligible for the placebo solution, significant reduc-

Table 3.

Interproduct Differences in CFU/ml Before and After Saliva Incubation for 6 Hours

Interproduct Comparison		CFU/ml of Incubated Saliva			
		Before Incubation		6 Hours of Incubation	
		Aerobic	Anaerobic	Aerobic	Anaerobic
CHX – Alc	CHX – CPC – Zn	0.132	**0.002**	0.606	0.996
CHX – Alc	AmF – SnF_2^{Mr}	0.052	**0.000**	**0.000**	**0.016**
CHX – Alc	AmF – SnF_2^{Sl}	**0.000**	**0.000**	**0.000**	**0.000**
CHX – Alc	Placebo	**0.000**	**0.000**	**0.000**	**0.000**
CHX – CPC – Zn	AmF – SnF_2^{Mr}	0.989	0.131	**0.002**	**0.034**
CHX – CPC – Zn	AmF – SnF_2^{Sl}	**0.007**	**0.000**	**0.000**	**0.001**
CHX – CPC – Zn	Placebo	**0.000**	**0.000**	**0.000**	**0.000**
AmF – SnF_2^{Mr}	AmF – SnF_2^{Sl}	**0.020**	0.171	0.411	0.588
AmF – SnF_2^{Mr}	Placebo	**0.000**	0.073	0.065	0.247
AmF – SnF_2^{Sl}	Placebo	0.515	0.990	0.818	0.964

Boldface type indicates a significant difference.

Table 4.

Power Analysis of Intraoral VSC Recording in Comparison to VSC Measurements of Incubated Saliva

Interproduct Comparison		Power Analysis for VSC Data			
		Intraoral Registration		Incubated Saliva	
		No Closure	2 Minute Closure	6 Hours	3 and 6 Hours
CHX – Alc	CHX – CPC – Zn	19.7	26.7	2.5	9.3
CHX – Alc	AmF – SnF_2^{Mr}	1.2	0.9	3.2	13.4
CHX – Alc	AmF – SnF_2^{Sl}	3.7	10.4	10.1	36.7
CHX – Alc	Placebo	33.8	31.3	**98.6**	**100**
CHX – CPC – Zn	AmF – SnF_2^{Mr}	9.9	46.6	1.0	0.5
CHX – CPC – Zn	AmF – SnF_2^{Sl}	54.7	**84.3**	1.7	3.9
CHX – CPC – Zn	Placebo	**89.9**	**93.6**	**90.4**	**99.9**
AmF – SnF_2^{Mr}	AmF – SnF_2^{Sl}	9.6	4.5	1.3	2.3
AmF – SnF_2^{Mr}	Placebo	44.7	14.7	**88.5**	**100**
AmF – SnF_2^{Sl}	Placebo	6.8	2.1	74.1	**99.8**

Boldface type indicates a significant difference.

tions were recorded for the CHX-Alc and CHX-CPC-Zn solutions, and to a lower extent for the AmF-SnF_2 rinse. These antibacterial effects corresponded well with the VSC reductions for both the incubated saliva and the intraoral registrations.

CONCLUSIONS

In the present study, the antimalodor effect on morning breath of 5 different mouthrinses was evaluated through both in vitro saliva incubation and intraoral measurements. The strong correlation between the results of odor production by incubated saliva and clinical assessments of morning breath odor suggests that the saliva incubation test may be used as an indirect method to measure oral malodor and the antimalodor effectiveness of oral hygiene products. This finding offers evident advantages for further clinical trials and diagnostics.

One should, of course, keep in mind that not all malodor complaints emanate from the oral cavity. The efficacy of this saliva test in a differential diagnosis between oral and extraoral causes (e.g., post-nasal drip, diabetes mellitus, liver insufficiency) for breath odor remains to be tested.

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Correspondence: Prof. M. Quirynen, Department of Periodontology, Kapucijnenvoer 7, B-3000 Leuven, Belgium. Fax: 32-16-33-24-84; e-mail: Marc.Quirynen@med.kuleuven.ac.be.

Accepted for publication January 10, 2003.