JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1975, p. 302-308

Vol. 1, No. 3
Printed in U.S.A.

Laboratory Experience with a Radiometric Method for Detecting Bacteremia

WILLIAM A. THIEMKE[1] AND KONRAD WICHER*

Division of Clinical Microbiology, Erie County Laboratories at the E. J. Meyer Memorial Hospital, Buffalo, New York 14215 and the Department of Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214*

Received for publication 13 December 1974

Two bacteriologic systems for detecting bacteria in blood were compared; the automated radiometric BACTEC and the conventional method used in our laboratory for many years. BACTEC consisted of two bottles with 30 ml and the conventional method with 50 ml of media for aerobes and anaerobes. The BACTEC bottles were inoculated with 2 to 3 ml and the conventional with 4 to 5 ml of blood at the patient's bedside. Out of the 3,045 blood specimens cultured (804 patients), 262 (117 patients) were positive by one or both methods. The conventional system detected 5% more cultures. The explanation of the differences is discussed. Positive blood cultures were detected by the BACTEC procedure as early as 6 h after the blood collection. In the first 24 h, on the average, 77% of aerobic organisms were detected by the BACTEC as compared to 48% by the conventional system. All anaerobic BACTEC cultures were positive within 4 days, whereas the conventional system detected at that time 74%. At day 4, 67% of fungi were detected by the BACTEC and only 27% by the conventional system. Of the 3,045 blood cultures examined by the BACTEC, 208 were recorded as false positive with growth index readings ranging from 30 to 59.

The rising morbidity and mortality attributed to bacteremia, it's causes and trends, was recently reviewed (12). The time of detection of causative microorganisms is of essence in controlling bacteremia. Recently a radiorespirometric blood culturing system designed for more rapid detection of positive cultures has been introduced (6). This system employs ^{14}C-labeled substrates in a culture medium; microbial metabolism converts some of the substrate to $^{14}CO_2$ and the instrument detects the $^{14}CO_2$ as an indicator of growth. Both automated and semiautomated radiometric blood culturing systems have been used in this country for the past several years. Comparative studies with conventional systems have reported either favorable results for the radiometric system (3, 6, 11) or some indifference towards it (4, 10, 13). The way in which each system is set up and run and the media each system consists of will undoubtedly influence the final evaluation. This paper represents a comparative evaluation of the radiometric system BACTEC (Johnston Laboratories Inc., Cockeysville, Md.) and the conventional blood culture system used in our laboratory, and it analyzes available data obtained by various researchers.

MATERIALS AND METHODS

Blood samples from patients suspected of bacteremia were examined by two culturing systems run in parallel. Each system employed two bottles of media which were inoculated on the floors by the physicians and then sent, within 1 h, to the laboratory. The hour at which the laboratory received the specimen was considered to be zero time.

Conventional system. The conventional system consisted of two bottles: for aerobes, Trypticase soy broth, and for anaerobes, thioglycollate medium without indicator-135 C (Baltimore Biological Laboratories, Cockeysville, Md.) Each bottle was under a vacuum and contained 50 ml of medium, polyanetholesulphonate, and CO_2. Each medium was inoculated with 4 to 5 ml of blood. The inoculated media were incubated at 37 C for a total of 14 days with daily visual examination. Gram-stained smears and subcultures of the Trypticase soy broth to chocolate agar plates, incubated in a CO_2 incubator at 37 C, were routinely done on day 1 (macroscopic positive or suspicious cultures) and days 2, 7, and 12. The first three subcultures were held for 24 h and the last for 48 h. The thioglycollate cultures were examined similarly using vitamin K heme blood agar plates incubated in a Gas-Pak jar (Baltimore Biological Laboratories) for 48 h.

BACTEC system. The BACTEC system consisted

[1] Present address: Veterans Administration Hospital, West Roxbury, Mass. 02132.

of two media (Johnston Laboratories, Inc.,). One bottle (no. 6A) contained 30 ml of enriched tryptic soy broth (for aerobes) and the other bottle (no. 7A) contained 30 ml of prereduced tryptic soy broth (for anaerobes). Both media contained 1.5 μCi of ^{14}C-labeled substrates, polyanetholesulphonate, and CO_2 and were inoculated with 2 to 3 ml of blood. BACTEC bottles were incubated at 37 C for a total of 7 days with daily visual examination. The aerobic bottles (no. 6A) were continuously examined on a 3-h cycle for the first day using the BACTEC-225 automated instrument (Johnston Laboratories, Inc.). The gas used to flush the bottles was a mixture of 90% air and 10% CO_2. This was followed by daily growth index (GI) readings for 5 days and subcultured on day 5 to chocolate agar incubated in a CO_2 incubator for 48 h. (The GI is an arbitrary scale ranging from zero to 100 which is linearly proportional to the amount of ^{14}C detected. GI readings of 30 or greater were considered, in our experiment conditions, positive for blood cultures.) The anaerobic bottles (no. 7A) were examined daily in the BACTEC-225 machine for 5 days. The gas used in the sampling procedure was a mixture of 85% nitrogen, 10% CO_2, and 5% hydrogen which had passed through a DEOXO catalyst (Englehard Industries, E. Newark, N.J.). The medium was then subcultured to a vitamin K heme blood agar plate and incubated in a Gas-Pak jar at 37 C for 48 h. Aerobic and anaerobic media having a GI reading of 30 or greater were immediately subcultured and smeared for Gram staining.

Single blood cultures containing coagulase-negative staphylococci, *Bacillus* species, or catalase-positive anaerobic diphtheroid organisms were considered contaminants, even though it is understood that under certain circumstances these organisms may be considered pathogens. When multiple blood samples from a single patient contained these organisms, the clinical significance of the isolates was determined on the basis of the patient's condition.

A total of 3,045 blood samples were examined during a 6-month period (August 1973 through January 1974). All organisms recovered were isolated and identified to the species level.

RESULTS

The 3,045 blood specimens cultured represented 804 patients suspected of bacteremia. Of these, 262 cultures were positive by one or both methods indicating 117 patients (15%) with positive cultures or an overall positive rate of 8.6%.

The number and percentage of positive blood cultures, patients, organisms isolated, and contaminants are summarized in Table 1. BACTEC recovered 206 (79%) of the total positive cultures and the conventional system 219 (84%), indicating a 5% greater efficiency by the conventional system. The latter system also detected 11 more positive patients than the BACTEC system. Since 18 specimens contained multiple isolates, the total number (262) of clinically significant positive cultures is lower than the total number (280) of organisms isolated. Contaminants as defined above consisted of 91 isolates which were not included in the clinically significant isolates. The BACTEC system demonstrated a slightly lower number of contaminants.

The number of significant isolates and their mean detection times in days is illustrated in Table 2. There was an equal rate of recovery of gram-positive aerobes. The conventional system detected 13 more strains in the gram-negative aerobic group; however, the BACTEC system did recover four more fungi. There was a significant difference in the isolation rate in the anerobic group, with the BACTEC recovering significantly fewer organisms. Chi square analysis (5) gave a P value of less than 0.01. The mean detection time for all groups of isolates was in favor of the BACTEC system. On the average the BACTEC detected gram-negative aerobes about one-half a day earlier, gram-positive aerobes and anaerobic organisms 1 day, and fungi 3 days earlier than the conventional system.

The cumulative percentages of the organisms isolated by each system are demonstrated in Table 3. It has to be stressed that in the

TABLE 1. *Summarizing data of 3,045 blood culture specimens from 804 patients examined in parallel by the BACTEC and conventional system*

System	Positive cultures		Positive patients		Organisms[a]		Contaminants	
	No.	%	No.	%	No.	%	No.	%
BACTEC	206	79	97	83	214	77	52	57
Conventional	219	84	108	92	235	84	59	65
One or both methods[b]	262	100	117	100	280	100	91	100

[a] Each organism isolated was counted separately; therefore these numbers will be greater than the positive cultures due to 18 cultures with multiple isolates.

[b] Positive results obtained by both methods were considered as 100%. The percentages of positive findings in BACTEC or conventional systems were calculated on this basis.

conventional system only macroscopically positive or suspicious media were subcultured; therefore the results of the first 24 h might subjectively favor the BACTEC. Considerably more gram-positive and gram-negative aerobic bacteria were detected during the first 24 h of incubation by the BACTEC. The differences in the anaerobic isolates are less pronounced; 100% of the BACTEC isolates were detected by day 4 as opposed to 74% of the conventional isolates. In the fungi group, 67% of the BACTEC isolates were seen by day 4 as compared to 27% for the conventional system.

The rate of recovery of individual organisms is given in Table 4. The greatest differences are observed with *Escherichia coli*, *Bacteroides*, and *Peptococcus*, which are more frequently isolated by the conventional system. There were no substantial differences between the two systems with the other microorganisms.

In a certain number of the inoculated bottles no organisms were detected on smear or subculture from media with a GI of 30 or greater. These samples are referred to as false positive. One of the several plausible explanations of this phenomenon is a high leukocyte count in the patient's blood at the time of culture. Of the 3,045 blood cultures examined, 208 were recorded as false positive with GI readings ranging from 30 to 59. The majority of these false positive cultures were sporadic and had GI values in the low thirties. Patients with two or more false positive specimens and whose blood cultures remained false positive had their leukocyte count determined at the time of culturing. A comparison of leukocyte counts in 24 false positive reactors with more than one false positive reading is given in Table 5. The data are arranged according to the day at which the first false positive reading was obtained. Also in the table are two groups of controls; one consists of 19 patients with two or more negative blood samples and the other with 15 healthy volunteers with a duplicate set of blood cultures. The highest mean cell count of leukocytes was observed in the patients whose blood samples give false positive readings from the first day of culture. Lower cell counts were seen in patients whose false positive readings began on days 2 and 3. The higher mean leukocyte counts of the false positive reactors strongly imply that a high leukocyte count at time of culture might be responsible for the false positive results, although other factors may also contribute. One of the reactors from day 1 had acute myelomonocytic leukemia and the other had anaplastic carcinoma of the lung with metastases. The other reactors had a variety of illnesses ranging from urinary tract infections and pneumonia to chronic alcoholism, pancreatitis, ulcers, chronic heart failure, and adenocarcinoma. One-third of the false positive reactors, nine of the 19 patients with negative results, and none of the healthy volunteers were on antibiotic therapy 4 days prior to the blood collection. One healthy volunteer with a leukocyte count of 8.4×10^3 per mm^3 did have false positive readings ranging from 30 to 35 on days 3, 4, and 5 of culture. This individual admitted having chronic gingivitis.

TABLE 2. *Number of significant isolates and mean detection time of groups of isolates*

Group	No. of isolates[a]			Mean detection time (days)	
	B	C	T	B	C
Gram-positive aerobes	67	67	79	1.2	2.2
Gram-negative aerobes	101	114	135	1.2	1.8
Anaerobes	15	27	29	2.5	3.4
Fungi	31	27	38	4.0	7.1

[a] B, BACTEC; C, conventional; T, total isolates by one or both systems.

TABLE 3. *Cummulative percentages of isolates*

Group	System[a]	Days					
		0.5	1	2	3	4	≥5
Gram-positive aerobes	B	50	76	85	91	94	100
	C	5	44	70	86	91	100
Gram-negative aerobes	B	45	79	89	93	98	100
	C	10	55	79	93	94	100
Anaerobes	B	0	7	60	87	100	100
	C	0	4	52	70	74	100
Fungi	B	0	3	22	56	67	100
	C	0	0	0	15	27	100

[a] B, BACTEC; C, conventional.

The number of BACTEC-225 automated instruments in use in diagnostic laboratories is still limited and only a few reports evaluating the rapid method are available. A comparison of our results with those of two previously published studies is compiled in Table 6. The results of Brooks and Sodeman (3) and Caslow et al. (4) are not included here because these authors used different media. In all of the blood cultures there was approximately a 1:10 ratio of blood to culture medium. Trypticase soy broth was used for all aerobic cultures, and thioglycollate broth, THIOL-broth, or thioglycollate medium-135 C and no. 7A medium were used for anaerobic cultures. Two to four times more blood per culture bottle was used in the conventional than in the BACTEC system. The percentages of positive cultures and positive patients in the present study and that of Renner et al. (10) were comparable and both are substantially higher than in the study of DeBlanc et al. (6). The percentages comparing the BACTEC and conventional systems in the three studies are comparable and portray similar overall evaluations in the rates of recovery. The most striking and convincing data indicating employment of the radiometric system is the percent of isolates detected in the first day. In all studies the BACTEC system demonstrates a substantially higher percentage of positive samples during this period.

TABLE 4. *Rates of recovery of individual organisms*

Organism	BACTEC	Conventional	One or both methods
Escherichia coli	59	69	77
Staphylococcus aureus	37	36	40
Candida	24	21	31
Streptococcus pneumoniae	19	18	22
Bacteroides	12	18	19
Proteus	14	15	19
Streptococcus (group A, B, and D)	8	10	14
Klebsiella	9	12	13
Pseudomonas	8	7	10
Enterobacter	5	5	7
Peptococcus	1	5	5
Cryptococcus	4	3	4
Clostridium	2	3	4
Torulopsis	3	3	3
Listeria	3	3	3
Flavobacterium	2	2	2
Serratia	1	2	2
Haemophilus	0	2	2
Neisseria	2	0	2
Peptostreptococcus	0	1	1
Citrobacter	1	0	1

DISCUSSION

In evaluating the results we have to make clear that we had compared two systems for detecting bacteria in blood without adjusting the protocol to each other. The inoculated media of both systems started to be examined at similar times; however, the conventional media were examined visually at the first day of inoculation and the BACTEC media by the machine. The inoculated media of neither system were vented (unless one considers the flushing of the BACTEC media as venting procedure).

It is evident from the presented data that neither system was capable of recovering all of the clinically significant isolates. This suggests that the recovery rate to some degree depends upon the volume of blood cultured and the quality of the media used. In looking at the rates of recovery of the aerobic, gram-negative isolates, the conventional system had an advantage expecially in the recovery of *E. coli*. This can be partially explained by the larger volume of blood used by this system (the conventional bottles contained twice the volume of blood as did the BACTEC bottles). This explanation may be supported by the observation of Fine-

TABLE 5. *Comparison of false positive BACTEC readings with leukocyte counts*

Group	False positive from day:	No. of individuals	Leukocyte count[a]	
			Range	Mean
Patients with false positive results	1	2	50-109.2	79.6
	2	11	11.9-29.6	18.4
	3	11	10.3-23.6	16.6
Patients with negative results		19	4.3-17.9	10.3
Healthy volunteers[b]		15	4.4-8.4	6.1

[a] Leukocyte counts are expressed as $10^3/mm^3$.
[b] The volunteer with the leukocyte count of 8.4 did have false positive cultures on days 3, 4, and 5.

TABLE 6. *Data of three studies evaluating a radiometric blood culture system as compared to that of a conventional system*

Determinants	System	DeBlanc et al. (2)	Renner et al. (6)	Present study
Aerobic set up (ml blood/ml media)	BACTEC	2/30 TSB[a] (JLI)[b]	3/30 (6A) (JLI)	2-3/30 (6A) (JLI)
	Conventional	8/70 TSB (BBL)	10/100 TSB (Difco)	5/50 TSB (BBL)
Anaerobic set up (ml blood/ml media)	BACTEC[a]		3/30 (7A) (JLI)	2-3/30 (7A) (JLI)
	Conventional	2/20 Thioglycollate (BBL)	10/100 THIOL (Difco)	5/50 Thioglycollate-135 C (BBL)
	Total samples	2,967	1,445	3,045
	Patients	1,280	484	804
	Positive cultures	138 (5%)	106 (7%)	262 (9%)
	Positive patients	57 (4%)	56 (12%)	117 (15%)
Positive cultures	BACTEC	111 (80%)	84 (79%)	206 (79%)
	Conventional	125 (91%)	85 (80%)	219 (84%)
Positive patients	BACTEC	48 (84%)	43 (77%)	97 (83%)
	Conventional	50 (88%)	48 (86%)	108 (92%)
Isolates detected first day (%)	BACTEC	65%	20%	62%
	Conventional	4%	7%	39%

[a] This system consisted of only one bottle for both aerobes and anaerobes. The medium was Trypticase soy broth (TSB) which contained ^{14}C-labeled glucose as the only labeled substrate and used room air as culture gas.

[b] JLI, Johnston Laboratories, Inc.

gold et al. (7) who reported that in gram-negative bacteremia the number of organisms in the blood can be very low, frequently less than 1 colony-forming unit per ml of blood. In our experimental conditions we were unable to control the volume of blood inoculated per bottle, since all bottles were inoculated on the floors by the physicians. It is justifiable to assume that some variations in the volume of blood used as inoculum existed which could influence the results of the study. We have performed some additional experiments in which decreasing numbers of *E. coli* were mixed with blood of healthy donors and inoculated into BACTEC no. 6A medium. The *E. coli* in numbers from 1 to 10 colony-forming units per bottle multiplied and gave positive GI readings within 24 h of incubation. This indicates that the medium is suitable for the growth of *E. coli* and confirms that, in all likelihood, the volume of inoculated blood may be the variable factor in the discrepancies. Since the BACTEC system requires only 2 to 3 ml of blood for each bottle, one may expect that, when only the radiometric system is used for blood culture examination, multiple specimens will be taken and the microorganisms present in the blood will be detected and recovered.

Larson and associates (9) have reported a low recovery rate of *Haemophilus influenzae* in the BACTEC system. We had a similar experience; one *H. influenzae* and one *H. parainfluenzae* were detected by the conventional system only. Since that study we have recovered by the BACTEC method 12 isolates from two patients; three *H. influenzae* from one and nine *H. parainfluenzae* from another patient.

The frequency of detecting anaerobic organisms is significantly higher in the conventional system and only a part could be explained by the use of large volumes of blood. The organisms in this group most often missed by the BACTEC are the *Bacteroides* and *Peptococci*. Our data are in agreement with those reported by Renner et al. (10). The plausible explanation for it is the quality of the anaerobic BACTEC medium, which is not as good as the BBL thioglycollate medium-135 C without indicator in recovering anaerobes. (Johnston Laboratories have since replaced anaerobic medium no. 7A with a new medium, no. 7B. We have not yet evaluated this new medium.)

The BACTEC system is more efficient in isolating fungi from blood and substantially more rapid in their detection. The results of the positive cultures were available to the clinicians 3 days earlier as compared to the conventional system. Similar results were obtained by Renner et al. (10).

In 14 blood cultures, the GI readings were <30 throughout the examination period; however, microorganisms were detected after subsequent subcultures. Among the 14 isolates, nine were fungi (four *Cryptococcus neoformans*,

four *C. albicans*, and one *C. parapsilosis*), three were gram-positive cocci (*Streptococcus* group D, *S. pneumoniae*, and *S. aureus*), and two were gram-negative bacilli (*E. coli* and *K. pneumoniae*). The BACTEC has not been credited with the detection of these organisms. From the group of 14 isolates four were not recovered by the conventional system.

The false positive cultures in the radiometric system do present a problem as yet not solved. Our data suggest a partial correlation of the false positive results with high leukocyte counts. It is feasible that this is only one of multiple factors influencing the high GI readings. Zwarun (14) showed that 10% sucrose in hypertonic medium no. 8A (Johnston Laboratories, Inc.), which is used to detect and recover bacteria with cell wall deficiencies, lowers the maximum GI readings of whole blood. Although this medium is as yet not extensively used, it may be a possible answer in controlling the number of false positive cultures, as has been implied in the report of Bannatyne and Hartnett (1). The November 1974 Newsletter of the Johnston Laboratories states that lowering the CO_2 concentration to 5% in the gas used to flush the bottles will lower the number of false positive samples. In no one case of the BACTEC false positive cultures did the conventional system detect a microorganism. The false positive cultures do not jeopardize the laboratory results or cause unnecessary antibiotic treatment since the physicians are not contacted until the Gram stain or subculture is positive. The false positive samples are troublesome only because they increase substantially the number of samples to be examined.

It has to be stressed that our results obtained before day 2 are based on comparison of the macroscopic examination in the conventional system with the reading of the BACTEC-225 instrument and subculture. In spite of that, only small discrepancies may be expected, since in our experience, during the first 24 h, approximately 75% of the positive cultures could be recognized macroscopically. Blazevic et al. (2) reported that 65% of their cultures were first detected by macroscopic examination, whereas Hall et al. (8) found 81.9%. Rosner (11) found only 29% of positive cultures by macroscopic examination. The variation of these results will depend, among other things, on the media used and the number of organisms present in the blood sample.

It is apparent from our study and that of DeBlanc et al. (6) that the advantage in employing the radiometric system is the time saving factor in detection of positive cultures.

In our hospital (680 bed certified general hospital) approximately 50 to 60 blood samples are received daily for examination. For the examination of the blood cultures by the conventional system generally two to three technicians were needed, whereas one technician (sometimes additional help is needed) is sufficient to examine the same number of samples by the radiometric system. It is obvious that if approximately 20% of the cultures are positive, including false positive results, the technician processes only 20% of the total number of specimens. Furthermore, the clinician may receive preliminary results of the culture and antibiograms in approximately 50% of the cases with aerobic bacteremia within 24 to 36 h. This is an indisputable advantage of the radiometric system.

The major concern is the number of positive cultures which were not recognized by the instrument and were detected only after subculture on day 5 and the microorganisms which were not detected at all. However, the conventional system also failed to detect some microorganisms. At present, there is no one single system which could secure a 100% recovery of microorganisms in the blood.

ACKNOWLEDGMENTS

We express appreciation to Geraldine Karny and Deborah Bistrick for their skillful technical assistance.

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