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Radiometric Detection of Bacteremia in Neonates

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The predicted prevalence of false positive blood cultures due to hyperactive neonatal blood cells in a radiometric detection system was confirmed. Suppression of this blood background radioactivity in the system was achieved by using a hypertonic medium containing 10% sucrose. The radiometric system produced accurate results as fast as the conventional blood culturing method, saved labor and minimized the recovery of extraneous contaminants.

The radiometric detection of bacteremia in adults by measuring the $^{14}CO_2$ resulting from bacterial metabolism of ^{14}C-labeled substrates in the blood-culture medium is faster than conventional methods and as accurate (4, 5, 9).

One of the early troubles of radiometric techniques was the occurrence of false positive results due to the metabolism of the ^{14}C-labeled substrates by the blood. Because neonatal blood cells are known to be metabolically hyperactive (2, 3, 6, 7), we predicted that the detection of bacteremia in this age group would be hindered and the usefulness of the technique would be reduced by the high incidence of false positives.

A study was undertaken to confirm these predictions and to seek possible solutions to the problem. At the same time the radiometric method was compared in terms of isolation rates, incidence of contaminants, and speed of detection with the existing blood-culturing system operative in our laboratory.

MATERIALS AND METHODS

Five hundred and fifty-nine blood cultures were performed in neonates with suspected sepsis. Since obtaining duplicate blood cultures was impractical, individual samples were processed by one or the other method on an alternating 4-day basis for a period of 7 months. All the patients were under one month of age and were nursed in the Neonatal Unit of The Hospital for Sick Children, Toronto. In most instances the site of the venipuncture was the antecubital fossa. Skin preparation consisted of thorough swabbing of the site with a 1:1,000 tincture of Benzalkonium (Winthrop Laboratories, Aurora, Ontario) chloride followed by drying with a sterile cotton ball. The blood obtained for culture varied in volume from less than 1 to 3 to 4 ml, and it was inoculated directly into the bottles through a sterile rubber diaphragm in the cap.

Conventional processing system. The laboratory's conventional blood-culture medium consisted of 80 ml of brain heart infusion broth. This was examined at 1, 2, 4, and 7 days by an open procedure. The bottle cap was unscrewed, the neck was flamed, and a loopful of fluid was removed for a Gram-stained smear and subculture to 10% horse blood agar with a *Staphylococcus aureus* streak both aerobically (with 10% CO_2) and anaerobically. The time and labor required did not permit the regular examination of cultures within the first 10 h. Extended incubation and examination for 3 weeks was reserved for selected diseases such as congenital heart disease.

Radiometric processing system. Radiometric blood-culture medium was of two sorts: (i) 6A vials (Johnston Laboratories, Cockeysville, Md.) containing 30 ml of tryptic soy broth with added hemin, vitamin K, and sodium polyanethol sulfonate; (ii) 4A vials of the same composition as 6A vials but with an added 10% sucrose. Each radiometric medium also contained ^{14}C-labeled substrates, mainly glucose, to a total activity of 1.5 μCi. A magnetic stirring bar occupied each vial. Inoculated cultures were incubated at 37 C and stirred during the first 24 h. Cultures were tested on receipt in the laboratory, at 10 h where possible, and daily for 7 days. When indicated, cultures were maintained and tested daily for 3 weeks. Testing was performed with the Bactec 301, an instrument which measures the amount of $^{14}CO_2$ evolved from the metabolism of ^{14}C-labeled substrates and released into the gas space above the culture fluid. The results are expressed visually on a panel meter printed on a scale of 0 to 100 and termed "Growth Index" (GI). A peak GI of 20 to 30 any time after inoculation is taken as a presumptive positive and a peak GI >30 as a definite positive test result. Vials giving results in this range at any time were sampled by needle aspiration of fluid through the rubber diaphragm. Smears were stained and the fluid was subcultured as described previously. All vials were similarly examined prior to discard.

For the purposes of the study a false positive result was defined as one in which a GI reading in the presumptive or definite positive range was not substantiated by smear or subculture.

RESULTS

Table 1 shows the high incidence of false positive results with the 6A vials at two differ-

ent GI break points. 4A vials, by contrast, were not subject to such aberrations. The incidence of true positive cultures is comparable irrespective of the vial used (Table 2).

The isolation rates by our routine method and by the radiometric method are displayed in Table 3. The increased isolation rate by the routine blood-culture method is largely accounted for by the higher incidence of *Staphylococcus epidermidis* (Table 4). Many of these represent extraneous contaminants. Isolation rates for recognized neonatal pathogens are equivalent or minimally better by the radiometric method. Speed of detection of positive cultures is set out in Table 5.

Of the 21 positive cultures in the radiometric series, it was possible to test 16 within the first 12 h and 4 of these (25%) were positive. At 24 h, detection rates between the two systems are virtually identical, but at 48 h they favor the radiometric method.

DISCUSSION

The predicted prevalence of false positive blood cultures from neonates with Bactec medium 6A in the radiometric system was confirmed. A hypertonic medium (Bactec 4A), containing 10% sucrose and designed originally as an osmotic stabilizer to promote the recovery of cell wall-damaged organisms (8, 10), successfully suppressed blood background radioactivity and eliminated false positive results. This was achieved without any apparent reduction in the incidence of true positives. Radiometric detection of bacteremia in neonates is therefore feasible.

The present study also endorses the advantages claimed by other investigators for the radiometric system over standard methods (4, 5). It is accurate and detects a broad range of bacteria. Because it is simple to operate and fast, considerable technician time is saved by the elimination of the routine subculturing of negative specimens. The time thus saved can profitably be used in retesting samples within the first 12 h, a desirable improvement which present conventional methods preclude. In this way the speed of detection is accelerated and the clinician is provided with important information at the earliest opportunity.

Because the system is closed, laboratory contamination is minimized. In the age group studied this is particularly appreciated by the clinicians since the common contaminant *Staphylococcus epidermidis* is sometimes pathogenic in neonates. (1) In the present study, except in one instance where *S. epidermidis* was recovered from the same patient on two separate occasions, all other isolations of this organism represent single isolations from individual patients, some of whom were cultured many times. In no case were these isolates considered to be clinically significant.

TABLE 1. *False positive blood cultures by radiometric method*

Vials	Peak GI > 20	Peak GI > 30
6A (100)	67	15
4A (100)	0	0

TABLE 2. *True positive blood cultures*

Radiometric method	
6A Vials (100)	12
4A Vials (100)	9

TABLE 3. *Neonatal blood cultures*

Method	Negative	Positive	Total
Routine	306	52 (17%)	358
Radiometric	180	21 (11.6%)	201

TABLE 4. *Positive blood cultures*

Organism	Routine (52)	Radiometric (21)
E. coli	6	4
P. aeruginosa	1	1
P. mirabilis	1	0
Citrobacter species	1	0
K. aerogenes	3	0
Enterococcus	1	1
Group B streptococcus	2	2
Pneumococcus	2	1
H. influenzae	0	1
S. aureus	3	2
C. albicans	0	1
Acinetobacter/Alcaligenes species	0	3
Bacillus species	2	0
Diphtheroid	5	0
S. epidermidis	25	7

TABLE 5. *Speed of detection of positive blood cultures*

Method	12 h (%)	24 h (%)	48 h (%)
Routine		46	73
Radiometric	25 (4/16)	48	86

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