JCM
Journals.ASM.org

Performance of Two Resin-Containing Blood Culture Media in Detection of Bloodstream Infections and in Direct Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) Broth Assays for Isolate Identification: Clinical Comparison of the BacT/Alert Plus and Bactec Plus Systems

Barbara Fiori,[a] Tiziana D'Inzeo,[a] Viviana Di Florio,[a] Flavio De Maio,[a] Giulia De Angelis,[a] Alessia Giaquinto,[a] Lara Campana,[b] Eloisa Tanzarella,[c] Mario Tumbarello,[b] Massimo Antonelli,[c] Maurizio Sanguinetti,[a] Teresa Spanu[a]

Institutes of Microbiology[a] and Infectious Diseases[b] and Department of Clinical Anesthesiology and Intensive Care,[c] Catholic University of the Sacred Heart, Gemelli Hospital, Rome, Italy

We compared the clinical performances of the BacT/Alert Plus (bioMérieux) and Bactec Plus (Becton Dickinson) aerobic and anaerobic blood culture (BC) media with adsorbent polymeric beads. Patients ≥16 years old with suspected bloodstream infections (BSIs) were enrolled in intensive care units and infectious disease wards. A single 40-ml blood sample was collected from each and used to inoculate (10 ml/bottle) one set of BacT/Alert Plus cultures and one set of Bactec Plus cultures, each set consisting of one aerobic and one anaerobic bottle. Cultures were incubated ≤5 days in the BacT/Alert 3D and Bactec FX instruments, respectively. A total of 128 unique BSI episodes were identified based on the recovery of clinically significant growth in 212 aerobic cultures (106 BacT/Alert and 106 Bactec) and 151 anaerobic cultures (82 BacT/Alert and 69 Bactec). The BacT/Alert aerobic medium had higher recovery rates for Gram-positive cocci ($P = 0.024$), whereas the Bactec aerobic medium was superior for recovery of Gram-negative bacilli ($P = 0.006$). BacT/Alert anaerobic medium recovery rates exceeded those of the Bactec anaerobic medium for total organisms ($P = 0.003$), Gram-positive cocci ($P = 0.013$), and *Escherichia coli* ($P = 0.030$). In terms of capacity for diagnosing the 128 septic episodes, the BacT/Alert and Bactec sets were comparable, although the former sets diagnosed more BSIs caused by Gram-positive cocci ($P = 0.008$). They also allowed earlier identification of coagulase-negative staphylococcal growth (mean, 2.8 h; $P = 0.003$) and growth in samples from patients not on antimicrobial therapy that yielded positive results (mean, 1.3 h; $P < 0.001$). Similarly high percentages of microorganisms in BacT/Alert and Bactec cultures (93.8% and 93.3%, respectively) were identified by direct matrix-assisted laser desorption ionization–time of flight mass spectrometry assay of BC broths. The BacT/Alert Plus media line appears to be a reliable, timesaving tool for routine detection of BSIs in the population we studied, although further studies are needed to evaluate their performance in other settings.

Throughout the world, the number of patients at risk for bloodstream infections (BSIs) continues to rise (1). BSIs are associated with high rates of morbidity and mortality, and they markedly increase the costs of hospital care (1, 2). Prompt identification of the causative agent(s) and rapid initiation of appropriate antimicrobial therapy are critical for reducing mortality, especially in patients with septic shock (2–4). Blood culture (BC) remains the gold standard for diagnosing BSIs (5, 6). Over the decades, improvements in culture media and the availability of software-assisted, automated growth detectors have enhanced the recovery of bloodstream pathogens and decreased the time to detection (TTD) of microbial growth. The time-consuming process of isolate identification with conventional culture-based methods has also been improved, thanks to the development of new methods that can be used directly on broth samples from BC bottles, such as matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) (7). Various studies have shown this approach to be a reliable, cost-effective, timesaving alternative for routine identification of bacteria and yeasts causing BSIs (7–10). Its routine use can increase the proportion of patients who receive effective antimicrobial treatment within 24 h of BC positivity, and its potential impact on outcome is even greater than that of Gram-stain reporting (4, 11).

Despite these advances, however, limitations persist. One of the most important involves the diagnostic performance of BCs collected from patients who are already on antimicrobial therapy. In up to 87% of patients with severe sepsis, empirical antimicrobial therapy is started before blood samples for cultures are drawn, and this practice can reduce or delay pathogen recovery (12–15). Increasing the diagnostic yield of positive BCs in this setting would allow more effective management of a highly vulnerable patient population (12, 16).

To this end, several manufacturers have designed BC media containing resins (Bactec Plus Aerobic/F and Anaerobic/F bottles; Becton Dickinson Instrument Systems, Sparks, MD) or charcoal

Received 22 April 2014 Returned for modification 14 May 2014
Accepted 11 July 2014
Published ahead of print 16 July 2014
Editor: K. C. Carroll
Address correspondence to Maurizio Sanguinetti, msanguinetti@rm.unicatt.it.
B.F. and T.D. contributed equally to this article.

doi:10.1128/JCM.01171-14

(BacT/Alert FAN, FA, and FN bottles; bioMérieux, Marcy l'Etoile, France) designed to adsorb antimicrobial drugs present in the blood. The ability of these media to improve the detection of bacteremia and fungemia in patients on antimicrobial therapy has been widely documented (13, 14, 17–28). In earlier studies, resin- and charcoal-based systems displayed similar recovery rates and TTDs, regardless of whether antibiotics were being administered when the BCs were drawn (13, 27, 28), but two more recent studies suggested that, in the presence of antibiotics, resin-containing media are superior (14, 15). In several studies, both types of media improved recovery and reduced the TTDs of significant pathogens in simulated BCs inoculated with blood containing therapeutic levels of commonly used antibiotics and antifungal agents (29–37). However, significant differences emerged between the resin- and charcoal-based media relative to specific microorganism-antimicrobial combinations. Charcoal particles also appear to interfere with the reading of Gram-stained smears, and charcoal-containing broths must be subjected to more complex, time-consuming extraction protocols before undergoing direct MALDI-TOF MS assay for species-level isolate identification (28, 38, 39).

Three recent studies (40–42) found that the new bioMérieux BacT/Alert FA Plus, FN Plus, and PF Plus BC media, which contain adsorbent polymeric beads, improve and accelerate the detection of bloodstream pathogens compared with standard BacT/Alert media and with BacT/Alert media containing charcoal particles. The present study represents the first attempt to evaluate the performance of the new BacT/Alert FA Plus and FN Plus media compared with Becton Dickinson's Bactec Plus Aerobic/F and Anaerobic/F media, which also contain antimicrobial-binding resins.

(Portions of the data from this study were presented at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, Germany 2013.)

MATERIALS AND METHODS

Design, setting, and population. This prospective study was conducted, with institutional review board authorization (approval no. P/403/CE/2012), from 1 May 2012 through 31 October 2013 at the Catholic University of the Sacred Heart Medical Center, a 1,200-bed tertiary-care hospital in Rome, Italy. The entire medical center is served by a central microbiology laboratory, which is open from 7:00 a.m. to 7:00 p.m., Monday through Saturday. For adult patients with suspected BSIs, the center's standard of care requires the sequential collection at 30-min intervals of at least three sets of aerobic and anaerobic BCs (43–46). For each set, a 20-ml blood sample is collected via a single venipuncture or intravascular line access. Skin or access ports are disinfected with alcohol and povidone iodine. The sample is used to inoculate one Bactec Plus Aerobic/F bottle and one Bactec Plus Anaerobic/F bottle (10 ml of blood each). The bottles are brought to the laboratory and incubated up to 5 days in the Bactec FX automated blood culture instrument. (Cultures arriving when the laboratory is closed are stored at room temperature in accordance with manufacturers' instructions.)

The present study focused on BCs submitted during the study period as part of routine care for patients ≥16 years old who were hospitalized in the medical center's three intensive care units (ICUs) or two infectious disease wards. For each suspected BSI episode, we comparatively analyzed one set of Bactec Plus cultures consisting of 1 Aerobic/F and 1 Anaerobic/F bottle (hereafter referred to as the Bactec set) and one set of BacT/Alert Plus cultures (1 FA Plus [aerobic] and 1 FN Plus [anaerobic] bottle, hereafter referred to as the BacT/Alert set). The staffs of participating wards were given written instructions for proper collection of blood samples.

Blood sample collection and processing of cultures. For study patients, three blood samples were collected as described above. The third sample, which was used in the study, was always drawn percutaneously (for the sake of uniformity), and the amount drawn was increased to 40 ml (bringing the total amount of blood collected per episode to 80 ml). The final sample was divided equally (10 ml/bottle) among the two Bactec Plus bottles and the two BacT/Alert Plus bottles. The order in which the two sets were inoculated was reversed each month. The paired BacT/Alert and Bactec BC sets were sent to the central microbiology laboratory and incubated in the BacT/Alert and Bactec FX automated BC systems, respectively. Submissions were excluded from the study if one or both culture sets were incomplete or if any of the 4 bottles contained less than or more than 10 ml of blood, assessed on the basis of volume markers (5-ml increments) on the bottles.

Isolate identification and *in vitro* antimicrobial susceptibility assays. When the growth index of a bottle was positive, broth aliquots were collected for standard identification studies, which entailed Gram staining (the results of which were immediately reported to the patient's physician), routine subculture, and MALDI-TOF MS (MALDI BioTyper; Bruker Daltonik GmbH, Leipzig, Germany) analysis of culture samples, supplemented when necessary with additional biochemical methods and/or 16S rRNA gene sequencing (5, 47, 48). Bottles flagged as positive that were smear and subculture negative were reincubated. Those still negative at the end of the fifth day were also terminally subcultured. Those that were persistently negative were classified as false-positive detections and excluded from analysis.

A second 8-ml broth sample was prepared for direct analysis in the Bruker MALDI BioTyper (study identification method) using an in-house extraction protocol. In brief, the aliquot was centrifuged (3,500 rpm for 15 min at room temperature [RT]) in an 8-ml Vacuette Z Serum Sep clot activator tube (Greiner Bio-One GmbH, Frickenhausen, Germany). The supernatant was discarded and the sediment was used to make a bacterial suspension adjusted to a McFarland standard of 2. After centrifugation, the resulting pellet was washed twice in 1 ml of pure water, centrifuged, and suspended in 300 μl of pure water plus 900 μl of absolute ethanol. After centrifugation, 30 μl of 70% formic acid plus 30 μl pure acetonitrile were added to the pellet. The solution was thoroughly vortexed and then subjected to a final centrifugation at maximum speed (2 min at RT). The supernatant was collected, and a 1-μl sample was applied in quadruplicate to a steel target plate (Bruker Daltonics, Inc.). The spots were dried, overlaid with 1 μl MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid), and air dried at RT for 5 min.

Mass spectrometry was performed with the Microflex MALDI-TOF MS (Bruker Daltonics GmbH). Captured spectra were analyzed with MALDI BioTyper 3.0 software and compared with those in the BioTyper database (Bruker Daltonics GmbH). Matches were ranked by log identification scores, which ranged from 0 to 3, and the match with the highest score was used for species identification. Identifications were considered valid at the species level when ≥2 of the 4 spectra had log scores of ≥1.9 or when all 4 spectra had log scores of ≥1.2 (7, 48). Identification of yeasts directly from BC bottles was performed as previously described (9). All isolates were subjected to *in vitro* antimicrobial susceptibility assays and the results were classified in accordance with EUCAST breakpoints (document version 3.1, February 2013) (http://www.eucast.org/clinical_breakpoints).

Definitions and data analyses. A septic episode was defined as the recovery from BacT/Alert and/or Bactec BCs of at least one organism, which at the time of the positive culture was judged to be clinically significant by an infectious disease consultant (28, 49). The judgment was based on the results of all three BC sets. Commensal organisms, such as coagulase-negative staphylococci and viridans group *Streptococcus* isolates, were considered clinically relevant pathogens when they were recovered from >2 BCs in patients with clinical manifestations of infection that were not explained by other causes.

TTDs were measured (in hours) from the time bottles were placed in the automated BC system. BC sets were classified as treatment positive if, at the time blood samples were drawn, empirical antimicrobial therapy

TABLE 1 Microorganisms recovered from aerobic BCs performed with BacT/Alert and Bactec culture media[a]

Microorganism (no.)	No. (%) of isolates recovered from indicated aerobic BC bottles			
	BacT/Alert and Bactec[b]	BacT/Alert only	Bactec only	P^c
Gram negative (65)	58 (89.2)	0	7 (10.8)	0.006
Escherichia coli (22)	19 (86.4)	0	3 (13.6)	NS
Klebsiella pneumoniae (14)	13 (92.9)	0	1 (7.1	NS
Morganella morganii (2)	2	0	0	NA
Proteus mirabilis (3)	2	0	1	NA
Pseudomonas aeruginosa (23)	21 (91.3)	0	2 (8.7)	NS
Stenotrophomonas maltophilia (1)	1	0	0	NA
Gram positive (48)	37 (77.1)	9 (18.7)	2 (4.2)	0.024
Enterococcus faecalis (3)	1 (33.3)	2 (66.6)	0	NA
Staphylococcus aureus (19)	14 (73.7)	3 (15.8)	2 (10.5)	NS
Coagulase-negative staphylococci (19)[d]	17 (89.5)	2 (10.5)	0	NS
Streptococcus anginosus (3)	2	1	0	NA
Streptococcus constellatus (1)	1	0	0	NA
Streptococcus pneumoniae (3)	2	1	0	NA
Yeasts (10)	7 (70)	1	2	NS
Candida albicans (7)	5	0	2	NS
Candida parapsilosis (1)	0	1	0	NA
Candida tropicalis (2)	2	0	0	NA
Total isolates (123)	102 (82.9)	10 (8.1)	11 (8.9)	NS
Treatment positive (32)	31	0	1	NS
Treatment negative (91)	71	10	10	NS

[a] Analysis was limited to the 212 aerobic BCs (BacT/Alert FA Plus and Bactec Plus Aerobic/F) that grew clinically relevant microbial species. The terms treatment negative and treatment positive refer to cultures drawn before and after initiation of empirical antimicrobial therapy, respectively (see Materials and Methods). Abbreviations: BC, blood culture; NS, not significant; NA, not applicable. Percentages were not calculated when ≤5 microorganisms were isolated.

[b] Isolates of the same species recovered from BacT/Alert and Bactec bottles in a given episode were counted as 1 isolate.

[c] Statistical significance was assessed by comparing the percentage of isolates recovered from BacT/Alert bottles (column 2 + column 3/column 2 +column 3 + column 4) versus percentage of isolates recovered from Bactec bottles (column 2 + column 4/column 2 +column 3 + column 4) (two-sample test of proportions).

[d] Includes *Staphylococcus epidermidis* ($n = 16$), *Staphylococcus haemolyticus* ($n = 2$), and *Staphylococcus hominis* ($n = 1$).

had already been started with at least one drug to which the isolate(s) subsequently displayed *in vitro* susceptibility.

For each septic episode, the BacT/Alert and Bactec sets were compared at three levels, aerobic bottles versus aerobic bottles, anaerobic bottles versus anaerobic bottles, and complete set versus complete set. The endpoints considered were rates of recovery and TTDs of clinically significant microorganisms, performance under treatment-positive and treatment-negative conditions, medium suitability for direct identification of pathogens using MALDI-TOF MS with the Bruker BioTyper, and (set versus set analysis only) correct diagnosis of BSI episodes.

For each clinically relevant species recovered, the yields of the BacT/Alert and Bactec bottles/sets were classified as concordant when the same species was detected in both. In this case, the two isolates were considered identical and counted as one isolate. Yields were considered discordant when the species was recovered from the BacT/Alert bottle/set alone or the Bactec bottle/set alone. The septic episode itself was ultimately attributed to all noncontaminant species recovered, including those classified as discordant detections. The diagnostic capacity of a BC set (BacT/Alert versus Bactec) was calculated as the percentage of sets with aerobic and/or anaerobic yields demonstrating the involvement of all causative organisms.

For each isolate recovered by the BacT/Alert and/or Bactec system, we compared standard (culture-based) identifications with those generated by direct broth analysis with the Bruker MALDI BioTyper. Discrepancies that emerged were resolved with additional biochemical tests or 16S rRNA gene sequencing (5, 47, 48). The results obtained with the direct broth assay were classified as correct only when they corresponded at the species level with the identification obtained by the standard method.

The two-sample test of proportions was used to assess differences between the BacT/Alert and Bactec systems in terms of isolate recovery rates and rates of correct identifications based on direct Bruker MALDI BioTyper broth assay. Differences between the mean TTDs for the two BC systems were assessed with the Wilcoxon matched-pairs signed-rank tests. Differences were considered statistically significant at P values of <0.05. All statistical analyses were performed with the Intercooled Stata program, version 11, for Windows (Stata Corporation, College Station, TX).

RESULTS

During the 18-month study, the microbiology laboratory received 1,456 paired (BacT/Alert plus Bactec) BC sets from patients who met the inclusion criteria. A total of 424 sets were excluded from analysis because they were incomplete or because one or more bottles contained <10 ml of blood. A total of 1,032 paired BC sets were left for comparative analysis.

Isolate recovery from BC bottles. A total of 411 bottles (216 BacT/Alert bottles and 195 Bactec bottles) were flagged as positive by their automated growth detection systems. Gram stain and subculture results were persistently negative for 7 (3.2%) of the 216 BacT/Alert bottles, which were classified as false positives. Twenty-one (10%) of the remaining 209 positive BacT/Alert bottles and 20 (10.3%) of the 195 Bactec bottles yielded isolates ($n = 48$) classified as contaminants (coagulase-negative staphylococci in most cases).

The rest of the study focused exclusively on the remaining 363 BC bottles with confirmed, clinically significant growth (monomicrobial in 343 [95%] cases). They included 212 aerobic cultures (106 BacT/Alert bottles and 106 Bactec bottles; $P =$ not significant

TABLE 2 Times to detection of aerobic BC positivity for BacT/Alert and Bactec culture bottles[a]

Microorganism(s)	No. of isolates	Times to detection (h) of aerobic BC positivity in:				
		BacT/Alert bottles		Bactec bottles		
		Median	Mean (IQRs)	Median	Mean (IQRs)	P^b
Monomicrobial cultures	90	12.4	14.7 (8.2–19.9)	12.3	15.4 (8.2–19.7)	NS
Gram-negative	48	10.8	12.1 (7.0–15.3)	10.2	12.7 (6.6–14.1)	NS
Escherichia coli	17	6.5	6.8 (5.6–8.1)	6.0	8.5 (4.3–9.0)	NS
Klebsiella pneumoniae	10	10.8	10.8 (8.9–12.2)	10.7	10.7 (8.3–13.1)	NS
Morganella morganii	2	7.8	7.8	8.3	8.3	NA
Pseudomonas aeruginosa	19	17.3	18.0 (13.4–18.2)	15.3	18.0 (12.2–21.2)	NS
Gram positive	36	14.0	16.1 (10.7–22.0)	14.8	16.5 (9.8–24.2)	NS
Enterococcus faecalis	1	21.1	21.1	NA	15.6	NA
Staphylococcus aureus	14	10.8	10.8 (8.2–12.1)	9.4	9.7 (7.9–12.7)	NS
Coagulase negative staphylococci	16	19.9	21.6 (15.1–24.2)	25.0	22.7 (16.9–28.6)	0.003
Streptococcus anginosus	2	21.7	21.7	18.3	18.3	NS
Streptococcus constellatus	1	NA	21.4	NA	20.4	NA
Streptococcus pneumoniae	2	12.3	12.3	11.4	11.4	NA
Yeasts	6	21.8	23.3 (12.5–28.4)	26.9	30.8 (14.5–51.3)	NS
Candida albicans	4	22.4	28.4 (15.6–47.3)	17.4	20.4 (13.1–30.7)	NS
Candida tropicalis	2	27.7	27.7	52.0	51.6	NA
Polymicrobial cultures	12	15.2	21.5 (7.9–32.4)	17.2	18.5 (7.2–28.5)	NS
Total isolates	102	12.4	15.1 (8.3–20.8)	12.3	15.6 (8.1–20.8)	NS
Treatment positive	31	18.0	19.8 (10.2–25.4)	15.2	18.7 (8.9–26.0)	NS
Treatment negative	71	12.0	13.2 (8.0–18.0)	12.1	14.3 (8.1–20.4)	NS

[a] Analysis was limited to the 192 aerobic bottles in which BacT/Alert and Bactec BC yields were concordant at the species level. The terms treatment negative and treatment positive refer to cultures drawn before and after initiation of empirical antimicrobial therapy, respectively (see Materials and Methods). Abbreviations: BC, blood culture; IQR, interquartile range (calculated only when ≥5 isolates were evaluated); NA, not applicable; NS, not significant ($P > 0.05$).
[b] Statistical significance of differences between mean TTDs for BacT/Alert and Bactec bottles (Wilcoxon matched-pairs signed-rank test).

[NS]) and 151 anaerobic cultures (82 BacT/Alert bottles and 69 Bactec bottles; P = NS).

(i) Aerobic cultures. Table 1 shows the 123 clinically significant isolates recovered from the aerobic BC bottles. For most isolates (102 [82.9%]), BacT/Alert and Bactec culture growths were concordant; the other 21 were recovered only from the BacT/Alert bottle (10 [8.1%]) or the Bactec bottle (11 [8.9%]). Gram-positive bacteria grew better in the BacT/Alert bottles (recovery rates, 95.8% versus 81.2% in Bactec bottles; $P = 0.024$), while the Bactec medium had higher recovery rates for Gram-negative bacteria (100% versus 89.2%; $P = 0.006$). At the single-species level, there were no significant differences between the two aerobic media. The BacT/Alert and Bactec media recovery rates were similar for treatment-positive and treatment-negative cultures.

Analysis of the 212 positive aerobic culture bottles revealed virtually identical TTDs for the BacT/Alert bottles ($n = 106$) and the Bactec bottles ($n = 106$) of 16.1 h (median, 14 h; interquartile range [IQR], 8.6 to 21.2 h) and 16.0 h (median, 12.4 h; IQR, 8.3 to 21.8 h), respectively (P = NS). When TTD analysis was restricted to the 192 bottles with concordant growth (Table 2), the BacT/Alert medium was significantly more rapid for detecting coagulase-negative staphylococci ($P = 0.003$).

(ii) Anaerobic cultures. As shown in Table 3, a total of 92 isolates were recovered from one or both of the anaerobic BC bottles, and the overall recovery rate for the BacT/Alert vials was significantly higher than that of the Bactec bottles (93.4% versus 78.2%, $P = 0.003$). The BacT/Alert FN Plus medium had higher recovery rates for *Escherichia coli* (90.9% versus 63.6%; $P = 0.030$), Gram-positive bacteria (95.9% versus 79.6%; $P = 0.013$), and isolates present in treatment-negative cultures in general (91.8% versus 74% for Bactec medium; $P = 0.004$).

The mean TTDs for the positive BacT/Alert ($n = 82$) and Bactec ($n = 69$) bottles were similar (16 h [median, 12.2; IQR, 8.3 to 22.6] versus 16.9 h [median, 13.8; IQRs, 9.7 to 22.5], respectively; P = NS). As shown in Table 4, the similarity also emerged when analysis was restricted to the 126 bottles with concordant growth (16.2 versus 17.1 h, respectively; P = NS). There were no significant species- or group-related differences between the two systems, but growth in treatment-negative cultures was detected 1.3 h earlier in the BacT/Alert bottles ($P = 0.007$).

Isolate identifications based on direct MALDI-TOF MS analysis of BC broths. Direct BC broth assays were performed on samples from all 363 BCs yielding clinically significant growth (188 BacT/Alert bottles and 175 Bactec bottles). As shown in Table 5, the results were fully concordant with standard culture-based identifications for 88.9% of the isolates grown in the 188 BacT/Alert bottles and 88.1% of those in the 175 Bactec bottles ($P = 0.998$), with mean (IQR) log score values for the best hits of 2.09 (1.99 to 2.20) and 2.05 (1.98 to 2.18), respectively. For the remaining 44 isolates (22 of which were from BacT/Alert cultures), Bruker MALDI BioTyper broth analysis yielded no identification. Of these, 22 (50%) were from polymicrobial cultures. When analysis was restricted to the monomicrobial cultures, rates of concordance between broth-based and culture-based identifications rose to 93.8% and 93.3% for BacT/Alert and Bactec bottles, respectively (P = NS). Mean (IQR) log score values for Gram-negative bacteria recovered from BacT/Alert and Bactec bottles were 2.15 (2.08 to 2.23) and 2.13 (2.02 to 2.23), respectively; similar values were observed for Gram-positive cocci (2.06

TABLE 3 Microorganisms recovered from anaerobic BCs performed with BacT/Alert and Bactec culture media[a]

Microorganism(s) (no.)	No. of isolates recovered from the indicated anaerobic BC bottles			
	BacT/Alert and Bactec[b]	BacT/Alert only	Bactec only	P^c
Gram negative (43)	29 (67.4)	10 (23.3)	4 (9.3)	NS
Escherichia coli (22)	12 (54.5)	8 (36.4)	2 (9.1)	0.030
Klebsiella pneumoniae (11)	9 (81.8)	1 (9.1)	1 (9.1)	NS
Morganella morganii (2)	1	1	0	NA
Proteus mirabilis (1)	0	0	1	NA
Anaerobic Gram negative (7)[d]	7 (100)	0	0	NS
Gram positive (49)	37 (75.5)	10 (20.4)	2 (4.1)	0.013
Enterococcus faecalis (6)	3 (50)	3 (50)	0	0.04
Enterococcus faecium (1)	1	0	0	NA
Staphylococcus aureus (18)	16 (88.9)	2 (11.1)	0	NS
Coagulase-negative staphylococci (19)[e]	13 (68.4)	4 (21.1)	2 (10.5)	NS
Streptococcus anginosus (2)	1	1	0	NA
Streptococcus constellatus (1)	1	0	0	NA
Streptococcus pneumoniae (1)	1	0	0	NA
Anaerobic Gram positive (1)[f]	1	0	0	NA
Total isolates (92)	66 (71.7)	20 (21.7)	6 (6.5)	0.003
Treatment positive (19)	18 (94.7)	1 (5.3)	0	NS
Treatment negative (73)	48 (65.8)	19 (26.0)	6 (8.2)	0.004

[a] Analysis was limited to the 126 anaerobic BCs (BacT/Alert FN Plus and Bactec Plus Anaerobic/F) that grew clinically relevant microbial species. The terms *treatment negative* and *treatment positive* refer to cultures drawn before and after initiation of empirical antimicrobial therapy, respectively (see Materials and Methods). Abbreviations: BC, blood culture; NA, not applicable; NS, not significant ($P > 0.05$). Percentages were not calculated when ≤5 microorganisms were isolated.

[b] Isolates of the same species recovered from BacT/Alert and Bactec bottles in a given episode were counted as 1 isolate.

[c] Statistical significance was assessed by comparing the percentage of isolates recovered from BacT/Alert bottles (column 2 + column 3/column 2 +column 3 + column 4) versus percentage of isolates recovered from Bactec bottles (column 2 + column 4/column 2 +column 3 + column 4) (two-sample test of proportions).

[d] Includes 6 isolates of *Bacteroides fragilis* and 1 of *Campylobacter rectus*.

[e] Includes 17 isolates of *Staphylococcus epidermidis* and 2 of *Staphylococcus haemolyticus*.

[f] Includes 1 *Parvimonas micra* isolate.

[1.98 to 2.10] and 2.03 [1.98 to 2.09], respectively), whereas those for *Candida* species isolates were substantially lower (1.84 [1.98 to 2.10] and 1.88 [1.98 to 2.09], respectively).

Diagnostic capacities and TTDs of BacT/Alert and Bactec BC sets. A total of 128 BSI episodes were diagnosed (including 11 that were polymicrobial). A total of 112 (87.5%) episodes were correctly diagnosed by both the BacT/Alert and Bactec culture sets. The remaining 16 episodes included 15 that were missed entirely by either the BacT/Alert set ($n = 5$) or by the Bactec ($n = 10$) (P = NS) and 1 episode incompletely diagnosed by both sets. For the 122 episodes identified by the BacT/Alert sets, diagnoses reflected growth in both the aerobic and anaerobic bottles in 65 (53.2%) cases; in the remaining 57 cases, they reflected growth in the aerobic ($n = 41$ [33.6%]) or anaerobic ($n = 16$ [13.1%]) bottle alone. Corresponding figures for the 117 episodes identified by the Bactec sets were as follows: aerobic and anaerobic bottles ($n = 57$), 48.7%; aerobic bottle alone ($n = 48$), 41%; and anaerobic bottle alone ($n = 12$), 10.3%. The BacT/Alert and Bactec sets were similar in terms of the types of BSIs they diagnosed, although the former identified more BSIs involving Gram-positive cocci (50/51 [98% of all such episodes] for the BacT/Alert system versus 42/51 [82.3%] for the Bactec system; $P = 0.008$). Of the 57 Gram-negative BSIs, the BacT/Alert sets identified 56 (98.2%) and the Bactec sets all 57 (100%) (P = NS). Of the 9 candidemias we found, 7 were identified by BacT/Alert and 8 by the Bactec system (P = NS). The diagnostic capacities of the two sets were similar, regardless of whether antimicrobial treatment had been started when cultures were drawn.

The mean TTDs for the episodes diagnosed by the BacT/Alert ($n = 123$) and Bactec ($n = 118$) sets were not significantly different (16.1 h [median, 14.2 h; IQR, 8.2 to 21.3] versus 16.9 h [median, 13.1 h; IQR, 8.3 to 24]). When TTD analysis was restricted to the 112 cases diagnosed by both sets (Table 6), the only difference that emerged involved coagulase-negative staphylococcal BSIs, which were detected faster by the BacT/Alert system (mean, 2.8 h; $P = 0.003$). There were no significant differences between BacT/Alert and Bactec set TTDs when cultures were drawn after the initiation of antimicrobial therapy, but the BacT/Alert sets provided diagnoses 1.3 h earlier in treatment-negative cases ($P < 0.001$).

In 92.7% (114 of 123) of the episodes diagnosed by BacT/Alert BC sets and 90.7% (107 of 118) of those identified with the Bactec sets, all causative organisms were correctly identified by direct Bruker MALDI BioTyper analysis of BC broths.

DISCUSSION

The aim of this study was to compare the clinical performances of two widely used lines of aerobic and anaerobic BC media containing resin-based systems for neutralizing antimicrobial molecules and other growth inhibitors (22, 50). Each paired (BacT/Alert Plus and Bactec Plus) BC set we analyzed was inoculated with 40 ml of blood collected from the same patient via a single percutaneous venipuncture (10 ml per bottle). This allowed us to compare the two medium lines not only in terms of aerobic bottle or anaerobic bottle yields, but also at the level of the two-bottle BC sets themselves, which is more relevant for the clinical microbiology laboratory (13, 27). The BacT/Alert Plus aerobic medium recovered 14% more Gram-positive organisms but

TABLE 4 Times to detection of anaerobic BC positivity for BacT/Alert and Bactec culture bottles[a]

Microorganism(s)	No. of isolates	Times to detection (h) of anaerobic BC positivity in:				
		BacT/Alert bottles		Bactec bottles		
		Median	Mean (IQR)	Median	Mean (IQR)	P^b
Monomicrobial cultures	60	12.1	15.9 (8.9–23.3)	14.2	16.8 (8.9–22.8)	NS
Gram negative	29	10.5	12.4 (6.4–15.7)	11.1	13.3 (7.1–21.2)	NS
Escherichia coli	11	6.2	6.4 (4.2–7.0)	6.3	8.0 (2.2–12.5)	NS
Klebsiella pneumoniae	8	11.0	11.6 (10.4–11.8)	11.0	11.7 (10.4–11.4)	NS
Morganella morganii	1	NA	7.4	NA	12.2	NA
Bacteroides fragilis	5	28.0	25.1 (23.2–28)	27.3	27.8 (26.1–29.7)	NS
Gram positive	37	19.2	18.6 (11.5–23.3)	18.2	19.4 (11.5–23.9)	NS
Enterococcus faecalis	3	12.3	19.3	11.5	13.6	NS
Enterococcus faecium	1	NA	11.5	NA	9.0	NA
Staphylococcus aureus	16	11.6	17.0 (9.0–23.3)	14.7	17.4 (9.0–18.2)	NS
Coagulase-negative staphylococci	13	21.3	21.3 (17.2–25.3)	24.3	24.3 (24.6–24.9)	NS
Streptococcus anginosus	1	NA	17.5	NA	21.1	NA
Streptococcus pneumoniae	1	NA	13.2	NA	13.1	NA
Polymicrobial cultures	6	26.1	21.4	26.3	21.6	NS
Total isolates	66	12.1	16.2 (8.6–23.3)	14.2	17.1 (9.0–23.9)	NS
Treatment positive	18	13.2	16.6 (8.3–25.6)	17.3	16.1 (9.2–21.6)	NS
Treatment negative	48	12.0	16.1 (9.0–23.5)	13.4	17.4 (8.5–24.2)	0.007

[a] Analysis was limited to the 126 anaerobic bottles in which BacT/Alert and Bactec BC yields were concordant at the species level. The terms *treatment-negative* and *treatment-positive* refer to cultures drawn before and after initiation of empirical antimicrobial therapy, respectively (see Materials and Methods). Abbreviations: BC, blood culture; IQR, interquartile range (calculated only when ≥5 isolates were evaluated); NA, not applicable; NS, not significant ($P > 0.05$).

[b] Statistical significance of difference between mean TTDs for BacT/Alert and Bactec bottles (Wilcoxon matched-pairs signed-rank test).

11% fewer Gram-negative bacteria than its Bactec counterpart. However, the BacT/Alert anaerobic medium outperformed its Bactec counterpart in the recovery of all microorganisms (+15%), of Gram-positive cocci (+16%), and of *E. coli* (+27%). As a result, the diagnostic capacities of the sets as a whole were quite similar (95.3% for the BacT/Alert versus 91.4% for the Bactec system; P = NS), even for Gram-negative infections (98.2% for the BacT/Alert versus 100% for the Bactec system; P = NS). The only residual difference involved infections caused by Gram-positive cocci, which were diagnosed more frequently by the BacT/Alert Plus BC set (98% for the BacT/Alert versus 82.3% for the Bactec system; P = NS). The two media lines were also comparable in terms of TTDs for positive cultures drawn after antimicrobial drugs had been started and performance in direct-broth MALDI-TOF MS assays for isolate identification.

In accordance with CLSI guidelines (45) and the recommendations of various other authors (43, 44), each BC bottle in our study was inoculated with 10 ml of blood. Because the number of microorganisms circulating in the blood may be relatively small, inoculum volume strongly affects BC sensitivity and incubation time requirements (40, 44, 46). Increasing this volume from 5 to 10 ml has been shown to significantly improve the overall yields of standard BacT/Alert aerobic BC bottles and accelerate the detection of pathogenic growth, especially that of *E. coli* and other *Enterobacteriaceae* (43). This effect was confirmed in a more recent study of Bactec Aerobic Plus and Anaerobic Plus media inoculated with 7 to 10 ml blood per bottle (49), where the likelihood of BC set positivity increased by 3.3% for every additional milliliter of blood cultured.

Using a standard inoculum volume obviously simplified our comparison of the yields and TTDs of the two media lines. However, we cannot exclude the possibility that the same results would have emerged if we had used a lower volume cutoff. Indeed, smaller inoculum volumes may be necessary owing to poor vein quality and/or concerns related to the risk of hypovolemia/anemia, and the positivity rates for such samples may actually be higher because they frequently come from critically ill patients at relatively high risk for BSIs (49).

Our experience suggests that the BacT/Alert anaerobic medium may be associated with higher yields and shorter incubation times in some settings. The diagnostic gains offered by anaerobic BCs have been attributed by some to the fact that their inclusion requires the use of larger blood volumes (13, 26, 27, 43), but Patel et al. (46) showed that BC sets comprising one aerobic bottle and one anaerobic bottle have higher pathogen yields than two sets of aerobic bottles. Anaerobic bottles provide conditions that facilitate not only the growth of obligate anaerobes but also that of facultatively anaerobic and aerotolerant species (13, 26, 27, 43, 46, 51). In our study, the inclusion of anaerobic cultures significantly improved the sensitivity of the BacT/Alert system (+13.8%) and to a lesser extent that of the Bactec system (+10.2%) over that achieved with aerobic cultures alone, and in both cases, almost 60% of the diagnostic gains were related to infections caused by facultative rather than obligate anaerobes. Medium composition differences probably account for the higher Gram-positive yields of the BacT/Alert Plus anaerobic bottles.

Time to detection of growth can naturally have a major impact on the clinical outcomes of BSIs. The BacT/Alert Plus system detected clinically relevant BSIs after a mean incubation of 16 h (IQR, 9 to 21 h), and for certain species, TTDs were frequently <12 h, which is consistent with previous reports (40–42). The

TABLE 5 Isolate identities furnished by direct MALDI-TOF MS analysis of BacT/Alert and Bactec BC broths[a]

Microorganism(s)	BacT/Alert bottles (n = 188)		Bactec bottles (n = 175)		P
	No. of standard method IDs[b]	Correct IDs by direct broth analysis[c]	No. of standard method IDs[b]	Correct IDs by direct broth analysis[c]	
Monomicrobial cultures	178	167 (93.8)	165	154 (93.3)	NS
Gram-negative	81	79 (97.5)	83	81 (97.6)	NS
Bacteroides fragilis	5	5	5	5	NA
Escherichia coli	35	35 (100)	34	34 (100)	NS
Klebsiella pneumoniae	18	18 (100)	20	20 (100)	NS
Morganella morganii	4	3	3	3	NA
Proteus mirabilis	0	0	1	0	NA
Pseudomonas aeruginosa	19	18 (94.7)	20	19 (95)	NS
Gram-positive	90	83 (92.2)	74	67 (90.5)	NS
Enterococcus faecalis	9	8 (88.9)	4	4	NA
Enterococcus faecium	1	1	1	0	NA
Staphylococcus aureus	35	35 (100)	31	31 (100)	NS
Staphylococcus epidermidis	32	28 (87.5)	27	24 (88.9)	NS
Staphylococcus haemolyticus	2	2	2	1	NA
Staphylococcus hominis	1	1	1	1	NA
Streptococcus anginosus	5	3	3	1	NA
Streptococcus constellatus	1	1	1	1	NA
Streptococcus pneumoniae	4	4	3	3	NA
Yeasts	7	5 (71.4)	8	6 (75)	NS
Candida albicans	4	3	6	5	NA
Candida parapsilosis	1	1	0	0	NA
Candida tropicalis	2	1	2	1	NA
Polymicrobial cultures	10/10	6/3 (60/30)	10/10	6/3 (60/30)	NS
Staphylococcus haemolyticus/Proteus mirabilis	1/1	0/1	1/1	0/1	NA
Pseudomonas aeruginosa/Proteus mirabilis	1/1	1/0	2/2	2/0	NA
Escherichia coli/Klebsiella pneumoniae	4/4	4/2	3/3	3/2	NA
Pseudomonas aeruginosa/Stenotrophomonas maltophilia	1/1	1/0	1/1	1/0	NA
Parvimonas micra/Campylobacter rectus	1/1	0/0	1/1	0/0	NA
Bacteroides fragilis/Streptococcus constellatus	1/1	0/0	1/1	0/0	NA
Klebsiella pneumoniae/Candida albicans	1/1	0/0	1/1	0/0	NA
Total	198	176 (88.9)	185	163 (88.1)	NS

[a] BacT/Alert broths (FA Plus and FN Plus); Bactec broths (Aerobic/F and Anaerobic/FN). Abbreviations: BC, blood culture; ID, identification; MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; NS, not significant. NA, not applicable. Percentages were not calculated when ≤5 microorganisms were isolated.
[b] Standard method, microscopic examination, routine subculture, and MALDI-TOF MS analysis of colony samples (supplemented when necessary with additional biochemical methods and/or 16S rRNA gene sequencing).
[c] No. (%) of identifications (IDs) furnished by direct Bruker MALDI BioTyper analysis of BC broths that were concordant with standard IDs at the species level. (Percentages are not reported for individual species recovered from polymicrobial cultures.)

TTDs for positive BacT/Alert and Bactec cultures drawn after antimicrobial drugs had been started were comparable, but the former system was faster for treatment-negative samples and those containing coagulase-negative staphylococci. These findings probably also reflect medium composition differences.

Isolate identification by direct MALDI-TOF MS analysis of positive BC broths has been a part of our laboratory's BC protocol since 2010. In 2013, the U.S. Food and Drug Administration approved MALDI-TOF MS for identifying Gram-negative isolates grown on solid media, but to date, direct assay of signal-positive BC broths remains an off-label indication for the use of this technology throughout the world. However, compared with conventional culture-based methods, this approach improved species-level identification of bloodstream isolates in terms of time, accuracy, and costs (7–11). In a previous study, direct assay of Bactec Plus broths were more successful that assays of BacT/Alert media containing charcoal (38), but bioMérieux's introduction of adsorbent polymeric bead-based media has evened the score for the two systems on this count. Bruker MALDI BioTyper assays of BacT/Alert Plus and Bactec Plus broths yielded isolate identifications that were concordant with culture-based identifications in almost 90% of all cases.

Among the limitations of our study are the relatively small numbers in our sample of patients with polymicrobial BSIs, those with BSIs caused by yeasts or anaerobic bacteria, and those whose cultures were treatment positive. It is also important to recall that this was a single-center study that examined samples from patients ≥16 years old who were hospitalized in ICUs and infectious disease wards. Therefore, our results are not necessarily applicable to other settings and populations. However, the bloodstream isolates we encountered are representative of those commonly reported in Italian hospitals and in other parts of the world as well (52, 53). Almost half (47.9%) of the microorganisms responsible for our patients' BSIs were Gram-negative bacilli, many of which were multidrug-resistant (data not shown).

Failure to detect an infecting bloodstream pathogen can have

TABLE 6 Times to detection of bloodstream infections by BacT/Alert and Bactec BC sets[a]

Etiology(s)	No. of episodes	Times to detection (h) for the indicated BC sets				P^b
		BacT/Alert		Bactec		
		Median	Mean (IQR)	Median	Mean (IQR)	
Monomicrobial cultures	103	14.0	15.1 (8.0–23.0)	12.8	16.3 (8.1–23.0)	NS
Gram negative	56	12.3	14.4 (6.4–16.1)	11.0	13.8 (6.5–17.8)	NS
Bacteroides fragilis	5	28.0	27.8 (27.6–28.0)	27.3	27.8 (26.1–29.7)	NS
Escherichia coli	21	6.2	7.2 (4.6–8.1)	6.0	9.1 (2.4–9.5)	NS
Klebsiella pneumoniae	10	10.4	10.3 (8.2–11.0)	10.7	10.3 (8.3–11.1)	NS
Morganella morganii	2	NA	7.4	NA	8.3	NA
Pseudomonas aeruginosa	18	16.8	16.6 (13.3–18.2)	14.4	18.1 (12.2–21.9)	NS
Gram positive	41	15.7	16.8 (10.9–21.9)	15.5	17.6 (10.2–24.2)	NS
Enterococcus faecalis	2	NA	16.7	NA	13.5	NA
Staphylococcus aureus	17	11.0	14.0 (7.9–14.0)	10.0	14.0 (7.6–14.0)	NS
Coagulase-negative staphylococci[c]	18	20.3	19.1 (16.5–22.4)	24.0	21.9 (18.9–26.0)	0.003
Streptococcus anginosus	2	NA	19.9	NA	18.3	NA
Streptococcus pneumoniae	2	NA	12.3	NA	11.4	NA
Yeasts	6	27.7	28.1 (16.0–34.8)	26.9	30.8 (14.5–51.3)	NS
Candida albicans	4	22.4	28.4 (15.6–47.3)	17.4	20.4 (13.1–30.7)	NS
Candida tropicalis	2	NA	27.7	NA	51.6	NA
Polymicrobial[d]	9	16.0	17.2 (9.8–24.6)	20.0	18.9 (7.9–28.0)	NS
Total episodes	112	13.7	15.3 (8.2–21.0)	12.4	16.5 (8.1–23.7)	NS
Treatment positive	33	18.0	18.3 (10.2–25.4)	15.6	18.6 (8.9–25.2)	NS
Treatment negative	79	12.0	14.4 (7.4–20.2)	12.1	15.7 (8.1–22.5)	<0.001

[a] BacT/Alert culture set, Bact/ALERT FA Plus and FN Plus bottles; Bactec culture set, Bactec Plus Aerobic/F and Anaerobic/FN bottles. Analysis was limited to the 112 episodes in which BacT/Alert and Bactec set yields were concordant at the species level. The terms treatment negative and treatment positive refer to cultures drawn before and after initiation of empirical antimicrobial therapy, respectively (see Materials and Methods). Abbreviations: IQR, interquartile range (calculated only when ≥5 BSIs were evaluated); NA, not applicable; NS, not significant ($P > 0.05$).

[b] Statistical significance of difference between mean TTDs for BacT/Alert and Bactec sets (Wilcoxon matched-pairs signed-rank test).

[c] Includes 16 *Staphylococcus epidermidis*, 1 *Staphylococcus haemolyticus*, and 1 *Staphylococcus hominis* bloodstream infections.

[d] Includes 1 *Staphylococcus haemolyticus* plus *Proteus mirabilis*, 1 *Enterococcus faecium* plus *Pseudomonas aeruginosa*, 1 *P. mirabilis* plus *P. aeruginosa*, 1 *P. aeruginosa* plus *Stenotrophomonas maltophilia*, 1 *Campylobacter rectus* plus *Parvimonas micra*, 1 *Streptococcus constellatus* plus *Bacteroides fragilis*, 2 *Escherichia coli* plus *Klebsiella pneumoniae*, and 1 *K. pneumoniae* plus *Candida albicans* bloodstream infections.

disastrous consequences. Three recent studies (40–42) have demonstrated the superiority of the new resin-containing BacT/Alert media (FA Plus, FN Plus, and PF Plus) over standard and charcoal particle-containing media of the same line.

In our study, the performance displayed by BacT/Alert Plus media was similar to that of resin-containing media in the Bactec line. Although further efforts are needed to evaluate their overall performance in different settings, our experience indicates that the new BacT/Alert FA Plus and FN Plus media are reliable, time-saving tools for routine identification of BSIs in patients in ICUs and infectious disease wards.

ACKNOWLEDGMENTS

The manuscript was edited by Marian Everett Kent with active input and full approval of all authors.

bioMérieux provided reagents and funding for this study, participated in the study design, and was permitted to review and comment on the manuscript prior to submission.

REFERENCES

1. **Goto M, Al-Hasan MN.** 2013. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin. Microbiol. Infect. **19:**501–509. http://dx.doi.org/10.1111/1469-0691.12195.
2. **Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro B, Fiori B, Citton R, D'Inzeo T, Fadda G, Cauda R, Spanu T.** 2007. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-β-lactamase-producing *Enterobacteriaceae*: importance of inadequate initial antimicrobial treatment. Antimicrob. Agents Chemother. **51:**1987–1994. http://dx.doi.org/10.1128/AAC.01509-06.
3. **Funke DJ, Kumar A.** 2011. Antimicrobial therapy for life-threatening infections: speed is life. Crit. Care Clin. **27:**53–76. http://dx.doi.org/10.1016/j.ccc.2010.09.008.
4. **Barenfanger J, Graham DR, Kolluri L, Sangwan G, Lawhorn J, Drake CA, Verhulst SJ, Peterson R, Moja LB, Ertmoed MM, Moja AB, Shevlin DW, Vautrain R, Callahan CD.** 2008. Decreased mortality associated with prompt Gram staining of blood cultures. Am. J. Clin. Pathol. **130:**870–876. http://dx.doi.org/10.1309/AJCPVMDQU2ZJDPBL.
5. **Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW (ed).** 2011. Manual of clinical microbiology, 10th ed. ASM Press, Washington, DC.
6. **Weinstein MP, Doern GV.** 2011. A critical appraisal of the role of the clinical microbiology laboratory in the diagnosis of bloodstream infections. J. Clin. Microbiol. **49:**S26–S29. http://dx.doi.org/10.1128/JCM.00765-11.
7. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4:**e8041. http://dx.doi.org/10.1371/journal.pone.0008041.
8. **Stevenson LG, Drake SK, Murray PR.** 2010. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization time of flight mass spectrometry. J. Clin. Microbiol. **48:**444–447. http://dx.doi.org/10.1128/JCM.01541-09.
9. **Spanu T, Posteraro B, Fiori B, D'Inzeo T, Campoli S, Ruggeri A, Tumbarello M, Canu G, Trecarichi EM, Parisi G, Tronci M, Sanguinetti M, Fadda G.** 2012. Direct MALDI-TOF mass spectrometry assay of blood culture broths for rapid identification of *Candida* species causing bloodstream infections: an observational study in two large microbiology laboratories. J. Clin. Microbiol. **50:**176–179. http://dx.doi.org/10.1128/JCM.05742-11.

10. **Lagacé-Wiens PR, Adam HJ, Karlowsky JA, Nichol KA, Pang PF, Guenther J, Webb AA, Miller C, Alfa MJ.** 2012. Identification of blood culture isolates directly from positive blood cultures by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry and a commercial extraction system: analysis of performance, cost, and turnaround time. J. Clin. Microbiol. **50:**3324–3328. http://dx.doi.org/10.1128/JCM.01479-12.
11. **Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G.** 2013. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. Clin. Infect. Dis. **56:**1101–1107. http://dx.doi.org/10.1093/cid/cis1204.
12. **Kang H, Kim S.** 2012. Clinical features associated with blood cultures according to the use of antimicrobial agents prior to blood collection. Korean J. Clin. Microbiol. **15:**21–26. http://dx.doi.org/10.5145/KJCM.2012.15.1.21.
13. **Pohlman JK, Kirkley BA, Easley KA, Basille BA, Washington JA.** 1995. Controlled clinical evaluation of Bactec Plus Aerobic/F and BacT/Alert Aerobic FAN bottles for detection of bloodstream infections. J. Clin. Microbiol. **33:**2856–2858.
14. **Roh KH, Kim JY, Kim HN, Lee HJ, Sohn JW, Kim MJ, Cho Y, Kim YK, Lee CK.** 2012. Evaluation of Bactec Plus aerobic and anaerobic blood culture bottles and BacT/Alert FAN aerobic and anaerobic blood culture bottles for the detection of bacteremia in ICU patients. Diagn. Microbiol. Infect. Dis. **73:**239–242. http://dx.doi.org/10.1016/j.diagmicrobio.2012.03.022.
15. **Zadroga R, Williams DN, Gottschall R, Hanson K, Nordberg V, Deike M, Kuskowski M, Carlson L, Nicolau DP, Sutherland C, Hansen GT.** 2013. Comparison of 2 blood culture media shows significant differences in bacterial recovery for patients on antimicrobial therapy. Clin. Infect. Dis. **56:**790–797. http://dx.doi.org/10.1093/cid/cis1021.
16. **McDonald LC, Fune J, Gaido LB, Weinstein MP, Reimer LG, Flynn TM, Wilson ML, Mirrett S, Reller LB.** 1996. Clinical importance of increased sensitivity of BacT/Alert FAN aerobic and anaerobic blood culture bottles. J. Clin. Microbiol. **34:**2180–2184.
17. **Appelbaum PC, Beckwith DG, Dipersio JR, Dyke JW, Salventi JF, Stone LL.** 1983. Enhanced detection of bacteremia with a new BACTEC resin blood culture medium. J. Clin. Microbiol. **17:**48–51.
18. **McGuire NM, Kauffman CA, Hertz CS, Kovach JM.** 1983. Evaluation of the Bactec antimicrobial removal system for detection of bacteremia. J. Clin. Microbiol. **18:**449–451.
19. **Doern GV, Gantz NM.** 1983. Detection of bacteremia in patients receiving antimicrobial therapy: an evaluation of the antimicrobial removal device and 16B medium. J. Clin. Microbiol. **18:**43–48.
20. **Hopfer RL, Moore DG, Fainstein V, Watkins S, Wenglar M.** 1983. Routine use of Bactec 16B bottles to remove antibacterial and antitumor agents from blood cultures of cancer patients. J. Clin. Microbiol. **18:**759–764.
21. **Weinstein MP, Mirrett S, Reimer LG, Wilson ML, Smith-Elekes S, Chuard CR, Reller LB.** 1995. Controlled evaluation of BacT/Alert standard aerobic and FAN aerobic blood culture bottles for the detection of bacteremia and fungemia. J. Clin. Microbiol. **33:**978–981.
22. **Gibb AP, Hill B, Chorel B.** 1998. Comparative study of BacT/Alert FAN bottles and standard BacT/Alert bottles. Diagn. Microbiol. Infect. Dis. **32:**159–163. http://dx.doi.org/10.1016/S0732-8893(98)00094-7.
23. **Wilson ML, Weinstein MP, Mirrett S, Reimer LG, Feldman RJ, Chuard CR, Reller LB.** 1995. Controlled evaluation of BacT/Alert standard anaerobic and FAN anaerobic blood culture bottles for the detection of bacteremia and fungemia. J. Clin. Microbiol. **33:**2265–2270.
24. **Mirrett S, Petti CA, Woods CW, Magadia R, Weinstein MP, Reller LB.** 2004. Controlled clinical comparison of the BacT/Alert FN and the standard anaerobic SN blood culture medium. J. Clin. Microbiol. **42:**4581–4585. http://dx.doi.org/10.1128/JCM.42.10.4581-4585.2004.
25. **Riley JA, Heiter BJ, Bourbeau PP.** 2005. Comparative recovery of microorganisms from BacT/Alert plastic and glass FA and FN blood culture bottles. J. Clin. Microbiol. **43:**3244–3246. http://dx.doi.org/10.1128/JCM.43.7.3244-3246.2005.
26. **Mirrett S, Joyce MJ, Reller LB.** 2005. Validation of performance of plastic versus glass bottles for culturing anaerobes from blood in BacT/Alert SN medium. J. Clin. Microbiol. **43:**6150–6151. http://dx.doi.org/10.1128/JCM.43.12.6150-6151.2005.
27. **Ziegler R, Johnscher I, Martus P, Lenhardt D, Just HM.** 1998. Controlled clinical laboratory comparison of two supplemented aerobic and anaerobic media used in automated blood culture systems to detect bloodstream infections. J. Clin. Microbiol. **36:**657–661.
28. **Jorgensen JH, Mirrett S, McDonald LC, Murray PR, Weinstein MP, Fune J, Trippy CW, Masterson M, Reller LB.** 1997. Controlled clinical laboratory comparison of Bactec Plus aerobic/F resin medium with BacT/Alert aerobic FAN medium for detection of bacteremia and fungemia. J. Clin. Microbiol. **35:**53–58.
29. **Spaargaren J, van Boren CPA, Voorn GP.** 1998. Effectiveness of resins in neutralizing antibiotic activities in Bactec Plus Aerobic/F culture medium. J. Clin. Microbiol. **36:**3731–3733.
30. **Nzeako BC, Al-Qasabi SSH.** 2004. Evaluation of the neutralising capacity of Bactec medium for some antibiotics. Br. J. Biomed. Sci. **61:**171–174.
31. **Viganò EF, Vasconi E, Agrappi C, Clerici P, Melloni P.** 2004. Use of simulated blood cultures for antibiotic effect on time to detection of the two blood culture systems BacT/Alert and BACTEC 9240. New Microbiol. **27:**235–248.
32. **Horvath LL, George BJ, Murray CK, Harrison LS, Hospenthal DR.** 2004. Direct comparison of the Bactec 9240 and BacT/Alert 3D automated blood culture systems for *Candida* growth detection. J. Clin. Microbiol. **42:**115–118. http://dx.doi.org/10.1128/JCM.42.1.115-118.2004.
33. **Flayhart D, Borek AP, Wakefield T, Dick J, Carroll KC.** 2007. Comparison of Bactec Plus blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J. Clin. Microbiol. **45:**816–821. http://dx.doi.org/10.1128/JCM.02064-06.
34. **Mitteregger D, Wolfgang B, Nehr M, Kundi M, Zeitlinger M, Makristathis A, Hirschl AM.** 2013. Neutralization of antimicrobial substances in new BacT/Alert FA and FN Plus blood culture bottles. J. Clin. Microbiol. **51:**1534–1540. http://dx.doi.org/10.1128/JCM.00103-13.
35. **Jekarl DW, Lee SY, Lee S, Park YJ, Lee J, Baek SM, An YJ, Ock SM, Lee MK.** 2012. Comparison of the Bactec Fx Plus, mycosis IC/F, mycosis/F lytic blood culture media and the BacT/Alert 3D FA media for detection of *Candida* species in seeded blood culture specimens containing therapeutic peak levels of fluconazole. J. Clin. Lab. Anal. **26:**412–419. http://dx.doi.org/10.1002/jcla.21535.
36. **Köck R, Eißing LC, Boschin MG, Ellger B, Horn D, Idelevich EA, Becker K.** 2013. Evaluation of Bactec mycosis IC/F and Plus aerobic/F blood culture bottles for detection of *Candida* in the presence of antifungal agents. J. Clin. Microbiol. **51:**3683–3687. http://dx.doi.org/10.1128/JCM.02048-13.
37. **Sullivan KV, Turner NN, Lancaster DP, Shah AR, Chandler LJ, Friedman DF, Blecker-Shelly DL.** 2013. Superior sensitivity and decreased time to detection with the Bactec Peds Plus/F system compared to the BacT/Alert Pediatric FAN blood culture system. J. Clin. Microbiol. **51:**4083–4086. http://dx.doi.org/10.1128/JCM.02205-13.
38. **Szabados F, Michels M, Kaase M, Gatermann S.** 2011. The sensitivity of direct identification from positive BacT/Alert (bioMérieux) blood culture bottles by matrix-assisted laser desorption ionization time-of-flight mass spectrometry is low. Clin. Microbiol. Infect. **17:**192–195. http://dx.doi.org/10.1111/j.1469-0691.2010.03229.x.
39. **Wüppenhorst N, Consoir C, Lörch D, Schneider C.** 2012. Direct identification of bacteria from charcoal-containing blood culture bottles using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Eur. J. Clin. Microbiol. Infect. Dis. **31:**2843–2850. http://dx.doi.org/10.1007/s10096-012-1638-2.
40. **Lee DH, Kim SC, Bae IG, Koh EH, Kim S.** 2013. Clinical evaluation of BacT/Alert FA Plus and FN Plus bottles compared with standard bottles. J. Clin. Microbiol. **51:**4150–4155. http://dx.doi.org/10.1128/JCM.01935-13.
41. **Kirn TJ, Mirrett S, Reller LB, Weinstein MP.** 2014. Controlled clinical comparison of BacT/Alert FA Plus and FN Plus blood culture media with BacT/Alert FA and FN blood culture media. J. Clin. Microbiol. **52:**839–843. http://dx.doi.org/10.1128/JCM.03063-13.
42. **Doern CD, Mirrett S, Halstead D, Abid J, Okada P, Reller LB.** 19 March 2014. Controlled clinical comparison of new pediatric medium with adsorbent polymeric beads (PF Plus) versus charcoal-containing PF medium in the BacT/Alert blood culture system. J. Clin. Microbiol. http://dx.doi.org/10.1128/JCM.00175-14.
43. **Weinstein MP, Mirrett S, Reimer LG, Wilson ML, Reller LB.** 1994. Controlled evaluation of five versus ten milliliters of blood cultured in aerobic BacT/Alert blood culture bottles. J. Clin. Microbiol. **32:**2103–2106.
44. **Cockerill FR III, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, Schleck CD, Ilstrup DM, Washington JA II, Wilson WR.** 2004. Optimal testing parameters for blood cultures. Clin. Infect. Dis. **38:**1724–1173. http://dx.doi.org/10.1086/421087.

45. **Clinical and Laboratory Standards Institute.** 2007. Principles and procedures for blood cultures; approved guideline. CLSI document M47-A. Clinical and Laboratory Standards Institute, Wayne, PA.
46. **Patel R, Vetter EA, Harmsen WS, Schleck CD, Fadel HJ, Cockerill FR III.** 2011. Optimized pathogen detection with 30- compared to 20-milliliter blood culture draws. J. Clin. Microbiol. **49:**4047–4051. http://dx.doi.org/10.1128/JCM.01314-11.
47. **Drancourt M, Berger P, Raoult D.** 2004. Systematic 16S rRNA gene sequencing of atypical clinical isolates identified 27 new bacterial species associated with humans. J. Clin. Microbiol. **42:**2197–2202. http://dx.doi.org/10.1128/JCM.42.5.2197-2202.2004.
48. **Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D.** 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization–time of flight mass spectrometry. Clin. Infect. Dis. **49:**543–551. http://dx.doi.org/10.1086/600885.
49. **Bouza E, Sousa D, Rodríguez-Créixems M, Lechuz JG, Muñoz P.** 2007. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? J. Clin. Microbiol. **45:**2765–2769. http://dx.doi.org/10.1128/JCM.00140-07.
50. **Jungkind DL, Thakur M, Dyke J.** 1989. Evidence for a second mechanism of action of resin in Bactec NR 16A aerobic blood culture medium, abstr C-225, p 430. Abstr 89th Annu. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.
51. **Grohs P, Mainardi JL, Podglajen I, Hanras X, Eckert C, Buu-Hoï A, Varon E, Gutmann L.** 2007. Relevance of routine use of the anaerobic blood culture bottle. J. Clin. Microbiol. **45:**2711–2715. http://dx.doi.org/10.1128/JCM.00059-07.
52. **Luzzaro F, Ortisi G, Larosa M, Drago M, Brigante G, Gesu G.** 2011. Prevalence and epidemiology of microbial pathogens causing bloodstream infections: results of the OASIS multicenter study. Diagn. Microbiol. Infect. Dis. **69:**363–369. http://dx.doi.org/10.1016/j.diagmicrobio.2010.10.016.
53. **Albrecht SJ, Fishman NO, Kitchen J, Nachamkin I, Bilker WB, Hoegg C, Samel C, Barbagallo S, Arentzen J, Lautenbach E.** 2006. Re-emergence of Gram-negative health care-associated bloodstream infections. Arch. Intern. Med. **166:**1289–1129. http://dx.doi.org/10.1001/archinte.166.12.1289.