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Performance of a Research-Use-Only Prototype Highly Multiplexed Sample-to-Answer Molecular Diagnostics System for Identification of Bloodstream Infections and Antimicrobial Resistance Genes from Positive Blood Culture Samples.

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Background

Rapid pathogen identification followed by timely and targeted therapy can positively impact patient outcomes and improve antimicrobial stewardship in cases of bloodstream infections (BSI). Substantial expansion of the BioFire® FilmArray® Blood Culture Identification (BCID) Panel menu with 15 additional targets (6 bacterial, 2 fungal, & 7 antimicrobial resistance (AMR) genes) to the BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel (BioFire Diagnostics, LLC) improves the ability to detect BSI pathogens from positive blood cultures (PBC). Notable additions include the emerging yeast, *Candida auris*, the anaerobe, *Bacteroides fragilis,* and an expanded AMR gene menu that provides accurate methicillin-resistant *Staphylococcus aureus* (MRSA) results plus detection for *mcr-1*, bla_{CTX-M}, bla_{IMP}, bla_{NDM}, $bla_{OXA-48-like}$, and bla_{VIM} genes. This study compared the performance of research-use-only (RUO) prototypes of the BioFire BCID2 Panel with standard of care (SoC) and independent PCR results with emphases on polymicrobial BSI (pBSI) and AMR gene detections.

The BioFire FilmArray 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*

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

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)

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

Methods

649 de-identified residual PBCs, 54 from 1 Greek site and 595 from 4 US sites with clinician-ordered SoC tests were prospectively tested with prototype BioFire BCID2 Panel pouches. Frozen aliquots of PBC and isolates were used to verify AMR gene detection and for discrepancy investigations by independent molecular comparator methods (comp-PCR).

Performance of the Prototype BioFire BCID2 Panel

432 of 649 samples evaluated with the most recent Panel prototype yielded an overall percent agreement of 99.8%.

Gram-Positive Bacteria						
Target	**TP**	**FN**	**PPA**	**TN**	**FP**	**NPA**
E. faecalis	26	0	100.0%	406	0	100.0%
E. faecium	9	0	100.0%	422	1	99.8%
L. monocytogenes	0	0	-	432	0	100.0%
Staphylococcus spp.	145	0	100.0%	281	6	97.9%
S. aureus	82	0	100.0%	347	3	99.1%
S. epidermidis	39	1	97.5%	384	8	98.0%
S. lugdunensis	7	0	100.0%	425	0	100.0%
Streptococcus spp.	64	1	98.5%	366	1	99.7%
S. agalactiae	7	0	100.0%	425	0	100.0%
S. pneumoniae	12	0	100.0%	420	0	100.0%
S. pyogenes	13	0	100.0%	419	0	100.0%
Gram-Negative Bacteria						
Target	**TP**	**FN**	**PPA**	**TN**	**FP**	**NPA**
Enteric bacteria	148	0	100.0%	283	1	99.6%
E. cloacae complex	18	0	100.0%	413	1	99.8%
E. coli	77	0	100.0%	355	0	100.0%
K. aerogenes	4	0	100.0%	428	0	100.0%
K. oxytoca	7	1	87.5%	423	1	99.8%
K. pneumoniae group	33	0	100.0%	398	1	99.7%
Proteus spp.	8	0	100.0%	424	0	100.0%
Salmonella spp.	1	0	100.0%	431	0	100.0%
S. marcescens	9	0	100.0%	423	0	100.0%
A. calcoaceticus-baumannii complex	5	0	100.0%	426	1	99.8%
B. fragilis	1	0	100.0%	430	1	99.8%
H. influenzae	5	0	100.0%	427	0	100.0%
N. meningitidis	0	0	-	432	0	100.0%
P. aeruginosa	17	2	89.5%	413	0	100.0%
S. maltophilia	2	0	100.0%	429	1	99.8%
Yeast						
Target	**TP**	**FN**	**PPA**	**TN**	**FP**	**NPA**
C. albicans	4	0	100.0%	427	1	99.8%
C. auris	0	0	-	432	0	100.0%
C. glabrata	4	0	100.0%	427	1	99.8%
C. krusei	1	0	100.0%	431	0	100.0%
C. parapsilosis/ C. tropicalis	1	0	100.0%	431	0	100.0%
C. neoformans/gattii	0	0	-	432	0	100.0%
Overall Performance	**749**	**5**	**99.3%**	**###**	**28**	**99.8%**

Five false negative (FN) results were encountered
- Comp-PCR assays showed that 2 *P. aeruginosa* and 1 *Streptococcus spp.* FNs were due to low titer levels in polymicrobial PBCs
- *K. oxytoca* and *S. epidermidis* FN were resolved as true negative results due to SoC misidentifications

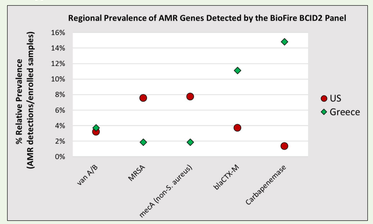
In 27/28 false positive (FP) results, the presence of the detected analyte in the PBC sample was substantiated by comp-PCR assays
- One *S. aureus* FP could not be reproduced upon retest

The BioFire BCID2 Panel Antimicrobial Resistance (AMR) Gene Detections and Concordance to Standard of Care (SoC) Antimicrobial Susceptibility Test (AST) Results

The Panel detected a corresponding AMR gene in **88% (99/112)** of cases with antimicrobial resistance by AST. Comp-PCR assays were used to confirm 41/49 Panel-only detections .

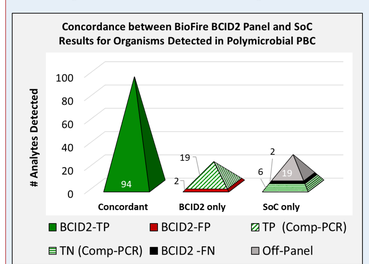
- The Panel detected **vanA/B gene** in all 11 PBCs where vancomycin resistant *E. faecium* and *E. faecalis* were reported by SoC ; additional detections were verified by comp-PCR
- The Panel detected **MRSA** in all 31 cases with an AST MRSA result; 4/14 additional detection remain unverified
  - In 54/55 (98%) of methicillin susceptible cases, the Panel algorithm accurately identified absence of MRSA
- AST was not performed in 17/41 (41.5%) detections of **non-MRSA mecA/C** gene (all from *S. epidermidis*)
- In 23/26 PBCs with **extended spectrum beta-lactamase (ESBL) producers** identified by AST, the ***bla*$_{CTX-M}$** gene was detected by the Panel; 3 confirmed negative by comp-PCR assay
- The Panel detected 12 **carbapenemase genes** (**5 *bla*$_{KPC}$, 5 *bla*$_{NDM}$, 2 *bla*$_{VIM}$**) in 11 of 16 PBCs where SoC reported **carbapenem resistant isolates;** all were verified by comp-PCR. $bla_{OXA-48-like}$ and bla_{IMP} genes were not detected
  - In the remaining 5 samples, comp-PCR did not detect any on-panel carbapenemase genes
- **Mobile colistin resistance gene**, *mcr-1*, was not detected in samples with AST colistin resistance results

Regional Differences in AMR Gene Prevalence as Detected by BioFire BCID2 Panel



- 15% of US PBCs had methicillin resistant *Staphylococci* (4% in Greek PBCs)
- *vanA/B* prevalence ~3-4% in both regions
- Relative prevalence for bla_{CTX-M} and carbapenemase genes higher in Greece
  - bla_{CTX-M} (11% Greece vs 4% in US)
  - Carbapenemase genes (15% Greece vs. 1.3% in US)

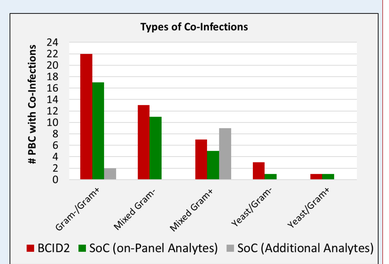
Comparison of Polymicrobial Bloodstream Infections Detected by The BioFire BCID2 Panel and SoC



56 samples were polymicrobial by either SoC (46, 7.1%) or the Panel (46, 7.1%).
- 36 polymicrobial by both methods

92% (94/102) of SoC on-panel organism detections in pBSI cases were correctly identified by the BioFire BCID2 Panel
- 6 on-panel SoC detections missed by the Panel were also not detected by comp-PCR assay
- 19 of 21 Panel-only detections were confirmed by comp-PCR assays
- 2 of 21 Panel-only detections were not confirmed by comp-PCR assays

For all combinations of on-panel co-infections, the BioFire BCID2 Panel detected equal or more pBSI than SoC.
- Mixed Gram+/Gram- infections were the most commonly identified type of pBSI by both methods.
- 2-organism infections were the most frequent type of pBSI detected by both methods



Majority of SoC results with off-panel organisms were found to be mixed Gram+ infections

Study Sites And Demographics

Prospective Pilot Sites	Sample Contribution	Sex		Age					
		Female	Male	<1 y	1-17 ys	18-44 ys	45-64 ys	65-84 ys	85+ ys
Primary Children's Hospital	136 (21%)	68 (50%)	68 (50%)	70 (52%)	64 (47%)	2 (1%)	0 (0%)	0 (0%)	0 (0%)
Loyola University Medical College	227 (35%)	98 (43%)	129 (57%)	0 (0%)	5 (2%)	37 (16%)	73 (32%)	97 (43%)	15 (7%)
Northwell Health Laboratories	63 (10%)	25 (40%)	38 (60%)	0 (0%)	0 (0%)	17 (26%)	23 (37%)	23 (37%)	0 (0%)
Ohio State University, Wexner Medical Center	169 (25%)	64 (38%)	105 (62%)	1 (1%)	2 (1%)	11 (7%)	39 (23%)	80 (47%)	36 (21%)
National and Kapodistrian University of Athens	54 (8%)	21 (39%)	33 (61%)	2 (4%)	0 (0%)	2 (4%)	12 (22%)	32 (59%)	6 (11%)
Overall	**649 (100%)**	276 (43%)	373 (57%)	73 (11%)	71 (11%)	69 (10%)	147 (23%)	232 (36%)	57 (9%)

- Of 649 samples, 270 (42%) were anaerobic & 379 (58%) were aerobic PBC
- Only Becton Dickinson blood culture media were used at all sites

Conclusions

With robust overall performance as well as pBSI and AMR gene detection capabilities comparable to SoC, the BioFire BCID2 Panel should expedite diagnoses and implementation of appropriate therapy; thus improving patient care and application of better antimicrobial stewardship practices.

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