Medical Applications of SIFT-MS in New Zealand

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Introduction

Selected ion flow tube mass spectrometry (SIFT-MS) is a relatively new analytical technique which offers real time identification and quantification of trace gases. It has the ability to detect and quantify the volatile organic compounds (VOCs) in various media such as liquid headspace and breath, thus opening up many new opportunities in medical research. NZ was a recipient of one of the world's first miniaturized SIFT-MS instruments that has been situated at the Christchurch Hospital since 2005. Herein we report on progress in the medical applications of SIFT-MS in this country.

The selected ion flow tube technique was developed initially in England in 1976 from modifications to a flowing afterglow system for studying gas phase kinetics. SIFT methodology was applied to investigate reactions between ions and neutral molecules in the gas phase.[1] A swarm of mass-selected reagent ions are carried by fast flowing helium into a flow tube where reactant neutral molecules are introduced at a controlled flow rate into the carrier gas. An ion-neutral reaction takes place and product ions are formed with all ions detected and counted via a second analytical mass spectrometer located downstream from the sample inlet. Under tightly controlled conditions, the rate coefficients for the reaction can be calculated from the decay of the reagent ion and the growth of the product ions.[2] This technique superseded flowing afterglow to become a standard method to study ion-neutral reactions at thermal interaction energies that are applicable in naturally occurring ionized media such as interstellar clouds and our ionosphere. Several laboratories around the world, including ones in NZ have established a large kinetics database and a better understanding of ion-neutral reactions using SIFT technique.[3]

The potential for adapting SIFT methodology to the trace gas analysis of air and breath (now known as SIFT-MS) was realised in 1996 by combining the use of NO^+, O_2^+ as well as H_3O^+ ions as the chemical ionization agents.[4] These three ionic species can be created simultaneously by microwave discharge using humid air as the ion source gas. They do not combine with major ambient gases, but react rapidly with most VOCs within milliseconds. This allows for the detection of rapid changes in test sample analyte concentrations in real time. Since then, the relevant kinetic parameters obtained from measurements of the reactions of these reagent ions with a number of organic molecules have been obtained. These results provide a database for SIFT-MS trace gas analysis in a large variety of applications.[5] Therefore, accurate identifications, and absolute concentrations can be detected using SIFT-MS under well-defined experimental conditions and with calculations utilizing the database of reaction rate coefficients.[6]

Herein we report the development of SIFT-MS in New Zealand, with special focus on medical applications. The first reported physiological study using SIFT-MS in this country[7] measured breath trace gases during exercise in 2000. However, the expansion of SIFT-MS applications here had a much earlier beginning.

After attending an enlightening lecture by David Smith (University of Keele) on the new SIFT-MS technique for breath analysis at the Christchurch School of Medicine, Randall Allardyce of Christchurch School of Medicine (Otago University) began cooperative studies in 1998 with Murray McEwan of this Department in the medical applications of SIFT-MS. They began studying the plume gas of bowel cancer patients using SIFT-MS with Big Bertha (the version of the SIFT instrument at Canterbury University) which took up an entire five meter long laboratory. In 2000, David Smith, Patrik Španěl, and Murray McEwan cooperated in constructing a smaller version of a SIFT-MS and in 2002 *Canterprise* (the technology transfer office of the University), founded Syft Technologies Ltd. to commercialize the technology and develop SIFT-MS applications. The first commercial VOICE100™ version of the instrument (about the size of a photocopier) was launched in March 2005. One of the major applications of this instrument has been to detect and measure fumigant levels within transport containers shipped into seaports. Other applications include the analysis of soil and seabed hydrocarbons in oil and gas exploration,[8] environmental monitoring,[9] occupational hazards,[10] food and flavour analysis,[11] detection of explosives,[12] chemical weapons, narcotics,[13] and bio-security risks.

Since receiving a FRST *Research for Industry* (RFI) grant to develop medical application products, SIFT-MS has made significant contributions to the real time detection of biomarkers in the clinical areas and it is these applications that form the focus of this article. In July 2007, a new generation of the SIFT-MS instrument, the VOICE200®, was released, which is lighter, more sensitive, and more portable than its predecessor. It has been fitted in a van and driven to local primary schools to survey the breath profiles of 230 school children. Additional cooperative studies are currently being carried out between Syft Technologies and renal, respiratory, intensive care, and surgical clinical research groups at the Christchurch School of Medicine.

Selected Ion-Flow Tube Mass Spectrometry (SIFT-MS)

The technique, principles, and theories of SIFT-MS have been detailed in other papers and in several reviews.[2,14] A brief description of the technique is presented here and a schematic diagram of the SIFT-MS device is shown in Fig. 1. The reagent ions H_3O^+, NO^+ and O_2^+ are produced by a microwave discharge of humid air and then focused by

ion lenses into a quadrupole mass spectrometer and mass selected. The mass-selected reagent ions are then injected into a fast-flowing stream of carrier gas through a Venturi orifice. The gas containing the analyte or VOC is introduced through a heated sample inlet into the flow tube at a known flow rate via a calibrated capillary. The precursor ion then undergoes a chemical reaction with the sample forming new product ions. At the downstream end of the flow tube, both the precursor and product ions are focused via electrostatic lenses into a second quadrupole mass spectrometer for mass analysis, and subsequently counted for identification and quantification. The analyte in the sample can be identified by comparing the observed product masses with the database of precursor ion-VOC reaction products. The concentration of the analyte in the sample can be calculated from the ratio of the number densities of ion products to precursor ions and the known experimental parameters and reaction rate coefficients. An example of breath profiles measured in the SIM mode comparing the concentrations of acetonitrile, acetone, hexanal, and isoprene in exhalations of a smoker versus a non-smoker is shown in Fig. 2.

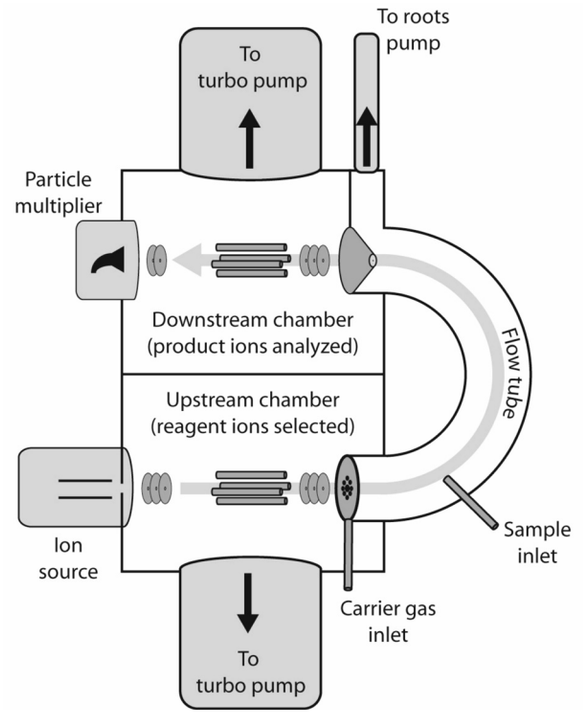

Fig. 1. Schematic diagram of SIFT-MS (reproduced with the permission of Syft Technologies Ltd.).

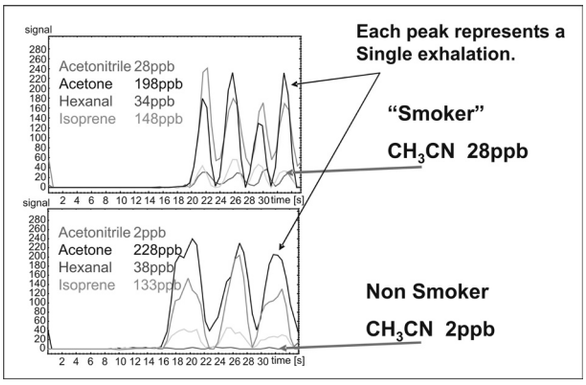

Fig. 2. Breath profile of smoker verse non-smoker (reproduced with the permission of Syft Technologies Ltd.).

For medical applications, SIFT-MS has several major advantages over other analytical techniques, such as GC-MS, ion-mobility spectrometry (IMS) and proton transfer reaction mass spectrometry (PTR-MS).[15] It offers real time detection and quantification of test samples regardless of humidity, and without any requirement for pre-concentration or sample preparation. It can detect several target compounds simultaneously or record full profiles over a selected mass range. The combination of three precursor ions, which react differently with the sample molecules, provides internal verification for accurate compound identification and the ability to distinguish between some isobaric and isomeric compounds.[16] For example, acetone and propanal (C_3H_6O) both result in product ions at *m/z* 59 $(M+H)^+$ by proton transfer from the H_3O^+ ion. However, NO^+ reacts with acetone via addition to form *m/z* 88 $(M+NO^+)$, but reacts with propanal via hydride ion transfer to form $(M-H)^+$ at *m/z* 57. O_2^+ offers further verification because acetone undergoes a charge transfer reaction with partial dissociation resulting in $MeCOMe^+$ (*m/z* 58) and $MeCO^+$ (*m/z* 43). The O_2^+ precursor characteristically undergoes charge transfer reactions with some compounds which do not react with either H_3O^+ or NO^+, *e.g.* NO, NO_2 and some smaller hydrocarbons.[17] Moreover, it reacts with NH_3 in a range of humid samples such as urine, blood headspace, and breath to provide verification for ammonia quantification using the H_3O^+ precursor. With H_3O^+ precursor ions, hydrated ions like $[H_3O^+.(H_2O)_n]$ (n = 1-3) and $(MH^+.H_2O)$ are also formed in a sample of air. These provide extra assistance in compound identification and establishing the humidity of the sample.[18]

The SIFT-MS instrument usually operates in one of two modes. The first is the selected ion monitoring mode (SIM), where only count rates of selected precursor ions and product ions are monitored simultaneously. The concentration of the target compound is calculated each time all the precursor and product ions are counted, resulting in a real time response to any changes in the target concentration. The sampling time between each data point depends largely on the collective monitoring time of the precursor ions (typically 25 ms each) and the product ions (typically 100 ms each). It is possible to quantify a large selection of target compounds; however, the more ions that are included per cycle, the longer the sampling time required between each cycle. This SIM mode is ideal for monitoring specific biomarkers in breath, such as ammonia, acetone, and isoprene.

When a large number of product ions are required to be monitored, or potential biomarkers are unknown, it can be more practical to operate under the second mode, which is the full mass-scan mode (MS). In the MS mode, a complete mass spectrum is obtained by sweeping the downstream quadrupole over a selected mass-to-charge (*m/z*) range for a chosen time with a chosen precursor ion. In this mode, the electronic settling time for the quadrupole to switch between difference masses is minimized. As the full mass spectrum of the sample gas is collected, it is possible to compare the *mass profile* of different samples using classifying algorithms.[19] A study on the use of VOC profiles obtained from breath samples analysed by GC-MS has

been reported for the diagnosis of lung cancer.[20] Studies are currently underway utilizing a classifying algorithm to compare mass profiles of control groups of breath samples in order to establish possible *mass profile* classifications and identify potential biomarkers for clinical conditions.

Applications to Medical Diagnosis

As discussed, the high sensitivity (detection range: 50 pptb to 40 ppmv), and real-time non invasive monitoring of breath samples by SIFT-MS make the methodology particularly applicable to medical testing.[21] Since the establishment of SIFT-MS testing capabilities in NZ, there have been several studies carried out which highlight the advantages of this technique.

Identification of infection

SIFT-MS can be used to quantify a number of VOCs in a single assay; this trait can be used to identify organisms or diseases by characterizing a specific VOC fingerprint. For example, Scotter *et al*.[22] found that it was possible to distinguish between several medically important fungi because the presence and quantity of the VOCs produced during culture varied. Although the presence of ethanol, methanol, acetone, acetaldehyde, methanethiol, and crotonaldehyde was dependant on the culture medium, there is potential for species specific identification which would enable targeted treatments.

Similarly SIFT-MS has been used to detect VOCs produced by bacteria. The high sensitivity of SIFT-MS compared to conventional blood culture systems, *e.g*. BacT/ALERT, makes earlier diagnosis of bacteremia (the presence of viable bacteria in the circulating blood) and identification of bacteria from the metabolic VOC fingerprints possible, if several VOC are analysed.[23] The early detection and identification of aerobic and anaerobic blood infections based on SIFT-MS technology provides clinical advantages over conventional methods. It may also be possible to extend this application to predict antibiotic susceptibility by monitoring changes to the bacterial VOC profile in the presence of antibiotics.[24] Potentially, SIFT-MS breath testing of patients could also be used to diagnose the presence of bacterial or fungal infection.

Monitoring exposure

The measurement of exposure to solvents in the workplace is receiving increasing attention by occupational health and safety regulators. SIFT-MS provides a rapid, accurate, and inexpensive method to monitor biological levels of solvents in the headspace of urine, saline, whole blood, red cells in saline, and plasma.[25] The instrument has sufficient sensitivity so that no pre-concentration of the samples are required, and several solvents can be monitored simultaneously if required. This leads to results being reported much more rapidly than by existing GC-MS methods.[26]

We have used SIFT-MS to quantify the amount of methanol, methyl ethyl ketone (MEK), and acetone in the headspace above urine that had known amounts of the solvents added. As expected, there is a linear relationship between the concentration in the urine and the amount measured in the headspace.[26] Similarly, Wilson *et al*.[27] measured the ethanol present in the headspace of blood and aqueous samples that contained known amounts of ethanol and found a linear correlation.

Real time analysis by SIFT-MS allowed this last group[28] to follow the decay of solvent levels after controlled exposure. The decay of xylene and mesitylene quantified in breath samples was fitted to a two compartment model. Concurrent blood samples were taken and it was shown that the amounts measured in the blood headspace and breath samples correlated. The amounts measured in the breath samples were about two-fold higher, which probably reflects the low solubility of the chemicals in blood.[28]

Commercially available Tedlar bags have been used to collect then transport samples to the SIFT-MS instrument when direct analysis is not possible. This technique was used to determine the VOC present in surgical plumes. Mass scans were used to determine VOC of interest, followed by SIM scans to accurately quantify the concentrations present of these and previously determined compounds. The VOCs produced included cardio-toxic HCN and carcinogenic buta-1,3-diene. However, the concentrations of these compounds within the surgical plume were less than those produced by a cigarette.[10]

Breath Analysis

Respiratory inflammation

Volatile halo-amines have been proposed as markers of eosinophil and neutrophil inflammation, however, their low concentration and reactivity in breath had previously made detection difficult. SIFT-MS has been used to measure several highly reactive, rapidly decomposing VOCs of interest. In addition, it has been shown that SIFT-MS has the sensitivity to quantify monobromamine (NH_2Br), monochloramine (NH_2Cl) and dichloramine ($NHCl_2$) in breath.[29]

Dialysis efficacy

As discussed previously, two modes of operation are possible with SIFT-MS: a screening mode, which uses a full mass scan, or the selected ion monitoring (SIM), which targets VOCs. The mass scan mode of SIFT-MS lends itself to the identification of volatile biomarkers, often present in trace amounts that are not detectable by other methods. Comparison of mass scans collected at different stages of a disease, or before and after interventions, can result in the identification of biomarkers for these conditions. The development of a classification algorithm has simplified this process.[19]

The mass scan algorithm has been used to determine which VOCs are most affected by dialysis. Change analysis of density profiles of mass scan VOCs pre- and post-haemodialysis showed that, in breath samples, ammonia was the marker exhibiting the greatest change. Breath ammonia was then monitored by SIM scan prior to and after the completion of dialysis. Traditionally dialysis efficacy is monitored by measuring the urea reduction ratio which requires measurement of plasma urea concentrations pre- and post-dialysis. Laboratory turn-around times for this test means that results are not available until the following dialysis session, and the invasive nature of the blood samples makes moni-

toring dialysis during the session difficult. SIFT-MS analysis of breath ammonia occurs in real time during the dialysis session, and is non-invasive so can be used to optimize the length of dialysis treatments.[30]

The mass scan mode has also been used to determine potential sources of interference in breath analysis. Epton *et al*.[31] investigated the *m/z* values most likely to appear after the use of CFC inhalers after noticing new masses present in the mass scan from a volunteer who had recently used the medication. The ions present were consistent with the predicted spectra of several freons present in the inhalers. The measurement of VOCs with similar masses would be affected by the presence of these compounds, and without the mass scan ability of SIFT-MS this interference may have gone undetected.[31]

Biological processes

The rapid analysis achieved by SIFT-MS is beneficial for monitoring the changes in biological processes. Changes to isoprene, ethanol, and acetone were quantified after cigarette smoking, and the effects of exercise on breath VOC have also been investigated.[13]

Ammonia, acetone, and isoprene vary with time during exercise, in particular breath acetone increased for most subjects and isoprene concentrations decreased.[7]

Conclusion

Real time analysis of breath samples, and rapid analysis, without sample preparation, of the headspace of blood or urine by SIFT-MS are proving to be valuable tools for medical diagnosis and the monitoring of disease. The SIM mode is ideal for quantifying known biomarkers, and the mass scan mode has made it possible to identify previously unknown markers. Bedside testing will become a reality when the instrument is further reduced in size thereby increasing the possible applications of SIFT-MS by allowing breath analysis of immobile patients. Since its establishment in NZ, SIFT-MS has proved to be a useful technique in medical applications, and future work will expand upon these to build the capabilities of SIFT-MS in the medical field.

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References

1. Adams, N.G.; Smith, D. *Int. J. Mass spect. Ion Phys.* **1976**, *21*, 349-359.
2. Smith, D.; Spanel, P. *Mass Spectrom. Rev* **2005**, *24*, 661-700.
3. McEwan M.J. In *Adv. Gas Phase Ion Chem.* Vol. 1, Adams N.G. and Babcock L.M. (Eds.), JAI Press, Greenwich, CN, 1992, 1-42; Knight, J.S.; Freeman, C.G.; McEwan, M.J.; Adams, N.G.; Smith, D. *Int. J. Mass Spectrom. Ion Proc.* **1985**, *67*, 317-330; MacKay, G.I.; Vlachos, G.D.; Bohme, D.K.; Schiff, H.I. *Int. J. Mass Spectrom. Ion Proc,* **1980,** *36*, 259-270; Copp, N.W.; Hamdan, M.; Jones, J.D.C.; Birkinshaw, K. *et al*. *Chem. Phys. Lett.* **1982**, *88*, 508-511; Glosik, J.; Twiddy, N.D.; Javahery, G.; Ferguson, E.E. *Int. J. Mass Spectrom. Ion Proc.* **1991**, *109*, 75-81; Squires, R.R. *Int. J. Mass Spectrom. Ion Proc.* **1992**, *118*, 503-518; Tsuji, M.; Aizawa, M.; Nishimura, Y. *J. Phys. Chem.* **1995**, *99*, 3195-3200.
4. Spanel, P.; Smith, D. *J. Chem. Phys.* **1996**, *104*, 1893-1899; Smith, D.; Spanel, P. *Rapid Comm. Mass Spectrom.* **1996**, *10*, 1183-1198.
5. Fairley, D.A.; Scott, G.B.I.; Freeman, C.G.; Maclagan, R.G.A.R. *et al*. *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 1305-1309; Matthews, K.K.; Adams, N.G.; Fisher, N.D. *J. Phys. Chem. A* **1997**, *101*, 2841-2847; Spanel, P.; Ji, Y.F.; Smith, D. *Int. J. Mass Spectrom,* **1997**, *165*, 25-37; Wang, T.S., *Spectrosc. Spectr. Anal*., **2006**, *26*, 747-752; Schoon, N; Amelynck, C; Debie, E; Bultinck, P; Arijs, E. *Int. J. Mass Spectrom*. **2007**, *263*, 127-136; Francis, G.J.; Milligan, D.B.; McEwan, M.J. *J. Phys. Chem. A* **2007**, *111*, 9670-9679.
6. Spanel, P.; Dryahina, K.; Smith, D. *Int. J. Mass Spectrom.* **2006**, *249*, 230-239.
7. Senthilmohan, S.T.; Milligan, D.B.; McEwan, M.J.; Freeman, C.G. *et al*. *Redox Rep*. **2000**, *5*, 151-153.
8. Milligan, D.B.; Wilson, P.F.; Mautner, M.N.; Freeman, C.G. *et al*. *J. Environ. Qual.* **2002**, *31*, 515-524; Clough, T.J.; Sherlock, R.R.; Mautner, M.N.; Milligan, D.B. *et al*. *Aust. J. Soil Res.* **2003**, *41*, 421-438.
9. Wilson, P.F.; Freeman, C.G.; McEwan, M.J. *Int. J. Mass spectrum.* **2003**, *229*, 143-149.
10. Moot, A.R.; Ledingham, K.M.; Wilson, P.F.; Senthilmohan, S.T. *et al*. *ANZ J. Surg.* **2007**, *77*, 20-23.
11. Davis, B.M.; Senthilmohan, S.T.; Wilson, P.F.; McEwan, M.J. *Rapid Comm. Mass Spectrom.* **2005**, *19*, 2272-2278; Davis, B.M.; McEwan, M.J. *J. Agric. Food Chem.* **2007**, *55*, 3334-3338.
12. Wilson, P.F.; Prince, B.J.; McEwan, M.J. *Anal. Chem*. **2006**, *78*, 575-579.
13. Senthilmohan, S.T.; McEwan, M.J.; Wilson, P.F.; Milligan, D.B. *et al*. *Redox Rep.* **2001**, *6*, 185-187
14. Smith, D.; Spanel, P. *Int. Rev. Phys. Chem.* **1996**, *15*, 231-271.
15. Critchley, A.; Elliott, T.S.; Harrison, G.; Mayhew, C.A. *et al*. *Int. J. Mass Spectrom.* **2004**, *239*, 235-241. Wilson, H.K.; Monster, A.C. *Occup. Environ. Med.* **1999**, *56*, 753-757.
16. Spanel, P.; Smith, D. *Int. J. Mass Spectrom.* **1999**, *187*, 139-147; Midey, A.J.; Williams, S.; Viggiano, A.A. *J. Phys. Chem. A* **2001**, *105*, 1574-1578.
17. Spanel, P.; Smith, D. *Rapid Comm. Mass Spectrom.* **2000**, *14*, 646-651.
18. Spanel, P.; Smith, D. *Rapid Comm. Mass Spectrom.* **2000**, *14*, 1898-1906.
19. Moorhead, K.T.; Lee, D.; Chase, J.G.; Moot, A.R. *et al*. *Comput. Methods Prog. Biomed* **2008**, Jan 8, E-public. ahead of print.
20. Phillips, M.; Gleeson, K.; Hughes, J.M.B.; Greenberg, J. *et al*. *Lancet* **1999**, *353*, 1930-1933.
21. Freeman, C.G.; McEwan, M.J. *Aust. J. Chem.* **2002**, *55*, 491-494.
22. Scotter, J.M.; Langford, V.S.; Wilson, P.F.; McEwan, M.J. *et al*. *J. Microbiol. Methods* **2005**, *63*, 127-134.
23. Allardyce, R.A.; Langford, V.S.; Hill, A.L.; Murdoch, D.R. *J. Microbiol. Methods* **2006**, *65*, 361-365; Scotter, J.M.; Allardyce, R.A.; Langford, V.S.; Hill, A.L. *et al*. *J. Microbiol. Methods* **2006**, *65*, 628-631.
24. Allardyce, R.A.; Hill, A.L.; Murdoch, D.R. *Diagn. Microbiol. Infect. Dis*. **2006**, *55*, 255-261.
25. Wilson, P.F.; Freeman, C.G.; McEwan, M.J.; Allardyce, R.A. *et al*. *Appl. Occup. Environ. Hyg.* **2003**, *18*, 759-763.
26. Storer, M.; Senthilmohan, S.; George, P. In *Harnessing New Technology for Clinical Diagnostics*, Hyatt Regency: Union Station, St. Louis, Missouri, 2007.
27. Wilson, P.F.; Freeman, C.G.; McEwan, M.J.; Milligan, D.B. *et al*. *Rapid Comm. Mass Spectro.* **2001**, *15*, 413-417.
28. Wilson, P.F.; Freeman, C.G.; McEwan, M.J.; Milligan, D.B. *et al*. *Rapid Comm. Mass Spectrom.* **2002**, *16*, 427-432.
29. Senthilmohan, S.; Kettle, A.; McEwan M.J.; Dummer, J. *et al*. *Rapid Comm. Mass Spec* **2008**, *22*, 677-681.
30. Scotter, J.; Moorehead, K.; Allardyce, R.A; Storer, M. *et al*. Int. Assoc. Breath Research *Breath Analysis Summit 2007: Clinical Applications of Breath Testing* Cleveland, Ohio, 2007.
31. Epton, M.J.; Ledingham, K.; Dummer, J.; Rhodes, B. *et al*. Int. Assoc. Breath Research *Breath Analysis Summit 2007: Clinical Applications of Breath Testing: 2007*, Cleveland, Ohio, 2007.