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ASSESSMENT OF HEAD-SPACE GAS—LIQUID CHROMATOGRAPHY FOR THE RAPID DETECTION OF GROWTH IN BLOOD CULTURES

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SUMMARY

Blood for transfusion was inoculated with between 10^0 and 10^2 colony-forming units (CFU) per ml of each of 59 microbial isolates and added to cooked meat broth. At intervals up to 72 h incubation, the cultures were examined by conventional visual inspection and automated head-space gas—liquid chromatography (HS-GLC). Forty-six isolates including all those examined of *Staphyloccoccus aureus, Streptococcus pyogenes, S. pneumoniae, S. faecalis, S. milleri, S. mitior, S. mitis, S. salivarius, S. sanguis, Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Morganella morganii, Serratia* sp., *Enterobacter cloacae, Bacterioides fragilis, Clostridium perfringens, Candida albicans, C. krusei* and *Torulopsis glabrata,* and three isolates of *Staphylococcus epidermidis,* were detected by HS-GLC. HS-GLC failed to detect the growth of eleven isolates including all those of *Pseudomonas aeruginosa, Acinetobacter calcoaceticus, Haemophilus influenzae, Corynebacterium* sp. and two isolates of *S. epidermidis.* The growth of all 59 isolates was detected by visual inspection. No significant difference was found between HS-GLC analysis and visual inspection in the speed of detection of bacterial isolates. All the yeast isolates were detected by HS-GLC after 24 h incubation, indicating that it may be possible to detect fungemias earlier by HS-GLC analysis than by other methods.

INTRODUCTION

One of the highest priorities of bacteriology today is the early detection of bacteremia and septicemia. In most clinical laboratories the detection of growth in blood cultures still depends on the classical method of visual inspection. Despite close attention to macroscopic changes, Gram staining and subculturing, only 50% of positive blood cultures are detected within 24 h of blood collection [1], and therefore results of positive cultures may not be available to the clinician until 72 h after the blood was taken for culture.

Various techniques such as radiometry [2, 3], impedance measurement [4] and centrifugation—filtration [5] have been recently developed for the rapid

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diagnosis of bacteremia and septicemia. Analysis by head-space gas—liquid chromatography (HS-GLC) to detect volatile microbial metabolic products has been proposed as a method for the diagnosis of bacteremia [6, 7], an approach that has already proved useful for the rapid detection of urinary tract infection [8].

The present report compares the rapidity and sensitivity of the HS-GLC method with that of visual inspection for detecting growth in simulated blood cultures.

MATERIALS AND METHODS

Microorganisms

A total of 59 isolates was used in this investigation including five strains each of *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*, four each of *Haemophilus influenzae* and *Proteus mirabilis*, three each of *Klebsiella pneumoniae* and *Candida albicans*, two each of *Streptococcus pyogenes*, *S. pneumoniae* and *Klebsiella oxytoca*, and one each of *Streptococcus milleri*, *S. mitior*, *S. mitis*, *S. salivarius*, *S. sanguis*, *Corynebacterium* sp., *Morganella morganii*, *Serratia* sp., *Enterobacter cloacae*, *Acinetobacter calcoaceticus*, *Bacteroides fragilis*, *Clostridium perfringens*, *Candida krusei* and *Torulopsis glabrata*. These microorganisms were identified using standard methods [9] and the API 20E system (Analytab Products, Plainview, NY, U.S.A.). The microorganisms were then cultured on appropriate solid media and stored at 4° C.

Simulated blood cultures

The medium used was brain—heart infusion broth (OXOID) containing 0.05% sodium polyanethol sulphonate and cooked bullock's heart. The medium was dispensed to give a final volume of 35 ml in 60-ml capacity glass screw-capped bottles and autoclaved at 121° C for 30 min. Immediately before incubation, 5 ml of whole human blood containing 0.327% (w/v) citric acid, 2.63% (w/v) sodium citrate, 0.251% (w/v) sodium acid phosphate and 2.32% (w/v) dextrose (Red Cross Blood Bank, Melbourne, Australia) were inoculated with one of the 59 microbial isolates and added to the culture medium. Inoculated blood media and control media containing uninoculated blood were incubated at 37° C in air and examined by both HS-GLC and visual inspection after 6, 9, 12, 15, 18, 24, 48 and 72 h incubation.

Quantitation of inoculum sizes

Viable counts on overnight cultures in brain—heart infusion broth of the microorganisms under test were performed to determine the inoculum size necessary to provide 10^0—10^2 colony-forming units (CFU) per ml blood in the simulated blood cultures. On the basis of these experiments, each 5-ml aliquot of blood for blood cultures was inoculated with 50 μl of a similar overnight culture diluted 10^{-6} for bacteria and 10^{-4} for yeasts. Confirmatory viable counts to determine the actual size of each inoculum were performed on the culture dilutions used to inoculate blood.

Analysis by HS-GLC

The GLC conditions were as described previously [8], except that the analysis time was 1.5 min. Head-space vapour was released from simulated blood cultures for GLC analysis by adding 2 ml blood culture supernatant to 3 g potassium carbonate in a glass vial provided with the automatic head-space injector, sealing immediately, shaking on a vortex mixer and holding in the injector turn-table at 60°C for injection. The column packed with 0.4% Carbowax 1500 on graphite 60—80 mesh was a new one and the retention times of ethanol, *n*-propanol and trimethylamine were 0.4 min, 0.675 min and 0.55 min, respectively.

Method of visual inspection

The inoculated blood cultures were inspected for the following signs of macroscopic growth: (1) gas bubbles; (2) lysis of blood; (3) reduction of hemoglobin; (4) turbidity; and (5) digestion of meat. Cultures exhibiting any of these features were stained by Gram's stain and subcultured onto appropriate media.

RESULTS

HS-GLC analysis was assessed by comparison with visual inspection for (a) its effectiveness in detecting a range of species (sensitivity) and (b) its speed in detecting the species (rapidity).

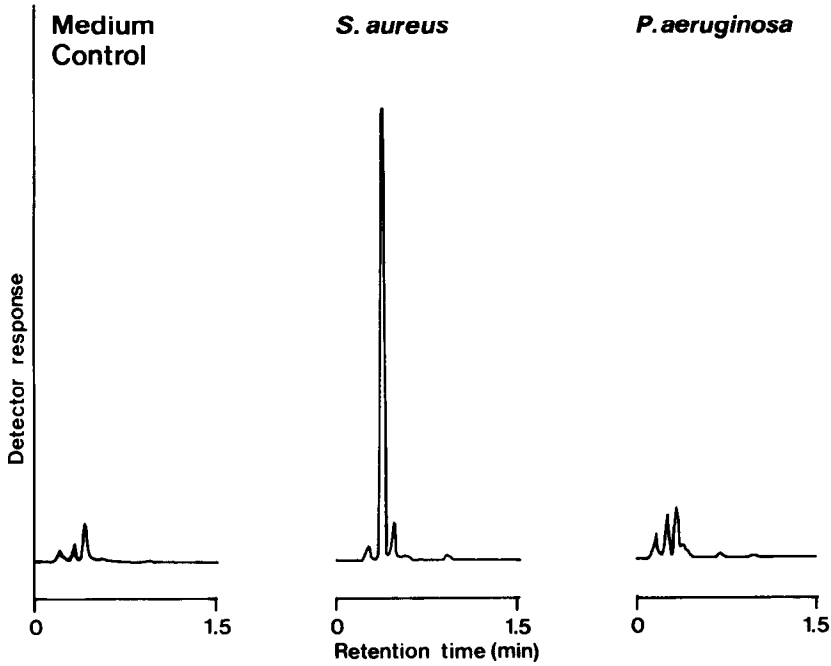

Fig. 1. Chromatograms of incubated, uninoculated blood culture medium (medium control) and of simulated blood cultures inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The principal peak in the chromatogram of the *S. aureus* blood culture is ethanol, with a retention time of 0.4 min.

Range of species detected by HS-GLC

Simulated blood cultures from all 59 microbial isolates, comprising 22 species, showed growth by visual inspection. HS-GLC analysis of uninoculated simulated blood culture media yielded chromatograms in which the peaks were small both before and after incubation (Fig. 1) compared with the size of the ethanol peaks in chromatograms of cultures in which growth was detected by HS-GLC (Fig. 1). Ethanol was detected in 46 of the 59 cultures (Table I) viz. 22 isolates of Gram-positive cocci, including three isolates of *S. epidermidis*, all seventeen isolates of *Enterobacteriaceae*, both isolates of anaerobes and all five isolates of yeasts. Ethanol was not detected in thirteen cultures including all five isolates of *P. aeruginosa* (Fig. 1), one isolate of *A. calcoaceticus*, all four isolates of *H. influenzae*, one isolate of *Corynebacterium* sp. and two isolates of *S. epidermidis*.

After 24 h incubation, a peak with the same retention time as *n*-propanol

TABLE I

RANGE OF SPECIES DETECTED BY HS-GLC IN SIMULATED BLOOD CULTURES

Ethanol in cultures	Species of microorganism	No. of isolates
Produced	*Staphylococcus aureus*	5
	Staphylococcus epidermidis	3
	Streptococcus pyogenes	2
	Streptococcus pneumoniae	2
	Streptococcus faecalis	5
	Streptococcus milleri	1
	Streptococcus mitior	1
	Streptococcus mitis	1
	Streptococcus salivarius	1
	Streptococcus sanguis	1
	Escherichia coli	5
	Klebsiella pneumoniae	3
	Klebsiella oxytoca	2
	Proteus mirabilis	4
	Morganella morganii	1
	Serratia sp.	1
	Enterobacter cloacae	1
	Bacteroides fragilis	1
	Clostridium perfringens	1
	Candida albicans	3
	Candida krusei	1
	Torulopsis glabrata	1
Not produced	*Pseudomonas aeruginosa*	5
	Acinetobacter calcoaceticus	1
	Haemophilus influenzae	4
	Corynebacterium sp.	1
	Staphylococcus epidermidis	2
	Uninoculated culture medium	10

was detected in twelve cultures, including all five isolates of *E. coli*, all three isolates of *K. pneumoniae*, both isolates of *K. oxytoca* and the isolates of *M. morganii* and *C. perfringens*. After 24 h incubation, a peak with the same retention time as trimethylamine was detected in all four isolates of *P. mirabilis*.

Speed of detection by HS-GLC

The inoculum sizes (10^0—10^2 CFU/ml of blood) were chosen to simulate the low order of magnitude of microorganisms generally found in the blood of patients with bacteremia [10].

TABLE II

HS-GLC COMPARED WITH VISUAL INSPECTION FOR THE DETECTION OF GROWTH IN SIMULATED BLOOD CULTURES

	Detection time (h)				No. of isolates
	HS-GLC		Visual		
	Range	Median	Range	Median	
Enterobacteriaceae	9—15	9	9—12	12	17
Bacteroides fragilis		48		48	1
Clostridium perfringens		12		12	1
Candida albicans		24		48	3
Candida krusei		24		72	1
Torulopsis glabrata		24		72	1
Staphylococcus aureus	15—18	15	12—48	18	5
Staphylococcus epidermidis	18—24	18	18—24	18	3
Streptococcus pyogenes		15, 18		18	2
Streptococcus pneumoniae		15, 18		18	2
Viridans *Streptococci*	15—24	18	12—15	12	5
Streptococcus faecalis	9—12	9	9—12	9	5

The times at which growth was detected by an ethanol peak in HS-GLC analysis and by visual inspection, are shown in Table II. HS-GLC analyses and visual inspections were done at 3-h intervals between 6 and 18 h. Consequently, if times of detection by HS-GLC and visual inspection are recorded in Table II as differing by 3 h, i.e. one inspection interval, this was the maximum difference and a smaller difference might have been found if the inspection intervals had been shorter. Further analyses and inspections were done at 24 h, i.e. an inspection interval of 6 h, and at 48 and 72 h, i.e. inspection intervals of 24 h. Consequently, differences of 6 h, between 18 and 24 h, or 24 h in times of detection after 24 h, by HS-GLC and visual inspection were maximum differences.

Of the 41 bacterial isolates that showed growth by both HS-GLC analysis and visual inspection, 17 were detected at the same time by both methods, 15 were detected earlier by HS-GLC analysis and 9 earlier by visual inspection. In all but three instances of earlier detection, by one method or the other, the difference was only one inspection interval, usually a maximum of 3 h. In cultures of one isolate of *S. aureus*, growth was detected by HS-GLC analysis

more than one inspection interval earlier than visual inspection; and in the cultures of *S. mitis* and *S. sanguis*, growth was detected by visual inspection more than one inspection interval earlier than by HS-GLC analysis.

The five yeast isolates showed growth by both HS-GLC analysis and visual inspection. All were detected earlier by HS-GLC analysis, three by a maximum of 24 h (one inspection interval) and two by more than 24 h.

DISCUSSION

The assessment of HS-GLC analysis was based upon the sensitivity and rapidity of the technique compared with that of the conventional blood culture method. No false positive results were obtained in our investigation. With HS-GLC analysis, the detection of ethanol production during incubation of blood cultures always indicated microbial growth. The likely source in the blood culture medium from which ethanol is produced is glucose, a carbohydrate that is known to be fermented by all of the detected isolates [9]. However, other compounds present in both the brain—heart infusion broth and the transfusion blood may also be possible sources from which ethanol could be produced. It should be noted that the transfusion blood used for the simulated blood cultures was buffered and contained added dextrose and citrate. Both additives are energy sources for microbial growth. In addition, the inocula for the simulated blood cultures were organisms that had been maintained in the laboratory and thus may have been physiologically different from organisms in the blood of a bacteremic patient. Therefore the sensitivity and rapidity of HS-GLC analysis and the rapidity of visual inspection for the detection of growth in simulated blood cultures may differ from routine blood cultures.

The sensitivity of HS-GLC in detecting a range of species when compared to visual inspection, was 78%. The variety of species detected was similar to those of previous studies [6, 7]. Species detected included *S. aureus*; eight species of *Streptococcus*; seven species of *Enterobacteriaceae*; three species of yeast including *C. albicans*, *C. krusei* and *T. glabrata*; and two anaerobic species, *B. fragilis* and *C. perfringens*.

Production of *n*-propanol by *E. coli* or a related species, and trimethylamine by *P. mirabilis*, have been previously reported [7]. In this investigation, small amounts of *n*-propanol and trimethylamine, compared with ethanol, were detected in 24-h cultures of *E. coli* and *P. mirabilis*, respectively, and may have been detected in early cultures if an integrator had been used. It appears that *n*-propanol and trimethylamine could be useful as markers for the presumptive identification of certain species of *Enterobacteriaceae*.

Species not detected by HS-GLC analysis included two established pathogens, *P. aeruginosa* and *H. influenzae*; an opportunistic pathogen, *A. calcoaceticus*; and a non-pathogenic *Corynebacterium* species. Detection of *S. epidermidis* by HS-GLC analysis was variable, confirming the results of previous studies [6, 7].

Overall, there was no significant difference in rapidity between HS-GLC and visual inspection. Detection of the *Enterobacteriaceae* and anaerobes by HS-GLC was either marginally quicker than, or at the same time as, visual inspection. With the exception of the viridans *Streptococci*, which were

detected earlier by visual inspection than by HS-GLC analysis, there was no significant difference in the detection times of the Gram-positive cocci by either method.

The most promising results in terms of rapidity occurred with the yeast isolates. Systemic fungal infections have become an increasing problem in immuno-compromised patients. A short detection time is very important for better management of this type of patient, since fungemia is often detected late in the course of the disease or after death [11]. In this investigation all the yeast isolates were detected by HS-GLC analysis after only 24-h incubation of blood cultures. Bille et al. [11], in a ten-year study on the detection of yeast in blood cultures, found that the recovery time of *Candida* sp. from vented biphasic brain—heart infusion blood culture medium ranged from 1 to 28 days, with mean recovery time of 3.5 days for *C. albicans*, 2.8 days for *C. krusei* and 8.3 days for *T. glabrata*. The average detection times of *C. albicans* and *T. glabrata* by the BACTEC radiometric system have been reported as 2.7 and 4.7 days, respectively [12].

Attempts to detect bacteremia rapidly by other gas chromatographic techniques, including analysis of acidified blood cultures [13] and direct analysis of patients' sera [14], have not been an improvement on traditional methods. Perhaps the use of a more sensitive technique, such as frequency-pulsed, electron-capture GLC [15], might expedite the detection of growth in blood cultures.

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