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Comparison of Macroscopic Examination, Routine Gram Stains, and Routine Subcultures in the Initial Detection of Positive Blood Cultures

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Blood was cultured in two vacuum bottles containing Columbia broth with sodium polyanethol sulfonate and CO_2. Filtered air was admitted to one bottle, and the bottles were incubated at 35 C until growth was detected or for a maximum of 7 days. Bottles were examined daily for macroscopic growth. Gram stains were made routinely on the 1st, 4th, and 7th days, and samples were routinely subcultured to sheep blood agar (incubated in GasPak jar) and chocolate agar (incubated in CO_2) on the 1st and 4th days of incubation. Of 1,127 positive blood cultures, 65% were first detected by macroscopic examination, 23% were first detected by Gram stain, and 12% were first detected only by subculture.

There are many methods recommended for the routine culture and examination of blood samples. There is agreement that blood cultures should be observed at least daily for macroscopic growth, but suggestions as to the need for routine Gram stains and blind subcultures vary from author to author. We are not aware of any published report comparing the efficacy of these procedures in the initial detection of positive blood cultures. Therefore, a comparative study was carried out to assess the value of the three approaches to detection of initial microbial growth in blood cultures.

MATERIALS AND METHODS

Blood cultures were obtained from patients in the University of Minnesota hospitals (approximately 800 beds) and were processed in the Diagnostic Microbiology Laboratory, which receives about 700 blood cultures per month.

Blood was cultured in two vacuum bottles containing 100 ml of Columbia broth with 0.03% sodium polyanethol sulfonate and 10% CO_2 (B-D Division of BioQuest). The blood was drawn by physicians, and the amount inoculated into each bottle varied from a few drops to approximately 10 ml. When the bottles were received in the laboratory, filtered air was admitted to one bottle by using a blood collection set (B-D Division of BioQuest); the collection set was removed from the bottle before incubation. The other bottle was considered to be anaerobic. Penicillinase (Difco) was added when indicated. The blood cultures were incubated at 35 C for 7 days or until growth was noted. Cultures from patients with suspected bacterial endocarditis or brucellosis were held for 2 to 3 weeks.

Cultures were examined macroscopically for growth in the morning and afternoon on the 1st day of incubation and in the morning of each day thereafter. Cultures that appeared positive were Gram stained immediately, and subcultures were made according to the types of organisms seen.

Gram stains were performed on all bottles that appeared macroscopically negative on the 1st, 4th, and 7th day of incubation. Blind subcultures were also made on the 1st and 4th days to a sheep blood agar plate (incubated anaerobically) and to a chocolate agar plate (incubated in CO_2). Subculture plates were held for 2 days before they were discarded as negative.

Each procedure was performed in the routine laboratory by a total of 13 microbiology technologists on a rotation basis.

RESULTS

The method of first detection of growth is shown in Table 1. There were a total of 7,357 blood cultures examined over a period of 10.5 months, and 1,127 were positive. Of these, 734

TABLE 1. *Method of first detection of growth in blood cultures*

First detection of growth	No.	%
Macroscopic exam	734	65
Gram stain	254	23
Subculture	139	12

(65%) were first detected by macroscopic examination, 254 (23%) were first detected by Gram stain, and the remaining 139 (12%) were first detected only by subculture.

Table 2 shows the day on which cultures were noted to be positive by three methods of detection. Forty-seven percent of those first detected by macroscopic examination were found on the 1st day. Of those first detected by Gram stain, 49% were found on the 1st day, 28% were found on the 4th day, and 23% were found on the 7th day. Of the positive cultures first detected by subculture, 76% were detected on the 1st day and 24% were detected on the 4th day. One hundred twenty-five positive cultures were not apparent macroscopically on the 1st day, and 106 positive cultures were not detected by Gram stain on the 1st day, nor were 33 positives detected by Gram stain on the 4th day.

Of the 1,127 positive blood cultures, 467 (41.4%) were detected on the 1st day either macroscopically or by Gram stain.

The numbers and types of organisms isolated, along with the mean times for detection (by all methods) are shown in Table 3. Of all the organisms isolated, *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella* sp., and *Neisseria gonorrhoeae* were detected first only by subculture. These organisms were never detected first by macroscopic examination or Gram stain, although approximately one-half of the *Haemophilus* cultures appeared macroscopically positive subsequent to subculture.

Of the *Pseudomonas aeruginosa* isolated, only one-third were detected first macroscopically, one-third were detected first by Gram stain, and the remaining one-third were detected first only by subculture.

Anaerobic organisms were almost always detected either by macroscopic examination or Gram stain. Only four strains of *Bacteroides* were first detected on the anaerobic subculture plate.

The organisms detected first by the 7th-day Gram stain included *Propionibacterium acnes*, *Candida*, *Corynebacterium*, *Peptococcus*, *Pseudomonas*, *Staphylococcus epidermidis*, and *Torulopsis glabrata*, although some strains of these bacteria were also detected by the other methods.

TABLE 2. *Method and day of detection of growth in blood cultures*

Method	Day first detected	No.	%
Macroscopic exam	1	342	47
	2	206	28
	3	81	11
	4	26	3
	5	12	2
	6	29	4
	7	38	5
Gram stain	1	125	49
	4	72	28
	7	57	23
Subculture	1	106	76
	4	33	24

TABLE 3. *Organisms isolated and mean time for detection*

Organism	No. isolated	Mean (days)
Acinetobacter	6	2.5
Bacillus	11	2.2
Bacteroides	74	2.4
Bifidobacterium	3	3.0
Candida	28	3.6
Citrobacter	8	1.0
Clostridium	44	1.2
Corynebacterium	25	5.1
Cryptococcus neoformans	1	3.0
Diplococcus pneumoniae	18	1.1
Enterobacter	49	1.3
Escherichia coli	191	1.4
Eubacterium	1	4.0
Haemophilus	18	2.8
Klebsiella	85	1.3
Lactobacillus	1	2.0
Moraxella	1	5.0
Neisseria gonorrhoeae	1	6.0
Peptococcus	4	4.5
Propionibacterium	60	6.5
Proteus	36	1.6
Pseudomonas	91	2.4
Salmonella	6	1.6
Serratia	18	1.5
Staphylococcus aureus	136	1.9
Staphylococcus epidermidis	147	2.9
Streptococcus, group D	86	1.3
Streptococcus, viridans	71	1.6
Streptococcus, beta	21	1.1
Torulopsis glabrata	3	6.3
Miscellaneous gram-negative rod	6	4.7

DISCUSSION

The data presented indicate that, for optimal speed in detection and identification of organisms from positive blood cultures, both routine Gram stains and blind subcultures should be performed in addition to daily visual inspection of cultures. If routine Gram stains and subcultures had not been performed, the detection of a positive blood culture or presumptive identification of the organism would have been delayed by at least 1 day in 35% of the cultures. If, in

addition to macroscopic inspection, only Gram stains were done, there would have been a delay in 12% of the cultures. If only subcultures had been performed, 23% of the positive reports would have been delayed at least 1 day. One might make the point that results of subcultures themselves were delayed by 1 day and that the culture in some cases may have been positive macroscopically the next day; however, even though this may be true, at the time of reading the subcultures a more definite identification of the organism could be given to the physician rather than just its Gram stain morphology. Subcultures were especially important in the more rapid detection of *Haemophilus*, since these organisms were all detected first only by this means. Both Gram stains and subcultures were also valuable in the more rapid detection of *Pseudomonas*, as two-thirds of those isolated were detected first only by Gram stain or subculture. Our experience with *Pseudomonas* bears out the study by Slotnick and Sacks (3), who stated that the use of visible growth or Gram stains alone are not sufficient to detect the presence of *Pseudomonas* in blood culture media.

Although there is no question about the importance of a Gram stain to detect positive blood cultures on the 1st day, the value of Gram stains on the 4th day in relation to the amount of work involved and the clinical importance might be questioned. In this study, approximately 6% of the positives were first detected by Gram stains on the 4th day. Individual judgments would have to be made as to whether detection of the positive on the 4th day would be that much more important than detection by subculture the following day.

The blood cultures in this study were incubated for a maximum of 7 days, except in cases of suspected brucellosis or endocarditis. This incubation period was based on the results of previous unpublished studies in our laboratory which demonstrated the rarity of isolation of clinically significant organisms after 1 week of incubation. Effersoe (1) has also shown that incubation for longer than 7 days is not necessary, especially if "control" Gram stain and subcultures are performed.

It was not the intent of this study to assess the overall rapidity of organism detection. However, the information in Table 3 does allow for comparison with other recently published studies (2, 4) on this subject. On the basis of these comparisons, we feel that the spacing of the procedures evaluated in our study are appropriate and practical for the clinical laboratory.

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