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Clinical Evaluation of the FilmArray Blood Culture Identification Panel in Identification of Bacteria and Yeasts from Positive Blood Culture Bottles

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The FilmArray platform (FA; BioFire, Salt Lake City, UT) is a closed diagnostic system allowing high-order multiplex PCR analysis with automated readout of results directly from positive blood cultures in 1 h. In the present study, we evaluated the clinical performance of the FilmArray blood culture identification (BCID) panel, which includes 19 bacteria, five yeasts, and three antibiotic resistance genes. In total, 206 blood culture bottles were included in the study. The FilmArray could identify microorganisms in 153/167 (91.6%) samples with monomicrobial growth. Thirteen of the 167 (7.8%) microorganisms were not covered by the FilmArray BCID panel. In 6/167 (3.6%) samples, the FilmArray detected an additional microorganism compared to blood culture. When polymicrobial growth was analyzed, the FilmArray could detect all target microorganisms in 17/24 (71%) samples. Twelve blood culture bottles that yielded a positive signal but showed no growth were also negative by FilmArray. In 3/206 (1.5%) bottles, the FilmArray results were invalid. The results of the FilmArray were reproducible, as demonstrated by the testing and retesting of five bottles in the same day and a longitudinal follow-up of five other blood cultures up to 4 weeks. The present study shows that the FilmArray is a rapid identification method with high performance in direct identification of bacteria and yeasts from positive blood culture bottles.

Despite the increased knowledge in pathogenesis of microbial diseases and effective treatment, bloodstream infections (BSIs) remain a leading cause of death and high health care-related costs worldwide (1, 2). Appropriate antimicrobial therapy significantly lowers the mortality rate for patients with BSI (3). Initial antimicrobial treatment often includes the use of a broad spectrum of antibiotics, a strategy often used due to the lack of specific identification of the causative infectious agent. Conventional microbiological methods for identification of microorganisms from blood cultures, such as agar-based culture techniques, take a considerable time, from 12 to 72 h. In response, several microbiological methods for rapid and specific identification of infectious agents from positive blood culture bottles have been suggested, including pathogen-specific real-time PCR (4), fluorescence *in situ* hybridization using peptide nucleic acid probes (PNA-FISH) (5), PCR coupled to high-resolution melting curve analysis (6), and direct matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (7, 8). These methods are, however, relatively labor-intensive and in some instances have a narrow diagnostic spectrum. Moreover, none of them has the capacity to evaluate important antimicrobial susceptibility markers, including *mecA*, *vanA*, and *vanB*. There is a need for reliable, simple, and direct identification methods with short hands-on time involving limited expertise. BioFire Diagnostic's FilmArray system (FA; BioFire, Salt Lake City, UT) is a PCR-based platform developed and tested for the diagnosis of several infectious agents involved in different diseases, including respiratory viruses, *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis* (9–11). Recently, the FilmArray blood culture ID (FA BCID) panel was introduced (12). This panel was later improved, and the current panel includes 27 targets (Table 1). The FA BCID uses high-order multiplex PCR analysis to identify a number of pathogens and susceptibility markers directly from positive blood culture bottles in 1 h. The aims of the present study were (i) to analyze the performance of the FA BCID panel in prospective clinical samples and (ii) to analyze the effect of different parameters, including blood culture bottle type, time to detection in blood culture bottles, and reproducibility of results after long-term storage.

MATERIALS AND METHODS

Blood cultures. The study was performed between April 2013 and June 2013 at Karolinska University Laboratory in Huddinge, Sweden, which serves the southern part of the greater Stockholm area and surrounding cities and suburbs. The laboratory receives blood culture specimens from three tertiary-care hospitals: Karolinska University Hospital in Huddinge, Stockholm, South General Hospital, Stockholm, and Södertälje Hospital in Södertälje, with a total of 1,569 patient beds. The total number of blood culture bottles processed each year is ca. 75,000. The blood cultures were collected at the clinical wards and then transferred to the laboratory. Four different blood culture bottles from two different blood culture systems were used in the study. The Bactec Mycosis IC/F bottles are used for selective culture and recovery of yeasts and fungi. BacT/Alert FA Plus aerobic, BacT/Alert PF Plus pediatric, and BacT/Alert FN Plus anaerobic bottles contain nonspecific media that are used to detect yeasts, bacteria, and anaerobic bacteria. The BacT/Alert FA, -N, and PF Plus bottles were incubated in BacT/Alert 3D (bioMérieux, Durham, NC) and the Bactec Mycosis IC/F bottles in the Bactec 9240 (BD Diagnostic Systems, Sparks, MD) blood culture systems until they yielded a positive signal or for a final period of 5 days. When blood culture bottles yielded a positive signal, they were removed from the system, and the microorganisms were identified

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TABLE 1 FilmArray BCID panel targets[a]

Category	Target
Gram-negative bacteria	*Enterobacteriaceae*
	Escherichia coli
	Enterobacter cloacae complex
	Klebsiella oxytoca
	Klebsiella pneumoniae
	Serratia marcescens
	Proteus spp.
	Acinetobacter baumannii
	Haemophilus influenzae
	Neisseria meningitidis
	Pseudomonas aeruginosa
Gram-positive bacteria	*Staphylococcus* spp.
	Staphylococcus aureus
	Streptococcus spp.
	Streptococcus agalactiae
	Streptococcus pyogenes
	Streptococcus pneumoniae
	Enterococcus spp.
	Listeria monocytogenes
Fungi	*Candida albicans*
	Candida glabrata
	Candida krusei
	Candida parapsilosis
	Candida tropicalis
Antibiotic resistance markers	*mecA*
	vanA/*vanB*
	KPC

[a] In total, the panel includes 27 targets, namely, 11 Gram-negative and 8 Gram-positive bacteria, 5 *Candida* spp., and 3 antibiotic resistance markers.

by both FilmArray and conventional methods. Only one blood culture per patient was used in the prospective evaluation of the FA BCID panel.

Conventional microbiological methods. Gram stains were done directly from positive blood culture bottles. According to the results of the staining, specimens from the positive bottles were subcultured onto relevant agar plates. The microorganisms grown on the agar plates were identified by Bruker MALDI-TOF MS (Bruker Daltonics, Bremen, Germany), by Vitek2 XL (bioMérieux, Marcy l'Etoile, France), and by a panel of validated desktop spot tests, including catalase, oxidase, indole spot, and L-pyrrolidonyl-β-naphthylamide (Remel Inc., Lenexa, KN) tests and agglutination tests for *Staphylococcus aureus* (Staphaurex latex test; Remel Europe Ltd., Dartford, United Kingdom), group A, B, C, D, and G streptococci, *Streptococcus pneumoniae* (Oxoid, Basingstoke, United Kingdom), and *Salmonella* sp. (Reagensia, Stockholm, Sweden). The susceptibility testing was performed by disc diffusion according to the EUCAST method. In three cases of discordant *mecA* results between FA and disc diffusion, conventional *mecA* PCR was performed.

FilmArray BCID. The FilmArray BCID panel received a Conformité Européenne In Vitro Diagnostics (CE IVD) marking in June 2013, authorizing it to be used in Europe for the purpose of *in vitro* diagnostic examination. The panel includes 19 bacteria, five yeasts, and three antibiotic resistance genes: *mecA*, *vanA*/*vanB*, and the KPC gene (Table 1). Briefly, it is a closed diagnostic system that combines nucleic acid extraction from clinical specimens, high-order nested multiplex PCR, and post-PCR DNA melting curve analysis. When the blood culture bottle yielded a positive signal in the blood culture system, 100 μl of broth from the positive culture was diluted in 500 μl sample dilution buffer, and then 300 μl of this sample solution was injected into the FA pouch for analysis. Extraction, amplification, detection, and analyses were completely automated within the pouch. Results of the assay were provided by the software only if the quality control reactions were appropriately detected. Each pouch includes two internal run controls for both the primary amplification and the analyte-specific detection stages. When either of the two control fails, the result is listed as "invalid."

Reproducibility tests. Five positive blood cultures, one of each with *S. aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (CoNS), *Escherichia coli* plus alpha-hemolytic streptococci, and *Escherichia coli* plus *Klebsiella pneumoniae*, were tested with the FilmArray twice during the same day.

Longitudinal follow up with FilmArray. In order to evaluate the performance of FilmArray in positive blood cultures that had been stored at room temperature (RT) over a longer period, five blood culture bottles growing *S. aureus*, CoNS with *mecA*, *E. coli*, *Klebsiella oxytoca*, and *Candida glabrata* were tested with FilmArray repeatedly in the 3 to 4 weeks following blood culture positivity (day 1). The blood culture bottles were taken out of the blood culture system when they yielded a positive signal and stored at RT during this period.

Statistical analysis. The detection rates of FilmArray and conventional microbiological methods were compared using Fisher's exact test.

RESULTS

In total, 206 blood culture bottles were included in the study. There were 167 and 24 positive blood cultures with mono- and polymicrobial growth, respectively. For 12 samples, Gram staining and cultures were negative despite the fact that bottles yielded a positive signal in the blood culture system. In 3/206 (1.5%) bottles, the FilmArray results were invalid. The longitudinal performance of the FA was evaluated in five positive blood cultures by a total of 29 tests during a period of 4 weeks. For another five samples, the FA results were repeated twice in the same day in order to evaluate the reproducibility of the method. During the study period, 236 FilmArray tests were performed and three were invalid, giving a 1.3% pouch failure rate.

Diversity of the clinical isolates. Thirty-five different species were isolated during the study period. The FilmArray BCID panel covered 24/35 (69%) isolates. The three *Enterococcus* spp., *Enterococcus faecalis*, *E. faecium*, and *E. avium*, were identified at the genus level by FilmArray. Interestingly, 175/191 blood culture bottles with positive growth had microorganisms that were included in the FilmArray BCID panel, covering 91.6% of the clinical isolates during the study period.

Monomicrobial growth. The FilmArray identified 153/167 (91.6%) samples with monomicrobial growth. Thirteen of the 167 (7.8%) microorganisms were not included in the FilmArray BCID panel and could not be identified (Table 2). These were three *Micrococcus* spp., two *Corynebacterium* spp., two *Peptoniphilus* spp., and one of each *Gemella* sp., *Bacteroides fragilis*, *Capnocytophaga canimorsus*, *Eggerthella lenta*, *Parvimonas micra*, and *Lactobacillus* sp. Interestingly, only 1/167 (0.6%) microorganisms, i.e., one coagulase-negative staphylococcus (CoNS), that was included in the FA BCID panel and positive in blood culture could not be detected by FilmArray. In contrast, in 6/167 (3.6%) samples, FilmArray detected an additional microorganism compared to blood culture. In four blood culture bottles with CoNS and in one bottle with *S. pneumoniae*, FilmArray simultaneously detected *Enterococcus* spp. In another blood culture bottle with *C. glabrata*, FilmArray detected also *Candida albicans* (Table 2).

Polymicrobial growth. When polymicrobial growth was analyzed, both FilmArray and blood cultures could detect all microorganisms in 17/24 (71%) samples (Table 3). In 6/24 (25%) polymicrobial cultures, FilmArray could not detect one or more of the

TABLE 2 Identification of bacteria and yeasts and detection of antibiotic resistance markers from 167 monomicrobial blood cultures by FilmArray

Identification	No. of samples		
	Blood culture and FA positive	Blood culture positive and FA negative	Blood culture negative and FA positive
Microorganisms included in FA BCID panel			
Gram-negative bacteria			
Escherichia coli	34		
Klebsiella pneumoniae	5		
Klebsiella oxytoca	2		
Proteus mirabilis	2		
Pseudomonas aeruginosa	2		
Haemophilus influenzae	2		
Enterobacter cloacae	1		
Enterobacter aerogenes	1		
Salmonella spp.	1		
Serratia marcescens	1		
Neisseria meningitidis	1		
Gram-positive bacteria			
Coagulase-negative staphylococci	37	1	
Staphylococcus aureus	19		
Streptococcus pneumoniae	13		
Enterococcus spp.	9[a]		4
Streptococcus agalactiae	5		
Alpha-hemolytic streptococci	4		
Streptococcus pyogenes	2		
Listeria monocytogenes	2		
Fungi			
Candida albicans	6		1
Candida glabrata	4		
Microorganisms not included in FA BCID panel			
Micrococcus spp.		3	
Corynebacterium spp.		2	
Peptoniphilus spp.		2	
Capnocytophaga canimorsus		1	
Bacteroides fragilis		1	
Eggerthella lenta		1	
Gemella spp.		1	
Lactobacillus spp.		1	
Parvimonas micra		1	
Antibiotic resistance markers			
mecA	15[b]	1	3[c]
vanA/*vanB*	0		0

[a] Six *E. faecalis* and 3 *E. faecium* isolates.
[b] One MRSA isolate and 14 methicillin-resistant CoNS.
[c] PCR showed that 1/3 CoNS was *mecA* positive, as shown by FilmArray, whereas 2/3 were *mecA* negative, as in the disc diffusion test.

microorganisms detected by blood cultures. In two samples, one with *E. faecalis* plus *Streptococcus pyogenes* and one with *E. faecalis* plus CoNS, FilmArray failed to detect *E. faecalis*. In two other polymicrobial samples, one with *E. coli* plus *Bacteroides fragilis* and one with *Propionibacterium acnes* plus *Micrococcus* sp., FilmArray could detect none of the four microorganisms. It is important to note that 3/4 microorganisms in these two samples were not included in the FilmArray BCID panel. In one sample with *S. pneumoniae* plus CoNS plus *Bacillus* sp., FilmArray could not detect the *Bacillus* sp., which is not in the FilmArray BCID panel. In another sample with *K. pneumoniae* plus *Clostridium perfringens* plus alpha-hemolytic streptococci, FilmArray could not identify *C. perfringens* plus alpha-hemolytic streptococci; *C. perfringens* is not in the FilmArray BCID panel (Table 3). In contrast, in one blood culture bottle with CoNS plus alpha-hemolytic streptococci, FilmArray additionally detected *Enterococcus* spp.

***mecA*, *vanA*/*vanB*, and *blaKPC*.** In total, there were 67 *mecA* results in the FilmArray analysis. In the 57 monomicrobial samples with *Staphylococcus* spp., one MRSA isolate, 18 methicillin-susceptible *S. aureus* (MSSA) isolates, 16 methicillin-susceptible CoNS, and 14 methicillin-resistant CoNS were detected both by FilmArray and blood cultures. Interestingly, three CoNS that were

TABLE 3 Direct identification of bacteria and yeasts and detection of antibiotic resistance markers in 24 polymicrobial blood cultures by FilmArray

Identification	Detection by[a]: Blood culture	FA
Organisms		
Enterococcus faecium, CoNS	1, 1	1, 1
Escherichia coli, *Klebsiella pneumonia*	1, 1	1, 1
Candida albicans, *Enterococcus faecalis*	1, 1	1, 1
Enterobacter cloacae, *Enterococcus faecium*	1, 1	1, 1
Enterococcus faecalis, *Pseudomonas aeruginosa*	1, 1	1, 1
Enterococcus faecium, alpha-hemolytic streptococci	1, 1	1, 1
Enterococcus faecium, CoNS	1, 1	1, 1
Escherichia coli, alpha-hemolytic streptococci	1, 1	1, 1
Escherichia coli, *Klebsiella pneumoniae*	1, 1	1, 1
Escherichia coli, alpha-hemolytic streptococci	1, 1	1, 1
Klebsiella oxytoca, *Enterococcus faecium*	1, 1	1, 1
Serratia sp., alpha-hemolytic streptococci	1, 1	1, 1
Staphylococcus aureus, CoNS	1, 1	1, 1
Staphylococcus aureus, alpha-hemolytic streptococci	1, 1	1, 1
Staphylococcus aureus, *Pseudomonas aeruginosa*	1, 1	1, 1
CoNS, alpha-hemolytic streptococci	1, 1	1, 1
Streptococcus pyogenes, *Enterococcus faecalis*	1, 1	1, 0
CoNS, *Enterococcus faecalis*	1, 1	1, 0
Escherichia coli, *Bacteroides fragilis*	1, 1	0, X
Propionibacterium acnes, *Micrococcus* sp.	1, 1	X, X
Escherichia coli, *Klebsiella pneumoniae*, *Enterococcus avium*	1, 1, 1	1, 1, 1
CoNS, alpha-hemolytic streptococci, *Enterococcus*	1, 1, 0	1, 1, 1
Klebsiella pneumoniae, *Clostridium perfringens*, alpha-hemolytic streptococci	1, 1, 1	1, X, 0
Streptococcus pneumoniae, CoNS, *Bacillus* sp.	1, 1, 1	1, 1, X
Antibiotic resistance markers		
mecA	5	5
vanA/*vanB*	0	0

[a] 1, detection of a microorganism or antibiotic resistance marker in the BCID panel; 0, failure to detect; X, the microorganism was not included in the panel.

methicillin susceptible according to the disc diffusion method were identified as *mecA* positive in the FilmArray. In order to evaluate the *mecA* result, conventional *mecA* PCR was performed. The PCR result showed that 1/3 CoNS were *mecA* positive, as determined by the FilmArray, whereas 2/3 were *mecA* negative, as determined by disc diffusion. The only CoNS that could not be detected by the FilmArray was methicillin resistant.

Ten polymicrobial samples, including at least one *Staphylococcus* sp., were also analyzed for *mecA* by FilmArray. In 5/5 samples that were methicillin resistant according to the disc diffusion method, FilmArray could detect *mecA*. In 4/5 methicillin-susceptible staphylococci, the FilmArray result were also *mecA* negative. In one sample with *S. aureus* plus CoNS, the FilmArray detected *mecA* without being able to distinguish MRSA or MSSA in the sample. Subsequent phenotypical tests later determined that the *S. aureus* isolate was MSSA and the CoNS was methicillin resistant.

None of the 24 *Enterococcus* spp. detected by the FilmArray was positive for *vanA* or *vanB*, which was in concordance with the 19 culture-positive enterococci that were susceptible to vancomycin by the disc diffusion method.

No microorganism was carbapenem resistant or *blaKPC* positive during the study period.

Overall performance of FilmArray. Table 4 shows the true- and false-positive and -negative results, sensitivities, specificities, and negative and positive predictive values for each microorganism included in the FA BCID panel.

FilmArray identified all microorganisms in 170/175 (97.1%) blood cultures positive for microorganisms that were included in the FA BCID panel. There was no statistical difference between FilmArray and conventional blood culture for detection of microorganisms that are in the panel. When all blood culture bottles were considered, FilmArray was able to identify all microorganisms in 170/191 (89.5%) blood culture bottles included in the study. Conventional cultures had a higher detection rate than FilmArray ($P < 0.001$).

Twelve blood culture bottles that yielded a positive signal in the blood culture system but showed no growth were also negative by FilmArray.

Time to detection. In total, the mean (standard deviation [SD]) time to detection (TTD) for positive blood cultures was 21.67 (18.56) h. The blood cultures that were positive for microorganisms not included in the FA BCID panel had a longer mean (SD) TTD, 53.92 (23.30) h. The blood culture bottle with CoNS that could not be detected by FilmArray yielded a positive signal after 42 h.

Reproducibility tests. There was no difference between the two FilmArray results for each of the five blood culture bottles tested during the same day, suggesting that the FA method is reproducible (data not shown).

When longitudinal follow-up of positive blood cultures was considered, the FilmArray was positive for the correct pathogen at all time points, i.e., for *S. aureus*, *E. coli*, and CoNS (with *mecA*) on days 1, 7, 14, 21, and 28 and for *C. glabrata* and *K. oxytoca* on days 1, 2, 3, 4, 11, 17, and 21 (data not shown).

Blood culture bottle type. In the monomicrobial group, 82 BacT/Alert FA Plus, 71 BacT/Alert FN Plus, five Bactec Mycosis IC/F, and nine BacT/Alert PF Plus blood culture bottles were included. The performance of the FilmArray was equally good in all four blood culture bottle types tested. No FilmArray detection was observed in nine BacT/Alert FA Plus and three BacT/Alert FN Plus blood culture bottles that were false positive; i.e., these bottles yielded a positive signal in the absence of growth. In 3/9 BacT/Alert PF Plus and 1/82 BacT/Alert FA bottles, FilmArray detected one *Enterococcus* sp. that was not detected by blood cultures. In contrast FilmArray failed to detect CoNS in 1/82 BacT/Alert FA Plus bottle that yielded a positive blood culture.

Ten BacT/Alert FA Plus bottles, 13 BacT/Alert FN Plus bottles, and one BacT/Alert PF Plus bottle were positive for multiple organisms. In 1/12 BacT/Alert FA bottles, FilmArray detected one *Enterococcus* sp. that was not detected by blood cultures. In 2/10 BacT/Alert FA, 3/13 BacT/Alert FN, and 1/1 BacT/Alert PF Plus bottles, FilmArray failed to detect one or more microorganisms that were detected by blood culture.

DISCUSSION

With extensive advances in instrumentation, clinical microbiology laboratories can dramatically improve sensitivity, specificity, and turnaround times in the diagnosis of invasive infections (13, 14). The aim of the present study was to evaluate one of the recently developed rapid methods in this field, the use of the FilmArray BCID panel.

The FilmArray BCID panel includes a limited number of

TABLE 4 Performance of FilmArray in identification of each microorganism[a]

Identification	No. of true/false positives	No. of true/false negatives	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Gram-negative bacteria						
Total	66/0	133/1	98.5	100	100	99.3
Escherichia coli	39/0	160/1	97.5	100	100	99.4
Klebsiella pneumoniae	9/0	191/0	100	100	100	100
Klebsiella oxytoca	3/0	197/0	100	100	100	100
Proteus mirabilis	2/0	198/0	100	100	100	100
Pseudomonas aeruginosa	4/0	196/0	100	100	100	100
Haemophilus influenzae	2/0	198/0	100	100	100	100
Enterobacter cloacae	2/0	198/0	100	100	100	100
Enterobacter aerogenes	1/0	199/0	100	100	100	100
Salmonella spp.	1/0	199/0	100	100	100	100
Serratia marcescens	2/0	198/0	100	100	100	100
Neisseria meningitidis	1/0	199/0	100	100	100	100
Gram-positive bacteria						
Total	117/5	74/4	96.7	93.7	95.9	94.9
Coagulase-negative staphylococci	44/0	155/1	97.8	100	100	99.4
Staphylococcus aureus	22/0	178/0	100	100	100	100
Streptococcus pneumoniae	14/0	186/0	100	100	100	100
Enterococcus spp.	16/5	177/2	88.9	97.3	76.2	98.9
Streptococcus agalactiae	5/0	195/0	100	100	100	100
Alpha-hemolytic streptococci	11/0	188/1	91.7	100	100	99.5
Streptococcus pyogenes	3/0	197/0	100	100	100	100
Listeria monocytogenes	2/0	198/0	100	100	100	100
Fungi						
Total	11/1	188/0	100	99.5	91.7	100
Candida albicans	7/1	192/0	100	99.5	87.5	100
Candida glabrata	4/0	196/0	100	100	100	100
Antibiotic resistance markers						
MecA	24/2	173/1	96	98.9	92.3	99.5
VanA/VanB	0				0	

[a] In total, 200 isolates that are included in the BCID panel were analyzed in the study.

pathogens. The choice of the microorganisms might be decisive in the performance of a diagnostic method in clinical practice (15). The FilmArray BCID panel covered all microorganisms in 91.6% of positive blood culture bottles included in the study. It is important to note that the microorganisms present in six blood culture bottles that are not included in the BCID panel were *Bacillus* spp., *Micrococcus* spp., and *Corynebacterium* spp., which are generally considered contaminants from normal skin flora. When the parallel blood culture bottles for these samples were analyzed in the laboratory information system, it was observed that these isolates were positive in only one blood culture bottle, supporting the likelihood of contamination. Thus, the FilmArray BCID panel could cover the vast majority of the isolates encountered during the study period.

The performance of the FilmArray was separately analyzed for mono- and polymicrobial growth. As expected, the FilmArray could identify 91.6% of microorganisms in blood culture bottles with monomicrobial growth. It was previously reported that direct MALDI-TOF MS had a high performance, >90%, in blood cultures that were positive for Gram-negative and -positive bacteria (16, 17). Similarly, the FilmArray had high performance in identification of both Gram-negative and -positive bacteria (Table 4). Identification of *Candida* spp. from positive blood cultures by direct MALDI-TOF has also been reported (18). However, the preparation steps for direct MALDI-TOF with growth of yeasts are different from those for the bacterial isolates and are time-consuming. The FilmArray could detect 9/9 *Candida* spp. that were cultured during the study period with less than 5 min of hands-on time.

In the present study, FilmArray identified 14/14 *S. pneumoniae* isolates from positive blood cultures, one of which had polymicrobial growth. In contrast, none of the 12 alpha-hemolytic streptococci was identified as *S. pneumoniae* by FilmArray, showing that the assay has high sensitivity and specificity in identification of this important pathogen.

The FilmArray detected 24 *Enterococcus* isolates during the study period. The FilmArray BCID panel does not include different types of *Enterococcus*. The choices of antimicrobial treatment for the two most common *Enterococcus* spp., *E. faecalis* and *E. faecium*, are different, i.e., ampicillin and vancomycin, respectively. It is desirable to choose a narrow spectrum of antimicrobial therapy in BSI patients with *E. faecalis*. Therefore, identification of *Enterococcus* spp. is a limitation in the FilmArray.

There were six microorganisms, five *Enterococcus* strains and one *C. albicans* strain, that were detected by FilmArray but not by blood cultures during the study period. Interestingly, all six microorganisms were from samples that eventually proved to be polymicrobial. In the evaluation of the preliminary FilmArray

BCID panel, it was shown that microorganisms that were detected by only by FilmArray and not by blood cultures could be detected by sequencing, showing that these were true positives (12). One limitation of the present study was that the blood broth samples from bottles with discordant results were not saved and could not be analyzed further.

The present study analyzed clinical samples prospectively. Therefore, the number of samples with particular microorganisms, including *Serratia* spp. and *Salmonella* spp., is rather limited. Further studies with larger clinical samples with different microorganisms might be helpful to define the performance of the FilmArray in identification of rare clinical isolates.

The numbers of patients with polymicrobial BSIs are increasing, possibly due to the advances in medicine that help extend survival of severely ill patients. Therefore, the ability to identify several different isolates is an important parameter for the modern rapid identification methods. The use of rapid identification methods, including direct MALDI-TOF, in identification of microorganisms in samples with polymicrobial growth is quite low (19). In the present study, FilmArray could detect all microorganisms in 16/24 and 1/24 blood cultures with two and three different isolates, respectively. In only 2/24 (8%) polymicrobial samples did the FilmArray fail to detect any of the four microorganisms. It is important to note that 3/4 microorganisms in these two samples were not included in the panel. The present study shows that FilmArray has the potential to identify multiple microorganisms simultaneously from positive blood cultures with polymicrobial growth. However, the general performance of the method is considerably lower in identification of microorganisms in blood culture bottles with polymicrobial than monomicrobial growth.

The lower limit of detection (LOD) of the FilmArray has not been analyzed. According to the instruction booklet for the FilmArray BCID panel, the bacterial concentrations at the time of blood culture positivity when detected by FilmArray were between 6.12×10^7 and 9.50×10^8 CFU/ml. Similar bacterial concentrations at the time of blood culture positivity have been reported by other investigators (20, 21). With the high detection rate of target microorganisms in the present clinical material, it is highly reasonable to suggest that the lower LOD of FilmArray is generally sensitive enough to detect the microorganisms that are in the BCID panel.

The total time to identification of a microorganism(s) from one blood culture using the FilmArray is 65 min. The short hands-on time of 5 min per sample with the FilmArray is an obvious advantage. However, the necessity of running one sample at a time might be a rate-limiting step for a rapid diagnostic method for positive blood cultures. The solution might be the establishment of several instruments that can be run in parallel, as was previously described in the case of implementation of the FilmArray respiratory panel in a laboratory (22).

In the present study, four different blood culture bottles were included. The FilmArray performed similarly with BacT/Alert FA Plus, BacT/Alert FN Plus, BacT/Alert PF Plus, and Bactec mycosis IC/F blood culture bottles. It is important to note that all four blood culture bottle types included resin-like particles and not charcoal.

Positive blood cultures are arguably the most important samples in a clinical microbiology laboratory and are therefore processed in a short period of time after positivity. However, there are clinical situations, including polymicrobial growth and no growth or suspected contamination of the agar plates on which the blood culture was subcultured, where the identification of microorganisms from previously positive blood cultures is still relevant. In order to evaluate the performance of the FilmArray in detection of microorganisms from such blood cultures, we analyzed five positive blood cultures longitudinally. Interestingly, the FilmArray could identify bacteria, yeasts, and polymicrobial growth and detect *mecA* over a period of ca. 4 weeks. Our results indicate that the FilmArray might be used in previously positive blood cultures with the microorganisms tested in the present study.

The economic pressure on laboratories is increasingly resulting in the consolidation of small to medium-sized laboratories into large central ones. One solution to the problem of long transport times for blood cultures could be the establishment of satellite blood culture systems in local laboratories such as biochemistry laboratories, as suggested previously (23). The new user-friendly closed identification systems that could work with short hands-on time may give small local laboratories the ability to start identification of microorganisms in situ at any time around the clock and then send the positive blood culture bottles to the central laboratory, where the susceptibility testing could be done. The results from the Gram staining might be important in selection of panels in some rapid identification methods, including PNA-FISH. Having a broad BCID panel, the FilmArray does not require prior Gram staining, which might be important in establishment of the method in local laboratories that harbor satellite blood culturing facilities. Regarding the cost of FilmArray, the current U.S. list price for the instrument is $39,500 (U.S. dollars [USD]), and the U.S. list price for the reagent is 129 USD per test, according to the manufacturer's website (www.filmarray.com).

The present study shows that the FilmArray is a reliable rapid identification system with high sensitivity and specificity in direct identification of bacteria and yeasts from positive blood culture bottles. Studies analyzing the clinical consequences of rapid identification of blood cultures isolates by FilmArray in samples from patients with BSIs and the cost effectiveness of the FilmArray are warranted.

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We have no conflicts of interest to declare.

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