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# Abstracts

## Oral Papers

### O1
### The ADA guidelines on oral malodor products

W Wozniak
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This presentation will concern the following: The ADA Acceptance Program, its history and value to ADA members and consumers, procedures for submission of products and role of ADA Council on Scientific Affairs, development of ADA Guidelines for Acceptance of Products, and finally the development of the Guidelines for Products for the Management of Oral Malodor.

### O2
### Oral malodor research sponsored by industry

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Hill Top Research has been performing oral malodor research for over 30 years, for commercial companies who use these independent clinical studies to support advertising messages. Much of the clinical data generated by Hill Top Research, Inc. does not reach the public domain of peer reviewed scientific journals because client sponsors often consider this research to contain proprietary information. Alternatively academic journals do not consider this type of clinical research to meet sufficiently high basic research standards for publication. This dichotomy results in a knowledge gap between industry and academia as to how malodor research is carried out by industry. The purpose of this presentation is to review the regulatory environments which govern Oral Malodor marketing claims in Europe and North America and thus the clinical research standards which are desirable to substantiate the data to a standard which will withstand competitive legal challenges and regulatory review. The impact of subject data protection laws, good clinical practice guidelines, IRB/IEC review, statistical sample sizes, dental association requirements, and the principles behind sensory research will be discussed in relation to Oral Malodor clinical research. Explanations will be given as to how some requirements may be relaxed depending up whether the purpose of the investigation is 'early stage proof of concept' research evaluating the feasibility of introducing new formulations or ingredients as compared to 'Safety and Efficacy' clinical research carried out on final formulations necessary to meet the requirements of a regulatory submission or challenge in the market place.

### O3
### Effects of training on experience and non-experience sensory odor judges

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In oral malodor research, sensory odor judging is considered the gold standard to evaluate and rate breath odor quality and intensity. Although training and calibration of sensory odor judges has been well documented in other scientific disciplines (foods/beverages, air/water quality, personal care products), little data have been published on training judges for bad breath assessment.

**Objective** The purpose of this pilot study was to evaluate the training process, with experienced and novice odor judges, for rating both pleasant and unpleasant odorants.

**Methods** Six oral malodor sensory judges four with prior experience and two novices were given verbal information on the oral malodor intensity scale (0-5) and were given the opportunity to sniff a reference sample of n-butanol, that had been declared to have a level 3 intensity. Subjects then independently assessed 20 odorant samples for odor intensity (pretest). Four unpleasant and two pleasant odorants, in a variety of intensity levels, and a water sample were included. Subjects then participated in a training program based on the American Society for Testing and Materials (ASTM) standards. Investigators assigned each odorant sample a 'true' intensity score. Subjects' intensity scores were then analyzed as the absolute difference from the 'true' intensity. This dependant variable was analyzed using repeated measures ANOVA.

**Results** Training significantly ($P = 0.02$) reduced (improved) odor judge error levels, irrespective of previous experience. When comparing improvement between experienced and novice odor judges, there was no significant ($P = 0.99$) difference in the size of deviations from the 'true' scores (errors) between experienced (0.948) and novice (0.950) judges. There was also no difference ($P = 0.94$) in improvement in error levels from pre- to post-test for experienced (pre = 1.13 and post = 0.76) or novice (pre = 1.13 and post = 0.78) judges. There was consistent improvement from pre- to post-test for all odorants ($P = 0.02$; pre = 1.10; post = 0.75) except for dimethylsulfide, which became worse ($P = 0.01$; pre = 0.97; post = 1.58). When comparing the unpleasant *vs* pleasant odorants, there was a significant ($P = 0.006$) difference in error levels (unpleasant 1.09; pleasant 0.70). After removing the water blank scores from the pleasant odorant category the difference was no longer significant ($P = 0.26$).

**Conclusions** A specific training program for odor judges improved subjects' ability to assess odor intensity irrespective of previous experience. This type of training is recommended prior to conducting oral malodor-related clinical research trials.

### O4
### An inventory study on a randomized group of 1000 patients visiting a multidisciplinary breath odor clinic at a university hospital

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In 1994 the first European multidisciplinary (periodontology, ENT, psychiatry and occasionally gastro-enterology) outpatient clinic was initiated at the University Hospital in Leuven, Belgium. The first 1000 patient files on the alphabetical list were analysed. It surprisingly consisted of 499 men and 501 women (2–90 years old, mean 38). The majority consulted

spontaneously (69%), others were referred by their house doctor (14%), general dentist (6%) or medical/oral specialists (11%). One-third had complaints since more than 5 years. Two-thirds were informed by confidents or colleagues about their breath odor while they were unaware of it, 15% of the patients noticed it themselves and 18% were informed by others besides noticing it themselves. Not a single patient was alcoholic but 14% were tobacco addicts. In 83.4% of the patients there was an oral problem. Mostly tongue coating or/and periodontitis, was evident. In 3.6% it was a combination of ENT/intra-oral causes. An ENT or gastro-enterologic cause was detected in 3.1 and 1.3% respectively. ENT causes involved postnasal drip, tonsillitis, sinusitis, rhinitis. Only 1% had a metabolic or hormonal problem. The latter regularly also had tongue coating or periodontitis, but the elimination of these causes did not solve the entire problem. On the other hand in 7.6% of the patients no physical cause could be detected, while psychological/psychiatric problems were clearly identified. After the first multidisciplinary examination and treatment initiation/instructions, a control visit was proposed 2–6 months later. All patients were meanwhile referred for follow-up to their general dentist or periodontologist or house doctor/medical specialist. Nearly 60% of the patients did not show up at the second appointment. When a sample was contacted by telephone ($n = 40$) all declared they were cured. 10% of the patients needed 3 appointments or more. From the 31% of the patients who came back for their control visit, 68.5% had experienced a (clear-cut) improvement, and 22.7% noticed a slight to strong improvement. The patients who did not report any improvement (8.8%) were nearly all identified as having psychological or psychiatric traits.

## O5

## Clinical association of volatile sulfur compounds, Halimeter values, organoleptic score and tongue coating in oral malodor

EG Winkel[1], A Tangerman[2]

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**Objective** Oral malodor is now generally accepted to be the main cause (about 90%) of bad breath. The objective of this study is to evaluate the potential power of volatile sulfur compounds (VSC), Halimeter values, organoleptic score (OLS) and tongue coating in assessing oral malodor.

**Methods** We investigated the mouth- and nose-breath of 42 subjects with oral malodor, visiting our bad breath clinic. The methods used were gas chromatography, Halimeter measurements, organoleptic scoring (OLS) and tongue coating measurements.

**Results** The concentrations of hydrogen sulfide ($H_2S$), methylmercaptan (MM, $CH_3SH$) and dimethylsulfide (DMS, $CH_3SCH_3$) in the mouth-breath of the 42 patients with oral malodor amounted to $0.74 \pm 0.95$ (s.d.), $0.42 \pm 0.30$, and $0.29 \pm 0.19$ nmol $l^{-1}$, respectively. $H_2S$ and MM were absent in nose-breath of all patients, indicating that the origin of these VSC is the mouth. DMS in nose-breath ($0.21 \pm 0.12$ nmol $l^{-1}$) was similar to that in mouth breath, indicating that the origin of DMS lies outside the mouth. The recognition threshold value of MM is four to 30 times lower than that of $H_2S$ and about three times lower than that of DMS, indicating that MM is the major source of oral malodor. A significant correlation ($r = 0.726$, $P < 0.001$) was found between mouth-$H_2S$ and mouth-MM. No significant correlations were found between these two VSC and DMS. The correlations of $H_2S$ and MM with the Halimeter measurements, with OLS and with tongue coating are all highly significant.

**Conclusions** Oral malodor is mainly caused by MM. The Halimeter is most sensitive for $H_2S$. Nevertheless, thanks to the good correlation between $H_2S$ and MM, the Halimeter remains a useful apparatus in predicting oral malodor. OLS and tongue-coating assessments are also useful methods in detecting oral malodor.

## O6

## The occurrence and cause of extra-oral halitosis

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**Objective** Oral malodor is now generally accepted to be the main cause (about 90%) of bad breath. Bacterial formation of the volatile sulfur compounds hydrogen sulfide ($H_2S$) and methylmercaptan (MM, $CH_3SH$) within the oral cavity is the main cause of oral malodor. The objective was to study the occurrence and cause of the less well-known extra-oral halitosis.

**Methods** We investigated the mouth- and nose-breath of 59 subjects complaining of bad breath and visiting our bad breath clinic. The methods used were gas chromatography and organoleptic scoring (OLS).

**Results** Six of 59 patients (10 %) were found to have extra-oral halitosis. The concentrations of $H_2S$ and MM in mouth-breath of patients with extra-oral halitosis were very low and below critical odorous levels. $H_2S$ and MM were absent in nose-breath of all patients. The concentration of dimethylsulfide (DMS, $CH_3SCH_3$) in mouth- and nose-breath in the six patients with extra-oral halitosis amounted to $1.57 \pm 1.31$ (s.d.) and $1.64 \pm 1.29$ nmol $l^{-1}$ (range: 0.50–4.50), respectively, compared to $0.29 \pm 0.19$ and $0.21 \pm 0.12$ nmol $l^{-1}$, respectively, in patients with oral malodor. The critical odorous concentration of DMS is about 0.5 nmol $l^{-1}$. Simulated DMS gas-mixtures had the same smell as observed in the extra-oral halitosis patients. In these six patients good to perfect correlations were found between the OLS of mouth- and nose-breath (simple correlation coefficient $r = 0.840$, $P < 0.05$), between mouth-OLS and the concentration of DMS in mouth-breath ($r = 0.794$, $P < 0.05$) and in nose-breath ($r = 0.824$, $P < 0.05$) and between nose-OLS and the concentration of DMS in mouth-breath ($r = 0.963$, $P < 0.001$) and in nose-breath ($r = 0.955$, $P < 0.001$). A perfect correlation was obtained between mouth- and nose-DMS ($r = 0.994$, $P < 0.001$), indicating that DMS does not

originate in the mouth . The origin of DMS must be found elsewhere, most probably in the blood.

**Conclusions** About 5–10% of patients with chronic halitosis has extra-oral halitosis. In all patients with extra-oral halitosis seen thus far, DMS was always the cause of bad breath. Other causes known, such as trimethylamine in the fish-odor syndrome, are very rare.

## O7
## The treatment of oral malodor

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**Objective** Because the increase in the bacterial load on the tongue is paramount in the etiology of malodor, we sought to reduce this load by debriding the tongue in combination with short-term usage of antimicrobial mouthrinses.

**Methods** Subjects with oral malodor had an OR score $>2$, had whole mouth volatile sulfur (VSCs) levels $\geq$200 ppb (Halimeter®), and had a positive tongue BANA test. The subjects were given a tongue scraper and a 16 oz bottle of mouthrinse (Listerine® or Ther-a-sol®, single blind design). They were instructed to debride their dorsal tongue surface, to brush and floss the teeth, to rinse with 1 oz of the mouthrinse for 1 min, instructed not to eat or drink for 2–3 h thereafter, and to repeat the protocol in the morning and evening for 7 days. After 1 week all malodor measurements were repeated. The patients were then given a zinc containing tongue gel and instructed to debride the tongue surface, repeating this procedure twice a day.

**Results and discussion** Thirty-two subjects entered this program, 31 returned 1 week later, and 20 returned for a follow-up examination (average of 37 weeks). The OR score decreased from 2.8 to 0.9 after 1 week of treatment, and to 0.6 for those who returned for the follow-up. The VSCs decreased from 339 ppb prior to treatment to 120 ppb after 1 week and to 53 ppb 37 weeks later. Eighty-one percent of the tongues were BANA positive prior to treatment, 16% after 1 week and 0% after 37 weeks. The microbial flora changed from one dominated by asaccharolytic species to one dominated by saccharolytic species such as *S. salivarius*.

**Conclusions** This report describes a long-term beneficial effect of treatment on oral malodor.

## O8
## Impact of periodontal therapy (including antiseptics) on tongue coating and malodour

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**Background** Bad breath is often caused by periodontitis and/or tongue coating. This study aimed to follow, over a 6 months period, the impact of periodontal therapy on several parameters linked with oral malodour.

**Methods** In this double-blind, randomized, long-term, parallel study 45 moderate periodontitis patients without obvious tongue coating were enrolled. Besides a 'one-stage, full-mouth' disinfection and oral hygiene improvement (including tongue scraping), patients were instructed to rinse with one of the following products (randomly allocated): CHX 0.2% + alcohol (Corsodyl®), CHX 0.05% + CPC 0.05% and no alcohol (Perio.Aid® Maintenance, a new formulation), or the placebo of the latter for 6 months. At baseline and after 3 and 6 months, a serious of parameters was recorded including VSC rating (Halimeter), tongue coating and an estimation of the microbial load (anterior & posterior part tongue, saliva, supragingival plaque).

**Results** Even though the initial VSC values were not high ($\pm$90, only 18 patients $>$100), significant ($P < 0.05$) reductions could be recorded in the CHX and CHX + CPC group, but only to a minor extent in the placebo group. Tongue scraping resulted in a significant reduction ($P \leq 0.05$) of the coating up to month 6 in the placebo and CHX + CPC group, but not in the CHX group. The microbial changes in the placebo group were never significant ($\leq$0.3 log values), even though the tongue had been scraped daily. The CHX and CHX-CPC group showed, in comparison to baseline, significant ($P < 0.001$) reductions in the number of anaerobic species in the supra gingival plaque, in the saliva and on the anterior part of the tongue. For the posterior part of the tongue the microbial changes remained $\leq$0.3 log values.

**Conclusions** The results of this study indicate that, in patients with moderate periodontitis, periodontal therapy including tongue scraping did not have a significant effect on the microbial load of the tongue or the VSC level, except when combined with a mouthrinse.

## O9
## Creation of oral care flavours to deliver breath freshening benefits – an *in vitro* method

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Flavours conventionally help to deliver breath freshening in oral care products via sensory masking. The aim of this study was to determine whether flavours could additionally inhibit formation of malodorous compounds by bacteria. Oral malodour is in large part related to the production of volatile sulphur compounds (VSCs) by Gram-negative organisms. We therefore developed a quantitative *in vitro* assay for hydrogen sulphide production by *Klebsiella pneumoniae*. The entire Quest dental flavour palette was screened for inhibition of $H_2S$ production. A database of flavour ingredient effects was created, and used to create VSC-inhibitory flavours. These flavours were assessed in the *in vitro* assay to confirm that the compounded flavour performed as expected. The best performing flavours developed in this way were selected for testing in the Quest Breath Freshness Panel (see poster, P21). Rules based on the performance of dental flavour ingredients, singly or in combination, were also used to develop a patent position. Flavours formulated using these rules (termed Q-fresh flavours) have been incorporated into a range of oral care products including toothpastes, mouthwashes, chewing gums and breath films. These products have been tested in the

Quest Breath freshness panel, and have been shown to deliver significant breath freshening benefits. An *in vitro* VSC-inhibition method has allowed reliable development of flavours with breath-freshening benefits.

## O10
## The technology behind Colgate® Total™ toothpaste

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In the early 1990s, a break-through toothpaste, known as Colgate® Total™, was launched with documented long-lasting activity against plaque, gingivitis, calculus, tooth decay and bad breath. The technology behind this toothpaste is the combination of triclosan, a polyvinyl methyl ether maleic acid (PVM/MA) copolymer and sodium fluoride (TCF). The function of the copolymer is to ensure optimal oral retention and prolonged release of the antibacterial triclosan. Effective levels of triclosan have been measured in the oral cavity 12 h after brushing the teeth. This allows prolonged control of oral bacteria that can lead to the formation of dental plaque and gingivitis and bad breath. Similarly, the retention of triclosan to oral surfaces during 2 years regular use of the product has led to a significant reduction in incremental coronal caries compared to an ADA-approved anti-cavity fluoride toothpaste. Furthermore, significantly less calcium phosphate has been shown to be present in dental plaque after brushing the teeth with the triclosan/copolymer toothpaste, and this has resulted in the reduced formation of tartar. A new variant of the triclosan/copolymer/fluoride toothpaste, having the numerous therapeutic and aesthetic benefits of the original formula, has been made available to consumers. The new dentifrice, which contains an impactful breath freshening flavor, has been documented to be significantly better ($P < 0.05$) than a control toothpaste in providing sustained control of bad breath over 12 h. After 12 h, breath odor was reduced from 51% compared to the control. The long term retention and subsequent release of triclosan by the copolymer in the TCF formula provide consumers protection against plaque, gingivitis, tartar, caries and bad breath.

## O11
## Effect of oral health care on the recovery from surgery in elderly patients by measuring volatile sulphur compounds

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Oral health care is suggested to be an effective method to decrease pathogenic microorganisms. The purpose of this study was to investigate the effect of oral health care on the recovery from gastrointestinal operations in elderly patients. For this objective, randomized control trials were used for 30 patients (70–80 years old) with gastrointestinal diseases. Infection control was done by antibiotics instillation before and after operations in general and by povidone–iodine solution in the oral cavity both in the intervention (test) group and the control group. For the intervention group, in addition, manual tooth brushing and tongue cleaning were advocated to reduce bacterial putrefaction. A simple gas chromatograph (Oral Chroma®, Abilit Co., Japan) was used to measure hydrogen sulphide, methyl mercaptan and dimethyl sulphide concentrations in the mouth air. Furthermore, oral microorganisms were isolated in tonsils. For 5 days after surgery, the increments of mean values of hydrogen sulphide, methyl mercaptan and dimethyl sulphide in the test group were $0.81 \pm 4.86$ ng/10 ml, $0.40 \pm 1.72$ ng/10 ml and $0.16 \pm 1.60$ ng/10 ml, respectively. On the other hand, those in control group were $2.93 \pm 8.09$ ng/10 ml, $1.10 \pm 2.30$ ng/10 ml and $0.36 \pm 1.13$ ng/10 ml, respectively. The differences in methyl mercaptan concentration between the test and the control groups were statistically significant ($P < 0.001$). In addition, the mean number of kinds of microorganisms in each patient's tonsils in the test and the control groups were $3.08 \pm 0.95$ and $3.64 \pm 1.34$ before surgery and were $2.62 \pm 0.65$ and $3.50 \pm 1.74$ 5 days after, respectively ($P < 0.05$). The oral cavity is a potential reservoir of pathogenic microorganisms. These results demonstrated that oral health care to remove pathogens from dental biofilm or tongue coating can decrease levels of oral malodor and may also reduce the risk of systemic diseases.

## O12
## Volatile markers of oral malodor in the breath

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**Objective** To identify the chemical composition of volatile organic compounds (VOCs) in the breath of patients with oral malodor.

**Methods** Patients with strong offensive oral malodor ($n = 4$) donated oral cavity breath into inflatable bags lined with polyethylene. Patients had grade ≥3 oral malodor on an organoleptic scale (0–5) as assessed by trained organoleptic judges. In the laboratory, each bag was heated to 38°C to vaporize condensed water. 150 ml breath was then removed from the bag and injected on to a sorbent trap (Carbotrap, Supelco) in order to capture the VOCs. Samples were analyzed by automated thermal desorption with gas chromatography and mass spectroscopy (ATD/GC/MS) and the VOCs were identified from a computer-based library of mass spectra.

**Results and discussion** The 10 most abundant VOCs observed were (in descending order): methylbenzene, 2,2-dimethyldecane, 2,2,3,3-tetramethylbutane, 2-propanone, 3-methyl-5-propylnonane, methylcyclohexane, 3-methylhexane, 2-methyl-1-propene, ethanol and methylcyclopentane. These VOCs may have been produced by bacterial metabolism.

**Conclusions** Assay with ATD/GC/MS identified the most abundant VOCs in oral cavity breath of patients with strong offensive oral malodor. Potential clinical applications of this technique include identification of the VOCs responsible for oral malodor in individual patients, and monitoring the effectiveness of treatment to reduce the abundance of these VOCs.

## O13

### Trimethylaminuria, diet and a 'fish-like' odour

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Trimethylaminuria, also known colloquially as the fish malodour syndrome, is a metabolic disorder characterized by the presence of abnormal amounts of the dietary-derived tertiary amine, trimethylamine, in the urine, sweat, expired air and other bodily secretions. Trimethylamine usually undergoes metabolism to form the innocuous metabolite, trimethylamine N-oxide. However, 'greater-than-normal' amounts of trimethylamine may accumulate within the body owing to a mismatch in the enzyme's capacity to undertake this N-oxidation reaction and the substrate load it has to process. Trimethylamine itself is a volatile substance and has the powerful aroma of rotting fish, conferring upon the sufferer a highly objectionable body odour, which can be destructive to the personal, social and work life of the affected individual. As more cases of this condition have been uncovered it has become apparent that there are several subtypes of this disorder, falling into two major categories, and this has allowed a clearer picture to emerge. First, there are those forms that are related to a dysfunction of the normal enzyme (flavin-containing monooxygenase 3; FMO3) activity owing to genetic, hormonal or inhibitory chemical influences. Secondly, there are those forms arising from substrate overload of enzyme activity (either in a normal or depressed state) such as an excess of dietary precursors or variations of gut microflora resulting in enhanced liberation of trimethylamine. Clearly, certain aspects of these two categories are intimately entwined and several factors may act together to give rise to the disorder. In recent years much progress has been made at all levels in our understanding of this condition. This presentation will summarize this progress, draw attention to the different types of the condition and highlight aspects that require further investigation. S.C. Mitchell, R.L. Smith (2001). Trimethylaminuria: the fish malodour syndrome. *Drug Metabolism and Disposition* **29**: 517–521.J.R. Cashman, K. Camp, S.S. Fakharzadeh, P.V. Fennessey, R.N. Hines, O.A. Mamer, S.C. Mitchell, G. Preti, D. Schlenk, R.L. Smith, S.S. Tjoa, D.E. Williams, S. Yannicelli (2003). Biochemical and clinical aspects of the human flavin-containing monooxygenase form 3 (FMO3) related to trimethylaminuria. *Current Drug Metabolism* **4**: 151–170.

## O14

### *In vitro* models for oral malodor

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We start with a description of what we mean by a model and introduce the idea of using different types of model to answer different types of questions. A model is a representation of some real phenomena and contains aspects or elements of the real system to be modelled. The model reflects (or duplicates) the type of behaviour (or mechanisms) seen in the real system. The main characteristic of any model is the mapping of elements or parameters found in the system being studied (e.g. tongue dorsum biofilm *in situ*) on to the model being devised (e.g. laboratory perfusion biofilm). Such parameters include correct physico-chemical (abiotic) conditions as well as biotic conditions that occur in both model and reality. The main purpose of a model is to provide information that better explains the processes observed or thought to occur in the real system. Such models can be abstract (mental, conceptual, theoretical, mathematical or computational) or 'physical' e.g. in the form of a real disaggregated *in vitro* system or laboratory model. A wide range of different model systems have been used in oral biofilm research. These will be briefly reviewed with special emphasis on those models that have contributed most to knowledge in breath odour research. The different model systems used in breath odour research will be compared with each other regarding their advantages, disadvantages and main applications. Finally, the requirements for developing an overall 'bad breath model' from considering the processes as a whole (real oral cavity; substrates in saliva;, biotransformation; odour gases in the breath) and extending this to the detection of malodour by the human nose (and all stages in between) will be outlined and discussed.

## O15

### Use of molecular identification techniques to study oral microbes and microcosms

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Over the last 100 years traditional culturing techniques have shaped our understanding of the oral microflora. However it is generally accepted that around 50% of the oral microbiota is unculturable. Recent advances in molecular biology have given rise to numerous techniques which form the basis of a number of strategies that can be used to detect and identify microorganisms to levels and specificities unattainable by conventional culture techniques. The molecular biology basis of these techniques can be conveniently split in to two broad groups. (1) DNA hybridization techniques: these are used for slot blot and checkerboard analysis and when combined with microscopy form a powerful visualization technique – fluorescent *in situ* hybridization (FISH). (2) PCR based approach: development of the PCR concept has lead to techniques such as global PCR, specific PCR, nested PCR, PCR-cloning, reverse transcriptase PCR, real time PCR and PCR-DGGE. The above techniques can usually be used on their own however they provide even more power when used in conjunction with each other. For example PCR-cloning or PCR-DGGE can be used to analyse a whole community. From this, un-named or unculturable taxa can be 'identified' and targeted for further characterisation. This may involve the use of PCR specific for the taxon in question, to survey a large number of samples to obtain some prevalence data or in a real time setting to quantify the taxon. Indeed, a fluorescently

labelled probe could be synthesized and used to visualize the taxon in an appropriate environmental sample or microcosm using confocal laser scanning microscopy. All the above techniques add up to a powerful set of tools which if selected appropriately have the potential to revolutionize our understanding of the microbial world.

## O16

## Organoleptic assessment of pure odour compounds representative of the metabolic end-products of oral microbes

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We have measured various production rates of volatile compounds in tongue scrape cell suspensions, oral biofilm models and the oral cavity *in situ* and wish to relate these to equivalent levels of oral malodour as assessed by the human nose.

**Objectives** To assess the relationship between measured concentrations of pure odourants and resultant organoleptic scores (using a defined 0–5 score).

**Methods** Seven odour judges, all with experience of organoleptic assessment, were used in this study for all experiments carried out at a single site (UWE, Bristol). A wide range of pure odourants were prepared at a range of concentrations (from a stock of 0.1 M) and were dispensed as 12 ml volumes into 'universal' bottles (24 ml capacity) leaving a headspace of 12 ml. The bottles were secured with rubber-lined metal caps, labelled with a randomized code and were presented to the odour judges for assessment using the Rosenberg 0–5 scale. The volatile sulphur compounds ($H_2S$ and $CH_3SH$) were presented as gases with dilutions being prepared through a dynamic gas flow rig (using oxygen free nitrogen as the diluent) and presented to the odour judges at a flow rate of 200 ml $min^{-1}$. Group means of determinations for each odourant were plotted against log10 of the concentration. Linear regression analysis was used to measure the gradient, 95% CI, and the scatter of the points around the gradient ($R^2$ value).

**Results** Organoleptic scores were proportional to the log10 concentration of odourant. Detection thresholds (expressed as mol $dm^{-3}$) were skatole ($7.2 \times 10^{-13}$) < methyl mercaptan ($1.0 \times 10^{-11}$) < trimethylamine ($1.8 \times 10^{-11}$) < butyrate ($2.3 \times 10^{-10}$) < hydrogen sulphide ($6.4 \times 10^{-10}$) < putrescine ($9.1 \times 10^{-10}$) < dimethyldisulphide ($5.9 \times 10^{-8}$). Hydrogen sulphide showed highest olfactory power whilst putrescine was the lowest.

**Conclusions** The olfactory response is exponential in nature. For any single compound, its contribution to malodour depends on: odour (olfactory) power, threshold (of detection) and volatility in addition to the concentration. It is now possible to relate production rates of VC's in models to typical levels of oral malodour perceived by the human nose.

## O17

## Effect of Probiotic *Streptococcus salivarius* K12 on oral malodour parameters

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**Objective** Recent non-cultural-based studies of the oral microbiota indicate that some subjects with halitosis are less likely to have the usually predominant commensal bacterium *Streptococcus salivarius* detected [*J Clin Microbiol* (2003) **41**(2): 558–563.]. The aim of this study was to determine whether the oral delivery of viable bacteriocin-producing *S. salivarius* could influence the composition of the oral microbiota implicated in halitosis and impact upon measurable parameters of malodour.

**Methods** Thirteen subjects with volatile sulphur compound (VSC) breath readings of >200 ppb on two separate visits were recruited for the study. The subjects undertook a 3-day regimen of chlorhexidine rinses followed at intervals by the sucking of four lozenges, each containing $>1 \times 10^9$ colony forming units of the bacteriocin-producing *S. salivarius* K12. The subjects then ceased using the chlorhexidine and took just two lozenges per day for a total of 2 weeks. At each pre-treatment visit and at 1 and 2 weeks after treatment initiation the subjects were tested for VSC and both saliva samples and tongue swabs were subjected to BANA testing, microbial culture and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. All measurements were taken in the morning prior to the subjects eating, drinking or using any oral care. The ability of the bacteriocins produced by *S. salivarius* K12 to inhibit bacteria implicated in halitosis was tested *in vitro* by deferred antagonism tests using pure cultures and saliva samples.

**Results and discussion** The VSC levels of eight subjects were significantly lower when tested 1 and 2 weeks after commencing treatment, three subjects had lower readings only after 1 week and two subjects maintained high levels throughout the study. Reduction of BANA activity and changes in the PCR-DGGE profiles occurred in most subjects following treatment. In deferred antagonism studies, *S. salivarius* K12 inhibited some of the bacterial species implicated in halitosis, and significantly inhibited black-pigmented colony types in saliva samples.

**Conclusions** The replacement of bacteria implicated in halitosis with the bacteriocin-producing commensal bacterium *S. salivarius* K12 appears to provide an alternative therapy for the long-term reduction of halitosis.

## O18

## Lethal photosensitization of oral pathogens via red-filtered halogen lamp

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**Objectives** The ability of laser irradiation in the presence of photosensitizing agent to induce lethal effect on oral bacteria is

well documented. We designed an *in vitro* experiment to achieve phototoxic results on two common oral pathogens, using a high intensity, red filtered halogen lamp. Our goal was to determine the minimum duration of light exposure and drug dilution to achieve at least 50% reduction in bacterial counts.

**Methods** Two common oral pathogens, *Porphyromonas gingivalis* and *Prevotella intermedia* were used in this experiment. The source for light energy was continuous working, high intensity, red filtered, halogen lamp (HL). The light was transmitted through a flexible light guide over petri dishes containing live bacteria. Microorganisms were exposed to halogen light for 5, 10 and 20 min. Methylene Blue (MB) in concentrations of 0.1, 0.075, 0.05, 0.025 and 0.01% was used as a photosensitizing agent. Light energy alone and MB alone was used as controls.

**Results and discussion** Optimum lethal photosensitization (50% or more bacteria killing) of oral pathogens was achieved under following conditions.1. Halogen light illumination for 5 min and longer with 0.05% MB.2. Exposure to light for 20 min in the presence of 0.025 and 0.01% MB.Light exposure of 20 min in the absence of MB was not effective in killing bacteria. In the absence of light, MB at concentrations of 0.025 and 0.001% was not effective. Reduction of bacteria with the use of 0.05% MB alone was also insignificant. However, 0.075 and 0.1% MB, even in the absence of light was found to be bacteriocidal.

**Conclusions** Our *in vitro* data indicates that we were able to achieve lethal photosensitization of two common oral pathogens with high intensity red filtered HL in the presence of diluted MB. In this era of increased incidence of antibiotic resistance, bacterial killing with laser or light energy in the presence of photosensitizing agents, can prove to be a valuable treatment modality.

## O19
## $H_2S$ inhibits $O_2^-$ scavenger and causes oxidative damage *in vivo*

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**Objective** Volatile sulphur compounds (VSCs), namely hydrogen sulfide and methyl mercaptan have been demonstrated to initiate or progress periodontal disease by increasing the permeability of sulcal epithelium and the degradation of extracellular matrices. Superoxide dismutase (SOD) is one of the most important antioxidative enzymes in intracellular protection against superoxide free radicals which may cause cancer or aging process. The objectives of this study were to demonstrate that hydrogen sulfide, a major contributor to oral malodor, inhibits SOD activity, and to determine if VSCs cause oxidative damage.

**Methods** Cu,Zn- and Mn-SOD, and human gingival fibroblast (HGF) SOD activities were examined with the determination of the inhibitory activity of the production of 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium formazan. Western blot analysis was employed to determine the effect of hydrogen sulfide on SOD molecules. Oxidative damage was determined with comet, caspase III and other assays.

**Results and discussion** The activity of Cu,Zn-SOD (0.01 U $ml^{-1}$) was found to decrease by 83% when exposed to 52 ng/10 ml of hydrogen sulfide for 60 min ($P < 0.0001$ by ANOVA and $P < 0.001$ by Tukey's multiple comparison test) and that of HGF-SOD decreased by 46% ($P < 0.0001$ by ANOVA and $P < 0.001$ by Tukey's multiple comparison test). The inhibition of SOD by $H_2S$ was both time- and concentration-dependent. Following exposure to VSC, SOD activity resumed after incubation in air. The results suggested that the inhibition might be reversible. VSC might cleave the disulfide bonds and might produce monomeric SOD resulting in reversible inhibition. However, Western blot analysis has not demonstrated monomeric SOD. Comet assay and other examination demonstrated that VSC caused oxidative damage and apoptosis.

**Conclusion** SOD is the body's major defence against oxidative damage. Cu,Zn- and Mn-SOD, and human gingival fibroblast (HGF) SOD activities were strongly inhibited by hydrogen sulfide. The inhibition of SOD resulted in oxidative damage and apoptosis.

## O20
## Psychophysical aspects of odour

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The history of psychology during the last century should certainly include a statement about the neglect of the crucial topic of human emotion and also ignorance concerning the role of the olfactory sensory system in human behaviour. During my research career, together with my research students, these are topics I addressed. The sense of smell is not a vestigial system but a sensory input of vital importance in human behaviour. Our experiments involved behaviour and psychophysiological and brain activity studies. Some of these studies involved collaboration with members of Quest working in sensory evaluation. Currently it is slowly being recognized that all human sensory input is integrative and each of the sensory systems is important.

## O21
## Malodour in denture wearers

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A significant proportion of the adult population wear complete or partial dentures, a proportion increasing with age. Denture plaque has not been studied to the same extent as dental plaque, and although there are many similarities in microbial composition, there are some significant differences. Plaque on denture fitting surfaces, particularly of the upper denture, is associated with denture-associated stomatitis, with the aetiological agent generally acknowledged as the yeast *Candida albicans*. In addition, poor denture hygiene and a more acidogenic denture plaque appear to be factors contributing to the condition. Anaerobic microorganisms, although present

in denture plaque, have been specifically investigated rarely. Teeth adjacent to partial dentures are also more susceptible to caries and periodontal diseases, perhaps due to an increased plaque buildup at the prosthesis/tooth interface. Dentures are fabricated from polymethylmethacrylate, using casts constructed from impressions taken in the mouth. The denture-fitting surface is not smooth (although the lingual/labial surfaces are polished) hence plaque accumulates more readily at the relatively stagnant sites. Over time, the epithelial surfaces in the edentulous mouth alter in shape, and dentures may fit less well. Soft liners, or more temporary tissue conditioners are applied to the prosthesis to improve comfort and fit. These materials, are more porous, and may thus entrap and accumulate more plaque biofilm. Little work has been done on malodour associated with denture wearers. There may be a number of reasons for this: (i) the population is deemed to be one which is less cosmetically aware, less likely to invest in products to enhance aesthetics. As the global population ages, and lifespan extends along with enhanced finance, leisure time and health status in selected developed countries, this presumption is perhaps inappropriate.(ii) The nature of malodour in denture wearers is ill defined. The wide age and health range presented by denture wearers poses problems in terms of defining the target populations and specific conditions.(iii) The origin of malodour in denture wearers – and indeed in the elderly in general – is varied: underlying serious illness (malignancy), chronic illness, ulcers, increased medication etc. are all likely to affect breath odour – ammonia, ketones etc.(iv) The increased likelihood of the presence of *Candida* spp. in the mouth may contribute 'yeasty' odours.(v) Poor denture hygiene, accumulation of calculus on prostheses, deterioration and colonization of soft liners will cause significant increases in plaque quantity. Products of deterioration of inert materials in the mouth may also contribute to odour.This presentation will review current status in the area, suggest possible causes of malodour in denture wearers, and consider the need for further work.

## O22
## Eating behaviour of institutionalized elderly people in relation to tongue cleaning

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**Introduction** Backing the oral hygiene of elderly people in need of care is an important task for geriatric nursing personal. Tongue cleaning has been generally recommended to improve the oral hygiene status, to prevent the development of oral malodour, and to improve the sense of taste. In Germany, no official recommendations for oral hygiene procedures in hospitals or nursing homes are available and if tongue cleaning is performed a regular toothbrush or a wooden spatula are mainly used. So far, only limited data have been published regarding the influence of tongue cleaning on taste sensation.
**Aim** Therefore, the aim of this study was to investigate, whether a regime of daily tongue cleaning with a specially designed tongue cleaner could improve the eating behaviour of institutionalised elderly patients.
**Methods** The study population consisted of 150 edentulous occupants (mean age 74 ± 4 s.d.) of a nursing home in Goettingen. Each subject served as its own control. During the test phase of 2 weeks each participant received five times daily a tongue cleaning procedure using the One Drop Only tongue cleaner (One Drop Only, Berlin; Germany; a combination of a tongue brush and a tongue scraper). During the control run the tongue cleaning was carried out also five times daily over a 2-week period using a regular toothbrush. All procedures were performed by a carefully instructed geriatric nurse. As a measure for the eating behaviour the amount of spurned and returned drinks and food was determined by weighing. The individual impression of taste was also determined by using a questionnaire. All subjects were able to eat and drink without assistance.
**Results** During the test period the subjects reported an increased appetite and only 18.3% of the served food and 17.1% of the served drinks were spurned and returned. In contrast, during the control phase the subjects returned 52% of the food and 53.2% of the drinks. These differences were statistically significant ($P < 0.05$, $t$-test n. FERGUSON: $\alpha = 1\%$; $f = 8$; $t = 3.37$).
**Conclusion** From our data we can conclude that the daily use of a specially designed tongue cleaner (One Drop Only) was capable of improving the appetite of institutionalised elderly patients. (This study was carried out without knowledge and support of the One Drop Only Company).

## O23
## Predicting bad breath in the non-complaining population

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Relatively few studies have addressed the prevalence and attributes of oral malodor among subjects who do not complain of bad breath. In the present investigation, 88 Israeli adults (mean age 37 ± 9 years) undergoing routine medical checkups, were asked to fill out a questionnaire including 38 questions on general and oral health, dietary habits, and their opinions regarding their own oral malodor levels. Oral malodor assessments included whole mouth odor judge scores on a 0–5 malodor intensity scale, as well as volatile sulfide levels (Halimeter®, Interscan Corp.), and salivary β-galactosidase (OK to Kiss, InnoScent Ltd.). Based on an odor judge cutoff score of 2, the prevalence of bad breath was found to be 29.8 ± 9.6%. Odor judge scores were significantly correlated with both Halimeter® ($r = 0.55$, $P < 0.01$; Pearson) and OK to Kiss ($r = 0.59$, $P < 0.01$; Spearman). Dichotomous analysis of odor judge scores, as compared with detection of salivary β-galactosidase (OK to Kiss) yielded sensitivity, specificity and accuracy of 89, 75 and 79%, respectively. Among the questionnaire results, 10 responses were significantly associated with odor judge scores ($P < 0.05$, unpaired

*t*-test). When the 10 responses were factored into linear multiple regression analysis to account for odor judge scores, a multiple $R$ of 0.846 was obtained. The results suggest that (i) the prevalence of bad breath in the general population is at least 20%; (ii) the OK to Kiss is a valid test for measuring the presence of oral malodor; and (iii) a simple questionnaire can be used to determine the degree of oral malodor among subjects without a complaint of bad breath.

## Poster Papers

### P1
### Bad breath among young Israeli recruits

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The purpose of the present study was to evaluate the extent of self-reported bad breath in an Israeli population of young army recruits and to assess its relationship with other self-reported parameters, as well as general dental status. Self-reported parameters included smoking status, bad taste, gingival bleeding and the presence of tonsilloliths. The study comprised 426 young adults recruits (ages 18–19), almost all males (95%), all of whom agreed to answer a questionnaire. All participants underwent a dental screening and were divided into three groups regarding to their dental status (do not need treatment, need moderate treatment, need extensive treatment). Statistical analysis included Pearson's chi square test of association using BMDP statistical software. Among the recruits, 142 (33 %) were active smokers. Thirty-five participants (8.2%) reported bad breath as well as bad taste. Twenty-seven (6.3%) reported being told that they had bad breath. Tonsilloliths were reported by 31 participants (7.3%) and gingival bleeding by 80 (18.8%). Self-reported bad breath was positively associated with bad taste, gingival bleeding, the presence of tonsilloliths and general dental status ($P < 0.05$). The study indicates that self reports of bad breath are associated by objective factors (e.g. dental status, tonsilloliths) as well as subjective parameters (bad taste). To our knowledge this is the first report indicating that one young adult in thirteen may suffer from tonsilloliths.

### P2
### Association between oral malodor and other body odors

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The purpose of the present study was to determine to what extent bad breath is associated with other body odors (axillary odor and pedal odor) in a group of 38 healthy adults. Two odor judges scored malodor from the mouth, tongue dorsum (spoon test), both axillae and shoes of the participants. Volatile sulfides were checked intraorally, as well as by sampling the subjects' shoes. In addition, microbial counts of tongue dorsum (spoon), axillae and shoe insole were assessed using DiaSlides™. In general, significant correlations ($P < 0.05$; Pearson) were found comparing underarm and shoe odor parameters. For example, a correlation of $r = 0.360$ ($P = 0.026$) was obtained comparing left axilla odor score and left shoe odor score of judge 2. Similarly, a correlation of $r = 0.534$ ($P = 0.027$) was found between volatile sulfide levels measured in left shoe, and left axilla odor score of judge 1. In contrast, comparisons of oral malodor parameters did not correlate significantly with the other odors tested. The results do not support the hypothesis that subjects suffering from bad breath have elevated levels of other body odors, as the result of poor personal hygiene habits. Rather, the correlations observed comparing axillary and pedal parameters suggest that these two areas share factors which are not exhibited in the oral cavity, such as similar skin microbiota and perspiration levels, which are not relevant in the case of oral malodor.

### P3
### The analysis of characteristics of the elderly people with high VSC level

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**Objective** The purpose of this study is to examine characteristics of the elderly people who had objectionable level of VSC.
**Methods** In 2002, a total of 115 people (mean age = 85 years) in Japan were surveyed. In the survey, we carried out oral examination, collecting tongue coat, measurement of the volatile sulphur compounds (VSC) levels in mouth air by VSC monitor (Oral Chroma™, Takasago, Japan), and assessment of the QOL using SF-36.
**Results and discussion** Average number of present teeth among subjects was 4.30 ± 7.11, and edentulous subjects were 66 persons and dentulous subjects were 49 persons. We divided subjects into two groups by VSC levels: the bad breath group ($H_2S$ >112 ppb and $CH_3SH$ >26 ppb) had seven persons, and the group without bad breath had 108 persons. It was shown that the amount of tongue coat was significantly higher in bad breath group ($P = 0.020$), while there was no significant difference in the amount of tongue coat between edentulous subjects and dentulous subjects. And, it was shown that the proportion of dentulous subjects in bad breath group was significantly higher ($\chi^2 = 5.664$; $P = 0.041$). Further, the analysis was performed for the result of SF-36 scores. It was shown that the scores of vitality (VT), social function (SF) and mental health (MH) were significantly higher in bad breath group. Although the levels of QOL had a tendency to be higher in dentulous subjects, there was no significant difference.
**Conclusion** These results showed that the elderly people with bad breath had tendency to be dentulous and had high deposit of tongue coat. And in this elderly group, people with bad breath tended to have mentally and physically improved.

## P4

## Parameters related to oral malodor in a population of 71 Israelis

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Bad breath is a common problem which is associated with a variety of factors. The contribution of dental factors related to periodontal health with bad breath remains controversial. Further, because of the problems inherent in odor judge scoring of oral malodor, other adjunct techniques have been proposed. We have recently developed one such colorimetric technique (the 'OK2KISS' test), based on the level of beta-galactosidase in saliva.

**Aims** The aim of the present study were (i) to determine the factors associated with oral malodour in a group of 71 Israeli subjects, and (ii) to compare existing testing methodologies with a new technique, recently developed in this laboratory (the 'OK2KISS' test).

**Methods** The study population consisted of a convenience sample 71 Israeli subjects (mean age 36.2 ± 38.4; ages ranging from 15 to 65) who were either undergoing a standard medical ($n = 14$), checkup, were about to commence orthodontic treatment ($n = 12$ ), or were siblings, or parents of orthodontic patients at Tel Aviv University ($n = 55$). Parameters measured included whole mouth odor judge scoring, halimeter, OK2KISS, gingival index, plaque index and probing depth.

**Results** Odor judge scores were significantly associated with halimeter ($r = 0.55$; $P < 0.001$), as well as the OK2Kiss test ($r = 0.52$; $P < 0.001$). However, neither gingival index, plaque index, nor probing depth was significantly associated with odor judge scores or halimeter scores. Logistic regression analysis showed that both halimeter and OK2Kiss scores factored significantly ($P = 0.005$ and 0.018, respectively, odds ratios 14.9 and 2.7, respectively) in predicting the severity of oral malodour.

**Conclusion** The study suggests that (i) in this population, periodontal health and oral malodour were not related; and (ii) that the OK2Kiss, together with halimeter scores, can serve to predict the severity of oral malodour levels.

## P5

## Halitosis and related factors in a Chinese general population

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The purpose of this study was to estimate the distribution of halitosis in a general Chinese population and assess the relationship between halitosis and oral health status and other social or behavioral factors. The subjects were 1000 males and 1000 females aged from 15 to 64 years in urban and rural area of Beijing, China. Questionnaire survey and oral examination was conducted. Questionnaire items included socio-economic status (income and education), oral habits (brushing), knowledge for oral health, dental attendance, life habits (smoking), medical history and self-assessment of halitosis. Oral examination comprised of DMFT, plaque index (PI), calculus index (CI), pocket depth (PD), modified sulcus bleeding index (mSBI) and tongue coating score (TCS). Volatile sulfur compounds (VSC) concentrations in mouth air were assessed by Halimeter. If VSC was 75 ppb and above, the subject was diagnosed halitosis. As a result, the prevalence of halitosis was 35.4% for the total population. Age and area of residence did not related to the VSC value. The levels of VSC were different among the period of assessment time. In the 15–24, 25–34 and 55–64-year-old groups, VSC values were significantly higher in the late afternoon. DMFT was not related with VSC. General health, social and behavioral factors did not relate to VSC. According to the logistic regression analysis, TCS, gender, CI and mSBI was significantly correlated with VSC. TCS had the highest OR value among them. The prevalence of halitosis was high in this population. Tongue coating played the most important role in determining VSC level, followed by periodontal status. However DMFT, smoking, social economic status, oral habits, general health and other social factors did not contribute to the level of VSC. The effective oral health promotion programs would be necessary to improve the poor oral health status and decrease the level of VSC for this study population.

## P6

## Complaints of halitosis related to the oral cavity. A preliminary report of 700 consecutive patients

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Patients with complaints of halitosis do seek treatment from physicians and dental practitioners, because of the fear that their halitosis may interfere with their social activities. Although the prevalence of halitosis has been reported to be as high as 50%, most physicians and dental practitioners are poorly informed about the causes and treatments of halitosis. In order to care for patients with complaints of halitosis a multidisciplinary team was established at the Erasmus Medical Centre, Rotterdam, The Netherlands. The team included a dental hygienist, an otorhinolaryngologist, and a dentist, who developed a special halitosis programme. One short press release regarding the establishment of the team, was provided to the national press-centre. In the out-patient clinic more than 700 patients were seen by the team. Using a structured questionnaire fed to a PC, patients answered questions regarding complaints about the oral cavity, the upper respiratory tract, the throat, their general health, their cleansing habits of the oral cavity, and prior experiences with general physicians, dental practitioners, and medical specialists. They underwent examinations of the extent of their halitosis, of the perioral and neck region, the oral cavity, the upper respiratory tract, and the upper digestive tract. Finally, the members of the team came to a joint diagnosis and a joint treatment plan for every individual patient. Of the first 700 consecutive patients

around 90% had a natural dentition without removable partial dentures. More than 60% were diagnosed as having periodontal disease with pockets of 4 mm or more in the maxilla. This figure was more than 50% in the mandible. Around 95% had tongue coating.

## P7
## Complaints of halitosis related to the extraoral cavity. A preliminary report of 700 consecutive patients

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Patients with complaints of halitosis do seek treatment from physicians and dental practitioners, because of the fear that their halitosis may interfere with their social activities. Although the prevalence of halitosis has been reported to be as high as 50%, most physicians and dental practitioners are poorly informed about the causes and treatments of halitosis. In order to care for patients with complaints of halitosis a multidisciplinary team was established at the Erasmus Medical Centre, Rotterdam, The Netherlands. The team included a dental hygienist, an otorhinolaryngologist, and a dentist, who developed a special halitosis programme. One short press release regarding the establishment of the team, was provided to the national press-centre. In the out-patient clinic more than 700 patients were seen by the team. Using a structured questionnaire fed to a PC, patients answered questions regarding complaints about the oral cavity, the upper respiratory tract, the throat, their general health, their cleansing habits of the oral cavity, and prior experiences with general physicians, dental practitioners, and medical specialists. They underwent examinations of the extent of their halitosis, of the perioral and neck region, the oral cavity, the upper respiratory tract, and the upper digestive tract. Finally, the members of the team came to a joint diagnosis and a joint treatment plan for every individual patient. Of the first 700 consecutive patients 57% were women. More than 80% were between 20 and 59 years old. One-third reported that they were never having breakfast or ate only soft food in the morning. Only 2% were diagnosed as having chronic sinusitis, 11% as having pharyngitis, 3% as having laryngitis, and 3% as having tonsillitis.

## P8
## Halitosis and periodontal disease in subjects with mental disabilities

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Halitosis has been correlated with the concentration of volatile sulfur compounds (VSC) produced in the oral cavity by metabolic activity of bacteria colonizing the periodontal area and the dorsum of the tongue. The aim of this investigation was to study the association among the presence of BANA-positive species (*Treponema denticola*, *Bacteroides forsythus*, *Porphyromonas gingivalis*), periodontal condition and halitosis in subjects with mental disabilities. This population (17–49 years of age) consisted of 17 Down Syndrome (DS) patients, 17 with Mentally Retarded (MR), and 17 mentally health (control group). The VSC in the human mouth was determined by a portable sulfide monitor (Halimeter®). Clinical parameters (Plaque Index – PlI, Probing Depth – PD and Bleeding on Probing – BOP) were obtained from six reference teeth (# 3, 8, 14, 19, 24, 30). In the same way, subgingival plaque samples were taken from the same selected teeth and from dorsal surface of the tongue and evaluated by BANA™ Test (BANAMet). The results were analyzed by Mann–Whitney *U* Test. PlI and BOP were higher in DS patients ($P < 0.01$) and PD was similar in DS and health patients ($P > 0.05$) but higher than MR. No difference was found among three groups for the presence of BANA-positive species, however the VSC levels were lower ($P < 0.01$) in DS (log1.9 ± 0.2) than in MR (log 2.2 ± 0.3) and health (log 2.2 ± 0.2) patients. Although the presence of anaerobic periodontal infection was similar in all groups the VSC levels were lower in Down syndrome patients.

## P9
## Screening for severe periodontal disease in an elderly population using methyl mercaptan concentration ratio

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**Objective** The purpose of this study was to examine the usefulness of methyl mercaptan concentration ratio on mass screening for periodontal disease in an elderly population.
**Methods** One hundred and twenty-two individuals (73 males and 49 females) aged 75 years who had at least 20 teeth in 2003 were chosen from participants of the Niigata Elderly Study (longitudinal study since 1998). Volatile sulfur compounds (VSC) in their mouth such as hydrogen sulfide ($H_2S$), methyl mercaptan ($CH_3SH$) and dimethyl sulfide ($(CH_3)_2S$) were measured, using portable gas chromatography (Oral Chroma®, Abilit Co Ltd, Osaka, Japan). The subjects were divided into periodontitis/no periodontitis groups based on having at least one site of pocket depth with more than 6 mm (PD 6+ mm). A $CH_3SH$ concentration ratio was defined as $CH_3SH/(CH_3SH + H_2S)$. The receiver operating characteristic curve was plotted to decide the best cut off point of the $CH_3SH$ concentration ratio for screening of subjects with PD 6+ mm.
**Results and discussion** The best cut-off point of the $CH_3SH$ concentration ratio was 0.35, and the sensitivity and specificity of this cut-off point were 58.7 and 68.4%, respectively. The percentage of subjects with PD 6+ mm in the group of $CH_3SH$ concentration ratio ≥0.35 was 52.9 % (27 of 51) and that in the <0.35 group was 26.8 % (19/71), and, the groups were significantly different ($P = 0.0059$, $\chi^2$ test). It had been reported that VSC, in particular $CH_3SH$ concentration, was significantly higher in persons with periodontal disease than in

periodontally healthy persons. Furthermore, in this study, the use of a portable gas chromatography enabled the VSC measurements for periodontal disease screening outside of the clinic.

**Conclusion** These findings suggest that $CH_3SH$ concentration ratio may be useful on mass screening for periodontal disease in an elderly population.

## P10

## Is transmission of bacteria that cause halitosis from pets to humans possible?

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**Objective** The aim of the study was to determine whether the level of salivary amines in halitosis patients depends on regular contact with pets (dogs and cats) in childhood or at present. It is believed that bacteria metabolizing amine compounds into compounds causing fetor ex ore may be transmitted to humans from animals.

**Methods** The study covered 84 patients suffering from halitosis and 40 healthy controls aged 20–62 (avg. 39.7). Each person completed a questionnaire and was then examined for organoleptic score, VSC by halimeter and evaluation of low molecular mass amines by a ninhydrine colourimetric reaction. Halitosis was diagnosed if the average level of VSC measured by the halimeter was $>125$ ppb and the organoleptic measurement using a 5-point scale was $>2$. Statistical analysis was performed using Wilcoxon's and Chi square tests.

**Results** Analysis showed a statistically significant correlation between halitosis and regular contact with pets at present ($P < 0.001$) or in childhood ($P < 0.001$).

**Conclusions** Pets (dogs, cats) owned in childhood or at present may transmit bacteria that cause halitosis.

## P11

## Correlation of headache and problems of vision with halitosis

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**Objective** Headaches are a common problem and may occur in response to several factors, e.g. high blood pressure, emotions, changes of atmospheric pressure. It is believed, that amines appearing due to halitosis may take part in this process. Different factors may cause problem of vision among humans. It is presumed that amines detected in halitosis chemically act on the neurological system. This process is sustained and the central nervous system is chemically intoxicated.

**Methods** Eighty-four patients suffering from halitosis and 40 healthy subjects aged 20–62 (avg. 39.7) participated in this study. Each subject completed a questionnaire and was then examined for organoleptic score, VSC by halimeter and evaluation of low molecular weight amines by ninhydrine colourimetric reaction. Halitosis was diagnosed if the average level of VSC as measured by the halimeter was $>125$ ppb and the organoleptic measurement using a 5-point scale was $>2$. Statistical analysis was performed using Wilcoxon's and Chi square test.

**Results** About 68% of respondents suffering from halitosis had problems with vision ($P = 0.035$). Headaches occurring among halitosis males were rare (20.0%), but were more common in females (43.0%). Differences in the incidence of headaches according to gender and diagnosis of halitosis were not statistically significant ($P = 0.8$).

**Conclusion** Problems with vision are more common in patients with halitosis. Such problems may be caused by metabolites of low molecular weight amine compounds produced during halitosis, which chemically act on the central nervous system.

## P12

## Halitometry in patients with chronic caseous tonsilitis prior to $CO_2$ laser treatment

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**Objective** Halitosis is a very common complaint at the ENT clinics. The aim of this study is to assess a population with chronic caseous tonsilitis (CCT) and complaint of halitosis prior to criptolisis with $CO_2$ laser treatment.

**Methods** Thirty patients were selected for $CO_2$ laser criptolisis. All patients underwent anamnesis, physical examination and halitometry prior to laser treatment. Halitometry technique followed the *Halimeter Interscan*™ manufacturer's instructions. Results $<150$ ppb of Volatile Sulphidric Compounds (VSC) were considered normal.

**Results** Thirty patients, nine male (30%) and 21 female (70%), average age 27.7 years ($\pm 10.4$) underwent halitometry. The patients were divided into two groups: Group A – abnormal halitometry ($>150$ ppb) – five patients (16.66%); Group B – normal halitometry ($<150$ ppb) – 25 patients (83.33%). Group A results: one male, four female, average age 25.2 ($\pm 5.6$). Halitometry was 315.8 ppb ($\pm 164$), three patients had caseum at examination (60%). Group B results: eight male, 17 female, average age 28.7 ($\pm 11.2$). Halitometry was 47.9 ppb ($\pm 18.6$), three patients had caseum at examination (12%).

**Discussion** Halitosis is a very common complaint among patients with CCT. Only 16% of the patients with CCT and complaint of oral malodor had abnormal halitometry. Nevertheless this population had a high incidence of tonsillary caseum (60%) comparing to a normal halitometry population (12%).

**Conclusions** Halitometry of VSC is useful to detect halitosis in patients with CCT. The presence of caseum at the examination is a predictor factor of abnormal halitometry. Further studies must be done to assess how $CO_2$ laser criptolisis could benefit this population.

## P13

Withdrawn/transferred/reclassified

## P14

### Characteristics of a cohort of patients suffering from halitosis and effect of a short-term treatment

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**Objectives** The aim of this study was to describe the clinical features of a cohort of patients suffering from bad breath observed at the Halitosis Unit of the Department of Stomatology – University of Milano. Moreover, the diagnostic and the short-term therapeutic approach to these patients has been evaluated.

**Methods** The clinical chart of patients have been reviewed and relevant data collected to be analyzed with an Apple Powerbook G4 PC. Patients complaining of bad breath were admitted to the halitosis unit. At first visit they were submitted to (i) clinical interview (ii) oral and periodontal examination (iii) tongue coating index recording (iv) hedonic breath testing (count-to-twenty test, wrist-lick test, spoon test, floss test) (v) measurement of oral VSC concentration with Interscan Halimeter®. By 1 week patients were submitted to professional prophylaxis and oral hygiene instructions with specific attention to tongue brushing. Patients were then prescribed with no-alcohol chlorexidine 0.2% for 30 s mouthrinse plus 30 s gargle twice a day after brushing. At the control appointment after 2 weeks the tongue coating index, the hedonic tests and the oral VSC measurements were recorded again.

**Results** One hundred and nine patients (F = 52; M = 59) were observed and treated at the halitosis unit in the period 2000–2002. Thirteen patients were halitophobic without any objective evidence of bad breath whereas 14 had causal ENT diseases. Of the remaining 82 patients with halitosis from oral causes, 60 showed no evidence of active periodontitis. In this latter group a significant reduction of the hedonic test scores and of the oral VSC concentration was found in 85% of patients. Eighty percent also declared a subjective improvement.

**Conclusions** The clinical protocol described was effective for the diagnosis and treatment of oral halitosis; the treatment with chlorexidine 0.2% was successful in the short-term control of the condition.

**Acknowledgement** This work was supported by the scientific fund FIRST 2000 – UNIMI.

## P15

### Non-surgical periodontal therapy reduces VSCs according to disease severity

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The objective of this study was to evaluate the effect of non-surgical periodontal therapy in VSC formation and its relationship with periodontal disease severity. Twenty-five periodontal patients without treatment and a control group with 10 subjects were selected. The patients were classified according to their periodontal diagnosis based on clinical attachment level (CAL): marginal gingivitis (MG), slight periodontitis-CAL = 3 mm (P1), moderate periodontitis-CAL = 5 mm (P2), severe periodontitis-CAL = 7 mm (P3) and terminal periodontitis-CAL > 10 mm (P4). At baseline, all patients received oral hygiene instructions and scaling/root planning. The periodontal parameters: plaque index, gingival index, probing depth and clinical attachment level were evaluated in two experimental periods (T1 = Baseline and T2 = 3 months recall). The Halimeter® was used to detect VSC formation in the same periods. The results after three months showed that VSC formation, probing depth and clinical attachment level decreased similarly in groups MG and P1 ($P < 0.05$). However, P3 and P4 groups showed the highest VSC formation and persistent bleeding in residual periodontal pockets. Within the limits of this study, it could be concluded that non-surgical periodontal treatment may interfere in VSC formation according to periodontal disease severity.

**Acknowledgement** This work was supported by FAPESP #44052-01.

## P16

### Effect of intraoral oxygen release device on breath odor

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**Objective** To assess the effect of intraoral oxygen release device on the reduction of breath odor.

**Methods** Twenty-two people (age ranged 17–81, mean age = 32, 11 males, 11 females) suffering from breath odor for average 8.3 years across the nation volunteered to participate in this clinical trial. Collection of baseline demographic information and clinical examination was conducted prior to the initial use of the intraoral oxygen release device. After having worn the device at night for 2 weeks, patients were recalled. Concentration of VSC (volatile sulfur compound) using Halimeter® (Interscan Corporation, Chatsworth, CA, USA) was measured and two independent examiners (one male, one female) performed organoleptic measurements, both before and after the use of the device. The two examiners were blinded to the use of device. Data were analyzed with non-parametric Wilcoxon Signed-rank test and proportion using statistical package JMP®5.0.1 (SAS Institute, Cary, NC, USA).

**Results and discussion** Following use of the device a change in breath odor severity was measured as a decrease of organoleptic breath odor by 58% with one examiner and by 79% with the other examiner. Median VSC level before device use was 111 ppb (parts per billion) and decreased to 75 ppb after device use. The VSC decrease was statistically significant ($P$-value = 0.004).

**Conclusions** The study indicated that the intraoral oxygen release device is effective in reducing Volatile Sulfur Compound level and organoleptic breath odor.

## P17

## Clinical comparison of a triclosan/copolymer/NaF dentifrice and a commercially available breath-freshening dentifrice in reducing breath volatile sulfur compounds overnight: a multiple-use study

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**Objective** The objective of this randomized, crossover study was to compare the effectiveness of the triclosan/copolymer/NaF dentifrice and a commercially-available breath freshening dentifrice containing fluoride for their ability to reduce volatile sulfur compounds (VSC) responsible for breath odor overnight.
**Methods** Following a one-week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or drinking in preparation for baseline breath sampling. Subjects were randomly assigned a test dentifrice and instructed to brush their teeth for 1 min, twice a day for 1 week. On the morning of the 8th day, subjects returned to the test site, having refrained from oral hygiene, eating and drinking, for overnight sampling. Following a second one-week washout period, subjects repeated the same regimen, but now using the other test product. At each measurement, the level of breath VSC was evaluated using a gas chromatograph equipped with a flame photometric detector. Measurements were taken in duplicate, and then averaged. The levels of VSC were expressed as parts per billion (ppb) in mouth air.
**Results** At baseline, the mean breath VSC levels for the TCF and breath freshening dentifrice groups were 618 and 581 ppb, respectively. There was no statistically significant difference between the groups. Overnight, the TCF and the breath freshening dentifrice reduced breath mean VSC levels to 267 and 521 ppb, respectively. This gave a 56.7 and 10.2% reduction in VSC levels for these two products, respectively, compared to baseline. The reduction for the TCF dentifrice was significantly different ($P < 0.05\%$) from that of the breath freshening dentifrice.
**Conclusion** The results of this randomized, double-blind, crossover study indicate that the TCF dentifrice was significantly more effective than a commercially-available breath freshening dentifrice containing fluoride in reducing breath VSC responsible for bad breath overnight.

## P18

## Clinical effectiveness of a triclosan/copolymer/sodium fluoride dentifrice in controlling oral malodor: a three-week clinical trial

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**Objective** The purpose of the study was to compare the effectiveness of a dentifrice containing 0.3% triclosan/2% polyvinylmethylether/maleic acid (PVM/MA) copolymer/0.243% sodium fluoride (TCF) to a commercially-available dentifrice containing 0.243% sodium fluoride (control) for the management of oral malodor in a 3-week, randomized double-blind, longitudinal clinical trial.
**Methods** A panel of four expert judges used a nine-point hedonic scale to evaluate breath odor using a protocol designed in accordance with the ADA Draft Acceptance Program Guideline for Products Used in the Management of Oral Malodor. Following a baseline evaluation, prospective subjects with hedonic scores above the threshold value for unpleasant breath were stratified by score and randomized into two treatment groups. Subjects brushed their teeth for 1 min with their assigned dentifrice, twice a day, for 3 weeks. Before oral malodor evaluations, the subjects refrained from eating odorigenic foods, from using mouthrinses and breath mints and from performing dental hygiene procedures.
**Results** Eighty-one adult male and female subjects completed the study. The baseline hedonic scores for the TCF and control dentifrices were 7.80 and 7.84, respectively, corresponding to unpleasant breath. The final mean oral malodor scores for the TCF dentifrice differed significantly from the baseline and control values ($P < 0.05$) for every time point examined (1.5-, 4.0-, 12.0-h and 1-, 2- and 3-week intervals). The mean final breath scores for the TCF dentifrice group were 3.06, 3.48, 3.42, 3.66, 3.42 and 3.36, respectively, for each time point. These scores correspond to pleasant breath. Conversely, the control dentifrice group scored at levels either above 5.0 (before 12 h) or above 7.0 (after 12 h) which corresponded to neutral or unpleasant breath.
**Conclusion** In conclusion, the results of this double-blind clinical study clearly indicate that a dentifrice containing triclosan/copolymer/NaF provides effective control of oral malodor for up to 12 h.

## P19

## A study for the effect of tongue cleaning

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The objective of this study was to evaluate breath odor reduction after giving tongue cleaning instruction to adults. The breath odor of 26 subjects (ages 19–77) was measured before and after tongue cleaning instruction. They were instructed to scrape away tongue coating with a Dental Pro multi-tufted tongue brush (Jacks co. Ltd.) once or twice a day. They used the tongue brush until they could see no more plaque on their tongue. Oral malodor was evaluated using: two portable sulphide monitors (Halimeter RH-17, Interscan Co.) and Brethtron, (Yoshida Co.), gas chromatography (GC-8A, Shimazu) and organoleptic measurements. Organoleptic measurements were estimated on a scale of 0 to 5 as follows : 0 = no odor, 1 = barely noticeable odor, 2 = slight odor, 3 = moderate odor, 4 = strong odor,

5 = severe odor. The subjects were asked to refrain from brushing, eating, drinking, smoking and using mouthwash 2 h before each examination. Tongue coating status was evaluated in five grades by inspection, according to distribution area and thickness of tongue coating. Probing depth, bleeding on probing and dental plaque were assessed. One week of tongue cleaning resulted in reductions in tongue coating status and breath odor. As a result of the before and after measurements, no correlation was observed between tongue cleaning as instructed and bleeding on probing or dental plaque. According to a questionnaire given 1 week after the study, tongue coating status had reverted to pre-cleaning condition in about 1 day. Therefore, we concluded that tongue cleaning should be done at least once a day to control breath odor.

## P20
## Effect of protease tablet on reduction of tongue coating

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**Objective** Practical treatment of halitosis requires tongue cleaning since volatile sulphur compounds (VSC) seems mainly to be from the tongue coating. From this point of view, mechanical tools such as tongue brushes or scrapers have been developed. However, approaches by chemical tongue cleaning have not been reported. Thus we developed tablets containing protease from kiwifruits, which could resolve tongue coating, and assessed the effects of the protease tablet to control tongue coating.

**Methods** Crossover studies and double blind experiments were designed using volunteers with informed consent. The trial was done twice per volunteer, that is, they had a tablet with or without the addition of protease from kiwifruits (test and placebo) with intervening washout periods of at least 2 weeks. The degree of change in tongue coating was evaluated visually using a tongue coating score which consisted of an area component (0–3) and a thickness component (0–3). An image analyzer was also used to measure the changing in actual area of coating.

**Results** The average value of the tongue coating scores after taking a test tablet (11.4 ± 5.2) was significantly smaller ($P < 0.01$) than before taking the tablet (18.8 ± 7.0). Image analyzer measurements also showed significant reduction ($P < 0.01$) of tongue coating by taking test tablet. On the other hand, a placebo tablet showed no significant effects in both analyses.

**Conclusions** This study indicated that taking protease tablets could reduce tongue coating. We are planning further clinical trials that can show reduced VSC concentrations in mouth air with decreasing tongue coating.

## P21
## Design and operation of a human breath freshness panel

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Breath freshening products are the fastest growing sector in the oral care marketplace. There is a clear need for robust testing protocols to evaluate the relative effectiveness of these products. The objective of this study was to design and set-up an organoleptically-assessed human breath freshening panel to allow reliable and robust comparative assessment of a range of oral care products. Initially, 10 prospective breath-odour judges were assessed in a Quest standard test for skills in recognition, differentiation, ranking and descriptive assessment of chemical odorants. The four highest scoring judges were further trained in assessment of chemical malodours relevant to breath freshening, using odorants including methyl mercaptan, indole, skatole, cadaverine, putrescine, butyric and isovaleric acids. An extensive programme of sniffing the breath odour of volunteers was undertaken, scoring breath using both the 'Rosenberg' (0–5) intensity scale, as well as a residual flavour (hedonic/pleasantness) scale. Comparative scores were analysed to ensure reasonably low inter-judge variability. A panel of members of the public were recruited as the Quest Breath Freshness panel. Panellists were assessed by a qualified dentist, and those with severe tooth decay, extensive dentures or severe periodontal disease were excluded, as were diabetics, pregnant women or those with any pre-disposition to respiratory illness. The panel has been used to conduct breath assessments prior to product use, and at a range of set time-points thereafter, up to 4 h. Results are routinely analysed by ANOVA. Trials were further optimized to use a 4- or 5-week, crossover, double-blind experimental design. Data from the panel has been used to support product claims, and to assess the efficacy of combinations of flavours, antimicrobial agents and oral product formats in delivering breath freshening benefits. The panel now runs routinely, and is used for research and commercial assessment of product benefits. The Quest breath freshness panel provides robust experimental data on the effectiveness of oral care products for breath freshening.

## P22
## Clinical efficacy of a triclosan/copolymer/NaF dentifrice and a commercially available breath-freshening dentifrice on hydrogen sulfide-forming bacteria

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**Objective** The objective of this double blind clinical study was to compare the 4-h and overnight effects of a triclosan/copolymer/fluoride (TCF) dentifrice and a commercially available breath freshening (CBF) dentifrice containing fluoride for their ability to control $H_2S$-forming bacteria.

**Methods** Following a one-week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or

drinking in preparation for baseline saliva sampling. After providing a baseline saliva sample, subjects were randomly assigned a test dentifrice and instructed to brush their teeth for one minute, twice a day for one week. On the morning of the 8th day, subjects returned to the test site having refrained from oral hygiene, eating and drinking for overnight sampling. Subjects then brushed for 1 min with the assigned dentifrice, and returned for 2- and 4-h postbrushing evaluations. Following a second one-week washout, subjects repeated the same regimen, but now using the other dentifrice. Oral microflora samples were collected by subjects rinsing with 10 ml of sterile water for 10 s. Each collected sample was serially diluted and plated in duplicate onto lead acetate agar. After incubating for 72 h, dark colonies were counted, expressed as log colony-forming units $ml^{-1}$, and reductions from baseline were calculated.

**Results** Compared to baseline, the TCF dentifrice reduced $H_2S$-forming bacteria 0.82, 0.80 and 0.22 log units at the 2-, 4- and 12-h time points compared to 0.60, 0.43 and 0.07 log units for the CBF dentifrice. The TCF dentifrice was statistically significantly better ($P < 0.05$) than the CBF dentifrice at 4 h and overnight after brushing the teeth.

**Conclusion** These results support data obtained in organoleptic and gas chromatography studies and strongly suggest that the TCF dentifrice is effective in controlling bacteria implicated in the formation of bad breath.

## P23

## Discrimination of oral-halitosis substance under humidity-rich environment by using QCM sensor array and a preconcentrator

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**Objectives** A halitosis test for objective evaluation is required. Halitosis is due to Volatile Sulfur Compounds (VSCs) produced by bacterial metabolism. In this study, we proposed a method for discriminating among sulfur compounds using a Quartz Crystal Microbalance (QCM) sensor array combined with a preconcentrator. The adjustment of the temperature profile, the optimization of the flow path sequence and the use of a dehumidification filter enabled VSC measurement under a high-humidity condition. Moreover, the result of principal component analysis indicated that three types of VSCs were separated using the sensor-array output pattern.

**Methods** Multiple QCMs with different coating films were used as a sensor array and the pattern recognition of eight different sensor responses enabled the odor identification. The preconcentrator was used not only for improving limit of detection but for eliminating the influence of humidity by adjusting the desorption temperature. The headspace over the liquid (water or ODO) where VSC was dissolved with the concentration of 0.1% (w/w) was used as the samples.

**Results and conclusion** An experiment on the discrimination among VSCs was performed. The headspace vapor of each sample was supplied to the preconcentrator for 30 s, followed by an exposure of the vapor thermally desorbed at 170°C to the QCM sensor array. The sensing films suitable for VSCs were selected among the various candidates and the peaks of the sensor responses to VSCs were analyzed using principal component analysis. Each sample was measured 10 times at the intervals of 30 min. As a result, it was found that three kinds of VSCs were successfully discriminated. Furthermore, it was found that the sensor responses increased in case of longer accumulation time. Thus, it is possible to detect the sample with lower concentration. This measurement method will be effective to diagnose halitosis objectively.

## P24

## Comparison of sampling bags used for measuring breath odor

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The use of sampling bags to collect breath odor has not been well established. The aim of this study was to compare and judge the quality and performance of three commercially available sampling bags. An aluminized polyethylene bag (Aluminum Bag, Tokyo Deodorant Inc., Japan), the Tedlar® bag (Tedlar® Bag, Tokyo Deodorant Inc.), and a polyester bag (Flek-Sampler® Nioibukuro; Omi Odorair Service Co. Ltd., Japan) were assessed. A GC instrument (GC14-B, Shimadzu, Japan) equipped with a flame photometric detection system were used to measure the concentrations of volatile sulfur compounds (VSC: hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) released from the sampling bags over time. An electronic nose (e-nose) (Odor Discrimination Analyzer FF-1, Shimadzu, Japan) was used to assess the characteristics of the gas eluted from the inner surfaces of the bags. The GC results showed that the Tedlar® bag had the highest performance, with 73% of the VSC concentration lasting for up to 4 days. The disadvantage of this bag, however, is that the Tedlar® bag itself has an odor that disturbs the e-nose's response and organoleptic evaluation. The polyester bags were only able to hold hydrogen sulfide for 2 h. The e-nose's responses were strongly associated with changes in humidity within the polyester bag. The humidity was more stable within the aluminized polyethylene bag, which was able to sustain 94% of the VSC concentration for up to 24 h. In conclusion, Tedlar® bags are beneficial for GC usage. Aluminum coated polyester bags are beneficial for GC measurements, e-nose measurements, and organoleptic judgements. Polyester bags have poor hold of VSC, but the low cost suits required disposability for the measurements of individual patients. Thus, breath odor sampling bags should be selected based on the specificity of the measuring instrument.

## P25

## Clinical assessment of oral malodor intensity expressed as absolute value using electronic nose

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We have previously reported that the score as determined under top-note mode with an electronic nose provides objective halitosis-related measurements, but those obtained by the multiple linear regression method (EM-MLR) show only relative and not absolute values. The purpose of this investigation was to assess clinically oral malodor intensity expressed as an absolute value using an electronic nose. Sixty-six patients who complained of oral malodor participated. Malodor intensity (EN-MI) calculated by totaling the contributions based on the measurement of multiple category gases and EM-MLR were measured by analyzing mouth air samples with an electronic nose system (FF-1, Shimadzu). Oral malodor was also assessed by an organoleptic test and volatile sulfur compound levels (VSC) using gas chromatography. Probing depth (PD), tongue coating score (TCS), and plaque control record (PCR) were used as oral health parameters. Receiver operating characteristics (ROC) procedures were applied to analyze the diagnostic accuracy of EM-MI using an organoleptic test as a gold standard. The area under the ROC plots for EN-MI (0.975) was significantly larger than that for logVSC (0.896) ($P = 0.036$), but did not differ significantly from that for EN-MLR (0.932). In logistic regression analyses of the possibility of subjects being in the upper 25th percentile of EN-MI distribution as a dependent variable, percentage of teeth with PD.4 mm, TCS, and PCR were each significantly associated with EN-MI and respective odds ratios were 13.0, 7.1, and 28.2. These results suggest that oral malodor intensity expressed as an absolute value using the electronic nose may be useful for evaluating clinically oral malodor.

## P26

## Breath odor evaluation by detection of volatile sulfur compounds – correlation with organoleptic odor ratings

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Detection of oral volatile sulfur compounds (VSCs) by gas chromatography (GC) is a widely used method for evaluating breath odor. Air aspirated from the mouth is injected into the GC column for analysis. To eliminate discrepancies caused by variations in operator sampling or injection techniques, a new GC system designed to aspirate breath samples directly into the GC was developed.

**Objective** A clinical study was performed to compare this new automated breath-sampling GC system to organoleptic evaluation by two-trained odor judges.

**Methods** A randomized, two-cell, double-blind, parallel design was used in which subjects were tested before and 3 h after using either a mouthrinse containing zinc or a matching placebo rinse. Thirteen subjects used the zinc mouthrinse, and twelve used the placebo. Subjects with a wide range of VSC levels were studied. The average organoleptic ratings for each subject at each time point were compared to the average VSC measurements made with the GC, and Pearson product–moment correlation coefficients between the corresponding GC and organoleptic measurements were determined.

**Results** The correlation between the GC and organoleptic assessment methods were highly significant ($P \leq 0.001$) for: total VSCs, 0.65; $H_2S$, 0.63; $CH_3SH$, 0.61; and $(CH_3)_2S$, 0.46. The correlation between the two judges was also highly significant (0.823, $P < 0.001$).

**Conclusion** These results demonstrate the utility of the automated gas chromatography method for evaluating breath odor.

## P27

## Comparison of ninhydrine methods of detecting amine compounds with other methods of detection in halitosis

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**Objectives** Availability of required substrate – amino acids is a factor determining the appearance of halitosis. The aim of the study was to determine whether a chemical test for low molecular weight amines in the saliva may be a new method of diagnosing halitosis.

**Methods** Eighty-four patients suffering from halitosis and 40 healthy volunteers aged 20–62 (avg. 39.7) participated in the study. Halitosis was diagnosed if the average level of VSC measured by a halimeter was $>125$ ppb and the organoleptic measurement using a 5-point scale was $>2$. In all subjects low molecular weight amines were evaluated by the ninhydrine method. Patients with halitosis were randomized into a treatment groups. Zinc tablets, tablets and mouthwash containing chlorhexidine or lyophilized lactic acid-forming bacteria were used. Test subjects were examined at day 0, 7, 14, 21 and after 3 months and controls on day 0, 21 and after 3 months. Statistical analysis was performed using Wilcoxon's test.

**Results** Analysis showed, that the level of amines was highest in subjects with halitosis (0.39, s.d. 0.06) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$). Reduction of amine levels after treatment was statistically significant (0.36, s.d. 0.06, $P < 0.001$). The amine levels in healthy controls was lower (0.29, s.d. 0.07) and remained at a similar, stable level.

**Conclusions** The ninhydrin method of detecting salivary amines may be one of the methods of diagnosing halitosis, because salivary amine levels significantly correlated with VSC levels measured by halimeter and organoleptic scores. This method may also be used to evaluate treatment efficacy.

## P28
## Analysis of 'Morning Breath'

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The objective of the study was to identify the volatiles responsible for the smell of oral malodour, the so called 'Morning Breath' or halitosis. Therefore the breath of volunteers was trapped on different SPME fibers and on Carbotrap 300 and analysed by Thermodesorption-GC-MS and Thermodesorption-GC-Sniffing Detection. So numerous odour active compounds were identified and their influence on malodour was determined. The identified substances belong to different chemical classes (S-compounds, N-compounds, alcohols, acids, phenols). An *in vitro* aromagram of the malodour was generated. The results indicate that the frequently mentioned 'Volatile Sulphur Compounds' (VSC) $H_2S$, methane thiol and dimethyl sulfide only play a minor role in the 'Morning Breath'. More important are other sulfur compounds, phenolic compounds (phenol, p-cresol) and N-compounds (indole, scatole).

## P29
## The effects of essential oils on periodontopathic bacteria and oral halitosis

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Oral halitosis has been associated with periodontal disease. Hydrogen sulphide, methyl mercaptan and dimethyl sulphide are the major components of halitosis originating in the oral cavity. These compounds are mainly produced by periodontopathic bacteria in the oral cavity. In the absence of the bacteria, the odoriferous compounds are not generated. We have demonstrated the antibacterial effects of essential oils on the periodontopathic bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*. As essential oils, manuka oil, tea tree oil, eucalyptus oil, lavandula oil, and romarinus oil were used, and their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The essential oils inhibited the growth of the bacteria tested, and manuka oil did so most effectively. MBC values showed that lavandula oil acted bacteriostatically, and the remaining oils bactericidally. Bacterial strains tested were killed completely by exposure for 30 s to 0.2% manuka oil, tea tree oil or eucalyptus oil. This study showed that among the essential oils tested, tea tree oil and manuka oil, particularly the latter, had strong antibacterial activity against periodontal disease associated bacteria. In the present study, we investigated the clinical effects of essential oils in the treatment of oral halitosis. When the degree of halitosis before and after the treatment was evaluated, the essential oils demonstrated efficacy in reducing the level of the three major components of halitosis. From the data of the present study, we consider that essential oils can be used in oral health management. This study was supported by grant from Kanpou Science Foundation.

## P30
## Relationship of oral malodor with tongue microbiota analyzed with real-time PCR

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Oral malodor is thought to mainly originate from the bacterial population present on the surfaces of tongue. However, the relationship between oral malodor and tongue microbiota has been little studied. In the present study, we analyzed tongue microbiota with real-time PCR and examined an association of oral malodor with tongue microbiota. The subject population consisted of 37 patients who complained of oral malodor. Oral malodor was assessed by an organoleptic test (OLT) and volatile sulfur compound (VSC) levels by gas chromatography. Real-time PCR was carried out in tongue biofilm samples using a LightCycler$^{TM}$ system as previously described (Kuboniwa *et al*. 2002) and the bacterial proportion in clinical sample was used as a quantitative parameter. The correlation coefficient between the proportion of total five Gram-negative anaerobes (*P. gingivalis*, *T. forsythensis*, *P. intermedia*, *P. nigrescens*, and *T. denticola*) and total VSC level ($r = 0.84$) was higher than that between the bacterial proportion and OLT score ($r = 0.24$). In these five Gram-negative anaerobes, the proportions of *P. intermedia*, *P. nigrescens* and *T. denticola* were correlated with the concentration of hydrogen sulfide ($r = 0.79$, 0.73 and 0.50, respectively). The proportions of *P. gingivalis*, *P. intermedia* and *P. nigrescens* were also correlated with the concentration of methyl mercaptan ($r = 0.91$, 0.40 and 0.71, respectively). When a linear regression analysis was conducted using the proportion of each of five Gram-negative anaerobes as independent variable, the explanatory power of these independent variables showed 77% for total VSC level and 10% for OLT score. These results suggest that Gram-negative anaerobes in tongue microbiota may contribute VSC production and may be also related to oral malodor.

## P31
## The relationship between the relative amount of *Porphyromonas gingivalis* in saliva and halitosis

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**Objectives** *Porphyromonas gingivalis* is one of the microorganisms that most actively produce $CH_3SH$ and we have reported that subjects with *P. gingivalis* have higher $CH_3SH$ levels than subjects without *P. gingivalis*. However, little is known about the relationship between *P. gingivalis* levels in saliva and the condition of oral malodor. In this study, we evaluated the association between the relative amount of *P. gingivalis* in saliva and halitosis in mouth air.
**Methods** All of the subjects were patients at the Preventive Dentistry and Breath Odor Clinic of Kyushu Dental College, where they received a periodontal examination. Volatile

sulfur compounds (VSC: hydrogen sulfide and methyl mercaptan) were measured using gas chromatography. Saliva samples were collected in a sterile plastic tube over a period of 5 min while the subject chewed on paraffin wax, and were then immediately stored at –80°C until use. Template DNA was obtained from the stored saliva using an Easy-DNA Kit (Invitrogen, CA, USA) according to the manufacturer's instructions. Conventional PCR assays were used to confirm the presence of *P. gingivalis*. A 5' nuclease TaqMan PCR was used to quantify *P. gingivalis* in saliva. The relative numbers of bacteria were measured using the comparative threshold cycle method.

**Results** We found a quantitative relationship between the *P. gingivalis* levels in saliva and the condition of halitosis in mouth air.

**Conclusion** We analyzed the relationship between the relative amount of *P. gingivalis* in saliva and oral malodor.

## P32

## Saliva incubation as replacement for intra-oral malodour evaluation

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**Background** Breath odor is scored by different techniques, each with its own shortcomings. Organoleptic ratings are uncomfortable for the patient, subjective, influenced by external parameters including food and cosmetics, and lack of international calibration. An intra-oral VSC rating (Halimeter) or gas chromatography can overcome the embarrassing situation for the patient, but not every clinician has this equipment. This project evaluated the reliability of saliva incubation as an indirect, *in vitro*, test for bad breath.

**Methods** In a double-blind, randomized, long-term, parallel study 45 moderate periodontitis patients that received a 'one-stage, full-mouth' disinfection, oral hygiene instruction (including tongue scraping) and antiseptics. The intra-oral VSC ratings (at baseline, and at 3 and 6 months) were compared to *in vitro* VSC recordings of the headspace air from saliva (0.5 ml, 37°C, 1 and 2 h, anaerobic chamber). The latter recordings were further correlated to the number of CFU in the saliva. At the same time the headspace also received an organoleptic rating.

**Results** Even though the VSC values remained within a narrow range (90% observation 10–200), a strong correlation was found between the intra-oral VSC ratings and the 1 h ($r = 0.48$, $P < 0.0001$) and 2 h ($r = 0.43$, $P < 0.0001$) VSC production of saliva. The VSC values of incubated saliva correlated strongly ($r = 0.71$) with the CFU for anaerobic species in the saliva. The VSC values and organoleptic ratings of the incubated saliva also correlated strongly ($r = 0.64$ after 1 h and 0.73 after 2 h incubation).

**Conclusions** Saliva incubation can be used as an indirect way to score bad breath. Due to its simplicity it can be useful in longitudinal studies as alternative for direct organoleptic recordings.

## P33

## *In vitro* perfusion biofilm model for the growth of oral microbes associated with oral malodour

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**Objectives** Oral malodour is mainly due to the generation of volatile sulphur compounds (VSC) by oral anaerobes present in the tongue biofilm. The aim of this study was to evaluate the use of a biofilm system to model the ecology and VSC physiology of tongue biofilm microcosms. Of particular interest was the use of the model to maintain a high diversity of bacterial groups and study their response to environmental changes including exposure to VSC substrates and inhibitors.

**Methods** Biofilms were grown using a Sorbarod perfusion model modified by supply of anaerobic gas to grow oral anaerobes. Sorbarods were inoculated using tongue scrape suspension containing $3 \times 10^8$ cfu ml$^{-1}$ and pre-incubated anaerobically for 24 h at 37°C to promote cell adhesion. After 24 h, the biofilm system was perfused at 12 ml h$^{-1}$ with BHI (0.74 g L$^{-1}$) supplemented with haemin, cysteine and/or DTT. Biofilms and eluates (released cells) were sampled up to 96 h and numbers of broad bacterial groups enumerated by selective media. VSC production was measured using a Halimeter$^{TM}$.

**Results** Repeated analysis of biofilms showed that a quasi-steady state was achieved between 48 and 96 h. The mean specific growth rate was 0.018 h$^{-1}$. Ecological analysis showed all broad groups were maintained at a constant proportion reflecting the original inoculum for both biofilms and eluates. VSC specific activity was upregulated when biofilms were perfused with appropriate VSC-substrates (cysteine, glutathione and methionine). *In situ* exposure of the biofilms to pulses of putative inhibitors (Zn, $H_2O_2$ and chlorhexidine) at a range of concentrations typically used *in vivo* gave dose-dependent inhibition of VSC production (for Zn and $H_2O_2$) and reduced viable numbers by one to two log-fold ($H_2O_2$ and chlorhexidine).

**Conclusions** The modified Sorbarod perfusion model is suitable for maintaining high diversity biofilms representative of the tongue flora in terms of both ecological behaviour and response towards VSC substrates and inhibitors.

## P34

## Effect of a triclosan/PVM/MA copolymer/fluoride dentifrice on volatile sulfur compounds *in vitro*

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**Objective** The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of $H_2S$-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for

the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

**Methods** An *in vitro* method was developed for the purpose of screening new technologies for their ability to control VSC formation and for determining bio-equivalence of efficacy when implementing changes in existing formulations. The method combines basic microbiological methods, dynamic flow cell techniques and head space analysis. The *in vitro* VSC method was validated by comparing the efficacy of two dentifrices containing TCF with a control fluoride dentifrice as the TCF products have been clinically proven to control oral malodor.

**Results** In the validation studies, the TCF-containing dentifrices were significantly better ($P < 0.05$) than the control dentifrice in inhibiting VSC formation and reducing $H_2S$-producing bacteria. For example, when compared to baseline, the TCF dentifrices reduced VSC formation between 42 and 49% compared to the control dentifrice which reduced VSC formation 3%. There was no significant difference ($P > 0.05$) between the two TCF dentifrices.

**Conclusion** Using an *in vitro* breath VSC model, it has been demonstrated that two variants of a dentifrice containing triclosan, PVM/MA copolymer and fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

## P35

Withdrawn/Transferred/Reclassified

## P36

## Use of n-butanol as an odourant to standardize the organoleptic scale of breath odour judges

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The alcohol n-butanol has been recommended for use as a standard odorant by various groups for the training or standardization of breath odor judges and sensory evaluation panels.

**Objective** To assess the use of n-butanol as a suitable odorant for use in organoleptic training of breath judges.

**Methods** One judge with full smell acuity was trained in the method of organoleptic assessment using odorant solutions of all chemical classes (acids, amines, indole, sulphides) with the exception of alcohols. The subject was proficient in scoring odorant solutions, standard gas mixtures and human breath using the Rosenberg 0–5 organoleptic scale. The judge had gained over 5 years experience of assessment and for the purposes of this work was considered to be the gold standard. A wide range of n-butanol solutions were prepared from 0 up to 90 000 ppm and dispensed as replicate 12 ml volumes in Universal bottles (24 ml) leaving a headspace of 12 ml. Sets of odorants were prepared, labelled by code, randomised and presented to the judge in a completely blind fashion. The judge scored each concentration. This process was repeated on 32 occasions over a period of 12 weeks. Means of data for each determination for each concentration series were plotted against the log concentrations of odorant. Linear regression slope analysis was used to measure slope, the 95% CI of slope and the scatter of points ($R^2$ value). Head space concentrations of odorant were determined using GC analysis.

**Results** The n-butanol regression slope gave a high $R^2$ value (0.971) and low scatter. However, the data did not correspond to that of other workers using an ASTM method where the range of recommended butanol concentrations was insufficient at the high end to determine the top of the organoleptic scale and insufficient at the low end to determine threshold. Moreover, headspace analysis using GC confirmed the published gas concentrations to be in error by a factor of 10. It was also observed that high concentrations of odourants were irritant causing desensitization if used for prolonged periods.

**Conclusion** A previously described method had erroneous headspace calculations listed and n-butanol could not be recommended as a training odorant because of its irritancy.

## P37

## Differences in score and inter examiner variation between four organoleptic methods

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**Objective** Organoleptic measurements are important tools for measuring halitosis. Many different methods have been applied, but an internationally recognized standardized method has not yet been agreed on. We hypothesized that four various organoleptic methods: (A) the open mouth method, (B) the counting method, (C) the 'HA-HA-HA' method (determining breath while the subject says HA-HA-HA), and (D) the glass straw method, may produce different scores and inter examiner variations. Thus the aim of this study was to compare the scores and inter examiner variations obtained by these four methods.

**Methods** Five trained (UWE Bristol) odour judges (OJs) assessed the breath of 20 test persons (17–71 years) by the methods A to D (see above), in a randomised and blinded set up. The 0–5 organoleptic scale by Rosenberg *et al*. (1991) was used for determining the intensity of oral malodour. OJs were allowed to score in fractions of 0.25 score. Inter examiner variation was calculated as the average percentage deviation for the scores of each OJ (computed from the mean score of the five OJs).

**Results** The mean organoleptic score of the 20 subjects differed significantly depending on the method applied ($P < 0.001$). Method (A) produced a score of $1.20 \pm 0.88$, (B) $1.69 \pm 0.83$, (C) $1.80 \pm 0.89$, and (D) a score of $2.11 \pm 0.92$. Further a significant difference was obtained for the inter examiner variation between the four methods ($P < 0.001$). The inter examiner variation for method (A) was

55%, for (B) 30%, for (C) 31%, and for (D) 26%. Finally a significant correlation was obtained between the organoleptic score and the inter examiner variation ($r_s = -0.70$, $P < 0.001$) becoming lower with higher scores.

**Conclusion** Due to the higher mean scores and the lower inter examiner variation the glass straw method may be preferable for organoleptic research studies of halitosis.

## P38

Transferred to oral session

## P39

## Evaluation of patient oriented outcome using SF-36 in halitosis therapy

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**Objective** To evaluate the patient oriented outcome of medical interventions, Medical Outcome Study Short Form-36 (SF-36) is a widely used, self-administered questionnaire. The SF-36 has eight dimensions of health status consisting of Physical Function (PF), Role- Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), SF (Social Function), Role-Emotion (RE) and Mental Health (MH). Furthermore, each dimensional score and two summary scores about physical and mental regions can be calculated by the algorithm. The aim of this study was to examine the application of SF-36 to the clinical parameter of halitosis.

**Subjects and methods** Seventy patients visiting our special clinic for halitosis were the subjects. In this study, we defined the healed persons as follows. They received our therapy two or more times, and the duration was over 2 months. After our therapy, they declared that the oral malodor of themselves could not bother them. In addition, they accepted to receive the general dental check-up regularly after the initial therapeutic period. The SF-36 was filled by the subjects at the first visit of our clinic and the first time of the regular check-up. The content of our therapy was mainly involved measurement of oral malodor, supporting for oral self-care, professional oral cleaning and counseling. All management concerning SF-36 was performed according to the manual of SF-36, version 1.2, Japanese edition.

**Results and discussion** Among all 70 subjects, GH, VT, SF, RE, MH and mental summary scores were significantly lower than national averages. Among the healed persons ($n = 17$), only SF score significantly rose after receiving our therapy. These results suggest that the consciousness of the improvement in social function could be an important outcome of halitosis therapy. In conclusion, measuring health related QOL using SF-36 might be useful for evaluating the efficacy of halitosis therapy.

## P40

## Measurement of VSC and organoleptic scores and subjective patients' opinion about halitosis – a questionnaire study

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**Objectives** The aim of the study was to evaluate the level of interest in fetor ex ore among respondents, patients of the Department of Conservative Dentistry in Warsaw.

**Methods** A questionnaire was completed by 295 patients, 202 female and 93 male aged 18–74 (avg 38.73). Each person was also examined for organoleptic score and VSC by halimeter. Halitosis was diagnosed if the average level of VSC measured by halimeter was ≥125 ppb and the organoleptic measurement using a 5-point scale was ≥2. Statistical analysis was performed using Chi square test.

**Results** Incidence of halitosis was greatest in age ranges 25–34 (29.68%) and 45–54 years (24.52%). People from these age groups are more careful about oral heath, so they are more likely to be interested in checking for halitosis. Among 295 respondents 25.42% often had a problem with bad breath, 39.66% sometimes, and 34.91% rarely or never. Persons with diagnosed halitosis frequently reported having a problem with bad breath (47.73%), and only 5.76% persons with halitosis did not notice this problem ($P < 0.001$). Among 76 healthy persons five (6.6%) reported having a problem with halitosis ($P < 0.001$). Clinical tests for halitosis allowed a diagnosis of halitophobia in four cases. Respondents noticed a problem with bad breath in their relatives in 46.0%. The frequency of malodor observed in relatives of patients with halitosis was similar to that in relatives of healthy subjects ($P = 0.58$). No correlation was found between cigarette smoking and a diagnosis of halitosis ($P = 0.22$).

**Conclusions** A statistically significant correlation was found between clinical diagnosis of halitosis and VSC level by halimeter, organoleptic measurement and subjective patients' opinion.

## P41

## Tongue cleaning habits in Italians: results of a survey in a sample population

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**Background and objectives** Mechanical cleaning of the tongue is suggested as an effective method for reducing cultivable flora of the tongue and teeth and decreasing oral malodor. The aim of this study was to evaluate the general attitude, knowledge and behaviour of a representative sample of Italian population regarding the hygienic tongue procedures.

**Methods** The survey has been conducted in January and February 2003 through structured phone interviews using CATI (computer aided telephone interviews) methods by EuriskoOmnibus, a specialized agency, on commission by GlaxoSmithKline Consumer Healthcare S.p.A.-Italy. The

sample has been randomly chosen among urban and rural residents from age 15 onwards with a nationwide distribution in proportion to the last demoskopic feature of general Italian population. Relevant data has been processed with MSExcel and StatView software in an Apple G4 Powerbook PC.

**Results** The sample is composed by 2000 individuals, 52% females and 48% males. Of these, 6% have an academic degree, 23% high school and 71% lower middle school Over one-third (39%) of Italians interviewed declared to perform specifically tongue cleaning: 40% of them every time they perform oral hygiene twofold or more daily, 24% once a day and 36% less than daily; the tongue cleaning habits are more diffuse in younger people. Seventy-one per cent of tongue cleaners perform the procedure with an ordinary toothbrush, 22% declared to clean tongue with an oral rinse and only 4% utilize specific tongue instruments. In our sample main reasons for tongue cleaning practices were reaching a better oral hygiene (60%) and fighting bad breath (25%).

**Conclusions** In Italians tongue cleaning is more diffuse than expected but the reasons are not principally related to the reduction of halitosis.

## P42
## Subjective symptoms and knowledge of oral malodor on dental students in Thailand

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The purpose of this study was to investigate subjective symptoms about oral malodor and the knowledge and attitude for bad breath on dental students. The subjects were 213 students from 1st to 6th year undergraduate course of one dental school in Thailand (male: 70, female: 143). Their mean (s.d.) age was 21.0 (1.9). Questionnaire survey was conducted in November 2003. Questionnaire items were subjective symptoms about oral malodor and dry mouth, the knowledge and attitude for bad breath, oral hygiene practice, smoking/drinking habits, and life stress. Five students (2.3%) answered that they concerned their oral malodor often and 87.8% reported sometimes. Of them, 72.9% wanted to receive treatment for their malodor and 70.8% said they suffer from bad breath in daily life. Two-thirds of students perceived strong oral malodor when waking up. There were no significant differences of the self-perceived malodor rates between gender or among year of study. More than half of the students did not know that oral diseases and poor oral hygiene are the major causes of bad breath. The students' attitudes for bad breath were different if the target was family members or friends. The majority of the students (88.7%) answered they perceived dry mouth. Perception of dry mouth was significantly related with self-reported oral malodor prevalence ($P < 0.05$). However other oral or behavioral factors did not relate to the self-reported malodor rates. In this study, dental students perceived bad breath in high prevalence. However the knowledge for bad breath was not enough even the students who already provided dental treatment to the patients. It would be necessary to give them adequate knowledge and methods in education program for managing not only patients' but also their own oral malodor problem.

## P43
## Effectiveness of a mechanic-chemical method in reducing malodor of dentures

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**Objective of investigation** To assess effectiveness of a mechanic-chemical denture cleansing to reduce malodor in denture wearers in comparison with a brushing only method.

**Methods** Twenty-nine denture wearers between 50 and 60 years old, 6 months after denture delivery were trained to clean their dentures with denture brush and soak in normal saline at nights for 1 month. The malodor of dentures was detected with a bad breath detector (VSC monitor: Tanita, Japan). In the following month the subjects were trained to clean with a denture brush but additionally soak in antiseptic mouthwash (Listerine, USA) each night and soak in 5% sodium hypochlorite twice a week for 5 min. At the end of 1 month the malodor of dentures was again assessed using the VSC monitor.

**Results and discussion** For denture brush alone all cases showed severe malodor VSC levels. Following the mechanic-chemical cleansing, 20 cases gave moderate and nine cases gave mild malodor VSC levels. Statistical analysis with Wilcoxon test determined the mechanic-chemical method to have been significantly effective ($P$-value $< 0.05$). The results show that for reducing malodor of dentures, denture brush alone is insufficient and use of antiseptic materials are necessary. Some studies have stated that allergic reactions to sodium hypochlorite are possible and in addition denture cleansing may be difficult for disabled or elderly patients. Therefore, future studies should focus on non-allergic disinfectants and/or simpler/easier methods (recommended protocols) to carry out effective disinfection.

**Conclusion** The mentioned mechanic-chemical method proved to be effective at reducing malodor of dentures whilst brushing alone was insufficient.

## P44
## Management of volatile sulphur compounds with ozone

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Volatile sulphur compounds (VSCs) are responsible for oral malodour (bad breath). There are many otc products for people for use, based on an oxidative reaction system. This paper examines the effect of ozone on VSCs.

**Objective** To test the efficacy of Ozone ($O_3$) dissolved in water on VSCs and the management of bad breath in a small pilot study in a general dental practice environment, test the Halimeter, and test a new bad breath Index, the Ks Index.

**Method** Twenty subjects were recruited into this study. After olfactory assessment, they were assigned to a Ks Index (The Kissability Index) and measured with the Halimeter (Interscan Corp, USA) after calibration, to establish their base-line VSCs. Each subject gargled with ozonated water for 30 s, and were re-assessed with the Halimeter. This was repeated after 5-, 30- and 60-min intervals. Ozone ($O_3$) was bubbled under pressure into 1 l of cooled, distilled water at a concentration of 2100 ppm for 1 min for each subject.

**Results** After just 1 min of gargling and mouth rinsing with 100 ml ozonated water, there was an average VSC reduction of 42.5% from the pretreatment baseline value. Five minutes after treatment, the VSCs rose an average of 14.2%; after 30 min, a further 18.9%, and after 60 min, the VSC values had risen by a further 24.6%. Ozone is a powerful oxidant, and in gaseous form, is used to reverse caries (1). When dissolved in water (maximum 5% volume in distilled, cooled and pressurised water) ozone is safe, and has been extensively used in reducing cfu's in dental water lines (2). Studies in Russia (3) have shown ozone to be an effective oral hygiene adjunct. This study showed that low concentrations of ozonized water is cheap and easy to make. It is a very effective product to reduce VSC's, and further research is proposed in this area.

**Conclusion** Ozone dissolved in water may offer a cheap alternative to the more expensive and less effective commercial mouth rinses to combat bad breath. However it is unstable (half-life of about 2–3 h if kept cold) and needs to be manufactured when required. It may have a greater role in the control of oral hygiene or as a presurgical mouth rinse.

## P45
## Microbiological culture analysis of the tongue microflora in subjects with and without halitosis

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**Objective** Determination of the microflora present on the tongue dorsum of subjects with and without halitosis using conventional microbiological culture methods.

**Methods** Twenty-one halitosis and 20 control patients were recruited using a strict clinical protocol. Samples were collected from the posterior dorsum of the tongue using a sterile brush. Each sample was vortex mixed for 30 s and serial 10-fold dilutions to $10^{-7}$ were carried out. Samples were plated onto fastidious anaerobe agar (FAA) and FAA enriched with vancomycin. These were incubated under anaerobic conditions for 10 days at 37°C. Strict anaerobes were identified by metronidazole sensitivity and bacteria were identified to genus level by a combination of colony morphology, Gram staining and biochemical and enzymatic tests (rapid ID 32 A).

**Results** The predominant species in test and control groups were *Veillonella* and *Prevotella*. Greater species diversity was found in the halitosis samples compared to controls. The halitosis samples contained a larger number of unidentifiable gram-negative rods, gram positive rods and gram negative coccobacilli.

**Conclusions** There was no obvious association between halitosis and any specific bacterial genus. The increased species diversity found in halitosis samples suggests that halitosis may be the result of complex interactions between several bacterial species. The role of uncultivable bacteria may also be important in contributing to this process.

## P46
## Stable storage of chlorine dioxide mouthrinse solutions

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Chlorine dioxide ($ClO_2$) is a highly effective yet non-toxic malodor counteractant and germicide that is listed as an ingredient in many mouthrinses. It is a water-soluble gas whose purported chemical instability and permeability through common storage containers has resulted in its inability to be provided as a shelf-stable, marketable solution of significant concentration. Thus $ClO_2$ has had to be generated *in situ*, or shortly before use, which has limited its utility. The object of the present investigation was to evaluate $ClO_2$ concentrations over time in aqueous, near neutral pH, $ClO_2$-$ClO_2^-$ (1:1–1:14) solutions (32–40 ppm $ClO_2$) prepared according to the method of Richter in containers made from a variety of materials under ambient, accelerated storage and other conditions. [$ClO_2$] was measured with standard spectrophotometric technique. All containers tested were standard 16 oz. bottles. The materials tested were glass of various colors and opacities, polyvinyl chloride (PVC), high-density polyethylene (HDPE), fluorinated HDPE, high-density terephthalate (PETE) of various colors and fluoridated polyolefins. We discovered that certain materials, such as glass, fluoridated HDPE, clear and white PETE and fluorinated polyolefins can maintain relatively constant levels of $ClO_2$ in pre-prepared solutions and in some cases for periods of up to several years under ambient and normal shipping conditions. In one series of studies, the mean loss of $ClO_2$ per day from solutions prepared to approximately 35 ppm was 0.8 ppm (s.d. = 2.9) (2.2%) for HDPE (32 days); 0.8 ppm (s.d. = 2.2)(2.16%) for PVC (30 days); 0.4 ppm (s.d. = 0.71)(1.20%) for fluoridated HDPE (45 days); 0.02 ppm (s.d. = 0.63) (0.045%) for PETE (45 days) and 0.14 ppm gain (s.d. 0.54)(0.31%) for clear glass (31 days). The s.d.s reported here are skewed high because they assume a linear loss of $ClO_2$ over time when in fact the actual measured loss (or gain) was in all cases nearly perfectly parabolic. In another study, one supplier's white opaque PETE actually showed a 6.2% overall gain in 252 days of storage. These results dispel the popular notion that $ClO_2$ always decomposes so rapidly that fresh solutions must be made up frequently to maintain effective $ClO_2$ concentrations. $ClO_2$ production from the reservoir of $ClO_2^-$ in these solutions is apparently sufficient to equal or exceed losses of $ClO_2$ from decomposition, diffusion through the container wall, possible reaction with the container material and escape during sampling for some of the materials tested. Previously observed losses of [$ClO_2$] from $ClO_2$-$ClO_2^-$ solutions were apparently due primarily to diffusion out of the containers rather than

from decomposition within the containers. This achievement now allows for the broad distribution of pre-prepared $ClO_2$ solutions for a wide range of oral care and other deodorant and germicidal applications.

## P47
## Creation of longer-lasting (substantive) oral care flavours

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Mainstream oral care flavours are primarily designed to provide hedonic (taste) benefits and to promote breath freshness. In particular, a key criterion for a commercially successful toothpaste flavour is a long lasting reduction in the perception of mouth odour. Various additives can help deliver this benefit, in addition to flavours formulated according to patented guidelines. The residence time (substantivity) in the mouth of flavours and additives is clearly critical with respect to the longevity of the breath-freshening benefit, and little data are available in the literature to guide the selection of substantive components. The aim of this project was to investigate the dynamics of flavour loss from the buccal cavity following brushing using a mass spectrometer equipped with an atmospheric pressure headspace sampler which enabled real-time determination of flavour components in mouth air. A number of flavour ingredients found in peppermint- and spearmint-based oral care flavours were studied. The *in vivo* decay kinetics of flavour ingredient loss were quantified and found to be strongly related to the physicochemical properties of ingredients, except in the case of esters where a more complex dependence was observed arising from chemical transformation occurring in addition to physical transportation away from the mouth. Surprisingly, some materials were discovered to undergo rapid degradation with a half-life of minutes. Confirmatory studies of the decay kinetics of such materials were carried out *in vitro,* and structural features were identified which were associated with the observed hydrolytic vulnerability. This work has allowed the development of new guidelines to enable the creation of longer-lasting oral care flavours.