Diagnosis of bacteraemia by automated head-space capillary gas chromatography

L LARSSON, P-A MÅRDH, G ODHAM,* M-L CARLSSON

From the Department of Medical Microbiology, University of Lund, Sölvegatan 23, S-223 62 Lund, and the *Laboratory of Ecological Chemistry, University of Lund, Lund, Sweden

SUMMARY Blood cultures from 196 patients with suspected bacteraemia or sepsis were analysed by automated head-space gas chromatography, using a 25 m fused silica capillary column, when turbidity indicated growth. Gas chromatography correctly identified 105 cultures as positive and 71 correctly as negative. No false-positive results were obtained. Of the 20 false-negative chromatographic results, Staphylococcus spp accounted for 14. Automated head-space gas chromatography is quicker, easier and more efficient than other gas chromatographic techniques for the evaluation of blood cultures.

Head-space gas chromatography (HSGC) has been applied in analyses of volatile acidic, neutral and alkaline bacterial metabolites. It makes extraction and derivatisation procedures unnecessary. It is non-deleterious to the gas chromatographic (GC) column and is suited to automation. Because it is quick and simple to perform, the method is an attractive alternative to conventional GC techniques in the clinical microbiological laboratory for the demonstration of volatile bacterial products.

In recent years, efforts have been made to speed up the culture diagnosis of bacteraemia by new techniques, such as radiometry and impedance measurements. Gas chromatography has also proved valuable for the demonstration of anaerobes by analysis of diethyl ether extracts of blood cultures.

The present report describes an application of HSGC analysis for the demonstration of aerobic, facultatively and strictly anaerobic bacteria in blood cultures. The system includes an automatic head-space injection unit, a 25 m fused silica capillary column to obtain an efficient separation of the bacterial metabolites and a computer terminal for evaluation of the results.

Material and methods

CULTURE MEDIA
Two blood culture media were used. Their composition has been described in detail elsewhere. Briefly, they consisted of Trypticase soy broth, sucrose, glucose, and sodium polyethanol sulphonate in distilled water. When used for the isolation of anaerobic bacteria, in an atmosphere of 90% CO₂ and 10% H₂, sucrose was replaced by cysteine hydrochloride. Portions of the media (45 ml) were transferred to 100 ml glass bottles which were sealed with rubber membranes and aluminium crimp caps, and used within one week.

CULTURE TECHNIQUE
Five ml of blood sample, collected from patients with suspected sepsis, was injected through the rubber membrane of each sealed medium-containing glass bottle. Blood for both aerobic and anaerobic culturing was collected from every patient. The bottles were incubated at 37°C and examined daily. When turbidity suggested bacterial growth, subculturing on blood and haematin agar was carried out. The haematin agar plates were incubated in a 5-10% CO₂ atmosphere. From the anaerobic blood culture bottles, subcultures to freshly prepared blood agar plates were incubated anaerobically. Simultaneously, a 1 ml sample was taken for HSGC analysis.

Apart from being subcultured when growth was suspected, all aerobic blood cultures were routinely plated on blood and haematin agar after one day of incubation and the anaerobic cultures on fresh blood agar plates after three days' incubation. The isolated organisms were identified according to established routines in the Bacteriological Laboratory, University Hospital, Lund, Sweden.

PREPARATION OF SAMPLES FOR ANALYSIS
The samples (1 ml) to be analysed by HSGC were
Results of automated head-space gas chromatography (HSGC) analysis of blood cultures

<table>
<thead>
<tr>
<th>Organisms recovered from blood culture</th>
<th>No of cultures where growth indicated by:</th>
<th>No false-negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turbidity</td>
<td>HSGC</td>
</tr>
<tr>
<td></td>
<td>TOT</td>
<td>AE*</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Enterobacter cloaceae</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Acinetobacter sp</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Campylobacter fetus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella oranienburg</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Diphtheroid rods</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fusobacterium necrophorum</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Yeasts (Candida spp)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Culture-negative bottles</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Negative controls</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>116</td>
</tr>
</tbody>
</table>

TOT = total number of patients from whom cultures were studied.

*Number of cultures incubated under aerobic (AE) or anaerobic (ANA) conditions, suspected of containing micro-organisms by turbidity and HSGC, respectively.

Transferred to 12 ml glass vials fitting the automatic turntable of the HSGC analyser (see below), to which approximately 2 g of anhydrous sodium sulphate, 10 drops of diluted (25%, vol/vol, in water) sulphuric acid, and pivalic acid, used as internal standard, had been added. The vials were then sealed prior to HSGC analysis as previously reported.1

Samples from a total of 196 patients were analysed. In 146 cases, the blood cultures showed turbidity in either the aerobic or the anaerobic culture bottle. The cultures from the remaining 50 patients showed no turbidity. These patients served as controls.

Automated head-space analysis

A Perkin-Elmer model F45 gas chromatograph equipped with a device for automatic head-space injection was employed. The glass vials were kept at 75°C in the automatic turntable of the apparatus for at least 20 min prior to analysis to ensure temperature equilibration. The temperature of the injection needle and flame ionisation detector was 150°C and that of the column 117°C. A 25 m fused silica capillary column coated with SP-1000 (id 0.2 mm), at a nitrogen carrier gas flow rate of 1.5 ml/min, was used. The injections were carried out at a split ratio of 1:20, and the make-up gas flow rate was 20 ml/min. The attenuation was kept at 2 in all analyses, and the injection period was 7 s.

A Perkin-Elmer chromatography terminal, model Sigma 10, was used to calculate relative peak areas.

Mass spectrometry

Peak identification was accomplished using a Ribermag model R10-10 GC/MS combination. The GC conditions were those described above. The analyses were carried out by manual head-space injection of 0.5 ml of vapours using a 1 ml gas-tight syringe.1 The ion source was held at 220°C and the electron energy was 70 eV.

Results

Identity of strains

The identities of the strains recovered from the blood cultures tested are given in the Table. Of the total number of 125 strains isolated, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus and S. epidermidis accounted for 102 (82%). No cases of mixed infections were detected.

HSGC studies on blood cultures

Chromatograms of the 71 sterile culture media studied contained one major peak with a retention time of approximately 2.8 min about equal in size to that of the internal standard, which eluted after 4.7 min. The former peak was found by mass spectrometric analysis to represent ethanol. A culture bottle was regarded as "HSGC-positive" if the area of the ethanol peak was at least three times that of the internal standard of pivalic acid, as calculated by the electronic integrator.

Examples of the chromatographic results...
Diagnosis of bacteraemia by automated head-space capillary gas chromatography

obtained are given in the Figure. The specific identities of the causative organisms by direct HSGC study of the blood cultures only were not established. Apart from ethanol, most of the organisms also yielded acetic acid. *Proteus mirabilis* and most of the strains of *Enterobacter cloacae* and *K pneumoniae* were further characterised by the presence of a peak eluting after 2-8 min, found by mass spectrometry to represent isoamyl alcohol. The culture media containing anaerobes—namely, *Clostridium perfringens* and *Fusobacterium necrophorum*, were easily distinguished from the remaining cultures by the presence of large amounts of *n*-butyric (*C perfringens*), and propionic and *n*-butyric (*P necrophorum*) acids, in corresponding chromatograms.

The results of the HSGC analyses are summarised in the Table. Of the total cultures tested (196), 105 were correctly assigned as positive and 71 correctly as negative. Twenty false-negative and no false-positive HSGC results were obtained. Eighteen aerobic and 14 anaerobic turbid culture bottles were culture-negative. HSGC analysis of these blood cultures indicated that all these bottles were sterile.

**Discussion**

Gas chromatography has recently been reported useful for the diagnosis of anaerobic bacteria in blood cultures.8-11 The technique employed in these studies involves both extraction of acidified culture media using diethyl ether followed by GC analysis of the ether phase, and methylation of less volatile acids to form methyl esters. By this method, it is possible to detect fatty acids in most of the cultures containing anaerobes. On the other hand, the presence of facultatively anaerobic and aerobic bacteria can usually not be established by this technique. Since anaerobic bacteria account only for about 10% of all positive blood cultures, GC analysis of ether extracts would therefore be unsuitable for diagnosis in the vast majority of bacteraemia cases.

In contrast to analysis of solvent extracts, the use of HSGC allows detection of compounds with very high volatilities—for example, short-chain alcohols.13 Such compounds are produced by several aerobic and facultatively anaerobic organisms. The peaks corresponding to these compounds are more or less hidden under the large solvent peak when analysing solvent extracts. Consequently, HSGC has an advantage over currently widely used gas chromatographic methods for the study of microorganisms.

Impedance measurements and radiometry have also been used to detect growth in blood cultures.7,8 In contrast to HSGC as described in the present study, these two techniques have the advantage of also detecting bacteria not producing much of volatiles, such as *Pseudomonas* and *Staphylococcus* spp.

Peaks representing short-chain fatty acids indicated presence of anaerobes, and a large peak of isoamyl alcohol indicated *E cloacae*, *K pneumoniae* or *P mirabilis*. Such specificity is lacking in radiometry and impedance techniques.

In the present study, the growth medium for the blood cultures was the standard medium used at the

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*Representative chromatograms obtained by the analysis of blood culture media containing Escherichia coli, Clostridium perfringens and Proteus mirabilis, and of sterile media. Pivalic acid, eluting after 4-7 min used as internal standard, was added to all samples prior to analysis. See text for specifications of analytical test conditions.*
Bacteriological Laboratory, University Hospital, Lund. Modification of the composition of blood culture medium might render HSGC capable of detecting even *Pseudomonas* and *Staphylococcus* spp with high accuracy. This approach has proved useful in the analysis of other clinical samples—for example, urine specimens. The clinical microbiological laboratory might then be able to provide the clinician with a preliminary answer regarding blood culture results more rapidly than when using conventional culture techniques only. However, the present study was not designed to study this aspect.

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Requests for reprints to: Dr Lennart Larsson, University of Lund, Department of Medical Microbiology, Solvegatan 23, S-223 62 Lund, Sweden.
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L Larsson, P A Mårđh, G Odham and M L Carlsson

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