

# Effectiveness of the BACTEC™ Blood Culture Resin-based Antimicrobial Removal System with Fluoroquinolones, Antifungals, Daptomycin, Tigecycline and Polymyxin B.

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## ABSTRACT

**BACKGROUND.** Antimicrobials in blood samples can cause blood culture recovery failures. BACTEC™ blood culture system Plus media contain resins to adsorb antimicrobials and allow bacteremia detection in such circumstances. This study assessed resin activity against previously untested antimicrobials.

**METHODS.** Newer fluoroquinolones levofloxacin (LEV), sparfloxacin (SPX), gatifloxacin (GAT), garenoxacin (GRN), gemifloxacin (GEM) and moxifloxacin (MOX), antifungals amphotericin B (solubilized, AMB, and lipid complex, l-AMB), fluconazole (FLZ), ketoconazole (KTZ), flucytosine (5-FC), voriconazole (VRZ), itraconazole (ITZ) and griseofulvin (GRF), the lipopeptide daptomycin (DAP), tigecycline (TGC), polymyxin B (PMB), or TGC + PMB (empiric therapy in some ICUs), were added at peak, mid, or trough serum level to 5-15 Plus Aerobic/F (resin) and 1-10 Standard/10 Aerobic/F (non-resin) blood culture bottles per condition, each with 10 mLs banked blood and 10-100 cfu of appropriate reference organism. Growth detection in < 5 days in a BACTEC™ 9240 blood culture instrument for resin but not corresponding non-resin bottles indicated effective inhibitory antimicrobial adsorption.

**RESULTS.** 100% detections with resins and fluoroquinolones, but no non-resin detections except 4 of 5 mid and 3 of 5 LEV trough and 1 of 5 GEM trough bottles. Detections with antifungals: 100% with resins; 100% non-resin with AMB, l-AMB, FLZ, 5-FC, GRF, or combinations of AMB, FLZ and 5-FC; no non-resin recoveries with KTZ or VRZ, or 2 of 3 peak ITZ bottles. DAP: 100% mid and trough and 13 of 15 peak detections with resins; 0% peak and mid, and 9 of 10 trough non-resin detections. TGC and PMB: 100% detection with resins; non-resin detections only with trough TGC and trough PMB.

**CONCLUSION.** BACTEC™ Plus medium resins were effective for the prevention of blood culture recovery failures due to clinically-relevant levels of fluoroquinolones, KTZ, VRZ, ITZ, DAP, TGC, PMB, or TGC + PMB.

## INTRODUCTION

The carryover of antimicrobials into blood culture from patients receiving chemotherapy has long been of concern (1, 2). Therapeutic failures can occur despite adequate serum concentrations of appropriate antimicrobials with susceptible organisms, due to factors such as ineffective tissue penetration (3). In such situations, antimicrobials carried into blood culture bottles with blood samples can effectively prevent the growth and detection of infectious organisms in culture despite being ineffective in patients. Such blood culture recovery failures prevent the timely detection of bacteremia and delay the identification of infectious agents and subsequent therapeutic responses.

Antimicrobial removal systems are incorporated into blood culture media to negate the effects of antimicrobial carryover. The ion exchange and nonionic adsorbent resins in BD BACTEC™ Plus blood culture media have been previously shown to effectively adsorb the broadest range of antimicrobials among antimicrobial removal systems, including ciprofloxacin (4, 5). An examination of BACTEC™ Plus medium resin performance against representatives of the newer, extended spectrum fluoroquinolones is worthwhile in light of the expanding clinical importance of this antimicrobial class (6). Likewise, increasing rates in mortality from invasive mycoses, alarming clinical rates of breakthrough candidemia, and reduced blood culture sensitivity for patients on antifungal prophylaxis prompted the testing of this antimicrobial removal system with a variety of antifungal agents (7, 8, 9).

Additionally, BACTEC™ Plus media resins were also challenged with three other previously untested antimicrobials. Daptomycin is a recently available cyclic lipopeptide promoted as an alternative to vancomycin and semi-synthetic penicillins for Gram-positive pathogens (10). Polymyxin B was selected due to the resurgence of interest in the polymyxins for combating multi-drug resistant Gram-negative organisms. Tigecycline, a new tetracycline analog, is active against MRSA and enterococci as well as many Gram-negative rods. Polymyxins and tigecycline are also of interest because of their use against extended-spectrum beta-lactamase producers, notably species of *Klebsiella*, *Enterobacter*, and other *Enterobacteriaceae*, as well as *Acinetobacter* species (11, 12).

## MATERIALS AND METHODS

**Antimicrobials and organisms.** Antimicrobial stock solutions were prepared that delivered in a 0.1 mL injection the peak, mid, or trough levels expected in 10 mLs of blood (see Table 1). Antimicrobial susceptibility testing strains (*Staphylococcus aureus* ATCC 29213, *Candida parapsilosis* ATCC 22019, and *Escherichia coli* ATCC 25922) were selected and maintained, and antimicrobials handled, in accordance with CLSI and manufacturers' guidelines (13, 14). Trough values were calculated from published peak serum levels, half-lives, and dosing regimes (15). Mid levels were averages of peak and trough values.

**Blood addition and inoculations.** Prior to the addition of antimicrobial stocks, each blood culture bottle received 10 mLs banked blood drawn not more than five days prior to use and stored at 4°C. After antimicrobials were added, bottles were inoculated with 0.1 mL each of suspensions delivering 10-100 cfu susceptible reference organism from overnight growth on TSA II with 5% sheep blood agar or Sabouraud dextrose agar (BBL). Inoculated Plus Aerobic/F BACTEC Medium (with antimicrobial-adsorbing resins) without antimicrobials served as positive controls, and inoculated Standard/10 Aerobic/F BACTEC Medium (no resins) with antimicrobials served as negative controls.

**Incubation.** After inversion to mix contents, bottles were immediately entered into a BD BACTEC™ 9240 blood culture instrument and allowed to incubate over a five-day protocol. Replicate bottles for each condition were split between two or more experiments started on different days. Bottles flagged as negative for growth after five days were considered recovery failures.

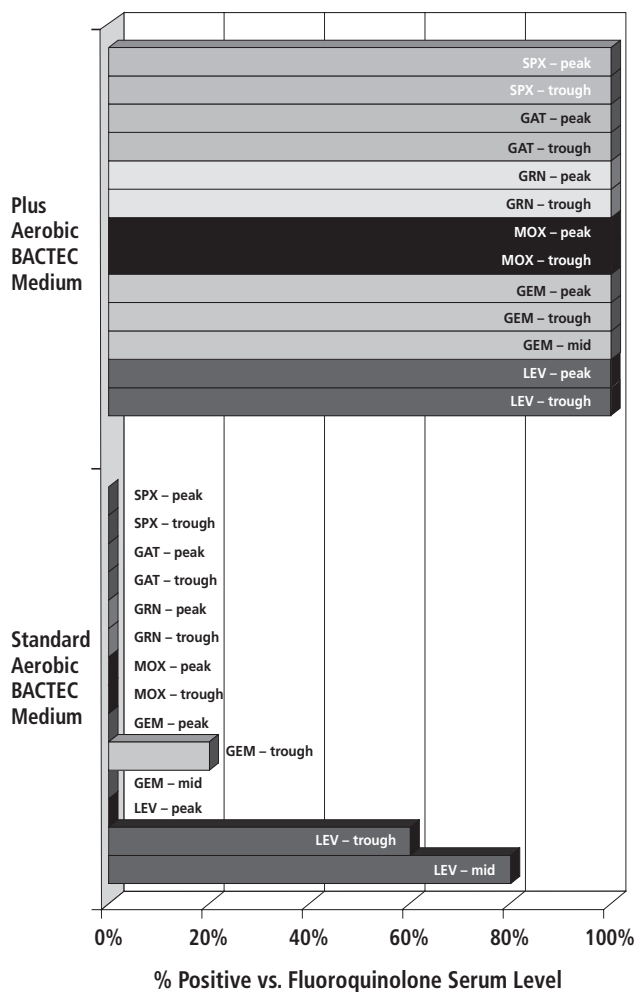
Table 1. Antimicrobials and their test concentrations, and number of Plus Aerobic/F and Standard/10 Aerobic/F BACTEC bottles per test condition.

Antimicrobial and Source	Serum Concentration, µg/ml (# Plus, # Standard Bottles Tested)		
	Peak	Mid	Trough
LEV: Levofloxacin, Chem-Impex Int'l, cat. 15155.	6.2 (10, 5)	3.4 (0, 5)	0.58 (5, 5)
SPX: Sparfloxacin, Sigma-Aldrich, cat. 56968.	2.0 (10, 2)	not tested	0.97 (3, 2)
GAT: Gatifloxacin, Chem-Impex Int'l, cat. 15156.	4.5 (10, 2)	not tested	0.35 (3, 2)
GRN: Garenoxacin, Toyama Chemical Co.	5.9 (10, 2)	not tested	1.9 (3, 2)
GEM: Gemifloxacin, Oscient Pharmaceuticals.	1.4 (10, 2)	0.77 (5, 2)	0.13 (10, 5)
MOX: Moxifloxacin, Bayer Healthcare.	4.5 (10, 2)	not tested	0.84 (3, 2)
AMB: Amphotericin B for injection, X-Gen, NDC 39822-1055-5.	2.0 (10, 6)	1.25 (5, 0)	0.5 (7, 0)
I-AMB: Amphotericin B lipid complex, Enzon, NDC 57165-101-41.	1.7 (3, 3)	not tested	0.85 (3, 1)
FLZ: Fluconazole, U. S. Pharmacopeia, cat. 1271700.	6.72 (10, 6)	not tested	4.18 (3, 2)
KTZ: Ketoconazole, MP Biomedicals, cat. 159158.	4.2 (10, 2)	not tested	0.57 (10, 2)
5-FC: Flucytosine (5-Fluorocytosine), Sigma-Aldrich, cat. F7129.	45.0 (10, 5)	not tested	12.6 (3, 2)
VRZ: Voriconazole for injection, Pfizer, NDC 0049-3190-28.	4.7 (6, 3)	not tested	3.06 (6, 3)
ITZ: Itraconazole for injection, Ortho Biotech, NDC 50458-297-10.	2.9 (6, 3)	not tested	1.81 (6, 3)
GRF: Griseofulvin, Sigma-Aldrich, cat. G4753.	2.0 (10, 5)	not tested	1.03 (3, 2)
AMB + 5-FC + FLZ	2/45/6.7 (3, 2)	not tested	not tested
DAP: Daptomycin, Cubist Pharmaceuticals.	54.6 (15, 3)	31.6 (10, 5)	8.6 (10, 10)
TGC: Tigecycline for injection, Wyeth, cat. 0108837.	0.87 (10, 5)	0.8 (0, 5)	0.72 (6, 5)
PMB: Polymyxin B sulfate, Sigma-Aldrich, cat. 405-20-5.	5.0 (10, 5)	2.5 (5, 5)	0.0023 (0, 5)
TGC + PMB	0.87/5.0 (10, 5)	0.8/2.5 (5, 5)	0.72/0.0023 (10, 5)

## RESULTS

**Effect of resins on recovery rates in the presence of newer fluoroquinolones.** A recovery rate of 100% was obtained for *S. aureus* in Plus Aerobic medium (with antimicrobial-adsorbing resins) in the presence of trough, mid, or peak serum levels of any of the six newer fluoroquinolones tested (Graph 1). By contrast, in medium without resins four of the fluoroquinolones caused 100% recovery failures (SPX, GAT, GRN, and MOX) at any serum level tested. Of the remaining two antibiotics, only two of 10 cultures with GEM at trough concentration detected and none did so at mid or peak levels; three of five trough, four of five mid, and no cultures with the peak level of LEV were positive for growth. Recovery failures in the presence of fluoroquinolones occurred in none of the 92 resin medium bottles (0%), but occurred in 31 of the 40 cultures without resins (77.5%). This is noteworthy given the increasing use of newer fluoroquinolones as alternatives to beta-lactams or macrolides (6).

Graph 1. Recovery of *S. aureus* ATCC 29213 in the presence of fluoroquinolones and 10 mLs blood from blood culture bottles with (Plus Medium) or without (Standard Medium) antimicrobial-adsorbing resins.

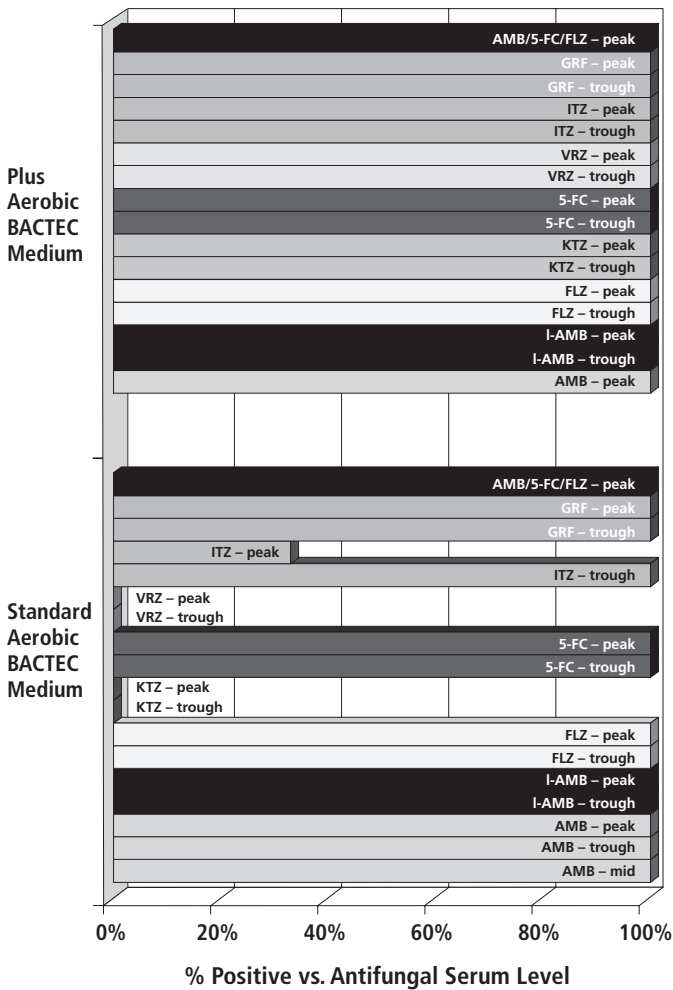


RESULTS (continued)

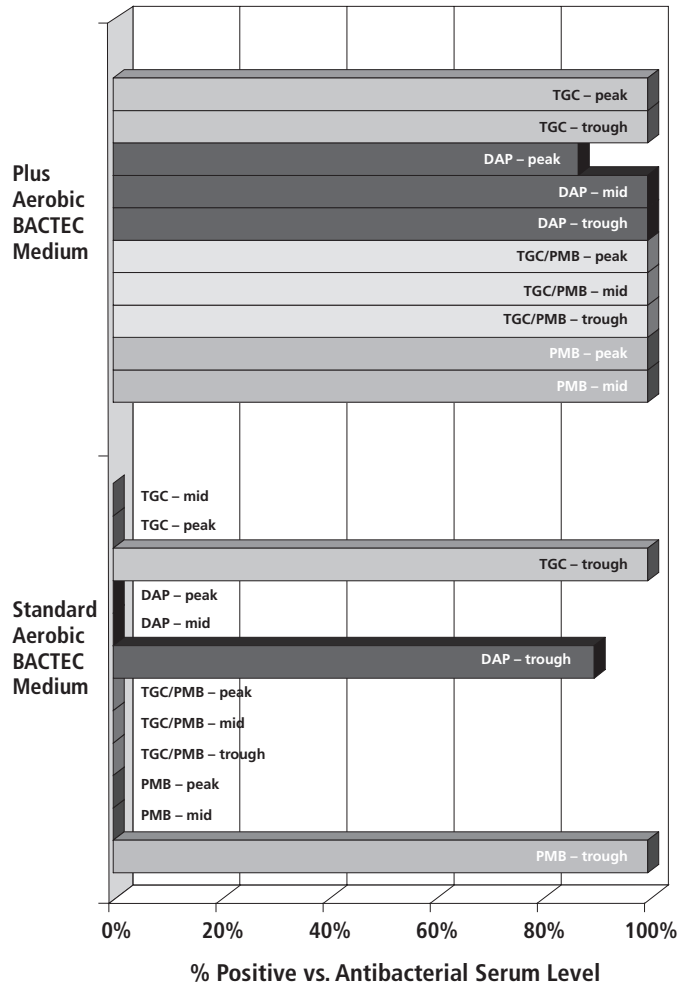
**Effect of resins on recovery rates in the presence of antifungals.** Resin medium was unnecessary for the recovery of *C. parapsilosis* in the presence of trough, mid, or peak serum levels of FLZ, 5-FC, GRF, either form of AMB tested, or a combination of AMB, 5-FC and FLZ (Graph 2). The results were the same when these tests were carried out in growth medium that did not contain a CO<sub>2</sub> sensor at the bottom of each bottle (data not presented). It was surprising that the dilution of blood samples by growth medium allowed *C. parapsilosis* growth in the presence of these antifungals, in light of the general ineffectiveness of dilution by culture medium with carryover antibacterials (4). However, ITZ caused a recovery failure rate of 33% at peak level without resins, and VRZ and KTZ caused 100% recovery failures at peak or trough levels in non-resin medium. There were no recovery failures among any of the 114 resin medium bottles with antifungals.

**Effect of resins on recovery rates in the presence of daptomycin, tigecycline, or polymyxin B.** All 91 resin medium cultures with these antibacterials were positive for growth except two of the 15 bottles with peak level DAP, for a total resin medium recovery rate of 97.8% (Graph 3). Only 19 of 63 bottles of non-resin medium were positive for growth, a 30.2% recovery rate, with 100% recovery only with TGC or PMB at trough levels and 100% recovery failure at mid or peak levels of DAP, TGC, PMB, or TGC + PMB. Overall rates of recovery failure with these three antibacterials were 69.8% without resins versus 2.2% with resins. TGC alone was tested with *S. aureus*, but in combination with PMB was tested with *E. coli*. The trough level of PMB is trivial, so the trough TGC + PMB condition was a de facto test of TGC alone against *E. coli*. *S. aureus* grew without resins in trough TGC alone, but trough TGC + PMB non-resin bottles had no detections. Since *E. coli* was more sensitive than *S. aureus* to TGC, recovery failures due to TGC may be more likely with *E. coli* than *S. aureus*.

Graph 2. Recovery of *C. parapsilosis* ATCC 22019 in the presence of antifungals and 10 mLs blood from blood culture bottles with (Plus Medium) or without (Standard Medium) antimicrobial-adsorbing resins.



Graph 3. Recovery of *S. aureus* ATCC 29213 (TGC, DAP) or *E. coli* ATCC 25922 (PMB, TGC + PMB) in the presence of antibacterials and 10 mLs blood from blood culture bottles with (Plus Medium) or without (Standard Medium) antimicrobial-adsorbing resins.



## CONCLUSION

- Drawing blood for blood cultures just before the administration of antimicrobials is good hospital practice, but a blood culture antimicrobial removal system is still necessary even with trough antimicrobial serum levels to prevent recovery failures due to antimicrobial carryover.
- At trough serum levels four of six newer fluoroquinolones caused 100% recovery failures and the other two had unreliable recovery. The resin-based antimicrobial removal system of BACTEC Plus blood culture media allowed 100% recovery with trough or peak levels of these potent antimicrobials.
- An antimicrobial removal system is necessary for patients receiving several important antifungals (voriconazole, ketoconazole, and itraconazole). This is of significance given the frequency and problematic nature of fungal infections among cancer and other immunocompromised patients, use of VRZ as a salvage therapy for treatment failures of invasive candidiasis, and use of ITZ and KTZ in combination with other antifungals (16, 17, 18).
- Recovery failure rates in non-resin medium were 100% for tigecycline or polymyxin B at mid and peak levels, and for trough, mid, or peak TGC + PMB. There were no recovery failures with these two antimicrobials in resin medium. Resin medium was also required for reliable detection of *S. aureus* in the presence of clinically relevant concentrations of daptomycin.

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