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Influence of a Blood Culture Inoculation Technique on Detection of Bacteremia by the BACTEC System

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A 2-year study compared the influence of blood culture inoculation technique on the detection of bacteremia by an automated radiometric system (BACTEC; Johnston Laboratories, Inc.). A total of 4,690 specimens (20 ml each) were collected. Of each sample, 10 ml was inoculated into a pair of Bactec bottles at the bedside (BACTEC system). The remaining 10 ml was placed in an evacuated blood collection tube (VACUTAINER; Becton Dickinson VACUTAINER Systems) and transported to the laboratory for subsequent inoculation into an identical set of vials (VACUTAINER-BACTEC system). A total of 309 cultures grew organisms considered to be clinically significant. The recovery rate, time to positivity, and spectrum of isolates were similar for the two methods. There were substantially more sporeforming "contaminants" isolated in the VACUTAINER-BACTEC system.

Blood culture specimens may be inoculated directly into media at the bedside or transported in an anticoagulant for later inoculation in a laboratory. The introduction of the evacuated blood collection tube (VACUTAINER; Becton Dickinson VACUTAINER Systems) considerably simplified the latter procedure and popularized its use among blood-drawing teams. To the best of our knowledge, however, there have been no controlled-perspective studies comparing the bacterial recovery rates of immediate versus delayed medium inoculation. This study was designed specifically for this purpose.

Blood (20 ml) was collected with a syringe and needle by ward physicians from patients with suspected bacteremia. When possible, three specimens were collected at 30- to 60-min intervals before the administration of antibiotic therapy. Of each specimen, 10 ml was injected into a sterile VACUTAINER blood collection tube. The remaining 10 ml was equally divided and added to 30-ml aerobic (6B) and anaerobic (7B/7C) tryptic soy broth BACTEC bottles (Johnston Laboratories, Inc.). All three containers were inverted several times to mix and sent to the laboratory within 30 min.

In the laboratory, the contents of the VACUTAINER tube were aspirated, measured, and injected into a second set of BACTEC bottles. All four BACTEC bottles were then incubated at 35°C for 8 days. Aerobic vials were shaken for 24 to 36 h. Routine final subcultures of aerobic vials to a chocolate blood agar plate, incubated at 35°C for 48 h in 7% CO_2 and 75% humidity, and anaerobic vials to a prereduced Columbia blood agar plate, incubated at 35°C for 48 h in a GasPak (BBL Microbiology Systems) jar, were done on day 7.

The bottles were tested for growth radiometrically on a BACTEC 460 instrument (Johnston). The headspace replacement gases were 5% CO_2 in air for the aerobic vials and 5% CO_2, 10% H_2, and 85% nitrogen for the anaerobic vials. The aerobic bottles were tested each morning and afternoon for the first 48 h and daily for the next 4 days. The anaerobic bottles were tested daily for 4 days beginning on day 3. The anaerobic bottles were tested once again on day 8.

Bottles having growth index values exceeding the preset threshold limits (aerobic, 40; anaerobic, 13) and those grossly positive were immediately Gram stained and subcultured. Media included a chocolate blood agar plate, a prereduced blood agar plate, and appropriate additional media if microorganisms were seen on the stain.

The organisms isolated were identified by using conventional media and standard identification techniques (2, 7). Patient diagnosis and the clinical significance of each isolate were determined by the Infectious Disease staff. The hypothesis of equal proportions of positivity was tested, using the sign test as recommended by Ilstrup (6a) and *P* values obtained from a distribution table (1).

During the 2-year study (April 1980 to January

TABLE 1. Isolates from 309 clinically significant cultures demonstrating differences in detection rates between BACTEC and VACUTAINER-BACTEC systems

Organism (no. of isolates)	BACTEC and VACUTAINER-BACTEC[a]	BACTEC only	VACUTAINER-BACTEC	P
Streptococcus pneumoniae (28)	20	6	2	>0.1
S. faecalis (10)	6	0	4	0.062
Klebsiella spp. (18)	11	6	1	0.062
Proteus mirabilis (14)	11	0	3	>0.1
Haemophilus influenzae (15)	12	3	0	>0.1

[a] BACTEC, Ward-filled bottles; VACUTAINER-BACTEC, laboratory-inoculated vials.

1982), 4,690 samples were cultured. Of these, 533 were found positive in one or more of the four bottles. The 309 positive cultures determined to be clinically significant represented 176 bacteremic episodes in 160 patients.

Of these 176 episodes, 147 were detected in ward-filled BACTEC bottles (BACTEC system), and 146 were detected in the laboratory-inoculated bottles (VACUTAINER-BACTEC system). This similarity in detection rate between the two systems was surprising in view of the several theoretical advantages of direct inoculation, including earlier initiation of growth and immediate dilution of the natural bactericidal activities of the serum and any antimicrobial agents that might be present. In addition, collection into the sodium polyanetholesulfonate (SPS)-containing VACUTAINER tube with subsequent inoculation into SPS-containing medium might result in concentrations of this substance inhibitory to some organisms (3–6, 8, 10).

Since SPS is known to inactivate a number of antibiotics, it is possible that an increased isolation rate in our BACTEC system might have been masked by a balanced improvement in bacterial isolation in the VACUTAINER-BACTEC system if patients were receiving antimicrobial agents at the time of specimen collection. However, detection rates between the two systems were identical (108 versus 108) when the results from patients who were receiving antibiotic therapy at the time of specimen collection were deleted from the calculations.

Time to detection was assessed by examining the 203 isolates from cultures positive in both the BACTEC and the VACUTAINER-BACTEC systems. Within this set, 29 isolates were detected first by VACUTAINER-BACTEC, 16 by the BACTEC bottles, and 158 by both methods at the same time ($P = 0.036$). The bulk of this difference could be accounted for by staphylococci. Twelve *S. aureus* isolates and one *S. epidermidis* isolate were detected earlier by the VACUTAINER-BACTEC method, whereas five *S. aureus* isolates were detected first by BACTEC ($P = 0.048$). Of the 13 staphylococcal isolates detected first in the VACUTAINER-BACTEC system, 11 were found in the BACTEC system within the next 24 h.

Variations in the range of organisms isolated were limited. *Klebsiella* spp. were isolated more frequently from the BACTEC bottles, and *Streptococcus faecalis* was isolated more often from the VACUTAINER-BACTEC bottles (Table 1). The results also suggest that the BACTEC method is more sensitive in detecting *Streptococcus pneumoniae* and *Haemophilus influenzae* and that the VACUTAINER-BAC-

TABLE 2. Isolates from 224 clinically insignificant cultures by medium

Organism (no. of isolates)[b]	BACTEC and VACUTAINER-BACTEC[a]	BACTEC only	VACUTAINER-BACTEC only	P
Nonspore forming				
Micrococcaceae (198) (coagulase-negative)	38	73	87	>0.1
Propionibacterium acnes (9)	3	4	2	>0.1
Other (24)	9	6	9	>0.1
Sporeforming				
Bacillus spp. (11)	0	2	9	0.033
Clostridium perfringens (5)	1	1	3	>0.1

[a] BACTEC, Ward-filled bottles; VACUTAINER-BACTEC, laboratory-inoculated vials.
[b] Multiple organisms were isolated from 15 of the cultures.

TEC system is more sensitive in detecting *Proteus mirabilis* bacteremia. Whether the lack of statistical significance in any of these differences is due solely to small numbers is unknown.

There were 224 positive specimens that were thought to be clinically insignificant. They yielded 247 individual isolates. A total of 196 isolates were detected in only a single system, 110 from the VACUTAINER-BACTEC and 86 from the BACTEC (Table 2). More sporeforming organisms, particularly *Bacillus* spp., were seen in the former system (12 versus 3, $P = 0.018$). Although our study was not specifically designed to detect differences in contamination rates between direct and delayed inoculation, it seems likely that these *Bacillus* isolates represented contamination rather than true, but clinically insignificant, bacteremias. Whether this was the result of inadequate sterilization of the VACUTAINER tubes during production or exogenous contamination during the processing of the blood specimen is unknown. Washington (9), reporting on the microbiology of evacuated blood collection tubes, noted that *Bacillus* spp. were the most common organisms isolated from nonsterile tubes (101 of 235). Although he did not recover *Bacillus* from any of the irradiation-sterilized blood collection tubes, it is conceivable that the sterilization procedure utilized occasionally fails to eliminate all viable spores.

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