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Rapid Identification of Pathogens from Positive Blood Cultures by Multiplex PCR using the FilmArray System

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Abstract

Sepsis is a leading cause of death. Rapid and accurate identification of pathogens and antimicrobial resistance directly from blood culture could improve patient outcomes.

The FilmArray® (FA; Idaho Technology, Inc., Salt Lake City, UT) Blood Culture (BC) panel can identify > 25 pathogens and 4 antibiotic resistance genes from positive blood cultures in 1 hour. We compared a development version of the panel to conventional culture and susceptibility testing on 102 archived blood cultures from adults and children with bacteremia. Of 109 pathogens identified by culture, 95% were identified by FA. Among 111 prospectively collected blood cultures, the FA identified 84 of 92 pathogens (91%) covered by the panel. Among 25 *Staphylococcus aureus* and 21 *Enterococcus* species detected, FA identified all culture-proven MRSA and VRE.

The FA BC panel is an accurate method for the rapid identification of pathogens and resistance genes from blood culture.

Keywords

polymerase chain reaction; sepsis; bacteremia; blood culture; molecular diagnostics; bloodstream infection; FilmArray

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Conflict of Interest: CH, EB, NFG, ST, GDA, KMR, IO and MAP are or were at the time of the study employees of Idaho Technology, Inc. AJB, CLB, JAD and ATP collaborate with Idaho Technology, Inc., on several NIH and CDC-funded projects (see funding above).

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Introduction

Sepsis is a leading cause of death in U.S. adults and children (Kochanek, et al., 2011). Prompt initiation of appropriate antimicrobial therapy is associated with improved outcomes (Bochud, et al., 2001; Dellinger, et al., 2008; Kumar, 2009). Definitive identification of the causative organism through traditional culture methods often requires 24–72 hours. This delay can lead to administration of either inadequate or overly broad antimicrobial therapy. Inappropriate antimicrobial selection may result in therapy-related complications, emergence of antimicrobial resistance, and increases in patient morbidity, mortality, and costs (Bauer, et al., 2010; Bochud, et al., 2001; Dellinger, et al., 2008; Kumar, 2009).

Continuously monitoring automated blood culture systems have enabled the detection of most blood stream pathogens within 24 hours of inoculation (Cockerill, et al., 2004). Automated systems for identification and susceptibility testing are available, however the organism must be isolated in pure culture prior to using them (Jorgensen and Ferraro, 2009). This requires a minimum of 12–24 hours after the blood culture becomes positive, and methods to improve bacterial growth rates are lacking (Mancini, et al., 2010).

Identification of pathogens directly from positive blood culture is one proposed solution. Ideally for such identification, rapid, species-specific identification of a wide range of pathogens would be provided. For PCR-based systems, this requires a high level of multiplexing that has been difficult to achieve (Mackay, 2004; Persson, et al., 2005). Here we describe the preliminary development of such an assay based on Idaho Technology's FilmArray® Platform (Poritz, et al., 2011), a closed diagnostic system allowing high-order multiplex PCR analysis with automated read-out of results. The assay described can identify 15 species-specific and 6 genus level targets, encompassing almost 95 percent of organisms commonly found in positive blood cultures (Biedenbach, et al., 2004). Four antibiotic resistance determinants are also included. Design of the assay panel as well as initial testing of a development version with clinical samples is presented.

Materials and Methods

The Institutional Review Boards of the University of Utah and Primary Children's Medical Center (PCMC, Salt Lake City, UT) approved this study. Informed consent was waived.

FilmArray®

The FDA approved FilmArray (FA) System has been described (Poritz, et al., 2011). Briefly, it is a closed diagnostic system that combines nucleic acid extraction from clinical specimens, high-order nested multiplex PCR, and post-PCR DNA melt curve analysis.

Panel Design

Nested and nested multiplex PCR—The FA system performs a “nested multiplex” PCR (nmPCR) within the instrument (Poritz, et al., 2011). Briefly, nmPCR is a 2-stage PCR in which the first-stage reaction is a multiplex that produces a 200–500 bp amplicon for each target. The second-stage primers are “nested” within this amplicon and amplify a shorter product. Nested PCR provides increased sensitivity compared with conventional PCR; the specificity is similar to probe-based assays (Narayanan, 1996). Assays were developed to function within the context of nmPCR, with first-stage multiplex amplification performed for 20 cycles and the array of second stage singleplex nested PCRs performed for 25 cycles.

Panel Design—Organisms were chosen for the BC panel based on laboratory data provided by our collaborating microbiology laboratory director (JAD) and through review of

SENTRY data regarding organism prevalence in blood cultures (Biedenbach, et al., 2004). Both organisms with a high frequency and low pathogenicity [i.e. coagulase-negative staphylococci (CONS)] and those with a low frequency and high pathogenicity (i.e., *Neisseria meningitidis*) were included. The panel used in the studies presented here includes 15 species-specific targets and 6 genus-level assays. This panel (Table 1) represents ~90–95% of all pathogens identified from participating laboratories and the SENTRY database.

Antibiotic resistance targets were chosen based on frequency of detection, implications for change in antibiotic therapy, and the likelihood of a direct correlation between gene presence and antibiotic resistance. The genes *mecA*, *van A*, *van B* and the *Klebsiella pneumoniae* carbapenemase (*bla_{KPC}*) were included in the panel (Cole, et al., 2009; Fluit, et al., 2001). In the version of the panel used for clinical validation, the *vanC* gene was also included, however this gene was removed from the panel used for the prospective study due to its low prevalence and association with a variable resistance phenotype (Clark, et al., 1998).

For species-specific bacterial targets such as virulence factors, genes were chosen based on a review of the literature. For genus (i.e. “*Streptococcus* spp.”) or family (i.e. “*Enterobacteriaceae*”) level assays, conserved housekeeping genes were targeted and degenerate primers were designed to encompass important species within the group. For example, the *Streptococcus* species assay targets the RNA polymerase β subunit (*rpoB*) gene and was designed to capture 7 species in the target alignment. Primers for antibiotic resistance genes are all located in conserved regions so that only one set contains degenerate bases (*vanA* gene; 2-fold degeneracy). The developmental panel used in these studies includes 50 first-stage primers in the multiplex and 65 second-stage singleplex PCRs.

Primer Design—Gene sequences were obtained through the National Center for Biotechnology Information’s GenBank Database (Benson, et al., 2011; Sayers, et al., 2011). Alignments were created and analyzed using Lasergene® software (DNASTAR, Madison, WI). Primers were designed using LightCycler® Primer Probe Design Software (Roche Applied Science, Indianapolis, IN). Degenerate primers were used in primer design in cases where species were divergent within the gene target and in genus-level assays.

Assay optimization

Assay optimization was performed as previously described for FilmArray system development (Poritz, et al., 2011).

Bacteria and Yeast Isolates—Bacteria and yeast isolates were obtained from the PCMC microbiology laboratory and from the University of Utah’s Associated Regional and University Pathologists (ARUP) Laboratories (Salt Lake City, UT). Clinical isolates were identified by standard microbiologic methods (see below). In addition isolates were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and from JMI Laboratories (North Liberty, IA).

Assay validation—All assays were validated for sensitivity and specificity as individual real-time PCR reactions and in the FA pouch with bacterial isolates and their extracted nucleic acids. For the Blood Culture (BC) panel described, targets analyzed in the FA included known positives for each pathogen at levels of approximately 100 – 10,000 genomes per pouch based on DNA fluorimeter measurements (Qubit Fluorimeter, Life Technologies, Grand Island, NY). Testing was also performed with bacteria/yeast isolates (2–3 colonies resuspended in 500 μ l of the sample buffer). For species-specific assays, testing was performed with at least 5 different isolates of each organism. Specific

amplification of targets was evaluated by testing against “nearest neighbors” (ie, testing of the *S. pneumoniae* assay against viridans streptococci, *S. pyogenes* and *S. agalactiae*). Genus or group level assays were tested with 1–5 isolates of each target organism within the group. All assays in the developmental panel had a sensitivity of <100 genomes per pouch and 100% species-or genus-level specificity for bacterial isolates tested (data not shown).

Blood Culture Analyses

Conventional microbiology at Primary Children’s Medical Center (PCMC) and the University of Utah’s Associated Regional and University Pathologists (ARUP) Laboratories

Both PCMC and ARUP are Clinical Laboratory Improvement Amendments (CLIA) certified. In both laboratories, blood culture is performed using the BACTEC automated blood culture system (BD Diagnostic Systems, Franklin Lakes, NJ). Cultures signaling positive are removed immediately from the instrument and Gram-stain is performed within 15 minutes. Isolates of bacteria and yeast are grown on solid media and at PCMC are identified by VITEK® (bioMerieux, Inc., Durham, NC) and by bench top biochemical testing. At ARUP, isolates are identified by Phoenix automated identification system (BD Diagnostic Systems), bench top biochemical testing and/or 16S rRNA gene sequencing. Antibiotic susceptibility is performed at PCMC by VITEK and by E-Test® (bioMerieux, Inc.) and at ARUP by the Phoenix (BD Diagnostic Systems), manual broth microdilution (Trek Diagnostic Systems, Inc., Cleveland, OH), and/or E-Test®. PCR is performed at ARUP for identification of the *mecA* gene.

Collection of Blood Culture Samples—1.5 ml samples from blood culture bottles (BD Diagnostic Systems, Franklin Lakes, NJ) signaling positive were collected from PCMC and from ARUP Laboratories. Sample collection was initiated at the onset of panel development and samples were stored frozen at –80 °C until testing.

Validation Study

A validation designed to demonstrate the accuracy of pathogen identification from clinical blood cultures was performed using blood culture specimens obtained and frozen in 2006–2007 from PCMC. De-identified samples were chosen to represent the majority of pathogens on the development version of the FA BC panel by an investigator (AJB) aware of the culture result, and were tested using the FA by an investigator who was blinded to the culture results (CH).

Prospectively Collected Sequential Samples

The utility of the panel for identification of pathogens commonly encountered in the clinical setting was evaluated through prospectively collected sequential samples from all positive blood cultures obtained between June and August 2010 from PCMC and ARUP. As the intended use of the panel in the clinical laboratory is to test samples that signal positive in the automated blood culture system, negative blood cultures were not specifically tested. Testing was performed, however, if a culture bottle signaled positive, even if organisms were not seen on Gram stain. When more than one sample was collected from a single patient, only the first positive sample was analyzed. Samples were de-identified and frozen within 2 hours of the blood culture signaling positive. All investigators testing samples on the FA were blinded to culture results.

Data Analysis

FA Results—Automated calls for the assays in the FA BC pouch were made using a beta version of the FA software (Poritz, et al., 2011) tuned for the BC pouch. Two investigators

(AJB and CH) confirmed all automated calls based on examination of amplification and melt curves.

In this developmental pouch version we observed low-level background contamination of reagents and the pouch. This is a common problem of broad-range PCR-based bacterial detection systems (Chang, et al., 2011; Corless, et al., 2000; Millar, et al., 2002; Philipp, et al., 2010; Sontakke, et al., 2009). Contaminants demonstrated late amplification [after 22 cycles in the second stage PCR (36 cumulative first + second stage cycles)] but generated positive calls by the software. Contaminants were consistent across a production lot of pouches and were identified by positive calls in the 3–5 negative control (water) runs performed for each lot. With this information, positive software calls were classified as contamination if a run was positive for a known lot-contaminating organism and the cycle crossing point was > 22 cycles in the second-stage PCR.

Statistical analysis—For both the validation and prospective studies sensitivity and specificity were calculated for the BC panel overall and for each pathogen on the BC panel independently. 95% confidence intervals were calculated according to the efficient-score method (corrected for continuity) (Newcombe, 1998). Blood culture was considered the gold standard. A FA test positive was identified when the FA BC assay was positive for a pathogen, and a true positive was identified when the blood culture was positive for the same pathogen. For specificity calculation, all samples negative for a particular pathogen were included.

Results

Clinical validation study using retrospectively collected positive blood cultures

Initial validation of the BC panel was performed on 102 clinical blood culture samples selected to be positive for organisms representing the majority of panel pathogens. These samples were tested retrospectively. Sixteen different pathogens were represented. No clinical specimens containing *Listeria monocytogenes*, *Acinetobacter baumannii* or *Candida* spp. were available in this study set and thus these are not included in Table 2. Due to the wide confidence intervals around the point estimates of sensitivity, these results are reported with an asterisk for organisms with < 5 clinical samples available (Table 2). Organisms containing *vanA/B/C* and *mecA* were tested, but no organisms were found either by culture or FA to be carbapenem-resistant or to contain *bla_{KPC}*.

From 102 clinical specimens, 109 pathogens were identified by culture (7 cultures were positive for more than one organism). Of the 109 pathogens identified by culture, 104 (95%) were also identified by FA. Three pathogens identified by FA were not identified by culture. Sensitivity for each pathogen ranged from 83–100% and specificity was 99% or greater for all. (Table 2)

The culture-positive/FA-negative discordant samples were analyzed using standard PCR. In 2 cases the specimen was also negative using both an alternative organism-specific assay as well as broad-range (16S) PCR, suggesting a mislabeled or incorrect culture sample. In two discordant samples in which culture and FA identified different pathogens, standard PCR and sequencing demonstrated that the organism identified by FA was correct. In one case of a culture-identified CONS missed by FA, the organism could not be detected using alternative PCR strategies. In the case of a FA-identified CONS not identified by culture, sequencing confirmed the presence of *S. epidermidis*.

Antibiotic resistance testing demonstrated good concordance with conventional susceptibility data. Thirteen *S. aureus* were identified by both culture and FA, of which 4

were methicillin-resistant *S. aureus* (MRSA) by susceptibility testing. The FA appropriately identified all *S. aureus* as MSSA (*mecA* negative) or MRSA (*mecA* positive). Of 13 enterococci identified, one was found by culture to be vancomycin-resistant (VRE) and positive for *vanA/B/C* by FA. Two enterococci tested susceptible to vancomycin in the microbiology laboratory but tested positive for *vanA/B/C* by FA. One of these isolates was identified by culture, standard PCR, and sequencing as *E. faecium*. PCR and sequencing also demonstrated the *vanA* gene to be present, with *E. faecium*-specific *vanA* polymorphisms based on alignment to sequences in GenBank. The other sample, identified by culture as vancomycin-susceptible *E. faecalis*, was identified by sequencing as *E. casseliflavus* containing the *vanC* gene.

Prospective analysis of sequentially collected samples

One hundred eleven blood culture samples were tested in this study (Table 3). Ninety-three were culture-positive. Eighteen were Gram-stain and culture-negative despite signaling positive on the automated blood culture machine. The 93 positive cultures grew 99 organisms (6 dual-positive cultures), of which 92 (93%) were pathogens included in the BC panel. Seven were positive for non-panel organisms [*Micrococcus* spp. (n=3); *Corynebacterium* spp., *Fusobacterium nucleatum*, *Propionibacterium acnes*, Diptheroids (n=1 each)]. In this study set there were no samples positive by culture or FA for *L. monocytogenes*, *E. cloacae*, *N. meningitidis* or *H. influenzae* and no pathogens contained *bla*_{KPC}.

The FA identified 84 of the 92 (91%) BC pathogens identified by culture. Sensitivity varied by organism, and some organisms were rarely seen, but all assays had high specificity (Table 3). The overall likelihood ratio positive for the test was 4 and the likelihood ratio negative was 0.11.

In two instances, a culture was positive for two different organisms of the same genus [2 different species of viridans streptococci (n = 1) and 2 species of CONS (n = 1)]. These could not be resolved by the genus-level PCR and were not counted as discordant results. FA accurately identified all *S. aureus* as MSSA or MRSA and all enterococci as susceptible or resistant to vancomycin. Except for one coagulase-negative staphylococcus detection by FA, all culture-negative samples and those positive for pathogens not in the BC panel were negative by FA.

FA did not identify eight pathogens identified in the 92 blood cultures. In six instances (6.5%) these were shown to be FA false-negatives. In three of these cases (2 yeast and 1 *Enterococcus* spp.) standard PCR with an increased number of cycles identified the organism. In two cases, samples positive for CONS by culture were negative by the CONS FA assay, although the *mecA* gene PCR was positive. In one case, *S. marcescens* identified in culture was not detected by FA or with an increased number of cycles in standard PCR, but was found by both culture and PCR in subsequent blood cultures from the same patient. Three of the 8 (37.5%) missed organisms were in dual-positive cultures; FA correctly identified one of the pathogens but did not detect the second. In 2 instances, primer cross-reactivity resulted in misidentification of organisms: in one culture positive for *Salmonella enterica* the FA misidentified the organism as *Klebsiella oxytoca*; in another case, FA identified CONS as well as *S. aureus* in a culture positive for *S. aureus* alone. The CONS identification was confirmed to be a cross-amplification of this particular *S. aureus* by the CONS primers.

Seven pathogens identified by FA were not identified by culture. While 2 were the result of the primer cross-reactivity described above, the other five organisms identified by FA but not by culture were likely true positives. In one case, Gram stain of BACTEC bottles

showed Gram-negative rods, but no growth was detected on culture. FA identified two organisms, *E. coli* and *Acinetobacter baumannii*, both of which were confirmed by standard PCR and sequencing. In two cases, the FA identified a second organism in cultures that yielded growth for only one organism. In the first, *S. aureus* was detected by FA in a sample also culture and PCR positive for GAS. In the second, *S. mitis/oralis* was detected by FA in a sample also culture and PCR positive for CONS. In each case, the presence of both pathogens was confirmed by PCR and sequencing. Finally the FA identified a CONS that was confirmed by PCR and sequencing to be present in a clinical specimen that was negative by culture.

Discussion

Rapid identification of organisms causing sepsis is critical to reducing inappropriate antibiotic use and infection-related morbidity and mortality. Here we describe preliminary development and clinical testing of a novel molecular diagnostic test for the identification of organisms in positive blood culture based on the FilmArray® System (Poritz, et al., 2011). The FA BC panel identifies a wide range of bacterial and fungal pathogens, accounting for 93% of organisms isolated from clinical specimens in our study. In addition, the panel identifies select antibiotic resistance genes, including those responsible for resistance to methicillin, vancomycin and carbapenems. Identification of pathogens and resistance markers was achieved from clinical samples with good sensitivity and specificity for all targets. An analysis of prospectively obtained blood cultures demonstrated accurate identification of 91% of pathogens and 100% of MRSA and VRE. When fully developed, this technology has the potential to contribute significantly to improvements in the management of adults and children with sepsis.

Rapid molecular identification of organisms from positive blood cultures is increasingly available in clinical laboratories. Both PCR and probe-based platforms are commercially available, but all have limitations. In the U.S., the Xpert® MRSA/SA BC assay (Cepheid, Sunnyvale, CA) and the fluorescence *in situ* hybridization method employing novel peptide-nucleic acids as probes (PNA-FISH®; AvanDx, Woburn, MA) are available, but both target limited numbers of organisms or require several panels (Hensley, et al., 2009; Morgan, et al., 2010; Shepard, et al., 2008). In Europe, bacterial detection methods from blood culture based on broad-range PCR are available that target a larger range of organisms in a single assay, but downstream identification using ELISA (Hyplex®; Cepheid Benelux, Bouwel, Belgium) or microarray hybridization (Prove-it™ Sepsis; Mobidiag, Helsinki, Finland) is required. One of the most comprehensive systems under investigation is based on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (La Scola and Raoult, 2009; Stevenson, et al., 2010). MALDI-TOF has the advantage of rapid turn-around time, modest sample preparation, and the ability to directly identify a large range of microorganisms. Disadvantages include the high start-up costs associated with the purchase of mass-spectrometry equipment and training of personnel.

Identification of blood culture pathogens using the FilmArray® System's BC panel has several advantages. A particular strength is the large number of targets, all evaluated in a single test, covering the majority of organisms detected in both adult and pediatric blood cultures. In this study, organisms identified by blood culture but not contained in the FA BC panel primarily included those often considered contaminants, such as diphtheroids and micrococcus. In addition, the FA System performs all steps of the assay, from nucleic acid extraction to interpretation of amplification data in closed system using a single pouch on a minimally processed clinical sample. The laboratory procedures required are not technologically complex and can be performed by persons who do not have training in molecular techniques (Poritz, et al., 2011).

Our clinical validation study of 102 retrospectively-collected blood culture samples demonstrated accurate identification of most pathogens. In the primary analysis there were 8 discordant identifications (5 culture+/FA-; 3 culture-/FA+), however all but one identification (of CONS) could be resolved in favor of the FA. Specificity was high for all pathogens, including species-specific and genus-level targets. Specificity was comparable to that of the VITEK2 for cultured organisms, for which the accuracy of identification ranges from 92–97% (Abele-Horn, et al., 2006; Nonhoff, et al., 2005; O’Hara and Miller, 2003; Rennie, et al., 2008).

The FA BC panel also contains antibiotic resistance markers. MRSA is a well-described health-threat (Klevens, et al., 2007), and a number of commercially available panels can distinguish MRSA from methicillin-susceptible strains through the identification of the *mecA* gene that is also identified in our panel. While in Europe a Nanosphere panel (Verigene System, Nanosphere, Northbrook IL) is available for detection of the primary genetic determinants of vancomycin-resistance in enterococci [*vanA* and *vanB*] (Courvalin, 2006), no commercially available panels approved for blood culture in the U.S. contain assays for *vanA* and *vanB* or carbapenem-resistance in enteric organisms [*bla_{KPC}*] (Nordmann, et al., 2009).

In choosing resistance targets for the BC panel, we chose genes in which detection of the resistance marker almost always indicates a phenotypically drug-resistant organism. We had one true genotype-phenotype discordance detected with regard to vancomycin susceptibility in our validation study: an *E. faecium* that was PCR positive for *vanA*, yet phenotypically vancomycin-susceptible. This discordance has been rarely reported (Choi, et al., 2011).

Our prospective study of consecutively collected samples was designed to evaluate the performance of the system on an unbiased selection of positive blood cultures. Overall, the system performed well, although the sensitivity was lower for *Serratia marcescens* (1/2), broad *Enterobacteriaceae* detection (3/4) and yeasts (4/6). The small numbers of samples preclude accurate assessment of the sensitivity for these organisms, however for others, sensitivity was good (88–100%). Specificity was >98% for all organisms.

There are limitations to the BC system at this point in development. While comprehensive, the FA BC panel does not detect all possible organisms. This reflects both a limitation in the number of assays that could be placed into the multiplex, and a conscious decision to primarily target organisms that occur at a high enough frequency to support FDA clearance. Sensitivity was not an issue for most organisms, however two of six *Candida* species detected in culture were not detected by FA. We hypothesized that yeast might signal “positive” at lower titers in automated blood culture systems. We performed growth studies in which the concentration of yeast at the time of culture positivity ranged from $10^3 - 10^7$ CFU/ml rather than the $10^7 - 10^{11}$ seen with bacteria (Tan, et al., 2008) similar to another published report (Chang, et al., 2000). For this reason we are evaluating multi-copy rather than single-copy targets for yeast detection.

Polymicrobial culture was the most common reason that a cultured organism went undetected by PCR. Approximately 7% of blood cultures in the prospective study were polymicrobial, however in three of eight (38%) instances in which the FA did not detect a pathogen, the blood culture was positive for two organisms. We speculate that in cultures positive for two organisms, one may grow more rapidly and trigger a positive signal in the culture system. At the time of the positive call, the second organism may be below the level of molecular detection.

As often reported for PCR-based detection systems that evaluate a broad range of bacteria, contamination of reagents and materials was an issue in this developmental version of the

system (Chang, et al., 2011; Corless, et al., 2000; Millar, et al., 2002; Philipp, et al., 2010; Sontakke, et al., 2009). This is most often reported for 16S PCR-based detection, however in our system we have specific assays for potentially contaminating bacteria such as CONS and *E. coli*. At the level of research and development this was not a significant issue for evaluation of the sensitivity and specificity of the panel. In positive blood cultures, second stage crossing thresholds for culture-identified organisms were generally in the range of 13–16 cycles. The level of contamination in the pouches was quite low, with second stage PCR crossing thresholds beyond 22 cycles [36 cumulative cycles] or signal only detected by the presence of a melting curve. Current development efforts are focused on eliminating detectable contamination through improvements in manufacturing as well as software cutoffs for commonly contaminating organisms. Overall system sensitivity must be maintained for the detection of yeasts and polymicrobial cultures as previously discussed.

A primary limitation of this study was the low number of samples positive for some organisms. While nucleic acid testing demonstrated good sensitivity and specificity of assays such as those for *L. monocytogenes*, *N. meningitidis* and *H. influenzae*, the low number of positive blood culture specimens makes clinical sensitivity hard to determine. In addition, there were no clinical specimens demonstrating carbapenem resistance or that were positive by PCR for *bla_{KPC}*. Additional studies, including at sites with higher rates of resistance will be necessary for clinical evaluation of these assays.

Overall the FA BC panel demonstrated accurate identification of pathogens found in positive blood cultures from both adult and pediatric patients. This panel identifies a larger range of pathogens than most currently available platforms in a system that is technically simple for the laboratory. The data presented here suggest that the FA BC system could be an important and useful tool for the management of bloodstream infection in the future.

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Table 1

Organisms Contained within the Developmental FilmArray BC panel

Organism	Gene 1	Gene 2
Gram Positive		
<i>Streptococcus pneumoniae</i>	<i>lytA</i>	<i>rpoB</i>
<i>Streptococcus agalactiae</i>	<i>sip</i>	
<i>Streptococcus pyogenes</i>	<i>speB</i>	
<i>Streptococcus spp</i> ¹	<i>rpoB</i>	
<i>Staphylococcus aureus</i>	<i>nuc</i>	<i>rpoB</i>
Coagulase-negative staphylococci ²	<i>rpoB</i>	
<i>Enterococcus spp</i> ³	<i>rpoB</i>	
<i>Listeria monocytogenes</i>	<i>rpoB</i>	
Gram-Negative		
<i>Escherichia coli</i>	<i>gyrB</i>	<i>ompA</i>
<i>Klebsiella oxytoca</i>	<i>gyrB</i>	<i>ompA</i>
<i>Klebsiella pneumoniae</i>	<i>gyrB</i>	<i>ompA</i>
<i>Enterobacter cloacae</i>	<i>gyrB</i>	<i>ompA</i>
<i>Serratia marcescens</i>	<i>gyrB</i>	<i>ompA</i>
Enterobacteriaceae ⁴	<i>ompA</i>	
<i>Neisseria meningitidis</i>	<i>ctrA</i>	
<i>Haemophilus influenzae</i>	<i>rpoB</i>	<i>bexA</i>
<i>Pseudomonas aeruginosa</i>	<i>gyrB</i>	
<i>Acinetobacter baumannii</i>	<i>tusA</i>	<i>rpoB</i>
Yeast		
<i>Candida albicans</i>	ACT	
<i>Candida non-albicans</i> ⁵	RPB-1	
Antibiotic Resistance		
Methicillin	<i>mecA</i>	
Vancomycin *	<i>vanA</i>	<i>vanB</i>
Carbapenem	<i>blaKPC</i>	

* *vanC* gene included in panel used for the validation study, but subsequently removed.

¹ Streptococcal species included for primer design were: *S. pneumoniae*, *S. parasanguinis*, *S. anginosus*, *S. salivarius*, *S. pyogenes*, *S. uberis*, *S. mitis*, *S. sanguis*, *S. mutans*, *S. oralis*, *S. equinus*, *S. gordonii*, *S. gallolyticus*

² Coagulase-negative staphylococci included for primer design were: *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. capitis*, *S. saprophyticus*, *S. warneri*, *S. xyloso*, *S. caprae*, *S. cohnii*, *S. koosii*, *S. saccharolyticus*, *S. nepalensis*, *S. microti*, *S. simiae*, *S. lugdunensis*

³ Enterococcal species included for primer design were: *E. faecalis*, *E. faecium*, *E. casseliflavus*, *E. durans*, *E. gallinarum*

⁴ Enterobacteriaceae included for primer design were: *E. coli*, *E. hermannii*, *E. fergusonii*, *Shigella sonnei*, *S. boydii*, *S. dysenteriae*, *S. flexneri*, *C. freundii*, *Salmonella enterica*, *S. typhimurium*, *Enterobacter aerogenes*, *E. cloacae*, *E. sakazaki*, *Klebsiella oxytoca*, *K. pneumoniae*, *Serratia marcescens*

⁵Non-albicans candida included are: *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. krusei*

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Table 2

Clinical Validation Study

Organism	Culture	FA	Culture+/FA-	Culture-/FA+	% Sensitivity (95% CI)	% Specificity (95% CI)
Total	109	106	5	3	95 (89-98)	N/A
<i>S. pneumoniae</i>	8	8	-	-	100 (60-100)	100 (95-100)
<i>S. agalactiae</i>	2	3	-	1 ^b	100* (20-100)	99 (94-100)
<i>S. pyogenes</i>	6	5	1 ^a	-	83 (36-99)	100 (95-100)
<i>Streptococcus spp</i>	7	7	-	-	100 (56-100)	100 (95-100)
<i>S. aureus</i>	14	14	1 ^b	1 ^c	93 (64-100)	99 (93-100)
CONS	16	14	2 ^{c,d}	1 ^e	88 (60-98)	99 (93-100)
<i>Enterococcus spp</i>	14	14	-	-	100 (73-100)	100 (95-100)
<i>E. coli</i>	10	9	1 ^a	-	90 (54-99)	100 (95-100)
<i>K. oxytoca</i>	7	7	-	-	100 (56-100)	100 (95-100)
<i>K. pneumoniae</i>	3	3	-	-	100* (31-100)	100 (95-100)
<i>E. cloacae</i>	5	5	-	-	100 (46-100)	100 (95-100)
<i>S. marcescens</i>	4	4	-	-	100* (40-100)	100 (95-100)
<i>Enterobacteriaceae</i>	4	4	-	-	100* (40-100)	100 (95-100)
<i>N. meningitidis</i>	3	3	-	-	100* (31-100)	100 (95-100)
<i>H. influenzae</i>	1	1	-	-	100* (5-100)	100 (95-100)
<i>P. aeruginosa</i>	5	5	-	-	100 (46-100)	100 (95-100)
AbxR						
mecA (MRSA)	4	4	-	-	100* (40-100)	100 (63-100)
vanaB/C (VRE)	1	3	-	2 ^f	100* (5-100)	85 (54-97)

* Less than 5 positive cultures tested

^a Sample negative by both alternative organism-specific PCR and by broad-range 16S PCR.^b Culture positive for *S. aureus*/FA positive for *S. agalactiae*; *S. agalactiae* confirmed by standard PCR and sequencing.^c Culture positive for CONS/FA positive for *S. aureus*; *S. aureus* confirmed by standard PCR and sequencing.

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- ^d Culture positive for CONS/FA negative; could not rule out a CONS species missed by the FA assay.
- ^e Culture negative, FA positive for CONS; *S. epidermidis* confirmed by standard PCR and sequencing.
- ^f One sequence positive for *E. faecium/vanA*; one sequence positive for *E. casseliflavus/vanC*

Table 3

Prospectively Collected Blood Culture Samples

Organism	Culture	FA	Culture+/FA-	Culture-/FA+	% Sensitivity (95% CI)	% Specificity (95% CI)
Total	92*	89	8**	7	91 (83-96)	77 (58-90)
<i>S. pneumoniae</i>	3	3	-	-	100 [§] (31-100)	100 (96-100)
<i>S. agalactiae</i>	2	2	-	-	100 [§] (20-100)	100 (96-100)
<i>S. pyogenes</i>	3	3	-	-	100 [§] (31-100)	100 (96-100)
<i>Streptococcus spp</i>	11	11	-	1 ⁱ	100 (66-100)	99 (94-100)
<i>S. aureus</i>	12	13	-	1 ^f	100 (70-100)	99 (94-100)
CONS	29	27	3^{d†}	2^{g,h}	89 (71-97)	98 (91-100)
<i>Enterococcus spp</i>	8	7	1 ^{a†}	-	88 (47-99)	100 (96-100)
<i>E. coli</i>	4	5	-	1 ^e	100 [§] (40-100)	99 (94-100)
<i>K. oxytoca</i>	0	1	-	1 ^c	-	99 (94-100)
<i>K. pneumoniae</i>	5	5	-	-	100 (46-100)	100 (96-100)
<i>S. marcescens</i>	2	1	1 ^{b†}	-	50 [§] (3-97)	100 (96-100)
<i>Enterobacteriaceae</i>	4	3	1 ^c	-	75 [§] (22-99)	100 (96-100)
<i>P. aeruginosa</i>	2	2	-	-	100 [§] (20-100)	100 (96-100)
<i>A. baumannii</i>	0	1	-	1 ^e	-	99 (94-100)
<i>C. albicans</i>	4	3	1 ^a	-	75 [§] (22-99)	100 (96-100)
<i>Candida non-albicans</i>	2	1	1 ^a	-	50 [§] (3-97)	100 (96-100)
Not in panel	7	All neg	7	-	N/A	N/A
Negative	18	21	4	1^g	N/A	N/A
AbxR						
<i>mecA</i> (MRSA)	3	3	-	-	100 [§] (31-100)	100 (63-100)
<i>vanA/B</i> (VRE)	4	4	-	-	100 [§] (40-100)	100 (31-100)

* not counted; organisms not in BCID panel

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^{**} in 2 cases, 2 organisms of one species were detected by culture (viridians streptococci/CONS). The species-level FA assay could not differentiate the two, but this was not counted as a missed organism.

- ^f Less than 5 positive cultures tested
- ^a Identified in the culture using standard nested PCR with an increased number of cycles.
- ^b Not identified in this culture with increased cycles, but identified in subsequent cultures from the same subject.
- ^c Culture positive for *Salmonella enterica*/FA positive for *Klebsiella oxytoca*, standard PCR and sequencing identified *S. enterica*.
- ^d 2 episodes in which CONS assay negative by FA, but *mecA* positive
- ^e Gram stain positive for 2 types of GNR; FA PCR positive for *E. coli* and *A. baumannii*, confirmed by standard PCR and sequencing.
- ^f *S. aureus* confirmed by standard PCR and sequencing.
- ^g Culture negative. CONS (*S. haemolyticus*) confirmed by standard PCR and sequencing
- ^h Cross-reactivity of *S. aureus* with CONS primers confirmed by sequencing
- ⁱ Confirmed by standard PCR and sequencing as *S. mitis/oralis*
- [‡] Missed organism in a dual-positive culture