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Clinical Comparison of a New Automated Infrared Blood Culture System with the BACTEC 460 System

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A new blood culture instrument, the BACTEC (Johnston Laboratories, Inc., Towson, Md.) NR-660, which utilizes infrared detection of carbon dioxide from microbial metabolism, was compared with the radiometric BACTEC 460 system. There were 1,554 isolates from 18,785 paired aerobic blood cultures. Of these isolates, 1,303 were isolated from the radiometric 6B medium, and 1,259 were isolated from the NR6A medium ($P = 0.06$). Analysis of the data indicated no significant differences in recovery when any individual species was considered. When organisms were considered as groups, there were no significant detection differences for gram-negative bacilli, yeasts, or anaerobes. For gram-positive cocci in aerobic medium, the BACTEC 460 detected 84.3% of the total isolates, and the BACTEC NR-660 detected 79.7% ($P = 0.04$). There were 891 isolates from 13,983 paired anaerobic blood cultures. Of these isolates, 725 were recovered from the radiometric 7D BACTEC medium, and 723 were recovered from the NR7A BACTEC medium ($P > 0.9$). In the anaerobic media there was no significant difference in detection of any organism group, including the gram-positive cocci. When the results of the aerobic and anaerobic media were combined, there was equivalence between the two systems for the detection of gram-positive cocci ($P > 0.2$) and other organism groups. When the ability to detect septic episodes was compared, there was no significant difference for any organism group ($P = 0.12$). For aerobic media, the mean times for detection were 30.5 and 29.5 h for the BACTEC 460 and NR-660, respectively. For anaerobic media, the mean times for detection were 39.8 and 41.6 h for the BACTEC 460 and NR-660, respectively. Compared with the BACTEC 460, the BACTEC NR-660 system had a greater ease of operation, faster test cycle, computerized data base, and equally rapid detection of positive cultures.

The detection of positive blood cultures is an important laboratory function that can be laborious and slow (6, 8, 11). The development of the BACTEC 225 and 460 instruments (Johnston Laboratories, Inc. Towson, Md.) allowed rapid detection of positive cultures and removed some of the subjectivity from the evaluation of blood culture bottles (4, 11). The BACTEC 460 system uses radioactive-labeled substrates in a rich bacteriological growth medium (9, 11). Metabolism by microorganisms present in the blood sample causes radioactive carbon dioxide to be released into the headspace of the closed bottles. A detector is used to quantitate the amount of radioactivity in the gas sample and to identify bottles as positive if the count is above a specified index value.

Although individual BACTEC bottles are considered exempt by the Nuclear Regulatory Commission (Title 10 of the Code of Federal Regulations, parts 30 through 34), we have noted some problems and inconvenience associated with the storage and use of radioactive media on patient wards, cleanup of broken bottles, disposal of the low-level radioactive wastes, and institutional surcharges related to the purchase, inventory, and disposal of these materials. In addition to the problems related to the radioactive isotope, the BACTEC 460 requires a significant amount of manual handling of bottles and recording of data.

An alternative blood culture instrument has been developed, which is similar in many ways to the radiometric BACTEC system. This new instrument is called the BACTEC NR-660 to differentiate it from the BACTEC 460. One key similarity is that a positive blood culture is identified by detecting an increase in carbon dioxide in the headspace of closed blood culture bottles. The increase in carbon dioxide is due to the metabolic action of microorganisms in culture medium. One key difference is that with the new system, quantitation of carbon dioxide is not made by using a radioactive label, but is done with an infrared spectrometer which is filtered and calibrated to selectively detect changes in total carbon dioxide concentration. The media for the BACTEC NR-660 are nonradioactive and are coded with the letters NR on the label. In addition, the bottles have a specially shaped notched base to differentiate them from the media for the BACTEC 460.

The purpose of this multicenter clinical trial was to determine whether the nonradiometric aerobic and anaerobic media tested on the NR-660 instrument would give results equivalent to those obtained with the radiometric media used for the BACTEC 460. Parameters evaluated were average time for detection of positive blood cultures, total numbers of organisms detected, and detection differences for individual species. Paired blood cultures were collected from patients when clinically indicated, and these cultures were tested simultaneously by each BACTEC system. In addition to evaluation of the performance characteristics of the media, the NR-660 instrument was evaluated regarding ease of operation, data management, and other performance characteristics.

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TABLE 1. Recovery of organism groups in aerobic media[a]

Organism group	Recovery from:		
	NR6A only	6B only	Both NR6A and 6B
Gram-positive cocci	117	151[b]	476
Gram-negative rods	78	86	389
Yeasts	24	28	103
Miscellaneous bacteria[c]	22	23	34
Anaerobic bacteria	10	7	6

[a] Microorganisms recovered from 18,785 aerobic bottle pairs.
[b] $P = 0.04$ for gram-positive cocci.
[c] Predominant organisms in the miscellaneous group in this and subsequent tables included *Listeria monocytogenes*, *Corynebacterium* JK and other *Corynebacterium* species, *Bacillus* spp., *Lactobacillus* spp., *Acinetobacter* spp., and *Neisseria* spp.

TABLE 2. Recovery of organism groups in anaerobic media[a]

Organism group	Recovery from:		
	NR7A only	7D only[b]	Both NR7A and 7D
Gram-positive cocci	79	69	289
Gram-negative rods	59	68	212
Yeasts	2	2	5
Miscellaneous bacteria[c]	4	4	10
Anaerobic bacteria	22	25	41

[a] Microorganisms recovered from 13,983 anerobic bottle pairs.
[b] $P > 0.9$.
[c] See Table 1, footnote *c*.

MATERIALS AND METHODS

Blood collection. A 12- to 20-ml sample of blood was drawn for each blood culture request, and equal portions were inoculated into each of four bottles of medium. The lower and upper limits for blood volume in each bottle were 3 to 5 ml, and the volumes were equal within each blood culture set. The total number of blood cultures included in the study was calculated to be 18,785 aerobic bottle pairs and 13,983 anaerobic bottle pairs.

Instrumentation and media. The BACTEC 460 system with standard radiometric aerobic 6B and anaerobic 7D media was used as the reference system. The BACTEC NR-660 with nonradioactive aerobic medium (NR6A) and anaerobic medium (NR7A) was the system under evaluation. Three clinical trial sites were involved in testing either the aerobic or anaerobic media. Two sites tested both media simultaneously.

Incubation and testing. The instrument protocols for each system were equivalent. Cultures were incubated at 35 to 37°C for 7 days. Aerobic cultures were shaken for 2 days, tested twice each day for the first 2 days, and then tested daily thereafter. Anaerobic media were tested once each day and were not shaken.

Detection of positive culture bottles. Examination of bottles was done according to the directions of the manufacturer of the systems. This examination involved testing each bottle with either the BACTEC 660 or 460 plus visually checking for bottles which were grossly positive. The two test systems were handled independently.

Data analysis. Statistical analysis of the paired blood culture data was performed with the McNemar test (2, 5).

A new clinical episode was considered to have occurred if there were no positive blood cultures in the preceeding 7 days. Only data from sites testing both NR6A and NR7A media on each patient were used in the analysis of data according to clinical episodes. Otherwise, independent comparisons were made between 6B and NR6A or 7D and NR7A media.

Corynebacterium sp., *Bacillus* sp., and coagulase-negative staphylococci were considered contaminants if they were isolated in only one bottle of the set and other sets did not grow the isolate. Probable contaminants were not included in the calculations.

RESULTS

Tables 1 and 2 show the incidence of recovery of all organism groups, including organisms which were recovered from only one system. For aerobic media, the McNemar test indicated that there was no significant difference in the overall isolation of organisms ($P = 0.06$), although the value approached significance. The organisms responsible for this approach to significance were the gram-positive cocci. The NR6A medium detected fewer organisms in this group ($P = 0.04$). There was no statistically significant difference in the frequency of recovery of various organism groups in the anaerobic media of the two blood culture systems ($P > 0.9$).

When the results of the paired studies with the aerobic and anaerobic media (Tables 1 and 2) were combined, the BACTEC NR-660 system missed a total of 463 isolates, and the BACTEC 460 missed 417 isolates ($P = 0.12$). For gram-positive cocci, the NR-660 missed 220 isolates, while the BACTEC 460 missed 196 ($P > 0.2$).

Tables 3 and 4 show the incidence of recovery of specific organisms, including those organisms recovered from only one system. Although a difference in the gram-positive cocci when considered as a group is indicated in Table 1, there was no significant difference when any individual gram-positive or -negative species was considered. All calculations for statistically significant differences for the individual organisms shown on Tables 3 and 4 gave P values of ≥ 0.1.

An important point for comparison of blood culture systems is the difference in detection of bacteremic episodes in

TABLE 3. Recovery of organisms in aerobic media[a]

Organisms	Recovery from:		
	NR6A only	6B only	Both NR6A and 6B
Staphylococcus epidermidis	60	76	167
Staphylococcus aureus	22	32	147
Streptococcus pneumoniae	3	2	23
Group D enterococcus	15	11	44
Alpha-hemolytic streptococci	13	21	53
Beta-hemolytic streptococci	3	5	25
Escherichia coli	27	29	140
Klebsiella pneumoniae	14	14	57
Enterobacter sp.	7	10	50
Salmonella sp.	1	1	5
Proteus mirabilis	6	3	27
P. aeruginosa	12	12	52
Haemophilus influenzae	1	2	5
Candida sp.	15	18	63
Cryptococcus neoformans	1	1	8
Miscellaneous organisms[b]	51	58	142

[a] Positives from 18,785 aerobic bottle pairs. $P \geq 0.1$ for all individual species and groups listed.
[b] See Table 1, footnote *c*.

TABLE 4. Recovery of organisms in anaerobic media[a]

Organisms	Recovery from:		
	NR7A only	7D only	Both NR7A and 7D
Staphylococcus epidermidis	32	25	83
Staphylococcus aureus	20	10	110
Streptococcus pneumoniae	5	5	25
Group D enterococcus	5	7	21
Alpha-hemolytic streptococci	8	11	21
Beta-hemolytic streptococci	7	10	23
Escherichia coli	39	31	92
Klebsiella pneumoniae	4	4	47
Enterobacter sp.	7	8	18
Proteus mirabilis	2	6	18
P. aeruginosa	5	11	16
Bacteroides fragilis	4	2	13
Bacteroides vulgatus	2	2	3
Clostridium perfringens	2	2	2
Clostridium innocuum	2	3	1
Eubacterium lentum	1	2	1
Propionibacterium acnes	4	6	5
Peptococcus sp.	2	1	1
Peptostreptococcus sp.	2	0	1
Miscellaneous organisms[b]	13	22	56

[a] Positives from 13,983 anaerobic bottle pairs. $P \geq 0.1$ for all individual species and groups listed.
[b] See Table 1, footnote *c*.

patients. This type of data analysis shows the overall system performance when multiple cultures on a patient and all media are taken into account. Table 5 shows the performance of the two systems regarding detection of bacteremic episodes. The data are from 7,768 blood cultures for which there were complete sets of aerobic and anaerobic media for each instrument. There was no statistically significant difference in the number of clinical episodes detected by the two systems ($P = 0.12$). The NR-660 system detected 84.5% of the episodes, and the BACTEC 460 system detected 88.7%. For episodes involving gram-positive cocci, the NR-660 system detected 84.2% and the 460 system detected 89.6% ($P = 0.12$).

TABLE 5. Detection of bacteremic episodes by the BACTEC 660 versus the BACTEC 460[a]

Organisms[b]	Detection by BACTEC:		
	660 only	460 only[c]	660 and 460
A	22	30	123
AE	0	2	5
AD	0	2	5
ADE	0	0	1
AC	0	0	3
ACE	0	0	1
AB	1	1	19
ABE	0	0	4
ABC	0	0	1
B	14	17	93
BE	0	0	4
BDE	0	0	1
BC	0	0	1
C	1	1	5
D	2	3	6
DE	0	0	1
E	4	4	11

[a] Positives from 7,768 paired blood culture sets.
[b] Organism key: A, gram-positive cocci; B, gram-negative rods; C, yeasts; D, other miscellaneous bacteria; E, Anaerobic bacteria.
[c] $P = 0.12$.

TABLE 6. Mean time to detection of microorganisms in aerobic media[a]

Organism group	No. of strains	Mean time (h)[b] to detection with:	
		6B	NR6A
Gram-positive cocci	481	30.2	29.6
Gram-negative rods	394	23.9	23.8
Miscellaneous bacteria[c]	35	44.5	36.1
Yeasts	104	51.8	48.0

[a] Positives from 18,785 aerobic bottle pairs.
[b] Average time to detection in hours when both types of aerobic media in the blood culture set were positive.
[c] See Table 1, footnote *c*.

Because early detection of positive blood cultures is a key characteristic of the BACTEC 460, it was considered important to determine how the BACTEC NR-660 system compared with the BACTEC 460 regarding average time to detect a positive culture with the various organism types. The results of these studies with the aerobic and anaerobic media are shown on Tables 6 and 7. These data were calculated in all instances where both the radiometric and the nonradiometric media in a blood culture set detected the organism.

Tables 6 and 7 show that the overall times for recovery of organisms from the aerobic and anaerobic media were equivalent when organisms were recovered from both systems. There were minor differences in the recovery times for individual organisms, but the only significant difference noted was with *Pseudomonas aeruginosa* in anaerobic media. The recovery times in anaerobic NR7A and 7D media were 72 and 40 h, respectively for 22 strains of *P. aeruginosa*. In aerobic media, which are the optimal growth environments for this oxidative organism, the mean times to detection for NR6A and 6B media were 27 and 24 h, respectively, for 53 isolates of *P. aeruginosa*.

DISCUSSION

Infrared spectroscopy has not been used before to routinely detect positive blood cultures. However, it is well documented that carbon dioxide can be measured by this procedure (1, 12). One previous clinical application of infrared spectroscopy has been the quantitative measurement of carbon dioxide and carbon monoxide concentrations in

TABLE 7. Mean time to detection of microorganisms in anaerobic media[a]

Organism group	No. of strains	Mean time (h)[b] to detection with:	
		7D	NR7A
Anaerobic bacteria	58	60.1	67.5
Facultative gram-positive cocci	317	41.5	39.9
Facultative and aerobic gram-negative rods	245	32.0	37.4
Miscellaneous bacteria[c]	11	58.2	50.2
Yeasts	7	39.4	41.0

[a] Positives from 13,983 anaerobic bottle pairs.
[b] Average time to detection in hours when both types of anaerobic media in the blood culture set were positive.
[c] See Table 1, footnote *c*.

exhaled breath samples during pulmonary function tests (3). To detect positive blood cultures, the BACTEC NR-660 uses infrared spectroscopy to periodically test the concentration of carbon dioxide in the headspace gas of a closed blood culture bottle. The bottle is flagged as a positive if the carbon dioxide concentration in the headspace increases to a point that exceeds a threshold value. Positives are also detected if there is a significant increase in carbon dioxide concentration over the previous reading, even if the threshold value has not been reached.

The recovery rates of microorganisms by the two aerobic media proved to be statistically equivalent when results for all organisms were combined. The NR6A medium recovered 81.0% of the total isolates, and the 6B medium recovered 83.8%. When subgroups of organisms were studied, there were no differences for gram-negative rods, yeasts, anaerobic bacteria, and other organisms, with the exception of the aerobic or facultative gram-positive cocci. When the gram-positive cocci were considered as a group, a statistically significant difference was seen between the two systems ($P = 0.04$), but there was not a statistically significant difference when the cocci were considered as individual species.

The staphylococci came closest to showing a significant difference in aerobic media when the coagulase-positive and -negative organisms were grouped ($P = 0.06$). There was no difference when the streptococci were considered separately or as a group ($P > 0.5$). Therefore this trend towards a difference in recovery in aerobic media was attributable mainly to the staphylococci for which the NR-660 detected 6% fewer isolates. In anaerobic media, however, the NR-660 detected 7% more staphylococcal isolates than the BACTEC 460.

Overall, the recovery rates of various organisms in the two anaerobic media were equivalent when results for all organisms were combined or were considered separately as organism groups or individual species. The two media each detected 81% of the 891 total isolates detected by either system.

In each of the types of anaerobic media, 19% of the total isolates were missed, and 16 and 19% were missed by aerobic 6B and NR6A media, respectively. These results are probably related to the fact that there were more total positives because an increased volume of blood was taken from each patient to allow inoculation of the extra bottles of NR aerobic or anaerobic media. All BACTEC bottles received the normal amount of blood (3 to 5 ml). The fact that a larger total volume of blood from each patient was cultured resulted in an increased number of total positives, but these positives were distributed among several types of medium (radiometric and nonradiometric). A positive from any bottle within a medium class (aerobic or anaerobic) counted as a positive culture for that medium class. It has been previously reported that the number of positive results increases with the number of cultures taken and the volume of blood cultured (6, 9, 10, 11). The recovery increase of 16 to 19% from the inoculation of two bottles rather than one of aerobic or anaerobic medium is very close to what is expected based on these previous studies. It is noteworthy that the distribution of organisms missed is relatively equal between the two blood culture systems, indicating that random chance and not an inherent difference in one system versus the other, was affecting the distribution.

When we evaluated the combined results from the paired aerobic and paired anaerobic bottles, there was no significant difference between the BACTEC 460 and NR-660 systems regarding recovery of total isolates ($P > 0.12$), gram-positive cocci ($P > 0.2$), or staphylococci ($P > 0.5$). Further studies are needed to determine whether the slightly lower detection of gram-positive cocci in the NR6A aerobic medium is reproducible at other institutions and clinically significant. Until then it would be prudent to subculture negative bottles in certain clinical situations such as endocarditis, or when one receives only enough blood for an aerobic bottle. Overall, a two-bottle NR-660 system with NR6A and NR7A media matched the performance of the 460 system with 6B and 7D media for the detection of positive blood cultures.

The detection of episodes of bacteremia in individual patients more closely measured differences that might be expected to have clinical significance rather than just differences between paired aerobic or anaerobic bottles. Multiple sets of blood cultures which include both aerobic and anaerobic bottles are often collected for each patient, and positive findings in any one set would result in detection of a positive episode. Minor differences between media or detection systems are compensated for to some extent by this multiplicity of testing. The two systems were statistically equivalent with regard to detection of clinical episodes of bacteremia, including those involving the gram-positive cocci.

An important aspect of the BACTEC radiometric system is the early detection of bacteria in blood cultures because of the sensitivity of this system for the detection of labeled carbon dioxide (4, 11). The mean time for detection of organisms by the radiometric BACTEC 460 versus the infrared detection system of the NR-660 was one of the most important points for comparison. Overall, the NR-660 system was equivalent to the BACTEC 460 with regard to the mean time for detection of bacteria and yeasts. For 1,652 isolates detected in both types of aerobic media or both types of anaerobic media, the mean time for detection in the BACTEC 460 system averaged 34.1 h and the NR-660 system averaged 34.2 h. For 1,014 isolates in aerobic media, the overall mean time for detection was 30.5 h for the BACTEC 460 and 29.5 h for the BACTEC NR-660. For 638 isolates in anaerobic media, the mean time for detection was 39.8 h for the BACTEC 460 and 41.6 h for the NR-660. With anaerobic NR7A media, there was a delay in detection of *P. aeruginosa*, but no significant difference in absolute recovery ($P > 0.13$). *P. aeruginosa* was recovered quickly in the aerobic medium of both the BACTEC 460 and NR-660. Overall, facultative organisms appeared to be detected faster in aerobic media than in anaerobic media. This result is due in part to the fact that in this study aerobic bottles were tested twice each day for the first 2 days of incubation, whereas the anaerobic bottles were tested only once.

There were other features of the BACTEC NR-660 that were noted during the study. Pseudobacteremias have been reported with other blood culture systems, including the radiometric BACTEC (7). In the case of the radiometric BACTEC system, inadequate sterilization of the sampling needle has been the cause of this occasional contamination. The NR-660 has an improved needle sterilization system that heats the entire length of the needle, and this system should reduce the occurence of contamination. The 35-s test cycle time of the NR-660 was faster than the 60-s cycle time of the BACTEC 460. This difference allows much faster completion of the daily workload. The computerized data base facilitated inquiry about the status of blood cultures on individual patients. The NR-660 computer prompts the user regarding quality control, test entry, and tray testing order.

This prompting made it easy to keep testing schedules in order. The use of large trays for incubating and testing bottles in groups of 60 resulted in less manual handling of individual bottles. This difference reduced the amount of work in the daily testing cycle, and there was less chance of test omission.

The use of a nonradiometric system offers definite advantages to those institutions operating under the more restrictive type of Nuclear Regulatory Commission license. It can simplify recordkeeping for media. Special radioactive waste disposal procedures are not necessary. Periodic monitoring of the work areas for isotope contamination can be eliminated. Moreover, some hospitals have a policy of collecting samples for blood cultures in a transport tube for later inoculation of media in the laboratory. This kind of sample collection is sometimes done because radiometric media are not allowed in patient care areas because of concerns regarding the reaction of patients to the special cleanup procedure required after accidental breakage of the bottles. Specimen collection can be simplified in those situations, and there may be less chance of bacterial contamination because blood specimens would be transferred once rather than twice to achieve inoculation of the BACTEC bottles.

In conclusion, the nonradiometric BACTEC NR-660 matched the overall performance characteristics of the radiometric BACTEC 460 system and was faster and more convenient to operate.

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