

# Ability of BD BACTEC Plus Blood Culture Bottles versus BacT/Alert FAN Blood Culture Bottles to Detect Bacterial Pathogens in Samples Containing Vancomycin

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## ABSTRACT

Bacterial blood cultures are often obtained after therapy has begun. One of the most frequently prescribed antimicrobial agents is vancomycin. Blood culture bottles with antimicrobial removal systems are recommended for patients who develop fever while on antibiotics. This study compared the effectiveness of the Becton Dickinson (Sparks, MD) BACTEC Plus bottles and the bioMerieux (Durham, NC) BacT/Alert FAN bottles to remove vancomycin and allow bacterial pathogens to grow. Each bottle was spiked with 10 ml of human blood, vancomycin, and vancomycin susceptible strains of methicillin susceptible *S. aureus*, methicillin resistant *S. aureus*, *E. faecium*, *E. faecalis*, *S. viridans*, and *S. pneumoniae*. Testing was completed in triplicate using 100-200 cfu/ml of organism with varying concentrations of vancomycin. Two rounds of testing were completed on different days. Bottles were mixed and loaded onto instruments per manufacturer's instructions. Vancomycin removal was evaluated on the basis of time to detection (TTD) up to five days of incubation. The results were as follows:

## 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 pathogens from these patients as isolation of the organism permits identification and antibiotic susceptibility testing. The identification of the isolate allows for proper antimicrobial therapy, targeting of broad spectrum empiric therapy, and if required, institution of Infection Control precautions. Vancomycin is a widely used antibiotic in hospitals. One study demonstrated a 20 fold increase in usage of vancomycin over 10 year period (4).

This study evaluated the ability of BACTEC Plus bottles (BD Diagnostics, Sparks, MD) and BacT/Alert FAN bottles (bioMerieux, Durham, NC) to remove vancomycin and allow bacterial pathogens to grow. Also analyzed was the reduction of vancomycin levels over time using a quantitative immunoassay.

	BACTEC Plus # of positive bottles; mean TTD	BacT/Alert FAN # of positive bottles; mean TTD
No Vancomycin	36/36; 11.4 hours	31/36; 13.4 hours
Vancomycin trough (10 µg/ml)	36/36; 15.9 hours	18/36; 29.4 hours
Vancomycin mid (25 µg/ml)	34/36; 18.8 hours	5/36; 35.8 hours
Vancomycin peak (50 µg/ml)	32/36; 19.5 hours	0/36
Total recovery	138/144; 13.4 hours	54/144; 20.8 hours

The BacT/Alert FAN system recovered 21% of the challenge organisms and only 86% of the antibiotic-free controls. At mid levels, only *E. faecalis* was detected. In contrast, the BACTEC Plus system recovered 95% of challenge and control organisms missing six *S. pneumoniae* isolates at the higher concentrations of vancomycin. This study demonstrates the superiority of the BACTEC Plus system compared to the BacT/Alert FAN system in recovering gram-positive pathogens in the presence of vancomycin.

## 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.

### Vancomycin

Antibiotic was diluted to concentrations that would result in final potencies of 10 µg/ml, 25 µg/ml, and 50 µg/ml. These potencies are the trough, mid and peak therapeutic serum levels (5). Antibiotic was measured and prepared on day of use.

### Organisms

Initial round of testing included ATCC and clinical strains of gram-positive pathogens. The second round of testing used the following ATCC strains: methicillin susceptible *S. aureus* ATCC 25923, methicillin resistant *S. aureus* ATCC 43300, *E. faecium* ATCC 35667, *E. faecalis* ATCC 49533, *S. viridans* ATCC 10551, and *S. pneumoniae* ATCC 49619. Serial dilutions of the organisms were completed to achieve a final concentration of 100 cfu/ml. Colony counts were completed to confirm concentrations. One diluted stock solution per organism was used for all testing/trial.

### 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 bottles. One set of each bottle type had no antibiotic added and were used as growth controls. 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.

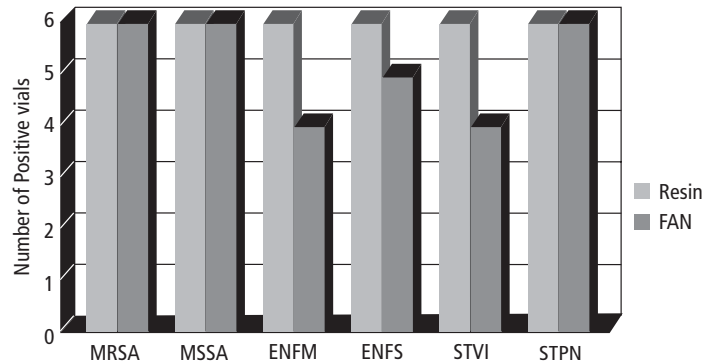
This protocol was repeated in duplicate on two different days. Data is shown as cumulative results obtained from both days of testing.

### Quantitative Immunoassay for Vancomycin

Bottles were inoculated with 10 mls of banked blood and varying concentrations of vancomycin. Two 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 vancomycin levels using the EMIT® 2000 Vancomycin Assay (Syva, Cupertino, CA). The assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of vancomycin in human serum or plasma.

## RESULTS

Figure 1. Recovery of Pathogens with No Vancomycin



MRSA= methicillin resistant *S. aureus*, MSSA=methicillin susceptible *S. aureus*, ENFM= *E. faecium*, ENFS=*E. faecalis*, STVI=*S. viridans*, STPN=*S. pneumoniae*

Figure 2. Recovery of Pathogens with Trough Levels of Vancomycin (10µg/ml)

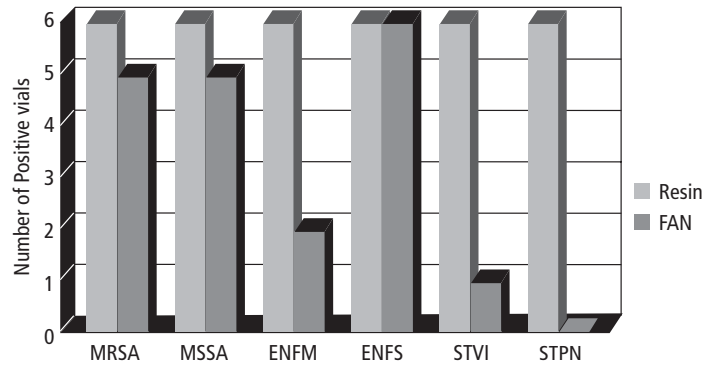


Figure 3. Recovery of Pathogens with Mid Levels of Vancomycin (25µg/ml)

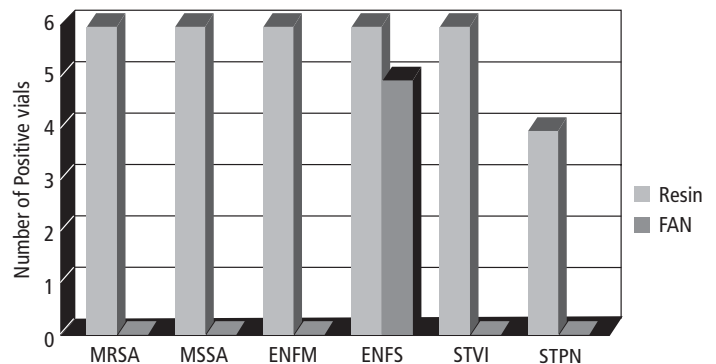


Figure 4. Recovery of Pathogens with Peak Levels of Vancomycin (50µg/ml)

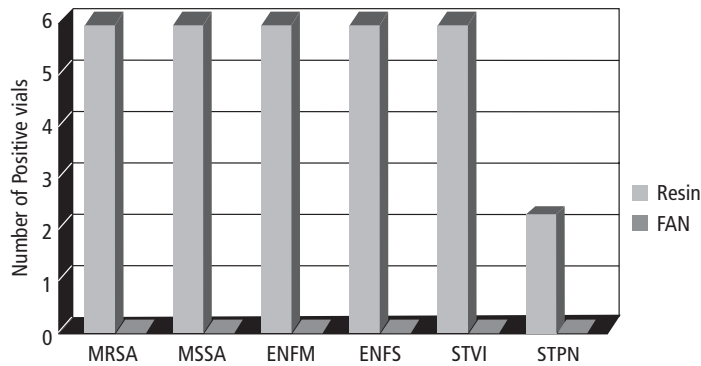


Figure 5. TTD based on Vancomycin concentrations

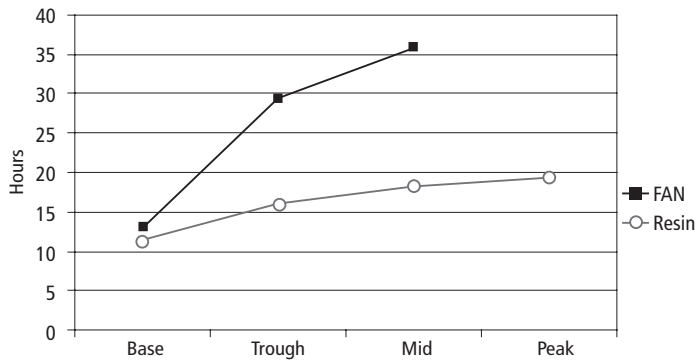


Table 1. Time to Detection (TTD) in hours

	Resin	FAN
<b>Base</b>	<b>11.48</b>	<b>13.41</b>
(No Vancomycin)	N=36	N=31
<b>Trough</b>	<b>15.98</b>	<b>29.37</b>
(10 µg/ml)	N=36	N=18
<b>Mid</b>	<b>18.18</b>	<b>35.8</b>
(25 µg/ml)	N=34	N=5
<b>Peak</b>	<b>19.45</b>	<b>n/a</b>
(50 µg/ml)	N=32	N=0

Table 2. Percent of Remaining Vancomycin Concentration after One Hour Incubation

	Resin	FAN
<b>Base</b>	<b>0</b>	<b>0</b>
(No Vancomycin)		
<b>Trough</b>	<b>0</b>	<b>88</b>
(10 µg/ml)		
<b>Mid</b>	<b>0</b>	<b>90</b>
(25 µg/ml)		
<b>Peak</b>	<b>30</b>	<b>72</b>
(50 µg/ml)		

### CONCLUSIONS

- The BacT/Alert FAN system recovered 21% of the challenge organisms and only 86% of the antibiotic-free controls.
  - At mid levels, only *E. faecalis* was detected.
- The BACTEC Plus system recovered 95% of challenge and control organisms.
  - Six *S. pneumoniae* isolates were missed at the higher concentrations of vancomycin.
- The quantitative analysis of the vancomycin 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 pathogens in the presence of vancomycin.

### 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 Infect 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. Ena, J., et.al., 1993, "The Epidemiology of Intravenous Vancomycin Usage in a University Hospital," JAMA, 269:598-602.
5. Mandela, G, et.al., 2000, "Principles and Practice of Infectious Disease," 5th edition, p. 578.

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