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WHITE PAPER

SIFT-MS: A new approach to the detection of microbial growth and identification of micro-organisms

Summary

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) provides a new approach to the quantitative detection of volatile microbial metabolites. Through the direct and highly-sensitive analysis of headspace samples, SIFT-MS rapidly and accurately detects the metabolites produced by a diverse range of microbial pathogens that have been cultured on a range of standard media. Comparison of the metabolite profiles has proven to be a reliable method for the early detection, quantification and identification of clinically important organisms.

SIFT-MS analysis of the headspace of microbial cultures can, therefore, significantly cut diagnosis time for microbial infections. Moreover, it provides a significant advance in the study of the metabolic processes that produce microbial volatile organic compounds (MVOCs).

Current methods of MVOC and microbe detection

It is well known that microbes produce volatile organic compounds (so-called microbial volatile organic compounds, MVOCs) as a result of their normal metabolic processes. MVOCs can therefore be used as indicators of active microbial growth. This has been used in diverse applications, such as analysis of microbial spoilage of foodstuffs [Gardener and Paterson (1975); Dainty *et al*. (1989); Borch *et al*. (1996)], studies of microbial contamination of residential dwellings [Wady *et al*. (2003)] and pathology [Larsson *et al*. (1978a, 1978b); Brogan *et al*. (1997); Kiviranta *et al*. (1998); Julak *et al*. (2000)].

While MVOCs provide a reliable indicator of microbial activity, traditional MVOC profiling methods suffer from low sensitivity and require sustained microbial growth, and substantial MVOC production, before microbes are detected. The resulting long culture times and possibility of infections moving into advanced stages before detection occurs and diagnosis is made, means MVOC analysis has not been suitable for routine use in time-critical clinical applications or high-throughput diagnostic laboratories.

For example, rapid and accurate laboratory diagnosis of bloodstream infections is critical for management of patients with suspected sepsis. Early diagnosis enables the clinician to apply appropriate antimicrobial therapy, or indicates proceeding with further investigations. Using current methods, a positive diagnosis usually requires at least 12 hours of primary culturing, followed by subculturing to determine which organism(s) are present. These delays greatly complicate treatment options and increase costs, patient recovery times and mortality rates.

A clinically-acceptable technique based on MVOC analysis must therefore supply an early positive result and identify which microbial organism(s) are present, even when minimal numbers of microbes are present in the sample. SIFT-MS fulfils these criteria.

SIFT-MS and the quantitative detection of MVOCs and microbes

SIFT-MS [Španel and Smith (1996); Freeman and McEwan (2002)] has four key features that provide significant benefits for MVOC analysis and the identification of microbes. These benefits are:

- Extremely fast analysis times (<20 seconds);
- Minimal sample preparation, with the ability to directly sample and analyse headspace gas;
- Sensitivity to very low part-per-billion (ppb) levels;
- Non-discriminatory (the ability to simultaneously quantify MVOCs with diverse chemical properties, as produced by microbes).

SIFT-MS analyses headspace gas samples for MVOCs produced by microbes that have been cultured in standard blood culture systems (standard blood culture bottles and growth media). The culture is prepared and incubated using normal microbial procedures. Headspace gas is extracted from the culture bottle by the vacuum of the SIFT-MS sampling system, once the culture bottle's septum has been pierced by the instrument's sampling needle. Since no sample preparation (beyond normal culturing) is required, the risk of sample contamination is low, while operator safety is high.

Similar procedures can be used in research laboratories, where microbes may be grown on a range of solid media, and headspace gases are analysed as above. Such research may involve, for example, investigation of variations in volatile metabolite production under different growth conditions.

The high sensitivity of SIFT-MS means that a wide range of MVOCs can be detected relatively early in the incubation cycle. Furthermore, the data generated allows identification of common microbes at around the same time that a positive culture is detected.

Figures 1 and 2 provide examples of results obtained using SIFT-MS. Figure 1 shows levels of MVOCs detected by SIFT-MS from two medically important bacteria, *Streptococcus pneumoniae* and *Neisseria meningitidis*. In this example, blood culture bottles were inoculated with five colony forming units (cfu) and incubated with agitation at 37°C for eight hours. SIFT-MS was then used to analyse the headspace, with positive results being recorded for a range of metabolites. Notice the very different volatile metabolite profile arising from the two species.

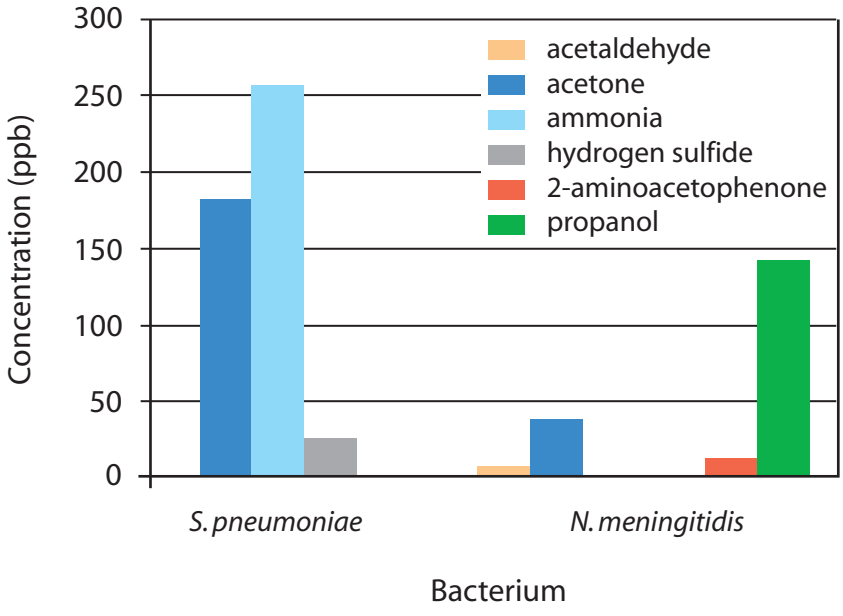

Figure 1: *MVOC concentrations (in parts-per-billion by volume, ppb) for two bacteria in blood after incubation for eight hours.*

Figure 2 shows levels of MVOCs detected by SIFT-MS from two fungi, *Aspergillus fumigatus* and *Mucor racemosus*, cultured on malt extract agar. Fungi were grown at 30°C for four days, then target headspace gases quantified by SIFT-MS.

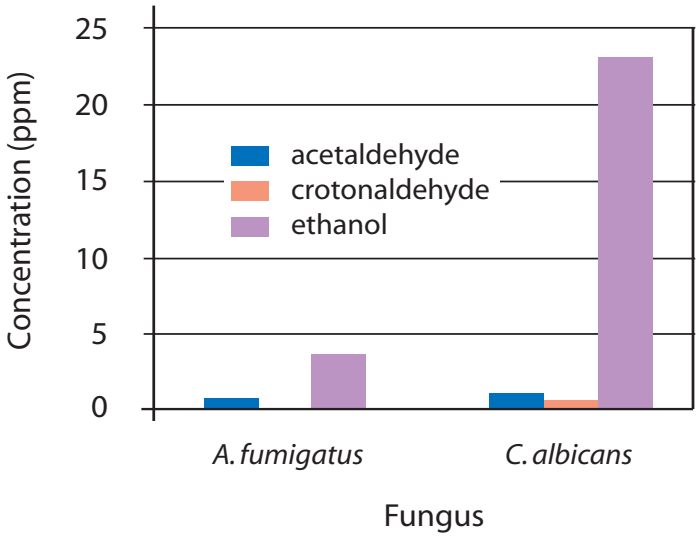

Figure 2: *MVOC concentrations (in parts-per-million by volume, ppm) for two fungi on malt extract agar, after incubation for four days.*

Conclusion

SIFT-MS eliminates many of the problems associated with existing MVOC analysis techniques – analysis is very rapid and highly sensitive, requiring no sample preparation or preconcentration of the headspace gas.

Rapid, quantitative detection of MVOCs using SIFT-MS, combined with the ability to directly sample headspace gas, allows timely clinical diagnosis of microbial infections. By analysing volatile profiles, SIFT-MS is also ideally suited to investigation of factors that impact microbial metabolic pathways.

SIFT-MS is a powerful new tool for clinical detection and identification of microbial infections.

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