

Ability of BD BACTEC Plus Blood Culture Bottles versus BacT/Alert FAN Blood Culture Bottles to Detect Bacterial Pathogens in Samples Containing Therapeutic Levels of Cefoxitin and Piperacillin/Tazobactam

D. FLAYHART, A. BOREK, T. WAKEFIELD, J. DICK, AND K.C. CARROLL

Division of Microbiology, Department of Pathology, The Johns Hopkins Hospital • Baltimore, MD

REVISED ABSTRACT

Blood culture bottles with antimicrobial removal systems are recommended for patients who develop fever while on antibiotics. This study compared the ability of the Becton Dickinson (Sparks, MD) BACTEC Plus bottles and the bioMerieux (Durham, NC) BacT/Alert FAN bottles to effectively remove cefoxitin and piperacillin/tazobactam thus allowing bacterial pathogens to grow. Each bottle was spiked with 10 mL of human blood, antibiotic, and strains of methicillin susceptible *S. aureus*, *S pneumoniae*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* susceptible to the antibiotic evaluated. *P. aeruginosa* was tested against piperacillin/tazobactam only. Testing was completed in triplicate using 10-100 cfu/mL of organisms with varying concentrations of each antibiotic. Bottles were mixed and loaded onto instruments per manufacturer's instructions. Antimicrobial removal was evaluated on the basis of time to detection (TTD) up to five days of incubation. The results were as follows:

	BACTEC Plus	BacT/Alert FAN		BACTEC Plus	BacT/Alert FAN
	# of positives;TTD (h)			# of positives;TTD (h)	
No Cefoxitin	24/24;9.21	24/24;13.03	No Pip/Tazo	30/30;10.46	30/30;13.48
Cefoxitin trough (10 µg/mL)	24/24;9.38	13/24;19.69	Pip/Tazo trough (5/0.7 µg/mL)	30/30;11.52	10/30;16.96
Cefoxitin mid (60 µg/mL)	24/24;9.99	0/24	Pip/Tazo mid (100/10 µg/mL)	30/30;12.85	2/30;16.97
Cefoxitin peak (110 µg/mL)	24/24;11.96	0/24	Pip/Tazo peak (240/24 µg/mL)	30/30;14.68	0/30
Total Recovery	96/96;10.14	37/96;15.36	Total Recovery	120/120;12.38	42/120;14.48

The BacT/Alert FAN system recovered 15% of the challenge organisms and 100% of the control organisms. *E. coli* was recovered at trough levels for both antibiotics and mid-level of piperacillin/tazobactam. *S. pneumoniae* was also recovered at the trough cefoxitin level. In contrast, the BACTEC Plus system recovered 100% of challenge and control organisms. This study demonstrates the superiority of the BACTEC Plus system compared to the BacT/Alert FAN system in recovering bacterial pathogens in the presence of cefoxitin and piperacillin/tazobactam.

INTRODUCTION

There are 200,000 bloodstream infections per year in the US. Bacteremia is associated with mortality rates from 20% to 50%. Nosocomial episodes are >50% in some hospitals and result in prolonged hospitalizations and increased mortality compared to community-associated episodes. (1)

28% to 63% of patients who have blood cultures obtained are on antibiotic therapy.(2,3) It is important to recover bacterial isolates from these patients because timely identification of the responsible pathogen permits targeted broad spectrum empiric therapy and if required, institution of Infection Control precautions. In addition, the organism is available for confirmatory susceptibility testing. Cefoxitin is approved for use in the treatment of lower respiratory tract infections and intra-abdominal and gynecological (gyn) infections caused by susceptible organisms. It is also approved for use for the prophylaxis of infections in patients undergoing uncontaminated gastrointestinal and gyn surgery. Piperacillin/tazobactam has broad spectrum activity against a variety of gram-positive and gram-negative bacteria. It is the most frequently used β -lactam agent for empiric treatment of patients with fever and potential serious illness prescribed in the JHH.

This study evaluated the ability of BACTEC Plus bottles (BD Diagnostics, Sparks, MD) and BacT/Alert FAN bottles (bioMerieux, Durham, NC) to remove cefoxitin and piperacillin/tazobactam and allow bacterial pathogens to grow. Also analyzed was the reduction of cefoxitin over time using an agar well diffusion method (bioassay).

MATERIALS AND METHODS

Media. BACTEC Plus bottles used for testing had an expiration date of 7/31/05 for first round of testing and 10/31/05 for the second round. BacT/Alert FAN bottles had an expiration date of 11/30/05 for the first round of testing and 2/28/06 for the second round.

Cefoxitin and Piperacillin/Tazobactam. Antibiotics were diluted to concentrations that would result in final potencies of 10 µg/mL, 60 µg/mL, and 110 µg/mL for cefoxitin and 5/0.7 µg/mL, 100/10 µg/mL, and 240/24 µg/mL for piperacillin/tazobactam. These potencies are the trough, mid and peak therapeutic serum levels.(4,5) Antibiotic was measured and prepared on each day of use.

Organisms. Both rounds of testing included ATCC type strains of gram-positive and gram-negative pathogens. The following ATCC strains were used: methicillin susceptible *S. aureus* ATCC 25923, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 33495, *P. aeruginosa* ATCC 23853. Serial dilutions of the organisms were completed to achieve a final concentration of 10 – 100 cfu/mL. Colony counts were completed to confirm concentrations. One diluted stock solution per organism was used for all testing/round.

Bottle Inoculation/Incubation. Bottles were inoculated with 10 mLs of banked blood drawn not more than five days prior to use and stored at 4°C. After inoculation of blood, antibiotic was added to the bottles. One set of each bottle type had no antibiotic added and was used as the growth control. After the addition of blood and antibiotic, organisms were added to all bottles. Bottles were inverted to mix. The four antibiotic concentrations were repeated in triplicate with each organism.

Immediately after inoculation, the bottles were loaded into the instruments for a five-day incubation protocol. When machines flagged bottles as positive, the bottles were pulled and a Gram stain and subculture was completed. If a bottle was a false positive, the bottle was reloaded for continued incubation.

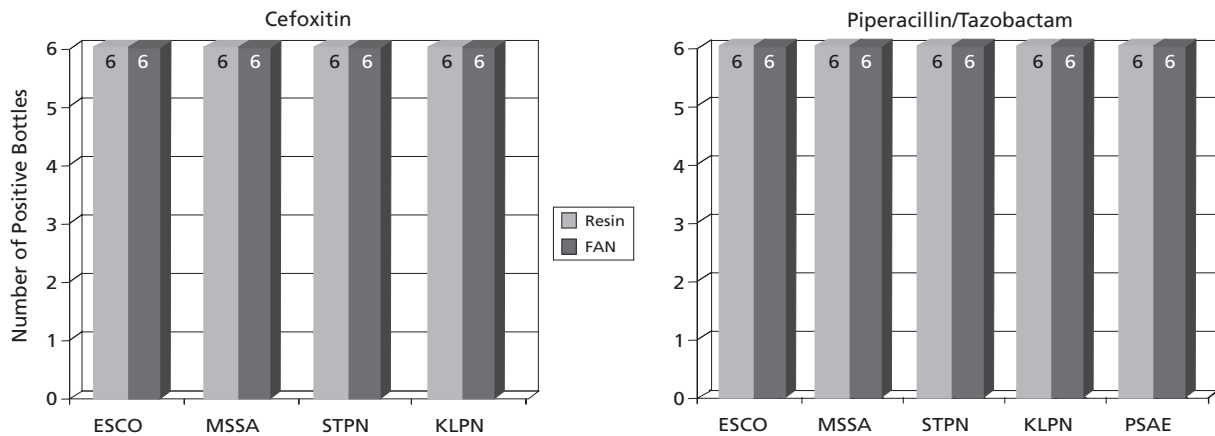
This protocol was repeated in duplicate on two different days. Data is shown as cumulative results obtained from both days of testing.

Agar Well Diffusion Method (Bioassay) for Cefoxitin. Bottles were inoculated with 10 mLs of banked blood and varying concentrations of cefoxitin. 2 mLs were immediately removed and spun at 1,400 rpm for 10 minutes. Bottles were loaded into

instruments and incubated for one hour. An additional 2 mLs were removed and spun. All samples were analyzed for antibiotic levels using the agar well diffusion method (bioassay). The bioassay was done according to established standard protocol (6). An ATCC strain of *S. aureus* (29213) was used for testing. Organisms were grown on blood agar media for 24h and inoculated into 5 mL of Mueller-Hinton broth (MHB). The MHB tubes were incubated in a water bath at 37°C until the turbidity reached a 0.5 McFarland standard concentration. Subsequently, a 1:10 dilution was made of *S. aureus*. Four milliliters of each dilution was added to a conical tube of liquefied media and inverted to mix. The media were then poured into 150-mm plates and allowed to harden. A 3-mm sterile, metal tube was used to punch holes in the media for testing. Each hole was then inoculated with 5 µL of cefoxitin (concentrations: 25, 50, 100, 200 µg/mL), controls, and samples from bottles in triplicate. All plates were incubated overnight at 37°C. Mean zone sizes were calculated. Sample concentrations were calculated using the cefoxitin standard curve. The lower limit of the assay was 10 µg/mL, therefore, trough levels weren't able to be determined.

RESULTS

Figure 1. Recovery of Pathogens from Blood Bottles with No Cefoxitin or Piperacillin/Tazobactam



ESCO= *E. coli*, MSSA= methicillin susceptible *S. aureus*, STPN= *S. pneumoniae*, KLPN= *K. pneumoniae*, PSAE= *P. aeruginosa*

Figure 2. Recovery of Pathogens at Trough Levels of Cefoxitin (10 µg/mL) and Piperacillin/Tazobactam (5/0.7 µg/mL)

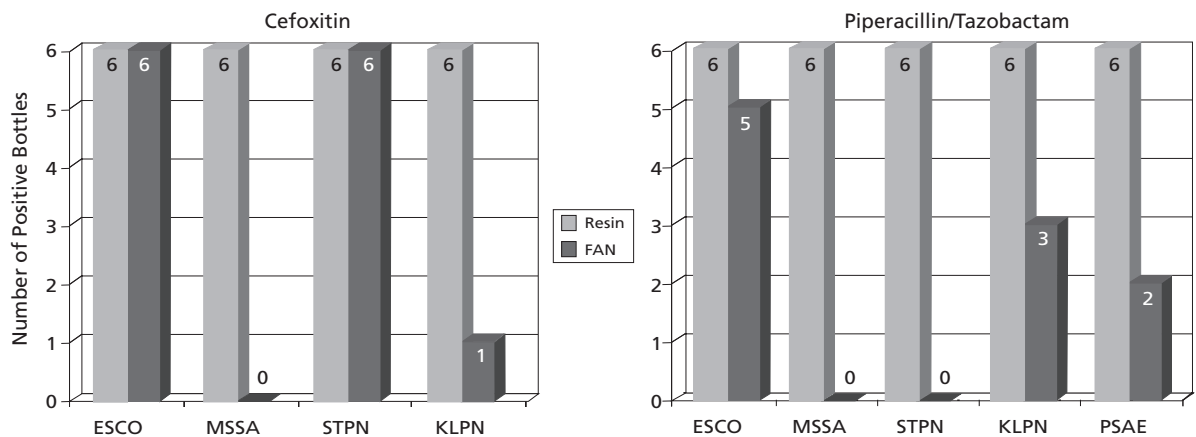


Figure 3. Recovery of Pathogens at Mid Levels of Cefoxitin (60 µg/mL) and Piperacillin/Tazobactam (100/10 µg/mL)

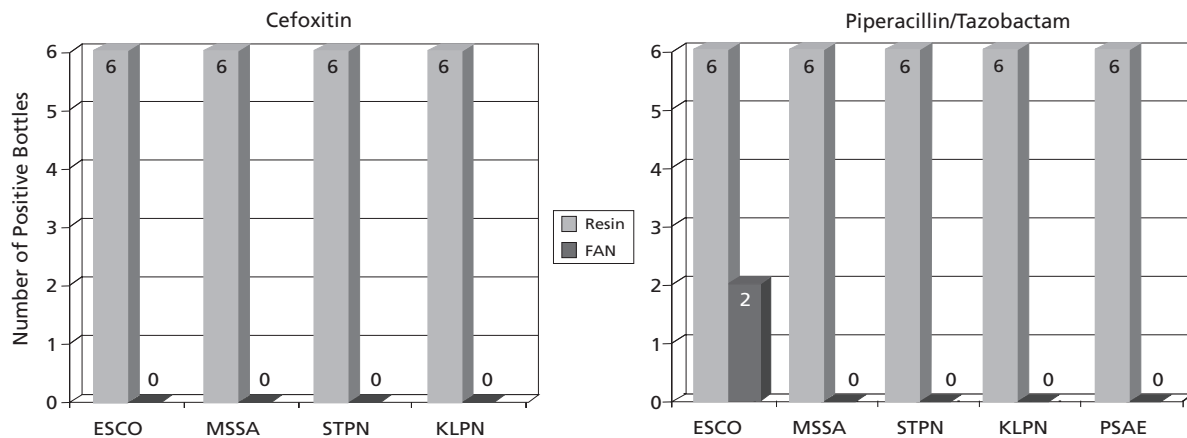


Figure 4. Recovery of Pathogens at Peak Levels of Cefoxitin (110 µg/mL) and Piperacillin/Tazobactam (240/24 µg/mL)

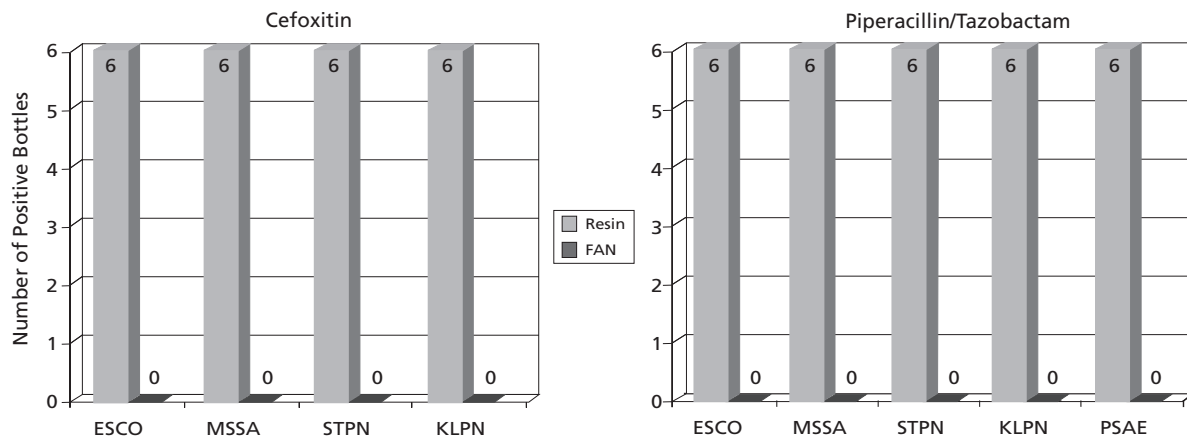


Table 1. Time to Detection (TTD) in hours for Bottles Containing Cefoxitin

	Resin	FAN
Base (No Cefoxitin)	9.21 N=24	13.03 N=24
Trough (10 µg/mL Cefoxitin)	9.38 N=24	19.69 N=13
Mid (60 µg/mL Cefoxitin)	9.99 N=24	n/a N=0
Peak (110 µg/mL Cefoxitin)	11.96 N=24	n/a N=0
Total	10.14 N= 96	15.36 N=37

Table 2. Time to Detection (TTD) in hours for Bottles containing Piperacillin/Tazobactam

	Resin	FAN
Base (No Pip/Tazo)	10.46 N=30	13.48 N=30
Trough (5/0.7 µg/mL Pip/Tazo)	11.52 N=30	16.96 N=10
Mid (100/10 µg/mL Pip/Tazo)	12.85 N=30	16.97 N=2
Peak (240/24µg/mL Pip/Tazo)	14.68 N=30	n/a N=0
Total	12.38 N=120	14.48 N=42

Table 3. Percent of Remaining Cefoxitin Concentration after One Hour Incubation

	Resin	FAN
Base No Cefoxitin	0	0
Mid (60 µg/mL)	0	71
Peak (110 µg/mL)	0	70

CONCLUSIONS

- BacT/Alert FAN system recovered 15% (25/162) of the challenge organisms and 100%(54/54) of the antibiotic-free controls. — At mid levels, only two *E. coli* were detected.
- The BACTEC Plus system recovered 100% of challenge and control organisms.
- The quantitative analysis of the cefoxitin levels correlated with the bacterial time to detection data.
- This study demonstrates the superiority of the BACTEC Plus system compared to the BacT/Alert FAN system in recovering gram-positive and gram-negative pathogens in the presence of cefoxitin and piperacillin/tazobactam.

REFERENCES

1. Magadia, et.al, "Laboratory Diagnosis of Bacteremia and Fungemia", 2001, *Infect Dis Clinics N Amer* 15:1009.
2. Weinstein, et.al., 1997, "The Clinical Significance of Positive Blood Cultures in the 1990s: A Prospective, Comprehensive Evaluation of the Microbiology, Epidemiology, and Outcome of Bacteremia and Fungemia in Adults," *Clin Infec Dis* 24:584-602.
3. Pohlman, et.al., 1995, "Controlled Clinical Evaluation of BACTEC Plus Aerobic/F and BacT/Alert Aerobic FAN Bottles for Detection of Bloodstream Infections," *J Clin Micro* 33:2856-2858.
4. Mandela, G, et.al., 2000, "Principles and Practice of Infectious Disease," 5th edition, p. 578.
5. Piperacillin/ Tazobactam *In* Physician's Desk Reference, 60th ed. 2006. Thomson Healthcare, Inc., Montvale, NJ, pp. 3492 – 3493.
6. Antibiotic Assays *In* Laboratory Procedures in Clinical Microbiology, 1974. By J.A. Washington, II, Little, Brown, and Co., Boston.