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Note

Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS)

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Abstract

To achieve faster bacteremia diagnosis, selected ion flow tube mass spectrometry (SIFT-MS) measured metabolic gases in the headspaces of BacT/ALERT® blood culture bottles. *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Neisseria meningitidis* growth and trace gas patterns were detected from 10 colony forming units after 6 h.

Keywords: Mass spectrometry; SIFT-MS; Bacteremia diagnosis; Blood culture; BacT/ALERT

In the United States, bloodstream infection incidence is estimated at about 500,000 per year (Centers for Disease Control and Prevention, 1992; Weinstein et al., 1997). In healthy subjects, a sudden release of bacteria is cleared from the blood within 30 to 45 min. Increasing bacteremia and consequential death in immunocompromised hosts (Hotchkiss and Karl, 2003) have resulted from increased chemotherapy, bone marrow transplants and prevalence of human immunodeficiency viral infection (Bryan, 1989; Bow, 1998; Tumbarello et al., 1998). The crude mortality rate due to bacteremia ranges from 25% to 50%, with nearly one third of deaths attributable to these infections (Berger et al., 1990; Bille et al., 1984; Birkenmeyer and Armstrong, 1992; Thomsen et al., 2005).

Suspected bacteremia is empirically treated with broad spectrum antibiotics because definitive diagnosis and antibiotic sensitivities require time consuming blood cultures. These delays significantly increase patient mortality, while empiric antibiotics result in greater expense (Pittet et al., 1994) and increasing antimicrobial resistance (Levy, 2005; Jones, 2001). Appropriate, early antibiotic treatment results in lower mortality rates (10.4%) than when given once blood culture results are known (25.8%) (Weinstein et al., 1997).

Volatile organic compound (VOC) metabolites shown by gas chromatography-mass spectrometry (GC-MS) (Larson et al., 1978a,b; Larsson and Holst, 1982; Julak

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Table 1
Volatile metabolites identified using SIFT-MS for five bacterial strains cultured in BacT/ALERT® FA blood culture bottles*

VOCs	*Escherichia coli*	*Neisseria meningitidis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pneumoniae*
Ethanol	XXX	XX		XXX	XXX
Pentanol isomer(s)	XX				
Formaldehyde	X	X			X
Acetaldehyde	XX			XXX	XX
Acetic acid	XX				
Hydrogen sulfide	XXX		X	XX	
Methanethiol (methyl mercaptan)	XXX	X	X	XXX	
Dimethyl sulfide	XX	XX	XX		X
Dimethyldisulfide	XX				
Trimethylamine	XX			X	X
Indole	XX	X		X	X
Propanol	XXX				
Aminoacetophenone	XXX			XX	XX
Hexanal	XX			X	X

*Relative levels of analytes present in bacterial cultures (test — medium/blood control).
X=<100 counts per second (cps); XX=100–1000 cps; XXX=>1000 cps.

et al., 2000; Würst, 1977) or selected ion flow tube mass spectrometry (SIFT-MS) (Wang et al., 2004) reflect bacterial growth in vitro. The commercial SIFT-MS produced by Syft Technologies Ltd (Christchurch, New Zealand) measures complex mixtures of gases regardless of the water vapor content in real time without sample preparation. Given the importance of early diagnosis and bacterial identification, this study describes SIFT-MS identification and quantitation of VOCs in the headspace above five clinically important bacterial species cultured in BacT/ALERT® media.

SIFT-MS has been described in detail previously (Spanel and Smith, 1996; Smith and Spanel, 2005). Briefly, the headspace of type strain-inoculated Biomerieux BacT/ALERT® blood culture bottles (Biomerieux, Durham, NC) is sampled through the sample inlet needle of the SIFT-MS. The sample reacts with quadrupole mass-selected H_3O^+, NO^+ or O_2^+ precursor ions. The resulting product ions are mass selected by a second "downstream" quadrupole and detected using a particle multiplier. The detection system is operated in either full mass scan or selected ion modes (SIM). Mass scans commonly identify compounds present in the sample, and were employed here for this purpose. SIM measures only pre-selected product mass(es) and is more rapid, sensitive and straightforwardly quantified than mass scans. It was used in this work to yield absolute concentrations of targeted VOCs in the culture headspaces.

Streptococcus pneumoniae (ATCC 49619), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Neisseria meningitidis* (NZESR 1033) were serially diluted for inoculation from initial suspensions defined by turbidometry and confirmed by quantitative plate counts (data not shown).

Triplicate test cultures were prepared in standard BacT/ALERT® FA aerobic culture bottles, containing 40 ml of medium, 9 ml of uninfected, heparinized human blood and 1 ml of each bacterial species in sterile saline. Control bottles were not inoculated. Cultures used to obtain 24-h SIFT-MS mass scans were inoculated with approximately 100 organisms of each species while cultures used to obtain SIM data at 6 and 24 h were inoculated with 10 bacteria each. Cultures

Table 2
Bacterial VOC concentrations at 6 h in BacT/ALERT® FA

6 h culture	Analyte VOC concentration (parts per billion v/v)*								
	Acetaldehyde	Acetic Acid	Ethanol	Acetone	Ammonia	H_2S	Methanethiol	DMS	DMDS
Control medium+blood	890	6900	1200	3600	1000	10	ND	690	200
Pseudomonas aeruginosa	1100	16,000	1500	6600	1100	40	ND	860	330
Streptococcus pneumoniae	5300	460	3000	5100	370	20	60	4300	180
Escherichia coli	11,000	5400	21,000	6100	500	4100	750	9600	430
Staphylococcus aureus	2400	1200	5800	3500	1800	60	180	2200	280
Neisseria meningitidis	350	870	770	7900	1200	ND	ND	320	360

*Analyte concentration minima and maxima did not exceed ±20% of triplicate mean values. ND=not detected.