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Relationship between sensory and instrumental evaluations of mouth odor

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Synopsis

Two methods are currently available to assess mouth odor: subjective evaluation (sensory rating) and gas chromatographic (GC) analysis of the volatile sulfur compounds (VSC), hydrogen sulfide, methyl mercaptan, and dimethyl sulfide in mouth air. The purpose of this study was to examine the correlation between these two methods in a human clinical study. Twenty adults participated in the study. The morning mouth odor of each subject was assessed by two expert sensory evaluators on a scale of 0 to 8 (showing increasing odor intensity). Following sensory evaluations, mouth air samples from each subject were then obtained and analyzed by GC for VSC. The study was divided into three phases, viz., a control, a test, and a recovery phase. Each phase lasted for three days, and two readings on the second and third day of each phase were obtained by both methods. During the test phase, the subjects brushed their teeth twice a day with a standard fluoride-containing dentifrice, and morning mouth odor determinations were conducted as described above. During the control and recovery phase, the subjects practiced oral hygiene *ad libitum*. Correlation coefficients (r) between sensory ratings and GC readings (VSC—ng/ml) of mouth air were 0.22, 0.77, and 0.78 during the control, the test, and the recovery periods, respectively. The latter two were significant at $p = 0.01$.

The ratios of the GC reading to the sensory rating were consistent over the range of values obtained; an average factor of 3.2 would predict the corresponding GC reading for a given sensory rating in this study. For example, VSC by GC of 25.8 ng/ml of mouth odor provided a sensory rating of 8 (strong odor). This indicates that the objective evaluation of mouth odor by GC does correlate with a subjective sensory rating of mouth odor. The GC method therefore provides us with useful instrumental measurements (ng/ml) that can be translated into a consumer-perceivable odor intensity.

INTRODUCTION

It has been previously shown that the volatile sulfur compounds (VSC) such as H_2S, CH_3SH, and $(CH_3)_2S$ are responsible for local mouth odor in healthy individuals (1). These VSC components arise from the putrefaction of salivary proteins and amino acids by gram-negative oral microorganisms (2) and can be detected in direct mouth air by a gas chromatographic method or by a sensory method (3). Since the sensory method is subjective, it is highly desirable to develop an objective instrumental method to assess offensive local mouth odor.

We have previously shown that the instrumental method is capable of assessing the effect of both rinse and dentifrice treatments on mouth odor in humans (4). The purpose of this study was to determine the correlation between the instrumental method and sensory evaluation in a controlled clinical trial.

MATERIALS AND METHODS

A 220 Tracor gas chromatograph equipped with a flame photometric detector, housing a 394 millimicron filter specific for sulfur, was used to measure the sulfur compounds responsible for mouth odor. A 25-ft teflon (FEP) Supelco column was also used (containing 0.05% polyphenyl ether and 0.25% phosphoric acid on a 30/60 mesh-chromosorb T) to specifically separate the primary sulfur components of mouth air. Air was used as a carrier gas to eliminate the air peak and to reduce the analysis time. The isothermal oven temperature was 60°C, with inlet and detector temperatures of 130°C. A standard gas SO_2 permeation tube was used to convert the results into nanograms/milliliter for the total sum of sulfides.

The mouth air sample was collected in a 24-ft teflon storage loop (⅛-in o.d. × 1/16-in i.d.) that was wrapped with teflon tape and had a Mininert teflon valve attached to each end (Figure 1). We have shown in previous studies that this loop is capable of storing mouth air without substantial sample loss. Each loop was calibrated to hold 10 cc of mouth air. When collecting the 10-cc sample, a 50-cc polyethylene syringe was attached to one end of the loop while a 3-in sterilized teflon tube was attached to the other end.

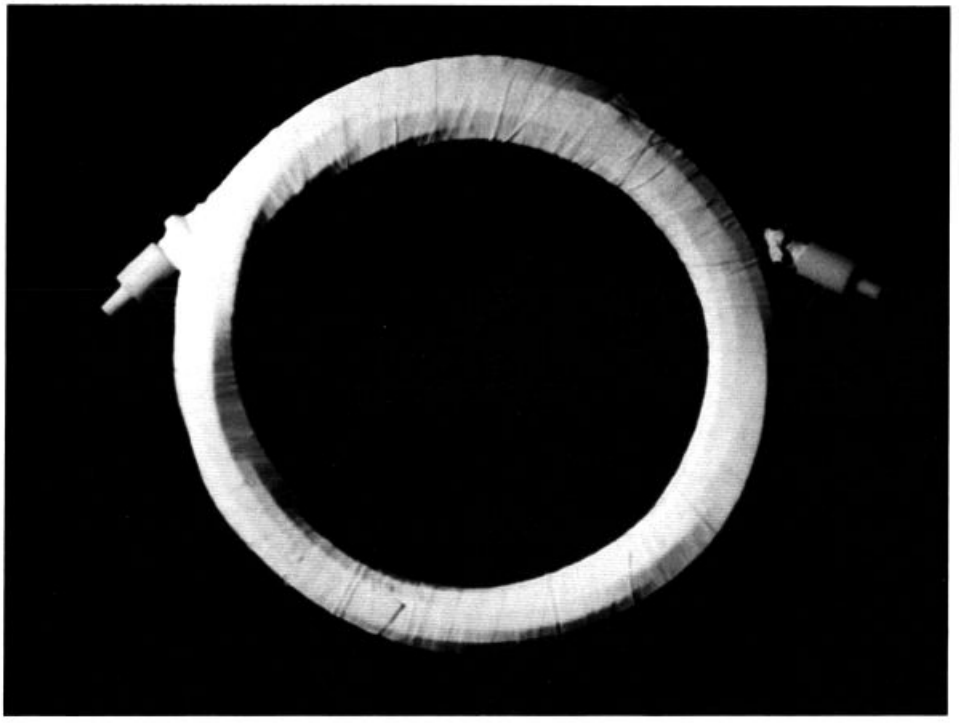

Figure 1. Storage loop for mouth air samples.

The tube was carefully inserted into the panelist's mouth, both teflon valves were opened, and the mouth air was drawn into the storage loop using the syringe. During the evaluation procedure, panelists were asked to sit for 10 minutes with their mouths closed prior to each evaluation to allow for the buildup of the sulfur volatiles.

The stored samples were transported to the laboratory in dry ice and analyzed within five to seven hours following collection. Two samples were obtained from each subject for analysis. The sample storage loop was attached to the gas chromatograph inlet gas sampling valve, where the stored mouth air sample was introduced into the GC.

The gas sampling valve apparatus was also calibrated for 10 cc of mouth air. A 50-cc syringe was attached to the outlet portion of the valve on the GC in order to draw the sample into the sampling valve. When the valve was turned, the air carrier gas swept the sample into the column. The instrumental readings were expressed in nanograms per milliliter (ng/ml).

The second method for the measurement of mouth odor is a subjective sensory evaluation. This evaluation was conducted by two professional judges from the La-Wall Harrison Research Laboratories immediately following the instrumental evaluations. Panelists waited for ten minutes with their mouths closed, similar to the instrumental procedure. A special booth was constructed that allowed the panelists to remain anonymous. A sterile thistle tube was inserted into the panelists' mouths while the bell-shaped end was inserted through the screen where the judge evaluated the mouth odor. Panelists were asked not to breath during this evaluation. Each of the two judges evaluated each panelist. The offensiveness of the odor was rated on a scale from 0 to 8 (Table I), where 0 is non-offensive and 8 is considered highly offensive. Two evaluations were conducted on each panelist, one evaluation by each judge.

STUDY DESIGN

Twenty panelists (ten men and ten women) were qualified to participate in the study by meeting the inclusion/exclusion characteristics of the protocol. The inclusion characteristics consisted of being of adult age, 20 years and above, having good general health, and having a total sulfur volatile baseline greater than 5 ng/ml of mouth air. This level

Table I
Sensory Evaluation Criteria for Mouth Odor

Sensory score	Offensiveness of mouth odor
8 7	Strong
6 5	Definite
4 3	Faint
2 1	Doubtful
0	None

was determined by a pre-study screening evaluation to identify the qualifying panelists. The early morning screening evaluations were conducted using the same method as described below in the test phase.

The exclusion characteristics consisted of being a smoker, having hard or soft tissue tumors of the oral cavity, having extensive caries or periodontal problems, and using full or partial dentures and/or orthodontic bands. Also excluded from the study were panelists who used medication such as antihistamines, antibiotics, or other types of medication for two weeks prior to or during the study.

The study consisted of three phases: control, test, and recovery. During the control phase, subjects used their normal hygiene practices for three days, with evaluations on the second and third days. During the test phase, subjects were asked to use a placebo dentifrice in the morning and prior to retiring at night for two days (days 1 and 2), with evaluations on the second and third day.

Subjects were asked to brush with the placebo dentifrice for 60 seconds and rinse with water. During the recovery phase subjects resumed their normal hygiene practices for two days as in the control phase, and were evaluated on the second and third days of the recovery phase. The purpose of this phase was to ascertain any carryover effect from the test phase. Spearman's rank correlation was computed to determine the relationship between the instrumental reading of VSC and the sensory ratings.

In all phases, subjects were asked to use their treatment twice a day, in the morning and at night before retiring (days 1 and 2). Following the final nighttime treatment on the morning of day 3, organoleptic evaluations and sample storage collections were conducted. For each morning evaluation subjects reported to the test site without brushing, rinsing, eating, or drinking in order not to influence or wash away the VSC formed in the mouth air. At this time, two baseline samples were evaluated organoleptically and two storage samples were collected from each subject. Subjects were then given breakfast and the appropriate treatment: normal hygiene in the control phase, the placebo dentifrice in the test phase, or normal hygiene in the recovery phase. Following the morning treatments, the subjects returned in three hours for the post-treatment organoleptic evaluations and sample storage collections. During this 3-hr interim between evaluations, subjects are asked not to eat or drink.

RESULTS

Table II shows both the mean instrumental and sensory mouth odor scores of subjects grouped according to treatment period of the study. For example, the mean and standard deviation for the sensory ratings for day 3 of the control period is 7.15 ± 0.42, while the mean for the instrumental evaluations is 21.86 ng/ml $\pm$ 4.18.

The results show that during the control period the correlation was poor ($r = 0.22$), while, during the test and recovery period, the values had improved with an r value of 0.77 and 0.78, respectively. These latter values were significant at a p-value of less than 0.01.

Table III shows the instrumental mean values that correspond to each mean integer of each sensory rating (i.e., 5, 6, 7, 8). All subjects with mean sensory ratings equal to 5, 6, 7, or 8 were identified, and their corresponding instrumental mean values were

Table II
Mean Instrumental Values vs Mean Sensory Ratings

Determination (mean ± S.D.)	N	Control period		Test period		Recovery period	
		Day 2	Day 3	Day 2	Day 3	Day 2	Day 3
Sensory rating	20	7.15 ± 0.42	6.98 ± 0.76	6.43 ± 0.73	6.45 ± 0.82	6.38 ± 0.70	6.55 ± 0.69
Volatile sulfur ng/ml	20	21.86 ± 4.18	22.77 ± 6.00	20.14 ± 6.12	20.16 ± 4.97	23.12 ± 6.13	22.22 ± 4.06
Correlation (r) coefficient		0.22 (N.S.)		0.77 ($p < 0.01$)		0.78 ($p < 0.01$)	

Table III
Calculation of Prediction Factor

Mean sensory ratings A	N*	Mean instrumental readings (total sulfur volatiles, ng/ml) B	Factor = B/A
8	40	25.85	3.23
7.0	31	22.70	3.24
6.0	21	19.78	3.29
5.0	28	16.59	3.32
			Average = 3.27

* Total of 120 determinations from all three phases of the study.

averages for each sensory rating (5, 6, 7, or 8). This allows us to calculate a prediction factor (B/A). Note that this prediction factor is quite consistent over the sensory and instrumentation values obtained in this study, providing another indication of correlation between sensory and instrumental scores.

A sensory rating of 5 was the lowest rating reported for any subject by the judges during this study. From Table I, a rating of 5 is the lowest rating for "definite" offensive mouth odor. From Table III, a rating of 5 corresponds to an instrumental score of 16.59. This instrumental value could be considered a threshold for odor offensiveness. In other words, if the total sulfide measurement in nanograms/milliliter for a given subject is below 16.59 ng/ml, it could be ascertained that the subject's mouth odor would not be of "definite" offensiveness (5 rating) but of "faint" offensiveness.

DISCUSSION

The organoleptic (sensory) rating is currently an acceptable *in vivo* procedure for determining the efficacy of mouth rinses and dentifrices in mouth odor reduction.

On the other hand, previous chemical, mass spectrometric, and gas chromatographic methods have established VSC as being the primary source of offensive local mouth odor (4). Therefore, a number of investigations have been conducted to establish a correlation between instrumental techniques and a subjective sensory method of evaluating mouth

odor. For example, Schmidt *et al.* (5) showed that VSC are detected instrumentally by GC and flame photometry, and correlated these scores with organoleptic ratings.

We have previously utilized the GC instrumental procedure to assess the efficacy of rinses against mouth odor in a clinical study. Antibacterial rinses significantly reduced VSC in mouth air samples obtained three hours post rinsing, while the placebo rinse did not significantly reduce VSC. These results indicated that this method could be used for assessing the effects of active agents against local mouth odor (6). It was of interest to correlate this instrumental (objective method) with the sensory (subjective method) rating procedure.

There were several obstacles in transforming this instrumental methodology into a practical tool for clinical trials in the field. The instrumental setup is large and cumbersome and thus cannot be transported readily to a clinical site. Also, the evaluations are normally done in the morning before subjects eat or drink. This makes the scheduling inflexible and restricts the number of subjects who can participate in the study. Lastly, no published correlation studies of this nature were conducted in a clinical study framework, between the sensory and instrumental evaluations. To facilitate this type of study, it was very useful to have a device to collect, store, and preserve the VSC mouth air samples. Such a device can then be used to analyze the stored samples upon returning to the laboratory. The storage loops described in this study fulfilled these requirements. The storage system also provided us an opportunity to conduct a true clinical study offsite and to correlate these instrumental measurements with a sensory evaluation done by expert judges prior to the instrumental VSC determination.

We obtained strong positive correlation ($r = 0.77–0.78$) during test and recovery periods of the study but less than a robust correlation ($r = 0.22$) during the control period. The latter is attributed to a lack of familiarity by subjects with the procedures in the early phase of the study. These subjects initially had difficulty with the organoleptic and storage sampling procedures. Based on previous studies, we are confident that the judges, sample collector, and analyst were not the factors contributing to the low correlation in the control phase. The noteworthy feature of this study is the development of a factor to predict sensory evaluations using the instrumental readings in this study. This provides us a useful tool to translate instrumental measurements into consumer-perceivable odor intensity.

Recently, Rosenberg *et al.* (6) measured the correlation between the sulfide measurement in the mouth air and sensory ratings in forty-one subjects using a portable sulfide monitor. The overall correlation coefficient obtained by the investigators ($r = 0.603$) was within the range of what we observed, although the instrument used was not of the sensitivity or specificity to sulfides as compared to the GC system used to measure sulfur volatiles in mouth air.

CONCLUSIONS

The clinical approach used to study the correlation between sensory measurements and instrumental measurements of mouth odor indicated that:

1. There was a good correlation between the instrumental and sensory evaluations for mouth odor. The correlation coefficients were 0.22 for the control and 0.77 and 0.78 for the test and recovery phases, respectively, between the two methods.

2. Results also indicated that the correlation improved as the subjects become familiar with the sampling procedures.
3. Both methods were useful for measuring the intensity of offensiveness of mouth odor.

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