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Comparison of Macroscopic, Microscopic, and Radiometric Examinations of Clinical Blood Cultures in Hypertonic Media

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Clinical blood cultures were collected in the Bactec 8A flask (Johnston Laboratories, Cockeysville, Md.) and examined macrosopically, microscopically, and radiometrically in an effort to determine which approach produced the fastest detection time. Of 360 blood cultures found to contain organisms by subculture, 334 were first detected by Bactec, 98 by macroscopic examination, and 68 by microscopic examination. Examination times were at 4, 8, 16, 24, 36, and 48 h after collection of the specimen. Sixteen hours after specimen collection, microscopic examination had detected 31 positive cultures, macroscopic examination had detected two positive cultures, and Bactec had detected 160 positive cultures. By the end of the first 24 h of incubation, Bactec had detected 313 (93%) of those cultures eventually found to be positive. Although Bactec produced the fastest detection time in an overwhelming majority of the cultures, it failed to detect three of three *Candida* spp. cultures, three of five *Bacteroides* spp. cultures, and six of 32 *Enterococcus* spp. cultures during the first 48 h of incubation.

The question of how best to first detect the presence of organisms in a blood culture system is of vital importance to the medical team. Some clinical microbiologists place major emphasis on the macroscopic examination of the flask, whereas others feel that automatic subculture is the best approach. The purpose of this study is to determine by parallel culture technique whether macroscopic, microscopic, or radiometric examination of clinical blood cultures produces the fastest detection time for a wide spectrum of organisms.

MATERIALS AND METHODS

For each blood culture requested, three identical Bactec 8A flasks (Johnston Laboratories, Cockeysville, Md.) were used. The Bactec 8A flask consists of 30 ml of tryptic soy broth made hypertonic by the addition of sucrose in a final concentration of 10%. In addition, this medium also contains sodium polyanethol sulfonate in a final concentration of 0.025% and radioactive material (^{14}C) in a final concentration of 1.5 μCi. Inoculation of each set of flasks was accomplished by obtaining a 10-ml blood specimen in a syringe and then placing 3-ml portions of the specimen into each of the flasks. The flasks were not inoculated in any special order. Once the set of flasks reached the laboratory, each flask was given a specific label. For any given set of flasks, one flask was labeled the V flask and was used solely for macroscopic examination. A second flask was labeled the M flask and was used only for microscopic examination. The third flask was labeled the B flask and was used for radiometric determinations. One technologist was assigned to process each type of flask, and each technologist was requested not to inform the others of his results. The final results were assembled by the chief technologist and the clinical microbiologist.

All flasks considered negative after 48 h of processing were automatically subcultured to two chocolate agar plates. One plate was incubated under 10% CO_2, and the other was incubated in the GasPak (BBL) under anaerobic conditions for 48 h. Whenever a positive flask was determined by the technologist, it was also subcultured as just described.

All flasks were examined at 4, 8, 16, 24, 36, and 48 h after collection of the specimen.

Processing the V flask. The V flasks were incubated at 35 C and carefully removed at each examination time. A positive result was recorded whenever the technologist assigned to this phase of the study noted colonies, gas formation, or cloudy broth. All positive flasks were subcultured immediately.

Processing the M flask. The M flasks were incubated as described and removed from the incubator for each examination time. Examination was accomplished by removing a small sample of the culture mixture with a syringe and preparing two slides, one for methylene blue stain and the other a wet mount for dark-field examination. Again, whenever the technologist decided a flask was positive, it was subcultured.

Processing the B flask. The B flasks were incubated on a 35-C magnetic stirring incubator. The Bactec model 301 unit was used to examine each flask at each examination time. The criteria for determining a positive result with this unit were not those sug-

gested by the manufacturer. Instead, a positive result was recorded whenever the reading on the growth index scale either exceeded 5 units at the first reading or exceeded any previous reading by 5 units. We used these criteria because the manufacturer informed us that the growth index scale on the model 301 was designed for nonhypertonic medium, which produces much higher readings than does hypertonic medium. The determination of whether any organisms recovered were clinically significant or contaminants was made solely by the attending physicians.

RESULTS

A total of 1,241 sets of flasks, representing 421 patients, were processed in this study. There were 360 sets of flasks, representing 91 patients, found to harbor clinically significant organisms and 108 flasks, representing 46 patients, found to harbor contaminating organisms by definition for this study. Those cultures considered to harbor contaminating organisms are not included in this report. Table 1 indicates (i) the number of confirmed positive flasks detected by each of the test methods for each of the examination times, and (ii) the number of false positive flasks considered positive by the individual examining them but found to be negative on subculture. The families *Enterobacteriaceae* and *Pseudomonadaceae* were not broken down to genus in order to reduce the length of the table. The *Enterobacteriaceae* included the following genera, with the number of samples in parentheses: *Klebsiella* (33), *Enterobacter* (17), *Serratia* (9), *Escherichia* (69), *Citrobacter* (3), *Proteus mirabilis* (11), and *Proteus morganii* (4). The *Pseudomonadaceae* consisted of the following genera: *Aeromonas* (3), *Pseudomonas* (31), and *Acinetobacter* (7). Of the 360 positive sets of flasks, 16 were found to be positive only on subculture.

The vast majority (63) of the false positives in the V flasks were due to cloudiness of the broth, whereas all of the M-flask false positives were due to artifacts resembling organisms. The 11 false positive results obtained from the B flasks were all due to differential readings that exceeded 5 units on the growth index scale.

DISCUSSION

The single most important factor to consider when selecting a blood culture system for use in a clinical laboratory is the ability of the system to allow the survival of a wide spectrum of organisms. When considering the best method for processing the blood culture system, however, there are two important factors. First, whatever protocol is selected, it must produce the maximal number of recoveries, and second, the time needed to first detect a positive flask should not be excessive.

There is no question that the faster a laboratory is able to detect the presence of organisms in a blood culture flask, the faster one can begin recovery and identification procedures. There is a question, however, of how many categories of organisms lend themselves to the various methods used for rapid detection. Some of the

TABLE 1. *Number of organisms detected by each study system at each examination time and the number of false positive detections*[a]

Organism	Total no. recovered	No. of organisms detected after:																	Total		
		4 h			8 h			16 h			24 h			36 h			48 h				
		B	V	M	B	V	M	B	V	M	B	V	M	B	V	M	B	V	M	B	V	M
Enterobacteriaceae	146	4	0	0	28	0	1	39	1	3	73	11	11	2	30	0	0	20	0	146	62	15
Pseudomonaceae	41	0	0	0	2	0	0	5	0	1	21	3	3	7	1	1	5	2	2	40	6	7
Staphylococcus aureus	27	1	0	0	9	0	2	13	0	7	4	12	3	0	0	0	0	0	0	27	12	12
Streptococcus viridans	36	0	0	0	3	0	0	15	0	2	16	7	7	2	2	2	0	0	0	36	9	11
Streptococcus pneumoniae	54	2	0	0	16	0	3	20	1	11	16	5	6	0	0	0	0	0	0	54	6	20
Streptococcus pyogenes	4	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	4	0	0
Enterococcus spp.	32	1	0	0	3	0	0	7	0	1	14	2	2	1	0	0	0	0	0	26	2	3
Bacteroides spp.	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	1	0
Anaerobic *Streptococci*	3	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	3	0	0
Neisseria meningitidis	3	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	0
Haemophilus influenziae	6	0	0	0	0	0	0	1	0	0	3	0	0	1	0	0	0	0	0	5	0	0
Candida spp.	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	360	7	0	0	51	0	6	102	2	25	153	40	32	14	33	3	7	23	2	334	98	68
False positives		0	0	0	1	0	2	1	1	9	4	16	11	2	37	6	3	21	7	11	75	35

[a] B, Flask tested by Bactec unit; V, flask used for macroscopic examination; M, flask used for microscopic examination.

recent literature (1, 2) compares microscopic and macroscopic examination and blind subculture for their ability to detect a positive culture. Blazevic et al. (1) found that 65% of their cultures were first detected by macroscopic examination, 23% by microscopic examination, and 12% by subculture. Hall et al. (2) reported that 81.9% of their cultures were first detected by macroscopic examination, whereas microscopic examination produced very few positive results. Our results indicated that macroscopic examination first detected only 29% and microscopic examination only 20%, whereas Bactec first detected 51%.

The large differences in the results of our study with those of the other two groups of investigators can be explained by the fact that our study used a hypertonic medium, whereas the other two did not. Hypertonic medium produces a cloudiness in the culture flask in most cases within 12 to 24 h, thus making macroscopic or microscopic examination very difficult. This is demonstrated by the fact that there were 75 false positive macroscopic and 35 false positive microscopic flasks. Thus it is really not possible to compare the results of our study with those of either Blazevic et al. or Hall et al.

Comparing the time needed to first obtain a detection in our study with that of either Blazevic et al. or Hall et al. is also very difficult, since Hall et al. simply reported mean detection times with no further definition and Blazevic et al. quoted their results in terms of "day first detected." In common practice, detection after "1 day of incubation" or "overnight incubation" can imply anywhere from 14 to 24 h after collection of the specimen. In our study, however, detection time is defined as the number of hours between collection of the blood culture and detection of the flask as positive. The various terms used by different investigators serve only to confuse the issue of detection time, and we therefore suggest that a single definition be used by all investigators.

In our study, 93% of all the flasks that were eventually found to harbor clinically significant organisms were detected by Bactec within the first 48 h of incubation. This compares with only 29% by macroscopic and 20% by microscopic examinations. The Bactec system failed to detect three of three flasks found by subculture to contain *Candida* spp., two of five with *Bacteroides* spp., six of 32 flasks with *Enterococcus* spp., one of six with *Haemophilus influenzae*, and one of three with *Neisseria* spp.

From the results of this study, it appears that macroscopic and microscopic examinations of blood culture flasks containing a hypertonic medium are not satisfactory methods for detecting positive cultures. It appears that the Bactec system is the best approach for the fastest detection of a positive flask; however, it must be remembered that the Bactec approach failed to detect any *Candida* spp.-positive cultures, along with some of the *Bacteroides* spp.- and *Enterococcus* spp.-positive cultures during the first 48 h of incubation. Because of these failures, the Bactec system cannot be used alone but should be coupled with subculture of all negative flasks.

LITERATURE CITED

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