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Comparison of the Lysis-Centrifugation and Agitated Biphasic Blood Culture Systems for Detection of Fungemia

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Although the detection of fungemia has been improved by the use of vented or biphasic blood culture bottles, the best recovery and earliest detection have been reported in the Isolator lysis-centrifugation system. It was recently demonstrated that improved detection of both bacteria and fungi was accomplished by mechanically agitating blood culture bottles for the first 24 h of incubation. In this study the detection of fungemia by use of the Isolator system was compared with that of an agitated biphasic system. A total of 182 fungi were isolated from blood specimens inoculated into both culture systems. No difference in the overall recovery of fungi or individual species of yeasts was observed between the two systems. However, all seven isolates of *Histoplasma capsulatum* were recovered in the Isolator system only. The time required to detect fungemia with each of the two systems was also compared. No statistically significant difference was observed. From the data collected during this 18-month study, it can be concluded that the overall recovery and time of detection of yeasts are equivalent in the lysis-centrifugation system and the agitated biphasic blood culture system. The lysis-centrifugation system is still superior for the detection of filamentous fungi such as *H. capsulatum*.

During the last few years, several new systems for culturing blood specimens have been introduced. Many of these systems, such as commercially prepared biphasic bottles, lysis-centrifugation, and nonradiometric detection of microbial metabolism, have improved our ability to detect both fungi and bacteria in septicemic patients (2, 4–6, 8, 11, 12, 14, 16, 18, 19, 23–25). It was previously demonstrated that the recovery of fungi in blood cultures was significantly improved when conventional culture bottles were aerated (9, 20) or biphasic bottles were used (3, 18, 19, 21, 23–25). However, the most significant improvement in the recovery of fungi was observed with the Isolator lysis-centrifugation system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Most clinical comparisons of the Isolator system with other culture systems, such as the Septi-Chek biphasic system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) and the BACTEC system (Becton Dickinson Diagnostic Systems, Rutherford, N.J.), have demonstrated that more fungal isolates were recovered and the time of detection was shortened in the Isolator system (2, 4–6, 8, 12, 14–16).

It was recently demonstrated that recovery of both bacteria and fungi was improved by agitating bottles constantly for the first 24 to 48 h of incubation (10, 17, 22). The Septi-Chek biphasic blood culture system has been used in my laboratory routinely since mid-1982, and in 1988 this system was supplemented with the Isolator system for patients with suspected fungal sepsis. In October 1988, workers in my laboratory started to agitate all biphasic blood culture bottles for the first 24 h of incubation. Corresponding to this last modification, a large increase in the recovery of fungi was observed (i.e., 86 fungemic patients were recognized in 1988 compared with 125 in 1989). Because the Isolator system has not been compared with an agitated biphasic system, the recovery of fungi in these two systems was analyzed.

MATERIALS AND METHODS

During the study period, approximately 20 ml of blood was collected aseptically by venipuncture from patients with suspected septicemia. An equal volume of blood was then inoculated into two blood culture broths: 70 ml of tryptic soy broth (TSB) and 70 ml of Columbia broth, each supplemented with 0.05% sodium polyanetholesulfonate (Roche Diagnostics). If fungemia was suspected, an additional 10 ml of blood was collected in an Isolator 10 tube. The broths and Isolator tube were then transported to the laboratory. At the time the bottles were received, the Septi-Chek slide unit was attached to the TSB bottle, the bottle was inverted to inoculate the agar surfaces, and then the bottle was placed in a 35°C incubator for a total of 2 weeks of incubation. For the first 18 to 24 h of incubation, the TSB bottles were placed on a mechanical mixer (150 rpm; capacity, 64 bottles per mixer; dimensions: width, 17 in. [ca. 43 cm]; depth, 20 in. [ca. 50 cm]; height, 12 in. [ca. 30 cm]; New Brunswick Scientific Co., Inc., Edison, N.J.). The Columbia bottle was placed into a 35°C incubator without the use of the slide unit or mechanical agitation. Because fungi grow poorly in unvented bottles, data collected pertaining to this bottle are not discussed in this report. For the duration of this study, the TSB bottle was examined twice on day 1, daily for the next 6 days, and then once more after an additional 1 week of incubation (18).

The Isolator system was processed as previously described (1). After the culture was concentrated, the sediment was inoculated onto Trypticase soy blood agar (BBL) (incubated at 35°C), chocolate blood agar (35°C), Sabouraud dextrose agar (30°C), and brain heart infusion agar with blood (30°C). All plates were examined daily for 7 days. The brain heart infusion agar plate was held an additional 3 weeks and examined twice per week.

The asymptotic chi-square test of McNemar as described by Ilstrup (13) and the Sign test (7) were used to compare the fungal recoveries of the two systems. The paired *t* test was

TABLE 1. Isolation of fungal pathogens in blood cultures collected during an 18-month period at Barnes Hospital

Organism	No. of isolates detected in:[a]	
	Septi-Chek	Isolator
Candida albicans	249	78
C. tropicalis	79	50
C. parapsilosis	36	17
C. krusei	7	1
Torulopsis glabrata	64	11
Cryptococcus neoformans	25	11
Histoplasma capsulatum	2	20
Blastomyces dermatitidis	0	1

[a] During this time period, a total of 48,446 Septi-Chek and 14,223 Isolator blood cultures were collected.

used to compare the times required for initial detection of fungi in the two systems.

RESULTS

The recovery of fungi in blood cultures received in Barnes Hospital was examined for an 18-month period, from October 1988 through March 1990. A total of 48,446 biphasic bottles and 14,223 Isolator cultures were received during this time period. The recovery of fungi in the two systems is summarized in Table 1. Fungi were isolated in 651 cultures from 184 patients, with 462 (0.95%) positive biphasic bottles and 189 (1.33%) positive Isolator cultures. The greater proportion of Isolator cultures positive for fungi was not unexpected because this system was selected when fungal sepsis was considered in the differential diagnosis. To determine the relative sensitivity of each culture system for fungal sepsis, blood cultures collected simultaneously in both systems were analyzed.

A total of 182 fungi were isolated from blood specimens inoculated into both culture systems (Table 2), with no difference in overall fungal recovery observed between the two systems. Fungi were recovered in only one of the two systems for 72 isolates (36 isolates in the Septi-Chek system only, 36 isolates in the Isolator system only), while 110 fungi were recovered in both systems. The detection of individual species of yeasts was not significantly different between the two systems. However, all seven isolates of the dimorphic fungus *Histoplasma capsulatum* were recovered in the Isolator system only ($P < 0.02$).

The time required to detect fungal pathogens in each of the two culture systems was also examined (Table 3). Of the 110 isolates that were recovered in both culture systems, 32 were detected on the same day, 31 were detected initially in the Septi-Chek system, and 47 were detected initially in the Isolator system ($P > 0.05$). The mean times of detection of the individual species of yeasts were essentially identical for the most common isolates (*Candida albicans* and *Candida tropicalis*), slightly earlier for *Candida parapsilosis* and *Cryptococcus neoformans* in the Isolator system, and slightly earlier for *Torulopsis glabrata* in the Septi-Chek system. These differences were not statistically significant.

TABLE 2. Comparison of the recovery of fungal pathogens from 182 specimens inoculated into two blood culture systems

Organism	No. of organisms detected in:			*P*
	Septi-Chek only	Isolator only	Both systems	
Candida albicans	23	19	48	NS[a]
C. tropicalis	4	5	37	NS
C. parapsilosis	2	1	10	NS
C. krusei	1	0	0	NS
Torulopsis glabrata	4	0	7	NS
Cryptococcus neoformans	2	4	8	NS
Histoplasma capsulatum	0	7	0	<0.02

[a] NS, Not significant.

TABLE 3. Comparison of the time of detection for fungal pathogens recovered from specimens inoculated into both blood culture systems

Organism	No. of isolates	Mean time of detection[a] (days)	
		Septi-Chek	Isolator
Candida albicans	48	3.1	3.0
C. tropicalis	37	2.6	2.5
C. parapsilosis	10	3.3	2.6
Torulopsis glabrata	7	3.6	4.1
Cryptococcus neoformans	8	3.9	3.6

[a] The differences in time of detection were not statistically significant.

DISCUSSION

The effect of aerating blood cultures on the recovery of fungi has been well documented. Significant increases in detection of fungemia were observed in vented versus unvented blood culture bottles (3, 9, 20, 21), biphasic versus vented bottles (3, 18, 20, 21, 24, 25) and the radiometric BACTEC system (23), and agitated versus stationary bottles (10, 17, 22). The time required for the initial detection of positive fungal cultures was also shortened by aeration. When compared with conventional vented bottles (16), the BACTEC system (5, 6, 14), and biphasic cultures (2, 4, 8, 12), the Isolator lysis-centrifugation system demonstrated the best recovery and earliest detection of fungi. The Isolator system was modified in 1986. The original system consisted of a double-stoppered tube containing, among other components, a dense, inert fluorochemical that served as a cushion during centrifugation. The modified Isolator tube consists of a single stoppered top and a round glass bottom with the fluorochemical removed. Although the modified Isolator tube has not been compared with alternative blood culture systems, Basille and associates (1) demonstrated that the recovery of fungi was essentially identical in the initial and revised Isolator systems. Thus, it would be expected that the modified Isolator system would remain superior to the alternative systems.

Despite the numerous evaluations of the lysis-centrifugation system, this system had not been compared with the agitated biphasic culture system. In the study reported herein, we observed equivalent overall recovery of yeasts in the two systems and slightly better recovery of *C. albicans* and *T. glabrata* (Table 2) in the biphasic blood culture system. Statistically insignificant differences in the time of detection were also noted (Table 3). The only group of fungi that was detected more efficiently in the Isolator system was *H. capsulatum*. All seven isolates of *H. capsulatum* from two patients were detected in the Isolator system only. These isolates were detected after incubation for 11 to 12 days. It was not surprising that they were not isolated in the Septi-Chek biphasic system, which was incubated for only 2

weeks during this analysis. Roberts and Washington (21) reported the mean recovery time for six isolates of *H. capsulatum* to be 16 days (range, 12 to 24 days) in a biphasic system with brain heart infusion broth and agar. In a comparison of the Isolator and Septi-Chek systems, Guerra-Romero et al. (8) isolated *H. capsulatum* from four blood cultures, all of which were detected in the Isolator only (detection time, 12 to 14 days). Bille et al. (4) recovered 15 isolates of *H. capsulatum* in the Isolator but only 7 in a biphasic brain heart infusion system. The mean detection times with the two systems were 8.0 and 24.1 days, respectively. Although it is possible that extending the incubation period with the agitated biphasic system would improve the recovery of *H. capsulatum* and other filamentous fungi, it is clear from this study and those reported previously that the Isolator is superior for the detection of this group of organisms.

The results of this study have practical ramifications. My laboratory currently processes approximately 95 to 100 paired aerobic-anaerobic (Septi-Chek) blood cultures each day as well as an additional 30 daily fungal (Isolator) blood cultures. With the agitation of the biphasic aerobic bottle, the use of the Isolator system for routine recovery of yeast cells is now unnecessary. The discontinuation of the Isolator system will result in a significant reduction in the time required to process and examine these cultures and their attendant expenses, without compromising the efficiency of detecting fungemia caused by yeasts. For the detection of filamentous fungi such as *H. capsulatum*, the Isolator system is still recommended. The incubation of the biphasic system could be extended to 3 weeks or more for the detection of these organisms. However, systematic studies to compare extended incubation of the agitated biphasic TSB system with the Isolator system have not been performed.

REFERENCES

1. **Basille, B. A., K. A. Easley, G. S. Hall, and J. A. Washington II.** 1987. Comparison of conventional and revised Isolator blood culture tubes. J. Clin. Microbiol. **25**:2221–2222.
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., G. D. Roberts, and J. A. Washington II.** 1983. Retrospective comparison of three blood culture media for the recovery of yeasts from clinical specimens. Eur. J. Clin. Microbiol. **2**:22–25.
4. **Bille, J., L. Stockman, G. D. Roberts, C. D. 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.
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. **Daniel, W. W.** 1978. Biostatistics: a foundation for analysis in the health sciences, 2nd ed. John Wiley & Sons, Inc., New York.
8. **Guerra-Romero, L., R. S. Edson, F. R. Cockerill III, 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.
9. **Harkness, J. L., M. Hall, D. M. Ilstrup, and J. A. Washington II.** 1975. Effects of atmosphere of incubation and of routine subcultures on detection of bacteremia in vacuum blood culture bottles. J. Clin. Microbiol. **2**:296–299.
10. **Hawkins, B. L., E. M. Peterson, and L. M. de la Maza.** 1986. Improvement of positive blood culture detection by agitation. Diagn. Microbiol. Infect. Dis. **5**:207–213.
11. **Henry, N. K., C. M. Grewell, P. E. Van Grevenhof, D. M. Ilstrup, and J. A. Washington II.** 1984. Comparison of lysis-centrifugation with a biphasic blood culture medium for the recovery of aerobic and facultative anaerobic bacteria. J. Clin. Microbiol. **20**:413–416.
12. **Henry, N. K., C. A. McLimans, A. J. Wright, R. L. Thompson, W. R. Wilson, and J. A. Washington II.** 1983. Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. J. Clin. Microbiol. **17**:864–869.
13. **Ilstrup, D. M.** 1978. Statistical methods employed in the study of blood culture media, p. 31–39. *In* J. A. Washington (ed.), The detection of septicemia. CRC Press, Inc., Boca Raton, Fla.
14. **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.
15. **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.
16. **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.
17. **Kim, M. J., R. L. Gottschall, L. D. Schwabe, and E. L. Randall.** 1987. Effect of agitation and frequent subculturing on recovery of aerobic and facultative pathogens by Roche Septi-Chek and BACTEC blood culture systems. J. Clin. Microbiol. **25**:312–315.
18. **Pfaller, M. A., T. K. Sibley, L. J. Westfall, J. E. Hoppe-Bauer, J. A. Keating, and P. R. Murray.** 1982. Clinical laboratory comparison of a slide blood culture system with a conventional broth system. J. Clin. Microbiol. **16**:525–530.
19. **Reimer, L. G., L. B. Reller, and S. Mirrett.** 1989. Controlled comparison of a new Becton Dickinson agar slant blood culture system with Roche Septi-Chek for the detection of bacteremia and fungemia. J. Clin. Microbiol. **27**:2637–2639.
20. **Roberts, G. D., C. Horstmeier, M. Hall, and J. A. Washington II.** 1976. Recovery of yeast from vented blood culture bottles. J. Clin. Microbiol. **2**:18–20.
21. **Roberts, G. D., and J. A. Washington II.** 1975. Detection of fungi in blood cultures. J. Clin. Microbiol. **1**:309–310.
22. **Weinstein, M. P., S. Mirrett, L. G. Reimer, and L. B. Reller.** 1989. Effect of agitation and terminal subcultures on yield and speed of detection of the Oxoid Signal blood culture system versus the BACTEC radiometric system. J. Clin. Microbiol. **27**:427–430.
23. **Weinstein, M. P., L. B. Reller, S. Mirrett, C. W. Stratton, L. G. Reimer, and W. L. Wang.** 1986. Controlled evaluation of the agar slide and radiometric blood culture systems for the detection of bacteremia and fungemia. J. Clin. Microbiol. **23**:221–225.
24. **Weinstein, M. P., L. B. Reller, S. Mirrett, W.-L. L. Wang, and D. V. Alcid.** 1985. Controlled evaluation of Trypticase soy broth in agar slide and conventional blood culture systems. J. Clin. Microbiol. **21**:626–629.
25. **Weinstein, M. P., L. B. Reller, S. Mirrett, W.-L. L. Wang, and D. V. Alcid.** 1985. Clinical comparison of an agar slide blood culture bottle with tryptic soy broth and a conventional blood culture bottle with supplemented peptone broth. J. Clin. Microbiol. **21**:815–818.