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# Diagnosis of Bacteremia in Children by Quantitative Direct Plating and a Radiometric Procedure

LEONARD J. LA SCOLEA, JR.,[1,2,3]* DIANE DRYJA,[1] T. DENNIS SULLIVAN,[2] LUIS MOSOVICH,[2] NORMAN ELLERSTEIN,[2] AND ERWIN NETER[1,2,3]

*Bacteriology and Serology Laboratories, The Children's Hospital of Buffalo,[1] and Departments of Pediatrics[2] and Microbiology,[3] The State University of New York at Buffalo, Buffalo, New York 14222*

During a 1-year period, three bacteriological systems for detecting bacteremia in children were analyzed, namely, the BACTEC system (Johnston Laboratories, Inc., Cockeysville, Md.), the Fisher/Lederle bottle (Lederle Diagnostics, Pearl River, N.Y.), and a direct plating method of blood, termed quantitative direct plating (QDP). Of 2,123 blood cultures, 135 (6.4%) were positive; *Haemophilus influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae* accounted for 3.4%, representing 61 patients, other pathogens accounted for 0.6%, and contaminants accounted for 2.4%. Of 72 cultures yielding *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*, 60 were recovered by both broth systems, 2 by BACTEC only and 10 by Fisher/Lederle bottle only. The BACTEC system failed to register a positive growth index reading by 24 h in 15 cultures which were positive for *H. influenzae*, even though growth had occurred, as shown by positive subculture and microscopy at this time. QDP detected 89% of the cultures positive for *H. influenzae* and *N. meningitidis*, of which 55% yielded results before either broth procedure. Only 50% of the cultures positive for *S. pneumoniae* yielded growth on QDP. This difference in the recovery rates probably is accounted for by the number of organisms in the blood. Thus, more than 100 organisms per ml of blood were found in 71% of cultures positive for *H. influenzae* and *N. meningitidis* but in only 7% of those positive for *S. pneumoniae*. These studies, then, have revealed that *H. influenzae*, which grew well in BACTEC broth, did not, however, give a significant growth index reading during day 1 of incubation, in contrast to *N. meningitidis* and *S. pneumoniae*. The QDP system not only provided information on the magnitude of bacteremia due to *H. influenzae* and *N. meningitidis* but frequently allowed earlier diagnosis and, thus, proved to be a valuable, simple, and inexpensive supplementary technique for broth cultures, although not for the diagnosis of *S. pneumoniae* bacteremia.

Only during the last several years has automation made a significant impact in the clinical bacteriology laboratory. Among these procedures is the automated BACTEC system (Johnston Laboratories, Inc., Cockeysville, Md.) for the detection of bacteremia. This method utilizes media containing $^{14}C$-labeled substrates which, when metabolized by bacteria, yield detectable levels of $^{14}CO_2$ (7, 8). The BACTEC system has been widely used and favorably evaluated (1, 4, 6, 12, 14, 16, 17), although some shortcomings have been identified (3, 5, 13, 18). Only limited information is available on the usefulness of the BACTEC system in pediatrics, particularly regarding the speed of detecting *Haemophilus influenzae* infection, which is so common in this age group (1, 11).

With the exception of urine cultures, quantitation of bacteria in body fluids by the pour plate method has found only limited application (2, 3, 10). This report describes a technique, termed quantitative direct plating (QDP), which involves the direct plating of patient's blood on chocolate and blood agar plates.

The two major aims of the study were (i) to determine the value of the QDP technique for providing information on the magnitude of bacteremia and early diagnosis, and (ii) to evaluate the BACTEC system for the diagnosis of bacteremia in children, with particular reference to *H. influenzae*, which is prevalent in this age group.

## MATERIALS AND METHODS

**Patient population.** A total of 2,123 blood samples were analyzed at the Children's Hospital of Buffalo from patients in the Outpatient Department and Intensive Care Unit for 1 year (22 January 1979 to 22 January 1980). The selection of these two units was based on the excellent level of cooperation of the

personnel and the feasibility in obtaining adequate volumes of patient's blood.

**Specimen collection and processing.** The Outpatient Department and Intensive Care Unit were supplied with special blood culture packages consisting of one Fisher/Lederle bottle of broth (Lederle Diagnostics, Pearl River, N.Y.), one BACTEC aerobic bottle, and one QDP heparin tube, with instructions for the inoculation of the patient's blood. Equal amounts (1 to 3 ml) of blood were injected into the BACTEC and Fisher/Lederle bottles, and 0.5 to 1 ml was inoculated into the QDP heparin tube. If only 2 ml was available, then 1 ml was injected into each bottle, and the QDP tube was not used. The time elapsed between the bleeding and laboratory arrival times was usually 1 h or less.

**BACTEC, Fisher/Lederle, and QDP techniques.** The radiometric system utilized the BACTEC model 460 for semiautomated detection of microbial growth. The aerobic medium (no. 6B) contained 30 ml of enriched tryptic soy broth with 2 $\mu$Ci of $^{14}$C-labeled substrates and polyanetholesulfonate. The blood cultures were monitored for microbial activity by growth index (G.I.) readings. Readings of 30 or higher were considered positive.

The Fisher/Lederle blood culture bottle contains broth supplemented with sodium polyanetholesulfonate and *p*-aminobenzoic acid.

The QDP procedure involved the injection of 0.5 to 1 ml of blood into a sterile heparin tube. Upon arrival at the laboratory, 0.2 to 0.5 ml each was directly pipetted onto sheep blood agar and chocolate agar plates and spread with a bacteriological loop. The plates were incubated at 37°C in 5 to 10% $CO_2$ and monitored for growth. The number of bacteria per ml of blood was calculated.

**Experimental design.** The BACTEC bottles were monitored every 4 h for the first 24 h, at 36 h, and on days 2, 3, 4, and 5. Repeated readings during day 1 of incubation were in accord with published practice (5, 12, 13, 17, 18). According to the manufacturer, "repeated testing of vials on days 1 and 2 is of real value." When a positive G.I. reading was obtained during the first 24 h, subcultures and Gram and methylene blue stains were performed at that time on both the BACTEC and the corresponding Fisher/Lederle bottles. If the G.I. reading was still negative at 24 h, "blind" subcultures and microscopic examinations were performed on both BACTEC and Fisher/Lederle bottles. When the G.I. reading became positive after 24 h, subcultures and microscopy were performed on both bottles. On day 5, all negative bottles were discarded. The QDP plates were inspected everyday at 8:00 a.m. and whenever the G.I. reading of the BACTEC system became positive.

## RESULTS

During a 1-year period, three procedures for the diagnosis of bacteremia in children, with particular reference to *H. influenzae* infections, were evaluated. To this end, the BACTEC system, the Fisher/Lederle bottle, and the QDP technique were employed. The results on 2,123 blood cultures are shown in Table 1. *H. influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* were recovered from 61 patients with sufficient frequency to permit meaningful analysis. Other pathogens, representing 0.6% or 12 cultures of *Streptococcus agalactiae*, *Staphylococcus aureus*, *Salmonella* spp., and *Candida* spp. were recovered too infrequently for evaluation. Bacteria considered to be contaminants, representing 2.4%, were *Staphylococcus epidermidis*, viridans streptococci, *Corynebacterium* spp., *Micrococcus* spp., and *Bacillus* spp.

Table 1 provides information on the recovery of *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* by both broth systems. Based on microscopic examination, subcultures, and G.I. readings of the BACTEC bottles, both broth systems yielded positive results with 60 cultures, BACTEC alone yielded positive results with 2, and the Fisher/Lederle bottle alone yielded positive results with 10 additional cultures. Thus, the radiometric system detected 62 out of a total of 72 positive cultures, representing an 86% recovery rate, and the Fisher/Lederle bottle detected 70 strains, representing a 97% recovery rate.

One of the major advantages of the BACTEC system is the automated G.I. readings at repeated intervals. Therefore, as shown in Table 2, it is of particular interest to note that at up to 24 h, there was only one culture with a positive

TABLE 1. *Positive blood cultures*

| Microorganism | No. of positive cultures | | | % of total blood cultures |
|---|---|---|---|---|
| | BACTEC and Fisher/Lederle | BACTEC | Fisher/Lederle | |
| *H. influenzae* | 32 | 0 | 4 | 1.7 |
| *N. meningitidis* | 12 | 1 | 0 | 0.6 |
| *S. pneumoniae* | 16 | 1 | 6 | 1.1 |
| Other pathogens | 12 | 0 | 0 | 0.6 |
| Contaminants | 17 | 25 | 9 | 2.4 |

TABLE 2. *Correlation among G.I. readings, films, and subcultures of BACTEC and Fisher/Lederle bottles at 24 h*

| Microorganism | No. of positive cultures | | | | |
|---|---|---|---|---|---|
| | BACTEC | | | Fisher/Lederle | |
| | Positive G.I. | Positive film | Positive subculture | Positive film | Positive subculture |
| *H. influenzae* | 1 | 9 | 16 | 11 | 16 |
| *N. meningitidis* | 12 | 12 | 12 | 5 | 11 |
| *S. pneumoniae* | 16 | 16 | 16 | 13 | 16 |

G.I. reading among 16 cultures positive for *H. influenzae*. Even microscopic examination revealed the presence of numerous bacteria in 9 out of 16 cultures. Of the 15 cultures with a negative G.I. reading at 24 h, in spite of growth of the microorganisms, 11 registered a positive G.I. reading at 36 h, 2 registered positive at 48 h, and 2 registered positive at 3 days. Furthermore, the G.I. reading of 14 out of the 15 cultures did not show a sequential increase before registering a reading of 30 and above. Failure of the G.I. reading to reflect growth in the BACTEC bottle at 24 h was observed only with *H. influenzae* and not with *N. meningitidis* and *S. pneumoniae* (Table 2). In fact, the G.I. readings for the latter two microorganisms were all positive before 24 h. Because the BACTEC broth was tested repeatedly during the first 24 h of incubation, resulting in removal of radioactive $CO_2$, the question arises whether repeated testing accounts for the failure to detect *H. influenzae* by means of the G.I. Accordingly, a few experiments were carried out in which BACTEC bottles were inoculated with a strain of *H. influenzae* type b, one bottle being tested every 4 h for 48 h, and the other being tested only at 24 and 48 h. Repeated testing did not result in significant differences in G.I. values.

The QDP technique yielded particularly interesting results. A comparison of the recovery rates with the QDP technique with the BACTEC and Fisher/Lederle broth procedures is shown in Table 3. The QDP detected *H. influenzae* and *N. meningitidis* in 89% of the positive broth cultures. This result is excellent in view of the fact that less blood was utilized for the quantitative procedure than for the broth techniques. In contrast, this procedure yielded positive results in only 50% of cultures of children with *S. pneumoniae* bacteremia.

Table 4 shows the detection times by QDP in comparison with the broth procedures. As shown, QDP detected *H. influenzae* and *N. meningitidis* earlier than did the BACTEC bottle in 69 and 42%, respectively. Comparable results were obtained with the Fisher/Lederle bottles. Thus, 55% of the QDP cultures were positive for *H. influenzae* and *N. meningitidis* before both broth procedures. Of these 18 cultures, QDP was positive earlier than the broth procedures in six instances between 6 and 12 h, in nine between 13 and 24 h, and in three between 25 and 36 h. In contrast, none of the cultures positive for *S. pneumoniae* were detected by QDP before the broth cultures.

TABLE 3. *Recovery rates of the QDP procedure*

| Microorganism | Total no. of positive cultures by: | |
|---|---|---|
| | BACTEC and Fisher/Lederle | QDP (%) |
| *H. influenzae* | 23 | 19 (83) |
| *N. meningitidis* | 12 | 12 (100) |
| *S. pneumoniae* | 14 | 7 (50) |

One of the major purposes of the QDP procedure was to determine the level of bacteremia (Table 5). It is evident that 71% of *H. influenzae* and *N. meningitidis* cultures had in excess of 100 organisms per ml. This was not the case with *S. pneumoniae*; only a single culture (7%) yielded more than 100 organisms per ml, and that was from a patient with sickle cell disease. As shown in Tables 3 to 5, the low level of *S. pneumoniae* bacteremia was reflected in the lower recovery rates and later detection by the QDP procedure.

## DISCUSSION

The two major aims of the present study were the determination of the usefulness of quantitation of blood cultures by the QDP procedure and the evaluation of the automated BACTEC system for the early diagnosis of bacteremia in children, with particular reference to *H. influenzae*, an organism which is prevalent in this age group (1, 11). The data suitable for analysis involved 72 cultures representing 61 patients positive for *H. influenzae* type b, *N. meningitidis*, and *S. pneumoniae*. Of the patients with blood cultures positive for the three aforementioned bacterial species, 89% were between the ages of 6 months and 6 years.

Surprisingly, quantitation is not widely used in the study of bacteremia (3, 10). Dietzman et al. (9) reported that in patients with neonatal *Escherichia coli* septicemia, the colony counts ranged from less than 1 (e.g., 1/5 ml) to more than 1,000/ml of blood. Santosham and Moxon (15), in a limited study, found the level of *H. influenzae* bacteremia to be predominantly in excess of 100 colony-forming units per ml, in contrast to cultures of *S. pneumoniae*. Furthermore, these investigators also noted that the direct plating of blood enhanced earlier detection of *H. influenzae* bacteremia.

In the present study, the QDP procedure, which uses less blood than the BACTEC and Fisher/Lederle broth procedures, recovered 89% of the blood cultures positive for *H. influenzae* and *N. meningitidis*; importantly, 55% were detected before both broth procedures. Thus, the data indicates that the direct plating of small quantities of blood allows the early detection of *H. influenzae* and *N. meningitidis* bacteremia in a significant percentage of cases. Therefore,

TABLE 4. *Detection times of BACTEC, Fisher/Lederle, and QDP techniques*

| Microorganism | Total no. of positive cultures | % of QDP cultures determined earlier than: | | % of QDP cultures determined simultaneously with: | | % of QDP cultures determined later than: | |
|---|---|---|---|---|---|---|---|
| | | BACTEC | Fisher/Lederle | BACTEC | Fisher/Lederle | BACTEC | Fisher/Lederle |
| *H. influenzae* | 19 | 69 | 58 | 5 | 16 | 26 | 26 |
| *N. meningitidis* | 12 | 42 | 50 | 0 | 0 | 58 | 50 |
| *S. pneumoniae* | 7 | 0 | 0 | 0 | 0 | 100 | 100 |

TABLE 5. *Quantitation of microorganisms in blood*

| Microorganism | No. of cultures with CFU/ml[a] of: | | | | % in excess of 100 CFU/ml |
|---|---|---|---|---|---|
| | 0 | 1-9 | 10-100 | >100 | |
| *H. influenzae* | 4 | 1 | 1 | 17 | 74 |
| *N. meningitidis* | 0 | 0 | 4 | 8 | 67 |
| *S. pneumoniae* | 7 | 4 | 2 | 1 | 7 |

[a] CFU, Colony-forming units.

the QDP is a valuable and inexpensive supplementary technique to broth procedures for the diagnosis of *H. influenzae* and *N. meningitidis* but not of *S. pneumoniae* bacteremia. These differences are probably due to patient selection. In addition, the QDP method yields growth on solid media, permitting earlier analysis of colony morphology, growth characteristics, serological typing, biochemical tests, and antibiograms. Interestingly, contaminants were not recovered on the QDP plates, in contrast to 2.4% for the two broth systems (Table 1).

The pour plate procedure for quantitation of bacteria in body fluids has found only limited application, with the exception of urine cultures (2, 3, 10). The QDP technique has several advantages in comparison with the pour plate procedure. It is less cumbersome. The bacteria are not exposed to heat of the melted agar, which may decrease the number of surviving organisms. The QDP surface plating prevents the impairment of growth below the surface of the agar of strictly aerobic bacteria. Furthermore, surface colonies are much easier to detect and enumerate than those below the surface.

The higher the counts of organisms per milliliter of blood the more likely QDP will yield positive results. This is clearly evident from the recovery rates of *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* (Tables 3 and 5).

The QDP method was positive in five out of six cultures of *S. aureus*, *S. agalactiae*, *Salmonella* spp., and *Candida* spp. These limited data suggest that this procedure may have value also with bacterial species other than those analyzed in this report. Investigations are in progress to evaluate the efficacy of QDP with other patient populations and to determine its clinical usefulness.

The study was not designed to evaluate BACTEC versus Fisher/Lederle broth. Differences observed between the two, in part at least, may be due to the fact that the BACTEC bottles are shaken repeatedly, in contrast to the Fisher/Lederle bottles. From the data obtained, it is evident that subcultures from either broth at 24 h often yielded positive results, and it is likely that even earlier subcultures may be useful.

Only limited information is available on the value of the BACTEC system in detecting *H. influenzae* by means of G.I. readings. Reports list either very few isolates of this microorganism or none at all (1, 4-6, 8, 12-14, 16-18). Furthermore, although detection times are given, information is not available as to when the cultures became positive by subculture or microscopy. In the present study, the BACTEC system detected a total of 32 cultures positive for *H. influenzae*. However, the radiometric procedure failed to register a positive G.I. reading by 24 h in 15 (47%) of the 32 cultures, even though they were positive by subculture or microscopy at this time. Furthermore, with the exception of a single culture, the G.I. readings did not show a sequential increase before registering a positive result of 30 or higher. The reason for the early negative G.I. readings was not due to lack of growth. Rather, it is suggested that the delay was due to production of inadequate amounts of labeled $CO_2$. If this interpretation is correct, then a need exists for modification of the BACTEC medium. The failure of the BACTEC system to detect *H. influenzae* at 24 h of incubation apparently also was not due to repeated testing of the bottle and the removal of radioactive gas. In contrast to *H. influenzae*, *N. meningitidis* and *S. pneumoniae* gave positive G.I. readings before 24 h, even though the number of *H. influenzae* per milliliter of blood exceeded that of *S. pneumoniae*. The possibility exists that the delay by the BACTEC system to detect *H. influenzae* by the G.I. reading may be shared also with other fastidious bacteria. BACTEC has been evalu-

ated thus far primarily in adult populations, and blind subcultures at 3, 5, or 7 days rather than at 24 h are recommended (5, 12–14, 17, 18). However, as shown here, for pediatric populations, due to the high incidence of *H. influenzae* bacteremia, subculture and microscopy should be done no later than 24 h after procurement of the blood specimens, particularly since repeated shaking of the BACTEC bottles may preclude the recognition of growth by gross inspection.

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## LITERATURE CITED

1. **Bannatyne, R. M., and N. Harnett.** 1974. Radiometric detection of bacteremia in neonates. Appl. Microbiol. **27**:1067–1069.
2. **Barry, A. L., P. B. Smith, and M. Turck.** 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coordinating ed., T. L. Gavan. American Society for Microbiology, Washington, D.C.
3. **Bartlett, R. C., P. D. Ellner, and J. A. Washington, II.** 1974. Cumitech 1, Blood cultures. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
4. **Brooks, K., and T. Sodeman.** 1974. Rapid detection of bacteremia by a radiometric system. Am. J. Clin. Pathol. **61**:859–866.
5. **Caslow, M., P. D. Ellner, and T. E. Kiehn.** 1974. Comparison of the BACTEC system with blind subculture for the detection of bacteremia. Appl. Microbiol. **28**: 435–438.
6. **DeBlanc, H. J., F. Deland, and H. N. Wagner, Jr.** 1971. Automated radiometric detection of bacteria in 2,967 blood cultures. Appl. Microbiol. **22**:846–849.
7. **Deland, F. H., and H. N. Wagner, Jr.** 1969. Early detection of bacterial growth with carbon-14-labeled glucose. Radiology **92**:154–155.
8. **Deland, F. H., and H. N. Wagner, Jr.** 1970. Automated radiometric detection of bacterial growth in blood cultures. J. Lab. Clin. Med. **75**:529–534.
9. **Dietzman, D. E., G. W. Fischer, and F. D. Schoenknecht.** 1974. Neonatal *Escherichia coli* septicemia—bacterial counts in blood. J. Pediatr. **85**:128–130.
10. **Isenberg, H. D., J. A. Washington, II, A. Balows, and A. C. Sonnenwirth.** 1974. Collection, handling, and processing of specimens, p. 59–88. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
11. **Larson, S. M., P. Charache, M. Chen, and H. N. Wagner, Jr.** 1973. Automated detection of *Haemophilus influenzae*. Appl. Microbiol. **25**:1011–1012.
12. **Randall, E. L.** 1975. Long-term evaluation of a system for radiometric detection of bacteremia, p. 39–44. *In* D. Schlessinger (ed.), Microbiology—1975. American Society for Microbiology, Washington, D.C.
13. **Renner, E. D., L. A. Gatheridge, and J. A. Washington, II.** 1973. Evaluation of a radiometric system for detecting bacteremia. Appl. Microbiol. **26**:368–372.
14. **Rosner, R.** 1974. Comparison of macroscopic, microscopic, and radiometric examinations of clinical blood cultures in hypertonic media. Appl. Microbiol. **28**:644–646.
15. **Santosham, M., and E. R. Moxon.** 1977. Detection and quantitation of bacteremia in childhood. J. Pediatr. **91**: 719–721.
16. **Smith, A. G., and R. R. Little.** 1975. The BACTEC 225—a radiometric technique for the detection of bacteremia. Lab. Med. **6**:18–26.
17. **Thiemke, W. A., and K. Wicher.** 1975. Laboratory experience with a radiometric method for detecting bacteremia. J. Clin. Microbiol. **1**:302–308.
18. **Washington, J. A., II, and P. K. W. Yu.** 1971. Radiometric method for detection of bacteremia. Appl. Microbiol. **22**:100–101.