JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1981, p. 681-683
0095-1137/81/120681-03$02.00/0

Vol. 14, No. 6

Comparative Recovery of Fungi from Biphasic and Conventional Blood Culture Media

TIMOTHY E. KIEHN,* CHERYL CAPITOLO, JOAN B. MAYO, AND DONALD ARMSTRONG

Diagnostic Microbiology Laboratory, Memorial Hospital and Infectious Disease Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 29 April 1981/Accepted 23 June 1981

A brain heart infusion broth and agar biphasic bottle was compared with a vented broth brain heart infusion bottle for the recovery of fungi from blood. A total of 40 fungi, all yeasts, were recovered from 5,000 blood cultures. The biphasic bottle slightly increased the overall recovery of six species of yeasts. In addition, yeasts were first detected more often in the biphasic bottle (73%) than in the vented broth bottle (38%). A routine early (6- to 24-h) or late (5-day) subculture of macroscopically negative cultures may not be required for yeast isolation when a biphasic medium is used. Of the yeasts initially detected in the biphasic medium, 83% were seen to be growing on the agar slant. Only four were detected from a 24-h subculture, and no biphasic isolate was recovered from a 5-day subculture. Only one yeast, a *Candida glabrata* of questionable clinical significance, was recovered after our routine blood culture period of 7 days; however, other fungi, not recovered in this study, require extended incubation periods.

Although there has been an increase in life-threatening invasive yeast infections (3, 8), there has been difficulty in diagnosing these infections. Patients with invasive candidiasis often have negative blood cultures (6). Even when cultures are positive, growth of the yeasts in the blood culture bottle may be so slow that recognition is delayed (10).

In recent years, several improvements in blood culture techniques have been developed. The requirement for a vented (aerobic) bottle for yeast recovery has been convincingly demonstrated (1, 4). Biphasic media (agar slant and broth) were shown to enhance significantly the recovery of fungi, but in that study the conventional broth bottles were not vented (10). In a subsequent report from the same laboratory, a biphasic bottle and a vented broth bottle were compared (9). The biphasic bottle did not significantly increased overall fungus recovery but did reduce the time before organisms were first observed. Other investigators have reported that there was a comparable recovery of six yeast species from brain heart infusion biphasic and Trypticase (BBL Microbiology Systems) soy biphasic media (2).

We tested a new commercial biphasic blood culture bottle to evaluate: (i) the overall recovery and the time to the first detection of fungi; (ii) whether the agar slant is an acceptable substitute for the routine blood subculture; and (iii) the need for extended incubation periods for fungal isolation. We were interested in the possibility of substituting the biphasic medium bottle for the conventional broth bottle in our two-bottle culturing set (7).

MATERIALS AND METHODS

The biphasic bottle (GIBCO Laboratories) is shown in Fig. 1. It contained 50 ml of brain heart infusion broth separated by a plastic partition from a brain heart infusion agar slant. The partition permitted the inoculated blood-broth mixture to be washed over the agar slant and then returned to the side of the bottle without agar, thus providing maximum aeration of the agar slant during incubation.

Blood specimens were drawn into Vacutainer tubes (165 by 16 mm) containing 3.4 ml of 0.35% sodium polyanetholesulfonate (Becton, Dickinson & Co.,). In the laboratory, equal volumes of blood were inoculated into three GIBCO bottles; biphasic, conventional brain heart infusion broth (50 ml) supplemented with cysteine (1 g/liter), and conventional brain heart infusion broth (50 ml).

The biphasic bottle and the conventional broth bottle without additional cysteine were transiently vented with a single-draw Vacutainer needle to release the vacuum. The agar slant in the biphasic bottle was washed with the blood-medium mixture immediately after incubation and once each day for 5 days. All bottles were incubated upright at 35°C, and the broth was routinely subcultured onto chocolate agar at 6 to 24 h and at 5 days. When the broth was turbid, subcultures were performed onto the appropriate media, as dictated by the Gram stain results. All subculture plates were incubated for 48 h in 10% CO_2.

After 5 days of incubation and subculture, culture-

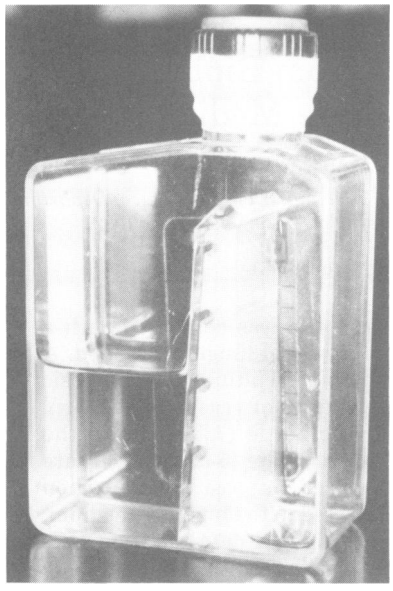

FIG. 1. *Biphasic blood culture bottle.*

negative biphasic and vented broth bottles were transferred to a 30°C incubator for the remainder of a 30-day incubation period. Both bottles of the set were inspected weekly for turbidity. Two additional subcultures onto chocolate agar were performed on all macroscopically negative bottles after 14 and 28 days of incubation.

The most rapid method for yeast detection was defined as the method which first yielded macroscopic growth. Yeasts were identified by conventional procedures.

RESULTS

During this study of 5,000 blood cultures, 40 fungi (all yeasts) were recovered from 20 patients. The species of yeasts and the numbers isolated were: *Candida tropicalis*, 15; *Candida albicans*, 10; *Candida parapsilosis*, 9; *Candida glabrata*, 3; *Cryptococcus neoformans*, 2; and *Rhodotorula rubra*, 1.

The yeast isolates eventually recovered in each of the three bottles, biphasic, vented broth, and unvented broth, are listed in Table 1. A total of 31 yeasts were recovered from the biphasic bottle, and 29 were recovered from the vented broth bottle. Of the total, 23 yeasts were isolated from both bottles, 6 were isolated from the biphasic bottle alone, and 3 were isolated from the vented broth bottle alone.

The bottles in which yeasts were first detected and the method of detection are shown in Table 2. Isolates that were first detected in more than one bottle appear more than once. Of the 40 yeasts, 29 were first detected in the biphasic bottle. Of these, 24 were first detected on the agar slant; 1 was initially observed macroscopically in the broth, and 4 were recovered from a 24-h subculture of the broths. A total of 17 yeasts were initially detected in the vented broth bottle; 14 of these isolates required either a 24-h or a 5-day blind subculture for detection. Of the 11 yeasts initially detected in the unvented broth bottle, 10 also required a blind subculture for initial detection.

The mean recovery time and range (in days) for each species of yeast is shown in Table 3. Only one yeast, a *C. glabrata* detected at 30 days, was recovered after the routine 7-day incubation period.

TABLE 1. *Number of yeast isolates from biphasic, vented broth, and unvented broth culture bottles*

Yeast isolate	Total no. for species	No. isolated from:		
		Biphasic medium	Vented broth	Unvented broth
C. tropicalis	15	9	11	9
C. albicans	10	9	8	4
C. parapsilosis	9	8	7	4
C. glabrata	3	3	1	1
C. neoformans	2	1	1	0
R. rubra	1	1	1	1

TABLE 2. *Initial method of detection of yeasts recovered from biphasic, vented broth, and unvented broth bottles*

Detection method	No. isolated from:		
	Biphasic medium	Vented broth	Unvented broth
Slant and 24-h subculture	8		
Slant	16		
Broth	1	3	1
24-h subculture	4	11	8
5-day subculture		3	2

TABLE 3. *Mean recovery time and range of 40 yeast isolates*

Yeast isolate	Mean recovery time (days)	Range (days)
C. tropicalis	2.1	1-6
C. albicans	2.9	1-6
C. parapsilosis	3.3	2-6
C. glabrata	12.0	2-30
C. neoformans	5.0	5
R. rubra	3.0	3

DISCUSSION

We sought to determine whether the addition of an agar slant to a broth culture bottle (bi-

phasic media) would affect the overall recovery, detection time, and requirement of blind subcultures for the recovery of fungi from blood.

The biphasic bottle only slightly increased the overall recovery of yeasts when compared with the vented broth bottle. Of the 40 yeasts eventually isolated, 78% were detected in the biphasic bottle and 73% were detected in the vented broth bottle.

The yeasts were detected earlier, however, more often in the biphasic than in the vented broth medium. A total of 29 (73%) of the isolates were initially detected in the biphasic bottle compared with 17 (43%) in the vented broth bottle ($P < 0.01$ by χ^2). The exact number of days to earlier detection was not quantifiable since broths which were not turbid were Gram stained when one bottle showed macroscopic growth. Earlier detection was attributable to the agar slant in the biphasic bottle. Of the yeasts observed initially in the biphasic medium, 83% were first detected on the agar slant.

The results also suggest that, when the biphasic bottle is used, 5-day blind subcultures are not necessary for the detection of the yeast species recovered in this study, since no biphasic isolate was obtained from a 5-day subculture. In addition, only 4 of the 31 biphasic isolates were initially detected by a 24-h subculture. Eliminating the blind subculture seems reasonable since a daily washing of the biphasic agar surface with the blood-broth mixture is, in fact, a subculture technique. Our findings support the work of Hall et al. (5), who have concluded that the use of their biphasic media, with possible enrichment, would eliminate the necessity of a routine subculture for microbial growth.

Only one yeast was isolated after the routine 7-day incubation period. This *C. glabrata*, detected at 30 days, was from a patient with invasive candidiasis. Only *C. albicans* was recovered from other blood cultures and autopsy cultures of the liver, spleen, and kidneys of this patient. Our results suggest that extended incubation periods may not be necessary for the species of fungi recovered in this study. However, some fungi such as *Histoplasma capsulatum* require an incubation period of more than 7 days for detection in blood cultures (10).

In conclusion, we have found that the biphasic blood bottle used in this study rapidly and efficiently reveals the growth of yeasts. It appears sufficiently better than the broth blood culture system tested; thus, we would recommend its use in laboratories anticipating a substantial number of yeast isolates. This bottle is not currently available; however, the company is attempting to resolve technical problems, and subsequent production is anticipated.

ACKNOWLEDGMENTS

The assistance of the technologists of the Microbiology Laboratory is gratefully acknowledged. Helen Rigopoulos was very helpful in the preparation of the manuscript. We thank Brian Wong for his critical review of the manuscript.

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