DIAGN MICROBIOL INFECT DIS
1986;5:207–213

Improvement of Positive Blood Culture Detection by Agitation

Bonnie L. Hawkins, Ellena M. Peterson, and Luis M. de la Maza

To evaluate the advantages of agitation in reducing the detection time and increasing the recovery rate of positive blood cultures, 1,000 three-bottle sets of tryptic soy broth on adult inpatients were analyzed. Two bottles were transiently vented, one of which was agitated (250 rpm) for 7–19 hr at 35°C. The other vented bottle and the anaerobic bottle were incubated stationary at 35°C. Smears and subcultures were performed 7–19 hr after collection on both agitated and nonagitated vented bottles. Subcultures were done on all bottles at 72 hr and smears were performed on the anaerobic bottle. There were 137 of 1000 (13.7%) positive cultures from 90 patients. The agitated bottle detected 112 of 137 (81.8%) positive cultures, was the first or only means of detection in 57 of 137 cultures (41.6%), and was the only positive bottle in 30 of 137 (21.9%) cultures. The nonagitated vented bottle detected 89 of 137 (65.0%) of positive cultures and was the only means of detection in 13 of 137 (9.5%), but was never the first means of detection. The anaerobic bottle detected 76 of 137 (55.5%) of positive cultures, was the first or only means of detection in 11 of 137 (8.0%), and was the first means of detection in one of 137 (0.7%) cultures. When both the agitated and nonagitated bottle were positive, the agitated bottle was positive on the average 35 hr earlier. We conclude that agitation of the vented bottle in a conventional blood culture system significantly decreases the detection time of positive blood cultures and increases the number of positive blood cultures detected.

INTRODUCTION

The detection of bacteremia and fungemia is one of the most important functions of a clinical microbiology laboratory. Blood cultures may be the only immediate source of the etiologic agent of serious infections and may also be the basis upon which diagnosis and institution of appropriate antimicrobial therapy is dependent. It is, therefore, imperative to provide the physician making therapeutic decisions with information at the earliest possible time concerning the detection of organisms in a patient's blood as well as the subsequent identification and antimicrobial susceptibility testing of the organisms.

Because conventional techniques are still in widespread use for processing blood cultures, it is important that detection procedures are optimal (Reller et al., 1982; Washington, 1978). Many studies have shown the value of performing early subcultures (6–18 hr after collection) to decrease detection time of positive blood cultures

From the Medical Microbiology Division, Department of Pathology, University of California, Irvine Medical Center, Orange, CA.

Address reprint requests to: Ellena M. Peterson, Ph.D., Assistant Director of Medical Microbiology, University of California, Irvine Medical Center, 101 The City Drive, Orange, CA 92668.

Received December 16, 1985; revised and accepted February 11, 1986.

0732-8893/86/$03.50

(Harkness et al., 1975; Silva and Washington, 1980; Todd and Roe, 1975). The usefulness of performing early smears (3–24 hr after collection) has also been shown to be beneficial for early detection (McCarthy and Senne, 1980; Mirrett et al., 1982). In fact, early smears and subcultures can detect approximately one-half of all positive blood cultures within 24 hr of collection (Hawkins et al., 1983; Silva and Washington, 1980). In a further effort to decrease detection time, we report on the results of a study using agitation of blood cultures in addition to performance of early smears and subcultures.

MATERIALS AND METHODS

Included in this study were 1,000 30-ml blood samples aseptically collected by venipuncture from adult patients in the acute care nursing units of the University of California Irvine Medical Center. Equal aliquots were inoculated into each of three 100-ml bottles containing tryptic soy broth (Difco Laboratories, Detroit, MI) with 0.025% sodium polyanetholesulfonate under vacuum with CO_2. In the laboratory, the set of three bottles was evaluated by visual inspection and only sets that contained adequate and approximately equal amounts of inoculum were included in the study. Two bottles of the three bottle set were aseptically transiently vented and the third bottle remained unvented. One vented bottle and the unvented bottle were placed in a stationary incubator at 35°C. The other vented bottle was incubated in a tabletop 35°C incubator shaker (Model G24, New Brunswick Scientific, NJ), which features a platform that holds bottles in place; the incubator was rotated at 250 rpm for 7–19 hr. After rotating the agitated bottle was incubated stationary at 35°C. All bottles were incubated for 7 days or until growth was detected.

Bottles were examined macroscopically for evidence of growth in the morning and afternoon on the first and second days of incubation and in the morning of each day thereafter. Gram stains and subcultures were performed on the vented bottles (agitated and nonagitated) of a set which appeared macroscopically negative after 7–19 hr of incubation. An additional subculture was performed after 72 hr of incubation on all macroscopically negative bottles and Gram stains were done only on the unvented bottle of a set. Subcultures were made to chocolate agar and incubated at 35°C in 5% CO_2 for 48 hr. Plates were inspected for evidence of growth in 12-hr increments.

The initial means of detection of microbial growth were classified as follows: macroscopic observation; the early Gram stain; the early subculture inspected after 12 hr, or $\geq$24 hr incubation; the 72 hr Gram stain and 72 hr subculture after 12 hr, or $\geq$24 hr incubation.

RESULTS

Of the 1,000 blood cultures included in this study, there were 137 (13.7%) positive cultures from 90 patients. All three bottles were positive in 57 (41.6%) cultures. The agitated bottle was the only positive bottle in 30 (21.9%), whereas the nonagitated vented bottle was the only positive in 13 (9.5%) cultures. The anaerobic bottle was the only positive bottle in 10 (7.3%) cultures, and there were 18 (13.1%) in which both the agitated and nonagitated vented bottle were positive and seven (5.1%) in which both the agitated and anaerobic bottle were positive. In two (1.5%) cultures the nonagitated vented and anaerobic bottles were positive. The agitated bottle was the most frequent bottle type positive. It was positive in 112 of 137 (81.8%) positive sets. This was statistically significant ($p < 0.01$) when compared with the nonagitated

vented bottle that was positive in 89 of 137 (65.0%) positive sets. The anaerobic bottle was positive in 76 of 137 (55.5%) positive sets.

Table 1 shows the first means of detection of positive cultures by method and type of bottle. Of the 137 positive cultures, the early Gram stain was the most frequent method of detection, 48 of 137 (35.0%), followed by macroscopic examination, 37 of 137 (27.0%), and the early subculture, 31 of 137 (22.6%). The 72 hr subculture detected 17 (12.4%) positives, whereas the 72 hr Gram stain detected only four (2.9%) positive cultures. When comparing the agitated and nonagitated vented bottles, each detected similar numbers of positives by each method of detection with the exception of the early Gram stain. Of the 48 positive cultures first detected by the early Gram stain, the agitated bottle detected 47 of 48 (97.9%) versus 28 of 48 (58.3%) detected by the nonagitated bottle. In 16 of 48 (33.3%) positives recognized by the early Gram stain, the agitated bottle was the first or only means of detection versus one of 48 (2.1%) detected by the nonagitated bottle.

There were 84 sets where two or more bottles were positive. Of these, there were 28 in which one bottle type was the first means of detection. The agitated bottle was the first means of detection in 27 of 28 (96.4%), the anaerobic bottle was the first means of detection in one of 28 (3.6%), and the nonagitated vented bottle was never the first means of detection when other bottles of a set were positive.

A comparison of the detection times of the 27 sets when the agitated bottle was positive before the nonagitated vented bottle shows an average detection time of 24 hr for the agitated bottle versus 59 hr for the nonagitated vented bottle.

The agitated and nonagitated bottles detected similar numbers of positive cultures by the early subculture (29 versus 24). The agitated bottle detected 17 positives after 12 hr of incubation versus eight positives which grew from the nonagitated bottle after 12 hr incubation.

Table 2 shows the types of organisms isolated and the types of bottles from which they were recovered. In addition, the numbers of bottles of each type which were the first or only means of detection are indicated for each organism. There were 153 organisms isolated from the 137 positive sets. Thirteen positive bottles yielded multiple organisms ranging from two to six isolates; in addition, there were seven positive sets with two or more positive bottles in which the organisms isolated from one or more bottles were different. This accounts for the larger number of organisms isolated from the 137 positive sets.

The agitated bottle recovered the largest number of organisms, 120 of 153 (78.4%), followed by the nonagitated vented bottle 98 of 153 (64.1%). This difference was found to be statistically significant ($p < 0.01$). The anaerobic bottle recovered 80 of 153 (52.3%) organisms. The majority of organisms recovered were *Enterobacteriaceae* (42), *Staphylococcus epidermidis* (33), and *Streptococcus* species (24); in all cases the agitated bottle recovered the greatest number of isolates. The agitated bottle detected 23 of 24 isolates of *Streptococcus* species and all isolates of *Staphylococcus aureus* (9), Streptococcus group D (6), *Candida albicans* (5), and *Enterobacter cloacae* (5), which the nonagitated vented bottle and anaerobic bottles failed to do. As expected, the agitated bottle was least successful in the recovery of anaerobic bacteria, two versus four anaerobic organisms detected by the nonagitated vented bottle and nine detected by the anaerobic bottle. *Corynebacterium* species were recovered in greater numbers from the agitated bottle (8) than either the nonagitated vented bottle (3) or the anaerobic bottle (1); however, it should be noted that three of the isolates were recovered from the same patient and were considered clinically significant and two of the three were isolated only from the agitated bottle. The thirty-three isolates of *S. epidermidis* were recovered in similar numbers from both the agitated bottle

TABLE 1. First or Only Means of Detection of Positive Blood Cultures[a]

Method	Specimens		Agitated		Nonagitated		Anaerobic	
	No.	%	Total number of specimens detected	First or only detection method	Total number of specimens detected	First or only detection method	Total number of specimens detected	First or only detection method
Macroscopic examination (≤24 hr)	7	5.1	5	2	2	0	5	2
Macroscopic examination (>24 hr)	30	21.9	19	13	19	5	17	7
Gram stain (7–19 hr)	48	35.0	47	16	28	1	27	0
Subculture (7–19 hr)	31	22.6	29	20	24	2	18	0
Gram stain (72 hr)	4	2.9	1[b]	0	2[b]	0	2	2
Subculture (72 hr)	17	12.4	11	6	14	5	5	0
Total	137		112	57	89	13	76	11

[a]First or only detection method refers to a bottle which was the only positive bottle in the set or a bottle which was positive at least 12 hr before the other bottles of a set.

[b]Gram stain was performed at 72 hr after the routine 72 hr Gram stain on the anaerobic bottle was positive.

TABLE 2. Organisms Isolated and Bottle From Which They Were Recovered

Organisms	Total number isolated	Agitated		Nonagitated		Anaerobic	
		Total	First means of detection	Total	First means of detection	Total	First means of detection
Enterobacteriaceae	42	34	11	31	4	27	4
Staphylococcus epidermidis	33	23	18	19	6	12	3
Staphylococcus aureus	9	9	4	5	0	4	0
Pseudomonas aeruginosa	8	6	5	6	2	5	0
Streptococcus species	24	23	8	19	1	18	0
Candida albicans	5	5	3	2	0	0	0
Corynebacterium species	10	8	7	3	1	1	0
Haemophilus influenzae	3	3	0	3	0	3	0
Anaerobes	11	2	0	4	1	9	7
Miscellaneous[a]	8	7	2	6	1	1	0
	153	120	58	98	16	80	14

[a]*Acinetobacter anitratus* (1), *Bacillus* species (2), *Brucella* species (2), *Campylobacter jejuni* (1), *Micrococcus* species (1), *Neisseria meningitidis* (1).

(23) and the nonagitated bottle (19); fewer isolates (12) were recovered from the anaerobic bottle. The agitated bottle and the nonagitated vented bottle were equally successful in the recovery of several fastidious isolates.

DISCUSSION

The results of our study clearly demonstrate the benefits of agitation and confirm previous studies, while including a wider number of organisms. Several investigators evaluating the use of agitation as applied to the BACTEC radiometric detection system in which blood cultures are agitated to increase the evolution of $^{14}CO_2$ from the substrate have shown that agitation produces more rapid detection for aerobic organisms, specifically *Pseudomonas aeruginosa* and has no adverse effects on bacterial growth (BACTEC, 1972, 1974, 1975). Agitation of aerobic cultures when using the BACTEC system demonstrated that agitation gave faster detection for *Bordetella bronchiseptica*, *N. meningitidis*, *S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, and *P. aeruginosa* (BACTEC, 1972, 1975). In a previous study, Ellner et al. (1976) have shown that agitation of conventional aerobic cultures improved recoveries of various organisms when compared with conventional systems not utilizing agitation. The overall recovery of aerobic and facultative organisms, principally *E. coli*, was markedly greater in the shaken bottle when compared with the stationary bottle (49 versus 28).

An earlier investigation to examine the utility of performing early smears and subcultures in which the processing protocol was identical to this study, with the exception of the agitated bottle, showed that the early Gram stain detected 14.0% positive cultures compared with 35.0% in the present study (Hawkins et al., 1983). This significant difference was the result of the greater number of positives detected by early Gram stain of the agitated bottle, 47 of 48 (97.9%), versus 28 of 48 (58.3%) detected by the nonagitated bottle in the present study. The 28 of 137 (20.4%) pos-

itives detected by the nonagitated bottle compares with the 14% positives detected by early Gram stain of a vented, nonagitated bottle in our previous study. The greater number of organisms that were present in the agitated bottles made detection of organisms much easier and reduced the time required for examining smears. The large number of positives [48 of 137 (35.0%)] detected by the early Gram stain was also responsible for the reduction in the average detection time of positives.

In our previous study the early subculture was the first means of detection in 31.0% positive cultures compared with 22.7% in the present study (Hawkins et al., 1983). The lower number of positive early subcultures was clearly a result of the increased number of positives detected by the early Gram stain of the agitated bottle. Of interest is the utility of inspecting early subcultures in 12 hr increments. In our previous study, 57.3% of positive cultures by early subculture were detected after 12 hr incubation, in the present study 19 of 31 (61.3%) positives were detected after 12 hr of incubation; of these the agitated bottle detected 18 of 19 versus four of 19 that grew from the nonagitated bottle after 12 hr incubation.

While offering decreased detection time and increased recovery rate, the disadvantages of using agitation are threefold: increase in the number of contaminants isolated; increased difficulty in assessing macroscopic changes in agitated bottles; necessity to purchase an incubator shaker.

Recently several new approaches have been introduced for the detection of bacteremia including the Dupont Isolator lysis centrifugation and Roche Septi-Chek biphasic systems, and although each has differing sensitivities with regard to certain groups of organisms, they all demonstrate an increase in the isolation of *S. epidermidis*, *Corynebacterium* species, and other organisms generally thought to be contaminants (Gill et al., 1984; Henry et al., 1983, 1984; Kelly et al., 1983; Pfaller et al., 1982; Thompson et al., 1984; Zierdt et al., 1982; Zierdt 1983). The question of the clinical significance of these organisms has yet to be satisfactorily resolved, particularly when the isolate is from a febrile immunocompromised host. One patient in our study was determined to have bacteremia associated with *Corynebacterium* species. The agitated bottle was positive in three sets, whereas the nonagitated bottle was positive in only one set.

As a result of the 7–19 hr of agitation, some bottles appeared partially hemolyzed and the broth had an opaque dark brown appearance as the incubation period progressed. The difficulty in reading these agitated bottles for macroscopic changes suggesting growth resulted initially in more Gram stains from agitated bottles being performed. Additionally for the first 6 months of the study, a terminal subculture was performed on any agitated bottle that was macroscopically difficult to interpret. None of these terminal subcultures were positive and the procedure was discontinued. As the study progressed the technical staff became more proficient at determining whether or not the agitated bottle was macroscopically positive.

The necessity to purchase at least one incubator shaker can present a problem for laboratories with limited equipment funds and workspace. However, the cost of approximately $2,500 for the incubator shaker seems reasonable when considering the cost savings to the hospital and patient afforded by earlier detection of bacteremia. Alternatively, purchasing a suitable shaker alone which could be placed inside an incubator would obviate the need for a more expensive piece of equipment.

This study reinforces the basic principles of aeration as a stimulant to the growth of microorganisms in broth and demonstrates the utility of agitating blood cultures in a clinical setting in order to reduce detection time and increase detection rate of positive blood cultures. In all cases, with the exception of anaerobes, bottles that were vented and agitated yielded more positive cultures and a shorter detection time

than bottles which were vented and not agitated. For laboratories using a conventional broth system for blood cultures, agitation is a cost effective and rational approach to the detection of septicemia.

REFERENCES

BACTEC Application Note, JLI-610B (December 1972) Johnston Laboratories, Cockeysville, MD.

BACTEC Application Note, JLI-617A (March 1974) Johnston Laboratories, Cockeysville, MD.

BACTEC Application Note, JLI-623 (January 1975) Johnston Laboratories, Cockeysville, MD.

Ellner PD, Kiehn TE, Beebe JL, McCarthy LR (1976) Critical analysis of hypertonic medium and agitation in detection of bacteremia. *J Clin Microbiol* 4:216.

Gill VJ, Zierdt CH, MacLowery JD (1984) Lysis-filtration and lysis-centrifugation methods for blood cultures. *J Med Tech* 1:621.

Harkness JL, Hall M, Ilstrup DM, Washington JA II (1975) Effects of atmosphere of incubation of routine subcultures on detection of bacteremia in vacuum blood culture bottles. *J Clin Microbiol* 2:296.

Hawkins BL, Peterson EM, de la Maza LM (1983) Rapid detection of positive blood cultures. *J Clin Microbiol* 18:716.

Henry NK, Grewell CM, McLimans CA, Washington JA II (1984) Comparison of the Roche Septi-Chek blood culture bottle with a brain heart infusion biphasic medium bottle and with a tryptic soy broth bottle. *J Clin Microbiol* 19:315.

Henry NK, McLimans CA, Wright AJ, Thompson RL, Wilson WR, Washington JA II (1983) Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. *J Clin Microbiol* 17:864.

Kelly MT, Buck GE, Fojtasek MR (1983) Evaluation of a lysis-centrifugation and biphasic bottle blood culture system during routine use. *J Clin Microbiol* 18:554.

McCarthy LR, Senne JE (1980) Evaluation of acridine orange stain for detection of microorganisms in blood cultures. *J Clin Microbiol* 11:281.

Mirrett S, Lauer BA, Miller GA, Reller LB (1982) Comparison of acridine orange, methylene blue, and Gram stains for blood cultures. *J Clin Microbiol* 15:562.

Pfaller MA, Sibley TK, Westfall LM, Hoppe-Bauer JE, Keating MA, Murray PR (1982) Clinical laboratory comparison of a slide blood culture system with a conventional broth system. *J Clin Microbiol* 16:525.

Reller LB, Murray PR, MacLowry JD (1982) Blood Cultures II. In Cumitech 1A, Ed., JA Washington II Washington DC: American Society for Microbiology, pp 1–11.

Silva HS, Washington JA II (1980) Optimal time for routine early subculture of blood cultures. *J Clin Microbiol* 12:445.

Todd JK, Roe MH (1975) Rapid detection of bacteremia by an early subculture technique. *Am J Clin Pathol* 64:694.

Thompson RB Jr, Vanzo SJ, Henry NK, Guenther KL, Washington JA II (1984) Contamination of cultures processed with the isolator lysis-centrifugation blood culture tube. *J Clin Microbiol* 19:97.

Washington JA II (1978) *The Detection of Septicemia*. West Palm Beach, FL: CRC Press.

Zierdt CH, Peterson DL, Swan JC, MacLowry JD (1982) Lysis-filtration blood culture versus conventional blood culture in a bacteremic rabbit model. *J Clin Microbiol* 15:74.

Zierdt CH (1983) Evidence for transient *Staphylococcus epidermidis* bacteremia in patients and in healthy humans. *J Clin Microbiol* 17:628.