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Development of o.a.s.i.s., a New Automated Blood Culture System in Which Detection Is Based on Measurement of Bottle Headspace Pressure Changes

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o.a.s.i.s. (Unipath Ltd., Basingstoke, United Kingdom) is a new automated blood culture system. The metabolism of microorganisms is detected by measuring changes in the pressure of the headspace of blood culture bottles. These changes are measured by monitoring the position of a flexible sealing septum, every 5 min, with a scanning laser sensor. This noninvasive system can detect both gas absorption and production and does not rely solely on measuring increasing carbon dioxide levels. A research prototype instrument was used to carry out an evaluation of the media, the detection system, and its associated detection algorithm. In simulated blood cultures, o.a.s.i.s. supported growth and detected a range of clinical isolates. Times to positivity were significantly shorter in o.a.s.i.s. than in the BACTEC 460 system. Results of a clinical feasibility study, with a manual blood culture system as a control, confirmed that o.a.s.i.s. was able to support the growth and detection of a variety of clinically significant organisms. On the basis of these findings, full-scale comparative clinical trials of o.a.s.i.s. with other automated blood culture systems are warranted.

Recently, new automated systems for analyzing blood cultures by continuous monitoring have been developed. Several of these measure the production of carbon dioxide by microorganisms growing in broths by colorimetric (16) or fluorometric (13) monitoring or by infrared spectroscopy (6). A system utilizing a fluorescent molecule which responds to carbon dioxide levels, pH, and oxidoreductant potential has been previously described (11). Changes in reducible substrates in the culture media are monitored in a further system (15). Another approach to detect microbial metabolism is to monitor gas consumption and production in culture bottles (12, 18). It was proposed that oxygen consumed during bacterial growth was replaced by an equivalent amount of carbon dioxide (4). However, Ahnell (1) demonstrated that when high numbers of oxygen-consuming bacteria were cultured in medium with agitation, gas production was occasionally preceded by gas absorption. Waters (18) investigated this phenomenon with low inocula, more applicable to the number of bacteria found in blood samples from patients with septicemia (17). In these studies, gas absorption always occurred when organisms grew aerobically and the rate of absorption could be used to detect growth. The rate of gas production, particularly for organisms growing anaerobically, was also detected in this instrument. Under both aerobic and anaerobic conditions, detection times similar to those achieved by the BACTEC 460 system were obtained.

In this paper we describe o.a.s.i.s. (Oxoid Automated Septicaemia Investigation System), a new automated blood culture system based on continuous monitoring of pressure changes taking place in a sealed culture bottle. These changes are expressed in the movements of a flexible septum which seals the bottle (2). We present data obtained with a prototype instrument used to demonstrate the principle of the detection system, the ability of the medium formulations to support growth of a wide range of clinically significant organisms, and the capacity of the system to detect early stages of microbial growth. In simulated blood cultures, the detection times of organisms grown both in o.a.s.i.s. and in the BACTEC 460 system were compared. Having established in these laboratory-based studies that microbial growth in o.a.s.i.s. aerobic and anaerobic media could be detected by the system, a clinical feasibility study was undertaken. Data from this study are also presented. The o.a.s.i.s. prototype instrument was run in parallel with the Oxoid Signal blood culture system (14) (Unipath Ltd., Basingstoke, United Kingdom) routinely used at the Queen Elizabeth Hospital, Birmingham, United Kingdom. The ability of o.a.s.i.s. to support the growth and detection of a variety of pathogenic microorganisms from clinical specimens was demonstrated.

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MATERIALS AND METHODS

Description of the prototype o.a.s.i.s. (i) Media and bottles. Two different broth formulations, one for growing aerobic and microaerophilic bacteria and fungi and the other primarily for the growth of anaerobic bacteria, were evaluated. Both media are based on tryptone soya broth supplemented with complex amino acids, cofactors, coenzymes, and carbohydrates. Sodium polyanethol sulfonate is used as an anticoagulant, with gelatin added to overcome inhibitory effects of sodium polyanethol sulfonate on the growth of certain organisms (8). The anaerobic medium contains a reducing agent, and the bottles are prepared with an anaerobic headspace. The formulations are designed to support the growth of a wide range of microorganisms and to ensure optimal gas production or utilization. Each glass culture bottle contains 90 ml of medium and is sealed with a rubber septum held in place by an aluminium crimp (Fig. 1). A partial vacuum in the bottle allows the

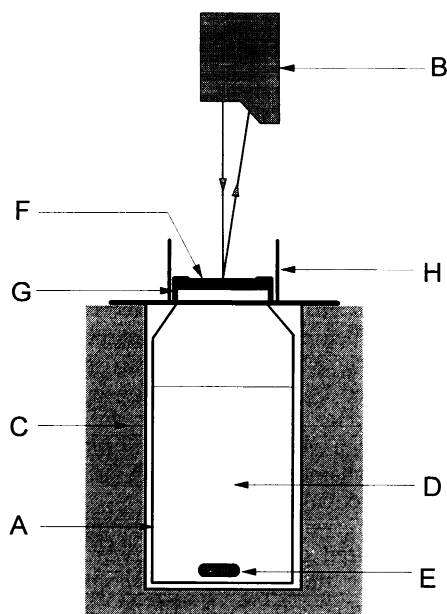

FIG. 1. Schematic view of an o.a.s.i.s. blood culture bottle (A) and laser sensor (B). The bottle is located in a temperature-controlled silo (C) and contains 90 ml of broth (D) and a magnetic stirrer (E). The rubber septum (F) is held in place by an aluminium crimp (G). The plastic overcap (H) forms a protective shield against needlestick injury and is where the bottle bar code is affixed.

addition of up to 10 ml of blood. Each bottle contains a magnetic stirrer. A bar code label on the bottle overcap facilitates easy sample management.

(ii) Detection system and computer algorithm. The detection system is based on the noninvasive measurement of pressure changes taking place in the headspace of the culture bottle. Metabolic activity due to growth of organisms in the bottle results in either the consumption or production of a variety of gases, giving rise to negative or positive pressure changes in the bottle headspace. In response to these changes the bottle septum undergoes conformational changes; the resulting movement is monitored by a high-resolution laser scanning a linear path across the septum. The septum height relative to reference points on the aluminium crimp is calculated. These readings, corrected for variations in barometric pressure, are used to produce a graph of septum displacement against time for each bottle. A detection algorithm analyzes the rate of change of septum displacement which corresponds to headspace pressure in each bottle. When a threshold rate is exceeded, a positive culture is indicated.

(iii) Instrument operation. Each prototype instrument was a self-contained incubator unit of 100-bottle capacity linked to a management computer for data processing and reporting. Within each module, space was subdivided into five sectors, each of 20-bottle capacity. After reconciliation of bottle bar codes and sample details, bottles were loaded into temperature-controlled silos, in the modules, set at 36 ± 0.5°C. The contents of the bottles were mixed by continuous magnetic stirring at 240 rpm. Bottle septa were scanned every 5 min by a laser sensor operating from a computer-controlled *x-y* mechanism. A graph of septum displacement against time was plotted for every bottle. A positive detection was shown by illumination of a light-emitting diode adjacent to the bottle and indicated on the management computer.

Laboratory evaluation of o.a.s.i.s. (i) Septum displacement with varying headspace pressures. A stainless steel test bottle, with inlet ports to permit pressurization or evacuation of the vessel, was used to measure septum conformational changes with varying pressures.

(ii) Septum displacement due to blood alone. Samples (10 ml) of fresh whole blood, collected from healthy volunteers, or of sterile defibrinated horse blood (MVS Ltd., Boltolph Claydon, Bucks, United Kingdom) were inoculated into aerobic and anaerobic o.a.s.i.s. bottles and tested on a prototype instrument for 7 days. Uninoculated bottles were simultaneously tested.

(iii) Preparation of inocula for simulated blood cultures. Organisms were grown on suitable solid media, and colonies were then emulsified in 3 ml of phosphate-buffered saline (PBS). Suspension density was measured with a Turbox nephelometer (Orion, Helsinki, Finland), and dilutions were made in PBS to achieve a target organism count. Viable counts were determined for the suspension.

(iv) Relationship between gas changes in the o.a.s.i.s. bottle headspace and septum displacement. The composition of headspace gases during growth of *Acinetobacter calcoaceticus*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Candida albicans* in o.a.s.i.s. aerobic media was investigated. Bottles were inoculated with between 5 and 50 CFU and 10 ml of defibrinated horse blood and then loaded into o.a.s.i.s. to obtain septum displacement curves. Two additional bottles per organism were placed on an orbital shaker and incubated at 36 ± 0.5°C. Headspace gas samples (100 μl) were withdrawn at intervals from these bottles and analyzed for oxygen and carbon dioxide levels by gas chromatography using a Shimadzu GC-8A gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with a thermal conductivity detector. A Porapak Z (80/100-mesh) column was used for separation of carbon dioxide, and a Carbosieve SII (80/100-mesh) column was used for separation of oxygen. Helium was used as a carrier gas.

(v) Comparison of detection times for simulated blood cultures in o.a.s.i.s. and the BACTEC 460 system. A range of fresh blood culture isolates, including fastidious organisms, was tested in o.a.s.i.s. and the radiometric BACTEC 460 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) (Table 1). The BACTEC 6B medium was compared to the o.a.s.i.s. aerobic medium, and the BACTEC 7D medium was compared to the o.a.s.i.s. anaerobic medium. Isolates were tested in only one medium type: aerobes, microaerophiles, and fungi in the aerobic media and anaerobes in the anaerobic media. Bottles were inoculated with 3 to 50 CFU and sterile defibrinated horse blood at 10% broth volume. BACTEC bottles were processed according to the manufacturer's instructions. All bottles were incubated until growth had been detected or for a maximum of 7 days. The times to detection were recorded. After detection, a purity check was carried out to ensure that no contaminants had been introduced. The sign test was used to compare detection times for simulated blood cultures in BACTEC 460 and o.a.s.i.s. (where $n \geq 6$).

(vi) Viability of *Streptococcus pneumoniae*. The long-term viability of 28 strains of *S. pneumoniae* in o.a.s.i.s. medium was also assessed. o.a.s.i.s. aerobic bottles were inoculated with 5 to 300 CFU and 10 ml of defibrinated horse blood. Once growth had been detected, bottles were subcultured onto solid media at 24-h intervals for a 5-day period to check that live organisms could be recovered.

Preliminary clinical evaluation of o.a.s.i.s. o.a.s.i.s. was compared with the Signal blood culture system in this clinical feasibility study. Signal is a single-bottle manual blood culture

TABLE 1. Number of strains tested in o.a.s.i.s. and BACTEC 460 in simulated blood cultures

Type and species	No. of strains
Gram-negative organisms	
Achromobacter sp. strain Vd	2
Achromobacter xylosoxidans	1
Acinetobacter calcoaceticus	10
Acinetobacter lwoffii	5
Acinetobacter spp.	8
Aeromonas caviae	1
Aeromonas hydrophila	2
Agrobacterium radiobacter	2
Alcaligenes denitrificans	2
Citrobacter diversus	1
Citrobacter freundii	1
Enterobacter cloacae	1
Enterobacter spp.	2
Escherichia coli	8
Flavobacterium meningosepticum	1
Flavobacterium odoratum	1
Haemophilus parainfluenzae	2
Haemophilus influenzae	22
Klebsiella aerogenes	6
Klebsiella oxytoca	2
Klebsiella spp.	2
Neisseria meningitidis	6
Pseudomonas acidovorans	3
Pseudomonas aeruginosa	39
Pseudomonas cepacia	1
Pseudomonas diminuta	1
Pseudomonas fluorescens	1
Pseudomonas paucimobilis	1
Pseudomonas spp.	6
Pseudomonas vesicularis	1
Salmonella bonariensis	1
Sphingobacterium multivorans	1
Xanthomonas maltophilia	6
Yersinia enterocolitica	1
Bacteroides distasonis	1
Bacteroides fragilis	10
Bacteroides loeschii	1
Bacteroides ovatus	3
Bacteroides thetaiotaomicron	3
Bacteroides uniformis	1
Bacteroides spp.	5
Gram-positive organisms	
Corynebacterium sp. strain JK	2
Corynebacterium spp.	4
Corynebacterium stratum	1
Corynebacterium xerosis	1
Enterococcus faecalis	1
Erysipelothrix rhusiopathiae	1
Listeria monocytogenes	5
Listeria monocytogenes 4B	2
Staphylococcus aureus	2
Staphylococcus epidermidis	7
Streptococcus group B	1
Streptococcus mitis	1
Streptococcus pneumoniae	10
Streptococcus sanguis	1
Streptococcus sp.	1
Clostridium fallax	1
Clostridium panci	1
Clostridium perfringens	13
Clostridium ramosum	1
Clostridium septicum	3
Clostridium sordellii	1
Clostridium spp.	2
Clostridium tertium	1
Eubacterium lentum	1
Peptostreptococcus spp.	3
Fungi	
Candida albicans	12
Candida glabrata	3
Candida parapsilosis	4
Candida sp.	1

system, designed to grow aerobic, anaerobic, and microaerophilic organisms. The medium is formulated to create pressure in the sealed bottle when organisms are growing. Positive pressure is detected by means of a growth indicator device which is connected to the bottle after the blood is added. Pressure results in a quantity of blood-broth mixture being displaced into the chamber of the growth indicator device as a sign of microbial activity (14).

Blood for culture was obtained from adult patients (>16 years of age) located on a variety of wards, including the renal and intensive therapy units. Each patient's skin was disinfected by standard methods. Blood samples were obtained from a single venipuncture site or via a central venous catheter. Up to 30 ml of blood was collected and equally distributed, in a random order, into the two o.a.s.i.s. bottles (aerobic and anaerobic) and a Signal bottle. Bottles were preweighed before distribution to the wards. On return to the laboratory, they were reweighed and the volume of blood added was calculated. Blood cultures obtained between 1700 and 0900 h were incubated at 37°C stationary in air and processed at 0900 h. Bottles were examined for any evidence of microbial growth, including turbidity, hemolysis, or a distended septum, before incubation in either system. Samples obtained from any visually suspected positives were Gram stained and subcultured. o.a.s.i.s. bottles were loaded into the instrument, and Signal bottles were placed in an incubator shaker for a minimum of 1 h at 37°C before the growth indicator device was inserted. Signal bottles were shaken at 150 rpm for the first 48 h of incubation. Thereafter they were kept stationary until the end of the incubation period. Signal bottles were visually monitored at 0900 h and at irregular intervals until 1700 h, according to normal laboratory practice. Signal bottles were considered positive if the blood-broth mixture appeared above the collar of the Signal growth indicator device (14). Bottles were incubated until a positive culture was detected or for 7 days. The period to positivity for both systems was defined as the difference between the time at which bottles were first entered into either system and when they were detected as positive. This information was recorded automatically by o.a.s.i.s. and manually for the Signal system. Samples from the suspected positive blood culture bottles were obtained with BCB vent subunits (Difco, East Molesey, United Kingdom) and were Gram stained and subcultured onto appropriate solid media. All negative bottles were terminally subcultured after 7 days of incubation. All organisms isolated were identified by standard microbiological techniques. The significance of all positive blood cultures was clinically assessed, according to standard practice. The organisms isolated were judged to be either clinically significant or contaminants. Only clinically significant organisms were included in the analysis. The paired Student *t* test was used to compare detection times for o.a.s.i.s. and Signal. The isolation rates of o.a.s.i.s. and Signal were compared by the McNemar test (10).

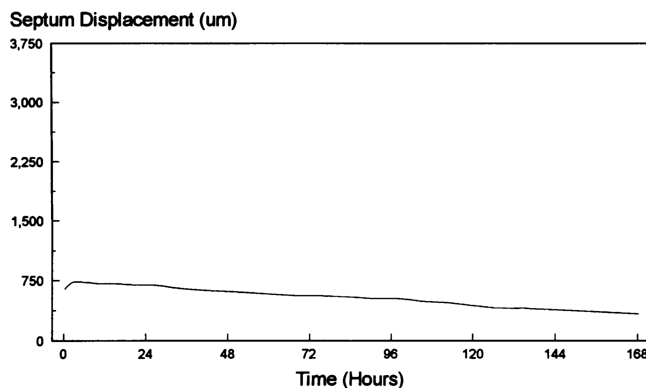

FIG. 2. Septum displacement curve of an aerobic o.a.s.i.s. bottle containing 10 ml of fresh whole human blood.

RESULTS

Laboratory evaluation of o.a.s.i.s. (i) Septum displacement with varying headspace pressures. Small increases or decreases in pressure were detected by the laser sensor as micromovements of the septum. There was a linear response over a pressure range of −50 to +50 kPa; results are therefore presented as septum displacement measurements.

(ii) Septum displacement due to blood alone. The detection algorithm was able to readily distinguish septum displacement due to either sterile media or blood-medium mixtures from that due to microbial growth. The performances of defibrinated horse blood and human blood were comparable. The septum displacement curve of an aerobic bottle with added sterile human blood is shown in Fig. 2. The trace overall is stable, with only a slight negative drift.

(iii) Relationship between gas changes in the o.a.s.i.s. bottle headspace and septum displacement. The relationship between the composition of gases in the bottle headspace and the septum displacement curve for *A. calcoaceticus* is shown in Fig. 3. Similar results were obtained with *H. influenzae*, *N. meningitidis*, and *C. albicans*. Growth of each organism was detected primarily following consumption of oxygen from the headspace.

(iv) Comparison of detection times for simulated blood cultures in o.a.s.i.s. and the BACTEC 460 system. Rapid changes in pressure as measured by septum displacement were observed when microorganisms were grown in o.a.s.i.s. bottles (Fig. 4).

A wide range of clinical isolates was tested in simulated blood cultures in o.a.s.i.s. and the BACTEC 460 system. All organisms grew and were detected in o.a.s.i.s.; however, two strains of *Corynebacterium* sp. strain JK failed to grow in the BACTEC 460 media. The median detection times of these organisms in o.a.s.i.s. and BACTEC 460 are shown in Table 2; 73.7% of the isolates were detected by o.a.s.i.s. within 24 h, compared with 60.3% detected by the BACTEC 460 system. For each case analyzed (when $n \geq 6$), it was found that o.a.s.i.s. detection times were significantly shorter.

(v) Viability of *S. pneumoniae*. All *S. pneumoniae* strains tested remained viable for 5 days after detection by o.a.s.i.s.

Preliminary clinical evaluation of o.a.s.i.s. A total of 1,548 blood culture sets was examined. The average volume of blood added to the Signal bottles was 5.5 ml, that added to o.a.s.i.s. aerobic bottles was 6.0 ml, and that added to o.a.s.i.s. anaerobic bottles was 5.9 ml. From 111 positive blood cultures, 122 clinically significant microorganisms were isolated (Table 3). A total of 105 organisms was isolated from o.a.s.i.s., and 98 were isolated from Signal. There was no significant difference between the numbers of organisms isolated from either system. The cumulative percentage of positive cultures detected by each system with time is shown in Fig. 5. o.a.s.i.s. detected 65% of positive cultures within 12 h and 90% within 24 h. In comparison, Signal detected 10% within 12 h and 82% within 24 h. Overall, detection of positive blood cultures was more rapid with o.a.s.i.s. than with Signal ($P < 0.0001$). The median detection times for individual organisms detected by o.a.s.i.s. on more than six occasions are presented in Table 4. Ten o.a.s.i.s. sets (0.65%) remained negative but on terminal subculture yielded growth from one of the bottles. The organisms which were recovered from these false-negative sets were two *Staphylococcus aureus* strains and one strain of each of the

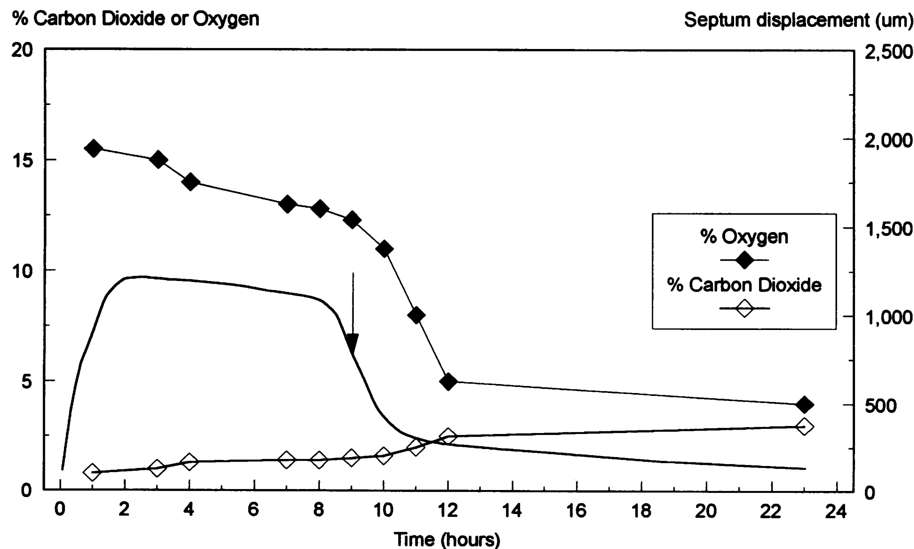

FIG. 3. Changes in the percent composition of oxygen and carbon dioxide in the headspace of an aerobic o.a.s.i.s. bottle during growth of *A. calcoaceticus*. The septum displacement curve obtained in parallel is also shown (solid line); detection time is indicated by an arrow.

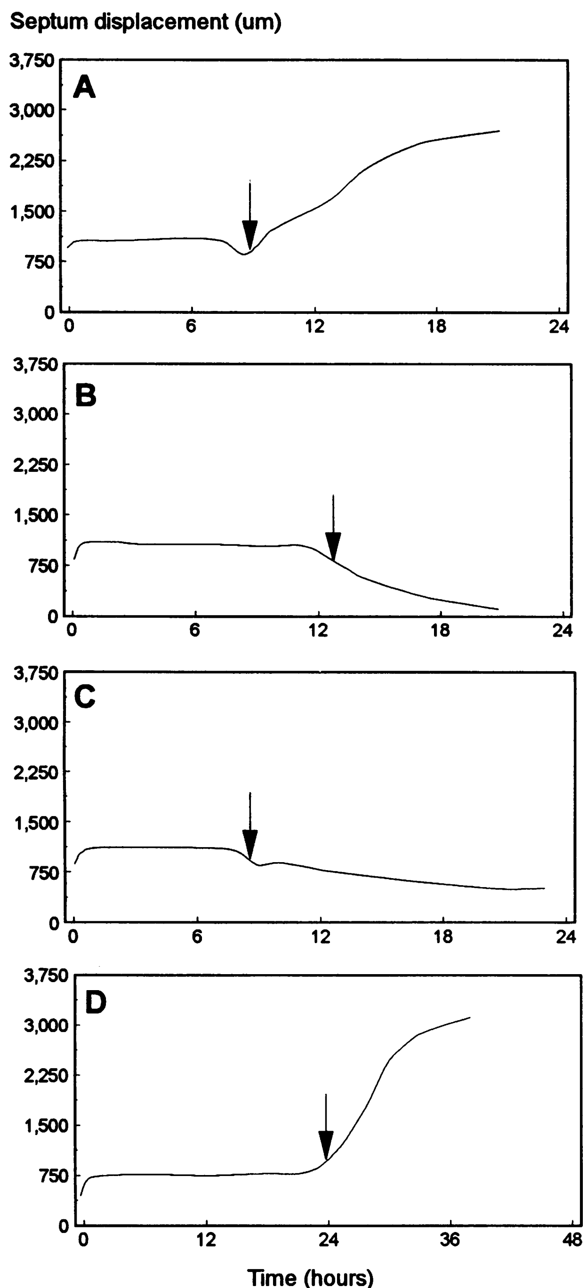

FIG. 4. Septum displacement curves during growth of *K. pneumoniae* (A), *Pseudomonas aeruginosa* (B), *Staphylococcus aureus* (C), and *Bacteroides fragilis* (D). Detection time is indicated by an arrow.

following species: *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus sanguis*, *Streptococcus agalactiae*, *Streptococcus mitis*, a *Corynebacterium* sp., and *Bacillus licheniformis*. The septum displacement curves were subsequently reanalyzed with revised, more sensitive parameters, and all organisms except *B. licheniformis* were then detected. There were three false-negative Signal bottles (0.2%), which yielded *S. epidermidis*, *S. aureus*, and *Xanthomonas maltophilia* on terminal subculture. There were eight false-positive o.a.s.i.s. bottles during the trial (0.3%). In comparison, there were two false-positive Signal detections (0.1%).

TABLE 2. Detection times for clinical isolates grown in o.a.s.i.s. and the BACTEC 460 system in simulated blood cultures

Organism	Total no. of strains	Detection time (h) in: o.a.s.i.s. Median	o.a.s.i.s. SD	BACTEC 460 Median	BACTEC 460 SD	*P*
Gram-negative organisms						
B. fragilis	10	30.4	4.0	43.0	0.6	<0.005
B. ovatus	3	35.6	4.3	43.0	0.0	—[a]
B. thetaiotaomicron	3	48.3	12.4	43.0	13.3	—
Bacteroides spp.	8	33.1	5.6	43.0	0.7	<0.050
Achromobacter spp.	3	30.4	2.5	70.0	13.1	—
A. calcoaceticus	10	11.6	1.5	19.0	2.4	<0.005
A. lwoffii	5	20.1	16.5	46.0	42.3	—
Acinetobacter spp.	8	13.9	7.8	22.0	21.3	<0.005
Aeromonas spp.	3	10.8	0.9	19.0	0.0	—
Enterobacter spp.	3	11.8	0.9	21.0	1.2	—
E. coli	8	10.7	1.0	21.0	0.0	<0.005
H. influenzae	22	12.6	1.1	21.0	9.1	<0.005
K. aerogenes	6	11.2	0.9	21.0	0.5	<0.025
Klebsiella spp.	4	11.8	1.4	21.0	0.0	—
N. meningitidis	6	14.6	4.7	86.5	0.0	<0.025
P. acidovorans	3	57.2	25.4	70.0	28.9	—
P. aeruginosa	39	15.3	5.8	21.0	27.1	<0.005
Pseudomonas spp.	11	19.0	15.5	46.0	55.5	<0.005
X. maltophilia	6	21.8	3.5	47.0	13.7	<0.025
Gram-positive organisms						
C. perfringens	13	10.5	6.5	21.8	0.4	<0.005
C. septicum	3	16.5	2.4	21.0	0.0	—
Clostridium spp.	7	13.8	7.0	21.8	44.6	<0.025
Peptostreptococcus spp.	3	29.5	10.4	46.0	14.6	—
Corynebacterium spp.	8/6[b]	30.0	8.2	48.0[b]	16.6[b]	<0.025
Listeria spp.	7	15.9	0.6	21.0	1.1	<0.025
S. epidermidis	7	13.2	1.0	21.0	0.2	<0.025
Streptococcus spp.	3	12.0	2.7	21.0	1.2	—
S. pneumoniae	10	11.4	1.7	21.0	9.3	<0.005
Fungi						
C. albicans	12	24.4	2.1	47.0	9.5	<0.005
C. glabrata	3	57.3	12.5	47.0	13.9	—
C. parapsilosis	4	32.1	1.5	70.0	12.1	—

[a] —, not tested.
[b] Two strains were not detected in the BACTEC 460 system.

DISCUSSION

Continuous monitoring of blood cultures offers the potential for a more rapid diagnosis of septicemia. The principle on which detection is based should be simple and robust and allow the construction of a fully automated instrument requiring a minimum amount of operator intervention. The o.a.s.i.s. prototype instrument described in this paper monitors pressure changes in the headspace of culture bottles, which occur as a result of microbial metabolism. Initial experiments confirmed that changes in bottle headspace pressure could be detected as micromovements of a flexible septum sealing the bottle. Indeed, there was a direct, linear relationship between pressure changes and septum displacement. This system is therefore able to monitor microbial metabolism noninvasively.

Septum displacement curves from bottles containing sterile media showed no change in septum position. Low levels of gas absorption were observed in sterile blood-broth mixtures, but these were easily distinguishable from those due to microbial metabolism by the detection algorithm. In comparison, when the two medium formulations were seeded with a wide variety of microorganisms, changes in septum displacement due to gas absorption or production were readily detected. The nature of gas absorption or production was investigated further. Oxygen

TABLE 3. Distribution of microorganisms isolated in o.a.s.i.s. and Signal during the preliminary clinical study

Microorganism	No. of strains isolated			
	In both systems	In Signal only	In o.a.s.i.s. only	Total
Staphylococcus epidermidis	24	6	4	34
Staphylococcus aureus	17	1	4	22
Streptococcus spp.	7	1	3	11
Enterococcus spp.	9	2	2	13
Other gram positives[a]	2	1	1	4
Escherichia coli	7	0	3	10
Klebsiella spp.	8	0	3	11
Other gram negatives[b]	4	5	3	12
Bacteroides fragilis	3	1	0	4
Candida albicans	0	0	1	1
Total	81	17	24	122

[a] Includes two *Corynebacterium* strains, one *Bacillus licheniformis* strain, and one *Staphylococcus hominis* strain.

[b] Includes four *Pseudomonas aeruginosa* strains, two *Xanthomonas maltophilia* strains, two *Acinetobacter calcoaceticus* strains, one *Proteus mirabilis* strain, one *Salmonella typhimurium* strain, one *Salmonella enteritidis* strain, and one *Campylobacter jejuni* strain.

absorption from the headspace was the predominant gas exchange occurring during growth of *A. calcoaceticus* in the aerobic medium (Fig. 3). As oxygen was consumed, the headspace pressure reduced, which was reflected in the position of the bottle septum. Detection of microbial growth occurred when the rate of oxygen utilization was at its greatest (8 to 12 h). Relatively small amounts of carbon dioxide had been produced by the organism at this time. Conversely, the growth of *Clostridium perfringens* is detected because of the production of hydrogen and hydrogen sulfide (data not shown). The ability of o.a.s.i.s. to therefore measure several types of metabolic activity gives it an advantage over other automated blood culture systems relying solely on measuring carbon dioxide levels.

It has been shown previously that by increasing the number of CFU inoculated into o.a.s.i.s. bottles (>50 CFU), the subsequent time to detection was reduced (5). However, high

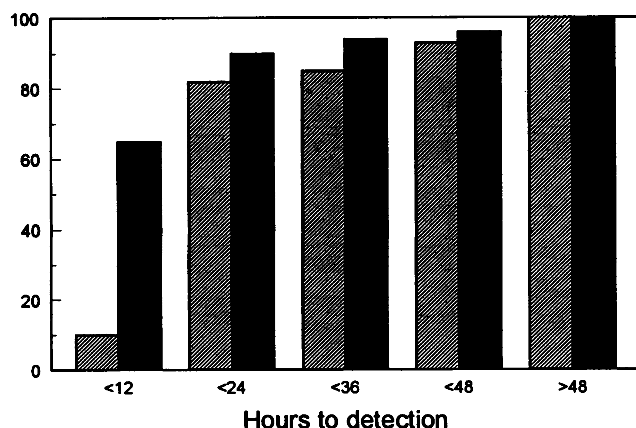

FIG. 5. Cumulative percentages of clinical blood culture isolates detected by o.a.s.i.s. (■) and Signal (▨) blood culture systems.

TABLE 4. Times to detection of positive blood cultures by o.a.s.i.s. in the preliminary clinical study

Organism	*n*	Detection time (h)	
		Median	SD
S. epidermidis	27	14.1	17.6
S. aureus	19	10.1	17.8
Enterococcus spp.	11	10.4	35.2
Streptococcus spp.	7	10.4	4.4
E. coli	10	10.3	3.8
Klebsiella spp.	10	9.1	11.9

inocula do not accurately reflect the clinical situation in adults (17). Consequently, in the o.a.s.i.s. and BACTEC 460 simulated blood culture comparison work, an inoculum of fewer than 50 CFU per bottle was used. This has not always been the case in simulated studies of other instruments (16). A wide range of clinical isolates, including fastidious organisms and those with low utilization of substrates, such as *Acinetobacter* spp. and *Bacillus cereus*, grew in the o.a.s.i.s. media. Microbial growth was readily detected by the algorithm. *S. pneumoniae* grew and remained viable after detection in the o.a.s.i.s. aerobic medium. This is particularly important with the emergence of antibiotic-resistant pneumococci (3). Live organisms can be recovered for identification and susceptibility testing from o.a.s.i.s. media.

In simulated blood cultures, o.a.s.i.s. detection times were found to be significantly shorter than those obtained with the BACTEC 460 system for all organism groups tested: gram-positive strains, gram-negative strains, and fungi. The frequency of monitoring (288 times per day) by o.a.s.i.s., coupled with the ability to monitor general metabolism, appears to offer a more rapid detection of positive blood cultures. It should be noted that the BACTEC 460 system has been superceded by the fully automated BACTEC 9240 system (13). However, the BACTEC 460 system has been used previously as a reference system for proving new blood culture systems (16, 18).

Following the laboratory findings, which indicated that o.a.s.i.s. could reliably grow and detect organisms, a clinical feasibility study was undertaken to confirm that o.a.s.i.s. could support the growth and detection of a wide variety of organisms in the clinical situation. This proved to be the case. Subsequent alteration to the sensitivity of the detection algorithm reduced the false-negative rate. Indeed, a more sophisticated algorithm has been developed for use in further clinical trials.

In the clinical study, frequent monitoring by o.a.s.i.s. resulted in the detection of positive blood cultures significantly faster than did that by the Signal system. Rapid detection of positive blood cultures offers an advantage in the clinical management of patients with sepsis (7). In suspected septicemia, treatment with broad-spectrum antimicrobial agents is often commenced empirically. However, directed therapy is preferable, and it is evident that o.a.s.i.s. offers this potential at an earlier stage, with concomitant improvement in patient outcome and the possibility of reduced hospital costs.

The modular format of o.a.s.i.s. enables the clinical microbiology laboratory to install a system meeting its current workload requirements, yet with the potential for expansion due to increased sample throughput. The use of magnetic stirrers in the bottles achieves the effective agitation necessary for rapid growth (9) and has enabled a compact instrument to be designed. The minimum number of moving parts also enhances instrument reliability and ease of service.

In conclusion, the findings of this study demonstrate that o.a.s.i.s. rapidly detects positive blood cultures. Further clinical trials, comparing o.a.s.i.s. with other automated blood culture systems, are therefore warranted.

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