JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1992, p. 1995–1998
0095-1137/92/081995-04$02.00/0

Vol. 30, No. 8

BioArgos: a Fully Automated Blood Culture System

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Received 5 March 1992/Accepted 14 May 1992

BioArgos (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) is a fully automated blood culture system that detects carbon dioxide production by infrared spectroscopy through a glass bottle. This hands-off system was compared with the BACTEC NR-660 system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.). A total of 336 microorganisms belonging to 74 taxa were tested in simulated blood cultures by both systems. Experimental data showed no significant differences between the two systems. The inclusive detection times (± the standard deviations) were 33.2 ± 28.7 and 35.0 ± 30.6 h with BioArgos and BACTEC, respectively. Anaerobes were detected earlier with BioArgos, whereas detection of some organisms that need oxygen to grow was slightly delayed. In conclusion, BioArgos is as reliable and accurate as BACTEC NR-660 and shows better practicability owing to noninvasive detection, reduction of vial manipulation, and absence of daily maintenance.

Several physical detection systems have been proposed to detect early growth of microorganisms in blood cultures. These systems are based on measurement of changes in impedance between two electrodes (11), measurement of ATP by bioluminescence (1), or detection of carbon dioxide generated during bacterial metabolism. DeLand and Wagner were the first to develop this latter method by using ^{14}C-labeled glucose (4).

Semiautomated detection of bacteria in blood cultures has been available with the BACTEC 460 system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) for about 20 years (3). BACTEC 460 is a radiometric system in which bacteria generate $^{14}CO_2$ during metabolism of [^{14}C]glucose and other ^{14}C-labeled substrates (3). The nonradiometric BACTEC NR-660 system was developed in 1985 (2). This system avoids the use of radioactive components and detects carbon dioxide production by infrared spectroscopy. In 1990, Thorpe et al. described another automated microbial detection system based on colorimetric detection of CO_2 produced by microorganisms: the BacT/Alert (Organon Teknika Corp., Durham, N.C.) (10).

In this report, we describe a new, fully automated blood culture system, BioArgos (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). We compared the sensitivity and practicability of this new infrared blood culture system with those of BACTEC NR-660, which on the basis of its wide acceptance and use, could be considered the reference blood culture detection system. An in vitro evaluation of 336 microorganisms was carried out with simulated blood cultures comparing BioArgos and BACTEC aerobic and anaerobic vials. The experimental data obtained are reported.

MATERIALS AND METHODS

Description of the BioArgos system. BioArgos is a self-contained and closed system consisting of four parts (Fig. 1). Part 1 is the sample loading unit, which includes a bar code reader. Up to 57 bar-coded vials can be introduced and stocked in this unit before transfer to the measuring and shaking unit. Part 2 is an infrared spectrometer built to detect carbon dioxide through the headspace of a glass bottle. Part 3 is an incubator, which accommodates up to 720 vials located on six thermostatic trays. The chosen temperature is under computer control (36 ± 1°C). Part 4 is a computer system (PS/2; IBM Corp., Paris, France) connected to the machine, with a video terminal, a keyboard, and a printer. The computer system manages all of the functions of BioArgos. Four utility programs are available: specimen processing, operating parameters, user maintenance, and assistance. As BioArgos is a hands-off system, no aerobic or anaerobic gas is injected into the blood culture vials during processing. The system needs neither needles nor a UV lamp to operate.

Instrument processing. Bar-coded vials are introduced into the sample loading unit. All subsequent operations are "walk away" for BioArgos. A motorized arm equipped with pliers takes vials at the bottleneck level and moves them from the sample loading unit to the reading unit. After each reading, blood culture vials are shaken for 12 s. No other shaking takes place during incubation. The arm then transfers the vials into the wells of the incubator tray assigned by the computer. The usual incubation time is 7 days. It can be either reduced by the microbiologist to as little as 5 days or extended to up to 14 days. When vials are considered positive, the machine automatically transfers them to the incubating box until further processing. The content of this box is limited to 50 positive vials. Negative blood culture vials are automatically discarded by the machine into a trash can on day 8. When a vial is considered positive by the machine, the well allocated by the computer remains unloaded for 24 h to allow reintroduction in case of false-positivity. No daily maintenance by technicians is required with the BioArgos system. Two sealed gas control vials with a calibrated atmosphere of carbon dioxide are incubated in the trays and read twice an hour. In case of deviation of the measurements of the control tests, adjustment of the spectrometer can be done either automatically or via the computer in case of complete failure.

Media. Two blood culture media are available. The media have been optimized to enhance carbon dioxide production

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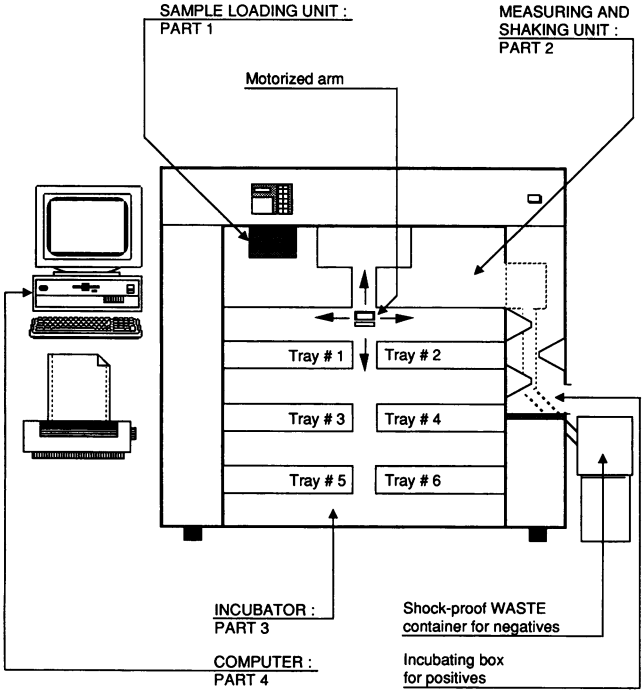

FIG. 1. Description of the BioArgos system.

by microorganisms. A special type of glass was selected to contain the medium to optimize carbon dioxide detection through the headspace of the vial. Aerobic and anaerobic blood culture bottles contain 25 ml of medium. Aerobic medium (BioArgos AER) is a brain heart infusion enriched with cysteine, hemin, and vitamins B1, B5, B6, and K that is suited for growth of aerobes and facultative anaerobes. Anaerobic medium (BioArgos ANAER) is prereduced Schaedler broth enriched with hemin, thiols, and vitamins B6 and K. The anticoagulants are 0.035% sodium polyanetholsulfonate in the aerobic medium and 0.02% sodium amylosulfate in the anaerobic medium. A partial vacuum (0.275 ± 0.025 atm [1 atm is 101.29 kPa]) is obtained in both types of blood culture media during the manufacturing process. The atmosphere is composed of carbon dioxide and reducing atmosphere in aerobic and anaerobic vials, respectively. The usual volume of blood injected into the blood culture vial is 3 to 5 ml. However, up to 7 ml of blood can be injected.

Criteria for positivity. The BioArgos system continuously monitors each blood culture bottle. The chronologies of readings is determined by the manufacturer. In this study, the first reading occurred at the second hour and the next occurred at the fifth hour. The first reading detected a putatively positive vial, whereas the second one gave the initial carbon dioxide concentration in the vial, which was considered the reference value. Successive levels of carbon dioxide in the vial were then compared to the baseline inferred from the reference value. Aerobic vials were read eight times on the first day, three times daily on days 2 and 3, and six times daily from days 4 to 7. Anaerobic vials were read four times on the first day, twice daily on days 2 to 4, and once daily on days 5 to 7. Positivity in the BioArgos system was assessed either upon variation from the baseline or by calculation of a change between two subsequent readings. The BioArgos positivity criterion was either a growth value that changed during a test day or a change in a growth value between two readings. When a vial was considered positive, it was moved to the incubating box until further processing. All of the information about positive vials was automatically printed out. Moreover, a light located on the system indicated the presence of positive vials to the technician.

Simulated blood cultures. Five-milliliter samples of sterile human blood from healthy donors were injected in equal parts into BioArgos AER and ANAER and BACTEC NR-6A and NR-7A. All vials were incubated for 5 days at 37°C. Strains recently isolated from clinical specimens were chosen to be representative in terms of species and numbers of microorganisms recovered from microbiology laboratories of 33 French university hospitals (9). We tested 270 aerobic and facultatively anaerobic bacterial strains, 45 anaerobic bacterial strains, and 21 fungal strains belonging to 74 taxa by both systems. The organisms and numbers of strains were as follows: *Achromobacter* sp., 1; *Acinetobacter* sp., 5; *Aerococcus viridans*, 2; *Aeromonas hydrophila*, 1; *Anaerobiospirillum* sp., 1; *Bacillus* sp., 6; *Bacteroides* sp., 5; *Bifidobacterium* sp., 2; *Bilophila wadsworthia*, 1; *Branhamella catarrhalis*, 1; *Brucella* sp., 3; *Campylobacter* sp., 2; *Candida albicans*, 6; *Candida* sp., 8; *Citrobacter diversus*, 1; *Citrobacter freundii*, 5; *Clostridium* sp., 11; *Corynebacterium* sp., 1; *Cryptococcus laurentii*, 1; *Cryptococcus neoformans*, 3; *Desulfomonas* sp., 1; *Eikenella corrodens*, 1; *Enterobacter aerogenes*, 2; *Enterobacter agglomerans*, 2; *Enterobacter cloacae*, 10; *Enterococcus faecalis*, 6; *Enterococcus faecium*, 10; *Erysipelothrix rhusiopathiae*, 1; *Escherichia coli*, 32; *Eubacterium* sp., 2; *Fusobacterium* sp., 3; *Gemella morbillorum*, 1; *Haemophilus influenzae*, 6; *Haemophilus parainfluenzae*, 1; *Hafnia alvei*, 1; *Klebsiella oxytoca*, 5; *Klebsiella pneumoniae*, 11; *Listeria monocytogenes*, 6; *Mobiluncus* sp., 1; *Moraxella* sp., 2; *Morganella morganii*, 3; *Neisseria meningitidis*, 3; *Neisseria* sp., 1; *Pasteurella multocida*, 4; *Peptostreptococcus* sp., 15; *Propionibacterium acnes*, 1; *Proteus mirabilis*, 9; *Proteus vulgaris*, 4; *Providencia stuartii*, 2; *Providencia rettgeri*, 2; *Pseudomonas aeruginosa*, 12; *Pseudomonas* sp., 2; *Rhodococcus equi*, 1; *Salmonella* sp., 11; *Sarcina lutea*, 1; *Serratia marcescens*, 11; *Shigella* sp., 7; *Staphylococcus aureus*, 20; *Staphylococcus epidermidis*, 8; *Staphylococcus saprophyticus*, 3; coagulase-negative staphylococci, 8; *Streptococcus agalactiae*, 2; *Streptococcus pneumoniae*, 7; *Streptococcus pyogenes*, 1; group C streptococci, 4; nonenterococcal group D streptococci, 4; group G streptococci, 2; nonhemolytic streptococci, 6; *Torulopsis glabrata*, 3; *Veillonella parvula*, 1; *Xanthomonas maltophilia*, 3; *Yersinia enterocolitica*, 4. A suspension of clinical isolates was made to obtain an inoculum concentration ranging from 0.2 to 100 CFU/ml of blood in the vial. Inocula were tested quantitatively on petri dishes. Prior to testing, all isolates were cultured in brain heart infusion and reisolated on agar plates to ensure purity and viability. The BACTEC NR-660 system was used to read BACTEC vials. Maintenance and processing of the BACTEC NR-660 system were carried out in accordance with the instructions of the manufacturer. Briefly, NR-6A vials were read twice daily on days 1 to 3 and once daily on days 4 to 5. NR-7A vials were read once daily on days 1 to 5. BioArgos and BACTEC vials were both discarded from the incubators on day 6 (2).

Analysis of data. A paired comparison of aerobic and anaerobic bottles from the two systems was performed. The time to positivity was the interval between the time of inoculation and the time of detection. False-negative vials were defined by negative detection by the instrument. The

TABLE 1. Isolates not recovered by the BioArgos and BACTEC systems in simulated blood cultures

Organism	No. of isolates not recovered by:	
	BioArgos	BACTEC
Acinetobacter junii	1	0
Bacillus sp.	3	0
Bifidobacterium dentium	0	1
Branhamella catarrhalis	1	0
Brucella sp.	0	3
Campylobacter fetus	0	1
Candida sp.	1	2
Clostridium sp.	2	0
Cryptococcus sp.	4	0
Eubacterium lentum	0	2
Fusobacterium nucleatum	0	1
Neisseria meningitidis	0	2
Peptostreptococcus sp.	0	9
Salmonella bovis morbificans	1	0
Staphylococcus aureus	0	1
Staphylococcus cohnii	1	0
Streptococcus pneumoniae	0	2
Torulopsis glabrata	0	3
Xanthomonas maltophilia	1	0
Yersinia enterocolitica	1	0
Total	16	27

time for negative or false-negative vials was equal to the maximum incubation time, which was 120 h. Statistical analysis was carried out with the Student *t* test and the chi-square test.

RESULTS

The mean of the inoculum was 14.3 CFU/ml of blood (standard deviation, ± 17.7 CFU/ml). Of the 336 microorganisms tested, 322 (95.3%) were detected with the BioArgos system and 311 (92.0%) were recovered with the BACTEC system. The difference in recovery rate was not statistically significant ($P > 0.05$). Table 1 shows the numbers of blood culture isolates unrecovered by both systems. BioArgos and BACTEC failed to recover 11 and 16 organisms, respectively. Moreover, false-negative results (failure of instrument detection) were more frequent with BACTEC (11 strains) than with BioArgos (5 strains). *Neisseria meningitidis* (two strains), *Brucella* sp. (three strains), and anaerobes (five strains) were not detected by BACTEC. *Cryptococcus* sp. (four strains) was not detected by BioArgos.

The times to recovery of microorganisms from aerobic and anaerobic cultures in both systems were also compared. The inclusive detection time with BioArgos was 33.2 ± 28.7 h, whereas it was 35.0 ± 30.6 h with BACTEC. Mean detection times for aerobic and facultatively anaerobic bacteria, anaerobic bacteria, and fungi are reported in Table 2. The differences were not statistically significant ($P > 0.05$). However, a more detailed study of the results showed several discrepancies in the recovery of some organisms (Tables 3 and 4). For instance, BioArgos detected *Haemophilus influenzae* and *Streptococcus pneumoniae* earlier ($P < 0.001$). On the other hand, BACTEC recovered *Acinetobacter* sp., *Xanthomonas maltophilia*, and *Yersinia enterocolitica* faster ($P < 0.001$). Other vial-versus-vial comparisons showed that recovery of *Candida albicans* and *Cryptococcus* sp. was better with BACTEC and recovery of *Torulopsis glabrata* and anaerobes, except for *Clostridium* sp., was significantly better with BioArgos ($P < 0.001$).

TABLE 2. Mean detection times for the BioArgos and BACTEC systems in simulated blood cultures

Organisms	Mean time (h) to detection (± SD) by:	
	BioArgos	BACTEC
Aerobic and facultative bacteria	26.1 ± 22.8	26.2 ± 20.7
Anaerobic bacteria	59.0 ± 29.2	74.9 ± 36.8
Fungi	68.5 ± 36.3	62.5 ± 38.3
Total	33.2 ± 28.7	35.0 ± 30.6

DISCUSSION

Evaluation of a new automated blood culture system, such as BioArgos, requires a thorough examination of the machine and blood culture media. Determination of the perfor-

TABLE 3. Isolates recovered by both the BioArgos and the BACTEC systems in simulated blood cultures

Organism(s)	No. of isolates[a]	Mean time (h) to detection by:		*P* value
		BioArgos AER	BACTEC NR-6A	
Gram-negative bacteria				
Acinetobacter sp.	5	42.9	23.3	<0.001
Brucella sp.	3	90.5	120	
Citrobacter freundii	5	18.4	18.0	
Enterobacter cloacae	10	14.9	19.1	
Escherichia coli	31	14.5	19.1	
Haemophilus influenzae	6	24.7	39.3	<0.001
Klebsiella oxytoca	5	16.8	18.4	
Klebsiella pneumoniae	11	16.0	19.4	
Morganella morganii	3	17.0	17.3	
Neisseria meningitidis	3	58.5	86.7	
Pasteurella multocida	4	15.7	20.7	
Proteus mirabilis	9	16.7	17.9	
Proteus vulgaris	4	19.0	18.2	
Pseudomonas aeruginosa	12	27.9	21.4	
Salmonella sp.	11	28.1	20.6	
Shigella sp.	7	18.0	18.3	
Serratia marcescens	11	15.6	19.4	
Xanthomonas maltophilia	3	57.5	21.7	<0.001
Yersinia enterocolitica	4	52.2	24.1	<0.001
Gram-positive bacteria				
Bacillus spp.	6	73.8	32.4	<0.001
Enterococcus faecalis	6	14.7	19.3	
Enterococcus faecium	10	14.4	18.6	
Listeria monocytogenes	6	26.9	21.3	
Staphylococcus aureus	20	25.3	26.7	
Coagulase-negative staphylococci	19	29.7	23.5	
Group C streptococci	4	26.2	21.5	
Group D streptococci	4	19.5	23.0	
Nonhemolytic streptococci	6	26.2	27.7	
Streptococcus pneumoniae	7	25.5	49.7	<0.001
Yeasts				
Candida albicans	6	65.2	38.3	<0.001
Candida sp.	8	58.1	52.3	
Torulopsis glabrata	3	54.7	120	<0.001
Cryptococcus sp.	4	120	74.2	<0.001

[a] Numbers of isolates that were three or greater are shown.

TABLE 4. Anaerobes recovered by both the BioAgros and the BACTEC systems in simulated blood cultures

Organism	No. of isolates[a]	Time (h) to recovery by:		*P* value
		BioArgos ANAER	BACTEC NR-7A	
Bacteroides sp.	5	48.4	53.2	<0.05
Clostridium sp.	11	44.0	36.6	<0.001
Fusobacterium sp.	3	47.3	66.3	<0.001
Peptostreptococcus sp.	15	62.4	98.5	<0.001

[a] Numbers of isolates that were three or greater are shown.

mance of the combined detection system and medium by using simulated blood cultures represents the first step in such an evaluation in comparison with a reference system.

In this study, blood culture vials were always inoculated with a concentration lower than 100 CFU/ml of blood. This value is more compatible with the physiological conditions of bacteremia (5, 7) than that used by Thorpe et al. in a similar study (10). The mean detection times obtained with the two systems were not significantly different. Some species were recovered earlier from either one or the other system. Because reading was done through a glass bottle, the BioArgos vial atmosphere was not vented during incubation, which accounts for the delay in the detection of aerobic microorganisms, such as *Acinetobacter* sp., *Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, *Bacillus* sp., and fungi. Detection of anaerobes almost always occurred earlier with BioArgos, despite the better anaerobic conditions encountered with the BACTEC system. Of the 27 strains not recovered with BACTEC, 13 were anaerobes. This discrepancy between the two systems could be explained either by the adequacy of the prereduced Schaedler broth for the growth of anaerobes or the presence of sodium amylosulfate instead of sodium polyanethol sulfonate as the anticoagulant. Graves et al. reported that the growth of *Peptostreptococcus* sp. was inhibited or delayed with sodium polyanethol sulfonate (6). We observed the same phenomenon with *Peptostreptococcus* sp. in this study. On the other hand, in another study we have reported poor recovery of anaerobes with anaerobic BACTEC medium (2).

BioArgos was the first hands-off prototype system described (8). Technicians found several advantages relative to BACTEC NR-660. For instance, there was no daily maintenance as with BACTEC NR-660: no gas to check, no needles to change, no controls to test. Manipulation of vials was reduced. (i) Bottles are handled only when they are introduced into the sample loading unit and left until the end of the incubation process. (ii) Negative blood culture vials are automatically transferred to a single-use trash can fitted to be autoclaved. The risk of vial-to-vial contamination is avoided, as reading is done through the glass bottle. The system is walk away, reducing the laboratory work load and the time needed to detect microbial growth. Like other new automated blood culture systems, the machine is connected to a powerful microcomputer that gives the vial history and epidemiological data. The microcomputer can be connected on line to a laboratory mainframe computer. Moreover, the work of secretaries is lightened owing to the existence of bar code labels on the vials.

In conclusion, our experimental study demonstrates that the BioArgos system is as reliable and accurate as the BACTEC NR-660 system. The overall recoveries of microorganisms from aerobic and anaerobic vials were not significantly different.

ACKNOWLEDGMENT

We thank Pierre Deroo for excellent technical assistance.

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