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Effect of Agitation and Frequent Subculturing on Recovery of Aerobic and Facultative Pathogens by Roche Septi-Chek and BACTEC Blood Culture Systems

M. JANE KIM,* RICHARD L. GOTTSCHALL, LYNN D. SCHWABE, AND EILEEN L. RANDALL

Division of Microbiology, Department of Pathology and Laboratory Medicine, Evanston Hospital, Evanston, Illinois 60201

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The positivity rate and time to recovery of pathogens were compared in Roche Septi-Chek (RSC-TSB) and BACTEC radiometric systems on 3,539 paired blood cultures. Both systems were steadily agitated, with frequent subculturing or processing of the RSC-TSB agar slides and BACTEC bottles, respectively, during the first 24 h of incubation. The RSC-TSB system recovered 249 pathogens (7.0% positivity rate), compared with 234 (6.6% positivity rate) isolates recovered from BACTEC. For the most common isolates, *Staphylococcus aureus* and the *Enterobacteriaceae*, the median time to detection was 15.8 h for BACTEC and 18.6 h for the RSC-TSB system. No statistically significant difference was observed in recovery of organisms from the two systems, except for *S. aureus* ($P < 0.05$). In the RSC-TSB system, 42% of *S. aureus*, 58% of the *Enterobacteriaceae*, and 45% of *Pseudomonas aeruginosa* isolates had sufficient growth on the agar slant to allow performance of rapid standardized identification and susceptibility studies. In comparison with other studies using static incubation, it appears that agitation and frequent subculturing of the RSC-TSB system during the first 24 h of incubation decreased the time to detection for the majority of significant blood culture isolates.

A number of studies have been performed comparing the Roche Septi-Chek (RSC-TSB) biphasic system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) with conventional and radiometric methods (1, 4–8) for the recovery of aerobic and facultative pathogens from blood cultures. Favorable results in these studies were noted with the RSC-TSB aerobic system when used with daily subculture and static incubation. The Roche system consists of a blood culture bottle with an attachable agar-containing chamber which allows easy subculturing by inversion of the bottle. Steady agitation of blood cultures, especially during the first 24 h, enhances the rapidity and rate of recovery of pathogens (2). The present study was conducted to compare the effects of agitation on the recovery of pathogens from RSC-TSB and BACTEC 460 (Johnston Laboratories, Inc., Towson, Md.) systems. In addition to agitation, the RSC-TSB bottles were subcultured four times during the first 24 h of incubation by the standard procedure in our laboratory for processing BACTEC aerobic bottles. The two systems were compared for sensitivity of recovery, time elapsed to initial detection, and time elapsed to availability of identification and susceptibility results.

MATERIALS AND METHODS

Two tubes (Becton-Dickinson Vacutainer Systems, Rutherford, N.J.) of equal volumes (6 to 8 ml) of blood were drawn from separate sites, one immediately after the other, from patients with suspected bacteremia and were sent to the laboratory. The two tubes, constituting a blood culture set, were inoculated into two blood culture systems (the total volume of blood going into each system being equal): one tube into an RSC-TSB aerobic bottle (containing 70 ml of tryptic soy broth) and the other tube divided equally between BACTEC bottles 6B and 7D (each containing 30 ml of tryptic soy broth). Under a laminar flow hood, the RSC-TSB slide chamber containing chocolate, malt, and MacConkey agars was attached immediately, and subculture was performed by inverting the bottle. The RSC-TSB and 7D anaerobic BACTEC bottles were incubated at 35°C with agitation (New Brunswick Scientific Co., Inc., Edison, N.J.; Shaker/Reciprocater R-2) at 200 to 250 rpm for the first 24 h and then stationary for 6 additional days. The 6B aerobic BACTEC bottles were incubated at 35°C with agitation for the entire 7 days. The 7D BACTEC bottles were tested on the BACTEC 460 once a day according to the recommended procedure. The 6B bottles were tested four times during the first 24 h, two times in the next 24 h, and once a day thereafter. Subculture by inversion was performed on the RSC-TSB bottles by the BACTEC 6B test schedule described above from 1 to 5 h postinoculation. All bottles from both systems were subcultured on day 7. Samples of blood were withdrawn from BACTEC bottles, and 0.1 ml was inoculated onto each of two plates (5% sheep blood and chocolate agars). The blood plates were incubated anaerobically, and the chocolate plates were incubated under 5% CO_2, both at 35°C. Final plate readings were made at 48 h. RSC-TSB bottles were subcultured by inversion of the bottles onto the agar slide chambers and incubated at 35°C, and final readings were made at 48 h.

Gram-stained smears were performed on BACTEC bottles if gas, turbidity, or hemolysis was observed or if growth indices of >30 (6B) or >20 (7D) or incremental changes in the growth indices of 10 (6B) or 5 (7D) were displayed. The RSC-TSB bottles were read for the presence of hemolysis, turbidity, or growth on the agar slant, and if positive, Gram-stained smears were performed. Statistical differences

* Corresponding author.

TABLE 1. Recovery of microorganisms from RSC-TSB, BACTEC bottles, and both systems

Microorganism	No. of isolates recovered from:			P
	RSC-TSB only	BACTEC only	Both	
Gram negative				
Enterobacteriaceae	36	27	73	NS[a]
P. aeruginosa	4	4	7	NS
Miscellaneous[b]	1	3	7	NS
Gram positive				
S. aureus	14	4	34	<0.05
CNS[c]	4	11	12	NS
S. pneumoniae	4	1	5	NS
Enterococci	3	0	13	NS
Streptococci[d]	7	7	17	NS
Corynebacterium sp.	0	1	1	NS
Yeasts	1	1	6	NS

[a] NS, Not significant. Overall, P was not significant.
[b] Includes *H. influenzae*, *C. jejuni*, *Neisseria meningitidis*, CDC-VB3, unidentified gram-negative bacillus, and *Aeromonas hydrophila*.
[c] CNS, Coagulase-negative staphylococci.
[d] Viridans group and beta-hemolytic streptococci.

in the isolation of organisms in the two systems were calculated by the modified chi-square test described by McNemar (3).

Times for detection in both systems were determined by demonstrating the presence of organisms in the broth bottles of BACTEC or, in the case of RSC-TSB, on the agar slant or in the broth bottle, whichever showed the presence of organisms first. In cultures displaying organisms in the broth from either system, samples were removed to harvest the organism for preliminary identification and susceptibility studies. With the slant-positive RSC-TSB cultures exhibiting sufficient growth, standardized identification and susceptibility studies were performed. All positive bottles, whether broth- or slant-positive, were subcultured for purity, for standardized identification and susceptibility studies, or for further definitive testing if indicated.

RESULTS

During a 17-month period, from June 1984 to October 1985, 3,539 blood culture sets were compared in the two systems. From these 7,078 blood cultures, 308 (4.4%) significant organisms were isolated; 249 isolates were recovered from RSC-TSB (7.0% positivity), and 234 isolates were recovered from BACTEC (6.6% positivity). From both systems, a total of 175 significant isolates were recovered; 74 were recovered from RSC-TSB only, and 59 were recovered from BACTEC only (Table 1). Anaerobic organisms isolated were excluded from the statistical data; however, excluding single isolates of *Propionibacterium* species (considered contaminants), BACTEC recovered a total of seven isolates of *Bacteroides* species, *Fusobacterium* species, and *Clostridium* species. No anaerobes were recovered from the RSC-TSB system. The contamination rates were 3.5% for RSC-TSB and 2.6% for BACTEC. Organisms considered contaminants, single isolates of coagulase-negative staphylococci, viridans group streptococci, *Bacillus* species, diphtheroids, and *Micrococcus* species, were excluded from the data. No statistically significant differences between the two systems were calculated in the recovery of the total organisms or individual organisms, with the exception of *S. aureus* ($P < 0.05$), of which 14 isolates were recovered by RSC-TSB only, 4 were recovered by BACTEC only, and 34 were recovered by both (Table 1).

The mean and median times to detection of organisms isolated by the two systems are shown in Table 2. Both the mean and median hours to detection were consistently shorter or the same with BACTEC than with RSC-TSB, although these differences, especially with the most commonly isolated organisms, *S. aureus* and the *Enterobacteriaceae* (63% of total organisms), consisted of 2 to 4 h. The mean and median times to detection for *Escherichia coli*, the organism which accounted for 61% of the total *Enterobacteriaceae* isolates, were approximately 4 h shorter with BACTEC. The differences between the two systems with the rest of the isolates varied from 4 to 42 h. The mean and median times to detection for the miscellaneous gram-positive organisms, consisting of beta-hemolytic streptococci, viridans group streptococci, and *Corynebacterium* species, were 12 and 20 h faster with BACTEC. The miscellaneous gram-negative organisms, consisting of *Haemophilus influenzae*, *Campylobacter jejuni*, CDC-VB3, and an unidentified gram-negative bacillus, were detected 42 h sooner by BACTEC. The mean and median detection times for *Streptococcus pneumoniae* were only 4 h faster with BACTEC, whereas the mean and median detection times for coagulase-negative staphylococci were 19 and 27 h quicker, respectively, with BACTEC. The mean detection times for *P. aeruginosa*, enterococci, and yeasts were identical for both systems.

Of 249 isolates from the RSC-TSB system, 117 (46.2%) showed sufficient growth on the agar slant at the time of detection for both standardized identification and susceptibility studies. Approximately 42% of *S. aureus* and *P. aeruginosa*, 44% of enterococci, and 58% of the *Enterobacteriaceae* exhibited sufficient growth for such studies. For some of the less commonly isolated organisms, i.e., *S. pneumoniae*, coagulase-negative staphylococci, miscellaneous gram-positive and gram-negative isolates, and yeasts,

TABLE 2. Mean and median times to detection of significant isolates by RSC-TSB and BACTEC

Organism	RSC-TSB			BACTEC		
	No. of isolates	Time (h) to detection		No. of isolates	Time (h) to detection	
		Mean	Median		Mean	Median
S. aureus	48	21.1	18.0	38	18.9	16.0
Enterobacteriaceae	109	20.5	19.1	100	17.1	15.6
E. coli	66	21.6	16.0	61	17.0	12.0
Klebsiella pneumoniae	18	20.1	17.0	16	13.7	12.0
P. aeruginosa	11	31.5	24.0	11	27.6	24.0
Enterococci	16	26.0	18.0	13	17.7	18.0
CNS[a]	16	50.8	45.0	23	31.5	18.0
S. pneumoniae	9	17.3	16.0	6	13.7	12.0
Miscellaneous gram positive[b]	25	45.8	43.6	26	32.9	21.8
Miscellaneous gram negative[c]	8	64.2	64.2	7	22.2	22.2
Yeasts	7	41.3	30.0	7	31.2	30.0
N. meningitidis	0			3	104.0	60.0

[a] CNS, Coagulase-negative staphylococci.
[b] Includes beta-hemolytic streptococci, viridans group streptococci, and *Corynebacterium* species.
[c] Includes *H. influenzae*, *C. jejuni*, CDC-VB3, and unidentified gram-negative bacillus.

the percentages of slant positives for complete standardized studies were 22, 69, 25, and 14%, respectively.

DISCUSSION

Although a greater number of clinically important organisms were isolated from RSC-TSB than from BACTEC, the difference was not statistically significant. As for individual organisms, more isolates of *S. aureus* were recovered from RSC-TSB than from BACTEC, and this did prove statistically significant ($P < 0.05$). In another study (8) which compared BACTEC with RSC-TSB, the authors stated that RSC-TSB isolated significantly more members of the *Enterobacteriaceae*, other then *E. coli*, than did BACTEC. In the present study, even though more of the *Enterobacteriaceae* were isolated from RSC-TSB ($n = 109$) than from BACTEC ($n = 100$), the difference was not statistically significant. The study of Weinstein et al. (8) included a higher number of paired blood cultures, and if we had studied an increased number of blood culture sets, this difference possibly would have been observed.

Almost 50% of RSC-TSB-positive cultures displayed sufficient growth on the agar slant at the time of detection to enable performance of identification and susceptibility studies. In many laboratories, including our own, by use of rapid methods, preliminary reports on gram-negative rods detected in broth blood culture bottles are available within 5 to 7 h. Standardized studies on colonies from subculture requiring an additional 24 h of incubation could be performed the next day. Even though organisms were detected a few hours earlier in the BACTEC broth bottles, final identification and susceptibility reports were generated more rapidly with RSC-TSB for nearly one-half of the pathogens, due to the availability of colonial growth on the RSC-TSB slant. With slide-positive RSC-TSB cultures, other easily performed tests, i.e., oxidase, coagulase, and rapid indole, can be performed which aid in the rapid identification of isolates.

Coagulase-negative staphylococci were detected approximately 24 h quicker by BACTEC than by RSC-TSB; however, almost 70% of these isolates were slant positive in the RSC-TSB system, thus allowing coagulase and standardized susceptibility studies to be performed at approximately the same time that standardized studies were being performed on the BACTEC isolates. A disadvantage of BACTEC, as with other broth blood culture systems, is that staphylococcal isolates must be subcultured to plate media for performance of standardized susceptibility studies, especially for detection of methicillin-heteroresistant staphylococci.

Substantial differences can be noted if the present RSC-TSB study is compared with other studies. In one study (5) which compared the Du Pont Isolator system (Du Pont Co., Wilmington, Del.) with RSC-TSB, the authors stated that the times to detection of *S. aureus* and *P. aeruginosa* were significantly shorter with the Isolator. In the present RSC-TSB study, the times to detection were 12 to 20 h shorter than the times to detection by the Isolator in that study. This difference may be because in the Du Pont Isolator–RSC-TSB study, the RSC-TSB cultures were incubated statically and examined for growth once a day for 7 days, whereas in the present study, the RSC-TSB bottles were agitated as well as subcultured and examined for growth six times within the first 48 h of incubation.

In another study (8) which compared BACTEC with RSC-TSB, the authors stated that statistically significant more organisms were isolated 24 h or greater more rapidly from BACTEC than from RSC-TSB. In our study, with agitation and frequent subculture of RSC-TSB, the time to detection for the majority of organisms was only 2 to 4 h more rapid with BACTEC. In addition, the previous study stated the mean time to detection of *S. pneumoniae* in RSC-TSB to be 41 h; our data showed a mean time of 17 h.

When cumulative percentages for detection times were calculated in the present study, by 24 h both systems had detected 90% of all *S. aureus* isolates and RSC-TSB and BACTEC had detected 84 and 93%, respectively, of isolates of the *Enterobacteriaceae*. It appears that agitation of the RSC-TSB system, at least during the first 24 h of incubation, and subculturing four times during the first 24 h have decreased the time to detection for the majority of significant blood culture isolates when comparing these times with those reported previously.

Frequent agitation and subculture as described are feasible in a laboratory setting. This study was conducted in a microbiology laboratory that routinely processes blood cultures on the BACTEC system four times in the first 24-h (2 a.m., 8 a.m., 2 p.m., 8 p.m.), two times in the next 24 h (2 a.m., 8 a.m.), and once a day thereafter. During each 24-h period, 35 to 40 new blood cultures are received, with each shaker-reciprocator unit capable of holding 32 RSC-TSB or 64 BACTEC bottles. To facilitate the frequent testing schedule, the laboratory is staffed as follows: one technologist is present in the blood culture area exclusively during the day shift, two technologists are present during the second shift, and one semitechnical person is present during the third shift. Their responsibilities include all specimen and blood culture processing. The time required to read the agar slants and subculture 40 negative RSC-TSB bottles is approximately 5 to 10 minutes (eight bottles in a plastic rack can be inverted simultaneously). Because the majority of the most common isolates are detected within the first 24 h of the process, shaking for this period only would minimize the number of shakers required, thereby making shaking a feasible alternative to static incubation for many laboratories.

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