P#1990

Reactivity and Specificity 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 diagnosis of causative agents of bloodstream infections improves patient outcomes and antibiotic stewardship. BioFire Diagnostics, LLC, is developing the BioFire® Blood Culture Identification 2 (BCID2) Panel, increasing the coverage of the BioFire® FilmArray® Blood Culture Identification (BCID) Panel for key pathogens and antimicrobial resistance (AMR) markers in aerobic and anaerobic positive blood culture (PBC). This revision expands the menu from 27 to 43 targets, with 26 bacterial (14 revised, 6 new) and 7 fungal analytes (2 revised, 2 new), as well as 9 AMR markers (1 revised, 6 new). Notable additions include the anaerobe *Bacteroides fragilis*, the emerging fungal pathogen *Candida auris*, and the mobile colistin resistance gene, *mcr*-1. This study details the reactivity and specificity of a RUO BioFire BCID2 panel.

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*

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
- $bla_{CTX\text{-}M}$
- bla_{IMP}
- bla_{KPC}
- *mcr-1*
- *mec*A/C and MREJ
- bla_{NDM}
- $bla_{OXA\text{-}48\text{-}like}$
- bla_{VIM}
- *van*A/B

Methods

The prototype panel was tested with fungal and bacterial isolates, some carrying AMR markers, at two sites by multiple operators. Reactivity was assessed at 10^5-10^7 colony-forming units/mL (CFU/mL) for 368 analytes, and specificity at $10^{7\text{-}9}$ CFU/mL for 31 on-panel and 160 off-panel strains. Evaluation included multiple strains for species level assays and AMR marker assays, as well as multiple species for family/genus level assays. Concordance with standard of care (SoC) results was examined for 157 archived PBC.

Enteric Bacteria Tested

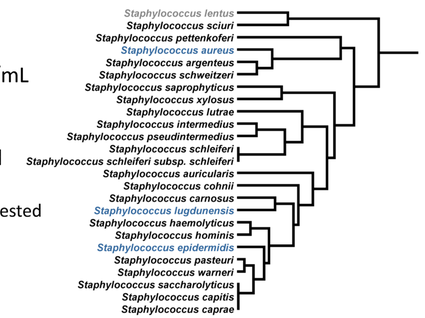
Inclusivity

- Gram-positive bacteria tested at 10^6 CFU/mL
  - Staphylococcus spp. non-reactive with 3/3 strains of *Staphylococcus lentus*
- Non-Enteric Gram-negative bacteria tested at 10^6 CFU/mL
- Enteric Gram-negative bacteria tested at $10^{6\text{-}7}$ CFU/mL
- Fungi tested at 10^5 CFU/mL
- Antimicrobial resistance (AMR) marker assays detected along with host organism assays
  - Reduced reactivity of MREJ assay against 5/25 MREJ types tested
  - Known limitation to less common MREJ variants

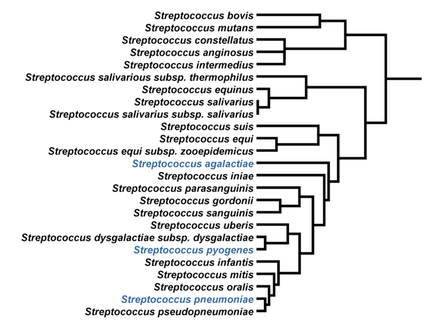
Inclusivity of Gram-Positive Bacteria (117/120 Strains Detected)	
Target	**Detected/Tested**
Enterococcus faecalis	5/5
Enterococcus faecium	5/5
Listeria monocytogenes	5/5
Staphylococcus spp.	45/48
Staphylococcus aureus	26/26
Staphylococcus epidermidis	5/5
Staphylococcus lugdunensis	5/5
Streptococcus spp.	57/57
Streptococcus agalactiae	5/5
Streptococcus pneumoniae	7/7
Streptococcus pyogenes	5/5
Inclusivity of Non-Enteric Gram-Negative Bacteria (40/40 Strains Detected)	
Target	**Detected/Tested**
Acinetobacter calcoaceticus-bamannii Complex	11/11
Bacteroides fragilis	5/5
Haemophilus infleunzae	7/7
Neisseria meningitidis	5/5
Pseudomonas aeruginosa	7/7
Stenotrophomonas maltophilia	5/5
Inclusivity of Enteric Gram-Negative Bacteria (152/152 Strains Detected)	
Target	**Detected/Tested**
Enteric Bacteria	152/152
Enterobacter cloacae Complex	8/8
Escherichia coli	14/14
Klebsiella aerogenes	4/4
Klebsiella oxytoca	6/6
Klebsiella pneumoniae Group	8/8
Proteus spp.	9/9
Salmonella spp.	9/9
Serratia marcescens	7/7
Inclusivity of Fungi (56/56 Strains Detected)	
Target	**Detected/Tested**
Candida albicans	7/7
Candida auris	12/12
Candida glabrata	7/7
Candida krusei	7/7
Candida parapsilosis	8/8
Candida tropicalis	6/6
Cryptococcus neoformans/gattii	9/9

Inclusivity of Antimicrobial Resistance Markers (112/117 Markers Detected)		
Target	**Detected/Tested**	**Host Organism(s)**
CTX-M	16/16	10
IMP	5/5	3
KPC	9/9	7
MCR-1	6/6	1
mecA/C	29/29	3
MREJ	20/25	2
NDM	10/10	8
OXA-48-like	5/5	4
vanA/B	7/7	2
VIM	5/5	3

Staphylococci Tested



Streptococci Tested



Exclusivity

- Panel exclusivity assessed against 191 species
  - On-panel organisms (intra-panel cross-reactivity)
  - Phylogenetic-neighbors
  - Representative subset of normal skin flora
  - Off-panel organisms likely to be encountered in PBC
- Bacteria tested at $10^{8\text{-}9}$ CFU/mL
- Fungi tested at 10^7 CFU/mL
- 100% specificity observed for 43/45 assays
- 2/45 assays were found to cross-react with near-neighbor species
  - Bfragilis assay with *Bacteroides xylanisolvens*
  - Ckrusei assay with *Candida inconspicua* and *Candida norvegensis*
  - Cross-reactivity only observed at high titers of organism

Phylogenetic tree of all bacteria tested constructed from target gene sequences obtained from the NCBI nr/nt and wgs databases. Organisms targeted by species/group-specific assays are highlighted in blue, and missed detections in grey.

Preliminary Concordance of BioFire BCID and BCID2 Panels with SoC in Archived Clinical Samples

Gram-Positive Bacteria								
Target	**TP**		**FN**		**FP**		**TN**	
	BCID	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**
Enterococcus faecalis	5	8	3	1	1	0	148	148
Enterococcus faecium	2	5	2	0	1	0	152	152
Listeria monocytogenes	0	0	0	0	1	0	156	157
Staphylococcus spp.	32	40	7	0	1	4	117	113
Staphylococcus aureus	9	9	2	2	2	2	144	144
Staphylococcus epidermidis	N/A	14	N/A	0	N/A	4	N/A	139
Staphylococcus lugdunensis	N/A	0	N/A	0	N/A	0	N/A	157
Streptococcus spp.	10	12	6	4	0	0	141	141
Streptococcus agalactiae	1	1	0	0	0	0	156	156
Streptococcus pneumoniae	2	2	0	0	0	0	155	155
Streptococcus pyogenes	2	2	0	0	0	0	155	155
Gram-Negative Bacteria								
Target	**TP**		**FN**		**FP**		**TN**	
	BCID	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**
Acinetobacter calcoaceticus-baumannii complex	0	2	2	0	1	1	154	154
Bacteroides fragilis	N/A	0	N/A	0	N/A	0	N/A	157
Enteric bacteria	26	30	9	5	1	1	121	121
Enterobacter cloacae complex	2	4	3	0	2	3	150	150
Escherichia coli	7	9	3	1	2	3	145	144
Klebsiella aerogenes	N/A	2	N/A	1	N/A	1	N/A	153
Klebsiella oxytoca	2	2	2	1	1	2	152	152
Klebsiella pneumoniae group	7	8	2	1	1	1	147	147
Proteus spp.	0	0	0	0	0	0	157	157
Salmonella spp.	N/A	0	N/A	1	N/A	1	N/A	155
Serratia marcescens	1	1	0	0	0	0	156	156
Haemophilus influenzae	0	0	0	0	0	0	157	157
Neisseria meningitidis	0	0	0	0	0	0	127	157
Pseudomonas aeruginosa	1	1	1	1	1	1	154	154
Stenotrophomonas maltophilia	N/A	2	N/A	0	N/A	1	N/A	154
Yeast								
Target	**TP**		**FN**		**FP**		**TN**	
	BCID	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**	**BCID**	**BCID2**
Candida albicans	8	8	0	0	1	1	148	148
Candida auris	N/A	0	N/A	0	N/A	0	N/A	157
Candida glabrata	2	3	0	0	2	1	153	153
Candida krusei	0	0	0	0	0	0	157	157
Candida parapsilosis/tropicalis	10	9	1	2	3	2	143	144
Cryptococcus neoformans/gattii	N/A	2	N/A	0	N/A	1	N/A	154

- BioFire detections are concordant with culture SoC results for the majority of analytes
  - True positive detections increased due to broader coverage of the BioFire BCID2 Panel
  - Reduction in false positive and false negative results due to improved sensitivity and specificity of the BioFire BCID2 Panel
    - Cparapsilosis & Ctropicalis assays have been re-configured to improve sensitivity
  - Discrepancies likely a result of misidentification due to phenotypic/morphological similarities
- Concordance of AMR markers not evaluated

Conclusions

Performance of the RUO BioFire BCID2 Panel shows that many key pathogens implicated in bloodstream infections can be identified with high sensitivity and specificity, and highlights the utility of the updated assays and the expanded menu to aid in the diagnosis of etiological agents of bloodstream infections.

All data presented were obtained with a Research Use Only (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.*