JOURNAL OF CLINICAL MICROBIOLOGY, June 1985, p. 955–958
0095-1137/85/060955-04$02.00/0

Vol. 21, No. 6

Evaluation of a Lysis Direct Plating Method for Pediatric Blood Cultures

DAVID F. WELCH,[1,2]* RONALD K. SCRIBNER,[2] AND DIANE HENSEL[1]

Oklahoma Children's Memorial Hospital[1] and Division of Pediatric Infectious Diseases, Oklahoma University Health Sciences Center,[2] Oklahoma City, Oklahoma 73126

Received 19 December 1984/Accepted 7 March 1985

The Isolator 1.5 Microbial tube (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) is a commercially available blood culture system for use in pediatrics. The methodology is based on blood lysis followed by direct plating of the sample on culture media to detect bacteria and fungi. Comparative recovery rates of pathogens from blood collected in this and a conventional broth system were similar. The Isolator detected 104 of 120 clinically significant isolates, whereas 106 of 120 isolates were detected by the broth system. The major advantage of the Isolator methodology was early detection of septicemia. Initial detection of gram-negative bacteria occurred an average of 14.2 h earlier by the Isolator system than by the conventional broth method. The Isolator also permitted quantitation of bacteremia and fungemia. Probable contaminants were recovered from 10.0% of the cultures processed by the Isolator, but steps which could be taken to minimize this problem were identified. The Isolator is a useful method for pediatric blood cultures.

Discovery of bacteria or fungi in blood elicits prompt consideration of therapeutic measures. The initial detection and etiological characterization of septicemia are critically important steps for optimal patient care. Diagnostic methods have been traditionally based on culturing blood in bottles containing a relatively large volume of a nutrient broth. Detection of positive blood cultures may be accomplished in the laboratory by observing growth macroscopically or microscopically in the broth or by obtaining growth from a subculture made onto an agar medium. A variation of the broth methodology (BACTEC system) employs radiometry for detection (9). These broth-dependent techniques are satisfactory but may not be optimal for use in pediatrics because the volume of blood cultured is disproportionately low and delay is often encountered in detection of the most commonly isolated pathogen, *Haemophilus influenzae*. Alternative approaches based on limited blood volume and direct plating of the specimen have been described previously (2, 7, 8). Recently such approaches have been combined with principles to eliminate the antibacterial properties of blood and have been introduced commercially as the Isolator 1.5 Microbial tube (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Lysis of blood in a medium of purified saponin and sodium polyanetholesulfonate occurs, and the sample is then directly plated on solid culture media to recover bacteria and fungi. In addition to facilitation of specimen handling and detection, this approach permits quantitation of bacteremia and fungemia. Sullivan et al. (11) have demonstrated that the magnitude of bacteremia in children is associated with severity of clinical disease. Thus, a reliable direct plating technique may be more clinically useful than conventional blood culture. A comparison of the Isolator 1.5 Microbial tube with the BACTEC system demonstrated no statistically significant overall difference in recovery rates (1). We compared the lysis direct plating (LDP) system in parallel with a conventional broth culture (CBC) method in hospitalized and nonhospitalized children. The LDP system permitted significantly earlier detection than the CBC method, although no major difference between the two systems was found in the recovery rates of pathogens.

* Corresponding author.

MATERIALS AND METHODS

Blood culture systems. The Isolator (LDP) consisted of a rubber-stoppered, evacuated tube (10 by 50 mm) containing purified saponin (1 U), sodium polyanetholesulfonate (0.96 mg), polypropylene glycol (0.8 μl), and water in a total volume of 0.1 ml. The reference system (CBC) employed 50-ml bottles of brain heart infusion broth with sodium polyanetholesulfonate, CO_2, and *para*-aminobenzoic acid (GIBCO Laboratories, Grand Island, N.Y.).

Study design. Blood for cultures was collected under the supervision of house staff physicians. Venipuncture sites and the outside of tube and bottle stoppers were prepared with a solution of povidone-iodine. Blood culture sets consisting of one LDP tube and one CBC bottle or one LDP tube and two CBC bottles were distributed for use to all patient-care areas of the hospital. All bottles were preweighed for subsequent verification of the quantity of blood cultured. Instructions on distribution of the blood sample were provided with each blood culture set: for blood volumes of 1 to 3 ml, the specimen was to be divided equally between the LDP tube and one CBC bottle; for blood volumes of >3 ml, the LDP tube was to be inoculated with 1.5 ml and the remainder divided equally between two CBC bottles. LDP tubes containing <0.5 ml of blood were excluded from the study, and for the purpose of analysis of positive blood cultures, only sets that received 0.5 to 2.0 ml of blood per system were included.

LDP tubes were processed upon receipt in the laboratory from 7 a.m. to 11 p.m. Those received from 11 p.m. to 7 a.m. were incubated at 35°C until after 7 a.m. All processing work was performed at a biological safety cabinet with vertical laminar air-flow. Blood was withdrawn aseptically from the LDP tube with a 3-ml syringe and a 20-gauge, 1-in. (2.54-cm) needle. The lysed blood was inoculated onto plate media in

TABLE 1. Clinically significant blood culture isolates recovered by the two systems

Organism	No. of isolates recovered by:		
	Both	LDP only	CBC only
H. influenzae	27	1	3
S. pneumoniae	19	2	7
N. meningitidis	2	0	0
Streptococcus spp.	5	2	3
Staphylococcus spp.	14	5	0
Enterobacteriaceae	8	1	2
Nonfermenters	7	0	0
Anaerobes	4	0	1
Candida spp.	4	3	0
Total %	75	12	13

amounts of approximately 0.3 ml per plate, including one anaerobic blood agar plate and one to four chocolate agar plates. The inoculum was dispersed in a straight line down the center of the plate, avoiding the edge. With a sterile wire spreader, the inoculum was distributed in three directions across the plate, keeping approximately 0.5 cm from the edge. The wire was not resterilized between plates. The plates were numbered in order of spreading to aid in interpretation of contamination. The blood agar plates were incubated in an anaerobic glove box at 35°C for the first 24 h and in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for the remainder of the incubation period (4 days). The chocolate agar plates were incubated upright in 5 to 10% CO_2 at 35°C. After 24 h, the plates were inverted and reincubated for a total of 4 days. CBC bottles were incubated at 35°C for 7 days. Bottles were left unvented unless two were received, in which case one bottle was then vented.

Agar and broth media were examined routinely for growth at 7:00 a.m. and 3:00 and 8:00 p.m. Blind subcultures from bottles were made to chocolate agar plates. An early subculture was performed at 4 to 14 h, depending on the time of receipt. Second and third subcultures were performed at 24 h and 5 days. The chocolate agar plates were incubated at 35°C in 5 to 10% CO_2 for 48 h. Gram stains were made from bottles at the time of the early and 24-h subcultures. Growth from positive CBC bottles or on plates from LDP tubes was Gram stained, subcultured, and identified by conventional methods. Positive CBC bottles were subcultured aerobically and anaerobically at 5 days to detect polymicrobial cultures.

Evaluation of isolates for clinical significance. *Staphylococcus epidermidis*, viridans group streptococci, *Corynebacterium* and *Bacillus* spp., and *Propionibacterium acnes* were considered contaminants, unless the same organism was isolated from more than one blood culture per patient and the clinical course of the patient was consistent with infection caused by one of these organisms. Single colonies outside the area of inoculation on agar plates were disregarded. The significance of other isolates was evaluated by review of the medical records of the patient and discussion with the clinical staff. Criteria used to designate an isolate as a probable cause of bacteremia included fever, leukocytosis, positive culture for the same organism from another source such as urine or wound, and response to specific antimicrobial therapy.

Statistical analysis. Comparisons of isolation rates and times to positivity were made by the methods of Ilstrup (4). The McNemar chi-square test was applied to the number of isolates recovered from the LDP system versus that recovered from the CBC system. A *t* test for paired data was applied to the difference between the time intervals for detection of positive results by the two systems.

RESULTS

During a period of approximately 1 year, 2,945 blood cultures were eligible for the study. There were 157 (5.3%) positive cultures that were deemed to be clinically significant; 116 of these met the criteria for analysis based on similar blood volume (0.5 to 2.0 ml per system). Table 1 lists the organisms recovered and proportion of isolates detected by the two systems. A total of 120 isolates were available for analysis. There was no statistically significant overall difference and no significant differences among individual groups of organisms in the positivity rate of the LDP system compared with the CBC method. *H. influenzae* and *S. pneumoniae* accounted for nearly one-half of the total number of isolates, and when these were analyzed separately, there was still no significant difference in sensitivity between the two systems. The LDP system detected 21 of 28 *S. pneumoniae* isolates, whereas 26 of 28 isolates were detected by the CBC method. That some organisms were recovered more often by CBC than by LDP may be due to a slightly larger quantity of blood allocated to CBC. The average volumes of blood cultured were 0.9 and 1.3 ml for LDP and CBC, respectively.

The majority of positive blood cultures were detected earlier by LDP than by CBC (Table 2). The most notable difference between LDP and CBC in average times to detection occurred with respect to *H. influenzae* ($P < 0.001$). Since the time of detection was the same as the time to isolation of colonies in the LDP system, an additional comparison could be made on the basis of the time to achieving isolated colonies in the CBC method. Colony isolation of *S. pneumoniae* and other gram-positive isolates, in addition to *H. influenzae*, was achieved significantly earlier by LDP than by CBC. There was no difference in times to isolation of gram-negative isolates other than *H. influenzae* from the two systems.

The magnitude of bacteremias ranged from <1 to >1,000 CFU/ml. A comparison between quantitation of *H. influenzae* and *S. pneumoniae* is shown in Fig. 1. This analysis was limited to blood samples that were processed within 2 h of collection to avoid spurious counts. Approximately two-thirds of the cultures that yielded *H. influenzae* contained >100 CFU/ml, whereas less than one-third of those that yielded *S. pneumoniae* were of the same magnitude. None of the staphylococci deemed to be clinically significant were present in quantities of <1 CFU/ml.

TABLE 2. Mean number of hours to detection of positive blood cultures and isolation of colonies

Organism	Mean time (h) to:				
	Detection			Isolation	
	LDP	CBC	*P* value	CBC	*P* value
H. influenzae	19.2	28.8	<0.001	34.9	<0.001
S. pneumoniae	21.5	22.9	NS[a]	32.5	<0.01
Other gram-negative bacteria	29.1	47.7	NS	56.0	NS
Other gram-positive bacteria	30.7	48.7	<0.01	56.4	<0.001
Candida spp.	42.4	102.7	NS	102.7	NS

[a] NS, Not significant.

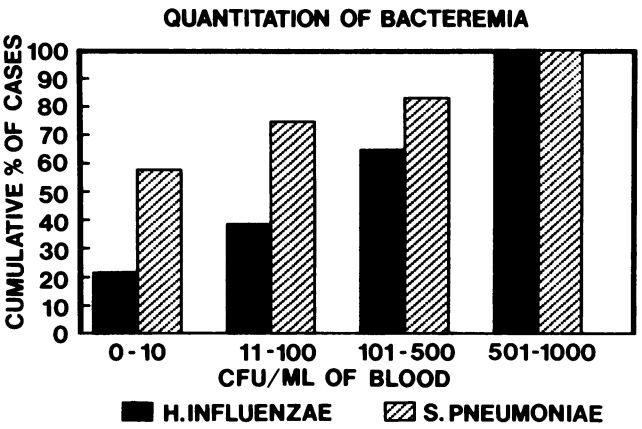

FIG. 1. Colony counts of *H. influenzae* and *S. pneumoniae* isolated from the blood of 35 patients (*H. influenzae*, 23; *S. pneumoniae*, 12).

LDP resulted in 294 cultures with clinically insignificant isolates, or a rate of 10.0%. The rate associated with CBC was only 1.8%. The incidence of *S. epidermidis*, *P. acnes*, and *Bacillus* spp. as contaminants was much higher from LDP than from CBC. The pattern almost always consisted of one or two colonies on a single plate, corresponding to <1 CFU/ml. Single colonies of coagulase-negative staphylococci that appeared after 2 days of incubation were likely to be clinically insignificant, suggesting that these organisms originated from handling plates in the laboratory. The incidence of *Streptococcus*, *Corynebacterium*, and gram-negative species as contaminants was comparable between the two systems.

DISCUSSION

The major finding of our study was an advantage of the LDP system over the CBC system for the early identification of blood culture isolates. Various improvements in blood culture systems for use in pediatrics have been described previously. These include direct plating of blood added to sodium polyanetholesulfonate (2) or heparin (8) as well as modifications in broth culture procedures (13). Generally, the aim is to accelerate detection or to enhance recovery rates of the most commonly encountered pathogens. In selecting a reference method for a controlled evaluation of the LDP technique, we attempted to optimize our broth culture system on the basis of results of previous studies. For example, the early subculture technique permits relatively rapid detection of bacteremia from conventional broth systems (13), and GIBCO brain heart infusion medium has been reported to be superior to 22 other types of broth media in laboratory testing for the recovery of *H. influenzae* (10).

The LDP technique is an adaptation of the Isolator system recently reported to be useful for adult blood cultures (3, 6). The pediatric version, however, is simplified in that centrifugation is not required as part of specimen processing. A possible limitation of the technique may be that the tube accomodates less blood than most broth systems, resulting in potentially inferior detection of low-magnitude bacteremias. Blood volume has been suggested as an important determinant in the diagnosis of bacteremia in both children (12) and adults (5). It has been unclear to what extent the volume effect observed in blood cultures from adults directly applies to pediatrics. Ilstrup and Washington (5) have reported that the yield of positive blood cultures from adults increased by an average of 38 and 61% when the volume of blood cultured was increased from 10 to 20 and 30 ml, respectively. Given blood volumes approximately 10% of these, pediatric blood culture studies are difficult to design without encountering potential bias because of unequal volumes. To minimize the effect of volume differences on blood culture results, we chose to base our study on only those sets in which 0.5 to 2.0 ml per system or a total of 1.0 to 4.0 ml of blood had been collected. Neither system yielded significantly more isolates than the other. That less blood was allocated to LDP (an average of 0.94 ml, compared with 1.3 for CBC) supports the efficacy of LDP compared with CBC. Although our results did not show an overall advantage in the recovery of organisms, selected organism groups were detected more often by LDP than by CBC. These included staphylococci and yeasts which were among those organisms also detected at a greater rate by the Isolator system than by a broth method in an adult patient population (3). Our results for staphylococci were remarkably consistent with those of Kelly et al. (6). Of 19 isolates, 5 (26%) were missed by the CBC system in our study, compared with 10 of 39 (26%) missed by a broth method in their study with the adult Isolator. Carey (1) reported no difference in the rate of recovery of staphylococci between LDP and the BACTEC system. In contrast to the results of our study, Carey (1) found better recovery of pneumococci by LDP than by the BACTEC system. This may actually reflect differences between the BACTEC system and our CBC system rather than the performance of the Isolator 1.5 Microbial tube in the two laboratories.

A major advantage of the LDP system was early detection of bacteremia and, hence, the ability to rapidly characterize and perform susceptibility testing on isolates. Preliminary information, such as the β-lactamase activity of *H. influenzae*, may be especially valuable in early selection of optimal antimicrobial therapy. Kelly et al. (6) reported that the lysis-centrifugation technique provided isolated colonies of all clinically significant organisms an average of 30 h earlier than a broth system. Though limited in number, *Candida* species in our study were isolated by LDP 60 h earlier, on the average, than by CBC. The times required to obtain isolated colonies of both *H. influenzae* and gram-positive isolates by LDP were also significantly less than those required by CBC.

Our results confirmed the potential usefulness of LDP for the quantitation of bacteremia. The technique was not prospectively studied, however, with the purpose of comparing bacterial counts with clinical manifestations. Despite this, our observations associated with selected patients during the study tended to support the findings of Sullivan et al. (11). Counts of >100 CFU of *H. influenzae* or *S. pneumoniae* per ml of blood were generally associated with meningitis, whereas low colony counts appeared to be most often associated with less serious manifestations, such as otitis media.

A high rate of contamination resulted from the use of LDP (10.0% versus 1.8% by CBC). This would be unacceptable without the ability to qualify interpretation of such cultures or to determine ways to reduce the rate. Two characteristics of the pattern associated with contamination in our study could be identified. *S. epidermidis*, which accounted for approximately two-thirds of the insignificant isolates, was commonly detected after 48 h of incubation and in quantities of <1 CFU/ml. Therefore, we believe that the total duration of incubation for routine cultures may be reduced generally from 4 to 3 days, while <1 CFU of *S. epidermidis* per ml could be regarded as probable contamination. Circum-

stances such as prior antibiotic therapy or a patient at a high risk for acquiring *S. epidermidis* bacteremia may warrant a more conservative approach to interpretation of the presence of this organism in blood cultures processed by LDP. Contamination occurring after 48 h of incubation is most likely due to shedding of organisms, in the case of *Staphylococcus* and *Propionibacterium* spp., by workers when the culture plates are handled in the laboratory. The appearance of *Bacillus* spp. on culture media suggests airborne contamination. Careful handling of culture plates is required to limit this problem. Another step to alleviate interpretative problems may be to incubate used Isolator tubes and stain their contents upon recognition of potential contaminants on agar media. Absence of bacteria on Gram stain in the presence of numerous colonies on plates would tend to support preexisting medium contamination rather than the source being the patient's skin or blood. It may also be noted that the contamination rate declined as experience was gained with LDP.

We conclude that the LDP method for pediatric blood cultures offers an advantage over broth systems in terms of timely detection and characterization of pathogens. A trend was noticed that suggests greater sensitivity of LDP than CBC for recovery of staphylococci, but the number of observations was few. Efficient recovery of staphylococci, in addition to the vulnerability of these cultures to contamination in the laboratory, account for a potentially high recovery rate of clinically insignificant organisms. LDP permits quantitation which may become clinically useful for diagnostic and prognostic purposes.

ACKNOWLEDGMENTS

The clinical microbiology staff of Oklahoma Children's Memorial Hospital, Harris Stutman, and Andrew Weber provided valuable assistance.

LITERATURE CITED

1. **Carey, R. B.** 1984. Clinical comparison of the Isolator 1.5 microbial tube and the BACTEC radiometric system for detection of bacteremia in children. J. Clin. Microbiol. **19**:634–638.
2. **Durbin, W. A., E. G. Szymczak, and D. A. Goldmann.** 1978. Quantitative blood cultures in childhood bacteremia. J. Pediatr. **92**:778–780.
3. **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.
4. **Ilstrup, D. M.** 1978. Statistical methods employed in the study of blood culture media, p. 31–39. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, West Palm Beach, Fla.
5. **Ilstrup, D. M., and J. A. Washington II.** 1983. The importance of volume of blood cultured in the detection of bacteremia and fungemia. Diagn. Microbiol. Infect. Dis. **1**:107–110.
6. **Kelly, M. T., M. F. Fojtasek, T. M. Abbott, D. C. Hale, J. R. Dizikes, R. Boshard, G. E. Buck, W. J. Martin, and J. M. Matsen.** 1983. Clinical evaluation of a lysis-centrifugation technique for the detection of septicemia. J. Am. Med. Assoc. **250**:2185–2188.
7. **La Scolea, L. J., Jr., D. Dryja, and E. Neter.** 1981. Comparison of the quantitative direct plating method and the BACTEC procedure for rapid diagnosis of *Haemophilus influenzae* bacteremia in children. J. Clin. Microbiol. **14**:661–664.
8. **La Scolea, L. J., Jr., D. Dryja, T. D. Sullivan, L. Mosovich, N. Ellerstein, and E. Neter.** 1981. Diagnosis of bacteremia in children by quantitative direct plating and a radiometric procedure. J. Clin. Microbiol. **13**:478–482.
9. **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.
10. **Schell, R. F., J. L. Le Frock, J. P. Babu, and D. B. Robinson.** 1979. Recovery of *Haemophilus influenzae* from twenty-three blood culture media. J. Clin. Microbiol. **9**:84–87.
11. **Sullivan, T. D., L. J. La Scolea, Jr., and E. Neter.** 1982. Relationship between the magnitude of bacteremia in children and the clinical disease. Pediatrics **69**:699–702.
12. **Szymczak, E. G., J. T. Barr, W. A. Durbin, and D. A. Goldmann.** 1979. Evaluation of blood culture procedures in a pediatric hospital. J. Clin. Microbiol. **9**:88–92.
13. **Winchester, P. D., J. K. Todd, and M. H. Roe.** 1977. Bacteremia in hospitalized children. Am. J. Dis. Child. **131**:753–758.