P# 1991

A Prospective Pilot Evaluation of a Research Use Only (RUO) Prototype of a Highly Multiplexed Sample-to-Answer PCR System for the Detection of Pathogens from Positive Blood Culture

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Background

Rapid identification of causative agents from positive blood culture (PBC) can aid earlier targeted therapy, as well as reduce mortality, length of stay, and costs associated with systemic infections. The BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel being developed by BioFire Diagnostics, LLC, aims to maintain or enhance the performance of the BioFire® Blood Culture Identification (BCID) Panel with 43 updated and novel assays. The 15 new analytes on the BioFire BCID2 Panel include 6 bacterial analytes, 2 fungal analytes, and 7 antimicrobial resistance (AMR) genes. Updated assays for retained analytes use current bioinformatics to expand coverage. Assay updates as well as modified algorithms confer improved specificity to the BioFire BCID2 Panel. The results from a prospective pilot study performed using a research-use-only (RUO) prototype of the BioFire BCID2 Panel are presented here.

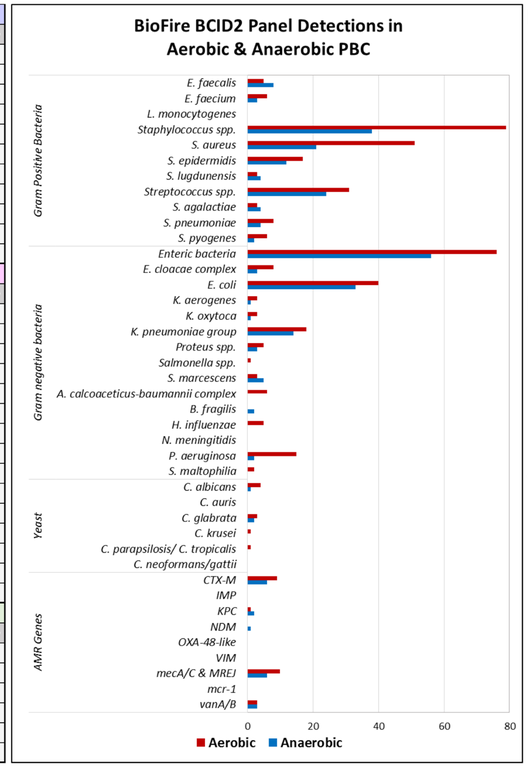
Methods

De-identified PBC samples (<24 hours post-positivity) for which clinician-ordered standard of care (SoC) tests had been performed were enrolled and tested with RUO versions of the BioFire BCID2 Panel. At all participating sites BD BACTEC™ PLUS Aerobic medium was used for aerobic blood cultures. Two types of anaerobic blood culture media, BD BACTEC™ Lytic Anaerobic medium (LUMC) and BD BACTEC™ Plus Anaerobic medium (all other sites), were used. Aliquots of residual PBC and isolates were frozen for discrepancy resolution and AMR gene verification. Clinician ordered BioFire BCID Panel tests were performed according to SoC practices at each site.

The BioFire BCID Panel was used as the secondary comparator to resolve discrepant results for all updated analyte assays. Alternate PCR (compPCR) assays followed by sequencing were used as comparators to verify detections of AMR genes and to resolve discrepant results for novel analytes. The BioFire BCID2 Panel MRSA algorithm was compared to the Cepheid Xpert® MRSA test for 30 select PBC samples.

Performance of BioFire BCID2 Panel Compared to SoC Culture Results for Bacteria and Yeast

Gram-Positive Bacteria						
Target	TP	FN	Sensitivity	FP	TN	Specificity
E. faecalis	13	0	100%	0	349	100%
E. faecium	8	0	100%	1	353	99.7%
L. monocytogenes	0	0	-	0	362	100%
Staphylococcus spp.	113	0	100%	4	245	98.4%
S. aureus	69	0	100%	3	290	99.0%
S. epidermidis	22	0	100%	7	333	97.9%
S. lugdunensis	7	0	100%	0	355	100%
Streptococcus spp.	53	0	100%	2	307	99.4%
S. agalactiae	7	0	100%	0	355	100%
S. pneumoniae	12	0	100%	0	350	100%
S. pyogenes	8	0	100%	0	354	100%
Gram-Negative Bacteria						
Target	TP	FN	Sensitivity	FP	TN	Specificity
Enteric bacteria	131	0	100%	1	230	99.6%
E. cloacae complex	11	0	100%	0	351	100%
E. coli	72	0	100%	1	289	99.7%
K. aerogenes	4	0	100%	0	358	100%
K. oxytoca	3	1	75.0%	1	357	99.7%
K. pneumoniae group	31	0	100%	1	330	99.7%
Proteus spp.	8	0	100%	0	354	100%
Salmonella spp.	1	0	100%	0	361	100%
S. marcescens	8	0	100%	0	354	100%
A. calcoaceticus-baumannii complex	5	0	100%	1	356	99.7%
B. fragilis	1	0	100%	1	360	99.7%
H. influenzae	5	0	100%	0	357	100%
N. meningitidis	0	0	-	0	362	100%
P. aeruginosa	23	0	100%	0	345	100%
S. maltophilia	1	0	100%	1	360	99.7%
Yeast						
Target	TP	FN	Sensitivity	FP	TN	Specificity
C. albicans	4	0	100%	1	357	99.7%
C. auris	0	0	-	0	362	100%
C. glabrata	4	0	100%	1	357	99.7%
C. krusei	1	0	100%	0	361	100%
C. parapsilosis/ C. tropicalis	1	0	100%	0	361	100%
C. neoformans/gattii	0	0	-	0	362	100%



Performance with 242 Aerobic PBC

- **388/388** BioFire BCID2 Panel detections in aerobic PBC were concordant with SoC positives
- 7/15 false positive (FP) detections were also positive by the BioFire BCID Panel assays (2˚ comparator)
  - Sequence confirmation by compPCR of FP results for 1 *A. baumannii,* 1 *K. oxytoca,* 1 *S. maltophilia* and 5 *S. epidermidis* are pending

Performance with 145 Anaerobic PBC

- **232/233** BioFire BCID2 Panel detections in anaerobic PBC were concordant with SoC positives
  - 1 false negative (FN) for *K. oxytoca* was also negative by the BioFire BCID Panel assay
- 7/11 FP detections were also positive by the BioFire BCID Panel assays
  - 1 *B. fragilis* FP result was confirmed by sequencing as TP (2˚ comparator)
  - Sequence confirmation of FP results for 1 *Staphylococcus* spp. and 2 *S. epidermidis* are pending

Overall Performance BioFire BCID2 Panel	SoC Culture Positive	SoC Culture Negative
BCID2 Positive	626	26
BCID2 Negative	1	10937
Overall Sensitivity	99.84%	
Overall Specificity	99.76%	

Overall Performance BioFire BCID Panel (SoC)	SoC Culture Positive	SoC Culture Negative
BCID Positive	289	64
BCID Negative	13	4016
Overall Sensitivity	95.70%	
Overall Specificity	98.43%	

- Greater coverage by updated genus-level assays improved concordance with SoC culture results
- No FP results of *Proteus* spp. with BioFire BCID2 Panel due to improved detection algorithms

BioFire FilmArray Blood Culture Identification 2 (BCID2) Panel

Gram-negative Bacteria
Acinetobacter calcoaceticus-baumannii complex
Bacteroides fragilis
Enteric bacteria
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Klebsiella aerogenes*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae* group
- *Proteus* spp.
- *Salmonella* spp.
- *Serratia marcescens*

Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia

Gram-positive Bacteria
Enterococcus faecalis
Enterococcus faecium
Listeria monocytogenes
Staphylococcus spp.
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus lugdunensis*

Streptococcus spp.
- *Streptococcus agalactiae* (Group B)
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes* (Group A)

Yeast
Candida albicans
Candida auris
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis
Cryptococcus neoformans/gattii

Antimicrobial Resistance Genes
bla_{CTX-M}
bla_{IMP}
bla_{KPC}
mcr-1
mecA/C and MREJ
bla_{NDM}
$bla_{OXA-48-like}$
bla_{VIM}
vanA/B

Specimen Enrollment & Demographics

- Prospective evaluation between April 2018 – August 2018
- Study had IRB approval at all participating sites
- 387 specimens tested with the most current RUO BioFire BCID2 Panel
  - 63% were aerobic PBC samples
    - 17% of data obtained from paired aerobic-anaerobic blood cultures
  - 60% specimens were collected from male patients
  - Only 1% of enrolled specimens were pediatric (< 18 y)

Prospective Pilot Site	Total	Aerobic	Anaerobic
Loyola University of Medical College (LUMC), Illinois, USA	133	73	60
National and Kapodistrian University of Athens (NKUA), Athens, Greece	17	10	7
Northwell Health Laboratories (NHL), New York, USA	62	40	22
Ohio State University (OSU), Ohio, USA	175	119	56

Demographics		Overall	LUMC	NHL	NKUA	OSU
Sex	Male	239 (62%)	83 (62%)	109 (62%)	10 (59%)	37 (60%)
	Female	148 (38%)	50 (38%)	66 (38%)	7 (41%)	25 (40%)
Age	< 1 y	1 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)
	1 - 17 ys	4 (1%)	2 (2%)	2 (1%)	0 (0%)	0 (0%)
	18 - 44 ys	43(11%)	14 (11%)	11 (6%)	1 (6%)	17 (27%)
	45 - 64 ys	111 (29%)	46 (35%)	39 (22%)	3 (18%)	23 (27%)
	65 - 84 ys	176(45%)	60 (45%)	85 (49%)	9 (53%)	22 (35%)
	85+ ys	52 (13%)	11 (8%)	37 (21%)	4 (24%)	0 (0%)

Performance of BioFire BCID2 Panel Compared to Molecular Comparators for AMR Genes

AMR Genes *	Host Bacteria	BioFire BCID2 Detection	SoC AST Data: Resistant	SoC AST Data: Susceptible	SoC AST Data: Not Available	Comparator PCR: Tested	Comparator PCR: Confirmed
bla_{CTX-M}	*E. coli*	16	8	2	6	7	7
bla_{KPC}	*K. pneumoniae*	5	3	0	2	4	4
bla_{NDM}	*K. pneumoniae*	1	1	0	0	1	1
bla_{OXA-48}	*K. oxytoca*	1	1	0	0	0	0
van A/B	*E. faecium*	9	2	2	5	3	3

** Data includes PBC tested with earlier RUO versions of the Biofire BCID2 Panel*

- 100% confirmation of the subset of BioFire BCID2 Panel AMR gene detections evaluated by compPCR and sequence

Concordance with SoC Susceptibility Testing						
BioFire BCID2 Panel Target	TP	FN	PPA	TN	FP	NPA
Staphylococcus aureus	24	0	100%	6	0	100%
MRSA	9	0	100%	9	1	90.0%
Concordance with Cepheid Xpert® MRSA Test						
BioFire BCID2 Panel Target	TP	FN	PPA	TN	FP	NPA
Staphylococcus aureus	24	0	100%	6	0	100%
MRSA	10	0	100%	20	0	100%

- BioFire BCID2 Panel MRSA algorithm is
  - ~95% concordant with Soc AST (only 19 samples with AST)
  - 100% concordant with the Xpert MRSA test

Co-Detections in PBC: BioFire BCID2 Panel Compared to SoC

Co-Detections	BioFire BCID2 Panel: Aerobic	BioFire BCID2 Panel: Anaerobic	SoC Culture: Aerobic	SoC Culture: Anaerobic
1 Analyte	216 (91.9%)	125 (91.9%)	215 (91.9%)	134 (92.4%)
2 Analytes	16 (6.8%)	9 (6.6%)	15 (6.4%)	7 (4.8%)
3 Analytes	1 (0.4%)	0 (0%)	4 (1.7%)	4 (2.8%)
4 Analytes	2 (0.9%)	2 (1.5%)	0 (0%)	0 (0%)

- BioFire BCID2 Panel and SoC have similar co-detection rates in aerobic and anaerobic PBC
- Enhanced coverage of the BioFire BCID2 Panel allowed detection of >98% of pathogens reported by SoC

Conclusions

The updated BioFire BCID2 Panel menu expands coverage to novel fungal and anaerobic pathogens, as well as additional AMR genes. Modified algorithms give the BioFire BCID2 Panel the ability to distinguish blood culture media contaminants such as *Proteus* spp. from actively growing pathogens in blood culture bottles and thus minimize FP results.

With >99% specificity, and >99% sensitivity, the BioFire BCID2 Panel is expected to provide accurate results for key pathogens associated with systemic infections, as well as important AMR genes with the same turn-around-time as the BioFire BCID Panel.

All data presented were obtained with a development (RUO) version of the panel. The BioFire BCID2 Panel has not been evaluated by the FDA or other regulatory agencies for In Vitro *Diagnostic use.*