Oral malodour– background and diagnostics

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Tiivistelmä – Referat – Abstract

Bad breath or oral malodour can be related to gingival diseases, trimethylaminuria, various inflammation diseases of upper respiratory tract, foreign bodies in nasal cavity etc. Bad breath is usually, in 85 % to 95 % of cases, inflicted by gram negative anaerobic bacteria in tongue coating. These bacteria have a tendency of producing foul-smelling sulphur containing gases called volatile sulphur compounds or VSC. Main cause of bad breath is parodontitis or postnasal drip into posterior part of the tongue.
Detecting bad breath is most efficiently done by organoleptic method. By skilled analyser the reason for oral malodour can be determined with great accuracy. For scientific study the most effective method is gas chromatography (GC) with flame photometric detector (FPD). With it almost every component of exhaled air can be detected both quantitative and qualitative. Effective chairside methods include portable sulphur monitors and saliva tests.

Avainsanat – Nyckelord – Keywords

Halitosis, Aetiology, Measurement

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

1. Introduction

1.1. Aim of the study and methods

The aim of the study was to summarize current knowledge concerning background of phenomena called bad breath and to get acquainted with clinically and scientifically relevant methods for diagnosing bad breath. This survey was carried out by means of literature search. The main sources of knowledge have been “Bad Breath - research perspectives” edited by Mel Rosenberg for scientific background of oral malodour and “Handbook of machine olfaction: Electric nose technology” edited by Pearce, Schiffman, Nagle and Gardner for principles of odor detection. Noticeable data sources have also been “Methods of Air sampling and analysis” edited by Katz and a review article by van den Broek, which is the main source of correlation coefficients for variable methods.

Hopefully the observations made in this review can be used for diagnosing and treating patients with bad breath more efficiently. For more basic information of bad breath please refer to the appendix I: British Dental Association fact file on bad breath.

Examination and thought progress has been the following: Figuring out the phenomenon --> caused by shifted balance of oral microflora --> historical background ---> Mediterranean region and ancient China --> Eating habits --> Mechanical cleaning and chemical cleaning--> Diagnosing of oral malodour is possible by analyzing exhaled air or bacterial flora --> Volatile sulphur compound detectors and saliva test are already in commercial use --> diagnosing methods should be based on the knowhow of the dentist and on the use of completing methods.

The structure of this document is following: first there is a wide and simplified look-over on oral malodour that hopefully explains basics and background of the phenomena and makes further reading easier. Then foul-odouring molecules are listed and it is explained why they smell. Further microbiological basis of

malodours is described. This section helps to understand analysis methods. Finally there is a review of laboratory and chairside methods for detecting malodour (molecules) and malodour sources (bacteria).

1.2. Bad breath in general and terminology

Halitosis means malodour in breath air or so called bad breath in common language. However, to be more specific, term halitosis is a fictional term made up by Listerine mouthwash company salesmen in 1920´s (Rosenberg 2009). More accurate term use would be *foetor ex ore* for only mouth air malodour and bad breath for malodour that is also present in nasal air (Imfeld 2008). In this review the terms bad breath and oral malodour are preferred to avoid any misunderstanding.

Bad breath is a common phenomenon worldwide. Depending on source about quarter to half of Western population suffers from it (Imfeld 2008, Laine et al., 2008, Bosy 1997). Bad breath is common in every race and social group. Bad breath has also a long history and it goes almost 4000 years back (Rosenberg 2009). Bad breath studies are being made worldwide and especially qualified background research is done in Canada, United States of America, Israel and Japan. Everybody has bad breath sometimes, but constant oral malodour is a thorny problem. In same cases halitosis or fear of oral malodours – halitophobia – can be crippling in social relationships (Rayman 2008).

One aspect in brad breath is the bad breath paradox. This paradox follows from the fact that a person cannot smell his/her own breath. Because of this people who have bad breath do not know about it and people who do not have it can be constantly worried about it. This brings us to the very point of this study. Therefore there is a huge demand for portable measurement device that would give correct results about breath air quality (Rosenberg 1997, 2009).

1.3. Aetiology of bad breath

1.3.1. **Physiological**

Everybody has bad breath sometimes. Morning breath is usually foul-smelling. This is due to decreased saliva flow in night-time. Main functions of saliva are to rinse the mouth, be antibacterial, transport oxygen into oral cavity, transport enzymes like amylase and to stabilize pH in the mouth. When saliva flow decreases due to medication, stress, sleep, long time lag between meals, not drinking enough, talking, hard exercise, drinking alcohol or coffee, the risk of bad breath increases. Physiological halitosis can be diagnosed by measuring saliva flow rate. It can be treated with mouth moistening sprays or more simplify by chewing something like sugarless chewing gum. If medication is the reason behind low saliva flow the current medicament should be replaced with another medicine with lower side effects if possible.

1.3.2. **Mouth based**

Bacterial population in the mouth and especially at the posterior parts of the tongue is the main explanation for halitosis according to the latest knowledge (Rosenberg 1997). There is a strong correlation between tongue coating and bad breath (Yasukawa 2010). Anaerobic bacteria of tongue coating causes bad breath in about 40-45 % of the cases (Asikainen 1996).

It is important to state that so-called normal flora is essential to immunological defence of the body and digestion. Abnormalities in this biofilm can lead to pathological states. Effectors to this bacterial population can be divided to stable and variable factors. Stable factors consist of innate factors like composition of saliva. Some of variable factors are more easily altered than others. Habits like oral hygiene, eating habits and diet are tough to alter. Some physiological factors like biofilm, bacterial families in mouth and salivary secretion rate in general alter between persons but for individuals they are quite constant (Rosenberg 1997, 2009).

Food remnants in mouth or food attached into braces are excellent nutrition for bacteria. Another sources are saliva, our own dead cells and especially in cases of periodontal disease – blood. According to some knowledge the most important amino acid source and reason behind oral malodour is postnasal drip. This occurs especially in cases when patients have taken good care of their oral hygiene. Postnasal drip is a phenomenon in which mucosa drops out of nasal passages into pharynx and posterior parts of the tongue. This mucus gets stuck into tongue and supplies bacteria with proteins. These proteins and single amino acids are then broken down by anaerobic bacteria. Bacteria then emit volatile sulphur compounds (VSC) as products of their metabolism (Rosenberg 1997, 2009).

Figure 1 gives a schematic picture of the tongue. Because of its spongiform nature different bacteria can easily attach to it and colonize its surface and crypts. Food remnants and postnasal drip can stay long times on the spongy surface of the tongue.

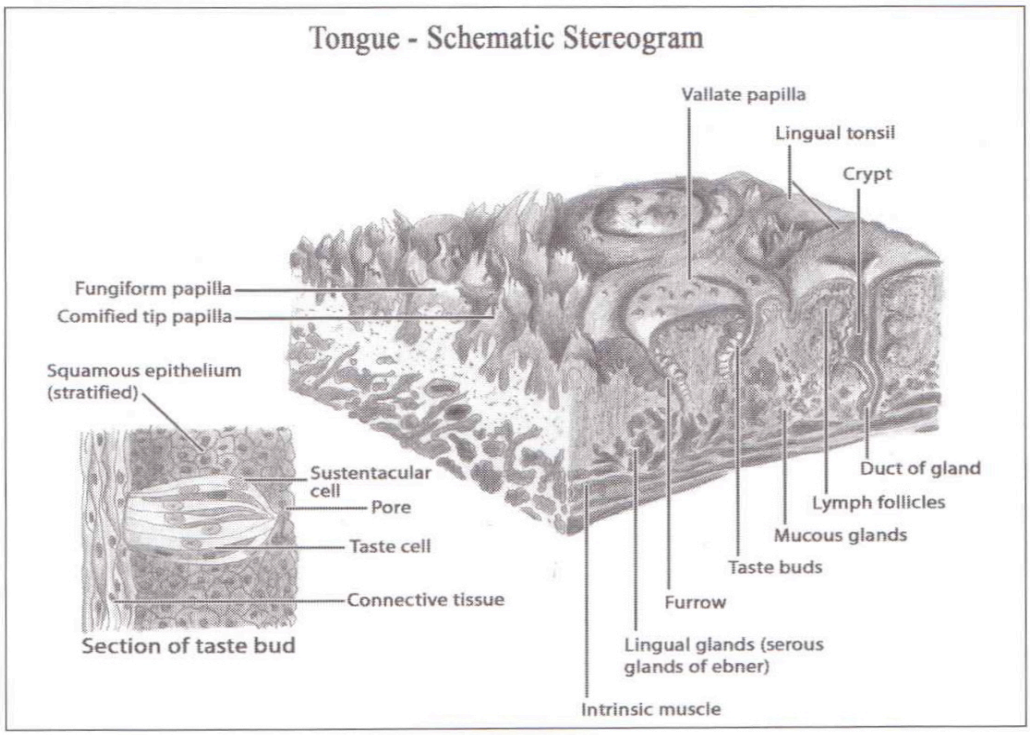


Figure 1. Schematic stereogram of tongue. Anaerobic bacteria colonises the nearest part of the tongue and gaps between papilla. There they consume amino acids of dying tongue epithelia and food remnants (Rosenberg 2009).

1.3.3. **Periodontitis and bad breath**

Periodontal diseases can cause bad breath. Studies give a strong correlation between bad breath and periodontal diseases (Miyazaki et al 1995, Yaegaki 1992,1997). However, periodontal disease is not always manifested by bad breath but it surely increases the risk of it (Rosenberg 1994). Some research studies give evidence that there is a straight connection between periodontal diseases and bad breath while others do not. In any case it is known that many of the bacteria linked to periodontal diseases are also related to bad breath (Kleinberg and Codipilly 1997). Usually when periodontal disease is healed the oral malodour also disappear (Rosenberg 1994, 2009). In a study by Yaegaki (1997) a link between gingival pockets and amount of VSC and CH_3SH/H_2S ratio was established. When pocket depth increases especially over 4 mm the VSC levels and methyl mercaptan/hydrogen sulphide ratio also increase. This means that when suffering from periodontitis the amount of VSC, especially that of methyl mercaptan, increases. Luckily these gases seldom escape from the periodontal pockets. There is also a link between the amount of the tongue coating and periodontal diseases. When patient has deep pockets he or she usually has a thick tongue coating also. This can lead to oral malodour. Furthermore, deep pockets can work as safe heavens for anaerobical bacteria that cause bad breath (Miyazaki et al., 1995, Yaegaki and Sanada, 1992, Yaegaki, 1997).

1.3.4. **Other reasons**

In minor part of cases bad breath can be caused by other reasons than tongue coating and periodontal disease (Asikainen 1996, Rosenberg 1994). When bad odours come from nasal passages, can the reason be foreign objects in nasal passages, sinusitis or polyps. Also throat diseases or tonsillitis can cause bad breath. This is most common in young children (Amir 1999). Tonsillar stones or tonsilloliths rarely cause bad breath even though they have a tendency of smelling very bad when removed from tonsils. This is because malodour ingredients are trapped inside these stones. When the tonsilloliths are crushed between fingers,

smell releases. In tonsils they do not smell because they are still stable structures. These conditions call for treatment of ear, nose and throat specialists, but dentists should be aware of them (Rosenberg 1994, 1997, 2009).

On rare conditions halitosis is blood borne. Even through it is commonly thought to be otherwise. As an example of food based halitosis – like in the case of garlic – most of the odour comes from food remnants in the oral cavity. Some odour is also blood borne, but it is barely noticeable. There is also a link between general health and oral malodours. These cases are often connected to blood-borne halitosis (Rosenberg 2009).

Uncontrolled diabetes mellitus can give rise to sweet ketonic breath (Rooth and Ostenson, 1966). The same effect is detected with lung carcinoma (Gordon et al. 1985). Also aniline and some other compounds can be detected in exhaled air when the patient suffers from lung carcinoma (Preti et al, 1988). Cancer in the upper respiratory tract or orapharyngeal region is combined with short chain fatty acids in breath air (McGregor et al, 1982).

Liver diseases often manifest in exhaled air. Friedman (1994) has done research about the connection between liver diseases and oral malodour. Due to disturbance in hepatic metabolism often many substance levels in the blood increase. This can cause blood borne oral malodour. Hepatic cirrhosis and other diseases in liver can increase levels of hydrogen sulfide, methyl mercaptan, short fatty acids, ethanethiol and dimethylsulfide in exhaled air (Friedman et al, 1994. Chen et al, 1970).

Ureamia or kidney disease can result in fishy odour in breathing air. This is due to dimethylamine and trimethylamine detected in exhaled air (Simenhoff et al, 1977). One disease - rare but still worth mentioning is trimethylaminuria (TMAU). The actual prevalence of trimethylaminuria is not known. The same holds true also for the biochemical nature of the disease. Trimethylaminuria is extremely rare in Finland. Trimethylaminuria usually manifests in fishy odour coming out of the whole body of the patient and oral malodour and bad taste in mouth. Malodour is due to raised levels of trimethylamine in exhaled air (Preti et al, 1993). Bad taste

may also result from ascended levels of trimethylamine in saliva (Preti et al, 1997). Recent studies state that TMAU is the main reason for undiagnosed body odours (Whittle 2007).

All these conditions mentioned above are rare, but they should still be kept in mind. Patients with these symptoms should be examined by a dentist and referred to the physician. Diagnosing these diseases in time can make a great difference to the patient's health and quality of life.

2. Human olfaction

Human nose is a very sophisticated instrument that can recognise over 400000 different chemicals (Boron et al, 2005). One main function of olfaction is to analyze the quality of food. Any over-activity of bacteria in food is easily detected by nose and so spoiled food is rejected. Also hazardous gases can be avoided due to the accurate olfaction. Of course there are exceptions like carbon monoxide. Some other poisons are also odourless and flavourless.

The olfaction process is based on chemoreceptor censoring and signal processing. Basic concept of olfaction is presented in figure 2.

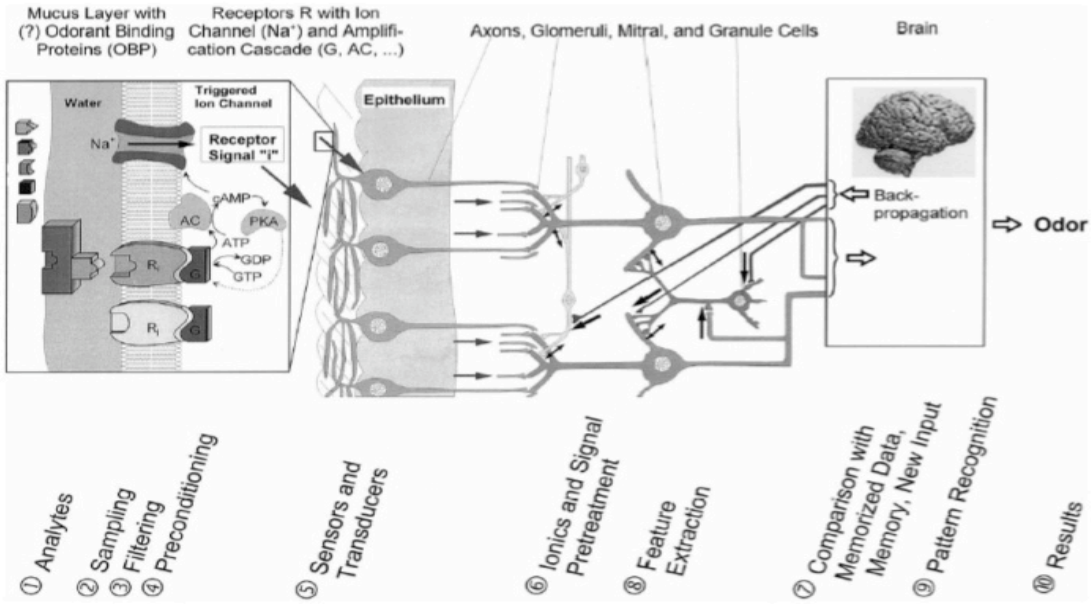


Figure 2. Concept of human olfaction [Ghasemi-Varnamkhasti 2002].

In the olfaction process first the airborne molecules are dissolved to aqueous mucosa of nasal passages. Then chemicals can absorb into specified receptors. When a chemical is bound to a receptor it activates and sends a signal via G-protein and nervous system to brain. The signal can be modified and adaptation is usually happening in olfaction system. Olfaction system adapts quickly and one of its main functions is to notice changes in smells (Boron et al, 2005).

3. Malodours

It is estimated that about 80 % of the 400000 different chemicals, that our olfaction system recognises smell bad (Boron et al. 2005). Bacteria breaking down amino acids or proteins produce the main parts of these. This can be related to spoiled food or any other source of disease. Humans classify almost every bacterial odour as foul. In table 1 there is a summary of organoleptic scores of substances applied to the skin of back of the hand. Back of the hand was then smelled and scored from 0 to 4. 0 stands for no odour and 4 for the foulest odour.

Table 1. Organoleptic scores of malodour sources applied to back of the hand in water solution. Back of the hand was smelled after listed times and repulsivity of smell was scored from 0 to 4 (0 means no odour and 4 stands for worst odour). Adapted from Kleinberg and Codipilly 1997.

	0 min	1 min	3 min	10 min
hydrogen sulfide	3	1	0	0
methyl mercaptan	3	1	0	0
acetic acid	3	4	0	0
propionic acid	3	4	0	0
butyric acid	4	4	2	2
valeric acid	4	4	3	2
indole	4	4	4	2
skatole	4	4	4	2
putrescine	4	4	3	1
cadaverine	4	4	2	1

[a] Solutions were 12.5 mM except for hydrogen sulfide and methyl mercaptan which were aqueous solutions obtained by bubbling these gases through distilled water for 2 minutes at 20°C.
Water evaporation was complete in all cases after 1.5 to 2.5 minutes. More than 50% evaporated within the first minute.

The main components of the bad breath are the so-called volatile sulphur compounds (VSC). They can be detected by olfactory system in very small

quantities in air. According to industrial data the levels are 5 ppb for hydrogen sulfide and methyl mercaptan in air (Martin, 1998). Hydrogen sulfide smells like rotten eggs and methyl mercaptan smells like rotten cabbage. They are products of degradation of sulphur containing amino acids (methionine -> methyl mercaptan and cysteine -> hydrogen sulfide). VSC are very toxic molecules for human cells and bacteria use them to break down gingival tissue in periodontal disease (Holt 2000).

Several other foul smelling components also exist. This is one reason why bad breath can occur while VSC levels are low. It also explains why licking of wrist and smelling it after 5 seconds usually gives a much worse picture of oral odour than it actually is. This happens because of indole, methylamine and cadaverine are not volatilized from the saliva (Tonzetich 1967). When saliva is allowed to dry on skin these odours are released (Kleinberg and Codipilly 1997). Hydrogen sulfide and methyl mercaptan are volatile in very low temperatures and they are also easily released from saliva. This can also be seen from the table above.

Many other foul smelling molecules have been identified. As already has been stated they are toxic substances itself or markers of rottening or spoiling. They are usually products of the degradation of amino acids or fatty acids.

Various foul smelling amino an acid derivative exists. One is indole that derives from tryptofan (organoleptic: feces). Another tryptofan derivative is skatole (organoleptics: feces). Putrescine derives from arginine and ornithine (organoleptics: putrefaction). Cadaverine derives from lysine (organoleptics: urine, semen, rottening tissue) (Anon, 2010a, Kleinberg and Codipilly 1997).

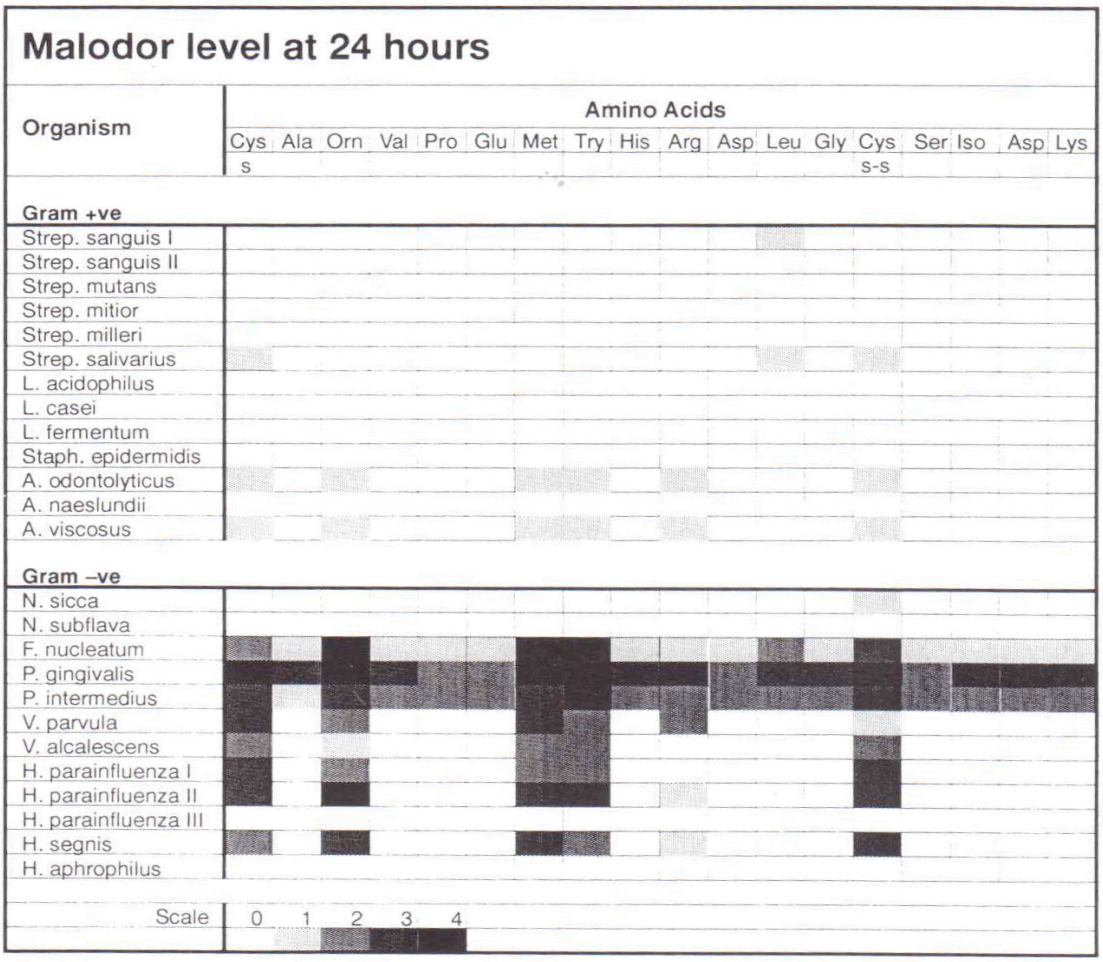
Small chain fatty acids are volatile and foul smelling. These are products of the degradation of longer fatty acids. Foul smelling acids are buturic acid (organoleptics: vomit, sharp acetic cheese, butter, fruit), acetic acid (organoleptics: pungent, sweet, sharp, pungent, sour vinegar), propionic acid (organoleptics: pungent acidic and dairy-like, sweat) and valerie acid or pentanoic acid

(organoleptics: acidic and sharp, cheese-like, sour milky, tobacco, with fruity nuances) (Anon 2010a, Kleinberg and Codipilly 1997).

4. Micro-organisms behind bad breath

Table 2 is adapted from a research investigating the relationship between oral bacteria and amino acids in vitro. Bacterial colonies in dishes were supplied with different amino acids and odour judges scored odours. Results are discussed at the end of this chapter.

Table 2. Organoleptic scores of an in vitro assay by Kleinberg and Codipilly (1997). Different bacteria where fed with different amino acids. This assay demonstrates the relationship between oral malodour and gram-negative anaerobic bacteria and amino acids. Organoleptic scale of odour is 0-4 and 4 stands for the worst smell.



Sources of amino acids for microorganisms are dead and peeling cells, food remnants, saliva, blood and postnasal drip. The most relevant group of

microorganisms to oral malodour is gram negative anaerobic bacteria. In oral cavity they live in the deepest part of biofilms. Anaerobes are usually related to gingival diseases and they can also survive in gingival pockets. There are also some data of the synergism between bacteria. Gram positive groups, like *Streptococcus salivarius* can metabolize carbohydrate parts of glycoproteins leaving the protein parts for gram negative groups (Sterer, Rosenberg, 2006). This can explain why high levels of gram negative bacteria do not always mean increased levels of VSC. According to some studies probiots like *Streptococcus salivarius* can inhibit oral malodour (Burton et al 2006). Roles of another bacteria and their relationships in biofilm clearly require more study.

There is no one specific bad breath bacterial species because mouth biofilm is a complex and dynamic entity. Six species of anaerobic bacteria are combined to periodontal disease and malodour production in oral cavity: *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia*, and *Treponema denticola* (Yasukawa 2010).

According to Kleinberg and Codipilly (1997) *Prevotella, Porphyromonas and Fusobacterium* are the main odour producers. This is also backed up by other studies (Kazor et al. 2003, Loesche 2002). This fact can prove helpful when talking about methods of oral malodour diagnostics.

Kleinbergs and Codipillys research also gives us data about foul smelling amino acids. According to figure 5 ornithine, cysteine, tryptofan and methionine are the main foul odour molecules. Also lysine seems to cause strong foul odour. This correlates with the foul smelling compounds mentioned earlier in this chapter.

5. Diagnostics

5.1. Anamnesis and dental examination

Daily habits and dental self-care should be mapped and corrected if they are found to be unsatisfactory. Within dental examination inspection of overall dental hygiene, taking note of any inflammation, probing of periodontal pockets and measurement of tongue coating is necessary. Also any bacterial colonized areas should be notified. The patient's own concepts about bad breath should be recorded as well. If the patient is not aware of his/her bad breath the dental professional should gently hint about this to the patient (Asikainen 1996, Rosenberg 1997, 2009).

5.2. Organoleptic method – the golden standard?

The oldest and most accurate method for evaluating if someone has bad breath or not is organoleptic method. Usually it is used by six step scale from 0 to 5 (Roseberg 2009):

0. No odour
1. Slight, barely noticeable odour, most likely not a concern
2. Slight, but noticeable odour. Most researchers think that this is the cut-off line
3. Moderate odour
4. Strong odour
5. Unbearably strong odour

Measurement with this method can be done in various ways, but a widely used method in bad breath clinics is the following: first the patient is asked to breathe out trough mouth. This breath is then smelled from close distance and scored. Secondly nose breath is measured. Also the difference between nostrils can be evaluated. Thirdly "count-to-twenty" -test can be applied. The patient is asked to count to twenty aloud and out flowing air through mouth is smelled during talk. This is a good method for evaluating oral malodour during conversation (Rosenberg 1991,1992,2009, Tonzetich 1977, van den Broek 2007).

Problem with the organoleptic method is mostly the poor reproducibility. Subjectivity is another drawback. Odor judges can be influenced by their physical state, mood and sex appeal of the patient. Also the measuring instrument – the nose – gets tired and adapted (Rosenberg 2009).

Organoleptic method is, however, extremely accurate and a skilled smeller can detect various kinds of odors in breathing air. This is a huge advantage because some diseases have special smells. According to this information an organoleptic examination should be done in all cases. It should be also backed up by some other methods (Rosenberg 1997).

Rosenberg has also published about organoleptic scoring. He has done a wide spectrum of test series and research that show correlation between skilled judges and common people. It is relieving to see that accurate detecting of bad breath does not need any training at all (Rosenberg 1994, 1997). Training can, however, help odour judges to make less mistakes (Nachnani et al. 2005). Another interesting finding is that people are never objective for their own body odours – they have a tendency of overestimating the bad smell of their breath (Eli et al. 1997).

5.3. Gas chromatography

5.3.1. **Principle**

Gas chromatography is the most accurate method for measuring chemistry of the breathing air. By using gas chromatography (later GC) the researcher or dentist can produce a large set of data concerning both qualitative and quantitative properties of mouth air. To gain reliable accuracy the GC must be equipped with flame photometric detector (FPD) (Tonzetich 1977).

5.3.2. **Application to mouth air detection**

Methods for mouth air sampling have been used for at least 30 years (Tonzetich 1971, 1977). They are well established and effective. Strength of GC lies in accuracy and comprehensiveness. When using GC the researcher can be certain that everything will be noticed. Major drawbacks are the very high price and the need of skilled staff for operating the device. GC cannot be used as a chairside

instrument in any case. Research made with GC is performed by collecting samples into teflon tubes and transporting them into laboratory in dry ice (Kleinberg and Codipilly 1997).

GC gives good correlation coefficients with low p-values. Multiple studies have been conducted for finding relationship between GC results and organoleptic scores. Usually r is over 0.60, thus the correlation and repeatability of CG is in high a level. The variation in the results of these 10 studies is outstanding small (van den Broek et al. 2007).

5.4. Concept of machine olfaction

Semiconductor-based breath detecting is based on chemical reactions, which alter current transporting properties of semiconductors. The current altering due to volatile chemicals in air then can be processed with computer and when correctly calibrated the electronical nose can give accuracy readings of molecule concentrations in air (Ghasemi-Varnamkhasti 2002).

The concept of an electronic nose is quite similar to human nose as presented in figure 2. The difference is that primary neurons are replaced with semiconductors and further signal processing is replaced with a computer program. Usually the electronic nose composes of several molecule specific receptors and recognized patterns can be linked to similar smells. Also the intensity of smell can be detected. Main applications for bad breath diagnostics have been developed for detecting sulphur compounds. Major drawback is that they do not have enough receptors to detect other foul smelling components as indole, skatole or putrescine (Ghasemi-Varnamkhasti 2002, Vestergaard et al. 2007).

5.4.1. **Halimeter™**

Halimeter™ was introduced in the early 1990´s. It has become a very popular instrument for verifying organoleptic scores in research. Correlations have been excellent and the device is very reliable. It is designed to detect sulphide in air and it detects hydrogen sulphide in good levels and also methyl mercaptan and dimethyl sulphide because of their sulphide group. Because it is mainly a VSC detector some molecules like trimethylamine and putrescine can escape its

detection. Other molecules like alcohol affect the readings and should be avoided before measuring. Halimeter™ is a rather cheap instrument costing less than 2000 euros and its running expenses are fairly low. The detector of Halimeter™ should be calibrated or replaced every 2-3 years. Cost of this is about 600 euros. In use Halimeter™ is a very simple device. The commercial brochure of Halimeter™ is available in the appendix for further reading (Rosenberg 1991).

The following readings taken from Halimeter™ website show a correlation between the concentration of sulphide molecules and the organoleptic score:

- At levels of 200-300 ppb, oral malodour is noticeable by an observer standing close to the patient. Organoleptics 2.
- At 350-400 ppb, the odor is noticeable by an observer standing several feet away from the patient. Organoleptics 3.
- At 500-700 ppb the odor is more noticeable not because it is "stronger," but because it is more foul. Organoleptics 4.
- At over 1000 ppb, the odor will linger for several minutes after the patient leaves the room. Organoleptics 5.

Multiple studies have been performed for measuring correlation between organoleptic scores and Halimeter™ scores. Article of van den Broek et al. (2007) summarizes scores of correlation coefficients of 11 studies. They vary from 0.37 to 0.78. So it can be stated that Halimeter™ works as a reliable tool for malodour detecting. This sulphide monitor correlates also strongly with gas chromatography ($r=0.70$, $p=0.01$ in average of 8 studies) (van den Broek et al. 2007).

5.4.2. **Oralchroma™**

According to marketing material Oralchroma™ is a simplified gas cromatograph. It is more likely to be a semiconductor-based system with wider detection of molecules in air. Oralchroma™ is also a chairside VSC monitor. It is more expensive than Halimeter™ and it appears to be as simple in use. Oralchroma™ comes with a computer program that can be used for calculating and processing the data. Oralchroma™ has proven to be a reliable test method (Tsai 2008,

Vandekerckhove 2009). The commercial brochure of Oralchroma™ is available in the appendix for further reading. Correlation coefficients are not yet available, but they should be at the same level as those of other VSC detectors.

5.5. Salivary tests

5.5.1. **General**

Salivary test are used for detecting bacterial load in the mouth. They can correlate strongly with organoleptic scores. However, some cases with oral maldour can still be undetected with these methods. Saliva tests are non-invasive, fast, chairside useable, cheap and easy to use. One or two of them used with another supplementary method can give excellent knowledge about the reasons behind oral malodour.

5.5.2. **BANA-test**

Some gram-negative anaerobic bacterial species like *Treponema denticola, Porphyromonas gingivalis* and *Tannarella forsythia* can be detected from plaque or gingival/tongue surfaces with their special ability to hydrolyse benzoyl-DL-arginine-a-naphthylamide (BANA). The test itself is very easy to use: the area in mouth is swept with a cotton swab or curette and this sample is then applied onto the paper strip and then incubated for 5 minutes in 55°C. If the test is positive the colour of the strip changes into blue. With BANA test, correlations with organoleptic methods have been good while with the sulphide monitors the correlations have been weaker. BANA-test information is given in appendix (Loesche et al. 1990, 1992, Kozlovsky et al. 1994, Figueiredo 2002). BANA-test correlations have been of the magnitude of $r = 0.40$ ($p = 0.003$) when compared with organoleptic scores. However, correlation between sulphide monitors is rather poor ($r < 0.30$). In a more resent multi-regression analysis between organoleptic measurements, peak values of sulphide and BANA-studies have given more significant correlation scores ($r = 0.50–0.59$; $p < 0.001$) (van den Broek et al. 2007).

5.5.3. **β-Galactosidase –test**

Deglycosylation of saliva based mucins may be a critical initial step leading to their subsequent proteolysis and putrefaction. Based on this knowledge, a simple saliva based test can be made which can correlate with the malodour levels. Simplified idea of the test is placing saliva onto paper discs with pre-applied β-galactosidase. The color change of the paper is then graded from 0-3 where 0 is no change and 3 is the deepest blue. Some researches have been successful in establishing good correlation coefficients between organoleptic scores and β-galactosidase activity (Sterer 2002, 2007, van den Broek 2007).

5.5.4. **Bacterial load test**

Oratest™ is a bacterial load test for estimating bacterial levels in saliva and mouth. Performing the test is simple. Patient rinses 10 ml of sterilized milk exactly 30 seconds and then spits it into a test tube. After this, 3 ml of milk-saliva mixture is added to a test tube with 0,12 ml of 0,1 % methylblue. The tube is then incubated in room temperature for 1-2 hours. If it takes less than 2 hours to accumulate a white area with a cross-section over 6 mm into the bottom of the test tube the bacterial load is high (Rosenberg 1990, Bhasin 2006, Tal 1990).

5.6. Other methods

5.6.1. **Colorimetric sensor**

One interesting method based on colorimetric sensor has been introduced recently (Alagirisamy 2010). Its effectiveness lies on simplicity and usability. This recent study is encouraging because of its novel character. However, light absorption relation to concentration has been widely used in industry to analyze exhaust gases for sulphide remnants in form of UV-spectrophotometry (Katz 1997).

The basic layout of this colorimetric sensor is presented in figure 3. Basic concept is following: the patient breathes mouth air through disposable plastic straw. The sample air is then conducted through sampling pump for stabilizing of sample flow. After that pump air is mixed with iodine for complex forming. Iodine reacts with hydrogen sulphide, which causes a measurable reduction of hydrogen

sulphide. In the light of this, the amount of intaken hydrogen sulphide can be concluded. Detection of iodine loss is made colorimetrically using starch. The starch works as fixative for iodine. The measurement itself is done by conducting monochromatic (570 nm) light into the sample. Then output is detected with special detector (radiometric sensitivity of 0.25 A/W, spectral range of 330–720 nm peaking at 580 nm). Detector readings are then filtered, amplified and printed out.

According to authors, the detection limit of the sensor is 0.05 μg/l of hydrogen sulphide, which means approximately 35 ppb. It has achieved good correlations between Halimeter™ (r = 0.934) and further discussion and studies are needed to assess its applicability for analyzing oral malodour.

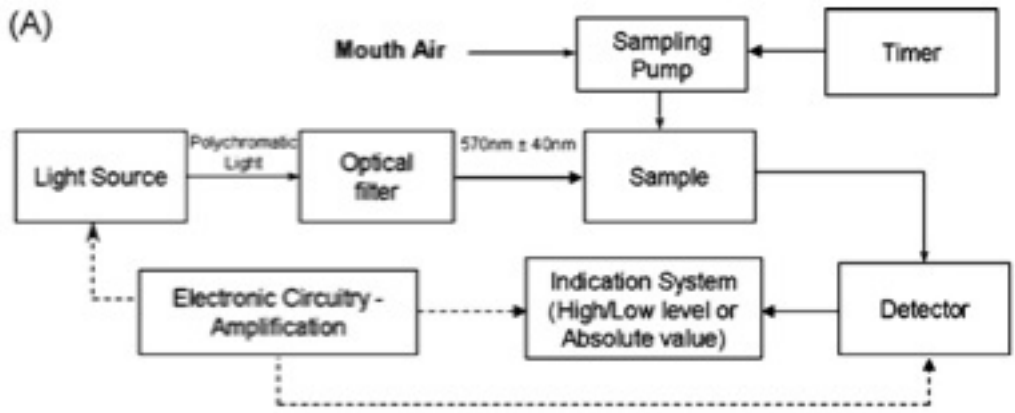


Figure 3. Schematic diagram of colorimetric sensor in assay made by Alagirisamy et al. [Alagirisamy 2009]

5.6.2. **Polymerase chain reaction**

Polymerase chain reaction (PCR) can be used for detecting bacterial species in mouth. This method is widely used in medicine and it can be a useful tool for also analyzing bacterial load in mouth (Kato et al. 2005, van den Broek et al. 2007).

6. Summary and the future of machine olfaction

6.1. Diagnostics

None of the above methods is a stand-alone test because of the nature of the bad breath. Routine dental examination for bad breath patients should include taking proper anamnesis, measurement of VSC-levels with a clinically effective method (Halimeter™ or Oralchroma™), examining dental pockets and tongue and organoleptic measurement of bad breath. After these, the dentist should be able to say whether or not the patient has bad breath. Wide spectrum of methods can also help the professional to specify the problem and make it easier to recommend an effective treatment strategy.

6.2. Future of machine olfaction

Semiconductor-based artificial noses should develop in the future similar to their applications in food, chemical and military industries. When technology is cost-effective enough, hopefully some devices can be created for chairside use that can detect all the main malodour causes from breath air. This can then possibly replace the unpleasant organoleptic method. Also possible occult diseases like TMAU or diabetes can be detected and treated. Quite an interesting Finnish invention of Environics Oy is an electronic nose for food industry. It has been successfully used in detecting putrescine in air near meat products and pizza slices. Combination of VSC detector and artificial nose should create more effective measurement device for malodour detecting (Vestergaard et al. 2007).

The urea test is also very interesting because it is one of the first clinically working breath test. In the urea test nonradioactive tracer ^{13}C-urea is taken per os, and later exhaled air is analyzed for ^{13}C-tracer (Yaegaki 1997). If the marker is found, then the result indicates *Helicobacter pylori* present in stomach. A similar kind of approach could work in bad breath diagnostics. The ideal method could be a combination of machine olfaction and BANA-type of test because of the tendency of foul-odour producing anaerobic bacteria to metabolize substances different from any other bacteria.

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British Dental Association fact file on bad breath

Dr. Mel Rosenberg

Bad breath (halitosis) is a common problem which often comes from the activity of bacteria in the mouth. Although there is no way of knowing for sure, most adults probably suffer from bad breath occasionally, with perhaps a quarter suffering on a regular basis. This fact file looks at the causes and at what dentists can do to help.

Is bad breath always treatable?

In the past, bad breath was often considered to be an incurable affliction. However, in recent years it has become increasingly evident that bad breath is usually treatable once a proper diagnosis is made.

The main problem is knowing whether we have it or not, because we are poor judges of our own breath odour. Some people suffer from bad breath without knowing it, while others build up exaggerated fears about breath odour even though they do not have it. The best way to find out whether we have bad breath is to ask for someone else's opinion. If we don't ask, other people are unlikely to tell us. And since bad breath can sometimes - fortunately rarely - be a sign of a significant general health problem, we should not be reluctant to tell people dear to us that they have a bad breath problem.

What should I do if I have bad breath?

If you have reason to believe that there is a problem, then see your dentist first, since bad breath often comes from the mouth itself.

When you see the dentist, it is a good idea to explain in advance that you will be asking for advice about bad breath. Also, try to go with someone who is familiar with the problem, to help give the dentist an objective picture of how bad the odour really is, how long it has been going on, and when it improves or

gets worse. Since bad breath often varies, a family member or friend can also help determine whether the odour at the time of the appointment resembles, both in character and intensity, the odour that is generally troublesome.

If the dentist knows that the consultation is about bad breath, you may be asked not to eat, drink, smoke, chew gum, suck confectionery, use mouthwashes, breath fresheners etc., so that the odour will be more typical. You should also avoid using perfumed cosmetic products, such as perfume, aftershave and scented lipstick prior to the appointment, since it can interfere with the odour assessment. If the dentist is not told about the reason for the consultation beforehand, do these things anyway and tell the dentist that you have prepared for the appointment in this way.

What will happen at my appointment?

Your dentist will ask questions to help determine the possible causes of the odour, and then compare the odour coming from your mouth and nose. In most cases (about 85-95%) , the odour comes from the mouth rather than the nose. This is an indication that bacterial activity somewhere in the mouth is responsible. If the odour comes mostly from the nose, then the nasal passages may be involved.

Your dentist may also make measurements using a sulphide monitor to help in diagnosis and treatment, since volatile sulphur compounds are often associated with bad breath.

The following table summarizes different odour-related problems, and their possible causes.

APPENDIX I

Problem	**Possible cause or source of malodour**
Odour after fasting, dieting, sleeping, taking medications, prolonged speaking, exercise	dryness in the mouth, insufficient saliva flow
Gums bleed and/or smell	gum problems, poor cleaning between teeth
Odour upon talking	postnasal drip on back of tongue
Odour at onset of menstrual cycle	swelling of gums
Small whitish stones with foul odour appear on tongue	tonsilloliths from crypts in tonsils
Odour appears suddenly from mouth of young children	onset of throat infection
Odour appears suddenly from nose or entire body of young children	foreign body placed in nose
Taste or smell of rotten fish	trimethylaminuria (rare)
Odour in denture wearers	dentures kept in mouth at night or not cleaned properly
Odour from nose	sinusitis, polyps, dryness, foreign body, hindered air or mucus flow
Bad taste all day long	poor oral hygiene, gum disease, excessive bacterial activity on tongue

Where does the odour come from?

Most cases of bad breath appear to be due to the breakdown of proteins by a variety of micro-organisms. Several of the breakdown products are foul smelling gases.

In people with healthy teeth and gums, the odour usually comes from the far back region of the tongue, and grows stronger when the patient starts talking. The dentist can sample this area using a plastic spoon. The odour coming from the spoon sample may then be compared to the overall odour. Although we do not know why, the very back of the tongue is an important source of bad breath, possibly as a result of postnasal drip, which can get stuck on the tongue and is then broken down by bacteria on the tongue surface.

If the back of the tongue is the problem, then the dentist can recommend a method of cleaning the area, either with a toothbrush, or a specially designed tongue scraper (in some countries, tongue cleaning is a common and ancient practice). It takes time and patience to overcome the gagging reflex. But, eventually, tongue cleaning becomes easy. Care should be taken to clean the back of the tongue thoroughly yet gently, without inflicting pain or sores.

Can gum disease cause bad breath?

In some people, bad breath is associated with gum disease, especially if rubbing the areas between the teeth and gums yields a foul odour. Your dentist can help prevent and treat gum diseases in various ways, depending on the type and extent of the problem, but your own daily home care makes all the difference in the world in maintaining gum health between appointments. Cleaning of the spaces between the teeth is of great importance. One home tip to healthy gums (and less bad breath) is to smell the odour coming from the dental floss, and to work to clean those areas more carefully. People with gum disease often have higher levels of odour coming from their tongue, as well.

What type of treatment is there?

Your dentist may recommend dental treatment, if there are other areas in which bacteria and food can become trapped and cause odour. The dentist may also suggest daily rinsing with one of several available mouthwashes which have been scientifically shown to reduce bad breath over time.

Your dentist may also refer you to clinics that specialize in identifying breath odours, or to other medical experts.

What can I do?

In all probability, professional diagnosis and treatment can help turn bad breath into good breath. However, it is sometimes difficult for us to sense the improvement ourselves. In this case, a family member or close friend can also provide important feedback and reinforcement.

Listed below are some of the Do's and Don'ts regarding bad breath. Remember, bad breath is a problem that needs professional attention. Don't mask it - deal with it.

Do's

- Visit your dentist regularly.
- Have your teeth cleaned periodically by a dental professional.
- Floss or otherwise clean between your teeth, as recommended by you dentist. Choose unscented floss so that you can detect those areas between your teeth that give off odours, and clean them more carefully.
- Brush your teeth and gums properly.
- Ask your dentist to recommend a toothbrush or scraper for your tongue.
- Clean your tongue all the way back gently, but thoroughly.
- Drink plenty of liquids.

- Chew sugar-free gum for a minute or two at a time, especially if your mouth feels dry. chewing parsley, mint, cloves or fennel seeds may also help.
- Clean your mouth after eating or drinking milk products, fish and meat.
- Unless your dentist advises otherwise, soak dentures overnight in antiseptic solution.
- Get control over the problem. Ask a family member to tell you whenever you have bad breath.
- If someone in your family or a close friend has bad breath, find a kind way to let them know. If you can't tell them directly, leave this fact file lying around.
- They may get the message.
- Ask your dentist to recommend a mouthwash which has been shown to be clinically effective in fighting bad breath.
- Use it most effectively right before sleeping.
- Eat fresh, fibrous vegetables such as carrots.

Don'ts

- Don't let your concern about having bad breath run your life. Don't be passive.
- Don't be depressed. Get help. Don't ignore your gums - you can lose your teeth as well as smell bad.
- Don't drink too much coffee - it may make the situation worse.
- Don't forget to clean behind the back teeth in each row.
- Don't brush your tongue with regular toothpaste - it's better to dip your toothbrush in mouthwash for tongue cleaning.
- Don't run to the gastroenterologist for concerns of having bad breath - it usually comes from the mouth and almost never from the stomach.
- Don't give mouthwash to very young children, as they can swallow it.
- Don't clean your tongue so hard that it hurts.
- Don't rely on mouthwash alone - practice complete oral hygienia

Bana-test

Chairside Test for Periodontal Risk

The BANA Test is a highly sensitive, inexpensive and easy-to-use chairside test for periodontal risk. In just 5-minutes, the BANA Test can detect the bacteria associated with periodontal disease simply by applying tongue swabbings or subgingival plaques to a small test strip.

The Science:

The BANA Test is a modification of the BANA hydrolysis test developed by Dr. Walter Loesche and colleagues at the Univ. of Michigan School of Dentistry. It exploits an unusual enzyme found in Treponema denticola, Porphyromonas gingivalis and Bacteroides forsythus, three anaerobic bacteria highly associated with adult periodontitis. Of 60 subgingival plaque species, only these three possess an enzyme capable of hydrolyzing the synthetic peptide benzoyl-DL-arginine-naphthylamide (BANA) present on BANA test strips. If any of the three species is present, they hydrolize the BANA enzyme producing B-naphthylamide which in turn reacts with imbedded diazo dye to produce a permanent blue color indicating a positive test. Socransky and Haffajee in an extensive study of over 10,000 plaque samples, found that these three BANA positive species were the most prevalent of over 40 plaque species evaluated by DNA probes (10,11) .

Malodour

About 90% of oral malodour originates from the tongue from proteolytic oral anaerobes. These bacteria degrade peptides and proteins releasing volatile sulfur compounds (VSC's), volatile fatty acids and other odiferous compounds such as putrescene that combine to create oral malodour. Volatile sulfur compounds can be detected with expensive sulfide monitors (halimeters), but until the BANA test, there was no practical chairside test for non-sulfurous malodourous compounds.

BANA positive bacteria (including the tongue species Stomatococcus mucinalagenous and Rothia dentocariosa) are known to produce a variety of foul smelling compounds including VSC's, valeric, propionic, butyric and other fatty acids. Several studies have demonstrated that tongue samples from malodourous individuals are usually BANA positive. The correlation between a positive BANA test and oral malodour is comparable to the use of sulfide detectors for a fraction of the cost.

How it works

To detect malodour, the tongue is wiped with a cotton swab. For periodontal risk assessment, subgingival plaque is obtained with a curette. The samples are placed on the BANA test strip, which is then inserted into a slot on a small toaster-sized incubator. The incubator automatically heats the sample to 55° for 5 minutes. If P. gingivalis, B. forsythus or T. denticola are present, the test strip turns blue. The bluer it turns, the higher the concentration and the greater the number of organisms. A color guide is printed on the container.

OralChroma™

< CHM-1 >

Breath-testing device to measure and classify oral gas concentrations into three major causal components of halitosis.

Overview

This testing device measures the chemicals in the breath; classifies the volatile sulfur compounds, a major component of halitosis, into three causal components (hydrogen sulfide, methyl mercaptan, and dimethyl sulfide); and provides a precise reading of each gas concentration. It is useful for identifying the cause of halitosis.

Purpose and effect

- As an indicator to identify the cause of halitosis
- As an auxiliary tool to control oral health

ABIMEDICAL

Basic function

- Classifies volatile sulfur compounds into three causal components (hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) and measures each gas concentration.
- Displays concentration values in standard units (ng/10 ml and ppb).
- Ensures precise measurement accuracy using semiconductor gas sensor and simple column.
- Not affected by ambient temperature and humidity.
- Short standby time after power-on (basically within 30 minutes).
- Short measuring time (8 minutes).
- Designed for silent operation.

Operability

- Operation time to collect oral breath sample is shortened by using small-capacity syringe for suction. (This lessens load on test subject.)
- Up to 99 measurement data values can be stored.
- Data can be stored and managed with a PC using specially designed software (option).
- Light and compact.

Maintainability

- Long-life sensor and column (maintenance free for approx. 2 years).
- Automated alarm function notifying when it is time to change the rubber plug for the gas inlet.

Specifications

Product name:	OralChroma	Type:	CHM-1
Detection system:	Simplified gas chromatography	Sample gas quantity:	0.5 cc
Detectable gas:	Following components included in volatile sulfur compounds 1) Hydrogen sulfide 2) Methyl mercaptan 3) Dimethyl sulfide	Measuring time:	8 minutes
		Detection unit:	ng/10 ml and ppb
		Gas collection method:	Manual use of syringe

Operating humidity range:	Relative humidity of 80% or less (no condensation)
Operating temperature range:	10～30℃
Storage humidity range:	Relative humidity of 20 – 90% (no condensation)
Storage temperature range:	–20～60℃
Power:	AC 100～220V, 50/60 Hz
Power consumption:	40 VA
Outer dimensions (mm):	280 (W) x 130 (H) x 400 (D)
Weight:	approx. 5.5 kg
Output terminal:	RS232C
PC connection software:	※1Windows 2000/NT4.0(SP5)/XP/Vista ※2Mac OS 9.1/9.2/X10～

※The OralChroma connects via the serial port (RS-232C) on the PC. Although it can be connected to a USB port, it requires an additional conversion cable, which must be purchased separately. ※1The OralChroma requires a display resolution of 1024 × 768 pixels or higher. ※2 The OralChroma requires a display resolution of 800 × 600 pixels or higher.

http://www.abilit-international.com

Manufacturing / Sales:

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HALIMETER®

Interscan's Halimeter® is the internationally recognized standard instrument for measuring oral malodor. Utilized extensively in academic, research, and clinical settings, it belongs in your office, too.

As thousands of dentists have already discovered, managing your patients' complaints of chronic halitosis with only empirical treatment (dispensing rinse and tongue scrapers) is not sufficient — for them or for you! Chronic halitosis should be treated as any other dental problem, and a diagnostic work-up is essential. After all, fully 80 percent of patients who present with oral malodor will have a gum condition etiology.

- Within seconds, the Halimeter® can confirm the typical finding of volatile sulfur compounds (VSC — hydrogen sulfide, methyl mercaptan, dimethyl sulfide) in the breath, produced by anaerobic bacteria on the tongue.

- What about imaginary halitosis, an all too common finding, and one that is often disputed by the patient? A rigorous protocol of Halimeter® testing, organoleptic measurements, and tongue bacterial cultures will absolutely confirm or reject this possibility.

- What if there is apparent oral malodor, but it does not originate in the mouth? Halimeter® testing of nasal air and lung air samples will localize the source, giving you the information you need for further work-up and patient referral.

- And, for those patients whom you diagnose as having the most common condition —tongue dorsum VSC derived chronic halitosis — the Halimeter® is your tool for monitoring the progress of treatment.

You know from your own experience that patients are much more likely to elect a treatment protocol in the first place, if they get unbiased feedback from an electronic instrument.

So don't just dispense. Practice evidence-based dentistry.

Ethically diagnose and treat chronic halitosis — with the Halimeter.®

INSTRUMENT SPECIFICATIONS

Sensor Principle of Operation	Electrochemical voltammetric *(U.S. Patent No. 4,017,373)*
Accuracy	± 5 ppb
Lag Time	< 1 second
Pump	Vibrating armature diaphragm
Internal Tubing	1/4" OD x 1/8" ID (6.35 x 3.18 mm) Polyethylene/ethyl acetate co-polymer
Tube Fittings	Polybutylene and polyethylene
Rotameter	Body—Styrene-acrylonitrile Float—Type 304 stainless steel
Digital Display	4 digit, 0.375 in. (9.5 mm) liquid crystal. Readouts provided for instantaneous concentration of volatile sulfur compounds in parts per billion (ppb), peak value, and average of up to three VSC measurements. Countdown timers also provided, assuring optimum breath sampling technique.
Enclosure	Aluminum, EMC-shielded
Dimensions	4 1/2" H x 10" W x 10 1/2" D (114 x 254 x 267 mm)
Weight	8 lb (3.6 kg)
Analog Output	0 -400 mV = 0 -1999 ppb. Interface at 1/4" (6.35 mm) phone plug connection
Power	105-125 VAC, 50/60 Hz, 1.5 A or 205-240 VAC, 50/60 Hz, 0.75A (Switch provided inside unit, but specify when ordering.)
Calibration	Against standard gas mixture, or via Interscan's Electronic Calibration Service

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