

Revised Abstract

Introduction. The detection of microorganisms in blood samples has been improved with automation of the blood culture systems, however, identification of the agents still requires culture based methods which can take several days. Faster identification of limited types of organisms is possible through FISH methods and a few organism targeted PCR assays. The ability to identify a large number of the most common pathogens would improve patient management by allowing greater ability to target antimicrobial therapy. The Idaho Technology FilmArray Blood Culture Identification (BCID) system is a multiplex PCR assay that targets 20 bacteria, 5 yeasts and 4 antimicrobial resistance genes. This assay is able to provide results in approximately 1 hour from the time a positive blood culture is flagged by the instrument.

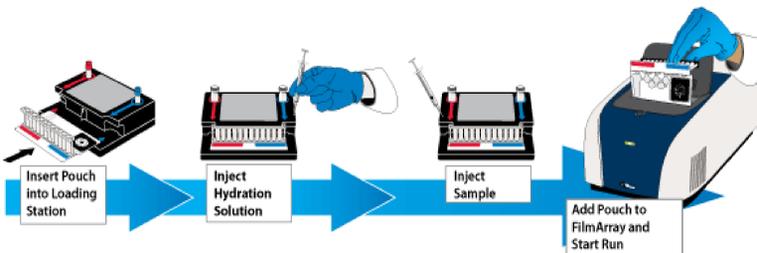
Methods. Blood was collected into BactAlert FAN (Biomérieux) bottles per standard of care and incubated in the BactAlert instrument. When a bottle was flagged as positive it was processed for gram stain and subculture. Next a 500 ul sample was collected by syringe, added to the BCID FilmArray diluent and transferred to the sample port of the pouch. The pouch reagents were rehydrated using the provided reagent solution. The pouch was scanned by the instrument and placed in the FilmArray processor. Sample information was entered and the assay run was started. The instrument performed the extraction and nested PCR on board.

Results. A total of 196 positive blood cultures have successfully been processed to date. The FilmArray correctly identified the pathogen(s) in 158 (81%). The FilmArray did not detect probable pathogens in 38 (19%) samples however all of these were organisms not included in the panel. The only misidentifications (3 agents) in which no other agents were identified occurred with *Enterobacter cloacae*, *Acinetobacter* species and *Pseudomonas* species which are known to be problems with this first generation system due to contaminants in the pouch assembly process. Later generation pouches were prepared from a different supplier of plastic and have begun to address this problem.

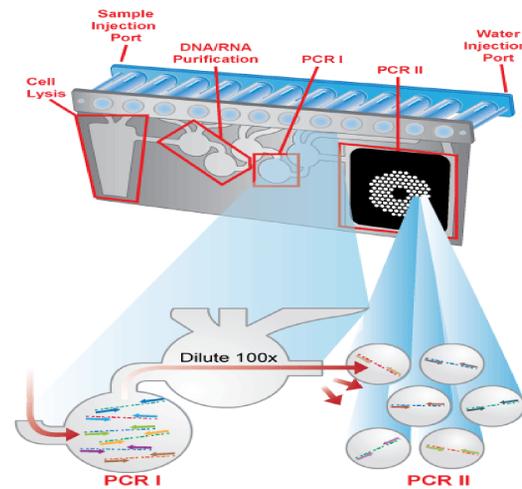
Conclusions. The Beta version of BCID FilmArray was able to detect 100% of the organisms included in the panel and 100% of the cases with antimicrobial resistance factors that were included in the panel within 1 hour. Once available for routine testing this system promises to provide significant improvement in managing patients with bloodstream infections.

FilmArray Workflow

From positive blood culture bottle to actionable results in approximately 1 hour compared to 48 to 72 hrs or more with standard culture methods



FilmArray Pouch



Targets on the Beta Version of the BCID FilmArray

Gram Positive	Gram Negative	Yeast	Antimicrobial Resistance
<i>Staph aureus</i>	<i>Enterobacteriaceae</i>	<i>C. albicans</i>	MecA
<i>Coag Neg Staph</i>	<i>E. cloacae</i>	<i>C. glabrata</i>	vanA
<i>L. monocytogenes</i>	<i>E. coli</i>	<i>C. krusei</i>	vanB
<i>Enterococcus sp</i>	<i>K. pneumoniae</i>	<i>C. parapsolosis</i>	KPC
<i>Streptococcus sp</i>	<i>S. marcescens</i>	<i>C. tropicalis</i>	
<i>S. Pneumoniae</i>	<i>Acinetobacter sp</i>		
<i>S. agalactiae</i>	<i>A. baumannii</i>		
<i>S. pyogenes</i>	<i>H. influenzae</i>		
	<i>H. Flu typeable</i>		
	<i>N. meningitidis</i>		
	<i>Ps. aeruginosa</i>		
	<i>K. oxytoca</i>		

Results

Pathogens Detected by culture and FilmArray

Gram Positive:

<i>Coag Neg Staph</i>	34
<i>Enterococcus</i>	21
<i>Staph aureus</i>	18
<i>Streptococcus sp</i>	11
<i>S. pneumoniae</i>	2
<i>S. pyogenes</i>	2
<i>S. agalactiae</i>	2

Gram Negative:

<i>Enterobacteriaceae</i>	55
<i>E. coli</i>	27
<i>K. pneumoniae</i>	18
<i>Ps. aeruginosa</i>	12
<i>Acinetobacter sp.</i>	10
<i>A. baumannii</i>	6
<i>E. cloacae</i>	4
<i>K. oxytoca</i>	1
<i>S. marcescens</i>	1
<i>N. meningitidis</i>	1

Yeast:

<i>C. albicans</i>	9
<i>C. parapsilosis/tropicalis</i>	6
<i>C. glabrata</i>	4
<i>C. krusei</i>	1

Antimicrobial Resistance:

mecA	45
VanA/B	6
KPC	1

Discrepant:

- Culture: *Enterobacter absurae*, FilmArray: *E. cloacae* (1) – awaiting sequencing for differentiation
- Early pouches had occasional issues with false positive for *Acinetobacter sp* (6), *Enterobacter cloacae* (18) and *Pseudomonas sp* (7) – all at high cT values and other true positive pathogens were detected.

Positive Cultures with Negative FilmArray

Organism	# of Cultures	<i>V. vulnificus</i>	2
<i>Bacillus spp</i>	8	<i>Morganella</i>	1
<i>P. mirabilis</i>	5	<i>Moraxella</i>	1
<i>Bacteroides spp</i>	4	<i>Rhodococcus</i>	1
<i>Diphtheroids</i>	3	<i>Eggerthella</i>	1
<i>Micrococcus spp</i>	3	<i>Eubacterium</i>	1
<i>Viridans strep</i>	2	<i>Elizabethkingia</i>	1
<i>B. cepacia</i>	2	<i>Cellulomonas</i>	1
<i>Lactobacillus</i>	2	<i>Pantoea spp</i>	1

None of the above organisms are targeted in the BCID FilmArray. *Proteus* target is being added to next version of the pouch.

CONCLUSIONS

1. The beta version of the BCID FilmArray was able to accurately detect and identify 100% of the 25 pathogens and 4 antimicrobial resistance factors in positive blood cultures with a time to result of about 1 hour.
2. There were 25 instances, especially with early pouch lots, where false positive results for some targets were detected in addition to other pathogens that were positive by FilmArray and culture. Less of an issue with more recent lots and is being addressed further by Idaho Technology.
3. Since the BCID FilmArray was able to reliably detect the targets included in the panel a no pathogen detected would indicate the presence of an organism not included as a target.
4. The BCID, once cleared for IVD use, has the potential to improve management of patients with sepsis – reducing the time to identification of organisms by 24-72 hours or more.

