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Comparison of Bactec 9240 and Difco ESP Blood Culture Systems for Detection of Organisms from Vials Whose Entry Was Delayed

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A comparison of the Bactec 9240 (Becton-Dickinson, Sparks, Md.) and Difco ESP (Difco Laboratories, Detroit, Mich.) instruments for the detection of organism growth from vials whose entry was delayed was evaluated. The instruments' capabilities for organism recovery, time to detection, rates of false-positive results, and numbers of vials in which growth was not detected were made by using seeded blood culture vial pairs and controls with and without delayed entry. Bactec 9240 and Difco ESP aerobic and anaerobic vials were inoculated with human blood and were seeded with organism growth from 18 species, including obligate aerobic, anaerobic, and facultative anaerobic organisms. Each organism was tested in triplicate at 0, 8, 24, 36, and 48 h and was incubated at both room temperature (RT) and 35°C. Two separate phases of the study were performed, each with a different version of Bactec 9240 software. Overall, detection of growth in vials with delayed entry into either the Bactec 9240 or the Difco ESP instrument resulted in an increased total time to detection with incubation at both RT and 35°C compared with the total time to detection for nondelayed vials. However, false-positive results and vials in which growth was not detected were minimal, and delayed entry did not require routine entry or exit subcultures for either system. Analysis of individual time points and incubation temperatures for the detection of all organisms suggested that Difco ESP vials delayed by up to 8 h may be incubated at 35°C (100% detection) and vials delayed for longer than 8 h may remain at RT. Bactec 9240 vials may be incubated at 35°C for up to 24 h with a minimal loss of detection (97.9% detection), and vials delayed for more than 24 h should remain at RT for optimal recovery of organism growth.

Recently, the introduction of fully automated, continuously monitoring blood culture systems has allowed for the faster detection of positive blood cultures (5, 7, 10, 11). These systems use detection algorithms based on assessments of changes associated with microbial growth. Two of the systems currently approved for use by the U.S. Food and Drug Administration are the Bactec 9240 (Becton Dickinson, Sparks, Md.) and the Difco ESP system (Difco Laboratories, Detroit, Mich.).

The Bactec 9240 system uses a pH-sensitive fluorescence sensor which detects increases in CO_2 as a result of microorganism growth in the medium. The Difco ESP system uses manometric principles to detect changes in the bottle's headspace pressure because of gas production and/or gas consumption by the microorganism.

For optimal recovery, freshly inoculated blood cultures should be transported to the laboratory and entered into the instrument as soon as possible. However, because of off-site collection or restricted laboratory operating hours, there may be a substantial delay between blood culture inoculation and entry into the instrument.

Dynamic changes in the blood culture bottle must occur to activate the positivity algorithms of both the Bactec 9240 and the Difco ESP systems. A prolonged delay in vial entry into a system may result in false-negative results because the organism may already be in or past the logarithmic phase of growth (Bactec 9240) and/or changes in gas production or gas consumption may be diminished and not detected (Difco ESP).

The purpose of the study described here was to evaluate the capabilities of the Bactec 9240 and Difco ESP blood culture systems at detecting microorganisms in cultures whose entry was not delayed and whose entry was delayed for 8, 24, 36, and 48 h between the time of inoculation and the time of entry into the instruments. Seeded blood culture vials in triplicate and controls whose entries into the instruments were and were not delayed as well as incubation at room temperature (RT) and 35°C were used. Recovery of organisms, time to detection (TTD), rates of false-positive results, and the numbers of seeded vials not detected by the instruments were compared between the two systems.

MATERIALS AND METHODS

Software and media. The study was performed in two phases. In the first phase, the Bactec 9240 Delayed Vial Entry (DVE) application was used. This required the use of specific DVE media (Bactec 9240 DVE Plus Aerobic/F and DVE Plus Anaerobic/F media) and special calibrator vials with threshold DVE positivity algorithms (version 3.06B software). In the second phase of the study, a newer version of the Bactec 9240 software (version 3.40H) was used. The newer version contains additional algorithms that do not require the threshold DVE algorithms and DVE media or calibrator vials for delayed entry. In phase 2, Bactec 9240 Plus Aerobic/F and Plus Anaerobic/F media were used. Both Bactec 9240 media are high-blood-volume resin media (the maximum volume per bottle is 10 ml).

For the Difco ESP system, the same Difco ESP algorithms (version 2.12) as well as the same media (Difco ESP Aerobic Broth 80A and Difco ESP Anaerobic Broth 80N media) were used for both phases of the study. These bottles are high-blood-volume nonresin media (the maximum volume per bottle is 10 ml).

Organisms. The organisms used in the study are listed in Table 1 and included 18 species. These organisms were from stock clinical isolates or were American Type Culture Collection (ATCC) strains. Organisms from stock clinical isolates included *Acinetobacter baumanii*, *Bacteroides fragilis*, *Enterobacter cloacae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. The ATCC strains used included *Candida albicans* ATCC 30449, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis*

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TABLE 1. TTD (Phase 2 data) of individual organism at each time point

Organism CFU/vial and system	TTD (h) at the following incubation temp and delay times:								
	0 h	RT				35°C			
		8 h	24 h	36 h	48 h	8 h	24 h	36 h	48 h
Streptococcus pyogenes (36)									
BD aerobic	10.12	6.14	2.41	1.46	1.12	3.74	ND[c]	ND[c]	ND[c]
Difco aerobic	9.53	6.47	ND[b]	ND[c]	ND[c]	6.40	ND[c]	ND[c]	ND[c]
BD anaerobic	9.14	5.97	2.11	1.29	1.06	3.74	0.91[d]	ND[c]	ND[c]
Difco anaerobic	13.80	10.80	7.27	4.40	5.60	10.80	11.0[d]	ND[c]	ND[c]
Streptococcus agalactiae (23)									
BD aerobic	10.69	8.39	2.95	1.06	1.00	5.60	0.83[d]	0.83[d]	ND[b]
Difco aerobic	10.33	8.53	56.73	ND[b]	ND[c]	6.40	ND[c]	ND[c]	ND[c]
Streptococcus pneumoniae (30)									
BD aerobic	12.90	11.09	8.83	6.03	6.98	7.70	0.85	19.22[e]	0.73[d]
Difco aerobic	15.73	14.13	10.93	8.73	9.07	10.67	ND[c]	ND[b]	ND[c]
BD anaerobic	17.39	14.98	12.77	8.27	9.54	12.67	1.13	1.0[d]	ND[c]
Difco anaerobic	21.87	20.30	41.4[d]	18.4[d]	27.47	17.73	10.40	10.8[d]	14.8[d]
Enterococcus faecalis (38)									
BD aerobic	11.23	8.72	3.77	2.53	1.17[b]	6.09	0.89	1.49[d]	ND[c]
Difco aerobic	11.60	8.60	6.00	5.30[e]	ND[b]	7.07	7.50[d]	ND[c]	ND[c]
BD anaerobic	11.68	8.77	3.88	2.65	1.17	7.38	0.73	0.71[d]	ND[c]
Difco anaerobic	13.33	10.73	10.53	5.60	5.93	10.60	10.80	11.47	14.87
Staphylococcus aureus (47)									
BD aerobic	11.79	8.72	3.68	2.66	1.61	5.83	1.88	1.00	1.11
Difco aerobic	11.67	8.87	6.00	4.67	3.33	6.67	3.60[e]	ND[c]	8.6[d]
BD anaerobic	11.57	9.99	4.51	3.99	2.45	13.46	2.16	1.99	1.77
Difco anaerobic	14.47	12.47	12.20	10.60	9.60	10.80	8.40	10.60	ND[c]
Staphylococcus epidermidis (50)									
BD aerobic	20.94	14.92	10.02	7.03	6.02	20.58	3.15	2.15	1.88
Difco aerobic	15.73	13.30	11.13	10.47	8.60	11.27	6.20	5.33	ND[b]
BD anaerobic	17.77	16.67	11.90	10.59	7.86	14.53	2.86	1.87	0.96[d]
Difco anaerobic	35.53	29.73	24.80	21.80	14.53	19.67	10.60	10.60	10.80
Bacteroides fragilis (110)									
BD anaerobic	22.80	22.17	18.07	13.55	12.88	19.17	12.11	113.98[e]	100.12
Difco anaerobic	22.73	19.07	14.67	10.40	10.47	17.67	9.00	10.40	10.40
Clostridium histolyticum (76)									
BD anaerobic	23.34	22.90	25.17	30.42	37.19	21.60	5.83[e]	8.06	7.21[e]
Difco anaerobic	19.13	16.27	10.67	10.60	10.80	14.80	10.60	10.60	10.80
Candida albicans (143)									
BD aerobic	26.16	20.66	11.37	7.84	5.60	18.92	11.92	3.95	3.91
Difco aerobic	21.60	17.27	11.33	9.47	8.60	15.27	9.87	9.13	10.40
Acinetobacter baumanii (70)									
BD aerobic	12.28	9.51	3.77	3.36	8.87	6.96	2.38	6.92	6.57[e]
Difco aerobic	11.76	10.60	10.33	10.40	6.27	10.47	7.47	8.20[e]	ND[b]
Haemophilus influenzae (101)									
BD aerobic	14.32	12.05	9.40	7.26	7.03[e]	9.60	3.90	3.75	2.40
Difco aerobic	13.73	12.70	9.80	8.73	8.33	9.93	6.20	6.67	11.40
Neisseria meningitidis (11)									
BD aerobic	19.83	20.19[d]	ND[b]	ND[c]	ND[b]	13.61	5.74	5.38[d]	4.39
Difco aerobic	19.00	17.93	21.33	20.27	22.27	17.07	23.87	17.73	ND[c]
Pseudomonas aeruginosa (71)									
BD aerobic	14.54	11.51	5.96	3.55	2.24	9.12	3.25	2.11	1.40
Difco aerobic	14.53	11.33	7.87	6.73	6.20[d]	8.93	6.40	ND[c]	ND[c]
Stenotrophomonas maltophilia (35)									
BD aerobic	32.37	28.55	12.73	5.49	3.56	19.81	5.56	4.15	1.17
Difco aerobic	22.73	16.93	12.90[e]	10.00	7.33	18.13	NA	23.07	ND[b]

Continued on following page

TABLE 1—*Continued*

Organism CFU/vial and system	TTD (h) at the following incubation temp and delay times:								
	0 h	RT				35°C			
		8 h	24 h	36 h	48 h	8 h	24 h	36 h	48 h
Enterobacter cloacae (80)									
BD aerobic	12.35	10.91	2.56	0.71	1.56	9.13	0.71	0.99	1.30
Difco aerobic	12.67	10.07	5.60	2.73	2.33	7.87	1.67	1.93	1.93
BD anaerobic	11.74	8.47	1.94	0.71	1.39	6.86	0.71	0.71	1.30
Difco anaerobic	16.80	14.60	9.27	5.73	3.47	14.00	4.20	3.20	3.67
Escherichia coli (40)									
BD aerobic	11.25	7.99	2.28	0.93	1.23	6.05	1.39	1.63	ND[b]
Difco aerobic	11.73	8.80	3.80	1.00	1.00	6.60	0.80	1.20	1.00
BD anaerobic	10.59	7.88	1.68	0.75	0.87[e]	5.38	0.67	10.46	10.80
Difco anaerobic	12.33	10.80	6.47	3.47	3.47	9.67	2.93	2.40	2.60
Klebsiella pneumoniae (61)									
BD aerobic	11.03	8.63	3.71	1.01	0.87	5.57	0.75	0.79	0.70
Difco aerobic	11.27	8.93	4.60	1.73	1.13	6.80	1.13	1.13	1.13
BD anaerobic	10.70	7.46	2.07	0.85	0.70	4.63	0.75	0.74	0.95[e]
Difco anaerobic	13.73	12.07	7.87	4.93	6.40	10.60	6.53	3.93	5.53
Proteus mirabilis (55)									
BD aerobic	15.20	11.89	3.90	1.03	1.07	10.18	1.25	1.03[e]	ND[b]
Difco aerobic	11.00	7.53	3.73	2.40	1.20	6.80	1.67	1.80	1.00
BD anaerobic	11.81	8.00	2.13	0.86	15.96	6.61	15.19	10.98	10.62
Difco anaerobic	16.27	11.67	7.67	3.53	3.33	10.80	5.07	3.93	3.20
Instrument TTD									
Bactec 9240	15.02	12.24	6.78	4.84	5.59	10.17	3.40	7.08	8.46
Difco ESP	15.73	12.89	12.40	8.75	7.83	11.24	7.21	7.68	6.98
Total TTD[g]									
Bactec 9240	15.02	20.24	30.78	40.84	53.59	18.17	27.40	43.08	56.48
Difco ESP	15.73	20.89	36.40	44.75	55.83	19.24	31.21	43.68	54.98

[a] BD, Becton-Dickinson.
[b] Growth in two of three vials was not detected.
[c] Growth was not detected in any of the three vials.
[d] Growth was not detected in one vial.
[e] Data for one vial were eliminated because of a twofold or greater difference in the TTD compared with those for the other two vials.
[f] NA, not available.
[g] Total TTD is instrument time plus delay time.

ATCC 44532. For *Clostridium histolyticum*, a clinical isolate was used in phase 1 and ATCC 19401 was used in phase 2 of the study.

Inoculum preparation. For all but *B. fragilis*, *C. histolyticum*, and *C. albicans* isolates, an organism suspension in Trypticase soy broth (TSB; Becton Dickinson) equivalent to a 0.5 McFarland standard was made from fresh subcultures. Three subsequent 1:100 dilutions were made in TSB to obtain a final suspension of 1.5×10^2 CFU/ml. From this final suspension, 0.3 ml was inoculated into each vial to obtain a final inoculum of approximately 45 CFU per vial. A calibrated culture of the final suspension used to inoculate the vials was made to determine the actual inoculum size. The actual numbers of CFU per vial for each organism are listed in Table 1.

For *B. fragilis* and *C. histolyticum*, organism suspensions were made in thioglycolate broth (Remel, Lenexa, Kans.). For *C. albicans*, a suspension equivalent to a 1.0 McFarland standard was made in TSB, and two subsequent 1:100 dilutions were made.

Vial inoculation. For each time point and delay incubation temperature, one blood sterility control vial and triplicate seeded vials were inoculated with a single strain of each organism tested. Thirty-six vials of each medium type were first inoculated with 8 to 10 ml of sodium polyanetholesulfonate (SPS) containing human blood (Interstate Blood Bank, Philadelphia, Pa.). After the addition of blood, 27 of the vials were inoculated with 0.3 ml of the organism inoculum. The other nine vials served as blood sterility controls for each time point.

Nondelayed vials were entered into the respective instruments immediately (0 h). Delayed vial sets were incubated at both RT and 35°C for 8, 24, 36, or 48 h prior to entry into one of the instruments. Difco ESP vials delayed at 35°C were allowed to equilibrate to RT before entry into the instrument for instrument quality control. When bottle incubation and monitoring begin, the headspace pressure increases directly as the temperature increases. If this initial pressure increase is not detected, a "no movement error" is signaled by the Difco ESP instrument. A total of 972 vials (729 seeded vials and 243 sterility control vials) were evaluated in each phase of the study.

Organism recovery. An entry and exit subculture with Gram staining was done for every vial to confirm that viable organisms were present. Each vial was also inspected for signs of visible growth before entry into and after removal from both systems. All vials remained in the instruments for a maximum of 5 days unless they were flagged as positive by the instrument.

Statistical analysis. Student's *t* test was used to analyze the differences in TTD from both instruments for both phase 1 and phase 2 by using SigmaPlot software.

RESULTS

Tables 1 to 4 show the different parameters evaluated in phase 1 and phase 2 of the study. The detection algorithms used for the Bactec 9240 system in phase 2 (version 3.40H) are available in the currently marketed version. The data from phase 1 are presented because the software (version 3.06B) continues to be used at some institutions in Asia, Canada, and Europe. Thus, comparative data between both phases are shown for specific parameters when appropriate. Data from both phases of the study are provided in Tables 2 and 3. Tables 1 and 4 provide data from phase 2 only, with differences between phase 1 and phase 2 described in the text.

TTD. TTD data are presented and compared in the following four ways: (i) individual species at each time point and

TABLE 2. Composite TTD for nondelayed vials (0 h) by organism group

Organism group	Vial[a]	Composite TTD (h)[b]			
		Phase 1		Phase 2	
		Bactec 9240	Difco ESP	Bactec 9240	Difco ESP
Streptococcus spp.	A	11.18 ± 1.62	12.38 ± 1.68	11.24 ± 1.12	11.80 ± 2.51
	N	10.95 ± 4.71	17.40 ± 3.05	12.73 ± 3.72	16.33 ± 4.29
Staphylococcus spp.	A	14.52 ± 1.54	14.37 ± 1.37	16.36 ± 5.52	13.70 ± 2.23
	N	16.05 ± 3.36	25.20 ± 0.42	14.67 ± 3.45	25.00 ± 12.22
C. albicans	A	34.23 ± 4.18	24.07 ± 0.64	26.16 ± 1.20	21.60 ± 0.53
Anaerobes[c]	N	26.60 ± 0.48[d]	21.47 ± 3.07	23.07 ± 1.07	20.93 ± 2.10
Aerobes[e]	A	17.63 ± 4.85	16.44 ± 3.24	18.67 ± 7.61	16.35 ± 4.32
Members of the family *Enterobacteriaceae*[f]	A	11.85 ± 0.93	12.12 ± 1.15	12.46 ± 1.81	11.67 ± 0.67
	N	10.59 ± 2.05	14.68 ± 2.25	11.21 ± 0.66	14.78 ± 1.92
All organisms combined	A and N	14.61 ± 6.24	16.20 ± 4.55	15.02 ± 5.74	15.72 ± 5.62

[a] A, aerobic vial; N, anaerobic vial.
[b] Values and means ± 1 standard deviation.
[c] Anaerobes included *C. histolyticum* and *B. fragilis.*
[d] Data include TTD for *B. fragilis* only, since none of *C. histolyticum* isolates grew in nondelayed vials in phase 1.
[e] Aerobes included *N. meningiditis*, *P. aeruginosa*, *S. maltophilia*, *H. influenzae*, and *A. baumanii.*
[f] Members of the family *Enterobacteriaceae* included *E. coli*, *P. mirabilis*, *E. cloacae*, and *K. pneumoniae.*

delay temperature (Table 1), (ii) total TTD for all organisms at each delay time point (Table 1), (iii) organism groups at 0 h (Table 2), and (iv) organism groups at all time points (Table 3).

Table 1 shows the TTD of each organism at each time point. The TTD for each set of three bottles into which organisms were inoculated is averaged for each type of media. For all of the organisms tested, the total TTD (instrument time plus delay time) increased as the delay time increased at both RT and 35°C (Table 1). For both instruments, growth in vials delayed for 8 and 24 h at 35°C was detected faster than growth in vials delayed at RT. The Bactec 9240 system detected growth in vials delayed for 24, 36, and 48 h at RT faster (5.7, 4.0, and 2.2 h, respectively) than the Difco ESP system.

Overall, the TTD for vials with nondelayed entry (0 h; Table 2) for all organisms tested was similar for both instruments: 14.61 ± 6.24 h for the Bactec 9240 system and 16.20 ± 4.55 h

TABLE 3. Composite TTD for all time points (including 0 h) by organism group

Organism group	Vial[a]	Composite TTD (h)[b]			
		Phase 1		Phase 2	
		Bactec 9240	Difco ESP	Bactec 9240	Difco ESP
Streptococcus spp.	A	8.15 ± 9.02	12.89 ± 11.47	5.36 ± 4.38	14.31 ± 19.38
	N	6.19 ± 5.99	14.39 ± 5.33	6.28 ± 5.21	13.23 ± 7.65
Staphylococcus spp.	A	7.03 ± 4.39	8.88 ± 3.60	6.94 ± 6.41	10.91 ± 16.45
	N	7.07 ± 4.76	16.63 ± 5.84	6.84 ± 5.34	14.48 ± 6.33
C. albicans	A	16.02 ± 9.81	19.33 ± 4.06	12.26 ± 7.73	12.55 ± 4.35
Anaerobes[c]	N	14.62 ± 8.95[d]	15.33 ± 5.68	27.90 ± 27.36	13.04 ± 4.11
Aerobes[e]	A	9.75 ± 6.51	12.82 ± 5.97	8.79 ± 7.07	12.85 ± 5.89
Members of the family *Enterobacteriaceae*[f]	A	4.72 ± 3.44	4.86 ± 3.95	4.43 ± 4.46	4.38 ± 3.75
	N	4.68 ± 3.25	8.91 ± 3.53	5.47 ± 4.83	7.39 ± 4.33
All organisms combined	A and N	7.72 ± 6.76	11.40 ± 6.95	8.26 ± 10.94	10.77 ± 9.64

[a] A, aerobic vial; N, anaerobic vial.
[b] Values and means ± 1 standard deviation.
[c] Anaerobes included *C. histolyticum* and *B. fragilis.*
[d] A total of 51.8% of the *C. histolyticum* isolates did not grow.
[e] Aerobes included *N. meningiditis*, *P. aeruginosa*, *S. maltophilia*, *H. influenzae*, and *A. baumanii.*
[f] Members of the family *Enterobacteriaceae* included *E. coli*, *P. mirabilis*, *E. cloacae*, and *K. pneumoniae.*

TABLE 4. Effect of delayed vial entry on organism detection (phase 2 data only)

Delay temp and time	Bactec 9240		ESP 384	
	Cumulative no. of vials in which growth was ND[a]	% Detection[b]	Cumulative no. of vials in which growth was ND	% Detection
35°C up to 8 h	0	100	0	100
35°C up to 24 h	5	97.9	11	95.5
35°C up to 36 h	17	94.7	33	89.8
35°C up to 48 h	40[c]	90.1	65[d]	83.9
RT up to 8 h	1	99.4	0	100
RT up to 24 h	3	99.2	3	99.2
RT up to 36 h	6	98.1	9	97.2
RT up to 48 h	8[e]	98.2	19[d]	95.3
Total no. (%) of cumulative vials ND	48 (6.6)[f]		84 (11.5)[g]	

[a] ND, not detected by instrument.
[b] Percent detection is calculated as follows: (number of vials in which growth was detected/number of vials tested) × 100.
[c] The majority (75%) of organisms not detected by the Bactec 9240 system at 35°C were *Streptococcus* spp.
[d] The majority (75%) of organisms not detected by the Difco ESP system at 35°C and RT were *Streptococcus* spp.
[e] All organisms in vials in the Bactec 9240 system at RT in which growth was not detected were *N. meningitidis*, which also did not grow on subcultures.
[f] A total of 67% of vials were visibly hemolyzed and/or turbid on entry into the instrument.
[g] A total of 88% of vials were visibly hemolyzed and/or turbid on entry into the instrument.

for the Difco ESP system in phase 1 ($P > 0.05$) and 15.02 ± 5.74 h for the Bactec 9240 system and 15.72 ± 5.62 h for the Difco ESP system in phase 2 ($P > 0.05$). The TTDs for nondelayed vials by organism group are listed in Table 2 for reference.

The composite TTD for all time points (Table 3) was faster for the Bactec 9240 system than for the Difco ESP system: 7.72 ± 6.76 and 11.40 ± 6.95 h for the Bactec 9240 and Difco ESP systems, respectively, in phase 1 ($P < 0.001$) and 8.26 ± 10.94 and 10.77 ± 9.64 h for the Bactec 9240 and the Difco ESP systems, respectively, in phase 2 ($P < 0.001$). The actual clinical significance of this difference in TTD between the instruments was not evaluated and would vary for each institution, especially in relation to the laboratory's hours of operation and policy for reporting positive blood culture results.

In phase 1, 14 of 27 (51.8%) *C. histolyticum*-seeded Bactec 9240 vials did not grow on subcultures (Table 2). These included the triplicate nondelayed vials. The *C. histolyticum* strain used was a clinical isolate from our hospital. The *C. histolyticum* isolate grew well in the Difco ESP media. An ATCC strain of *C. histolyticum* was used in phase 2 of the study, and it grew without difficulty in the Bactec 9240 media and was detected by the system.

When the TTD data were analyzed by organism group, the Bactec 9240 system showed a faster TTD than the Difco ESP system for *Streptococcus* spp. and *Staphylococcus* spp. by more than 4 h. The majority of vials in which growth was detected faster occurred for the anaerobic vials (Tables 2 and 3). In contrast, the Difco ESP system detected strict anaerobes faster than the Bactec 9240 system by a range of 1.5 to 103.5 h (mode, 3.1 h in phase 2). Statistical significance was not performed for individual organism groups.

Effect of delayed entry on organism detection. As the delay time increased, growth in more vials was not detected by either the Bactec 9240 or the Difco ESP instrument. A decreasing detection rate with an increasing delay time occurred more in the vials delayed at 35°C than vials delayed at RT (Table 4).

In both of the Bactec 9240 and Difco ESP systems, the majority (75%) of organisms not detected were *Streptococcus* spp. for vials delayed at 35°C (Table 1). In the Difco ESP system the majority of vials delayed at RT in which growth was not detected were also *Streptococcus* spp. (Table 1). The organisms in all of these vials in which growth was not detected grew either on entry or on exit subculture. However, in the Bactec 9240 system all vials delayed at RT in which growth was not detected were those inoculated with *N. meningitidis*. In addition, the organisms in these vials failed to grow on either entry or exit subculture (Table 4).

The total numbers of seeded vials in which growth was not detected were 51 of 729 (7.0%) for the Bactec 9240 system and 77 of 729 (10.3%) for the Difco ESP system in phase 1 (data not shown). In phase 2 the total numbers of seeded vials in which growth was not detected were 48 of 729 (6.6%) for the Bactec 9240 system and 84 of 729 (11.5%) for the Difco ESP system (Table 4). Of the seeded vials in which growth was not detected, the majority were visibly positive (67% in the Bactec 9240 system and 88% in the Difco ESP system) by turbidity and/or hemolysis. Visible growth was subjectively easier to determine in the Difco ESP media than in the Bactec 9240 media.

Rate of false-positive results. A vial with a false-positive result was a vial that tested positive by the instrument but whose contents did not grow on subculture and no organism was seen on Gram staining. These vials included unseeded sterility control vials as well as seeded vials in which the organism did not grow on subculture (all of the latter were *C. histolyticum* in the Bactec 9240 system in phase 1). Rates of false-positive results were 30 of 972 (3.1%) for the Bactec 9240 system and 1 of 972 (0.1%) for the Difco ESP system in phase 1 and 2 of 972 (0.2%) for the Bactec 9240 system and 0% for the Difco ESP system in phase 2.

DISCUSSION

Reductions in the numbers of technical personnel in the laboratory and in hours of operation, as well as off-site specimen collection, are becoming more common. Often, these measures result in delayed entry of blood cultures into instruments. Since the detection algorithms of continuously monitoring instruments are based on significant changes in microbial growth characteristics, multiple factors regarding these systems and delayed entry of bottles need to be addressed. These include the optimal bottle incubation temperature, the maximum time that a bottle can be delayed outside of the

system, and the necessity of performing entry and/or terminal subcultures.

The literature addressing the issue of delayed bottle entry for the most commonly used continuously monitoring instruments (Bactec 9240, Difco ESP, and BacTAlert [Organon-Teknica, Durham, N.C.] systems) is sparse. The only available information is in abstract form (1–4, 6, 8, 9, 12). In addition, software updates for the Bactec 9240 system and abstracts from studies of delayed vial entry which did not reference the software version used (3, 4, 12) make the application of findings from one study to the next difficult. This discussion summarizes selected information from these abstracts pertinent to the Bactec 9240 and Difco ESP systems in relation to the data obtained in the present study.

With delayed vial entry there were false-negative results for both the Bactec 9240 (48 of 729 [6.6%]) and the Difco ESP (84 of 729 [11.4%]) systems (phase 2 data). The majority of these vials tested with both instruments contained *Streptococcus* spp. This was also the case in the recent study by Muller-Serieys et al. (6), in which 7 of the 10 isolates not detected by the Bactec instrument were *Streptococcus* spp. The reason that the majority of organisms in the delayed vials not detected by both instruments were *Streptococcus* spp. can be attributed in part to the metabolic pathway of this organism group. Prolonged incubation results in the accumulation of acid, which compromises the further metabolism of streptococci in the vials. Thus, the positivity algorithms may not be triggered.

It is noteworthy that in our study most of the seeded vials in which growth was not detected by the instruments were visibly turbid and/or hemolyzed; 67% in the Bactec 9240 vials and 88% in the Difco ESP vials. In a laboratory that uses standard blood culture protocols, a visibly turbid and/or hemolyzed bottle would signal an automatic subculture and Gram stain on the part of the technologist, and these bottles would not be entered into the system. Thus, the numbers of false-negative results would be reduced for both systems.

The rate of false-positive results in tests with Bactec 9240 DVE media and software (version 3.06B) in phase 1 was high compared with that in tests with Difco ESP media and software: 3.1 versus 0.2%, respectively. Similarly, in a study by Bergogne-Berezin et al. (1) that described a study with seeded bottles that used Bactec 9240 DVE software and bottles, the overall rate of false-positive results was 3.7%. The latest software (version 3.40H) for the Bactec 9240 instrument uses kinetic algorithms that do not require threshold settings, calibrator vials, or DVE media. By using the new software in phase 2 of the present study, the rate of false-positive results by the Bactec 9240 instrument dropped to 0.1%. This is similar to the rate of false-positive results of 0.28% reported by Muller-Serieys et al. (6), who used software algorithms similar to those that we used. The rate of false-positive results in phase 2 for the Difco ESP system was 0%. Laboratories still using phase 1 software (version 3.06B) with Bactec 9240 DVE media need to evaluate the threshold setting to avoid a high rate of false-positive results.

A slight decrease in sensitivity for the overall detection of organisms by the Bactec 9240 system from delayed vials was seen in phase 2 compared with that seen in phase 1: 93.1% (data not shown) to 90.1% (Table 4) in vials delayed for up to 48 h at 35°C. These sensitivities are between those reported in the study by Bergogne-Berezin et al. (1) with seeded DVE vials, in which the sensitivities were 89.1% for vials delayed for 24 h and 100% for vials delayed for 48 and 72 h at 35°C. For the Difco ESP system, the sensitivities were 84.4% in phase 1 (data not shown) and 83.9% in phase 2 (Table 4) for vials delayed for 48 h at 35°C.

In the study presented here, with the Bactec 9240 system and software version 3.40H (phase 2), delayed vials could be held for 24 h at 35°C with no significant loss of detection (97.9%) or for up to 48 h at RT (98.2% recovery). These data are similar to the data presented by Muller-Serieys et al. (6), who reported 100% recovery from vials delayed for up to 30 h and 85.9% recovery from vials delayed for more than 30 h at 37°C. Their study used software version 3.07, which uses some of the detection algorithms used in phase 2. The slight difference in sensitivities between the two studies may be attributed to the use of seeded vials with a known inoculum and 8 to 10 ml of 48-h-old SPS-containing human blood per vial in our study. Clinical specimens (fresh blood) were used in the study by Muller-Serieys et al. (6), and information on the organism load, blood volume, and antibiotics present were not described and/or available in the abstract.

On the basis of the data from the present study, Difco ESP bottles delayed for up to 8 h may be incubated at 35°C (100% recovery) and those delayed for longer than 8 h and for up to 24 h may be incubated at RT (99.2% recovery). This recommendation is identical to the manufacturer's recommendation, based on the only other study on delayed vial entry with the Difco ESP system (9). In a seeded culture study by Sullivan et al. (9), each bottle was inoculated with $<10^2$ CFU of bacteria or yeasts, and the bottles were incubated at 4, 25, and 35°C for 0, 4, 8, and 24 h at RT before entry into the instrument. Ten of 52 strains tested were not detected by the instrument after 24 h of delay at 35°C. Five of the strains not detected were *Streptococcus* spp., yet all of the bottles in which growth was not detected were visibly turbid before entry into the instrument. There is no recommendation for 48 h for the Difco ESP system, but again, on the basis of the data presented here, bottles delayed for longer than 24 h should be held at RT (Table 4) and carefully viewed for turbidity and/or hemolysis.

By using the manufacturers' recommendations for incubation of delayed vials, the following sensitivities of detection were seen: 97.9% up to 24 h at 35°C and 98.2% up to 48 h at RT for the Bactec 9240 system and 100% up to 8 h at 35°C and 99.2% up to 24 h for the Difco ESP system. However, Becton-Dickinson recommends that vials only be held at 35°C for up to 20 h to yield 100% detection. Thus, the current recommendations of Becton-Dickinson for incubation of delayed vials are up to 20 h at 35°C and >20 h at RT.

Citing only instrument detection of vials with positive growth, the Bactec 9240 system offers the advantages of longer incubation times off-site: at 35°C for up to 20 h and at RT for 48 h. These time points may be beneficial for laboratories that are not open on weekends as well as laboratories that rely on less experienced technologists to load bottles onto the instrument, who may miss turbid and/or hemolyzed bottles. The new Bactec 9240 software eliminated the need for special DVE vials and calibrator vials with a minimal change in sensitivity and overall TTD, while it substantially reduced the number of vials with false-positive results.

Although the present study was limited to 18 species, the Difco ESP system was able to support the growth of all species tested. The study demonstrates that *N. meningiditis* delayed at RT (phase 2) and *C. histolyticum* (phase 1) did not grow well in the Bactec 9240 media yet were supported and detected in the Difco ESP system. In addition, turbid and/or hemolyzed bottles at all time points evaluated were subjectively more easily detected in the Difco ESP media than in the resin media of the Bactec 9240 system.

Overall, both the Bactec 9240 and the Difco ESP blood culture instruments showed increased total TTD for all delayed vials for incubation at both RT and 35°C compared with

that for nondelayed vials. Delayed entry did not result in significant rates of false-positive or false-negative results outside of the manufacturer's recommendations allowing the elimination of blind subculturing prior to entry of the vials into the instrument. Further studies evaluating clinical specimens whose entry is delayed with blind subcultures before and at exit from continuously monitoring instruments should delineate more specific guidelines for delayed vial entry for these instruments.

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