

# A new measurement protocol to differentiate sources of halitosis

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## Cited as:

Aydın M. Özen ME. Kırbıyık U, Evlice B, Ferguson M, Uzel I. A new measurement protocol to differentiate sources of halitosis. Acta Odontol Scand., 2016, 11:1-5 DOI: 10.3109/00016357.2016.1163732

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### **ABSTRACT:**

**Objective:** 3 sources of halitosis exist, potentially in any combination: mouth, nasal cavity or alveolar breath. There has been no universally accepted protocol which differentiates and quantifies each odor source separately. In this study a new gas measurement protocol is described and tested to determine whether each odor source can be separately detected without contamination.

**Material and Methods:** 90 healthy volunteers were divided into 3 groups. Hydrogen sulfide ( $H_2S$ ), volatile organic compounds (VOCs), and hydrogen ( $H_2$ ) were artificially generated in the mouth, nose and pulmonary alveoli respectively. VOC, ammonia ( $NH_3$ ), sulfur dioxide ( $SO_2$ ),  $H_2S$  and  $H_2$  gas readings from mouth, nose and alveolar air were measured and compared. Measurements were taken before and during gas generation.

**Results:** Contamination of nasal air (2.8%) and alveolar air (5.0%) by oral  $H_2S$ ; alveolar air (2.06%) and oral air (4%) by nasal organic gas; nasal air (18.43%) and oral air (9.42%) by alveolar  $H_2$  was calculated.

**Conclusion:** The results demonstrated that artificially generated oral  $H_2S$  nasal VOC and alveolar  $H_2$  can be individually quantified. This gas measurement protocol can be used diagnostically or to gauge response to therapy in any medical or dental setting.

**Key words:** Halitosis, oral and nasal malodor diagnose.

**Short Title:** To explore source of the halitosis

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## INTRODUCTION:

Halitosis is chronic, endogenous malodor and is etiologically classified into Type 0 (physiologic), Type 1 (oral), Type 2 (airway), Type 3 (gastroesophageal), Type 4 (blood-borne) and Type 5 (subjective). [1] The main halitosis gases are volatile sulfur compounds (VSC), volatile organic compounds (VOC) and nitrogen containing gases (amines); some gases fall into both groups. 3481 VOCs are detected in alveolar breath of healthy subjects, [2] 400-700 VOCs in breath at any time, [3] 694 VOCs in mouth air of persons with or without halitosis.[4] According to some, > 80% of oral odor is attributed to alkanes or methylated alkanes. [5] Typically, VSCs are the greatest contributors to odor, [6] especially hydrogen sulfide (H<sub>2</sub>S), or methylated sulfides.[7] Potentially other gases may contribute, e.g. indole, skatole, acetic acid and short chain acids. [8]

In any objective halitosis case, odor is emitted via nose, mouth or breath, irrespective of its origin. So analysis of exhaled oral air alone may fail to identify the source of volatiles, and exhaled air from mouth, nose, and lungs must be analysed separately. The nasal passages are the second most likely source. [9] Quantification of volatiles found in each air sample could reveal the source of malodor and facilitate diagnosis.[3]

The presence of a gas may have different implications depending on whether it comes from nose, mouth or breath. For example methylated sulfides in oral air shows type 1 halitosis associated with, e.g., tongue coating; however when present on the alveolar breath, it shows type 4 halitosis (e.g. hepatic pathology, hypermethioninemia). [10,11]

Breath ammonia is linked to blood urea or blood ammonia concentration, [12] digestion of proteins, [13] and *Helicobacter pylori* positivity (if recent ingestion of urea). [14] On the other hand, even in healthy individuals, a negligible amount (0.85- 5.5 mMol) of NH<sub>3</sub> is secreted into saliva from plasma [14] or can be produced by oral bacteria from arginine or urea. [15] Thereby, it would be necessary to separate the NH<sub>3</sub> origins in a gas sample to distinguish Type 1 and 4 halitosis.

This is why discrimination of gas origin is important. The clinician needs to precisely determine the origin of gases in order to correctly diagnose halitosis.

Some studies [16,17] aim to differentiate oral and nasal odors, but still no efficient protocol exists to distinguish gases emitted from halitosis patients (Table.1).

For more accurate information about the exact origin(s) of odor, there was need for a new measurement protocol to differentiate gas samples taken from different sources. The new protocol described below was tested clinically.

## METHODS AND MATERIALS

### Subject selection criteria:

90 volunteers (55 male, 35 female; age 19-56, median 23) were recruited. None complained of halitosis, all were periodontally healthy with no systemic disease. None were pregnant or expecting menstrual period, and none consumed medications, odorous foods. All subjects fasted from the previous midnight until time of the measurement. Subjects were randomly allocated into 3 groups. Ethical approval was granted by Mersin University Ethics committee (#78017789/050.01.04/61)

**Table 1.** Critique of existing gas measurement protocols

Ref#	Gas sampling protocol	Comment
[26]	Oral air assessed immediately following incubation period (3 minute) during which the subject has their mouth closed and breathes through their nose. The patient then exhales via the mouth, 4-6 inches away from the judge's nose. This is then repeated but with the nares occluded for 3 min while breathing through the mouth, then the judge assesses the breath exhaled via nose with mouth closed. The subject continues to expel slowly while the judge turns his/her head away and sniffs room air to avoid olfactory adaptation to any perceived odor by resetting his/her sense of smell to ambient room air. As the subject approaches the end of exhalation the judge turns back to the patient and assess the last sample of air that the patient can force out the lungs through the nose.	First gas sample truly represents oral halitosis. Second gas sample does not represent nasal odor but a mixture of alveolar and nasal odors. Content of third gas sample is no different from the second one. This protocol is not able to separate oral, nasal and alveolar odors
[27]	Subjects hold their breath for 15 s and then exhale. The first 500 ml of expired air is excluded, and the second 500 ml (alveolar air) is collected in a foil bag. The tube is placed in the mouth and the subject makes a seal around the tube with their lips. The subject breathes via nose for 30 s and then holds their breath for an additional 15 s. Mouth air is then aspirated into the syringe.	This protocol is suitable to collect mouth and alveolar air but no sampling protocol for nasal air was described
[28]	Air exhaled via mouth is collected in a sampling bag	This consists of a mixture of alveolar and oral air, and does distinguish odor sources
[10]	For evaluation of intraoral halitosis, subjects exhale via mouth, and then exhale via nose for extraoral halitosis	These gas samples do not represent intra/extra oral halitosis, but rather represents mixtures of alveolar and oral air, and alveolar and nasal air respectively
[24]	Air samples, mouth breath, nose breath or oral air (while subjects held their breath) were used	Oral air represents correct sample but breath exhaled via mouth and nose contains a large portion of alveolar gas
[29]	Subjects kept their mouths closed for 3 min prior to the measurement of oral malodor. Before measurement of exhaled air and nasal air, subjects breathed via mouth for 30 s	With this protocol it is difficult to separate nasal air and exhaled air. The exhaled gas sample will consist of alveolar and oral air, also, nasal air would consist of a mixture of oral, alveolar and nasal air
[30]	Subjects close their mouth, holding their breath in their mouth for up to 2 min while breathing via nose. Then, the subject opens their mouth and the judge smells the patients mouth from 5-10 cm away, sniffing 2-3 times. The subjects then breathe through their mouth for a time, then close their mouth and breath via nose. The judge then smells the breath exhaled via nose	This protocol gives a brief idea as to whether oral odor is different from a mixture of nasal and alveolar odor, but poorly separates nasal odor. Also organoleptic assessment is subjective, carrying a degree of inter-observer variation and is not particularly reproducible.

### **Gas reading instruments:**

Each participant's baseline VOC, NH<sub>3</sub>, SO<sub>2</sub>, H<sub>2</sub>S, H<sub>2</sub> levels in oral, nasal and alveolar air were used as individualized control data. Artificial odor or gas produced in mouth, nose and breath, gas levels were measured as described below and recorded for these 5 gases using a portable multigas detector (IBRID MX6 C526R311, IndSci). One sensor is a photoionization detector (PID) to read volatile organic compounds (VOCs), the NH<sub>3</sub>, SO<sub>2</sub>, H<sub>2</sub>S, H<sub>2</sub> sensors are electrochemical. Before each gas measurement, the detector was switched on for 20 minutes, zeroed to ambient air (to exclude background odorants), and data read within 1-2 minutes after the aspirating tube connected.

Gas profile of currently used halimeters (Halimeter or Oral Chroma ie) is restricted with sulfurous compounds. They can not read the gases mentioned in the study (H<sub>2</sub>, NH<sub>3</sub> or VOC). The Halimeter (Interscan, Chatsworth, CA) and MX6 have been connected to a H<sub>2</sub>S source with adjusted gas concentration to 400, 800, 1000, 1500, 2000, 2500, 3300 ppb in a separate experiment to validate the use of MX6. Successive readings were recorded as 400-410, 700-710, 1100-1107, 1600-1593, 2100-2111, 2400-2408, 3200-3212 ppb (first values were read by the MX6, second values by the Halimeter).

### **Oral H<sub>2</sub>S production with cysteine solution, Group A**

20 mMol (2.43 g/L) of aqueous solution L-Cysteine (#1.02839.0100, Merck) was used to generate H<sub>2</sub>S. 5 ml cysteine solution was held in contact with the dorsal tongue for 30 seconds. Gas detection was then carried out 3 minutes later. Oral bacteria act on cysteine substrate, releasing H<sub>2</sub>S in the oral cavity.

### *Oral gas measurement protocol:*

While breathing through the nose, the subject places his left index finger between the upper and lower left molars, and gently bites leaving a space between the anterior teeth. This degree of mouth opening is more representative of physiologic mouth position in the social environment. The aspirating tube is connected to the gas detector and the tip placed on the dorsal tongue. Subjects were instructed not to occlude the tube aperture with the tongue.

### **Nasal VOC production with menthol-eucalyptol containing ointment, Group B**

To obtain odorous gas in the nasal cavity, Sulfarhin nasal ointment was used (Santa Farma, Türkiye; containing ephedrine (100 mg), naphazoline (3 mg), antazoline (30 mg), chloretone (100 mg), menthol (100 mg) and eucalyptol (100 mg) per 12 g). The last two ingredients are odorous. (Any menthol or eucalyptol containing ointment can be used to reproduce the experiment). A 3 cm line of Sulfarhin (0.15 g) was applied on sterile paper which was then placed into the closed nostril with a sterile spatula. The nostrils were occluded for 3 minutes while subject breathed via their mouth. This helped the accumulation of gases released from the ointment into the nasal cavity.

### *Nasal gas measurement protocol:*

To avoid inclusion of oral gases reaching the nasal cavity via the nasopharynx, the subject rinsed his mouth with water for 30 seconds before nasal gas measurement.

The patent half of the nasal cavity physiologically alternates every few hours. Subjects were asked which nostril felt more patent and this half was occluded with cotton wool.

The tip of a Nelaton catheter #2 (Changhu medical, Ch) was lubricated with

odorless, non-irritant Anestol ointment (Sandoz, Türkiye); containing vaseline and lidocaine 5% to facilitate painless catheter insertion. The catheter was gently inserted into the non-patent nostril for 5 cm parallel to the floor of the nasal cavity. The subject squeezed both nostrils with digital pressure to stabilize the catheter, while breathing through his mouth continually. The catheter was then connected to the gas detector.

#### **Alveolar hydrogen gas production with lactulose, Group C**

Breath  $H_2$  is elevated when metabolism of sugars is impaired due to defect of specific digestive enzymes. Usually, undigested sugar reaches to colon within 60-110 minutes (orocecal transit time, OCTT), and is fermented by the gut microbiota.  $H_2$  is generated (although rarely some individuals produce methane instead of hydrogen), absorbed into the blood circulation and then is transferred to the exhaled breath during gas exchange in the pulmonary alveoli. [18,19] Humans lack enzymes to digest lactulose. Oral lactulose is used to obtain a measurable breath  $H_2$  (hydrogen breath test). Individuals in Group C took 10 g (15 ml) Laevolac syrup orally (Ulugay, Türkiye). (Any lactulose containing syrop can be used to reproduce the experiment). Since physical activity prolongs OCTT, subjects sat still for 30 minutes. Alveolar gas measurement was performed immediately before and 30 minutes after lactulose administration. If  $H_2$  was not detected, readings were repeated every 15 minute until 90 minutes had passed. Normally, fasting individuals have <10 ppm breath  $H_2$ . Subjects with >10 ppm breath  $H_2$  at baseline were excluded.

##### *Alveolar gas measurement protocol:*

To avoid inclusion of oral gases, the subject rinsed his mouth with water for 30 seconds, took a deep breath via the mouth (not nose), and held the breath for minimum

15 seconds. This period permits transfer of blood gases to alveolar air. Subjects then forcefully exhaled the initial 2/3 of vital capacity via the mouth, helping to exclude "dead space" air. Next, the remaining third of vital capacity was immediately exhaled into a Tedlar bag (CEL, USA). This gas sample represents the alveolar air, if its volume was too small, the above step was repeated until approximately 500 ml had been collected. The sample was then analysed by inserting an aspirating tube which was connected to the gas detector.

##### *Data analyses:*

Sample size is calculated as 25, for type 1 (0.05) error level, 80% power, with 0.01 ppm concentration difference ( $\pm 0.2$  SD), each group contained 30 individuals. Gas measurements were made blindly by the same investigator. Paired t-test was applied to each group's before and after gas measurements for each of the 5 gases tested. The p-significance cut off was calculated using *Bonferroni* correction for the 45 measurements (p-value cutoff set to 0.05/45~0.0011).

## **RESULTS and DISCUSSION**

In the literature [20], subjects closed their mouths for ~3 min prior to halitosis measurement. This artificially forces malodor generation, when there may not be malodor at other times. Such readings may not reflect the normal conditions of the patient's social environment. Fasting and avoiding oral hygiene also creates more malodor than actually exists. In the new measurement protocol, subjects kept their mouths slightly open, which is more representative of everyday life.

In this study, fasting was required before hydrogen breath testing, and toothbrushing avoidance was required in order to challenge  $H_2S$  production. If the

aim was to diagnose halitosis and not to demonstrate separation of different odor sources, it would have been preferable not to have the subjects fasted and having avoided oral hygiene.

*Group-A: Oral gas (H<sub>2</sub>S) discrimination from nasal and alveolar air*

In healthy individuals, 6 mMol cysteine oral rinse generates approximately 1.7 ppm H<sub>2</sub>S as degraded by oral bacteria. [20] In this study, mean 16.69 ppm H<sub>2</sub>S was generated with 20 mMol cysteine. Such H<sub>2</sub>S concentration is never seen in halitosis patients. The aim was to challenge gas contamination between odor sources by enhancing oral H<sub>2</sub>S, nasal VOC and alveolar H<sub>2</sub>.

Ideally, contamination between odor sources should be as low as possible, but realistically cannot be zero. Inevitably, some oral air will pass to the nasal cavity via the nasopharynx, and vice versa. Contamination of nasal air by oral H<sub>2</sub>S was calculated as

2.8% (maximum oral H<sub>2</sub>S increase taken as 100%; correlation coefficient r=0.364). Contamination of alveolar air by oral H<sub>2</sub>S was 5.0% (r=0.365).

There is no consensus for a “halitometric threshold” for halitosis. Thresholds have ranged from 75 to 250 ppb [1], 0–1.3 ppm. [21] Actually, oral H<sub>2</sub>S momentary alters. [22] In this study, the initial mean H<sub>2</sub>S level was 0.72 ppm (SD ± 0.36 ppm, median 0.70 ppm, n=90). (Table 2).

Any numerical threshold does not have significant diagnostic value,[23] and is only an estimation of the momentary level of odorants. There is greater diagnostic value in comparing initial and peak H<sub>2</sub>S levels after cysteine rinsing. The H<sub>2</sub>S peak after a cysteine rinse reflects the individual’s oral halitosis capacity. This information can be used to ascertain whether there may be type 1 halitosis at other times apart from the momentary halitometric examination.

	Cysteine	Change	p-value	Sulfarhin	change	p-value	Lactulose	change	p-value
VOC	Oral	0.117	0.0245	Oral	0.330	0.0000*	Oral	-0.303	0.0001*
	Nasal	0.203	0.0000*	Nasal	8.220	0.0000*	Nasal	-0.203	0.0000*
	Alveolar	0.097	0.0324	Alveolar	0.173	0.0000*	Alveolar	-0.163	0.0122
NH3	Oral	-0.100	0.1841	Oral	-0.10	0.0831	Oral	-0.133	0.0434
	Nasal	0.000	NS	Nasal	0.000	NS	Nasal	0.267	0.3860
	Alveolar	0.000	NS	Alveolar	-0.033	0.3256	Alveolar	-0.067	0.1608
SO2	Oral	0.003	0.7122	Oral	-0.003	0.3256	Oral	-0.040	0.0314
	Nasal	0.000	NS	Nasal	0.010	0.3256	Nasal	0.000	NS
	Alveolar	-0.007	0.3256	Alveolar	0.007	0.3256	Alveolar	-0.017	0.3619
H2S	Oral	18.923	0.0000*	Oral	-0.023	0.4997	Oral	-0.140	0.0084
	Nasal	0.523	0.0000*	Nasal	0.053	0.0399	Nasal	-0.180	0.0004*
	Alveolar	0.943	0.0000*	Alveolar	-0.007	0.7687	Alveolar	-0.090	0.0331
H2	Oral	0.667	0.0693	Oral	-0.800	0.1312	Oral	3.833	0.0000*
	Nasal	0.300	0.3697	Nasal	2.000	0.0000*	Nasal	7.500	0.0000*
	Alveolar	0.900	0.0130	Alveolar	0.333	0.3103	Alveolar	40.633	0.0000*

\*Statistically significant, NS, Not significant; VOC, Volatile Organic Compound

*Group-B: Nasal gas (VOC) discrimination from oral and alveolar air:*

Oral cysteine releases H<sub>2</sub>S and oral urea releases NH<sub>3</sub>. In the nasal cavity however, high concentrations of either cysteine (24 mMol) or urea (1.7 M) does not generate gas (data not shown). So it is difficult to challenge type 2 halitosis in the nasal cavity in the same way as can be done for type 1 halitosis. Sulfarhin was utilized as an exogenous odorant to simulate odor emission in the nose. Slight contamination with alveolar air (2.06%) and oral air (4%) occurred. It is expected that menthol and eucalyptol would arrive the oral cavity via the retronasal route because they are highly volatile. Many other gas measurement protocols (Table 1) don't utilize intranasal catheters to collect nasal air samples, and likely give significantly higher contamination.

*Group-C: Alveolar gas (H<sub>2</sub>) discrimination from oral and nasal air:*

H<sub>2</sub> isn't naturally generated by the body. It's an end product of bacterial breakdown of carbohydrates. Therefore, the oral or nasal H<sub>2</sub> may reflect local bacterial activity, while elevated breath H<sub>2</sub> is representative of all blood gases exhaled from alveolar air meanwhile Type 4 halitosis.

Fasting breath H<sub>2</sub> is normally <10 ppm, [19] or shows variation 0.3 - 34.1 ppm (mean 9.1 ppm) during the day. [21] In this study it was 1-17 ppm (mean 6.7 ppm, n=90). Mean OCTT was 75 minutes (range 60-90 minutes, n=30).

In the literature, serious contaminations of alveolar and mouth air are found [24]. This is potentially because the first half of exhaled breath was not excluded from the samples. In this study, alveolar H<sub>2</sub> contaminated nasal air (18.43%), oral air (9.42%) despite some lactulose may remain in the oral cavity while swallowing the

Laevolac syrup. Oral bacteria act on the lactulose and cause oral H<sub>2</sub> generation. The low molecular weight H<sub>2</sub> easily moves to the oronasal spaces while breathing. This may explain why nasal H<sub>2</sub> contamination was greater than oral H<sub>2</sub> contamination. H<sub>2</sub> (is lighter and more volatile than NH<sub>3</sub>), was successfully separated in group C individuals. Possibly this protocol also quantifies each NH<sub>3</sub> source separately.

In a valid protocol present in the literature [19], subjects hold their breath for 15 seconds, the first 500 ml of expired air is excluded, and the second 500 ml is collected in a foil bag. The second part of exhaled breath represents alveolar air as very similar to the protocol described in this study. Interpretation of the alveolar H<sub>2</sub> measured by this protocol, is a part of breath hydrogen tests [18][19][21] are acceptable to detect disaccharide intolerance / malabsorption. Some other gas sampling protocols widely used in the literature and their effectiveness are discussed in the third column of Table.1

Some individuals (n=7), who were excluded from the study on the grounds of poor periodontal health, had >40 ppm oral H<sub>2</sub> but <10 ppm alveolar H<sub>2</sub> (data not shown). Oral VOC in these individuals was also higher (>5 ppm) than the maximum value (1.7 ppm) of oral VOC found in all periodontally healthy individuals (data not shown), and there was good correlation between oral VOC and H<sub>2</sub> (but not H<sub>2</sub>S) and with periodontal score (R=0.93, n=7). The potential relationship between H<sub>2</sub> and periodontitis can be explained as periodontal inflammation causes oxidative stress in periodontal cells, [25] which generates alkanes in the mouth. [5] Hence, detection the level of oral VOC and H<sub>2</sub> may be useful to score or monitor periodontal healing.

The highest values of SO<sub>2</sub> (0.6 ppm), and H<sub>2</sub> (126 ppm) were detected in their baseline alveolar air (data not shown) of 2 excluded subjects who reported constipation

at the time of the study. Another excluded individual showed high breath H<sub>2</sub> (43.4 ppm) and also reported constipation. It is unknown whether constipation may contribute to Type 4 halitosis.

### **Conclusion:**

The protocol is summarized as follows:

1 - To more accurately model the emission of halitosis gases in the patient's social environment, the mouth is not kept closed before gas measurement. Instead, the lips are slightly open and the aspirating tube is placed on the tongue while reading momentary oral gases.

2 - The gas measurement protocol described here, does not depend on specificities of gas detector. Any multi-gas detector can be used, capable of detecting H<sub>2</sub>S, NH<sub>3</sub>, H<sub>2</sub> and VOC. This makes the protocol flexible, reproducible, easy to use at any condition in any medical or dental setting. This sensor configuration best fits the natural composition of halitosis gases. Measurement of a single gas is inadequate to interpret halitosis.

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3 - To challenge the capacity of producing Type 1 halitosis, oral gases are read before and after cysteine rinse to compare H<sub>2</sub>S increase. This gives most accurate evidence about Type 1 halitosis potential which momentary halitosis reading cannot demonstrate. This makes the measurement protocol lesser depend on mathematical thresholds.

4 - Oral, nasal and alveolar gas measurement is carried out as described previously. This gas measurement protocol can be applied by dentists without additional training, and successfully separates oral, nasal and alveolar gas samples. Therefore it can be used in diagnostically in clinical practice. A diagnostic method utilizing this protocol will be discussed in a future publication.

**Acknowledgement** There is no financial interest in products or companies mentioned in the article, this includes a salaried position in the company including consultant position. None to declare.

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