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Automated Radiometric Detection of Bacteria in 2,967 Blood Cultures

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A new radiometric method for the automatic detection of bacterial growth in blood cultures has been compared with conventional methods. A total of 2,967 cultures from 1,280 patients suspected of having bacteremia were studied. A 2-ml amount of blood was inoculated into culture media in which the glucose was labeled with carbon-14. The release of $^{14}CO_2$ by bacterial metabolism was checked hourly for 18 to 24 hr, daily for the next 2 days, and, on the 12th day, with an automated instrument. A 10-ml amount of blood was studied by conventional bacteriological techniques. In 125 cultures from 50 patients, there was bacterial growth in at least one of the routine media. Of these, the radiometric method detected 102 cultures from 40 patients. In 111 cultures from 48 patients, there was radiometric detection of bacterial growth. In all of these cultures, there was detection of bacterial growth in subcultures from the radioactive medium. Of these, the routine laboratory detected 98 cultures from 40 patients. Neither method detected all patients with bacteremia. Among the 57 patients positive by one or both methods, routine techniques detected bacteria in 87% and the radiometric method detected bacteria in 85%. Seventy per cent of the cultures were detected first by the radiometric method, 65% on the day of inoculation. Our results suggest that the radiometric method is faster than conventional techniques and comparable in accuracy. Its great advantage is that it is simple, automatic, and can be extended to automatic detection of bacterial sensitivity to antibiotics.

Current methods for detection of bacteria in blood cultures consist of visual and microscopic examination of culture media with subsequent subculturing when indicated. In most cases, detection is not possible for 18 to 24 hr. Cultures frequently require daily handling by technicians over a 12- to 14-day period before being discarded as negative. A previous report (1) describes our automated radiometric method for the detection of bacterial growth. The method is based upon the formation of radioactive CO_2 from ^{14}C-glucose by bacterial metabolism (2, 3). The present report describes the use of an improved instrument employing this technique in 2,967 blood cultures from patients at Johns Hopkins Hospital.

MATERIALS AND METHODS

The automated instrument (Bactec bacterial growth detector, Johnston Laboratories, Cockeysville, Md.) handles 25 samples simultaneously. It measures the amount of radioactive CO_2 generated by the bacterial metabolism of ^{14}C-glucose. Specimens to be tested are inserted into a circular holding tray. The tray is placed onto a revolving core in the center of an enclosed incubation chamber that is kept at a constant temperature of 37 C. As the tray revolves during a sampling cycle, each culture bottle is placed beneath an intense ultraviolet (UV) light for 70 sec. Immediately after UV irradiation, the vial moves into position underneath two sampling needles. The needles descend and penetrate through the rubber stopper into the air above the culture broth within the vial. Approximately 200 ml of filtered air is used to flush the air of the culture bottle into an ionization chamber. This process removes $^{14}CO_2$ that has been formed by bacterial metabolism since the last sampling.

Release of the $^{14}CO_2$ from the liquid broth into the air above is facilitated by the constant stirring action of a magnetic rod. The radioactivity present in the air of each sampling is measured by the ionization chamber, which produces an electrical current directly proportional to the amount of radioactivity detected. The electrical potential is converted to a digital index between 0 and 99 and is simultaneously printed on paper tape for a permanent record. The measurement cycle for each sampling requires 72 sec or 30 min for one full revolution of the holding tray containing 25 vials. A culture was considered positive for bacterial growth when the digital index reached a level of 20, which was at least twice the room background.

For each study, 8 ml of the patient's blood was placed into a flask containing 70 ml of Trypticase soy

broth (no. 11768 Trypticase soy broth, BBL), 2 ml was placed into a tube containing 20 ml of thioglycollate broth (no. 11720 fluid thioglycollate medium, BBL), and 2 ml was placed into a sealed vial (Bactec culture vial, Johnston Laboratories) containing 30 ml of glucose-free Trypticase soy broth, 0.5 μCi of ^{14}C-glucose (uniformly labeled), and a magnetic stirring rod. The cultures in the flask and tube were handled by personnel in the clinical microbiology laboratory according to the following protocol. After incubation for 12 to 18 hr, material from the flask was examined microscopically and subcultured onto chocolate-agar. Cultures were examined daily, and negative samples were again subcultured onto chocolate-agar at 10 days. A new tube containing thioglycolate broth was inoculated from the original one. Cultures were kept for 12 days before being discarded as negative.

In the automated radiometric system, cultures were automatically tested at hourly intervals for 18 to 20 hr and retested once on the 2nd, 3rd, and 12th days. Subcultures from the vials onto routine media were made on all cultures that were positive by either the routine or the automatic technique.

RESULTS

Of the 2,967 blood cultures, 138 from 57 patients were positive by one or both methods. A total of 111 cultures from 48 patients were positive radiometrically, and 125 cultures from 50 patients were positive by routine bacteriological testing. Table 1 lists the organisms detected radiometrically, and Table 2 lists those detected by routine testing.

Among the 111 cultures positive by the radiometric method, 98 from 40 patients were positive and 13 from 8 patients were negative in the routine laboratory. All of the 111 cultures were positive on subculture from the radiometric vials.

TABLE 1. *Organisms detected by the radiometric method*

Organism	No. of samples	No. of patients
Pneumococcus	34	15
Staphylococcus aureus	23	9
Escherichia coli	12	4
Klebsiella	10	6
Beta streptococcus	6	3
Gonococcus	2	1
Pseudomonas	1	1
Haemophilus	1	1
S. epidermidis	2	1
S. aureus and *Klebsiella*	4	2
S. aureus and beta streptococcus	2	1
Proteus and *Streptococcus faecalis*	3	1
Yeast	11	3
Total	111	48

TABLE 2. *Organisms detected by routine techniques*

Organism	No. of samples	No. of patients
Pneumococcus	32	14
Staphylococcus aureus	20	7
Escherichia coli	15	5
Klebsiella	11	7
Beta streptococcus	5	2
Gonococcus	3	1
Pseudomonas	1	1
S. epidermidis	2	1
Streptococcus faecalis	1	1
Microaerophilic alpha streptococcus	8	1
S. aureus and *Klebsiella*	4	2
S. aureus and beta streptococcus	4	2
Proteus and *S. faecalis*	3	1
Alpha streptococcus	3	1
Gamma streptococcus	2	1
Yeast	11	3
Total	125	50

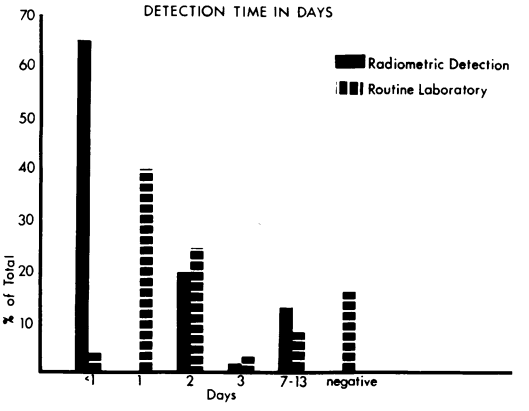

FIG. 1. *Time required for detection of bacterial growth by the radiometric and routine methods.*

One of the organisms detected by the radiometric method, but negative in the routine laboratory, was a wall-defective streptococcus confirmed by special culture techniques.

Among the 125 cultures positive in the routine laboratory, 102 were positive and 23 were negative by the radiometric method. The 23 negative cultures fall into two groups. In one group (11 cultures), both the radiometric results and the subculture from the radiometric vial were negative. Since there were no viable bacteria in the radioactive medium at the time of subculture, these disparities possibly represent sampling errors, as the volume of blood used with the con-

TABLE 3. *Results from simulated cultures of Escherichia coli and Pseudomonas aeruginosa in Trypticase soy broth*

Organism	Inoculum (organisms/ml)			
	10^1	10^2	10^3	10^4
Escherichia coli	8.1[a] (0.8)[b]	6.7 (0.8)	5.7 (0.8)	4.2 (0.6)
Pseudomonas aeruginosa	15.8 (1.0)	14.5 (1.2)	13.1 (0.9)	11.3 (0.8)

[a] Average number of hours after inoculation required for detection.
[b] Numbers in parentheses show one standard deviation.

ventional method (10 ml) was five times larger than that tested radiometrically (2 ml). In the other group (12 cultures), radiometric detection was negative, but the subcultures from the radiometric vial were positive. Eight of these cultures were from one patient, who had 18 blood samples tested, of which 8 were positive by conventional methods. The organism in all eight of these was a microaerophilic alpha streptococcus. Failure of the organism to metabolize glucose and release $^{14}CO_2$ was probably related to purely aerobic culture conditions. The organism grew only after transfer to more suitable conditions for growth (chocolate-agar under 10% CO_2). Among the other four cultures in this group, one contained a gonococcus from a patient who had two other samples containing gonococci that were radiometrically positive; another was a pneumococcus from a patient who had one other pneumococcus that was detected radiometrically. Two others were from another patient; each contained both *Staphylococcus aureus* and beta streptococcus.

The radiometric technique was faster than conventional techniques for the detection of bacteremia. Seventy per cent of the cultures were detected first by the radiometric method, 65% on the day of inoculation. Twenty-four per cent required the same time for detection by both methods. Six per cent were detected first by the routine method, 4% on the day of inoculation. Figure 1 compares the times in days required for detection by the two methods.

A total of 57 patients were found to have bacteremia by one or both methods, the radiometric and the conventional. Neither method detected bacteremia in all patients. The routine techniques were positive in 40 of 48 patients (83%) in whom bacteria were detected by the radiometric method. The radiometric method was positive in 40 of 50 patients (80%) positive by the routine techniques. Among the 57 patients positive by one or both methods, 48 (85%) were positive with the radiometric method and 50 (87%) were positive in the routine laboratory.

DISCUSSION

Our results seem to be in conflict with a recently published report by Washington and Yu (4). On the basis of their results from 59 simulated blood cultures and 65 blood cultures from patients with suspected bacteremia, they concluded that the radiometric method lacked the sensitivity required for a suitable alternative to conventional techniques. Our results in nearly 3,000 blood cultures indicate that our radiometric method is faster than conventional techniques for the detection of bacteremia and comparable in sensitivity. In our study, neither the conventional or radiometric method detected all patients with bacteremia. The advantages of the radiometric method are that it is simple, automatic, and can be extended to automated measurement of bacterial sensitivity to antibiotics.

The apparent discrepancy between our results and those of Washington and Yu may be explained as follows. In 59 simulated blood cultures containing between 4 and 4,250 bacterial organisms per ml, Washington and Yu were unable to detect bacterial growth within 6 hr after inoculation. At their next testing interval, 18 hr after inoculation, they detected growth in all of 13 cultures containing *S. aureus*, all of 14 cultures containing *Escherichia coli*, 13 of 17 cultures containing *Pseudomonas aeruginosa*, and 10 of 15 cultures containing group D streptococcus.

In a preliminary evaluation of our system, we tested hundreds of simulated cultures, with different-sized inocula and different bacterial species. Cultures containing *E. coli* were among those detected fastest. Those containing *P. aeruginosa* required the longest times for detection. Table 3 shows the average times required for detection of growth after inoculation of simulated cultures containing these two species. The sizes of the inocula are comparable to those used by Washington and Yu in their simulated blood cultures. These data suggest that our system is more sensitive and detects bacterial growth more rapidly.

The techniques used by Washington and Yu differ from those employed in our study. They

incubated their cultures on a rotary shaker. In our system, each culture is constantly stirred with a magnetic stirring rod. We have evidence that the method of agitation of the culture broth during incubation is critical for certain bacterial species. In experiments with *P. aeruginosa*, we found detection times to be considerably prolonged with the use of a smaller magnetic stirring bar that produced an active but very irregular stirring motion, as compared to the stirring produced by the magnetic bar we are currently using.

Washington and Yu monitored their cultures for the presence of radioactivity at 2, 4, 6, 18, 48, and 72 hr. In our system, each culture is automatically sampled hourly for the first 18 to 20 hr. It is possible that they would have detected growth in many of their simulated cultures before 18 hr had they monitored the samples hourly.

Washington and Yu obtained negative results in samples from patients with bacteremia caused by *P. aeruginosa* and group D streptococcus. Their other false-negative results were from samples containing *Bacteroides fragilis*, an anaerobe. In their study and in our system, only aerobic conditions were used in the radiometric method. To detect anaerobic and microaerophilic organisms, we are currently employing 8% carbon dioxide with and without anaerobic conditions instead of room air. We are also evaluating the use of substrates other than ^{14}C-glucose for the detection of unusual organisms that do not metabolize glucose.

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