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Optimum Detection Times for Bacteria and Yeast Species with the BACTEC 9120 Aerobic Blood Culture System: Evaluation for a 5-Year Period in a Turkish University Hospital

Gül Durmaz,* Tercan Us, Aydin Aydinli, Abdurrahman Kiremitci, Nuri Kiraz, and Yurdanur Akgün

Department of Clinical Microbiology, Medical Faculty Hospital of Osmangazi University, 26480 Eskisehir, Turkey

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We tracked and documented the time of positivity of blood cultures by using the BACTEC 9120 (Becton Dickinson Diagnostic Instrument Systems) blood culture system over a 5-year study period. A 7-day protocol of the incubation period was selected, and a total of 11,156 blood cultures were evaluated. The clinically significant microorganisms (32.95%) were isolated in 3,676 specimens. Gram-positive and -negative bacterial isolation rates were found to be 41.07 and 44.88%, respectively. Yeasts were found in 14.03% of all pathogens. Both the false-positivity and -negativity rates were very low (0.1 and 0.3%, respectively). The mean detection times for all of the pathogens were determined to be 19.45 h. Yeasts, nonfermentative gram-negative bacteria, and *Brucella melitensis* strains were isolated within 5 days. By taking these data into account, we decided to establish a 5-day-incubation protocol in our laboratory instead of the 7 days that are commonly used.

Bloodstream infection is one of the most serious problems in all infectious diseases. Despite recent developments, like nucleic acid probes, PCR, and other molecular techniques for microbiological diagnosis, blood cultures still remain the most practical and reliable method in the diagnosis of bloodstream infections. Blood culture is one of the most important tools in the clinical microbiology laboratory. Rapid isolation and identification of the microorganisms in blood samples and directing of the treatment accordingly are critically important in order to reduce the mortality rate (2). The fact that conventional methods are not sufficient for a low mortality rate has resulted in the extensive usage of various continually monitored blood culture systems in many clinical microbiology laboratories over the past 25 years. In the end, these systems were proven to be highly reliable. One of these, BACTEC 9120, is based on the monitoring of the CO_2 concentration produced by growing microorganisms with a fluorescent sensor located at the bottom of each bottle (8). The aim of this study was to determine the detection times and the distribution of the bacteria and yeasts isolated from the blood samples by using BACTEC 9120 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) over a 5-year period. We also had it in mind to analyze the data to decide which incubation protocol would be more suitable for practical purposes.

This study was conducted in the Clinical Microbiology Laboratory of Osmangazi University Medical Faculty Hospital from August 1996 to December 2001. Most of the patients were adults from intensive care and hematology units. Two bottles were used in the culturing for every patient. All of the phlebotomies were performed with peripheral sticks, and the blood samples were drawn by a clinician by the bedside after cleansing the skin with 70% isopropyl alcohol and applying 10% povidone-iodine for 1 min. The blood samples were inoculated at a volume of 1 to 5 ml into BACTEC Peds Plus/F and a volume of 10 ml into Plus+Aerobic/F blood culture bottles and were placed in the BACTEC 9120 blood culture instrument. Because anaerobic blood culture bottles were not used in our laboratory, anaerobic blood cultures were not prepared. All study bottles were incubated for a period of 7 days. Whenever there was a sign of microbial growth, the detection time was documented. The bottles that had a positive signal were smeared and stained with Gram stain. Subcultures on chocolate agar and Sabouraud dextrose agar plates were also used. Subcultures were incubated at 35°C for a duration of 48 h. When growth was detected, the bacteria were identified by using Sceptor (Becton Dickinson) or VITEK (Biomerieux, Marcy l'Etoile, France) identification panels and the yeasts were detected by using conventional microbiological procedures as well as the Mycotube method (BBL Diagnostics). Identification of brucellae was performed by H_2S, urease production, and dye tests. Instrument-negative bottles were Gram stained and subcultured at the end of the 7-day protocol to confirm negativity. False-positive cultures were defined as those that were indicated by the instrument to be positive but had revealed no microorganisms by Gram staining and subculture (14). All isolates were considered to be clinically significant, except that a single positive culture of *Corynebacterium* spp., coagulase-positive staphylococci, viridans group streptococci, *Neisseria* spp. (other than *N. gonorrhoeae* and *N. meningitidis*), *Bacillus* spp., and *Micrococcus* spp. was identified to be a contaminant.

The time of detection was measured in hours, beginning with the placement of the bottles in the instrument and ending with the positive signal of the instrument.

A total of 11,156 blood cultures were inspected during the

* Corresponding author. Mailing address: Department of Clinical Microbiology, Medical Faculty of Osmangazi University, 26480 Eskişehir, Turkey. Phone: 90-222-2267786. Fax: 90-222-2395681. E-mail: gdurmaz@ogu.edu.tr.

TABLE 1. Results for the 11,156 blood cultures

Group and microorganism	No. of samples	% of total
Gram-positive bacteria		
Coagulase-negative *Staphylococcus* spp.	900	24.48
Staphylococcus aureus	465	12.65
Streptococcus pyogenes	26	0.71
Streptococcus pneumoniae	24	0.65
Streptococcus agalactiae	10	0.27
Streptococcus spp. (viridans group)	36	0.98
Enterococcus faecalis	31	0.84
Enterococcus faecium	18	0.49
All gram-positive bacteria	1,510	41.07[a]
Enterobacteriaceae	1,067	29.03
Klebsiella pneumoniae	431	11.72
Escherichia coli	172	4.68
Serratia marcescens	94	2.55
Enterobacter aerogenes	88	2.39
Klebsiella oxytoca	85	2.31
Enterobacter cloacae	77	2.09
Edwardsiella ictaluri	41	1.11
Salmonella spp.	36	0.98
Proteus spp.	17	0.46
Enterobacter agglomerans	11	0.29
Alcaligenes spp.	9	0.24
Morganella morganii	2	0.05
Citrobacter freundii	4	0.10
Other gram-negative bacteria		
Pseudomonas aeruginosa	320	8.71
Stenotrophomonas maltophilia	76	2.06
Acinetobacter baumanii	146	3.97
Acinetobacter lwoffii	21	0.57
Brucella melitensis	20	0.54
All gram-negative bacteria	1,650	44.88[a]
Yeasts		
Candida albicans	237	6.45
Candida glabrata	121	3.29
Candida tropicalis	56	1.52
Candida parapsilosis	55	1.49
Candida kefyr	37	1.01
Candida krusei	10	0.27
All yeasts	516	14.03[a]
Contaminants	233	2.08[b]
False-positive	35	0.3[b]
False-negative	12	0.1[b]
No growth	7,235	64.85[b]

[a] Total, 3,676 specimens.
[b] Total, 11,156 specimens.

5-year period. Clinically significant microbial growth was detected in a total of 3,676 cultures. The BACTEC 9120 instrument had 35 (0.3%) false-positive signals. The false-negativity rate was 0.1%. The organisms that were recovered from false-negative cultures included 1 *Candida albicans* strain and 11 *Corynebacterium* strains. A total of 233 specimens were positive for contaminant organisms, including the *Corynebacterium* and *Micrococcus* species (Table 1).

Microorganisms that were recovered from the blood culture bottles and the mean times for their detection are shown in Table 2.

In the present study, the presence of pathogenic microorganisms was observed in 3,676 (32.95%) cultures taken from the 11,156 blood samples during the 5-year period. The gram-positive and -negative bacterial isolation rates were 41.07 and 44.88%, respectively. The members of the *Enterobacteriaceae* family were the most frequently isolated strains among the gram-negative bacteria. The gram-negative and -positive bacterial isolation rates obtained by Bayram et al. (2) with BACTEC 9240 were similar to our results in that *Enterobacteriaceae* were also found to be the most frequently isolated strains among the gram-negative bacteria. Tuncer et al. (12) concluded that the gram-positive bacteria were the most frequently found (70.7%) with BacT/Alert, but members of the family *Enterobacteriaceae* took first place among the gram-negative bacteria as in the present study.

Among the 1,510 gram-positive bacteria detected in our

TABLE 2. Detection times for pathogens isolated with 8ACTEC 9120

Group and microorganism (no. of samples)	Detection time (h)	Range
	Median (mean)	
Gram-positive bacteria		
Coagulase-negative *Staphylococcus* spp. (900)	24.34 (14.99)	0.21–158.31
Staphylococcus aureus (465)	17.22 (13.85)	1.21–89.44
Streptococcus pyogenes (26)	13.32 (8.30)	2.52–32.61
Streptococcus pneumoniae (24)	24.98 (5.70)	20.95–29.00
Streptococcus agalactiae (10)	12.73 (7.55)	2.05–35.60
Streptococcus spp. (viridans group) (36)	21.19 (2.02)	19.76–22.62
Enterococcus faecalis (31)	31.61 (28.16)	8.73–104.21
Enterococcus faecium (18)	28.12 (25.01)	7.51–96.52
All gram-positive bacteria (1,510)	18.83 (15.45)	0.21–158.31
Enterobacteriaceae (1,067)	11.91 (9.99)	0.67–57.35
Klebsiella pneumoniae (431)	11.32 (9.30)	0.67–45.65
Escherichia coli (172)	10.70 (9.49)	0.83–36.88
Serratia marcescens (94)	7.97 (3.99)	3.89–13.67
Enterobacter aerogenes (88)	12.85 (6.99)	3.77–22.26
Klebsiella oxytoca (85)	16.34 (9.15)	9.87–22.81
Enterobacter cloacae (77)	14.21 (12.58)	3.54–40.66
Edwardsiella ictaluri (41)	19.99 (11.16)	10.17–36.58
Salmonella spp. (36)	19.48 (6.53)	14.51–31.96
Proteus spp. (17)	6.94 (5.27)	1.02–19.72
Enterobacter agglomerans (11)	14.47 (10.00)	2.24–24.12
Alcaligenes spp. (9)	24.67 (6.05)	18.90–30.96
Morganella morganii (2)	7.76 (2.88)	5.72–9.79
Citrobacter freundii (4)	33.02 (7.85)	7.69–57.35
Other gram-negative bacteria		
Pseudomonas aeruginosa (320)	17.68 (21.32)	0.01–151.36
Stenotrophomonas maltophilia (76)	22.63 (20.00)	2.25–93.29
Acinetobacter baumanii (146)	11.94 (11.72)	2.05–64.14
Acinetobacter lwoffii (21)	39.68 (41.23)	10.52–68.83
Brucella melitensis (20)	69.87 (29.97)	31.22–117.54
All gram-negative bacteria (1,650)	15.67 (17.56)	0.1–167.17
Yeasts		
Candida albicans (237)	30.84 (16.56)	0.10–63.74
Candida glabrata (121)	31.13 (16.73)	5.55–78.46
Candida tropicalis (56)	28.91 (8.53)	19.10–120.23
Candida parapsilosis (55)	27.91 (15.30)	2.01–56.28
Candida kefyr (37)	17.94 (11.19)	3.19–75.65
Candida krusei (10)	18.60 (7.21)	10.54–27.26
All yeasts (516)	29.87 (15.68)	0.1–120.23
Contaminants (233)	40.21 (27.04)	1.05–143.60

study, 900 were determined to be coagulase-negative staphylococci. Gray et al. (5) drew attention to a striking increase in the isolations of coagulase-negative staphylococci (47.7%) and *Enterobacteriaceae* (56.8%) in their study, conducted using the BACTEC 9240 blood culture system. All of our yeast isolates were *Candida* species, and in 14.03% of them, pathogenic microorganisms were detected. In the previous studies, these rates were reported to be 3.23 and 5.5% lower than the respective rates determined in our study (2, 12). The yeast isolations may differ with respect to the clinics from which the samples were obtained. As an example, it can be easily demonstrated that fungemia incidence is higher in intensive care units and hematology clinics (13). Because of the fact that most of the blood samples were obtained from intensive care and hematology units in our study, our *Candida* isolation rate was found to be higher than the other researchers' results mentioned above.

We found that the mean detection times for the gram-positive bacteria, the gram-negative bacteria, and the yeasts in this study were 18.83, 15.67, and 23.87 h, respectively. Cockerill et al. (3) found the mean detection time to be 23.0 h for all pathogens. Smith et al. (9) reported that the detection time of the yeasts is 41.0 h. The growth determined by using BACTEC 9240 has been detected in about 87 to 90.2% of the samples within the first 24 h (5, 9). During the 5-year period, 68.77% of all cultures containing pathogens were detected within the first 24 h of incubation.

In our 20 *Brucella melitensis* isolates, the mean detection time was found to be 63.87 h. Thirteen of them were isolated within the first 72 h. Gedikoglu et al. (4) reported that 72 h is needed for 31 samples and 84 h is needed for the rest of the samples for the growth of 58 *B. melitensis* isolates, which were isolated with BACTEC 9120. The detection time of *B. melitensis* was reported to be between 2 and 7 days in various studies (1, 10, 15, 16). We found that the recovery time was significantly shorter with BACTEC 9120. *B. melitensis*, which is endemic in Middle Eastern countries, including Turkey, is more virulent for humans than other species. Diagnosis of the disease is usually difficult because brucellosis may imitate other clinical symptoms such as rheumatic disorders, and thus, brucellosis should be confirmed by laboratory methods. From the clinical point of view, the rapid detection of brucella with the BACTEC 9120 blood culture system may lead to an earlier diagnosis of the disease and may improve case management (17).

In this study, the false-positive rate was found to be 0.3%, while Smith et al. (11) had reported a rate of 0.5% and Cockerill et al. (3) had reported a rate of 1.3%, both with BACTEC 9240. The false-negative rate was found as 0.1% in our study. The false-negative rate was reported to be 0.12% by using BACTEC 9240 in the research of Lelievre et al. (7). The low rate of false positives and negatives in our laboratory, together with the routine confirmation of results by microscopy and cultures, prevents many of the complications that may arise in practice.

The fact that the blind subculturing procedure of conventional methods has not been applied in the automated systems minimizes the risks of contamination. The most important advantage of this system is the time it gains for treatment, due to the rapid isolation, especially for slow-growing microorganisms (6). In our study, the vast majority of pathogens were recovered within 5 days, including *Candida* and *Brucella* spp. Only four isolates (one strain each of coagulase-negative staphylococci and *Candidas tropicalis*, two isolates of *Corynebacterium* spp.) were recovered after 5 days. Even though there is not sufficient published data regarding the optimal incubation time for this system, the data in our study suggest that a reduction of the 7-day incubation period that was typically applied with the BACTEC 9120 system to 5 days is possible, which in turn renders the BACTEC 9120 system a more effective tool.

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