JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1488–1491
0095-1137/94/$04.00+0

Vol. 32, No. 6

Comparison of BACTEC Plus 26 and 27 Media with and without Fastidious Organism Supplement with Conventional Methods for Culture of Sterile Body Fluids

DEANNA D. FULLER,[1,2]* THOMAS E. DAVIS,[1,2] PAMELA C. KIBSEY,[3] LINDA ROSMUS,[3] LEONA W. AYERS,[4] MAURETA OTT,[4] MICHAEL A. SAUBOLLE,[5] AND DAVID L. SEWELL[6]

Wishard Memorial Hospital[1] and Indiana University Medical Center,[2] Indianapolis, Indiana; Grey Nuns Hospital, Edmonton, Alberta, Canada[3]; The Ohio State University Hospitals, Columbus, Ohio[4]; Good Samaritan Regional Medical Center, Phoenix, Arizona[5]; and Veterans Affairs Medical Center, Portland, Oregon[6]

Received 29 November 1993/Returned for modification 13 January 1994/Accepted 8 March 1994

We compared the BACTEC Plus 26/27 culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) with and without fastidious organism supplement with conventional centrifugation preparation and plating for the recovery and speed of detection of microorganisms. A total of 1,101 sterile body fluid specimens were collected and processed at five hospital laboratories, yielding 234 (21%) positive cultures. Of the 176 isolates considered clinically significant, 133 (76%) were recovered by both the BACTEC system and conventional culture, while 28 (16% [$P < 0.005$]) were recovered by BACTEC only and 11 (6%) were recovered by conventional culture alone. There were no statistically significant differences in the speed of detection of microbial growth. It was found that BACTEC, with or without the addition of fastidious organism supplement, exhibited improved sensitivity for the recovery of microorganisms, including fastidious bacteria.

The conventional method for examination of sterile body fluids (SBF) other than blood often involves centrifugation or filtration of the specimen, with subsequent staining, and/or inoculation onto solid media. Often, the number of organisms is low, and benefit can be gained from culturing a larger quantity of specimen to enhance recovery (4).

SBF submitted for microbiological culture include peritoneal, pleural, synovial, cerebrospinal, pericardial, amniotic, and other fluids. Recent reviews have discussed the merit of semiautomated blood culture systems for the culture of ascitic fluids from patients with spontaneous bacterial peritonitis and from patients with peritonitis secondary to continuous ambulatory peritoneal dialysis (CAPD) (3, 6, 12, 14, 17). Liquid culture of other SBF has been studied less often, although use of broth media for routine culture of cerebrospinal, joint, and pleural fluids has been reported (8, 15, 16, 18). Several investigators have utilized the BACTEC system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) for culture of CAPD fluid and/or ascitic fluid (1, 3, 5, 6, 10–12, 14, 15), yielding comparable results, but failure to grow certain fastidious microorganisms such as *Neisseria gonorrhoeae* without supplementing the broth with blood or serum has been a problem (9, 15, 16).

This multihospital study compared culturing of SBF using BACTEC nonradiometric (NR) aerobic (Plus 26) and anaerobic (Plus 27) resin-containing media with and without supplement with the conventional culture method. Fastidious organism supplement (FOS) was added to the BACTEC system to evaluate its ability to optimize the recovery of nutritionally fastidious bacteria such as *Neisseria* and *Haemophilus* species. FOS, developed by Becton Dickinson Diagnostic Instrument Systems, was designed to neutralize some of the toxic effects of sodium polyanethol sulfonate present in the BACTEC bottles in concentrations up to 0.05% and to provide growth factors X and V.

MATERIALS AND METHODS

We evaluated the recovery and speed of detection of microorganisms found in 1,101 SBF specimens collected from patients at Wishard Memorial Hospital, Indianapolis, Ind.; University of Alberta Hospital, Edmonton, Alberta, Canada; The Ohio State University Hospital, Columbus; Good Samaritan Regional Medical Center, Phoenix, Ariz.; and Veterans Affairs Medical Center/Portland Division, Portland, Oreg.

Specimen collection. A volume of 2.5 to 50 ml of each SBF was required. Fluids included were synovial, pleural, pericardial, peritoneal, cerebrospinal, amniotic, and CAPD. The patient specimens were collected in sterile containers according to local protocol and transported to the microbiology laboratory for processing. One SBF per patient per 24-h period was accepted, and each specimen was evaluated individually. All fluid culture sets were processed for purposes of patient care, but only those adequately filled and processed within 12 h after collection were included in the study.

Specimen processing. Equal volumes of SBF were inoculated into conventional media and into a combination of BACTEC broth media (Fig. 1). BACTEC Plus NR26 is an aerobic enriched soybean-casein digest broth with CO_2, and BACTEC Plus NR27 is a prereduced (anaerobic) enriched soybean-casein digest broth with CO_2. Conventional setup included the use of 1 to 20 ml of body fluid specimen. Specimens received for conventional setup with a volume greater than 10 ml were centrifuged at 1,500 × *g* for 15 min, and the sediment was plated onto the media. Those with a volume of 1 to 10 ml were plated directly onto the conventional media. Chocolate blood agar, 5% sheep blood agar, and anaerobic blood plate media along with thioglycolate and tryptic soy broths were inoculated. BACTEC Plus medium inoculation consisted of adding 0.5 to 10 ml of body fluid specimen each to NR26, NR27, and a second NR26 to which

* Corresponding author. Mailing address: Department of Pathology, Wishard Memorial Hospital, 1001 W. 10th St., Indianapolis, IN 46202. Phone: (317) 630-8060. Fax: (317) 630-7913.

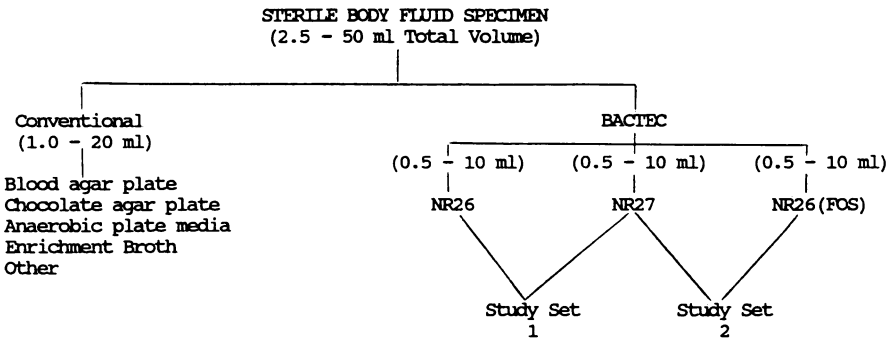

FIG. 1. Inoculation of conventional media and BACTEC bottles. In the conventional culture method, in most cases, if the fluid volume was >10 ml the specimen was centrifuged and the sediment was plated. Some institutions included additional media besides the ones specified here (Other). In the BACTEC system, the volume of specimen examined in each study set was equal to the conventional culture volume; for example, if 2.5 ml of specimen was received, 1.0 ml was inoculated onto conventional media and 0.5 ml was inoculated into each of the three BACTEC vials, although only one study set-conventional method pair was analyzed at a time.

2 ml of FOS was added (NR26FOS). Conventional culture was always compared with two vial sets: either NR26 without FOS and NR27 (NR26/27; study set 1) or NR26 with FOS and NR27 (NR26FOS/27; study set 2). The FOS was previously reconstituted and stored according to the manufacturer's instructions. BACTEC Plus NR26/27 and NR26FOS/27 were considered separately for statistical purposes. No specific order of inoculation for either method was introduced in order to eliminate inoculation order bias. The supplemented NR26 bottle was clearly marked to distinguish it from the companion unsupplemented bottle.

Incubation and testing. Conventional media were incubated at 35°C in an atmosphere containing 5 to 10% CO_2 for 7 days for negative cultures. All plates and broths were screened daily for growth, with identification and susceptibility testing of microorganisms performed according to standard laboratory procedures for each institution. For the BACTEC bottles, the 7-day testing protocol with the BACTEC NR660 instrument was followed. Bottles were placed in the 35°C incubator immediately after inoculation, and the aerobic bottles (NR26 and NR26FOS) were placed on the shaker shelf for the first 24 to 48 h of incubation. The aerobic bottles were tested twice on days 1 and 2 and once thereafter. The anaerobic bottles were tested once daily. A growth value (GV) of 30, a delta GV of 10, or visual signs of growth (turbidity, gas production, or floating colonial growth) were indicative of a positive BACTEC bottle. When one or more bottles within a given set became positive, the remaining bottles continued to be tested according to the standard protocol for the remainder of the 7 days. Fluid from each positive bottle was Gram stained and subcultured onto appropriate media for identification and susceptibility testing.

Reporting. All test results were recorded on the data report form. The following information was included for each positive culture: (i) specimen type; (ii) specimen identification; (iii) date and time of collection; (iv) conventional results, including time, date of detection, smear results, and subculture results; (v) BACTEC results, including GV or delta GV, time and date of detection, smear results, and subculture results; (vi) name(s) of organism(s) identified and significance; and (vii) description of patient's antimicrobial therapy. In addition, GVs for all negative BACTEC bottles were documented.

Clinical assessment. When possible, patients with positive SBF cultures were evaluated by an infectious disease consultant or pathologist to determine whether the microorganisms isolated were clinically significant (true-positive isolates) or were contaminants. This clinical assessment was made in accordance with published criteria (7).

Data analysis. The data were analyzed by the SPSS statistical program at Becton Dickinson Diagnostic Instrument Systems (13). McNemar chi-square analysis (8) was used to determine the statistical significance of differences in numbers of isolates determined by each system.

RESULTS

Classification of specimens and isolates. Of the 1,101 fluid specimens received for culture, 234 (21%) were positive either by the BACTEC system or by conventional culture techniques. One hundred fifty-five specimens yielded clinically significant isolates. The numbers of cultures (yielding clinically significant isolates) positive by each method were as follows: 140 (90%) from BACTEC Plus NR26, 139 (90%) from BACTEC Plus NR26FOS, 122 (79%) from BACTEC Plus NR27, 127 (82%) from conventional media, 147 (95%) from BACTEC Plus NR26/27, and 147 (95%) from BACTEC Plus NR26FOS/27. One hundred seventy-six isolates were considered clinically significant, while 89 isolates were considered contaminants and eliminated from further analysis.

The 176 clinically significant isolates were further categorized by source and method of recovery (Table 1). Two separate comparisons were evaluated: BACTEC Plus NR26FOS/27 versus conventional culture and BACTEC Plus NR26/27 versus conventional culture. A total of 28 microorganisms were isolated only from the BACTEC Plus NR26/27 system with FOS compared with 9 microorganisms isolated from conventional culture. Similarly, 28 microorganisms were isolated only from the BACTEC NR26/27 system without FOS compared with 11 microorganisms isolated from conventional culture. We analyzed the significance of fluid type versus recovery method and found that BACTEC had increased recovery of clinically significant isolates from all fluids. Clinically significant microorganisms recovered included staphylococci, streptococci, members of the family *Enterobacteriaceae*, nonfermenting gram-negative bacilli, yeasts, anaerobes, and miscellaneous bacteria (Table 2). The difference in time to detection for the clinically significant isolates recovered in both systems is also shown in Table 2. Although not statistically significant, conventional culture showed an earlier time to detection for more isolates than BACTEC (30 versus 22 isolates).

TABLE 1. Fluid source and recovery method for 176 clinically significant isolates

Culture system(s)	No. of isolates recovered from indicated source					Total no. of isolates
	Synovial fluid	Pleural fluid	Ascites fluid	CAPD fluid	Other[a]	
BACTEC Plus NR26/27 only	5	2	10	11	0	28[b]
BACTEC Plus NR26FOS/27 only	7	2	8	11	0	28[b]
Conventional only						
Compared with BACTEC Plus NR26FOS/27	1	0	5	3	0	9
Compared with BACTEC Plus NR26/27	1	0	5	4	1	11
Both BACTEC and conventional	8	10	15	40	7	80
Both BACTEC with FOS and conventional	8	10	16	40	7	81

[a] Includes two pericardial, three cerebrospinal fluid, and two amniotic fluid specimens.
[b] Chi-square = 9.76; $P < 0.005$; significant (BACTEC only versus conventional only).

DISCUSSION

The presence of microorganisms in normally SBF specimens is potentially life-threatening; however, recovery of microorganisms is problematic. First, large-volume specimens such as peritoneal fluid, ascitic fluid, and CAPD fluid often have very low numbers of microorganisms and require processing in order to concentrate the microorganisms in the specimen (4). Second, many fluids, such as cerebrospinal and synovial fluids, are received in very small volumes which may hinder recovery of microorganisms (18). Third, the initiation of antimicrobial therapy prior to collection of SBF specimens submitted for culture may produce falsely negative cultures (15, 16). Fourth, fastidious organisms may be missed if proper media or supplements are not incorporated into the culture method. Fifth, contaminant microorganisms may overgrow potential pathogens. Sixth, the delay in transport of the specimen to the microbiology laboratory may result in decreased recovery (1, 10).

The use of the BACTEC system for low-volume specimens aids in the recovery of microorganisms (2), and resins inactivate antimicrobial agents that could inhibit the growth of microorganisms on conventional culture media. The BACTEC system and the conventional culture technique yielded equivalent numbers of contaminant isolates. The addition of FOS to the BACTEC system resulted in the recovery of one *N. gonorrhoeae* isolate, two *Haemophilus influenzae* isolates, and one *Sphingobacterium multivorum* isolate that were not detected in BACTEC Plus NR26 without FOS. The use of FOS did not result in an increase in contaminant isolates in BACTEC bottles. Additionally, one isolate of *Staphylococcus aureus* was isolated from the BACTEC Plus NR26FOS that was not detected in either NR27 or conventional culture. Conversely, four isolates (one coagulase-negative *Staphylococcus* isolate, one viridans streptococcus, one *Klebsiella pneumoniae* isolate, and one *Candida parapsilosis* isolate) detected in BACTEC Plus NR26 without FOS were missed by use of BACTEC Plus NR26FOS, BACTEC Plus NR27, and conventional culture. This clarifies the discrepancy between the 172 significant isolates recovered from the comparison of BACTEC Plus NR26FOS/27 versus conventional culture and the 176 total significant isolates recovered from all methods (BACTEC Plus NR26FOS, BACTEC Plus NR27, conventional culture, and BACTEC Plus NR26 without FOS). Conventional culture also failed to detect one *N. gonorrhoeae* and one *H. influenzae* isolate.

BACTEC Plus NR26/27 with or without FOS improved the recovery of microorganisms from SBF; however, for simplicity, we will limit further discussion to BACTEC Plus NR26FOS/27 unless otherwise stated. If conventional cultures had been omitted, only 11 of 172 (6%) clinically significant isolates would have been missed, but if the BACTEC system had been omitted, 28 of 172 (16%) clinically significant isolates would have been missed. The remaining isolates showed equivalent results with both systems. Additionally, we found that the BACTEC system yielded increased recovery of clinically significant isolates in each fluid type analyzed. There were no statistically significant differences in time to detection for those microorganisms recovered by both BACTEC and conventional culture systems.

TABLE 2. Culture method, organism recovery, and time to detection

Organisms	No. (%) of isolates recovered		No. (%) of isolates with the indicated time to detection		
	Conventional method only	BACTEC only[a]	Shorter by conventional method	Shorter by BACTEC[a]	Same by the two methods
Staphylococcus spp. (n = 100)	3 (3)	10 (10)	23 (23)	14 (14)	50 (50)
Streptococcus spp. (n = 28)	3 (11)	5 (18)	4 (14)		16 (57)
Enterobacteriaceae (n = 24)	1 (4)	7 (27)	1 (4)	5 (19)	10 (38)
Nonfermenting gram-negative bacilli (n = 7)		2 (29)	2 (29)	1 (13)	2 (29)
Miscellaneous bacteria (n = 3)		1 (33)		2 (67)	
Yeasts (n = 4)	1 (14)	2 (29)			1 (14)
Anaerobes (n = 6)	3 (50)	1 (17)			2 (33)
Total (n = 172)[b]	11 (6)	28 (16)	30 (18)	22 (13)	81 (47)

[a] BACTEC Plus NR26FOS/27 study set.
[b] The only observed disparities in significant isolate recovery between BACTEC Plus NR26FOS and NR26 without FOS were (i) one *S. multivorum* isolate, two *H. influenzae* isolates, one *N. gonorrhoeae* isolate, and one *S. aureus* isolate recovered from NR26FOS only and (ii) one viridans streptococcus, one coagulase-negative staphylococcus, one *K. pneumoniae* isolate, and one *C. parapsilosis* isolate recovered from NR26 without FOS only.

Cost is almost always a determinant in evaluating diagnostic methods. Our cost of using the BACTEC system is greater than that of conventional culture methods; however, the necessity of using the anaerobic Plus NR27 bottle is debatable. If the anaerobic bottle had been eliminated from this study, 5 of 172 (3%) clinically significant isolates would have been missed (only one obligate anaerobe), but the cost would have been reduced to less than that of the conventional setup, while better recovery than that of the conventional culture alone would still have been achieved. These data suggest that if a laboratory wishes to address speed of recovery and its completeness as well as cost, a system of one plate plus one BACTEC bottle should be explored.

In agreement with others (10), we feel that future studies should be performed to evaluate the potential benefit of bedside inoculation versus laboratory inoculation for cultivation of microorganisms from SBF. We further conclude that the BACTEC Plus NR26/27 liquid culture system is an excellent alternative to conventional culture methods for SBF specimens, used with or without the addition of FOS.

ACKNOWLEDGMENTS

We gratefully acknowledge Diane Leland for her expertise and review of the manuscript.

This study was supported in part by Becton Dickinson Diagnostic Instrument Systems.

REFERENCES

1. **Blondeau, J. M., G. B. Pylypchuk, and J. E. Kappel.** 1993. Evaluation of BACTEC 16A media for the recovery of microorganisms causing peritonitis, abstr. C-388, p. 514. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993.
2. **Davis, T., D. Fuller, D. Davidson, K. Tabatowski, and R. Schuen.** 1992. Comparison of BACTEC Peds Plus, 13A and lytic culture media to conventional culture for cultivation of microorganisms in fine needle aspiration specimens, abstr. C-45, p. 428. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992.
3. **Doyle, P. W., E. P. Crighton, R. G. Mathias, and R. Werb.** 1989. Clinical and microbiological evaluation of four culture methods for the diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. **27:**1206–1208.
4. **Isenberg, H. D., J. A. Washington II, G. V. Doern, and D. Amsterdam.** 1991. Specimen collection and handling, p. 15–28. *In* A. Balows, W. J. Hausler, Jr., K. Herrman, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
5. **Maartens, G., and E. D. Bateman.** 1991. Tuberculous pleural effusions: increased culture yield with bedside inoculation of pleural fluid and poor diagnostic value of adenosine deaminase. Thorax **46:**96–99.
6. **Males, B. M., J. J. Walshe, L. Garringer, D. Koscinski, and D. Amsterdam.** 1986. Addi-Chek filtration, BACTEC, and 10-ml culture methods for recovery of microorganisms from dialysis effluent during episodes of peritonitis. J. Clin. Microbiol. **23:**350–353.
7. **Mandell, G. L., R. G. Douglas, Jr., and J. E. Bennett (ed.).** 1990. Principles and practice of infectious diseases, p. 33–922. Churchill Livingstone, New York.
8. **McNemar, Q.** 1962. Psychological statistics, 3rd ed., p. 209–239. John Wiley and Sons, Inc., New York.
9. **Reinhold, C. E., D. J. Nickolai, T. E. Piccinini, B. A. Byford, M. K. York, and G. F. Brooks.** 1988. Evaluation of broth media for routine culture of cerebrospinal and joint fluid specimens. Am. J. Clin. Pathol. **89:**671–674.
10. **Runyon, B. A., M. R. Antillon, E. A. Akriviadis, and J. G. McHutchison.** 1990. Bedside inoculation of blood culture bottles with ascitic fluid is superior to delayed inoculation in the detection of spontaneous bacterial peritonitis. J. Clin. Microbiol. **28:**2811–2812.
11. **Runyon, B. A., E. T. Umland, and T. Merlin.** 1987. Inoculation of blood culture bottles with ascitic fluid. Arch. Intern. Med. **147:**73–75.
12. **Saubolle, M. A., D. L. Sewell, M. D. Holland, and T. A. Golper.** 1989. Comparison of two commercial broth-culture systems for microbial detection in dialysates of patients on continuous ambulatory peritoneal dialysis. Diagn. Microbiol. Infect. Dis. **12:**457–461.
13. **SPSS Inc.** 1992. SPSS/PC+ V 3.0. SPSS Inc., Chicago.
14. **Vas, S. I., and L. Law.** 1985. Microbiological diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. **21:**522–523.
15. **Von Essen, R.** 1989. Letter. Am. J. Clin. Pathol. **91:**502.
16. **Von Essen, R., and A. Holtta.** 1986. Improved method of isolating bacteria from joint fluid by the use of blood culture bottles. Ann. Rheum. Dis. **45:**454–457.
17. **Von Graevenitz, A., and D. Amsterdam.** 1992. Microbial aspects of peritonitis associated with continuous ambulatory peritoneal dialysis. Clin. Microbiol. Rev. **5:**36–48.
18. **Yagupsky, P., R. Dagan, C. W. Howard, M. Einhorn, I. Kassis, and A. Simu.** 1992. High prevalence of *Kingella kingae* in joint fluid from children with septic arthritis revealed by the BACTEC blood culture system. J. Clin. Microbiol. **30:**1278–1281.