

Blood Culture Antimicrobial Removal Effectiveness of BD BACTEC Plus Media Versus BacT/ALERT FAN Media

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ABSTRACT

The antimicrobial removal effectiveness of two adsorbent systems, resins in Plus media of the BD BACTEC™ 9240 blood culture system and activated charcoal in FAN media for the Organon Teknika BacT/ALERT™ 3D instrument, were compared in side by side trials with Plus and FAN aerobic, anaerobic and pediatric formulations. Vials were entered into the instruments after each received banked blood at the maximum amount recommended per media type, the peak serum level of one of 22 test antimicrobials for a given blood volume, and 10–100 cfu of a susceptible reference organism. Growth detection by an instrument indicated effective antimicrobial removal. Both systems effectively removed hydrophobic antimicrobials. However, BacT/ALERT FAN media failed to effectively remove most penicillins or cephalosporins tested, and also failed to effectively remove the glycopeptide vancomycin, whereas the BACTEC Plus media effectively removed all penicillins, most cephalosporins, and vancomycin. Penicillins and cephalosporins together constitute approximately 40% of the antimicrobials currently in clinical use in the United States. In conclusion, the BD BACTEC Plus media showed superior antimicrobial removal performance due to the ineffectiveness of BacT/ALERT FAN media against penicillins, cephalosporins and vancomycin.

INTRODUCTION

Antimicrobials present in blood samples taken from patients undergoing antimicrobial chemotherapy can delay or prevent the detection of bacteremia in blood culture systems. To prevent this from happening, adsorbents are employed to remove antimicrobials from blood culture media. The BD BACTEC™ 9240 blood culture system (BD Diagnostics, Sparks, MD) utilizes ion exchange and nonionic adsorbent resins to remove antimicrobials introduced by blood samples, while the Organon Teknika BacT/ALERT blood culture system (bioMerieux, Durham, NC) uses activated charcoal for this purpose. Antimicrobial removal formulations of aerobic, anaerobic and pediatric media are available for both the BACTEC (Plus media) and BacT/ALERT (FAN media) systems. In this study, the two systems were compared in side by side trials with blood culture vials seeded with antimicrobial susceptibility reference organisms, to which banked blood and antimicrobials at or below peak serum levels were added.

MATERIALS AND METHODS

ANTIMICROBIAL ADDITION. Antimicrobial susceptibility testing strains were selected and maintained, and antimicrobials handled, in accordance with NCCLS guidelines (references 2 and 3). Injection of 0.1 mL per vial of potency-adjusted, filter-sterilized antimicrobial stock provided the amount of a given antimicrobial that would be found at the peak serum level for that antimicrobial in 10 mL blood (3 mL for pediatric media). Several antimicrobials were also tested at below their peak serum levels. The clinical formulation of aztreonam was used (Azactam for Injection, Bristol-Myers Squibb), as was the clinical quinupristin-dalfopristin blend Synercid (Catalytica Pharmaceuticals). Trimethoprim and sulfamethoxazole were injected into vials from separate stocks.

BLOOD ADDITION AND INOCULATION. Prior to the addition of antimicrobial stocks, each vial received 10 mL banked blood (3 mL for pediatric media) drawn not more than five days prior to use and stored at 4°C. After antimicrobials were added, each vial was inoculated with 0.1 mL of bacterial suspension in 0.85% saline that contained 10-100 cfu from overnight growth on TSA II with 5% sheep's blood agar (BBL). Vials to which 0.1 mL of sterile dH₂O was added in place of an antimicrobial served as negative controls, while Standard Aerobic BACTEC vials (no resins) with antimicrobials served as positive controls.

INCUBATION. After inversion to mix contents, vials were immediately entered into blood culture instruments and allowed to incubate over a five-day protocol. A total of 10 vials in each instrument were tested for each condition, split between two groups of vials in experiments started on different days. Results are presented as percent growth detection for the 10 vials of each condition, with growth detection by an instrument indicative of effective antimicrobial removal from the medium.

ANTIMICROBIALS (peak serum level, µg/mL). Amoxicillin (5), ampicillin (47), azithromycin (3.6), aztreonam (125), cefazolin (188), cefoperazone (93), cefoxitin (79), ceftriaxone (150), ciprofloxacin HCl (4.6), clindamycin phosphate (2.5), doxycycline HCl (2.1), gentamicin sulfate (8), imipenem (40), linezolid (15), metronidazole (25), oxacillin (57), penicillin G (20), piperacillin (400), quinupristin (2.7), dalbapristin (7.2), tetracycline HCl (2.2), trimethoprim (9), sulfamethoxazole (105), and vancomycin HCl (50). Reference 1.

RESULTS

AEROBIC MEDIA. Graph 1 shows that 100% detection was attained in both systems for eight of the 14 antimicrobials tested in aerobic media. Three of 10 Plus vials did not detect with quinupristin/dalbapristin versus ten detections in FAN medium. However, the BacT/ALERT system was ineffective in adsorbing five of the 14 antimicrobials tested (vancomycin and all four beta-lactams) with 0% of vials detecting growth versus 100% detection with the BACTEC system.

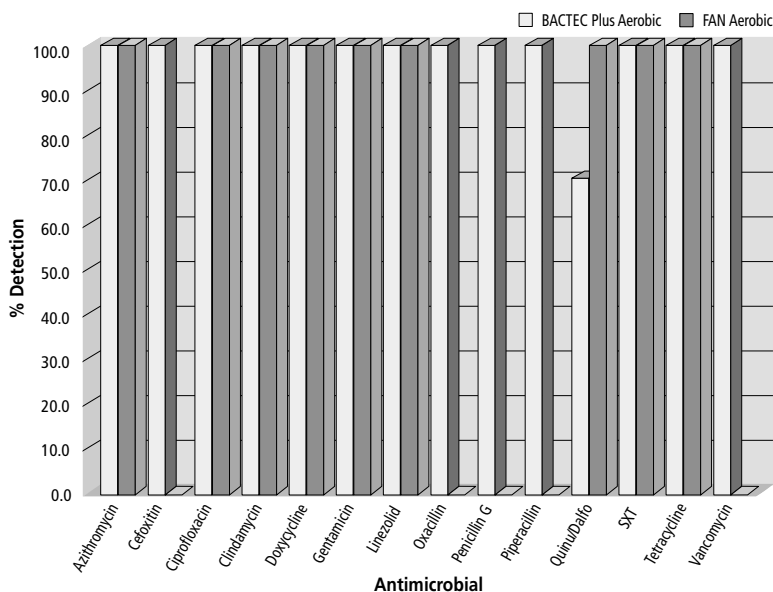
ANAEROBIC MEDIA. Graph 2 shows that 100% detection was attained in both systems for eight of the 12 antimicrobials tested in anaerobic media. Neither system was effective against metronidazole, with no detections in Plus medium and only one detection in FAN medium. The BacT/ALERT system registered 0% growth detection for three of the five beta-lactams tested, versus 100% detection with these in the BACTEC system.

PEDIATRIC MEDIA. Graph 3 shows that 100% detection was attained in both systems for five of the 13 antimicrobials tested in pediatric media. Neither system was effective against cefazolin, with no growth detection in any Plus or FAN vials, or aztreonam, where no Plus vials and only one FAN vial was positive for growth. Against vancomycin and the remaining five beta-lactams, detection was 90–100% in the BACTEC system versus no growth detection in the BacT/ALERT system, except for a single positive vial with ceftriaxone.

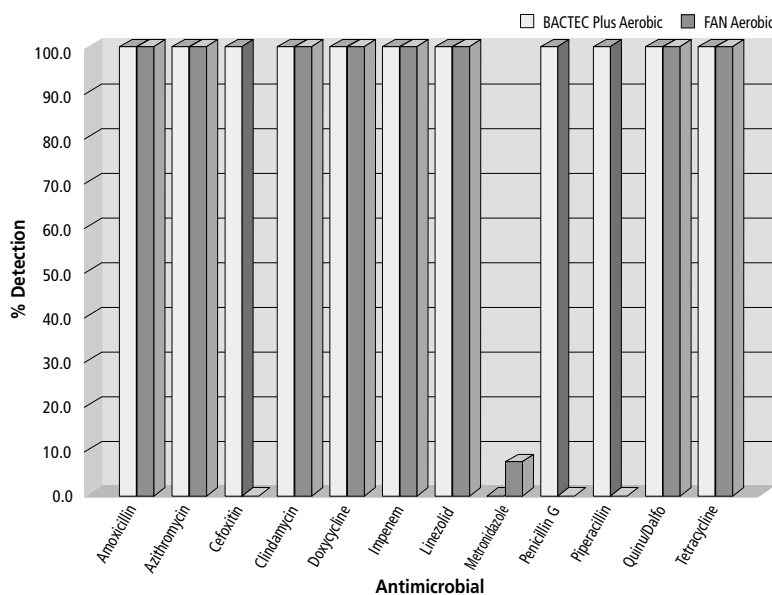
BETA-LACTAMS AND VANCOMYCIN. Graph 4 shows the results of tests in aerobic media provided with 10 mL blood per vial, reference organism, and either vancomycin or one of six beta-lactams at one-fourth to one-tenth the peak serum levels used in previous tests. Under these conditions, 100% detection was attained in the BACTEC system, as expected from previous results, while no growth was detected by the BacT/ALERT system.

Data is not presented for non-resin or antimicrobial-free controls.

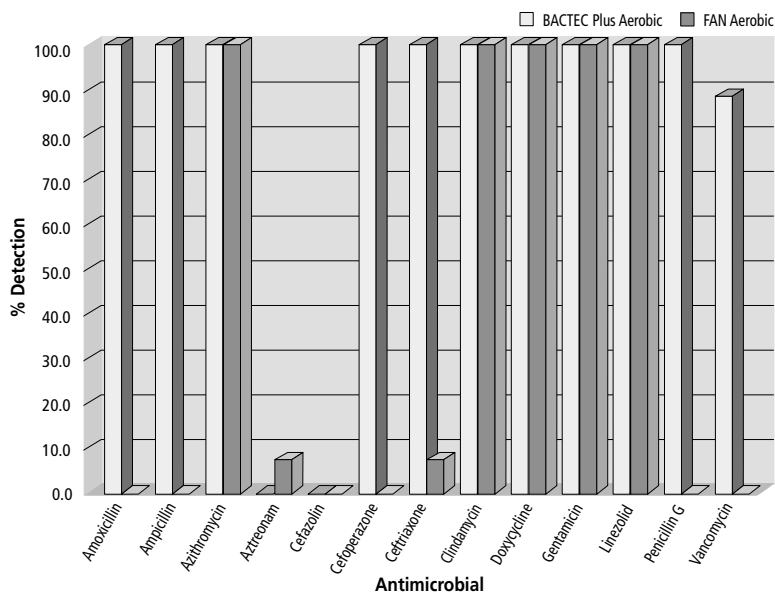
Graph 1. Antimicrobial Removal by BACTEC Plus Aerobic versus BacT/ALERT FAN Aerobic Media. *S. pneumoniae* ATCC 49619 was inoculated into vials containing linezolid and quinupristin/dalbapristin, and *S. aureus* ATCC 29213 into remaining vials. SXT = trimethoprim/sulfamethoxazole.



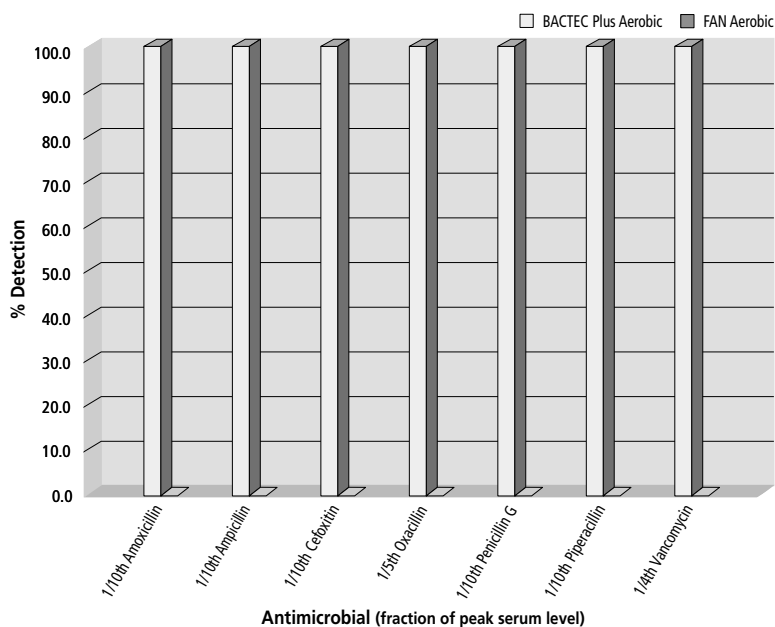
Graph 2. Antimicrobial Removal by BACTEC Plus Anaerobic versus BacT/ALERT FAN Anaerobic Media. *S. pneumoniae* ATCC 49619 was inoculated into vials containing linezolid and penicillin G, and *B. fragilis* ATCC 25285 into remaining vials.



Graph 3. Antimicrobial Removal by BACTEC Peds Plus versus BacT/ALERT Pediatric FAN Media. *S. pneumoniae* ATCC 49619 was inoculated into vials containing azithromycin, cefazolin, and linezolid, *E. coli* ATCC 25922 into vials containing aztreonam and cefoperazone, and *S. aureus* ATCC 29213 into remaining vials.



Graph 4. Removal of Beta-Lactams and Vancomycin at Less Than Peak Serum Levels by BACTEC Plus Aerobic versus BacT/ALERT FAN Aerobic Media. *S. aureus* ATCC 29213 was inoculated into all vials.



DISCUSSION

Antimicrobials in blood samples are diluted by the growth medium in blood culture vials. The fill volumes of the vials tested in this study ranged from 20–40 mL per vial, with 25 mL fills in BACTEC Plus aerobic and anaerobic media and 40 mLs per Peds Plus vial, versus 30-, 40-, and 20 mLs per BacT/ALERT FAN aerobic, anaerobic, and pediatric formulations, respectively. Only 3 mLs of blood per vial was added to pediatric media as these formulations are optimized for low blood volumes (versus an

optimal 10 mLs per vial for aerobic and anaerobic media). Although the pediatric FAN medium has only one half the fill volume of Peds Plus, the performance deficiencies of pediatric FAN (Graph 3) cannot be attributed to dilution since the amounts of antimicrobials added to pediatric media were also reduced by 70% along with the blood volume reduction from 10 to 3 mLs per vial. With aerobic and anaerobic media, the dilution aspect of fill volume differences between Plus and FAN vials favored the FAN media, yet BACTEC

Plus media antimicrobial removal was nonetheless effective across a wider range of antimicrobials than that of the BacT/ALERT media.

The results presented in Graphs 1–3 are from test conditions that represent the worst-case scenario: a highly susceptible microorganism in growth medium given the maximum recommended volume of blood that contains the maximum clinical concentration of an antimicrobial. Worst-case antimicrobial removal conditions were employed in order to fully test the antimicrobial removal capabilities of both blood culture systems. Antimicrobial removal effectiveness demonstrated by FAN anaerobic medium against amoxicillin and by both anaerobic media against imipenem (Graph 2) was overstated with *B. fragilis* versus results if the same tests had been performed in aerobic media with the more susceptible *S. aureus* (see amoxicillin results, Graphs 3 and 4). Both blood culture systems performed well against the more hydrophobic antimicrobials such as tetracyclines and the macrolide azithromycin.

Graph 4 shows results from test conditions that were far from rigorous, with antimicrobials added at one-fourth to one-tenth maximum amounts, yet with the dilution benefit provided by 10 mLs blood per vial. Under these undemanding conditions the FAN aerobic medium was still incapable of removing beta-lactams (penicillins and cephalosporins) or vancomycin sufficiently to allow growth to occur. This is cause for concern as beta-lactams account for approximately 40% of the antimicrobials prescribed in the United States, while vancomycin is the treatment of choice for methicillin-resistant *S. aureus* and of significant importance for prophylactic therapy.

REFERENCES

1. Gilbert, D.N., R.C. Moellering Jr., and M.A. Sande (editors). 2001. *The Sanford Guide to Antimicrobial Therapy*, 31st edition. Antimicrobial Therapy, Inc.
2. National Committee for Clinical Laboratory Standards, 2001. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 5th edition. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Villanova, PA.
3. National Committee for Clinical Laboratory Standards, 1997. *Methods for antimicrobial susceptibility testing of anaerobic bacteria*, 4th edition. Approved standard M11-A4. National Committee for Clinical Laboratory Standards, Villanova, PA.

CONCLUSION

- The resin-based antimicrobial adsorbent system in BACTEC Plus media is superior to the activated charcoal system of BacT/ALERT FAN media due to the inability of FAN media to effectively remove penicillins, cephalosporins and vancomycin from blood culture growth media, even at concentrations well below peak serum levels.