Detection of Bacteremia by an Automated Radiometric Method and a Tubed Broth Method

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ABSTRACT

As the result of a comparative study, it was found that a radiometric method utilizing an aerobic and an anaerobic vial was much more efficient than a tubed broth method in detecting bacteremia. The radiometric method detected 97 (92.4 percent) of 105 isolants as compared to 58 (55.2 percent) for the tubed broth method. Forty-seven (44.8 percent) of the isolants were detected only by the former system, whereas 50 isolants (47.6 percent) were detected by both systems. Of the media for the radiometric method, the aerobic vial detected 84 (91.3 percent) of 92 isolants detected by the combination of aerobic and anaerobic vials. The anaerobic vial detected 46 (50.0 percent) of these isolants. Strains of Escherichia coli, coagulase positive Staphylococcus and Pseudomonas sp. were much more efficiently isolated by the radiometric method, which also presented better isolation rates for fastidious organisms such as Diplococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae, the latter in the absence of added V factor in the media. Serious deficiencies of the radiometric method were the occurrence of a high rate of false positive results and occasional false negative results. These situations prevented the realization in a clinical environment of the method's potential efficiency in detecting bacteremia.

Introduction

The detection of bacteremia is a most important function of the clinical microbiology laboratory. Barring contaminants, any isolant cultured from the blood of an ill patient is potentially significant in making a definitive diagnosis. Therefore, the efficiency of the technique used in culturing blood specimens should be of primary concern; the incidence of recovery should be consistent with clinical expectations and experience, and recovery should be rapid. Conventionally, a blood specimen is added to a broth medium in a ratio of at least 1:10, in order to help counteract through dilution the natural bactericidal action of fresh blood and to increase the probability of recovering anaerobes, a medium such as thioglycollate broth is inoculated in parallel. The chances of detection are facilitated further if an anticoagulant is added to the specimen or is present in the culture
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medium. Currently, sodium polyanethol sulfonate* is the anticoagulant of choice, being less toxic for bacteria than citrate and other anticoagulants.4,7,8,12,13 Extant are commercial systems using large vials containing broths fortified with Liquoid, CO₂ and other additives, such as 10 percent sucrose. Some of these systems are under vacuum and provide for the direct inoculation of the patient's specimen into the culture medium. They increase the probability of recovering microorganisms by providing for a larger quantity of specimen. The pour plate technique is the least efficient method of recovering organisms from the bacteremic state.8,10

A recent advance in blood culturing utilizes a radiometric technique for detecting bacterial growth through the liberation of ¹⁴CO₂ by the bacterial metabolism of carbon 14-labeled glucose and other substrates in broth media.2,14 The technique has been shown to be sensitive, rapid, readily adapted to automation,¹⁵ and comparable in accuracy, if not more accurate, than conventional techniques.⁸,¹⁰,¹¹

This report presents the results of a comparative study in which a radiometric technique was evaluated clinically against a tubed broth method for the detection of bacteremia. The study is concerned with 1194 matched blood specimens cultured over a period of four months.

Materials and Methods

The radiometric technique was performed using the Bactec 225,* an automated instrument provided with a removable circular tray which contains receptacles for 25 culture vials. The testing cycle is begun with ultra-violet irradiation of the rubber septum of the first vial in the tray, after which the tray is rotated so that the vial is in position beneath twin heat-sterilized needles; these descend and penetrate into the atmosphere above the broth culture, which has been stirred constantly while in the instrument through the action of a contained magnetic stirring bar. The stirring bar helps release ¹⁴CO₂ from the culture. Through one needle filtered air containing 10 percent CO₂ is drawn into the vial, flushing the vial's atmosphere with its newly formed content of ¹⁴CO₂ out through the other needle into an ionization chamber. The air in the chamber is ionized by the ¹⁴CO₂ and produces an electric current directly proportional to the amount of radioactivity present. Following amplification, the electrical potential is converted to digital form to register between 0 and 98. There is both a read-out and a print-out of this “growth index.” A reading above 30 is considered indicative of bacterial growth. While a given vial is being so sampled, the next in line is being subjected to ultraviolet irradiation. The test cycle takes 20 minutes to complete and can be set to repeat at one, two or three hour intervals. Positive vials are identified additionally by a red light on a positive display panel marking their position in the tray. The positive vials are sampled aseptically by syringe and needle and subcultured to blood agar and chocolate agar plates, which are then placed in a CO₂ incubator; a drop of the culture is also smeared and Gram-stained.

Specimens were run in duplicate in an anaerobic vial which contained a preduced medium with ¹⁴C-labeled substrates, but no stirring bar. The composition of the gas for flushing these vials was 85 percent N₂, 10 percent CO₂ and 5 percent H₂. The H₂ was present for the catalytic removal of any traces of O₂ which might be present in the cylinder gas mixture. Aerobic vials were placed in the Bactec immediately upon receipt, or as soon thereafter as daily testing schedules would allow. On the first day, test periods were made at two hour intervals; on the second day test periods

* Liquoid from Hoffmann-LaRoche.
* Johnston Laboratories, Inc., Cockeysville, MD 21030.
TABLE I

COMPARATIVE SYSTEMS RESULTS: MATCHED CULTURALLY POSITIVE BLOOD SPECIMENS

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total Isolants</th>
<th>Number Detected By</th>
<th>Bactec No.</th>
<th>VCT No.</th>
<th>Both Systems Only</th>
<th>Only</th>
<th>Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>22</td>
<td>20.9</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Coagulase Staphylococcus</td>
<td>13</td>
<td>12.4</td>
<td>6</td>
<td>7</td>
<td>0</td>
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<td></td>
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<tr>
<td>Pseudomonas sp.</td>
<td>11</td>
<td>10.5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Candida albicans</td>
<td>9</td>
<td>8.5</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>8</td>
<td>7.6</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diplorococcus pneumonia</td>
<td>8</td>
<td>7.6</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>6</td>
<td>5.7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>6</td>
<td>5.7</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-Streptococcus pneumonia</td>
<td>5</td>
<td>4.8</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Proteus mirabilis</td>
<td>3</td>
<td>2.9</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td></td>
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<tr>
<td>Enterobacter aerogenes</td>
<td>3</td>
<td>2.9</td>
<td>1</td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>staphylococcus haemolyticus</td>
<td>3</td>
<td>2.9</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>2</td>
<td>1.9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>staphylococcus Group A</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>staphylococcus not A or D</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>γ-Streptococcus pneumonia</td>
<td>1</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Kima polymorpha</td>
<td>3</td>
<td>0.9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Herellea vagincola</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium paraputrificum</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>105</td>
<td>99.7</td>
<td>50</td>
<td>47</td>
<td>8</td>
<td></td>
<td></td>
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</table>

Categories | Number | Percent |
---|--------|---------|
Bactec, total isolants | 97 | 92.4 |
VCT, total isolants | 58 | 55.2 |
Bactec positive, VCT negative | 47 | 44.6 |
Bactec positive, VCT positive | 50 | 47.6 |
Bactec negative, VCT positive | 8 | 7.6 |

were at three hours; thereafter there were daily testings through the seventh day. Inasmuch as experience was being gained with an unfamiliar system, cultures were tested and cultured again on the 14th day before being discarded as bacteriologically negative. Anaerobic vials were tested for the first time two days following receipt. Thereafter, their testing schedule paralleled that for the aerobic vials.

The tubed broth method was performed using the Vacutainer Culture Tube. In this system a tube, containing 18 ml of a highly nutritive broth medium which has been flushed with 10 percent CO₂ in N₂, is under sufficient vacuum to draw two ml of blood by venipuncture when used in the prescribed manner. For the purpose of this comparative study, participating clinicians were directed to draw eight ml of blood, dispensing two ml into a VCT, three ml into a Bactec anaerobic vial and three ml into a Bactec aerobic vial. Participants were cautioned to perform a thorough skin preparation at the site of venipuncture and to disinfect stopper surfaces prior to inoculation of media. The volumes inoculated resulted in a 1:10 dilution of specimen in each case. Upon delivery to the laboratory, the VCT was fitted with a venting unit. The procedure for evaluating the VCT system was that in routine use, i.e., after overnight incubation, a sample was withdrawn for Gram-staining and subculturing onto a blood agar plate and a chocolate agar plate; incubation was in a CO₂ incubator. Thereafter, VCTs were inspected for turbidity on a daily basis for five days. When staining indicated a positive culture while the aerobically incubated plates were negative, repeat subcultures were incubated in the Gaspak anaerobic system.† On the fifth day, all apparent negatives were Gram-stained. Those with positive smears were subcultured as described in this paper; the remainder were discarded.

Results

The data in table I reveal on the basis of comparative systems results that the radiometric technique was much more efficient in detecting bacteremia than the tubed broth technique. Of 105 isolants, the Bactec detected 97 (92.4 percent); the VCT detected 58 (55.2 percent). Forty-seven (44.8 percent) of the isolants were (VCT, Becton-Dickinson, Rutherford, NJ 07070. † BBL-Bioquest, Cockeysville, MD 21030.)
detected by the Bactec only. Fifty isolants (47.6 percent) were detected by both systems. In only eight instances (7.6 percent) did the VCT detect bacteremia while the Bactec was negative; in two of these instances, either one or the other Bactec vial inadvertently had not been utilized. Of the Bactec media, the aerobic vial detected 84 (91.3 percent) of 92 isolants detected by the combination of aerobic and anaerobic vials. The anaerobic vial detected 46 (50 percent) of these isolants. In eight cases (8.7 percent) only the anaerobic vial was positive.

The commonest isolant was *Escherichia coli*; yet, surprisingly, only seven of the 22 strains were detected by the VCT. Likewise, only six of 13 strains of coagulase positive *Staphylococcus* and six of 11 *Pseudomonas* strains were detected by this system. In no instance where more than one isolant of an organism was detected did the VCT system recover more isolants than the Bactec system. Also, the latter system detected 100 percent of strains in 12 of 19 species recovered, whereas the VCT system detected 100 percent of only 4 species. It is of interest that the VCT missed one of two isolations of *Neisseria meningitidis*, one of three isolations of *Haemophilus influenzae* and the single anaerobic isolant, *Clostridium paraputrificum*.

In table II are compared the Bactec and VCT systems in the 54 matched culturally positive patients from whom were obtained the 105 isolants considered in table I. If any specimen from a patient was culturally positive by a given system, the system was rated positive for that patient. Encountered examples of this application were: in the case of patient A, one of three specimens were positive by VCT; patient B, one of three; patient C, one of four. All 10 specimens were positive by Bactec. The isolants were *E. coli*. On this basis the comparative efficiency of the Bactec system dropped slightly; that of the VCT increased by nearly 10 percent. There were nine patients for whom multiple specimens by the VCT remained negative: one had three specimens over four days; the Bactec detected *Candida albicans* in all; another had five specimens over three days; all were positive by Bactec for a coagulase positive *Staphylococcus*; the remaining seven had two specimens each, all positive by Bactec. For one patient, the opposite situation occurred: the VCT detected *Diplococcus pneumoniae* in one of two specimens, whereas the Bactec was negative in both.

A disturbing result was the incidence of false positives by the Bactec, i.e., a growth index reading above 30, but in the absence of bacterial metabolism, the $^{14}$CO$_2$ being evolved by some other process. The false positives outnumbered the true culturally positive specimens by 106 to 95. Of greater importance was the occurrence of false negatives; these involved a growth index reading of below 30 on the Bactec, followed by a subsequent positive isolation. Four of five specimens from one patient fell into this category; the isolant was an *Enterococcus*. From another patient one of five specimens was a false negative; all the specimens yielded *Candida albicans*. From a third patient a *Beta Streptococcus* (no. 15, table I) was cultured from the anaerobic vial of a specimen reading under 30, while the aerobic vial was both radiometrically and culturally negative. The total positive specimens missed by each system was in close agreement with the total iso-
lants missed as presented in table I, the data reaffirming the increased efficiency of the radiometric technique over the tubed broth technique.

**Discussion**

The disparity in results between the Bactec and VCT systems is due in part to the difference in blood specimen volumes tested. In the former, a total of six ml are processed, with three ml being present in each testing unit. This is 50 percent more specimen per unit than is present in the VCT system, which processes two ml. Overall the Bactec system processes three times as much specimen. On this basis alone, with other conditions being equal, the Bactec System would be expected to have a higher detection rate, especially where the bacteremia was of a low order, e.g., less than one organism per two ml of specimen. The random distribution of organisms in bacteremias of low order helps to explain some of the comparative systems results presented in table I. One of the organisms, *Diplococcus pneumoniae*, was never isolated under anaerobic conditions, and only four of 11 strains of *Pseudomonas* were detected in the anaerobic vial. However, an explanation based on something other than "volume of specimen" is needed to account for the Bactec system's detection superiority over the VCT, which amounted to a 37.2 percent increase in the total isolant detection rate. According to the data in table I, the VCT system is quite inadequate for the detection of some of the commoner etiologic agents of bacteremia, e.g., *E. coli*, coagulase positive *Staphylococcus* and *Pseudomonas* sp. What this inadequacy is remains to be determined, inasmuch as the VCT supplemented broth medium is far more complex in its formulation than what has been published for the Bactec media.

The isolation of *H. influenzae* by Bactec is of especial interest because of the absence of the V factor (pyridine nucleotide) in its medium; X factor (heme) is present. *H. influenzae* has been isolated from additional patients since the compilation of the above data (see addendum). It would seem as if the organism's requirement for V factor is adequately supplied by the patient's specimen. This result is in contrast to those obtained by Larson, et al who, with their simulated cultures in the absence of blood, recommended that both X and V factors be added to the radiometric medium when the detection of *Haemophilus* species is desired.

**False Positive**

The occurrence of false positives to such a great extent in the Bactec system is unfortunate in that it leads to a considerable waste of time and materials in the laboratory and raises false hopes among clinicians regarding a diagnosis. It is true that the radiometric profile of the false positive is, in many cases, readily recognized by the experienced technologists, but one cannot wait the several days necessary for its development before reaching a decision on subculturing; one must subculture as soon as the critical growth index is reached. The etiology of the false positive has not been diagnosed, and patients whose blood specimens give rise to this phenomenon have as yet not been shown to have anything in common in their clinical background.

**False Negative**

Of far less occurrence, but of greater importance, is the category of the false negative. Three of 54 culturally positive patients (5.5 percent) presented specimens which were radiometrically negative. This would seem to require that the spectrum of 14C-labeled substrates in Bactec media must be broadened to accommodate those strains of organisms which cannot produce 14CO2 from the present array of labeled compounds. In any event, the phenomena
of false positive and false negative situations requires that every blood culture must now be subcultured before being discarded as culturally negative regardless of its radiometric reading. Until these situations have been eliminated, radiometric read-outs cannot be accepted with complete confidence in the clinical laboratory environment, and certain advertised claims regarding the benefits of this automated system remain ideals yet to be achieved. Deland and Wagner reported finding no false positives nor false negatives in their series of 500 blood specimens containing 30 which were culturally positive. However, DeBlanc et al did report 12 false negative specimens in their series from 57 culturally positive patients.

Polymicrobial bacteremia is rather rarely reported; however, surveys indicate the occurrence is between 6 and 10 percent. Of the uncontaminated positive specimens in this report, 10.53 percent were polymicrobial with the same mixture of organisms being isolated from three patients in multiple specimens. This would tend to rule out contamination as an important factor in recovering more than one type organism from blood cultures. In the present series, the VCT was negative in two of 10 mixed infections, both from one patient. The organisms were E. coli and alpha Streptococcus, both poorly detected by the VCT system according to information in table I.

The contamination rate of 21 percent of culturally positive specimens in the present series was regrettably high. In addition to the usual causes of contamination which plague blood culturing, i.e., inadequate skin preparation at the venipuncture site and subsequent palpation of the prepared site by contaminated fingers, there is with the Bactec system the added possibility of contaminating the specimen when it is inoculated through a vial stopper whose surface is contaminated, and the analogous situation when the twin needles penetrate through a contaminated stopper prior to sampling the gas in a vial.

What the ultimate system will be that might detect all bacteremias, if this is possible, is an academic question at this time. Nevertheless, despite its present shortcomings in the form of false positive and false negative results, the Bactec, because of its speed and efficiency of detection, simplicity of operation and state of automation, would appear to be the preferred system for those clinical laboratories having a heavy blood culture load, i.e., 200 or more specimens per week. However, such laboratories would not be able to take advantage of the instrument's capabilities for continuous monitoring; e.g., on a one hour test cycle, one could theoretically continuously monitor a maximum of 75 vials; on a two hour cycle, 150 vials; on a three hour cycle, 225 vials. Even the three hour cycle is impractical when more than 200 vials are to be read, since the theoretical limitation is 600 readings per eight hour day, and these must be divided between aerobic and anaerobic monitorings. A laboratory receiving an average of 30 specimens per day and on a seven-day discard schedule, reading the aerobic daily and the anaerobic vials on days two through seven, would average 390 readings per day, a task that would require 5.2 hours at the maximum rate of 75 tests per hour. Hence, a laboratory processing more than 200 specimens per week would have to be content with one test per working day per vial, inasmuch as the technologist in charge must have time to continue on with the implied diagnostic challenges of the radiometrically positive specimens. The foregoing rationale would not apply to those clinical laboratories blessed with more than one shift per day of competent medical technologists. Here, more frequent monitorings would be possible and desirable, especially of those specimens acquired within 24 hours, inasmuch as the purpose
### Addendum

The etiologies of 174 bacteremias among 1857 additional consecutive specimens following the comparative study are summarized in Table III. The specimens were processed exclusively by the Bactec 225 and covered a period of 2.5 months. Precisely 9.37 percent of the specimens were culturally positive, exclusive of those judged to be contaminated, compared to the 7.95 percent positive rate recorded in the evaluation study. The results were interesting from several view points. The system continued to be effective in recovering a wide range of organisms, including fastidious, e.g., *H. influenzae*, as well as nonfastidious, anaerobic, e.g., *Bacteroides fragilis*, as well as aerobic, and those reported only rarely as etiologic agents of bacteremia, e.g., the *Lactobacillus*, No. 15 in the “singly infected” column. This strain was repeatedly isolated from one patient and, hence, could not be judged a contaminant.

The number and spectrum of polymicrobial specimens was unexpected. Their etiologies were similar in many cases to those recorded by Hermans and Washington. Of 18 polymicrobial etiologies recorded in Table III, 13 (72.2 percent) involved members of the *Enterobacteriaceae*. Of 26 poly-
microbial specimens, *E. coli* was a member in 12 (46.2 percent); *K. pneumoniae* in 9 (34.6 percent); *Enterococci* in 7 (26.9 percent); and *Enterobacter* spp. in 6 (23.1 percent). The commonest mixture was comprised of *D. pneumoniae* plus *K. pneumoniae* and occurred in 5 specimens (19.2 percent).

**References**