The correlation between different techniques for the evaluation of oral malodour in children with and without orthodontic treatment

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Abstract

Aim The aim of this work was to evaluate the correlation between different methods (organoleptic evaluation, gas chromatography, salivary β-galactosidases activity) for the evaluation of halitosis in children. The secondary purpose was to investigate the influence of orthodontic treatment on halitosis.

Materials and methods Study Design: Oral malodour was detected with different methods in 50 children in the Paediatric Dentistry Unit, University of Rome Tor Vergata, Rome, Italy. During the dental visit, level of oral hygiene, tongue coating scores and presence of an orthodontic device, fixed or mobile, were recorded. Two trained and calibrated operators performed the organoleptic evaluation; the Oral Chroma™ device was used for the volatile sulfur compounds (VSCs) quantification and salivary β-galactosidases (Sβ-g) activity was evaluated through the spectrophotometric method. Statistics**:** The Cohen's Kappa score was used to evaluate the level of agreement between the operators. The Pearson's correlation coefficient was used to evaluate the linear relationship between continuous variables (e.g. Sβ-g vs. VSCs values) and the Spearman's correlation coefficient was calculated for ordinal variables (e.g. organoleptic scores) vs. other parameters. The LSD test was used to compare the parameters analysed in the study.

Results A positive and significant correlation between the organoleptic evaluation, the Sβ-g, the levels of hydrogen sulfide (H2S) and methyl mercaptan (CH3SH) was found. The Spearman's correlation has shown that organoleptic scores were significantly correlated with Sβ-g (0.664, $p<0.001$) and the Oral Chroma™ measurements of H2S (0.538, $p<0.001$) and Ch3SH (0.316, $p=0.026$). The Pearson's correlation showed that Sβ-g was statistically significantly correlated with the Oral Chroma™ measurements of H2S (0.379 $p=0.007$) and Ch3SH (0.299, $p=0.0035$). Stratifying results for orthodontic treatment, it was possible to show that children under orthodontic treatment, both fixed or removable, were characterised by higher level of Sβ-g. The organoleptic evaluation and Oral Chroma™ measurements showed that children wearing fixed orthodontic were characterised by higher scores.

Conclusions There was a significant correlation between the three different techniques for the evaluation of oral malodour in children. Like in the adult population, the increase of Sβ-g activity was associated with oral malodour. The presence of fixed orthodontic appliances was correlated to increased scores of all methods for the evaluation of halitosis.

KEYWORDS Halitosis; β-galactosidases; VSCs; hydrogen sulfide

Introduction

Halitosis is defined as an unpleasant odor emanating from the mouth, and in 80-90% of subjects the causes lies in the oral cavity [Bollen and Beikler, 2012; van den Broek et al., 2007]. Literature is very variable about the prevalence of halitosis, because of the great heterogeneity of the studies, however, it has been estimated that about the 30% of the world population suffers from this problem [Outhouse et al., 2016; Sanz et al., 2001]. Halitosis affects also the paediatric population with a prevalence comprised between the 23 and 38 % of children [Villa et al., 2014; Lin et al., 2003].

Several causes of oral malodour have been described [Scully and Greenman, 2012]: lingual and/or dental plaque accumulations, periodontal disease [Scorzetti et al., 2013], caries [Li et al., 2015], abscesses, oral ulcers, bone diseases and hyposalivation.

Currently, the gold standard technique for the evaluation of oral malodour is still the organoleptic measurement [Nalçacı and Sönmez, 2008]. However, this method is susceptible to such bias, because is subjective and there is the risk to have imprecise scores if operators are not properly trained and calibrated. Moreover, this method cannot detect patients with pseudohalitosis and halitophobia that they are not really affected by oral malodour [Scully and Greenman, 2012]. For this reason, electronic devices and other methodologies have been developed in order to obtain an easy and objective evaluation of oral malodour [Rosenberg et al., 1991].

Oral Chroma™ is a portable gas chromatography device with semiconductor gas sensor that is able to quantify the three main volatile sulfur compounds (VSCs) that more frequently characterise the breath of subjects with oral malodour: hydrogen sulfide (H_2S), methyl mercaptan (Ch_3SH) and dimethyl sulfide (CH_3S_2) [Krespi et al., 2006; Szabó et al., 2015]. They are the final product of bacterial amino acids decomposition, however, their quantification in breath alone, give us no other information about the real origin of malodour, intra- or extra-oral [Scully and Greenman, 2012].

Currently, the quantification of salivary β-galactosidases is considered only an additional method for the assessment of halitosis, because it does not permit alone to obtain a direct evaluation of halitosis. However, the positivity of this test confirms us that the cause of oral malodour relies on, partially or totally, in the mouth [Scully and Greenman, 2012;

Petrini et al., 2012; Masuo et al., 2012; Sterer and Rosenberg, 2009]. In literature the correlation between β-galactosidases activity and VSCs level has been investigated only in the adult population [Masuo et al., 2012; Yoneda et al., 2010].

The aim of this work is to quantify oral malodour and to compare different techniques for evaluation of halitosis in a group of children wearing or not an orthodontic device.

Materials and methods

All subjects (50 children) were recruited in Paediatric Dentistry Unit, University of Rome Tor Vergata, Rome, Italy. The inclusion criteria were: children systemically healthy who did not assume any drugs in the 30 days before the consultation.

The study protocol was explained and written informed consent was received from the parent/guardian of each child before enrollment in the study. Medical and dental history were obtained for each child and then a trained paedodontist performed a standardised oral visit to all patient, as previously described [Petrini et al., 2014]. The level of oral hygiene, the tongue coating scores [Oho et al., 2001] and the presence of an orthodontic device, fixed or mobile, were recorded [Petrini et al., 2014]. We considered as fixed, each orthodontic device cemented in the oral cavity (e.g. multibracket or palatal expander), that could not be removed by the patients.

Halitosis evaluation

Two different trained operators evaluated and classified the quality of the breath of the patients using the organoleptic intensity scale, based on Rosenberg [Rosenberg et al., 1991], as follows: 0 = absence of odour; 1 = questionable malodour; 2 = slight; 3 = moderate; 4 = strong; and 5 = severe. In order to avoid the risk of bias, all measurements were carried out between 9 and 11 o'clock in the morning.

Organoleptic scores were assigned evaluating the intensity of oral malodour at a distance of 10 cm from the mouth of the patient that was invited to count until 10. Each operator was blind about the score expressed by the other. At the end of the organoleptic evaluation, the Cohen's kappa coefficient was calculated in order to evaluate the level of agreement between the two operators, and then, in cases of different scores, the higher value was reported.

Measurements of Volatile Sulphur Compounds (VSC)

The measurement of VSC was carried out through the use of portable gas chromatography (Oral Chroma™, Abilit, Osaka, Japan) that permitted to quantify the three sulfides: hydrogen sulfide, methyl mercaptan and dimethyl sulfide [Tangerman and Winkel, 2008].

Salivary β-galactosidase activity assay (Sβ-g)

Saliva was collected using the spitting method. Samples were obtained from the participants between 09:00 a.m. and 11:00 a.m. to minimise the effects of diurnal variability in the salivary composition. Sβ-g activity was evaluated spectrophotometrically, as previously described. [Petrini et al., 2012; Petrini et al., 2014]. Each sample was analysed three times, and the average value was considered.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 21 (IBM SPSS Inc., Chicago, IL, USA). The Cohen's Kappa Value was used to evaluate the level of agreement among the operators. The Pearson's correlation coefficient was used to evaluate the linear relationship between continuous variables (e.g. Sβ-g vs. VSCs values) and the Spearman's correlation coefficient was calculated for ordinal variables (e.g. organoleptic scores) vs. other parameters. The LSD test was used to compare the parameters analyzed in the study. Data were analyzed using linear regression and descriptive statistics. The significance threshold was set at 0.05.

		Sβ-g	H_2S	CH_3SH	CH_3S_2
Organoleptic s.	Spearman's Correlation	0.664*	0.538*	0.316*	0.147
	Sig. (2-code)	<0.001	<0.001	0.026	0.309
Sβ-g	Pearson's correlation	/	0.379*	0.299*	0.083
	Sig. (2-code)		0.007	0.035	0.568
H_2S	Pearson's correlation	/	/	0.268	0.239
	Sig. (2-code)	/	/	0.60	0.095

TABLE 1 The Spearman's and Pearson's correlations among the parameters analysed in the study. * The correlation is significant at 0,05 (2-code).

Results

The correlation between different techniques for the evaluation of oral malodour was calculated in a group of 50 children, 25 males and 25 females (mean age 8.7 ±2.1 years). The level of agreement between the two operators, calculated through the Cohen's kappa coefficient was 0.851.

The 54% of the patients were considered as affected of halitosis, because they received an organoleptic score > 2. The Spearman's correlation (Table 1) has shown that organoleptic scores were significantly correlated with Sβ-g (0.664, p<0.001) and the Oral Chroma™ measurements of H_2S (0.538, p<0.001) and Ch_3SH (0.316, p=0.026). The Pearson's correlation (Table 1) has shown that Sβ-g was statistically significantly correlated with the Oral Chroma™ measurements of H_2S (0.379 p=0.007) and Ch_3SH (0.299, p=0.0035). On the contrary, no association was found with the levels of CH_3S_2 (0.083, p=0.568). Also for operator scores, there was not a positive correlation with the level of CH_3S_2 (0.147, p=0.309).

Ten out of 50 participants were in treatment with orthodontic therapy: 6 with fixed and 4 with removable devices. The oral hygiene level and the tongue coating scores, were quite similar in all groups, independently from the presence of orthodontic devices (Fig. 1). However, stratifying results for the presence of an orthodontic treatment, it was possible to observe that the level of Sβ-g (Fig. 2) were significantly correlated with orthodontic appliances, independently if fixed or removable, Spearman's Correlation= 0.347. However, Fisher LSD found no statistically significant differences between the three groups on the level of this enzyme. On the contrary, higher operators' scores (Fig. 3) characterised children with fixed orthodontic appliances: results were statistically significantly higher than scores that characterised children with orthodontic removable devices (p=0.024 S).

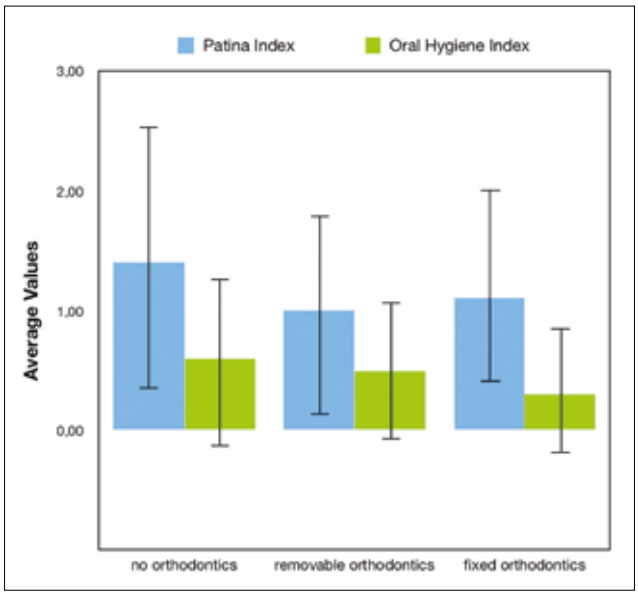

FIG. 1 The Oral Hygiene Level (0= insufficient, 1= sufficient, 2= good) and tongue coating scores (0 = no tongue coating to 3 = thin with more than two-thirds covered or thick with more than one-third covered) of the patients. Mean values (bars= standard deviation).

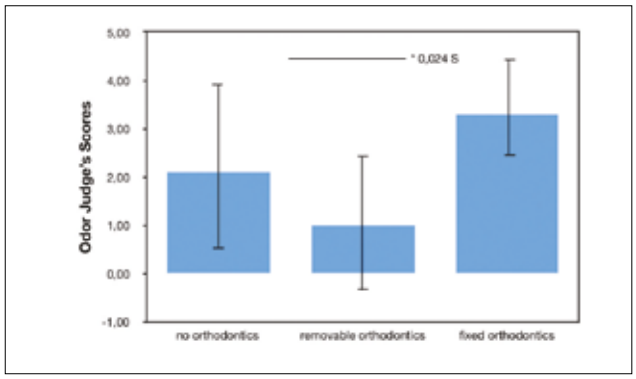

FIG. 3 Organoleptic scores stratified for orthodontic treatment, fixed or removable. Mean values (bars= standard deviation), * the correlation is significant at 0,05 (2-code).

The Oral Chroma™ measurements (Fig. 4) have confirmed the organoleptic scores: children with fixed orthodonticappliancess had higher levels of VSCs; in particular, they had a significantly higher level of H_2S than children with removable devices (p=0.045) and without orthodontic treatment (p=0.031).

Discussion

Different techniques were used to detect halitosis in a group of 50 children; a remarkable correlation between the quantification of β-galactosidases activity (Sβ-g), organoleptic scores and VSC concentration in the expired air was found (Table 1). These results are in accordance with Masuo et al. [2012] and Yoneda et al. [2010] who performed a similar research on an adult population without periodontitis. In our previous studies we found a similar correlation between Sβ-g and organoleptic scores in a group of children, however, we did not perform the Oral Chroma™ measurements [Petrini

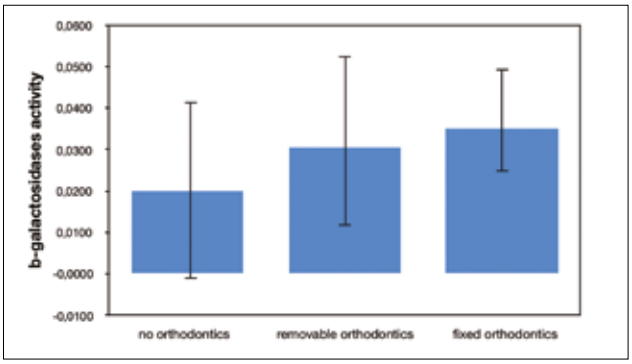

FIG. 2 The level of salivary β-galactosidases stratified for orthodontic treatment, fixed or removable. Mean values (bars= standard deviation).

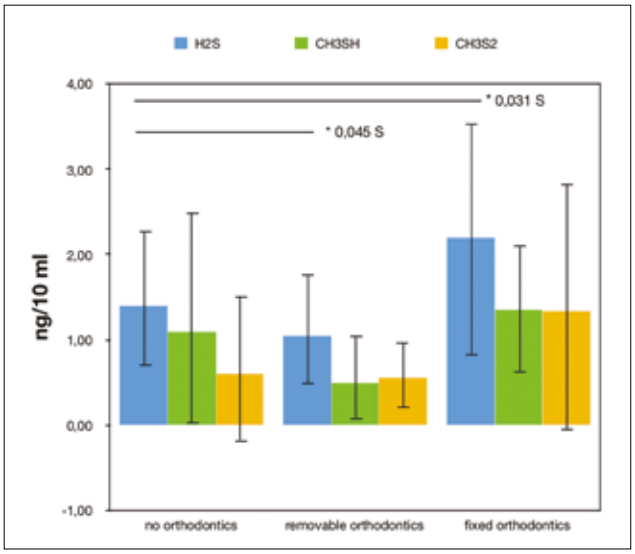

FIG. 4 The level of VSCc stratified for orthodontic treatment, fixed or removable. Mean values (bars= standard deviation), * the correlation is significant at 0,05 (2-code).

et al., 2014; Petrini et al., 2018]. We did not find any study that correlated VSC measurements with β-galactosidases activity in children. β-galactosidases are essential for the sulfur compounds increment because they deglycosylate salivary glycoproteins, facilitating their further degradation in VSCs by bacterial enzymes like cysteine proteases and methioninase [Chu et al., 1999; Yoshimura et al., 2000; Tanabe and Grenier, 2012; Sterer and Rosenberg, 2006]. It is very interesting to highlight that Sβ-g were statistically correlated with H_2S (P=0.379, p =0.007) and CH_3SH (R=0.299, p=0.035), that represent the 90% of VSCs in mouth air [Tonzetich, 1971].

Sterer et al. [2009] have shown that β-galactosidases activity and H_2S production occur simultaneously in an experimental biofilm but in different bacterial populations: β-galactosidases in the outer layers by gram-positive facultative bacteria and VSCs in the deeper ones by gram-negative anaerobic. It was shown that 0.1–0.2 mm of tongue coating are enough to provide an environment depleted of oxygen, allowing the onset of VSCs production [Krespi et al., 2006]. For this reason, β-galactosidases are considered the first step in VSCs production and the inhibition of their activity, for example as suggested by Morin et al. [2015] with natural compounds like green tea extract, could contribute to reducing the halitosis

occurrence.

We have also stratified results based on the presence of orthodontic treatment. Considering the very small number of children with orthodontic treatment in our population, our results could only be considered as preliminary. However, the Sβ-g were significantly correlated with orthodontics, independently if fixed or removable. On the contrary, the organoleptic evaluation found higher scores in children with fixed orthodontic device, and results were statistically significant with respect to children wearing removable devices. Similar results were found with Oral Chroma™ measurements that found a statistically significant higher level of H_2S in children wearing fixed appliances, with respect to those with removable devices and those without orthodontic treatment. The increment of H_2S is very important because many studies have suggested its contribution in the pathogenesis of connective breakdown and periodontitis, due to its cytotoxicity and ability to stimulate oxidative stress [Rizzo, 1967; Tonzetich, 1978; Greabu et al., 2016]. The increment of all halitosis indexes are in accordance with literature, which supports the role of fixed orthodontics in the increment of oral malodour. However Babacan et al. have suggested that the direct cause was the increment of plaque accumulation because of the increased difficulties in oral hygiene maneuvers in subjects with not aligned teeth and with fixed orthodontics, that contrast also the natural cleaning of the tongue [Sökücü et al., 2016; Babacan et al., 2011; Delli Mauri et al., 2015]. However, Figure 2 shows that all children included in this study were characterised by a similar level of tongue coating scores and oral hygiene level. The increment of VSCs in children with fixed orthodontics, in our population, could not be a consequence of an increased quantity of plaque accumulation, but of the different quality of bacteria; our theory is confirmed by the increased Sβ-g activity (Fig. 3). Also with an accurate oral hygiene programme, the presence of protected interdental sites difficult to clean in case of malocclusion and the retentive action of bracket and archwire could create a variation of dental plaque quality [Perinetti et al., 2004]. Indeed 8–14 hours of plaque accumulation are sufficient for plaque deposits to produce VSCs [Babacan et al., 2011].

Conclusions

The quantification of Sβ-g activity was significantly correlated with organoleptic scores and Oral Chroma™ measurements, especially H_2S and CH_3SH concentrations. For this reason, this analysis could permit to understand not only the aetiology of bad breath but also to provide the most effective therapy. The presence of fixed orthodontic appliances was correlated to increased VSCs, organoleptic scores and Sβ-g activity, with respect to patients with removable devices or without orthodontic treatment.

References

» Babacan H, Sokucu O, Marakoglu I, Ozdemir H, Nalcaci R. Effect of fixed appliances on oral malodor. Am J Orthod Dentofacial Orthop 2011;139(3):351-5.
» Bollen CM, Beikler T. Halitosis: the multidisciplinary approach. Int J Oral Sci 2012;4(2):55–63.
» Chu L, Ebersole JL, Kurzban GP, Holt SC. Cystalysin, a 46-kDa L-cysteine desulfhydrase from Treponema denticola: biochemical and biophysical characterization. Clin Infect Dis 1999;28(3):442–50.
» Delli Mauri A, Petrini M, Vitale D, Tecco S, Festa F, Barbato E, Spoto G. Alkaline phosphatase level in gingival crevicular fluid during treatment with Quad-Helix. J Biol Regul Homeost Agents 2015;29(4):1017-23.
» Greabu M, Totan A, Miricescu D, Radulescu R, Virlan J, Calenic B. Hydrogen Sulfide, Oxidative Stress and Periodontal Diseases: A Concise Review. Antioxidants (Basel, Switzerland) 2016 Jan 14;5(1).
» Krespi YP, Shrime MG, Kacker A. The relationship between oral malodor and volatile sulfur compound-producing bacteria. Otolaryngol Head Neck Surg 2006;135(5):671–6.
» Li MY, Zhi QH, Zhou Y, Qiu RM, Lin HC. Impact of early childhood caries on oral health-related quality of life of preschool children. Eur J Paediatr Dent. 2015 Mar;16(1):65-72.
» Lin MI, Flaitz CM, Moretti AJ, Seybold SV, Chen JW. Evaluation of halitosis in children and mothers. Pediatr Dent 2003;25(6):553–8.
» Masuo Y, Suzuki N, Yoneda M, Naito T, Hirofuji T. Salivary β-galactosidase activity affects physiological oral malodour. Arch Oral Biol 2012;57(1):87–93.
» Morin MP, Bedran TB, Fournier-Larente J, Haas B, Azelmat J, Grenier D. Green tea extract and its major constituent epigallocatechin-3-gallate inhibit growth and halitosis-related properties of Solobacterium moorei. BMC Complement Altern Med 2015;15:48.
» Nalçacı R, Sönmez IS . Evaluation of oral malodor in children. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106(3):384–8.
» Oho T, Yoshida Y, Shimazaki Y, Yamashita Y, Koga T. Characteristics of patients complaining of halitosis and the usefulness of gas chromatography for diagnosing halitosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91(5):531–4.
» Outhouse TL, Al-Alawi R, Fedorowicz Z, Keenan JV. Tongue scraping for treating halitosis. Cochrane Database Syst Rev 2016 26;(5):CD005519.
» Perinetti G, Paolantonio M, Serra E, D'Archivio D, D'Ercole S, Festa F, Spoto G. Longitudinal monitoring of subgingival colonization by Actinobacillus actinomycetemcomitans, and crevicular alkaline phosphatase and aspartate aminotransferase activities around orthodontically treated teeth. J Clin Periodontol 2004; 31(1):60–7.
» Petrini M, Costacurta M, Ferrante M, Trentini P, Docimo R, Spoto G. Association between the organoleptic scores, oral condition and salivary β-galactosidases in children affected by halitosis. Int J Dent Hyg 2014;12(3):213-8.
» Petrini M, Costacurta M, Biferi V, Benavoli D, Docimo R, Spoto G. Correlation between halitosis, oral health status and salivary β-galactosidases and time spent in physical activities in children. Eur J Paediatr Dent 2018; 19(4):260-264.
» Petrini M, Trentini P, Ferrante M, D'Alessandro L, Spoto G. Spectrophotometric assessment of salivary β-galactosidases in halitosis. J Breath Res 2012;6(2):021001.
» Rizzo AA. The possible role of hydrogen sulfide in human periodontal disease. I. Hydrogen sulfide production in periodontal pockets. Periodontics 1967;5(5):233–6.
» Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. J Dent Res 1991;70(11):1436–40.
» Rosenberg M, Septon I, Eli I, Bar-Ness R, Gelernter I, Brenner S, Gabbay J. Halitosis measurement by an industrial sulphide monitor. J Periodontol 1991; 62(8):487–9.
» Sanz M, Roldán S, Herrera D. Fundamentals of breath malodour. J Contemp Dent Pract 2001 Nov 15;2(4):1–17.
» Scorzetti L, Marcattili D, Pasini M, Mattei A, Marchetti E, Marzo G. Association between obesity and periodontal disease in children. Eur J Paediatr Dent 2013 Sep;14(3):181-4.
» Scully C, Greenman J. Halitology (breath odour: aetiopathogenesis and management). Oral Dis 2012;18(4):333–45.
» Sökücü O, Akpınar A, Özdemir H, Birlik M, Çalışır M. The effect of fixed appliances on oral malodor from beginning of treatment till 1 year. BMC Oral Health 2016;16:14.
» Sterer N, Rosenberg M. Streptococcus salivarius promotes mucin putrefaction and malodor production by Porphyromonas gingivalis. J Dent Res 2006;85(10):910–4.
» Sterer N, Shaharabany M, Rosenberg M. β-Galactosidase activity and H2S production in an experimental oral biofilm. J Breath Res 2009;3(1):016006.
» Szabó A, Tarnai Z, Berkovits C, Novák P, Mohácsi Á, Braunitzer G, et al. Volatile sulphur compound measurement with OralChroma(TM): a methodological improvement. J Breath Res 2015;9(1):016001.
» Tanabe SI, Grenier D. Characterization of volatile sulfur compound production by Solobacterium moorei. Arch Oral Biol 2012;57(12):1639–43.
» Tangerman A, Winkel EG. The portable gas chromatograph OralChromaTM: a method of choice to detect oral and extra-oral halitosis. J Breath Res 2008;2(1):017010.
» Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. Arch Oral Biol 1971;16(6):587–97.
» Tonzetich J. Oral malodour: an indicator of health status and oral cleanliness. Int Dent J 1978;28(3):309–19.
» van den Broek AM, Feenstra L, de Baat C. A review of the current literature on aetiology and measurement methods of halitosis. J Dent 2007;35(8):627–35.
» Villa A, Zollanvari A, Alterovitz G, Cagetti M, Strohmenger L, Abati S. Prevalence of halitosis in children considering oral hygiene, gender and age. Int J Dent Hyg 2014;12(3):208–12.
» Yoneda M, Masuo Y, Suzuki N, Iwamoto T, Hirofuji T. Relationship between the β-galactosidase activity in saliva and parameters associated with oral malodor. J Breath Res 2010;4(1):017108.
» Yoshimura M, Nakano Y, Yamashita Y, Oho T, Saito T, Koga T. Formation of methyl mercaptan from L-methionine by Porphyromonas gingivalis. Infect Immun 2000;68(12):6912–16.