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Routine Evaluation of the Nonradiometric BACTEC NR 660 System

RENÉ J. COURCOL,* ANNIE FRUCHART, MICHELINE ROUSSEL-DELVALLEZ, AND GUY R. MARTIN

Laboratoire de Bactériologie C, Hôpital A. Calmette, 59037 Lille, France

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The present study compares the sensitivity, accuracy, and practicability of a nonradiometric blood culture system with those of the standard blood culture system in use in our laboratory. A total of 1,080 sets of four blood cultures bottles were compared, giving 143 positive aerobic-medium associations and 139 positive anaerobic-medium associations. The conventional system recovered 171 isolates, whereas the BACTEC NR 660 system (Johnston Laboratories, Towson, Md.) recovered 155 isolates. There were no significant differences in total isolates with either system ($P > 0.05$). The BACTEC NR 660 system recovered a slightly lower number of *Serratia marcescens* and *Streptococcus faecalis* but a higher number of *Pneumococcus* spp. The mean detection times were 55.9 h with the conventional system and 31.6 h with the BACTEC NR 660 system ($P < 0.001$). Our results demonstrate that the BACTEC NR 660 system is suitable for routine use.

The rapid detection of bacteremia is one of the most important functions of bacteriology laboratories. Methods commonly in practice detect bacteremia by using conventional blood culture bottles with visual inspection. However, for about 15 years, automated detection of bacteria in blood cultures has been available with the BACTEC 460 system (Johnston Laboratories, Towson, Md.), a radiometric system in which bacteria generate $^{14}CO_2$ during bacterial metabolism of [^{14}C]glucose and other ^{14}C-substrates.

Many studies reported that the BACTEC 460 system produced accurate results faster than, or as fast as, the conventional blood culturing method, saved labor, and minimized the recovery of extraneous contaminants. However, in considering the use of radioactive components, the legislation in force in some countries, such as France or The Netherlands, does not allow the routine utilization of such apparatus in laboratories. This drawback is now avoided with the existence of a nonradiometric growth detection system, the BACTEC NR 660 (Johnston Laboratories). The present study compares the sensitivity, accuracy, and practicability of this automated infrared blood culture system with those of the standard system in use in our laboratory, consisting of diphasic medium with tryptic soy broth and of prereduced Schaedler broth medium in blood culture bottles.

MATERIALS AND METHODS

Blood sampling, inoculation, and incubation. The study was carried out in four departments of the A. Calmette Hospital and in a cancer center (Centre Oscar Lambret, Lille, France) from June 1985 to September 1985 (17 weeks). A total of 1,080 blood culture sets were collected and processed during the course of the study. Each set consisted of a pair of BACTEC which contained aerobic medium NR 6A and anaerobic medium NR 7A, and a pair of conventional bottles, which contained diphasic medium with tryptic soy broth and supplemental factors for aerobes and facultative anaerobes (kindly supplied by BioMérieux, Marcy-l'Etoile, France) and prereduced Schaedler broth medium for anaerobes (Pasteur Production, Paris, France). Blood samples were obtained at the bedsides of patients and were distributed as follows: 3 to 5 ml of each specimen was inoculated into each BACTEC and 5 to 10 ml was injected into each conventional bottle. Thus, the volumes of blood were those recommended by the blood culture bottle manufacturers.

Description of the BACTEC NR 660 system. The BACTEC NR 660 system made use of infrared detection of carbon dioxide from metabolism by microorganisms. It consisted of two parts. The first part was an incubator containing up to 600 vials located on 10 trays (5 aerobic and 5 anaerobic). At the bottom of the incubator, there were two rotary shakers for recently inoculated aerobic vials (the last 24 h). The second part was the test module connected to a video terminal, a keyboard, and a printer. This module contained the computer system, the measuring system, the tray transport, and the test head assembly. The video display showed the technologist the operating status of the system, the test in process, and the vial test data.

Maintenance and processing were carried out in accordance with the instructions of the manufacturer. Briefly, BACTEC vials were incubated for 7 days at 37°C. The NR 6A vials were read twice on days 1 to 3 and once on days 4 to 7. The NR 7A vials were read daily on days 1 to 7. They were both discarded from the incubator on day 8.

Criteria for positivity. Conventional blood cultures were inspected daily for evidence of growth. Positive criteria for diphasic blood cultures were the existence of colonies on the agar slide, the appearance of turbidity, hemolysis, gas production, and bacterial colonies in or on the blood layer (2). Positive criteria for prereduced Schaedler broth blood cultures were identical, except for the presence of colonies on the agar slide.

Positive BACTEC vial criteria were (i) a visual inspection of all vials prior to testing on the machine to detect evidence of microbial growth, such as bulging septa, hemolysis, or turbidity, and (ii) a growth value of $\geqq 30$ or a change in the growth value between two readings of $\geqq 15$. All information about positive vials was automatically printed out.

Processing of specimens. Direct smears were prepared from putative positive bottles and stained with May-Grunwald-Giemsa and Gram stains. Subculturing was carried out with media appropriate for the organism observed. Microorganisms were identified by standard methods. When bottles or vials were positive, according to the criteria described above for positivity, and when microscopic examination was negative, subculturing was carried out with the

* Corresponding author.

TABLE 1. Blood culture isolates recovered by the BACTEC and conventional systems (aerobic and anaerobic media)

Organism	No. of isolates recovered with indicated system	
	Conventional	BACTEC
Aerobic and facultative bacteria		
Gram negative		
Escherichia coli	15	13
Citrobacter freundii	3	3
Klebsiella pneumoniae	0	1
Klebsiella oxytoca	1	0
Enterobacter cloacae	3	2
Serratia marcescens	14	9
Proteus mirabilis	1	1
Haemophilis influenzae	1	1
Pseudomonas aeruginosa	11	12
Pseudomonas maltophilia	1	1
Acinetobacter spp.	9	8
Aeromonas hydrophila	1	1
Gram positive		
Staphylococcus aureus	36	35
Staphylococcus epidermidis	36	34
Micrococcus spp.	6	5
Streptococcus faecalis	10	6
Streptococcus faecium	1	1
Streptococcus sanguis	1	1
Streptococcus mitis	2	2
Nonhemolytic streptococci	2	3
Streptococcus pneumoniae	4	7
Corynebacterium spp.	1	2
Anaerobic bacteria		
Bacteroides fragilis	4	1
Clostridium perfringens	2	1
Yeast (*Candida albicans*)	6	5

following media: for aerobic bottles, chocolate agar with IsoVitalex enrichment (BBL Microbiology Systems); for anaerobic bottles, 10% blood–meat–yeast agar (Pasteur Production) and Rosenow medium (Pasteur Production) inoculated and then incubated at 37°C in a GasPak Jar (BBL Microbiology Systems), the latter medium also being overlaid with sterile paraffin. When one vial or several vials in a set were positive, processing of the negative vials was continued, and subculturing was carried out on day 8.

Recording and analysis of data. The following information was recorded on a report sheet for each set of positive bottles for both the conventional and BACTEC systems: bottle identification; time of sample collection; results for conventional bottles (i.e., growth on the agar slide, hemolysis, gas production, bacterial colonies in or on the blood layer, and turbidity); results for BACTEC vials (i.e., growth value or change in growth value and test number); subculture results; organism identification; existence of antimicrobial therapy; and time of antibiotic administration.

A paired comparison of the two blood culture systems was performed on positive bottles. The time to positivity was equal to the mean time of blood sampling in the ward plus the time until detection in the laboratory. As the quality of one anaerobic BACTEC medium batch was deficient, the corresponding data were reported but not analyzed. Statistical analysis was carried out with the Student *t* test.

RESULTS

During this study, 1,080 sets of blood cultures were obtained; 99 sets were incomplete, giving 1,048 associations of aerobic bottles and 1,013 associations of anaerobic bottles. There were 143 positive aerobic-medium associations (13.6%) and 139 positive anerobic-medium associations (13.7%). Sets containing *Staphylococcus epidermidis*, *Micrococcus* spp., or *Corynebacterium* spp. were considered to be contaminated if organisms were isolated in only one bottle of the set and if the other bottles of the set did not yield growth. This was the case in 26 positive sets (15 aerobic bottles and 11 anaerobic bottles).

Table 1 shows blood culture isolates recovered by the conventional and BACTEC systems. The conventional system recovered 171 isolates, whereas the BACTEC system recovered 155 isolates. This difference in the recovery rate was not statistically significant ($P > 0.05$).

For aerobic cultures, the time required for the detection of positivity by the two systems was compared in terms of the cumulative positive cultures per day and the cumulative percentage of paired positive cultures during the period of observation (Table 2): 34.0% of cultures detected by the BACTEC system were positive on day 1, and 92.8% were positive on day 2; the results for the conventional system were 6.2 and 63.9%, respectively. The recovery rates became equivalent on day 6 (97.9%). All fungi were detected on day 2 by the BACTEC system but not until day 3 by the conventional system. Moreover, the incubation times of organisms from positive aerobic cultures in both systems were classified. Thus, the mean detection times were 55.9 h (standard deviation, ±28.9) with the conventional system and 31.6 h (standard deviation, ±25.0) with the BACTEC system. This difference (24.3 h) between mean times was statistically significant ($P < 0.001$). For anaerobic cultures, most of the isolates detected were facultative bacteria. The incubation time required for these organisms was longer because culture conditions were more suitable for anaerobes. A comparison of the mean detection times was not performed for anaerobic cultures because there was a low number of anaerobes and because the performance of one batch of anaerobic vials was not suitable.

Organisms detected by only one system are shown in Table 3: 27 isolates were recovered by the conventional system, whereas 17 isolates were recovered by the BACTEC system. This system failed to detect some anaerobes because of the performance of one batch of anaerobic vials. Gram-negative bacteria were recovered better with conventional blood cultures. Major discrepancies between the two aerobic and anaerobic blood culture systems were detected. Thus, gram-negative bacteria such as *Serratia marcescens* seemed to be isolated better by the conventional system in three cases, whereas the recovery of *Pneumococcus* spp. was better in anaerobic and anaerobic vials with the BACTEC system (three isolates).

In many of the inoculated BACTEC vials with a growth value of ≧30, no organisms were detected, either on smears or on subcultures from NR 6A media. Thus, there were 199 aerobic vials (18.9%) with one or more false-positive readings. Of these, 162 (15.4%) ranged between growth values of ≧30 and <40, and 37 (3.5%) ranged between growth values of 40 and 85.

DISCUSSION

The BACTEC 460 system is a worldwide blood culture detection system. The sensitivity and accuracy of this sys-

TABLE 2. Cumulative positive cultures per day detected by both systems (aerobic bottles)

Organism	System[a]	No. of cumulative positive cultures detected on day:							
		1	2	3	4	5	6	7	8 (Subculture)
Gram-positive bacteria									
Staphylococcus aureus	C	1	18	25	27	28			
	B	8	26	27					28
Staphylococcus epidermidis	C		4	5	13		14	15	16
	B	3	11	12	13		15		16
Streptococci	C		11		12				
	B	4	12						
Other	C			1					
	B		1						
Gram-negative bacteria									
Enterobacteriaceae	C	3	16	18	19				
	B	12	19						
Pseudomonas aeruginosa	C	1	8	9		10			
	B	5	10						
Other	C	1	5	6	7				
	B	1	7						
Yeast (*Candida albicans*)	C			1	3	4			
	B		4						
Cumulative %	C	6.2	63.9	67.0	93.8	96.9	97.9	98.9	100
	B	34.0	92.8	94.8	95.9		97.9		100

[a] C, Conventional; B, BACTEC.

tem are already documented. However, the drawback of using C14-substrates in blood culture vials has limited the acceptance of this technique. With infrared detection of carbon dioxide from microbial metabolism, the BACTEC NR 660 system now avoids this drawback. From our experience, during a 4-month comparison of the BACTEC NR 660 system with the conventional blood culture system, we can draw some conclusions.

It is obvious that neither system was able to recover all of the clinically significant isolates. However, one of the main advantages of the BACTEC system was that it provided quicker detection of positive blood cultures. The BACTEC system detected positive blood cultures 24.3 h sooner than did the conventional system, and 92.8% of the isolates were recovered on day 2. The same percentage of recovery was obtained on day 4 with the conventional system. Thus, in connection with rapid methodologies for bacterial identification and susceptibility tests, this new system has an indisputable advantage for clinicians, who may receive quicker results concerning the culture. Some bacteria, such as *Pneumococcus* spp., enterococci, *Staphylococcus aureus*, *S. marcescens*, and *Pseudomonas aeruginosa*, were detected on day 1. However, our mean detection times were longer than those found by Jungkind and Millan in a previous comparison of BACTEC 460 and BACTEC NR 660 (D. Jungkind and J. Millan, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1068, 1984). This discrepancy might be explained by a longer mean time between blood sampling and blood culture incubation in our study.

Our results included the recovery of isolates considered to be contaminants (Table 1). These contaminations were, however, not due to a failure of the needle heater, as previously reported by other authors using the radiometric system. Moreover our pseudobacteremia was not due to cross contamination associated with the system (4).

As reported by some researchers, the number of organisms in gram-negative bacteremia is usually <2 bacteria per ml (3, 7). This observation might explain the fact that, in our study, gram-negative bacteria were sometimes recovered better by conventional bottles than by BACTEC vials. However, the lack of detection of aerobes and facultative anaerobes was equivalent in each aerobic system, whereas the detection of these bacteria was better with conventional anaerobic bottles than with BACTEC anaerobic vials. Thus, the use of prereduced Schaedler broth appeared to be more suitable than the use of anaerobic BACTEC vials for the growth of aerobes and facultative anaerobes and anaerobes. It is well known that isolate yield is predominantly dependent on specimen volume (2, 3, 5, 8). Thus, Plorde et al. (8) reported that the increase in the number of BACTEC vials improved the recovery rate of *Escherichia coli*, yeasts, and anerobes because the blood volume was increased. Therefore, in our study, the slight failure to detect gram-negative bacteria may have been due to the small volume of each blood sample. However, there was a nonsignificant difference in the recovery of gram-negative bacteria. On the other hand, with a view to increasing the recovery of bacteria in the blood, 60% of blood samples were drawn when the antimicrobial concentrations were at their lowest levels, in

TABLE 3. Organisms detected by only one system

Organism	No. of organisms detected			
	Aerobic vials		Anaerobic vials	
	Conventional system	BACTEC system	Conventional system	BACTEC system
Staphylococcus aureus	2	0	1	3
Staphylococcus epidermidis	1	3	1	2
Streptococcus faecalis			2	0
Streptococcus mitis	2	0	0	2
Escherichia coli	1	1	1	
Klebsiella pneumoniae	0	1		
Enterobacter cloacae	1	0		
Serratia marcescens	1	0	4	0
Pseudomonas aeruginosa			0	1
Acinetobacter spp.	1	2	2	0
Clostridium perfringens	1	0		
Bacteroides fragilis	2	0	2	0
Candida albicans	0	1	2	0

accordance with the recommendation of Rodriguez and Lorian (10). Concerning the recovery of fungi, our results correlated with those found with the BACTEC 460 system. Indeed, some authors reported that the BACTEC 460 system was more efficient in isolating fungi from blood and quicker in detecting fungi (around 3 days earlier) than the conventional system (9, 11).

The poor recovery of anaerobes in our study was really due to the performance of one batch of anaerobic BACTEC vials. However, this problem was rectified 1 month ago by the manufacturer. Therefore, this evaluation is still going on to establish if there is a significant difference.

Some authors reported that one of the problems with the radiometric system was the occurrence of false-positive results because of the metabolism of the ^{14}C-substrates by the blood (1, 9, 11). This phenomenon was more frequent with neonates because neonatal blood cells are known to be metabolically hyperactive, giving a high incidence of false-positives (1). Thus, as with BACTEC 460, we observed many false-positive cultures with a growth value of $\geqq 30$. Therefore, to avoid a great number of false-positive vials, it would seem necessary to modify the growth value. Thus, if the growth value was $\geqq 40$, the detection of true-positive cultures would not decrease, and false-positive cultures would still number 12 and be detected by a change in the growth value between two readings of $\geqq 15$. Furthermore, the number of false-positive cultures would be 37 (3.5%) instead of 199. This percentage is close to that found with conventional bottles. Indeed, technicians must frequently check conventional bottles because either the broth is slightly turbid or the blood is lacteous or lipemic. These events occur frequently in patients admitted to intensive-care units. Moreover, in our study, the intensive-care units of the hospital supplied more than 50% of the blood samples.

The practicability of the BACTEC NR 660 system was also evaluated. It met with a favorable reaction from technicians, despite the complexity of the study protocol and some difficulties at the beginning in understanding the sample testing schedules in correlation with the tray numbers. Furthermore, no problems were experienced with the standardized maintenance and testing procedures. All the secretaries used the system without any problem, entering patient data and operating the instrument. In fact, our technicians do not wish to go back to a conventional system because they feel that they save time, despite the number of false-positive cultures. Moreover, for examination of samples with the conventional system, two to three technicians were needed, whereas one technician seemed sufficient to examine the same number of samples with the BACTEC NR 660 system. The advantage for microbiologists was that the computer system managed the vial history, thus allowing them to check the latter at any time. Lastly, the BACTEC NR 660 system, a nonradiometric growth detection system, is available for use in laboratories or countries in which the routine use of radioactive components is forbidden. In conclusion, our results demonstrate that the BACTEC NR 660 system provides for the quicker detection of positive blood cultures and produces a highly acceptable level of reliability, in comparison with the conventional system.

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