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Controlled Comparison of the BACTEC High-Blood-Volume Fungal Medium, BACTEC Plus 26 Aerobic Blood Culture Bottle, and 10-Milliliter Isolator Blood Culture System for Detection of Fungemia and Bacteremia

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The BACTEC high-blood-volume fungal medium (HBV-FM) (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) was compared with the Isolator (IS) tube and the BACTEC Plus 26 (BP26) blood culture bottle for the ability to recover fungi from the blood of adult patients suspected of having fungemia. A total of 6,836 blood culture sets that fulfilled criteria for inclusion in the study were received. Three separate comparisons were performed: 4,907 HBV-FM versus IS, 4,886 BP26 versus HBV-FM, and 4,949 BP26 versus IS. For the HBV-FM versus IS comparison, 218 isolates were recovered: 125 (57.3%) were bacteria and 93 (42.7%) were fungi. HBV-FM was comparable to IS for recovery of yeasts, but IS was superior for recovery of *Histoplasma capsulatum* (25 versus 0 isolates recovered [$P < 0.001$]). Growth of *Torulopsis glabrata* was detected earlier ($P < 0.05$) in HBV-FM bottles. For the BP26 versus HBV-FM comparison, 229 isolates were recovered: 161 (70.3%) were bacteria, and 68 (29.7%) were fungi. HBV-FM was superior for recovery of *T. glabrata* ($P < 0.025$) and all fungi combined ($P < 0.025$). There were no statistically significant differences in the speed of detection of microbial growth. For the BP26 versus IS comparison, 251 isolates were recovered: 165 (65.7%) were bacteria, and 86 (34.2%) were fungi. IS was superior for recovery of *H. capsulatum* ($P < 0.001$), *T. glabrata* ($P < 0.05$), and fungi other than *H. capsulatum* ($P < 0.025$). BP26 was superior for recovery of all bacteria combined ($P < 0.001$) and viridans group streptococci ($P < 0.01$). Growth of *T. glabrata* ($P < 0.05$) was detected earlier in IS tubes. Growth of *Staphylococcus aureus* ($P < 0.01$), viridans group streptococci ($P < 0.01$), *Pseudomonas aeruginosa* ($P < 0.05$), and all microorganisms combined ($P < 0.05$) was detected earlier in BP26 bottles. For yeasts, 57 of 59 (96.6%), 79 of 80 (98.7%), and 64 of 67 (95.5%) were recovered from BP26 bottles, HBV-FM bottles, and IS tubes, respectively, by day 14; for *H. capsulatum*, 14 of 36 (38.9%) isolates were recovered from IS tubes by day 14. Mean times of recovery were similar for BACTEC bottles and IS. We conclude that (i) for recovery of fungi from blood cultures, HBV-FM is equivalent to IS (with the exception of *H. capsulatum*); (ii) for recovery of bacteria, BP26 is superior to IS; (iii) BP26 bottles are inferior to both HBV-FM bottles and IS tubes for recovery of *T. glabrata*; and (iv) HBV-FM bottles must be paired with another blood culture bottle or system to optimize detection of bacteremia.

Although laboratory detection of bacteremia has long been one of the most important roles of the clinical microbiology laboratory, laboratory detection of fungemia has become increasingly important in modern medical care. Disseminated fungal infections are common among patients with natural or acquired immunosuppression, particularly patients with AIDS and patients who are receiving exogenous steroid therapy and/or antineoplastic agents, and can occasionally occur even in patients without factors known to predispose them to disseminated fungal infections. Fungemia usually is caused by common pathogenic yeasts, such as *Candida* spp., but may also be caused by environmental molds, such as *Aspergillus* spp. (12, 21, 24, 26, 32). Disseminated infection caused by *Histoplasma capsulatum* is not uncommon among immunosuppressed patients in certain geographic areas. Moreover, the levels of morbidity and mortality associated with fungemia are high (8, 12, 21, 24) but may be improved with rapid diagnosis and administration of antifungal therapy early in infection (24).

Becton Dickinson Diagnostic Instrument Systems (Sparks, Md.) recently introduced a selective high-blood-volume fungal medium (HBV-FM) for use with their BACTEC nonradiometric 660, 730, and 860 blood culture instruments. This medium was formulated to enhance recovery of fungi and contains chloramphenicol and tobramycin to suppress bacterial growth. It also contains a lysing agent to release phagocytized fungi.

In a collaborative multicenter study performed at four university-affiliated hospitals, the HBV-FM was compared with the Isolator (IS) centrifugation-lysis blood culture system (Wampole Laboratories, Cranbury, N.J.) and the BACTEC Plus 26 (BP26) nonradiometric bottle for the ability to

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recover fungi from blood and for relative speed of detection of fungal growth in an adult patient population with an incidence of fungemia higher than that of a general adult inpatient population. The IS system was chosen as a reference method because it had previously been shown to be a superior method for recovering fungi relative to biphasic manual blood culture bottles (2, 3, 9, 11), BACTEC radiometric bottles (4–6, 13), and low-volume nonradiometric (14, 28) bottles. In addition, the IS system has been shown to be superior for recovering specific fungal pathogens, such as *Histoplasma capsulatum* (3), *Cryptococcus neoformans* (5), and *Coccidioides immitis* (29). Finally, the IS tube, like the HBV-FM bottle, accepts inocula of up to 10 ml of blood. The BP26 bottle, which previously has been shown to be an acceptable method for recovering common yeasts and bacteria (16, 19, 30), was included in the evaluation to (i) ensure that each patient in the study had an adequate blood culture for bacterial pathogens and (ii) assess the ability of the BP26 bottle to recover fungi in a timely manner. In addition, the BP26 bottle, like the HBV-FM bottle and IS tube, accepts inocula of up to 10 ml of blood.

MATERIALS AND METHODS

Patient selection. The study was performed at Duke University Medical Center, Durham, N.C.; Wishard Memorial Hospital and Indiana University Hospital, Indianapolis; and the University of Iowa Hospital and Clinics, Iowa City. At each study site, BACTEC HBV-FM bottles were made available only to those wards and/or clinics caring for patients with a higher probability of fungemia than would be expected in a general patient population.

Blood culture collection. Skin was disinfected by a standard technique (25). Following venipuncture, 30 ml of blood was withdrawn into a sterile syringe and 10 ml was randomly and aseptically transferred to the following three study bottles: one BP26 bottle, one HBV-FM bottle, and one IS tube. The two BACTEC bottles and the IS tube were gently rocked back and forth to mix the contents of each with the inoculated blood. The two BACTEC bottles and the IS tube were then transported to the clinical microbiology laboratories, where they were compared against standards filled with known volumes of fluid, and filling was recorded as inadequate (<8 ml), adequate (8 to 12 ml), or overfilled (>12 ml).

Blood culture processing. All blood culture sets were processed for purposes of patient care, but only those cultures received with at least two of the three bottles or tubes adequately filled and within 8 h of collection (10, 27) were included in the study. HBV-FM and BP26 bottles were processed on BACTEC 660 blood culture instruments. The bottles were left in a 35°C incubator throughout the 4-week incubation period. They were shaken at approximately 280 rpm during the first 24 to 48 h of incubation. Each bottle was tested twice on days 1 and 2 of incubation, once per day on days 3 to 7 of incubation, and twice weekly thereafter during the final 3 weeks of incubation. IS tubes were processed with an Isostat in accordance with the manufacturer's recommendations. The centrifuged pellet was transferred directly to four plates as follows: (i) one chocolate agar plate incubated at 35°C in an atmosphere containing 5 to 10% CO_2; (ii) two brain heart infusion agar plates containing 10% sheep erythrocytes, of which one was incubated at 35°C in an atmosphere containing 5 to 10% CO_2 and the other was incubated at 25°C in room air; and (iii) a Sabouraud dextrose agar plate incubated at 25°C in room air. All fungal media were incubated for 4 weeks and examined on days 1 to 4 and 7 and then twice weekly for 3 additional weeks on the same days that the BACTEC bottles were tested. All bacterial media were incubated in accordance with each laboratory's routine and standard microbiological techniques (1).

All bottles and plates were treated independently. That is, when any bottle or plate became positive (grew a microorganism), the other bottles or plates from that blood culture set continued to be processed as specified above for the remainder of the 4 weeks or until they became positive (i.e., Gram staining and subculturing were not performed on the other bottle and/or the IS tube unless evidence of growth was present). BACTEC bottles that became positive by any criterion (visual evidence of growth, growth value, or delta growth value) but were negative by Gram staining and/or subculturing were returned for additional incubation and testing until they became positive or until the end of the 4-week incubation period. At the end of the 4-week incubation period, terminal subculturing was performed on negative BACTEC bottles from those blood culture sets in which the IS tube and/or the other BACTEC bottle was positive. For each positive culture, the following were recorded: time to positivity, the means by which BACTEC bottles were determined to be positive, identity of any microorganisms recovered and from which bottle or plate they were recovered, the clinical significance of each microorganism (recorded as contaminant, indeterminate, or significant), and the presence of antimicrobial therapy at the time the blood culture was drawn.

Clinical assessment. When possible, patients with positive blood cultures were evaluated by an infectious disease consultant or pathologist to determine whether the microorganisms isolated from the blood culture(s) were clinically important (true-positive isolates), were indeterminate as the cause of sepsis, or were contaminants. This clinical assessment was made in accordance with published criteria (31).

Data analysis. Patient and culture data were collected at each study site and forwarded to the Clinical Microbiology Laboratory at Duke University Medical Center. The data were then collated and analyzed for three separate comparisons: HBV-FM versus BP26, BP26 versus IS, and HBV-FM versus IS. The two BACTEC bottles and the IS tube were compared for the ability to recover microorganisms (yield) and the time required for detection of microbial growth (speed of detection). Only adequately filled blood culture sets that were processed within 8 h of collection and grew clinically significant microorganisms were included in the comparisons. All comparisons were analyzed statistically with McNemar's chi-square test, modified, when necessary, with Yates' correction for small numbers (18). Differences in yield or speed of detection were considered statistically significant if the chi-square value was ≥ 3.84 (i.e., the P value was <0.05).

RESULTS

Of the 6,386 blood culture sets received during the study, 4,458 were received with both BACTEC bottles and the IS tube adequately filled, 428 were received with the two BACTEC bottles adequately filled, 491 were received with the BP26 bottle and the IS tube adequately filled, and 449 were received with the HBV-FM bottle and the IS tube adequately filled (Table 1). Therefore, a total of 4,886 BP26 versus HBV-FM comparisons were possible, as were 4,949 BP26 versus IS comparisons and 4,907 HBV-FM versus IS adequate comparisons.

Of the 251 isolates recovered from the BP26 versus IS

TABLE 1. Number of blood culture sets received for each two-bottle comparison

No. of bottles in set	No. of comparisons for:		
	BP26 vs HBV-FM	BP26 vs IS	HBV-FM vs IS
3[a]	4,458	4,458	4,458
2[b]	428	491	449
Total	4,886	4,949	4,907

[a] All of the sets in this row were received with both BACTEC bottles and the IS tube adequately filled with blood.
[b] All of the sets in this row were received with the BACTEC bottle(s) and/or IS tube shown in each column adequately filled with blood.

comparison, 136 were recovered from both BP26 bottles and IS tubes, 54 were recovered from BP26 bottles only, and 61 were recovered from IS tubes only. Of these, 165 (65.7%) were bacteria and 86 (34.3%) were fungi (Table 2). Overall

TABLE 2. Comparative yield of clinically important microorganisms recovered from BP26 bottles and IS tubes

Microorganism(s)	No. of isolates recovered from:			P value
	BP26 bottle + IS tube	BP26 only	IS only	
Fungi				
Torulopsis glabrata	6	1	8	<0.05
Candida parapsilosis	8	2	2	NS[a]
Candida tropicalis	3	4	3	NS
Cryptococcus neoformans	4	2	3	NS
Candida albicans	4	1	2	NS
Candida krusei	4	0	0	NS
Malassezia pachydermatis	0	0	2	NS
Histoplasma capsulatum	1	0	25	<0.001
Fusarium sp.	0	0	1	NS
Fungi other than *H. capsulatum*	29	10	21	<0.025
Aerobic + facultative bacteria				
Gram positive				
Staphylococcus aureus	25	5	2	NS
Coagulase-negative staphylococci	11	9	3	NS
Enterococci	7	1	0	NS
Viridans group streptococci	18	9	0	<0.01
Other streptococci[b]	4	3	0	NS
Other gram-positive bacteria[c]	4	3	1	NS
Gram negative				
Enterobacteriaceae[d]	19	1	5	NS
Pseudomonas aeruginosa	10	3	0	NS
Other gram-negative bacteria[e]	8	10	4	NS
All bacteria	106	44	15	<0.001
All microorganisms	136	54	61	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes three group A and four group B streptococcus isolates.
[c] Includes five *Corynebacterium* group J-K, one *Bacillus* sp., one *Lactobacillus* sp., and one *Mycobacterium avium* complex isolates.
[d] Includes 10 *Enterobacter cloacae*, eight *Klebsiella pneumoniae*, two *Escherichia coli*, two *Serratia marcescens*, one *Klebsiella oxytoca*, one *Proteus mirabilis*, and one *Salmonella* sp. isolates.
[e] Includes nine *Xanthomonas maltophilia*, six *Pseudomonas* sp., four *Acinetobacter*, two *Campylobacter jejuni*, and one *Pseudomonas fluorescens* isolates.

TABLE 3. Comparative speed of detection of clinically significant microorganisms recovered from BP26 bottles and IS tubes

Microorganism(s)	No. of isolates whose growth was detected in:			P value
	BP26 bottle + IS tube at same time	BP26 earlier	IS earlier	
Fungi				
C. parapsilosis	1	4	3	NS[a]
T. glabrata	0	0	6	<0.05
C. albicans	0	1	3	NS
C. krusei	2	2	0	NS
C. neoformans	1	1	2	NS
C. tropicalis	1	0	2	NS
H. capsulatum	0	0	1	NS
All fungi	5	8	17	NS
Gram-positive bacteria				
S. aureus	9	14	2	<0.01
Coagulase-negative staphylococci	3	5	3	NS
Enterococci	0	5	2	NS
Viridans group streptococci	3	13	2	<0.01
Other streptococci[b]	0	2	0	NS
Corynebacterium group J-K	0	1	3	NS
Gram-negative bacteria				
Enterobacteriaceae[c]	6	10	3	NS
P. aeruginosa	1	8	1	<0.05
Other gram-negative bacteria[d]	3	3	2	NS
All microorganisms	29	62	42	<0.05

[a] NS, not significant ($P > 0.05$).
[b] Includes two group B streptococcus isolates.
[c] Includes six *E. cloacae*, six *K. pneumoniae*, two *S. marcescens*, one *P. mirabilis*, two *E. coli*, one *K. oxytoca*, and one *Salmonella* sp. isolates.
[d] Includes four *Pseudomonas* sp., two *Acinetobacter* sp., one *P. fluorescens*, and one *X. maltophilia* isolates.

recovery of microorganisms was not significantly different between the two methods. Recovery of *Torulopsis glabrata* ($P < 0.05$), *H. capsulatum* ($P < 0.001$), and all fungi other than *H. capsulatum* ($P < 0.025$) was significantly better with IS tubes. Recovery of viridans group streptococci ($P < 0.01$) and all bacteria combined ($P < 0.001$) was significantly better with the BP26 bottle. Growth of *T. glabrata* ($P < 0.05$) was detected significantly earlier in IS tubes (Table 3). Growth of *Staphylococcus aureus* ($P < 0.01$), viridans group streptococci ($P < 0.01$), *Pseudomonas aeruginosa* ($P < 0.05$), and all microorganisms combined ($P < 0.05$) was detected significantly earlier in BP26 bottles.

Of the 229 isolates recovered from the BP26 versus HBV-FM comparison, 43 were recovered from both bottles, 156 were recovered from BP26 bottles alone, and 30 were recovered from HBV-FM bottles alone. Of these, 161 (70.3%) were bacteria and 68 (29.7%) were fungi (Table 4). Recovery of *T. glabrata* ($P < 0.025$) and all fungi combined ($P < 0.025$) was better from HBV-FM bottles. Recovery of bacteria ($P < 0.001$) was better from BP26 bottles. For the 43 isolates recovered from both bottles, there were no significant differences in the speed of detection of microbial growth (Table 5).

Of the 218 isolates recovered from the HBV-FM versus IS comparison, 54 were recovered from both HBV-FM bottles

TABLE 4. Comparative yield of clinically important microorganisms recovered from BP26 and HBV-FM bottles

Microorganism(s)	No. of isolates recovered from:			P value
	Both bottles	BP26 only	HBV-FM only	
Fungi				
T. glabrata	4	2	10	<0.025
C. parapsilosis	10	1	2	NS[a]
C. albicans	5	2	6	NS
C. tropicalis	5	3	3	NS
C. neoformans	3	2	4	NS
C. krusei	4	0	0	NS
H. capsulatum	0	1	0	NS
Petriellidium sp.	0	0	1	NS
All fungi	31	11	26	<0.025
Bacteria				
Gram positive[b]	6	95	1	<0.001
Gram negative[c]	6	50	3	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes 31 *S. aureus*, 27 viridans group streptococcus, 22 coagulase-negative staphylococcus, eight enterococcus, five *Corynebacterium* group J-K, four group B and three group A streptococcus, one *Bacillus* sp., and one *Lactobacillus* sp. isolates.
[c] Includes 13 *P. aeruginosa*, nine *X. maltophilia*, seven *E. cloacae*, seven *K. pneumoniae*, five *Pseudomonas* sp., five *E. coli*, four *Acinetobacter* sp., two *S. marcescens*, two *C. jejuni*, one *Citrobacter freundii*, one *K. oxytoca*, one *P. mirabilis*, one *Salmonella* sp., and one *P. fluorescens* isolates.

and IS tubes, 21 were recovered from HBV-FM bottles only, and 143 were recovered from IS tubes only. Of these, 125 (57.3%) were bacteria and 93 (42.7%) were fungi (Table 6). Recovery of *H. capsulatum* ($P < 0.001$) was significantly better with IS tubes. There were no other significant differences in the recovery of fungi, although more yeasts (16 versus 8) were recovered from HBV-FM bottles. Recovery of all bacteria ($P < 0.001$) was better with IS tubes. Growth of *T. glabrata* ($P < 0.05$) was detected significantly earlier in HBV-FM bottles (Table 7). There were no other significant differences in the speed of detection of microbial growth.

Of the 79 septic patients from whom fungi were isolated, only 5 (6.3%) had fungemia caused by more than one fungal species. These included two patients who had fungemia caused by *C. tropicalis* and *T. glabrata* and one patient each with fungemia caused by *C. albicans* and *T. glabrata*, *C.*

TABLE 5. Comparative speed of detection of clinically important microorganisms recovered from BP26 and HBV-FM bottles

Microorganism(s)	No. of isolates whose growth was detected in:			P value
	BP26 + HBV-FM at same time	BP26 earlier	HBV-FM earlier	
Fungi				
C. albicans	3	1	1	NS[a]
C. krusei	1	2	1	NS
C. neoformans	1	0	2	NS
C. parapsilosis	7	1	2	NS
C. tropicalis	4	0	1	NS
T. glabrata	0	0	4	NS
All fungi	16	4	11	NS
Bacteria				
Gram positive[b]	4	2	0	NS
Gram negative[c]	0	5	1	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes one *S. aureus*, one coagulase-negative staphylococcus, and four *Corynebacterium* group J-K isolates.
[c] Includes one *E. cloacae*, one *P. aeruginosa*, one *X. maltophilia*, and three *Pseudomonas* sp. isolates.

TABLE 6. Comparative yield of clinically important microorganisms recovered from HBV-FM bottles and IS tubes

Microorganism(s)	No. of isolates recovered from:			P value
	HBV-FM bottle + IS tube	HBV-FM only	IS only	
Fungi				
T. glabrata	11	4	2	NS[a]
C. albicans	5	5	2	NS
C. parapsilosis	10	1	0	NS
C. tropicalis	6	3	1	NS
C. neoformans	5	2	1	NS
C. krusei	5	1	0	NS
M. pachydermatis	0	0	2	NS
H. capsulatum	0	0	25	<0.001
Fusarium sp.	0	0	1	NS
Petriellidium sp.	0	1	0	NS
Fungi other than *H. capsulatum*	42	17	9	NS
Bacteria				
Gram positive[b]	6	1	67	<0.001
Gram negative[c]	6	3	42	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes 29 *S. aureus*, 18 viridans group streptococcus, 11 coagulase-negative staphylococcus, seven enterococcus, four *Corynebacterium* group J-K, four group B streptococcus, and one *M. avium* complex isolates.
[c] Includes nine *E. cloacae*, nine *P. aeruginosa*, eight *K. pneumoniae*, seven *X. maltophilia*, six *Pseudomonas* sp., four *E. coli*, two *S. marcescens*, two *Acinetobacter* sp., one *K. oxytoca*, one *P. mirabilis*, one *Salmonella* sp., and one *P. fluorescens* isolates.

TABLE 7. Comparative speed of detection of clinically significant microorganisms recovered from HBV-FM bottles and IS tubes

Microorganism(s)	No. of isolates whose growth was detected in:			P value
	HBV-FM bottle + IS tube at same time	HBV-FM first	IS first	
Fungi				
C. albicans	2	0	3	NS[a]
C. krusei	1	2	2	NS
C. neoformans	1	1	3	NS
C. parapsilosis	0	4	6	NS
C. tropicalis	1	3	2	NS
T. glabrata	5	6	0	<0.05
All fungi	10	16	16	NS
Bacteria				
Gram positive[b]	0	1	5	NS
Gram negative[c]	0	1	5	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes one *S. aureus*, one coagulase-negative staphylococcus, and four *Corynebacterium* group J-K isolates.
[c] Includes one *E. cloacae*, one *P. aeruginosa*, and four *Pseudomonas* sp. isolates.

TABLE 8. Comparison of mean times to recovery of yeasts

Microorganism(s)	Time to recovery (days) with:								
	BP26			HBV-FM			IS		
	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
C. albicans	17	1–10	3.3	19	1–14	3.1	14	1–5	2.7
C. krusei	4	2–5	2.7	6	1–10	3.2	5	2–7	3.6
C. parapsilosis	12	1–28	5.4	13	1–7	3.4	11	2–4	2.6
C. tropicalis	8	1–4	2.3	13	1–4	2.2	9	1–22	6.2
C. neoformans	6	5–28	10.0	8	4–19	8.0	8	3–15	5.7
T. glabrata	12	1–14	7.3	21	1–7	2.6	20	1–14	3.7
All yeasts	59	1–28	5.0	80	1–19	3.4	67	1–22	3.9

kruseii and *C. tropicalis*, and *C. tropicalis* and a *Fusarium* sp.

The mean times of recovery for yeasts varied between the two BACTEC bottles and the IS tube, although the differences generally were small (Table 8). There were relatively few isolates of some species, which caused several mean values to be disproportionately increased by one or more isolates recovered only after an extended period of incubation. For example, although the nine *C. tropicalis* isolates recovered from IS tubes had a mean recovery time of 6.2 days, seven were recovered between 1 and 5 days and the other two were recovered on days 19 and 22; if one calculates the mean for the first seven isolates alone, the mean value decreases from 6.2 days to 2.1 days.

For yeasts, all isolates of *C. albicans*, *C. krusei*, and *T. glabrata* were recovered by day 14 (Table 9). The HBV-FM bottle recovered the most yeasts by day 14 (79 of 80 [98.7%]), followed by the BP26 bottle (57 of 59 [96.6%]) and the IS tube (64 of 67 [97.1%]). For filamentous fungi, only *H. capsulatum* was recovered in numbers sufficient for calculation of a mean value, and then only from IS tubes, with a mean time to recovery of 15.3 days and a range of 3 to 28 days. In contrast to pathogenic yeasts, only 14 of 36 (38.9%) *H. capsulatum* isolates were recovered by day 14.

DISCUSSION

This multicenter evaluation compared the recently introduced BACTEC HBV-FM bottle with the BP26 bottle and the IS tube for the ability to recover microorganisms from the blood of patients suspected of having fungemia and for the relative speed of detection of microbial growth. The design of the study also allowed comparison of the BP26 bottle versus the IS tube for the same performance characteristics. HBV-FM is a selective medium containing chloramphenicol and tobramycin to optimize fungal growth, at least partially through suppression of bacterial overgrowth, in blood specimens containing both fungi and bacteria.

TABLE 9. Proportion of yeasts recovered by day 14

Microorganism(s)	No. recovered/total (%)		
	BP26	HBV-FM	IS
C. albicans	17/17 (100)	19/19 (100)	14/14 (100)
C. krusei	4/4 (100)	6/6 (100)	5/5 (100)
C. parapsilosis	11/12 (91.6)	13/13 (100)	11/11 (100)
C. tropicalis	8/8 (100)	13/13 (100)	7/9 (77.8)
C. neoformans	5/6 (83.3)	7/8 (87.5)	7/8 (87.5)
T. glabrata	12/12 (100)	21/21 (100)	20/20 (100)
All yeasts	57/59 (96.6)	79/80 (98.7)	64/67 (95.5)

HBV-FM was found to be superior to BP26 for recovering *T. glabrata* and all fungi combined. In addition, although the difference was not statistically significant, more yeasts other than *T. glabrata* (15 versus 8) were recovered from HBV-FM than were recovered from BP26. HBV-FM was found to be equivalent to BP26 in speed of detection of microbial growth. This was to be expected, since the two bottles were incubated under identical conditions and tested at the same time on the same instrument (i.e., any differences in the speed of microbial growth that occurred in a time frame less than that of the testing cycle would not be detected). With the exception of *H. capsulatum*, which was recovered more often from IS alone (25 versus 0), HBV-FM was found to be equivalent to IS for recovery of fungi. Although the difference was not statistically significant, more yeasts (16 versus 8) were recovered from HBV-FM than from IS. HBV-FM was also found to be equivalent to IS in speed of detection of microbial growth and detected growth of *T. glabrata* earlier than did IS. Therefore, compared with IS or BP26, HBV-FM is an acceptable method for recovering fungi other than *H. capsulatum* from the blood of septic patients. The antimicrobial agents present in HBV-FM were found to inhibit bacterial growth effectively.

In general, the mean times for recovery of yeasts from IS tubes and BP26 bottles were consistent with those previously published (3, 4, 9, 19, 21, 23, 29). The differences in mean recovery times generally were small and thus probably were not clinically important. It is of interest that although the range of recovery times was 1 to 28 days, only six isolates were recovered after day 14. Moreover, only one of these six isolates was not recovered from either the companion BACTEC bottle or IS tube in the same blood culture set within 14 days. Thus, the question of whether it is cost effective to incubate and/or test fungal blood cultures for more than 14 days must be asked, particularly since most pathogenic yeasts would probably be recovered from any other bacterial or fungal blood cultures drawn at the same time. Moreover, the question of whether isolation of yeasts 2 weeks after a blood culture was obtained would make any difference in therapy or outcome must also be asked. Consequently, the personnel of each laboratory must decide the most appropriate duration of incubation and testing for fungal blood cultures drawn from the particular patient population being served and the possible affect it would have on patient care and hospital and laboratory costs.

In contrast, most *H. capsulatum* isolates (22 of 36 [61.1%]) were recovered after day 14, with a mean recovery time of 15.3 days and a range of 3 to 28 days. These findings are in

general agreement with the findings of previous studies, in which *H. capsulatum* had mean recovery times of 9.4 to 13 days with a range of 6 to 28 days (3, 9, 22, 29). Therefore, unlike optimal recovery of yeasts, optimal recovery of *H. capsulatum* requires extended incubation and a testing period of up to 4 weeks. Because recovery of this microorganism from blood cultures can be delayed by up to 4 weeks, alternative methods of diagnosis, such as serologic tests, should be considered while awaiting blood culture results.

The reason(s) for the inability to recover *H. capsulatum* from HBV-FM (or BP26) was not apparent from this evaluation. Whether this phenomenon is unique to *H. capsulatum* or would occur with other dimorphic fungi is unknown; such isolates were not recovered during this study. Previous reports have shown that IS is superior to biphasic blood culture systems for recovery of *H. capsulatum* (2, 3, 8, 20), and one report has indicated that IS is superior to BP26 for recovery of unspecified molds (28). The inability of BP26 and HBV-FM to recover *H. capsulatum* appears to be a phenomenon common to most broth-based blood culture systems. Recovery of *H. capsulatum* is, therefore, optimized by use of the IS blood culture system, by culturing infected tissue in localized infections, or by culturing bone marrow in cases of disseminated histoplasmosis (22).

Although this study was intended primarily to evaluate the performance of the HBV-FM bottle for recovering fungi, the data indicate that most of the clinically significant isolates recovered from patients suspected of having fungemia, in fact, are bacteria. In the three comparisons, bacteria accounted for between 57.3 and 70.3% of the isolates while fungi accounted for between 29.7 and 42.7%. Therefore, use of the HBV-FM bottle alone would not be appropriate; the HBV-FM bottle, because it is selective for fungi, must be paired with another BACTEC bottle or blood culture system designed for optimal recovery of bacteria.

In the BP26 versus IS comparison, BP26 recovered significantly more viridans group streptococci and was superior to IS for overall recovery of clinically important bacteria (Table 2). IS previously has been shown to be inferior to radiometric BACTEC bottles for recovery of all streptococci combined and for viridans group streptococci (5). Although the IS tube is an acceptable method for detecting bacteria, this system has been reported to have a higher contamination rate relative to that of BACTEC bottles and to be inferior to other systems for recovery of certain bacteria (particularly streptococci, pseudomonads, and anaerobic bacteria) (5, 7, 14, 15, 17).

In summary, data from this study indicate that the BACTEC HBV-FM bottle is superior to the BP26 bottle and, with the exception of *H. capsulatum*, equivalent to the IS blood culture system for recovery of fungi from the blood of septic adult patients. Most pathogenic yeasts are recovered within the first 2 weeks of incubation and testing; in contrast, many *H. capsulatum* isolates are recovered only after day 14. Because HBV-FM contains antimicrobial agents to prevent bacterial growth, very few pathogenic bacteria grow in HBV-FM bottles. Therefore, since the majority of patients suspected of having fungemia actually have bacteremia, HBV-FM should not be used alone when culturing blood from such patients. Data from this study also indicate that BP26 is equivalent to IS for recovery of most bacteria and is superior to IS for recovery of viridans group streptococci and all bacteria combined. Although BP26 is equivalent to IS and HBV-FM for recovering most yeasts, it is inferior to both for recovering *T. glabrata*. Therefore, use of an HBV-FM bottle paired with a BP26 bottle would allow for optimal recovery of both fungi (other than *H. capsulatum*) and bacteria. Since both bottles are designed for use on the same group of blood culture instruments, use of this pair of blood culture bottles would be a practical alternative to the IS blood culture system for culturing blood from patients suspected of having fungemia.

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REFERENCES

1. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
2. **Bille, J., R. S. Edson, and G. D. Roberts.** 1984. Clinical evaluation of the lysis-centrifugation blood culture system for the detection of fungemia and comparison with a conventional biphasic broth blood culture system. J. Clin. Microbiol. **19:**126–128.
3. **Bille, J., L. Stockman, G. D. Roberts, C. Horstmeier, and D. M. Ilstrup.** 1983. Evaluation of a lysis-centrifugation system for recovery of yeasts and filamentous fungi from blood. J. Clin. Microbiol. **18:**469–471.
4. **Body, B. A., M. A. Pfaller, J. Durrer, F. Koontz, and D. H. M. Groschel.** 1988. Comparison of the lysis-centrifugation and radiometric blood culture systems for recovery of yeast. Eur. J. Clin. Microbiol. Infect. Dis. **7:**417–420.
5. **Brannon, P., and T. E. Kiehn.** 1985. Large-scale clinical comparison of the lysis-centrifugation and radiometric systems for blood culture. J. Clin. Microbiol. **22:**951–954.
6. **Brannon, P., and T. E. Kiehn.** 1986. Clinical comparison of lysis-centrifugation and radiometric resin systems for blood culture. J. Clin. Microbiol. **24:**886–887.
7. **Gill, V. J., C. H. Zierdt, T. C. Wu, F. Stock, P. A. Pizzo, and J. D. MacLowry.** 1984. Comparison of lysis-centrifugation with lysis-filtration and a conventional unvented bottle for blood cultures. J. Clin. Microbiol. **20:**927–932.
8. **Gold, J. W. M.** 1984. Opportunistic fungal infections in patients with neoplastic disease. Am. J. Med. **76:**458–463.
9. **Guerra-Romero, L., R. S. Edson, F. R. Cockerill, C. D. Horstmeier, and G. D. Roberts.** 1987. Comparison of Du Pont Isolator and Roche Septi-Chek for detection of fungemia. J. Clin. Microbiol. **25:**1623–1625.
10. **Hamilton, D. J., D. Amos, R. W. Schwartz, C. M. Dent, and G. W. Counts.** 1989. Effect of delay in processing on lysis-centrifugation blood culture results from marrow transplant patients. J. Clin. Microbiol. **27:**1588–1593.
11. **Henry, N. K., C. A. McLimans, A. J. Wright, R. L. Thompson, W. R. Wilson, and J. A. Washington.** 1983. Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. J. Clin. Microbiol. **17:**864–869.
12. **Horn, R., B. Wong, T. E. Kiehn, and D. Armstrong.** 1985. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. Rev. Infect. Dis. **7:**646–655.
13. **Kellogg, J. A., J. P. Manzella, and J. H. McConville.** 1984. Clinical laboratory comparison of the 10-ml Isolator blood culture system with BACTEC radiometric blood culture media. J. Clin. Microbiol. **20:**618–623.
14. **Kelly, M. T., F. J. Roberts, D. Henry, I. Geere, and J. A. Smith.** 1990. Clinical comparison of Isolator and BACTEC 660 resin media for blood culture. J. Clin. Microbiol. **28:**1925–1927.
15. **Kiehn, T. E., B. Wong, F. F. Edwards, and D. Armstrong.** 1983. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. J. Clin. Microbiol. **18:**300–304.
16. **Koontz, F. P., K. K. Flint, J. K. Reynolds, and S. D. Allen.** 1991. Multicenter comparison of the high volume (10 ml) NR BAC TEC PLUS system and the standard (5 ml) NR BACTEC system. Diagn. Microbiol. Infect. Dis. **14:**111–118.
17. **McLauglin, J. C., P. Hamilton, J. V. Scholes, and R. C. Bartlett.**

1983. Clinical laboratory comparison of lysis-centrifugation and BACTEC radiometric blood culture techniques. J. Clin. Microbiol. **18**:1027–1031.
18. **McNemar, Q.** 1962. Psychological statistics, 3rd ed., p. 209–239. John Wiley & Sons, Inc., New York.
19. **Muñoz, P., J. C. L. Bernaldo de Quirós, J. Berenguer, M. R. Créixems, J. J. Picazo, and E. Bouza.** 1990. Impact of the BACTEC NR system in detecting *Candida* fungemia. J. Clin. Microbiol. **28**:639–641.
20. **Murray, P. R.** 1991. Comparison of the lysis-centrifugation and agitated biphasic blood culture systems for detection of fungemia. J. Clin. Microbiol. **29**:96–98.
21. **Nielson, H., J. Stenderup, and B. Bruun.** 1991. Fungemia in a university hospital 1984–1988. Scand. J. Infect. Dis. **23**:275–282.
22. **Paya, C. V., G. D. Roberts, and F. R. Cockerill.** 1987. Laboratory methods for the diagnosis of disseminated histoplasmosis: clinical importance of the lysis-centrifugation blood culture system. Mayo Clin. Proc. **62**:480–485.
23. **Prevost-Smith, E., and N. Hutton.** 1992. Value of extended agitation and subculture of BACTEC NR 660 aerobic resin blood culture bottles for clinical yeast isolates. J. Clin. Microbiol. **30**:3239–3242.
24. **Rantala, A., J. Niinikoski, and O.-P. Lehtonen.** 1989. Yeasts in blood cultures: impact of early therapy. Scand. J. Infect. Dis. **21**:557–561.
25. **Reller, L. B., P. R. Murray, and J. D. MacLowry.** 1982. Cumitech 1A, blood cultures II. Coordinating ed., J. A. Washington II. American Society for Microbiology, Washington, D.C.
26. **Smith, R. L., S. M. Meixler, and M. S. Simberkoff.** 1991. Excess mortality in critically ill patients with nosocomial bloodstream infections. Chest **100**:164–167.
27. **Stockman, L., G. D. Roberts, and D. M. Ilstrup.** 1984. Effect of storage of the Du Pont lysis-centrifugation system on recovery of bacteria and fungi in a prospective clinical trial. J. Clin. Microbiol. **19**:283–285.
28. **Tarrand, J. J., C. Guillot, M. Wenglar, J. Jackson, J. D. Lajeunesse, and K. V. Rolston.** 1991. Clinical comparison of the resin-containing BACTEC 26 Plus and the Isolator 10 blood culturing systems. J. Clin. Microbiol. **29**:2245–2249.
29. **Telenti, A., and G. D. Roberts.** 1989. Fungal blood cultures. Eur. J. Clin. Microbiol. Infect. Dis. **8**:825–831.
30. **Weinstein, M. P., S. Mirrett, M. L. Wilson, L. J. Harrell, C. W. Stratton, and L. B. Reller.** 1991. Controlled evaluation of BACTEC Plus 26 and Roche Septi-Chek aerobic blood culture bottles. J. Clin. Microbiol. **29**:879–882.
31. **Weinstein, M. P., L. B. Reller, J. R. Murphy, and K. A. Lichtenstein.** 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev. Infect. Dis. **5**:35–53.
32. **Young, R. C., J. E. Bennett, G. W. Geelhoed, and A. S. Levine.** 1974. Fungemia with compromised host resistance. A study of 70 cases. Ann. Intern. Med. **80**:605–612.