



Efficient Inactivation of Clinically Relevant Antimicrobial Drug Concentrations by BacT/Alert or Bactec Resin-Containing Media in Simulated Adult Blood Cultures

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ABSTRACT We assessed the antimicrobial-inactivation capability of BacT/Alert (FA Plus and FN Plus) or Bactec (Plus Aerobic/F and Plus Anaerobic/F) media for 40 antibiotic-bacterium combinations in simulated adult blood cultures. Aside from high recovery rates (93.2% and 88.4%, respectively), we showed that at the lowest but clinically relevant antibiotic concentrations, both BacT/Alert and Bactec media recovered all the organisms tested with drugs except for *Escherichia coli*, which was tested in the presence of meropenem. Delayed recoveries were mainly associated with vancomycin.

KEYWORDS blood culture system, drug inactivation, personalized therapy

Bloodstream infections (BSIs) are associated with high rates of morbidity and mortality (1). Thus, timely and accurate diagnoses of BSIs are crucial to correctly manage infected patients (2), particularly those with infection-complicating sepsis and septic shock (3). Often, the physician in charge empirically administers broad-spectrum antimicrobial therapy before obtaining a microbiological diagnosis of the infection because of the patient's critical status (4). Blood culture (BC) is still the gold standard for BSI detection (5). Time to detection (TTD) with today's automated BC systems (BacT/Alert Virtuo [bioMérieux, Marcy l'Étoile, France] and BactecFX [Becton Dickinson, Sparks, MD]) may be significantly delayed or even unmeasurable because of slow or absent microbial growth due to antimicrobial activity in BCs (6).

In the early 1990s, BacT/Alert FAN bottles (Organon Teknika, Durham, NC) containing Fuller's earth and absorbent charcoal were invented to enhance recovery of microorganisms. The bottles were later improved and launched as charcoal-containing BacT/Alert FA and FN bottles (bioMérieux). Resin-containing media such as Bactec Plus Aerobic/F and Plus Anaerobic/F (Becton Dickinson) and, since 2013, BacT/Alert FA Plus (aerobic) and FN Plus (anaerobic) (bioMérieux) further improved the recovery of microorganisms from patient samples. Numerous studies evaluated a single bottle type or compared the performance of charcoal-containing bottles with that of resin-containing bottles in the presence of antimicrobials (7–10). In particular, a range of antimicrobial agents that were potentially active in BCs of patients treated for BSI was neutralized *in vitro* (11, 12).

Until now, few BC simulation studies (carried out using spiked BCs with whole blood, bacteria, and antibiotics) evaluated the antimicrobial-neutralizing capability of BacT/Alert FAN Plus media in comparison with Bactec Plus media in both anaerobic and aerobic BC bottles. In two independent studies (13, 14), researchers compared aerobic

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media performances of BacT/Alert FA Plus bottles with those of Bactec Plus Aerobic/F bottles by testing bacteria and antibiotics (*Pseudomonas aeruginosa* and antipseudomonal β -lactams, in one case [14]). However, investigating both aerobic and anaerobic BC bottles is important for improving treatment outcomes in septic patients (15). Therefore, provision of more *in vitro* data on the capability of BC bottles to overcome the inhibitory effect of preadministered antibiotics in contrived clinical scenarios is essential (16). With this in mind, we aimed to assess the effects of antimicrobial inactivation in BacT/Alert FAN Plus or Bactec Plus media by measuring the TTD and organism growth for several antibiotic-bacterium combinations in simulated adult aerobic and anaerobic BCs. Specifically, we mimicked antibiotic blood concentrations of patients under antimicrobial drug treatment.

(Part of this study was presented at the 28th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Madrid, Spain, 21 to 24 April 2018 [17].)

The ethics committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS approved the study (approval number 48781/17). We used resin-containing aerobic and anaerobic bottles in both BacT/Alert Virtuo (BacT/Alert FA Plus and FN Plus) and Bactec FX (Bactec Plus Aerobic/F and Plus Anaerobic/F) systems. We chose the 13 most commonly used antimicrobial drugs to treat specific BSIs and paired them with the most frequently isolated Gram-negative ($n = 4$) and Gram-positive ($n = 4$) bacterial species, for which we showed full susceptibility against specific antibiotics tested (Table 1). We obtained antibiotics from their respective manufacturers and the bacterial species from the American Type Culture Collection (ATCC) as reference strains. Each antibiotic was diluted in sterile water from freshly prepared stock solutions, so that 0.5 ml of a given dilution provided enough of the drug to reach the intended drug concentration in a bottle spiked with whole blood (see below). C_{\max} corresponded to the peak plasma concentrations achievable after standard dosage of the drug in a 70-kg adult with normal renal function (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf), and two other concentrations corresponded to one-half and one-quarter of the C_{\max} ($C/2$ and $C/4$, respectively) (Table 1). The C_{\max} concentrations were estimated and did not exactly reflect the actual concentrations *in vivo* (e.g., in patients with renal failure). Before testing, the bacterial strains were subcultured twice overnight on standard solid media at 37°C, and their MICs were determined with the broth microdilution method according to the ISO 20776-1/2006 procedure as recommended by EUCAST (18). In our BC simulation model (19), each bottle was spiked with 8-ml banked whole blood (obtained from the Division of Transfusion Medicine at our institution), 0.5-ml antibiotic solution (obtained as described above), and 0.5-ml bacterial suspension (50 to 100 CFU, achieved after diluting a suspension of $\sim 5 \times 10^8$ CFU/ml in phosphate-buffered saline). Resins are equally useful in the presence of lower and higher microbe quantities, as those present in blood during BSIs (20), but we decided on a more stringent experimental approach and used only one inoculum in the study. We included a positive (i.e., antibiotic-free bottle) and a negative (i.e., only blood-containing bottle) control for each simulated BC. The latter controls served to confirm the sterility of the blood, which we previously checked with a culture. In total, 40 antibiotic-organism combinations were tested in triplicate with each of the three different drug concentrations in both aerobic and anaerobic bottles, except for *Bacteroides fragilis* and *P. aeruginosa*, which were only tested under anaerobic or aerobic conditions, respectively. We immediately loaded the spiked bottles into the respective BC instruments and incubated them up to 5 days (120 h) or until they showed positive results. The positive (growth detection) or negative (no growth detection) signal was the endpoint used to calculate the TDD for each bottle (i.e., calculated from when the bottle entered into the BC system). After that, we subcultured the BC broth onto standard solid media to confirm positive or negative detections and/or to exclude contaminations. Statistical analyses were done using the Intercooled Stata program version 11 and GraphPad Prism 7. In each BC simulation, we calculated the number of positive bottles detected with both BC systems by the total number of bottles tested, as well as the mean TDD for the positive

TABLE 1 Organism and antimicrobial drug combinations tested by the simulated adult blood culture model

Species (strain)	Drug	MIC ($\mu\text{g/ml}$) ^a	Drug concentration ($\mu\text{g/ml}$) ^b		
			C _{max}	C/2	C/4
Gram-negative organism					
<i>Bacteroides fragilis</i> (ATCC 25285)	Meropenem	0.12	50	25	12.5
	Metronidazole	0.5	25	12.5	6.3
	Piperacillin-tazobactam	0.06	368/23	184/11.5	92/5.8
<i>Escherichia coli</i> (ATCC 25922)	Ceftaroline	0.06	39	19.5	9.75
	Ciprofloxacin	0.016	3.9	1.95	0.97
	Gentamicin	0.5	12	6	3
	Levofloxacin	0.06	9.5	4.75	2.38
	Meropenem	0.06	50	25	12.5
	Piperacillin-tazobactam	2	368/23	184/11.5	92/5.8
	Tigecycline	0.12	1.5	0.75	0.37
<i>Klebsiella pneumoniae</i> (ATCC 700603)	Ceftaroline	0.5	39	19.5	9.75
	Ciprofloxacin	0.25	3.9	1.95	0.97
	Gentamicin	2	12	6	3
	Levofloxacin	0.06	9.5	4.75	2.38
	Meropenem	0.12	50	25	12.5
	Piperacillin-tazobactam	8	368/23	184/11.5	92/5.8
	Tigecycline	0.5	1.5	0.75	0.37
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	Ciprofloxacin	0.5	3.9	1.95	0.97
	Gentamicin	1	12	6	3
	Meropenem	0.25	50	25	12.5
	Piperacillin-tazobactam	4	368/23	184/11.5	92/5.75
Gram-positive organism					
<i>Enterococcus faecalis</i> (ATCC 29212)	Ampicillin	0.5	47	23.5	11.75
	Linezolid	2	20	10	5
	Tigecycline	0.12	1.5	0.75	0.37
	Vancomycin	2	50	25	12.5
Methicillin-susceptible <i>Staphylococcus aureus</i> (ATCC 29213)	Ceftaroline	0.12	39	19.5	9.75
	Daptomycin	0.25	57	28.5	14.25
	Linezolid	2	20	10	5
	Tigecycline	0.06	1.5	0.75	0.37
	Vancomycin	0.5	50	25	12.5
Methicillin-resistant <i>Staphylococcus aureus</i> (ATCC 43300)	Ceftaroline	0.5	39	19.5	9.75
	Ciprofloxacin	0.5	3.9	1.95	0.97
	Gentamicin	1	12	6	3
	Linezolid	2	20	10	5
	Tigecycline	0.25	1.5	0.75	0.37
	Vancomycin	1	50	25	12.5
<i>Streptococcus pneumoniae</i> (ATCC 49619)	Ciprofloxacin	0.12	3.9	1.95	0.97
	Linezolid	0.5	20	10	5
	Penicillin G	0.25	40	20	10
	Vancomycin	0.12	50	25	12.5

^aMICs of the antimicrobial drugs for each organism tested were determined by the EUCAST broth microdilution method according to standard procedures (18).

^bFor each antimicrobial drug tested, concentrations corresponding to the plasma peak (C_{max}), half (C/2), and quarter (C/4) levels are indicated. The C_{max} values were those reported in online documents by EUCAST (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf).

bottles. The TTD for bottles that were negative at 5 days was expressed as >120 h. Thus, we compared the rates of detection and the differences in TTDs between BC systems and between aerobic (BacT/Alert FA Plus and Bactec Plus Aerobic/F) and anaerobic (BacT/Alert FN Plus and Bactec Plus Anaerobic/F) bottles with the McNemar's test or the paired *t* test, as appropriate. We considered all differences with *P* values of <0.05 statistically significant.

We obtained 1,752 BacT/Alert (FA Plus and FN Plus) or Bactec (Aerobic/F Plus and Anaerobic/F Plus) media-containing BC bottles to study 13 antibiotic and 8 bacterial organism combinations (Table 1). Of the bottles, 888 were aerobic (spiked with all the

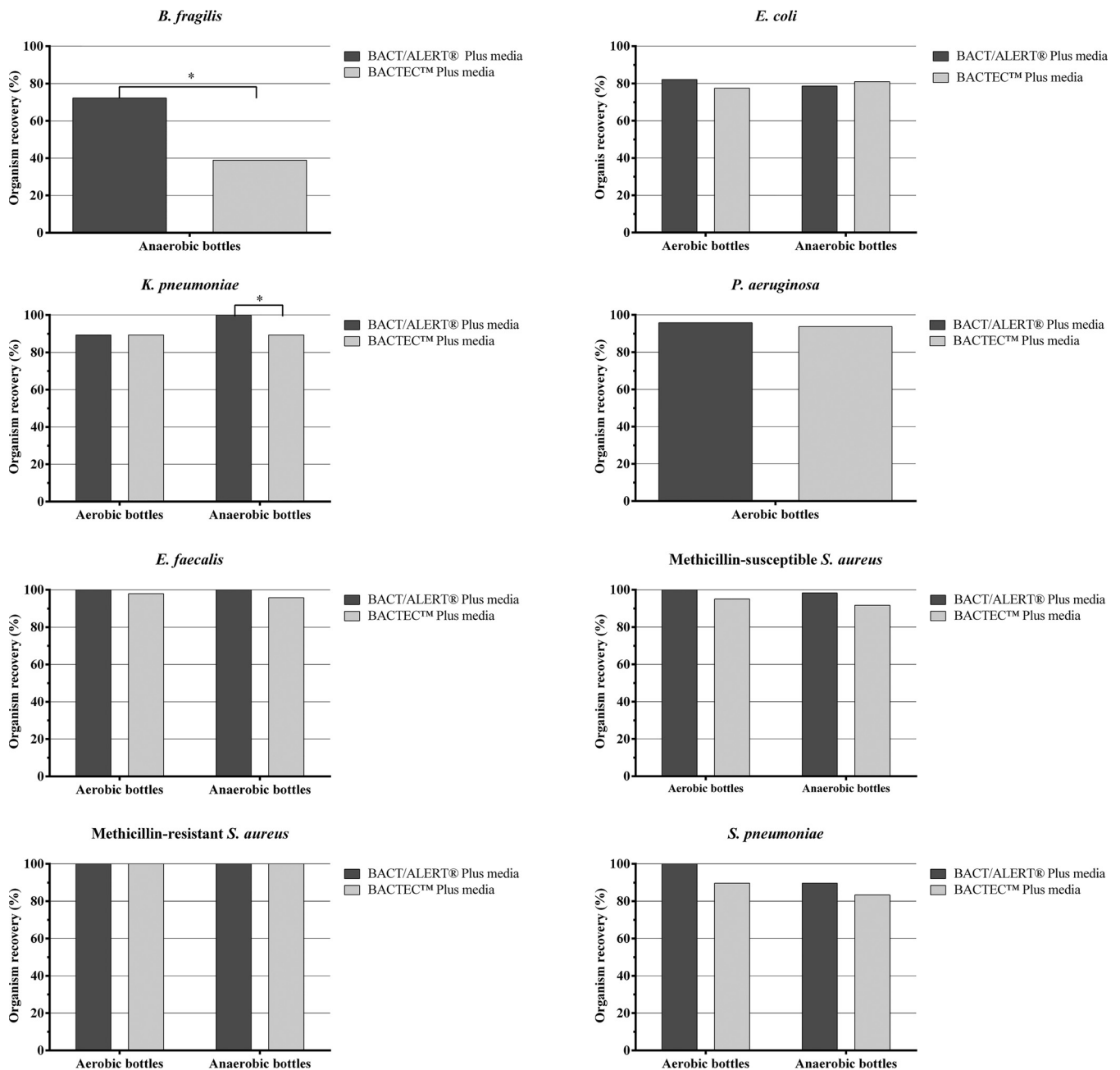


FIG 1 Percent recovery in aerobic (BacT/Alert FA Plus and Bactec Aerobic/F Plus) or anaerobic (BacT/Alert FN Plus and Bactec Anaerobic/F Plus) bottles containing antibiotics by organism.

organisms except for *B. fragilis*), and 864 were anaerobic (spiked with all the organisms except for *P. aeruginosa*). The overall percent recovery of organisms with BacT/Alert Plus media was 93.2% (816/876 bottles) and with Bactec Plus media was 88.4% (774/876 bottles). This overall difference was statistically significant ($P < 0.001$). Statistically significant differences were also evident between BacT/Alert and Bactec media in aerobic bottles (94.2% [418/444] versus 90.9% [404/444]; $P < 0.001$) and anaerobic bottles (92.1% [398/432] versus 85.6% [370/432]; $P < 0.001$), respectively. Of BC bottles without antibiotics (drug-free controls), 100% (480/480) yielded all the organisms tested. We further stratified the results by organism type (Fig. 1). Differences were statistically significant in favor of BacT/Alert Plus media, most notably for *B. fragilis* and *Klebsiella pneumoniae* in anaerobic BC bottles ($P < 0.05$). We did not observe statisti-

cally significant differences for *Escherichia coli*, *P. aeruginosa*, *Enterococcus faecalis*, methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), or *Streptococcus pneumoniae* organisms.

Tables 2 and 3 summarize the TTDs for the aforementioned Gram-negative and Gram-positive bacterial species, respectively, in both BacT/Alert and Bactec aerobic and anaerobic Plus media with antibiotics compared with the same media without antibiotics. The highest Δ TTDs (≥ 3 h) were noticed for meropenem-*P. aeruginosa* (aerobic), meropenem-*K. pneumoniae* (anaerobic), and piperacillin-tazobactam-*B. fragilis* (anaerobic) in BacT/Alert FAN Plus media and for meropenem-*P. aeruginosa* (aerobic) and ceftazidime-*E. coli* (anaerobic) in Bactec Plus media for Gram-negative organisms recovered at the antibiotic C_{\max} (Table 2). The highest Δ TTDs (≥ 3 h) in BacT/Alert or Bactec media, respectively, were noted for ampicillin-*E. faecalis* (aerobic/anaerobic), vancomycin-MSSA (aerobic/anaerobic), ceftazidime-MRSA (anaerobic or aerobic/anaerobic), tigecycline-MRSA (anaerobic or aerobic), and vancomycin-MRSA (aerobic/anaerobic) for Gram-positive organisms recovered at the antibiotic C_{\max} (Table 3). Furthermore, the highest Δ TTDs were evident for linezolid-*S. pneumoniae* (anaerobic), gentamicin-MRSA (anaerobic), and ciprofloxacin-MRSA (anaerobic) in only BacT/Alert FAN Plus media and for linezolid-MRSA (aerobic/anaerobic) in only Bactec Plus media. Interestingly, at the antibiotic C/4 ($1/4 C_{\max}$), Δ TTDs for the aforementioned antibiotic-Gram-negative organism combinations were nearly zero (Table 2), whereas Δ TTDs for the aforementioned antibiotic-Gram-positive organism combinations had values much higher than zero (Table 3). The only exceptions were ampicillin-*E. faecalis* and linezolid-*S. pneumoniae* combinations. More interestingly, only Δ TTDs for meropenem tested with *E. coli* and (only in aerobic media) *K. pneumoniae* at the three drug concentrations remained unmeasured, because no growth was detected in both BacT/Alert and Bactec media (Table 2). Finally, overall TTDs (aerobic/anaerobic) in BacT/Alert FAN Plus media were significantly shorter than in Bactec Plus media for both Gram-negative (11.08/11.85 h versus 13.28/14.77 h; $P < 0.0001$, for all comparisons) and Gram-positive (11.86/12.06 h versus 14.30/15.36 h; $P < 0.0001$, for all comparisons) bacterial species.

Our findings agree with the recent findings of other researchers that the present *ex vivo* (using human blood and simulated antibiotic concentrations)/*in vitro* study provides further support for a worthwhile use of BacT/Alert (FA Plus and FN Plus) or Bactec (Plus Aerobic/F and Plus Anaerobic/F) antimicrobial-inactivating BC media (13, 14). We tested a range of Gram-negative and Gram-positive bacterial species against clinically effective concentrations of three to seven antibiotics per species. Apart from the peak (P) concentration (i.e., C_{\max}), we selected for each antibiotic two descending concentrations (i.e., C/2 and C/4) that were higher than the midpoint (M) and/or through (T) concentrations tested elsewhere (8, 9, 14). Therefore, consistent with the study by Mitteregger et al. (10), we ensured that the antibiotic levels of C/2- or C/4-spiked BCs were still higher than the expected antibiotic-inactivating capability for aerobic/anaerobic BC media so that organism recoveries could occur in BC bottles containing still-active (above the MIC) antibiotic concentrations. Importantly, aside from the overall high recovery rates by both systems (93.2% and 88.4%, respectively), we showed that either BacT/Alert (FA Plus and FN Plus) or Bactec (Plus Aerobic/F and Plus Anaerobic/F) media allowed for the recovery of all organisms tested at the antibiotic C/4 in ≥ 1 replicate of 40 antibiotic-organism combinations (Tables 2 and 3). The only exceptions were that *E. coli* in the presence of C/4 meropenem failed to grow in any aerobic or anaerobic bottle, and *K. pneumoniae* in the presence of C/4 meropenem and *B. fragilis* in the presence of C/4 metronidazole grew only in BacT/Alert FN Plus. In fact, BacT/Alert FN Plus was able to recover *K. pneumoniae* isolates at all levels of meropenem, not just at C/4. In the study by Grupper et al. (14), *P. aeruginosa* ATCC 27853, the sole bacterial species tested and able to be compared, grew in < 26 h in all BacT/Alert FA Plus and Bactec Plus Aerobic/F bottles in the presence of meropenem T concentrations. In our study, the same species grew in ~ 13 and ~ 17 h in all BacT/Alert FA Plus and Bactec Plus Aerobic/F bottles, respectively in the presence of meropenem C/4 concentrations. Despite differences in the bacterial inoculum between that study and ours (7 to 30 and

TABLE 2 Times to detection of Gram-negative organisms recovered from BacT/Alert or Bactec aerobic (AE) and anaerobic (ANA) Plus media at each antimicrobial drug concentration tested in blood culture triplicates

Organism and drug	Concentration ^a	BacT/Alert Plus media						Bactec Plus media					
		Recovery (no. of replicates/ total)		Mean TTD ^b (h)		ΔTTD ^c (h)		Recovery		Mean TTD ^b (h)		ΔTTD ^c (h)	
		AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA
<i>B. fragilis</i>													
Meropenem	C _{max}	– ^d	3/3	–	30.87	–	2.35	–	0/3	–	>120	–	NA ^e
	C/2	–	3/3	–	29.32	–	0.80	–	0/3	–	>120	–	NA
	C/4	–	3/3	–	27.49	–	–1.03	–	1/3	–	33.26	–	1.03
	No drug	–	3/3	–	28.52	–	–	–	3/3	–	32.23	–	–
Metronidazole	C _{max}	–	0/3	–	>120	–	NA	–	0/3	–	>120	–	NA
	C/2	–	0/3	–	>120	–	NA	–	0/3	–	>120	–	NA
	C/4	–	1/3	–	36.08	–	5.62	–	0/3	–	>120	–	NA
	No drug	–	3/3	–	30.46	–	–	–	3/3	–	33.11	–	–
Piperacillin-tazobactam	C _{max}	–	1/3	–	32.59	–	3.57	–	0/3	–	>120	–	NA
	C/2	–	3/3	–	29.54	–	0.52	–	1/3	–	48.00	–	14.36
	C/4	–	3/3	–	28.52	–	–0.5	–	3/3	–	46.30	–	12.66
	No drug	–	3/3	–	29.02	–	–	–	3/3	–	33.64	–	–
<i>E. coli</i>													
Ceftaroline	C _{max}	3/3	1/3	11.11	11.02	1.75	2.07	0/3	1/3	>120	13.56	NA	3.24
	C/2	3/3	3/3	9.49	10.04	0.13	1.09	1/3	3/3	13.46	12.28	3.00	1.96
	C/4	3/3	3/3	9.28	9.37	–0.08	0.42	3/3	3/3	11.95	10.91	1.49	0.59
	No drug	3/3	3/3	9.36	8.95	–	–	3/3	3/3	10.46	10.32	–	–
Gentamicin	C _{max}	3/3	2/3	9.27	9.20	0.07	–0.08	3/3	3/3	11.15	12.96	0.46	1.71
	C/2	3/3	3/3	9.59	8.75	0.39	–0.53	3/3	3/3	11.12	11.43	0.43	0.18
	C/4	3/3	3/3	9.28	8.38	0.08	–0.9	3/3	3/3	10.72	11.18	0.03	–0.07
	No drug	3/3	3/3	9.20	9.28	–	–	3/3	3/3	10.69	11.25	–	–
Meropenem	C _{max}	0/3	0/3	>120	>120	NA	NA	0/3	0/3	>120	>120	NA	NA
	C/2	0/3	0/3	>120	>120	NA	NA	0/3	0/3	>120	>120	NA	NA
	C/4	0/3	0/3	>120	>120	NA	NA	0/3	0/3	>120	>120	NA	NA
	No drug	3/3	3/3	9.31	8.89	–	–	3/3	3/3	11.19	11.93	–	–
Piperacillin-tazobactam	C _{max}	3/3	3/3	10.35	9.95	0.64	0.80	3/3	2/3	13.69	14.50	2.40	2.14
	C/2	3/3	3/3	10.22	9.42	0.51	0.27	3/3	3/3	13.07	13.43	1.78	1.07
	C/4	3/3	3/3	10.30	9.31	0.59	0.16	3/3	3/3	12.37	12.43	1.08	0.07
	No drug	3/3	3/3	9.71	9.15	–	–	3/3	3/3	11.29	12.36	–	–
Tigecycline	C _{max}	0/3	0/3	>120	>120	NA	NA	0/3	0/3	>120	>120	NA	NA
	C/2	1/3	1/3	10.14	10.30	0.66	1.31	2/3	2/3	12.34	12.92	0.69	0.75
	C/4	2/3	2/3	9.50	10.15	0.02	1.16	2/3	3/3	11.95	12.48	0.30	0.31
	No drug	3/3	3/3	9.48	8.99	–	–	3/3	3/3	11.65	12.17	–	–
Ciprofloxacin	C _{max}	3/3	3/3	10.08	10.14	–0.01	1.52	3/3	3/3	11.40	11.32	0.19	0.58
	C/2	3/3	3/3	9.53	9.40	–0.56	0.78	3/3	3/3	11.18	11.26	–0.03	0.52
	C/4	3/3	3/3	10.09	8.91	0.00	0.29	3/3	3/3	11.18	11.19	–0.03	0.45
	No drug	3/3	3/3	10.09	8.62	–	–	3/3	3/3	11.21	10.74	–	–
Levofloxacin	C _{max}	3/3	3/3	10.21	9.34	0.37	0.28	3/3	3/3	12.15	12.10	0.74	0.73
	C/2	3/3	3/3	10.15	9.35	0.31	0.29	3/3	3/3	11.45	11.45	0.04	0.08
	C/4	3/3	3/3	9.46	8.69	–0.38	–0.37	3/3	3/3	11.48	11.32	0.07	–0.05
	No drug	3/3	3/3	9.84	9.06	–	–	3/3	3/3	11.41	11.37	–	–
<i>K. pneumoniae</i>													
Ceftaroline	C _{max}	3/3	3/3	11.16	11.20	0.12	0.47	3/3	3/3	13.15	13.08	0.98	0.23
	C/2	3/3	3/3	11.22	10.81	0.18	0.08	3/3	3/3	13.26	13.20	1.09	0.35
	C/4	3/3	3/3	11.07	10.57	0.03	–0.16	3/3	3/3	12.52	12.48	0.35	–0.37
	No drug	3/3	3/3	11.04	10.73	–	–	3/3	3/3	12.17	12.85	–	–
Ciprofloxacin	C _{max}	3/3	3/3	10.32	10.45	1.17	0.99	3/3	3/3	11.46	11.26	0.07	–0.11
	C/2	3/3	3/3	10.12	10.19	0.97	0.73	3/3	3/3	11.29	11.30	–0.10	–0.07
	C/4	3/3	3/3	9.57	9.90	0.42	0.44	3/3	3/3	11.38	11.28	–0.01	–0.09
	No drug	3/3	3/3	9.15	9.46	–	–	3/3	3/3	11.39	11.37	–	–
Gentamicin	C _{max}	3/3	3/3	10.10	9.85	0.25	0.53	3/3	3/3	11.43	11.39	0.24	0.11
	C/2	3/3	3/3	10.08	9.31	0.23	–0.01	3/3	3/3	11.49	11.38	0.30	0.10
	C/4	3/3	3/3	9.42	9.42	–0.43	0.10	3/3	3/3	11.21	11.19	0.02	–0.09
	No drug	3/3	3/3	9.85	9.32	–	–	3/3	3/3	11.19	11.28	–	–

(Continued on next page)

TABLE 2 (Continued)

Organism and drug	Concentration ^a	BacT/Alert Plus media						Bactec Plus media					
		Recovery (no. of replicates/total)		Mean TTD ^b (h)		ΔTTD ^c (h)		Recovery		Mean TTD ^b (h)		ΔTTD ^c (h)	
		AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA
Levofloxacin	C _{max}	3/3	3/3	10.61	10.30	0.33	-0.10	3/3	3/3	12.40	12.88	-0.01	0.53
	C/2	3/3	3/3	10.31	10.30	0.03	-0.10	3/3	3/3	12.37	12.43	-0.04	0.08
	C/4	3/3	3/3	10.16	10.39	-0.12	-0.01	3/3	3/3	12.19	12.35	-0.22	0.00
	No drug	3/3	3/3	10.28	10.40			3/3	3/3	12.41	12.35		
Meropenem	C _{max}	0/3	3/3	>120	14.92	NA	4.67	0/3	0/3	>120	>120	NA	NA
	C/2	0/3	3/3	>120	12.75	NA	2.50	0/3	0/3	>120	>120	NA	NA
	C/4	0/3	3/3	>120	11.60	NA	1.35	0/3	0/3	>120	>120	NA	NA
	No drug	3/3	3/3	10.14	10.25			3/3	3/3	12.28	12.55		
Piperacillin-tazobactam	C _{max}	3/3	3/3	9.46	9.43	0.07	0.11	3/3	3/3	11.37	10.87	1.08	-0.08
	C/2	3/3	3/3	9.92	9.44	0.53	0.12	3/3	3/3	10.84	10.87	0.55	-0.08
	C/4	3/3	3/3	9.27	9.13	-0.12	-0.19	3/3	3/3	10.42	10.75	0.13	-0.20
	No drug	3/3	3/3	9.39	9.32			3/3	3/3	10.29	10.95		
Tigecycline	C _{max}	3/3	3/3	11.16	11.20	0.28	0.36	3/3	3/3	13.15	13.08	0.87	0.81
	C/2	3/3	3/3	11.22	10.81	0.34	-0.03	3/3	3/3	13.26	13.20	0.98	0.93
	C/4	3/3	3/3	11.07	10.57	0.19	-0.27	3/3	3/3	12.52	12.48	0.24	0.21
	No drug	3/3	3/3	10.88	10.84			3/3	3/3	12.28	12.27		
<i>P. aeruginosa</i> Ciprofloxacin	C _{max}	3/3	-	15.33	-	1.89	-	1/3	-	17.15	-	1.23	-
	C/2	3/3	-	14.86	-	1.42	-	3/3	-	16.40	-	0.48	-
	C/4	3/3	-	14.75	-	1.31	-	3/3	-	16.07	-	0.15	-
	No drug	3/3	-	13.44	-			3/3	-	15.92	-		
Gentamicin	C _{max}	1/3	-	14.36	-	1.22	-	2/3	-	16.20	-	0.54	-
	C/2	3/3	-	13.54	-	0.40	-	3/3	-	15.33	-	-0.33	-
	C/4	3/3	-	13.19	-	0.05	-	3/3	-	15.07	-	-0.59	-
	No drug	3/3	-	13.14	-			3/3	-	15.66	-		
Meropenem	C _{max}	3/3	-	19.48	-	5.91	-	3/3	-	37.01	-	19.77	-
	C/2	3/3	-	14.32	-	0.75	-	3/3	-	20.14	-	2.90	-
	C/4	3/3	-	13.59	-	0.02	-	3/3	-	17.27	-	0.03	-
	No drug	3/3	-	13.57	-			3/3	-	17.24	-		
Piperacillin-tazobactam	C _{max}	3/3	-	14.20	-	1.03	-	3/3	-	16.80	-	1.55	-
	C/2	3/3	-	13.96	-	0.79	-	3/3	-	16.26	-	1.01	-
	C/4	3/3	-	13.34	-	0.17	-	3/3	-	16.14	-	0.89	-
	No drug	3/3	-	13.17	-			3/3	-	15.25	-		

^aFor each antibiotic tested, C_{max}, C/2, and C/4 drug concentrations are those specified in Table 1.

^bFor each organism-antibiotic combination tested, the mean time to detection (TTD) was calculated by summing the TTDs of single blood culture replicates. TTDs values are rounded to the nearest decimal.

^cFor each organism-antibiotic combination tested, ΔTTD is the difference of TTD of the antibiotic-containing blood culture by that of the antibiotic-free blood culture (no-drug control). Boldface values are the clinically significant ΔTTD values (≥3 h) obtained for some organism-antibiotic combinations at the C_{max}, C/2, and/or C/4 tested.

^d-, Lack of data because the organism was cultured only in anaerobic (ANA) (*B. fragilis*) or aerobic (AE) (*P. aeruginosa*) bottles.

^eNA, not applicable (no organism growth in any bottle).

50 to 100 CFU/bottle, respectively), we could equitably compare our results with those of Grupper et al. (14). Conversely, Mitteregger et al. (10) showed the recovery of meropenem-susceptible *P. aeruginosa* isolates (MIC, 0.094 μg/ml) in BacT/Alert FA Plus bottles containing meropenem P concentrations. If a larger inoculum (up to 100 CFU/ml) in the study by Mitteregger et al. contributed to these conflicting findings, it remains unknown. In one clinical study, Zadroga et al. (11) assessed the recovery of bacteria in patient BCs in relation to prior antimicrobial administration. Surprisingly, the researchers reported residual piperacillin concentrations above the MIC in BacT/Alert FAN bottles, which contain activated charcoal instead of the polymeric beads found in the FA Plus and FN Plus bottles, sampled near the P or M and not in those sampled near the T concentration. Notably, Zadroga et al. (11) observed the recovery of bacteria in Bactec Plus BC bottles from the same patients who were administered piperacillin-tazobactam before collection. Taken together, these data suggest that the variability in

TABLE 3 Times to detection of Gram-positive organisms recovered from BacT/Alert or Bactec aerobic (AE) and anaerobic (ANA) Plus media at each antimicrobial drug concentration tested in blood culture triplicates

Organism and drug	Concentration ^a	BacT/Alert Plus media						Bactec Plus media					
		Recovery (no. of replicates/ total)		Mean TTD ^b		ΔTTD ^c		Recovery		Mean TTD ^b		ΔTTD ^c	
		AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA
<i>E. faecalis</i>													
Ampicillin	C _{max}	3/3	3/3	18.75	17.79	9.08	8.06	2/3	1/3	15.28	16.50	3.17	4.23
	C/2	3/3	3/3	11.28	11.30	1.61	1.57	3/3	3/3	13.97	14.35	1.86	2.08
	C/4	3/3	3/3	10.95	10.32	1.28	0.59	3/3	3/3	12.28	13.29	0.17	1.02
	No drug	3/3	3/3	9.67	9.73			3/3	3/3	12.11	12.27		
Linezolid	C _{max}	3/3	3/3	9.66	9.81	0.20	-0.11	3/3	3/3	11.30	11.52	-0.01	0.14
	C/2	3/3	3/3	9.28	9.27	-0.18	-0.65	3/3	3/3	11.25	11.54	-0.06	0.16
	C/4	3/3	3/3	9.15	9.24	-0.31	-0.68	3/3	3/3	10.50	11.38	-0.81	0.00
	No drug	3/3	3/3	9.46	9.92			3/3	3/3	11.31	11.38		
Tigecycline	C _{max}	3/3	3/3	10.53	11.38	1.04	2.16	3/3	3/3	13.39	13.45	2.03	2.26
	C/2	3/3	3/3	9.32	9.84	-0.17	0.62	3/3	3/3	11.93	12.29	0.57	1.10
	C/4	3/3	3/3	9.21	9.27	-0.28	0.05	3/3	3/3	11.09	11.44	-0.27	0.25
	No drug	3/3	3/3	9.49	9.22			3/3	3/3	11.36	11.19		
Vancomycin	C _{max}	3/3	3/3	10.99	10.70	1.31	0.72	3/3	3/3	12.85	13.71	0.75	1.62
	C/2	3/3	3/3	10.86	10.42	1.18	0.44	3/3	3/3	12.39	13.40	0.29	1.31
	C/4	3/3	3/3	10.90	10.66	1.22	0.68	3/3	3/3	12.28	13.20	0.18	1.11
	No drug	3/3	3/3	9.68	9.98			3/3	3/3	12.10	12.09		
Methicillin-susceptible <i>S. aureus</i>													
Ceftaroline	C _{max}	3/3	3/3	11.82	12.20	0.89	2.73	0/3	0/3	>120	>120	NA ^d	NA
	C/2	3/3	3/3	10.94	10.45	0.01	0.98	3/3	3/3	16.93	16.77	5.45	4.32
	C/4	3/3	3/3	10.36	9.71	-0.57	0.24	3/3	3/3	14.34	13.78	2.86	1.33
	No drug	3/3	3/3	10.93	9.47			3/3	3/3	11.48	12.45		
Daptomycin	C _{max}	3/3	3/3	10.20	9.54	-0.02	0.48	3/3	3/3	12.76	12.62	1.33	0.29
	C/2	3/3	3/3	10.30	9.25	0.08	0.19	3/3	3/3	11.77	12.33	0.34	0.00
	C/4	3/3	3/3	10.40	9.16	0.18	0.10	3/3	3/3	11.43	12.40	0.00	0.07
	No drug	3/3	3/3	10.22	9.06			3/3	3/3	11.43	12.33		
Linezolid	C _{max}	3/3	3/3	11.70	10.49	1.52	1.24	3/3	3/3	11.97	13.08	0.61	1.08
	C/2	3/3	3/3	11.90	10.17	1.72	0.92	3/3	3/3	12.11	13.21	0.75	1.21
	C/4	3/3	3/3	10.46	9.83	0.28	0.58	3/3	3/3	11.46	12.38	0.10	0.38
	No drug	3/3	3/3	10.18	9.25			3/3	3/3	11.36	12.00		
Tigecycline	C _{max}	3/3	3/3	10.94	10.90	-0.18	0.88	3/3	2/3	12.28	12.85	0.66	0.29
	C/2	3/3	3/3	10.89	9.90	-0.23	-0.12	3/3	3/3	11.49	13.30	-0.13	0.74
	C/4	3/3	3/3	11.07	9.89	-0.05	-0.13	3/3	3/3	11.34	12.95	-0.28	0.39
	No drug	3/3	3/3	11.12	10.02			3/3	3/3	11.62	12.56		
Vancomycin	C _{max}	3/3	2/3	15.52	13.41	3.51	3.51	3/3	2/3	25.49	36.32	12.53	23.71
	C/2	3/3	3/3	14.57	13.24	3.34	3.34	3/3	3/3	21.45	23.94	8.49	11.33
	C/4	3/3	3/3	13.92	12.05	2.15	2.15	3/3	3/3	18.23	18.82	5.27	6.21
	No drug	3/3	3/3	11.19	9.90			3/3	3/3	12.96	12.61		
Methicillin-resistant <i>S. aureus</i>													
Ceftaroline	C _{max}	3/3	3/3	13.88	19.84	1.32	9.21	3/3	3/3	17.64	21.78	4.34	7.27
	C/2	3/3	3/3	13.84	17.60	1.28	6.97	3/3	3/3	17.35	18.28	4.05	3.77
	C/4	3/3	3/3	13.23	16.14	0.67	5.51	3/3	3/3	15.96	16.80	2.66	2.29
	No drug	3/3	3/3	12.56	10.63			3/3	3/3	13.30	14.51		
Ciprofloxacin	C _{max}	3/3	3/3	13.62	16.32	1.93	6.00	3/3	3/3	13.63	14.84	0.08	-0.06
	C/2	3/3	3/3	12.51	15.08	0.82	4.76	3/3	3/3	13.37	14.77	-0.18	-0.13
	C/4	3/3	3/3	12.55	14.20	0.86	3.88	3/3	3/3	13.32	14.32	-0.23	-0.58
	No drug	3/3	3/3	11.69	10.32			3/3	3/3	13.55	14.90		
Gentamicin	C _{max}	3/3	3/3	13.85	16.66	2.10	6.29	3/3	3/3	13.17	14.29	-0.37	0.10
	C/2	3/3	3/3	12.68	16.40	0.93	6.03	3/3	3/3	13.29	14.07	-0.25	-0.12
	C/4	3/3	3/3	12.55	15.40	0.80	5.03	3/3	3/3	13.20	14.08	-0.34	-0.11
	No drug	3/3	3/3	11.75	10.37			3/3	3/3	13.54	14.19		
Linezolid	C _{max}	3/3	3/3	12.80	12.02	1.37	1.37	3/3	3/3	18.57	19.52	5.27	4.44
	C/2	3/3	3/3	12.90	11.00	0.35	0.35	3/3	3/3	17.65	18.54	4.35	3.46
	C/4	3/3	3/3	11.46	11.09	0.44	0.44	3/3	3/3	16.20	18.47	2.90	3.39
	No drug	3/3	3/3	11.98	10.65			3/3	3/3	13.30	15.08		

(Continued on next page)

TABLE 3 (Continued)

Organism and drug	Concentration ^a	BacT/Alert Plus media						Bactec Plus media					
		Recovery (no. of replicates/ total)		Mean TTD ^b		ΔTTD ^c		Recovery		Mean TTD ^b		ΔTTD ^c	
		AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA	AE	ANA
Tigecycline	C _{max}	3/3	3/3	15.25	14.55	2.33	4.15	3/3	3/3	17.94	18.01	4.27	2.90
	C/2	3/3	3/3	14.46	13.88	1.54	3.48	3/3	3/3	18.15	17.34	4.48	2.23
	C/4	3/3	3/3	13.92	13.22	1.00	2.82	3/3	3/3	17.12	15.42	3.45	0.31
	No drug	3/3	3/3	12.92	10.40			3/3	3/3	13.67	15.11		
Vancomycin	C _{max}	3/3	3/3	16.19	20.27	4.15	9.69	3/3	3/3	25.26	38.05	11.63	22.48
	C/2	3/3	3/3	15.46	14.80	3.42	4.22	3/3	3/3	22.44	26.61	8.81	11.04
	C/4	3/3	3/3	14.00	13.89	1.96	3.31	3/3	3/3	20.33	19.22	6.70	3.65
	No drug	3/3	3/3	12.04	10.58			3/3	3/3	13.63	15.57		
<i>S. pneumoniae</i> Ciprofloxacin	C _{max}	3/3	3/3	12.42	12.24	1.19	0.99	3/3	3/3	13.55	13.51	0.48	0.13
	C/2	3/3	3/3	12.20	11.26	0.97	0.01	3/3	3/3	13.17	13.47	0.10	0.09
	C/4	3/3	3/3	11.88	11.24	0.65	-0.01	3/3	3/3	13.05	13.36	-0.02	-0.02
	No drug	3/3	3/3	11.23	11.25			3/3	3/3	13.07	13.38		
Linezolid	C _{max}	3/3	3/3	11.26	19.51	0.19	7.13	3/3	3/3	13.66	16.40	0.53	2.42
	C/2	3/3	3/3	11.89	13.83	0.82	1.45	3/3	3/3	13.29	14.81	0.16	0.83
	C/4	3/3	3/3	11.74	12.41	0.67	0.03	3/3	3/3	13.19	15.12	0.06	1.14
	No drug	3/3	3/3	11.07	12.38			3/3	3/3	13.13	13.98		
Penicillin G	C _{max}	3/3	3/3	12.50	14.95	1.63	2.52	3/3	2/3	16.71	17.40	2.60	2.95
	C/2	3/3	3/3	11.32	12.52	0.45	0.09	3/3	3/3	15.13	15.88	1.02	1.43
	C/4	3/3	3/3	11.63	12.26	0.76	-0.17	3/3	3/3	15.03	15.30	0.92	0.85
	No drug	3/3	3/3	10.87	12.43			3/3	3/3	14.11	14.45		
Vancomycin	C _{max}	3/3	0/3	11.32	>120	0.14	NA	0/3	0/3	>120	>120	NA	NA
	C/2	3/3	2/3	11.22	12.25	0.04	0.22	1/3	0/3	19.50	>120	7.06	NA
	C/4	3/3	2/3	11.28	12.19	0.10	0.16	3/3	2/3	16.43	17.35	3.99	4.25
	No drug	3/3	3/3	11.18	12.03			3/3	3/3	12.44	13.10		

^aFor each antibiotic tested, C_{max}, C/2, and C/4 are those specified in Table 1.

^bFor each organism-antibiotic combination tested, the mean time to detection (TTD) was calculated by summing the TTDs of single blood culture replicates. TTDs are rounded to the nearest decimal.

^cFor each organism-antibiotic combination tested, ΔTTD is the difference of TTD of the antibiotic-containing blood culture by that of the antibiotic-free blood culture (no-drug control). Boldface values are the clinically significant ΔTTD values (≥3 h) obtained for some organism-antibiotic combinations at the C_{max}, C/2, and/or C/4 tested.

^dNA, not applicable (no organism growth in any bottles).

BC performances across the studies may not only depend on antimicrobial-binding media but also on combinations of bacteria and antibiotics in BCs (16).

In terms of TTD, the other parameter evaluated here, at the antibiotic C/4, delays ≥3 h with respect to detection in control (antibiotic-free) bottles were noticed in 12 bacterium-antibiotic pairs across all Gram-positive bacteria except for *E. faecalis*. Among Gram-negative bacteria, *B. fragilis* with metronidazole (BacT/Alert FN Plus) and piperacillin-tazobactam (Bactec Plus Anaerobic/F) also showed delays of ≥3 h. Eight (66.6%) of the 12 Gram-positive cases included MRSA tested with ceftaroline, ciprofloxacin, gentamicin, or vancomycin in BacT/Alert FN Plus; linezolid or vancomycin in Bactec Plus Anaerobic/F; tigecycline in Bactec Plus Aerobic/F; and vancomycin in both Bactec Plus Aerobic/F and Plus Anaerobic/F. Two (16.7%) cases involved MSSA, and 2 (16.7%) involved *S. pneumoniae*, all tested with vancomycin in both Bactec Plus Aerobic/F and Plus Anaerobic/F. Looking at these cases, differences in TTD did not favor the Bactec Plus Aerobic/F and/or Plus Anaerobic/F media for 8 drug-organism combinations and BacT/Alert FN Plus media for 4 combinations. Notably, 7 (58.3%) of 12 cases involved vancomycin, i.e., 2 with MSSA, 3 with MRSA, and 2 with *S. pneumoniae*. Thus, it is plausible that the type/species of bacterium and/or its interaction with a specific antibiotic in a complex milieu such as that of the BC bottle can affect the outcome of antibiotic inactivation by the resin within the BC bottle media.

In conclusion, within the limitations of a simulated study (16), we showed a relative rather than an absolute advantage for BacT/Alert (FA Plus and FN Plus) media compared

with Bactec (Plus Aerobic/F and Plus Anaerobic/F) media. At concentrations almost equivalent to the lowest possible levels of antibiotics in plasma, both BacT/Alert and Bactec Plus media were efficient against 13 antibiotics, with the exception of *E. coli* with meropenem in all bottles and *K. pneumoniae* with meropenem in BacT/Alert FA Plus and Bactec Plus Aerobic/F and Plus Anaerobic/F bottles. Clinically relevant delays in TTD (≥ 3 h) were noticed with vancomycin, particularly when tested in Bactec Plus media. Ultimately, optimizing the time of draw for and the collection of BCs (i.e., just before the next antibiotic dose and with complete aerobic and anaerobic sets) may help maximize the efficiency of BacT/Alert or Bactec Plus media for the recovery of bloodstream bacterial pathogens.

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