

Evaluation of BacT/Alert FAN Plus Bottles for the Culture of Peritoneal Dialysate

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Background: A major complication of peritoneal dialysis (PD) is peritonitis, and bacterial culture of PD effluent in a blood culture bottle is the preferred technique for diagnosis of peritonitis. In this study, we compared dialysate inoculation and culture using the BacT/AlerT[®] Fastidious Antimicrobial Neutralization Plus blood culture bottles (FAN Plus; bioMérieux, France) to the conventional centrifugation culture method.

Methods: A total of 170 PD effluents were simultaneously processed by the conventional centrifugation culture method and by culture using FAN Plus media with two different inoculation procedures: inoculation after centrifugation and direct bedside inoculation.

Results: Of the 52 cultures that were positive on at least one of the culture methods, 27 samples were positive on conventional centrifugation. However, 46 samples showed growth following inoculation into the FAN Plus media after centrifugation, and 47 samples were positive on the direct FAN Plus inoculation

method. Using the case definition for PD peritonitis to classify samples, sensitivity of the conventional method was 50.0% (95% CI, 33.7-66.3%), whereas the sensitivity of the FAN Plus media was 78.9% (95% CI, 62.2-89.9%) by inoculation after centrifugation and 86.8% (95% CI, 71.1-95.1%) by direct inoculation. Use of both inoculation methods with FAN Plus media resulted in 92.1% sensitivity (95% CI, 89.2-99.9%).

Conclusion: Culture using FAN Plus media demonstrated a superior bacterial recovery rate to the conventional centrifugation culture method. A combination of the two inoculation methods with FAN Plus media is recommended for the best diagnostic yield, while direct inoculation alone can be useful due to its simplicity and cost-effectiveness. (*Ann Clin Microbiol* 2019;22:90-95)

Key Words: BacT/AlerT 3D system, Centrifugation, Culture media, FAN Plus blood culture bottle, Peritoneal dialysis, Peritonitis

INTRODUCTION

Continuous ambulatory peritoneal dialysis (PD) has become the preferred method of home dialysis for patients with end-stage renal failure [1]. The most concerning complication of PD is peritonitis, which is the direct or major contributing cause of death in about 16% of PD patients and accounts for antibiotic use in many patients undergoing PD [2,3]. Therefore, identification of the causative organism and its antibiotic sensitivities not only helps guide the choice of antibiotic, but also often sug-

gests the possible source of infection [3].

International Society for Peritoneal Dialysis (ISPD) guidelines recommend that blood-culture bottles be used for bacterial culture of PD effluent and also suggest that centrifugation of dialysate effluent prior to inoculation can increase the microbial yield [3]. Specifically, the ISPD 2016 guidelines suggest use of several specimen processing and culture methods including direct inoculation into blood-culture bottles, centrifuging PD fluid followed by inoculation into solid culture media or blood-culture media, and the lysis centrifugation technique [3].

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Numerous studies have documented the advantage of using blood-culture bottle kits to improve the microbial yield of peritoneal fluid culture [2,4-9]. The widely used BacT/AlerT[®] 3D system (BacT/AlerT system; bioMérieux, Marcy l’Etoile, France) is an automated blood culture system that can be used in combination with BacT/AlerT[®] Fastidious Antimicrobial Neutralization Plus blood culture bottles (FAN[®] Plus bottles; bioMérieux). FAN Plus is a resin-based media containing antibiotic-binding polymeric beads in place of charcoal-based antimicrobial neutralization (FAN blood culture media). Although various automated blood culture systems using blood-culture bottle kits have been evaluated for culture of PD fluid [2,4,7-11], studies assessing recently developed blood-culture bottles and media are lacking.

In this study, we evaluated the performance of inoculation and culture of PD effluent using FAN Plus blood culture media. We compared the outcome of culture in the FAN Plus media by two different inoculation procedures—inoculation after centrifugation and direct inoculation—to the conventional centrifugation culture method.

MATERIALS AND METHODS

1. Patient population

This study was conducted at a tertiary-care hospital in Seoul, Korea and was approved by the Institutional Review Board of Samsung Medical Center (approval number: 2018-07-174). PD effluent samples submitted to the laboratory between Jan 2014 to May 2018 were included in this study. A total of 170 effluents from 93 patients were evaluated. All samples were simultaneously processed by the conventional centrifugation culture method and by two different inoculation methods into the FAN Plus blood culture media: 1) inoculation after centrifugation and 2) direct bedside inoculation.

2. PD effluent processing methods and microbial identification

The conventional centrifugation culture method and inoculation into the FAN Plus after centrifugation were conducted using centrifuged sediment inoculation as follows. The 50 mL of PD effluents were centrifuged at 3,000×g for 15 minutes, followed by resuspension of the sediment in 3-5 mL of supernatant. The 1.5-2.5 mL of resuspended sediment was inoculated into FA Plus (aerobic) blood culture bottles and FN Plus (anaerobic) blood culture bottles, respectively. The remaining resuspended sediment was inoculated into conventional cul-

ture media (5% sheep’s blood agar, MacConkey agar, Brucella agar, and thioglycolate broth for anaerobes). The direct bedside FAN Plus inoculation was done by directly adding 5-10 mL peritoneal dialysis effluent into FA Plus and FN Plus blood culture bottles at bedside.

Conventional media cultures were examined by standard protocols and growth was monitored for up to 7 days [12]. FAN Plus bottles were incubated in the BacT/Alert 3D system at 36°C with continuous monitoring for 5 days. When bacterial growth was detected by CO₂ production, the broth was aspirated for Gram staining and subculture. An aliquot of the FA Plus media cultures was subcultured onto 5% sheep’s blood agar, MacConkey agar, and chocolate agar, and then incubated at 35°C in 5% CO₂. For FN Plus media cultures, an aliquot of the broth was subcultured onto 5% sheep’s blood agar, MacConkey agar, and brucella agar, and then incubated in an anaerobic chamber (Bactron II & IV, Sheldon Manufacturing Inc., Cornelius, OR, USA). Each isolated organism was identified using the Vitek 2 or Vitek MS system (BioMérieux, Marcy l’Etoile, France).

3. Diagnosis of peritonitis

All clinical findings of patients were assessed by medical record review. In accordance with the ISPD guidelines, cases were classified as having PD peritonitis when at least two of the following were true: 1) clinical features consistent with peritonitis, such as abdominal pain, cloudy PD effluent; 2) PD effluent with white cell count >100/μL and with more than 50% neutrophils; 3) positive PD culture [3]. Follow-up cultures for the same patient within the same infectious event were excluded from analyses of diagnostic sensitivity and specificity.

4. Statistical analysis

The recovery rates of microorganisms in culture using the FAN Plus media were compared to those using the conventional centrifugation culture method by McNemar’s test. Diagnostic sensitivity and specificity of each culture method were calculated on the basis of peritonitis diagnosis by the ISPD definition. A *P* value less than 0.05 was considered statistically significant. For statistical evaluations, VassarStats (<http://vassarstats.net>) was used.

RESULTS

Among 170 PD effluent samples, 52 showed culture-positive

results by at least one culture method. A total of 27 samples were culture-positive following the conventional centrifugation culture method, 46 samples showed growth in the FAN Plus media inoculated after centrifugation, and 47 samples were positive following direct inoculation into FAN Plus media (Table 1). The use of both inoculation methods with FAN Plus media resulted in culture positivity of five additional samples. Compared to the conventional culture method, significantly more isolates were recovered from each method using FAN Plus media: FAN Plus inoculation after centrifugation and direct FAN Plus inoculation (P value <0.001).

The microorganisms isolated by each method are listed in Table 2. Of the total 52 culture-positive samples, 75 different bacterial organisms were detected and polymicrobial growth occurred in 11 samples. None of the isolates from culture-positive samples were found only in the conventional culture method. However, a total of 40 isolates were found only in the cultures with the FAN Plus media. Among those, eight isolates were grown only using the method of FAN Plus inoculation after centrifugation. These included three *Enterobacteriaceae*, two enterococci, and three other bacteria. Fourteen isolates were recovered only from the direct FAN Plus inoculation method and in-

Table 1. The recovery of microorganisms following culture methods in FAN Plus media compared the conventional centrifugation culture method

Method	Result	Conventional centrifugation culture (no. of specimens)			P value
		Positive	Negative	Total	
FAN Plus inoculation after centrifugation	Positive	27	19	46	<0.001
	Negative	0	124	124	
	Total	27	143	170	
Direct FAN Plus inoculation	Positive	26	21	47	<0.001
	Negative	1	122	123	
	Total	27	143	170	
Combination of two methods using FAN Plus media	Positive	27	25	52	<0.001
	Negative	0	118	118	
	Total	27	143	170	

Table 2. Microorganisms isolated from each culture method

Organism	No. of organisms detected			
	Conventional centrifugation culture	FAN Plus inoculation after centrifugation	Direct FAN Plus inoculation	Total
Gram positive	12	27	29	34
<i>Staphylococcus aureus</i>	4	5	5	5
Coagulase-negative staphylococci	2	5	6	7
<i>Enterococcus faecium</i>	1	2	3	3
<i>Enterococcus faecalis</i>	1	4	5	6
Other <i>Enterococcus</i> spp.	1	6	4	6
<i>Streptococcus</i> spp.	2	2	2	3
Other	1	3	4	4
Gram negative	15	30	26	33
<i>Escherichia coli</i>	3	5	5	6
<i>Citrobacter freundii</i>	1	6	5	6
<i>Enterobacter</i> spp.	1	6	4	6
<i>Klebsiella pneumoniae</i>	0	0	1	1
<i>Pseudomonas aeruginosa</i>	7	8	7	8
Other	3	5	4	6
Anaerobes	4	4	4	8
<i>Clostridium</i> spp.	1	1	2	3
<i>Bacteroides</i> spp.	2	2	1	3
<i>Prevotella</i> spp.	1	1	1	2

Table 3. Diagnostic sensitivity and specificity of each method based on the diagnostic criteria of PD peritonitis

Tests	Result	PD peritonitis		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
		Positive (N=38)	Negative (N=56)		
Conventional centrifugation culture	Positive	19	1*	50.0 (33.7-66.3)	98.2 (89.2-99.9)
	Negative	19	55		
FAN Plus inoculation after centrifugation	Positive	30	1*	78.9 (62.2-89.9)	98.2 (89.2-99.9)
	Negative	8	55		
Direct FAN Plus inoculation	Positive	33	0	86.8 (71.1-95.1)	100 (92.0-100)
	Negative	5	56		
Combination of two methods using FAN Plus media	Positive	35	1*	92.1 (77.5-97.9)	98.2 (89.2-99.9)
	Negative	3	55		

**Enterococcus faecalis* from the same specimen.

cluded four anaerobes, three enterococci, two *Enterobacteriaceae*, two coagulase-negative staphylococci, and three other bacteria.

Of the total 170 PD effluent samples evaluated, 74 were considered follow-up cultures. After excluding two cases with insufficient medical information, 94 nonreplicate samples were classified according to the diagnostic criteria for PD peritonitis. A total of 38 were from patients classified as having peritonitis, and the remaining 56 were from patients without peritonitis (Table 3). On the basis of these classifications, sensitivity of the conventional method was 50.0% (95% confidence interval [CI], 33.7-66.3%). However, the sensitivity of inoculation after centrifugation using FAN Plus media was 78.9% (95% CI, 62.2-89.9%) and the sensitivity of direct FAN Plus inoculation was 86.8% (95% CI, 71.1-95.1%). Use of both inoculation methods with FAN Plus media resulted in 92.1% sensitivity (95% CI, 77.5-97.9%). Specificities of each method were over 98%.

DISCUSSION

Previous studies have shown that PD effluent culture methods using automated blood culture systems have several advantages, including increased sensitivity and earlier detection of microbial growth due to continuous monitoring [2,6,13-15]. Furthermore, several recent studies have reported that the use of resin-based blood culture media significantly improves recovery rates compared to charcoal-based media [16-19]. This has been attributed to the improved binding kinetics of the resin-based media for a variety of antimicrobial agents [19]. Based on these results, the resin-based medias, including the FAN Plus media, have been thoroughly evaluated for culturing of blood and several types of

sterile body fluids [20-24]. However, the utility of this method had not previously been evaluated for PD effluent culture. In the present study, culture using the FAN Plus media has superior recovery rates compared to the conventional culture method. Moreover, two methods using FAN Plus media, inoculation after centrifugation and direct inoculation, resulted in comparable recovery rates.

Recent ISPD guidelines suggest that sampling and culture methods be reviewed and improved if more than 15% of peritonitis episodes are culture-negative [3]. Also, the guidelines describe that bacterial yield from peritoneal fluid culture is enhanced by either inoculating the fluid directly into blood-culture bottle kits, centrifuging PD fluid and culturing the pellet, or the lysis centrifugation technique [3,8]. A number of novel diagnostic techniques have also been explored, including biomarker assays, nucleic acid amplification, and 16S rRNA gene sequencing, but these techniques are difficult to implement for routine testing in clinical laboratories [3,25,26]. In our study, the use of both inoculation after centrifugation and direct inoculation into FAN Plus media resulted in less than 8% of culture-negative peritonitis. Therefore, incorporation of the two culture methods using the FAN Plus media is recommended for diagnosis of PD peritonitis for the best recovery rate. In the practical field, single method of direct inoculation can be useful regarding of the simplicity and cost-effectiveness.

Consistent with previously reported results, coagulase-negative staphylococci were the most frequently isolated Gram-positive bacteria and *Enterobacteriaceae*, including *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Enterobacter* spp., were the most frequently isolated Gram-negative bacteria (data not shown) from nonreplicated peritonitis cases

[13,27,28].

This study has several limitations inherent to retrospective analyses. Specifically, we cannot compare the time required to detect growth by each culture method or draw conclusions regarding the appropriate incubation period in this study. Also, we cannot confirm the contamination at the bacterial culture procedure. It is difficult to distinguish true infection from contamination, especially when single culture method shows positive culture results. In this study, we considered the isolated bacteria as a causative organisms of PD peritonitis through clinical review of each case.

In summary, we compared culture using the FAN Plus media to the conventional centrifugation culture method for the diagnosis of PD peritonitis. The culture methods using the FAN Plus media—inoculation after centrifugation and direct inoculation—had a superior recovery rate compared to the conventional centrifugation culture method. The two different inoculation methods using FAN Plus media resulted in comparable recovery rates. Use of both inoculation methods with the FAN Plus media is recommended for the best diagnostic yield, and single method of direct inoculation can be useful regarding of the simplicity and cost-effectiveness.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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=국문초록=

BacT/Alert FAN Plus 혈액배양병을 이용한 복막투석액 배양능 평가

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배경: 복막투석의 가장 큰 합병증은 복막염이며, 복막염의 진단을 위해 혈액배양병을 이용한 투석액 배양이 권장되고 있다. 저자들은 BacT/Alert[®] Fastidious Antimicrobial Neutralization Plus 혈액배양병(FAN Plus; bioMérieux, France)을 이용한 투석액 배양법과 원심분리를 이용한 고식적인 배양 방법을 비교하고자 하였다.

방법: 총 170개의 투석액을 고식적인 원심분리 배양 방법과 FAN Plus를 이용한 배양 방법으로 동시에 배양하였다. FAN Plus를 이용한 배양 시 접종은 원심분리 후 접종과 환자 침상 옆 직접 접종 두 가지 방법으로 시행하였다.

결과: 배양양성인 52개 검체 중 고식적인 원심분리 배양 방법에서는 27개의 검체가 양성이었다. 원심분리 후 FAN Plus에 접종한 방법에서는 46개의 검체가 양성하였고, 직접 FAN Plus에 접종한 방법에서는 47개의 검체가 양성이었다. 복막염 진단에 대한 민감도는 고식적인 원심분리 방법에서 50.0% (95% CI, 33.7-66.3%), 원심분리 후 FAN Plus에 접종한 방법에서 78.9% (95% CI, 62.2-89.9%), 직접 FAN Plus에 접종한 방법에서 86.8% (95% CI, 71.1-95.1%)였다. FAN Plus 혈액배양용기를 이용한 두 가지 방법을 모두 이용하였을 때의 민감도는 92.1% (95% CI, 89.2-99.9%)였다.

결론: FAN Plus를 이용한 배양법은 고식적인 원심분리 배양법보다 우수한 양성률을 보였다. 또한 FAN Plus를 이용한 두 가지 접종방법을 동시에 시행할 때 가장 높은 배양률을 기대할 수 있다. [*Ann Clin Microbiol* 2019;22:90-95]

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