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Enhanced Detection of Bloodstream Pathogens from Positive Blood Culture Specimens with an Improved Multiplex PCR Molecular Diagnostic System

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Background

Timely bloodstream infection (BSI) pathogen identification requires robust sample purification and testing methods that can accommodate the wide variety of blood culture media used for growing positive blood culture (PBC) specimens. Sensitive molecular methods are needed for identification of all organisms present in PBC, especially polymicrobial cultures which can be difficult to identify with standard methods. Multiple types of BD and BioMérieux blood culture media commonly used in hospital labs were used to evaluate the performance of a prototype BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel with PBCs.

Methods

Fungi (7) and bacteria (19) were independently seeded in blood samples, inoculated into as many as 8 different types of blood culture bottles, and incubated on the recommended instrument. Time to detection (TTD) was recorded for all PBCs. Subsets of PBCs were enumerated and tested on the BioFire BCID2 Panel and BioFire® FilmArray® Blood Culture Identification (BCID) Panel. Polymicrobial testing was performed by seeding fast and slow growing organisms into the same bottles.

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

Conclusions

The data compiled here provides valuable insight regarding TTDs, titers, and FilmArray performance of PBCs in many different blood culture media bottles on multiple vendor platforms.

This study demonstrates that a prototype BioFire BCID2 Panel, as well as the BioFire BCID Panel, robustly detect and identify (100%) BSI pathogens over a multitude of common blood culture media and systems. Polymicrobial results highlight the systems ability to detect multiple pathogens in a single sample as well as demonstratimg that PBCs can be tested anywhere from 0 to 24 hours after positivity.

Results confirm PBC (single & polymicrobial) titers are above the levels of sensitivity for both BioFire BCID and BCID2 Panels. An expanded menu of targets (organism & resistance) and faster run time with the BioFire BCID2 Panel will offer a flexible and comprehensive aid in the diagnosis of BSIs.

Results

Average TTD of each organism in multiple bottle types was curated from the 1774 PBCs generated; ~900 PBCs enumerated; ~600 PBCs tested on BioFire BCID2 Panel, and ~300 tested on BioFire BCID Panel. Both BioFire BCID Panels resulted in correct pathogen identification in 100% of PBCs tested. Fungi grew to levels ranging from 8E+05 to 5E+07 CFU/mL, Gram+ bacteria titers ranged from 7E+06 to 2E+09, and Gram- bacteria titers ranged from 9E+07 to 3E+09. Vendor and formulation of blood culture media did not appear to impact final organism titers, but did affect TTDs for some organisms.

FilmArray Detections of Gram - PBCs

Organism	Blood Culture Media	Avg. TTD (hh:mm:ss)	Avg. Titer in PBC	Detections BCID	Detections BCID2
A. baumannii	BacT/ALERT SA	11:53:00	1.5E+08	√	√
	BacT/ALERT FA+	10:54:24	6.7E+08	√	√
	Bactec aerobic F	11:39:30	9.2E+08	√	√
B. fragilis	Bactec Anaerobic F	23:07:05	2.4E+09	N/A	√
K. aerogenes	BacT/ALERT SA	12:16:00	1.2E+09	√*	√
	BacT/ALERT SN	10:16:00	7.5E+08	√*	--
	BacT/ALERT FA+	13:46:00	1.3E+09	√*	√
	BacT/ALERT FN+	10:46:00	3.2E+08	√*	√
	Bactec aerobic F	11:46:00	1.5E+05	--	√
	Bactec Anaerobic F	11:56:00	5.5E+04	--	√
E. cloacae	BacT/ALERT SA	10:12:46	1.2E+09	√	√
	BacT/ALERT SN	9:21:09	8.9E+08	√	√
	BacT/ALERT FA+	9:42:05	1.3E+09	√	√
	BacT/ALERT FN+	8:45:31	2.8E+08	√	√
	Bactec aerobic F	13:24:19	7.9E+08	--	√
	Bactec Anaerobic F	22:54:45	3.8E+08	--	√
E. coli	BacT/ALERT FA+	15:45:48	6.2E+08	--	√
	Bactec aerobic F	11:50:07	9.7E+08	√	√
	Bactec Anaerobic F	14:14:00	3.9E+08	√	√
H. influenzae	BacT/ALERT SA	15:52:00	1.9E+08	√	√
	BacT/ALERT SN	14:22:00	2.2E+08	√	√
	BacT/ALERT FA	19:33:00	1.5E+09	--	√
	BacT/ALERT FA+	14:02:00	1.2E+09	√	√
	BacT/ALERT FN	40:57:12	4.4E+08	--	√
	BacT/ALERT FN+	12:32:00	1.4E+08	√	√
	Bactec aerobic F	18:21:11	5.1E+08	√	√
	Bactec Anaerobic F	18:02:00	1.0E+09	√	√
K. oxytoca	BacT/ALERT SA	10:23:00	2.3E+09	√	√
	BacT/ALERT SN	10:08:00	2.4E+09	√	√
	BacT/ALERT FA+	9:48:00	2.7E+09	√	√
	BacT/ALERT FN+	9:43:00	1.1E+09	√	√
	Bactec aerobic F	10:57:00	1.4E+09	--	√
	Bactec Anaerobic F	11:06:22	5.1E+08	--	√
K. pneumoniae	BacT/ALERT SA	10:05:19	1.1E+09	√	√
	BacT/ALERT SN	6:54:55	5.0E+08	√	√
	BacT/ALERT FA+	9:51:01	8.6E+08	√	√
	BacT/ALERT FN+	8:58:48	4.8E+08	√	√
	Bactec aerobic F	10:37:53	1.0E+09	√	√
	Bactec Anaerobic F	12:12:24	8.6E+08	√	√
N. meningitidis	BacT/ALERT SA	16:14:00	9.2E+07	√	√
	BacT/ALERT SN	15:33:00	1.2E+08	√	√
	BacT/ALERT FA+	15:44:00	2.8E+08	√	√
	BacT/ALERT FN+	16:08:00	1.6E+08	√	√
	Bactec aerobic F	14:15:00	5.2E+08	√	√
	Bactec Anaerobic F	17:00:00	5.7E+07	√	√
P. aeruginosa	BacT/ALERT SA	15:20:00	3.4E+08	--	√
	BacT/ALERT SN	14:20:00	2.0E+08	--	√
	BacT/ALERT FA+	13:50:00	2.8E+08	--	√
	Bactec aerobic F	14:55:00	4.6E+08	√	√
P. mirabilis	BacT/ALERT SA	12:34:12	9.6E+08	√	√
	BacT/ALERT SN	9:27:58	1.0E+09	√	√
	BacT/ALERT FA+	12:13:26	1.4E+09	√	√
	BacT/ALERT FN+	9:35:36	7.8E+08	√	√
	Bactec aerobic F	9:35:00	9.6E+08	√	√
	Bactec Anaerobic F	8:45:00	9.3E+08	√	√
S. enterica	BacT/ALERT SA	12:28:31	1.4E+09	√*	--
	BacT/ALERT SN	8:36:26	9.0E+08	√*	√
	BacT/ALERT FA+	10:32:02	9.2E+08	√*	--
	BacT/ALERT FN+	9:02:50	5.6E+08	√*	√
	Bactec aerobic F	11:55:16	9.7E+08	√*	√
	Bactec Anaerobic F	13:44:42	7.6E+08	√*	√
S. maltophilia	BacT/ALERT SA	52:26:36	--	N/A	√
	BacT/ALERT FA	21:38:56	1.2E+09		√
	BacT/ALERT FA+	44:05:16	4.3E+08		√
	Bactec aerobic F	24:24:22	6.4E+08		√
S. marcesens	BacT/ALERT SA	11:38:00	1.3E+09	--	√
	BacT/ALERT SN	10:58:00	1.6E+09	--	√
	BacT/ALERT FA+	11:28:00	1.8E+09	--	√
	BacT/ALERT FN+	11:08:00	1.3E+09	--	√
	Bactec aerobic F	9:30:00	3.2E+09	√	√
	Bactec Anaerobic F	9:30:00	1.3E+09	--	√

*Enterobacteriaceae reported by BCID

FilmArray Detections of Gram + PBCs

Organism	Blood Culture Media	Avg. TTD (hh:mm:ss)	Avg. Titer in PBC	Detections BCID	Detections BCID2
E. faecalis	BacT/ALERT SA	18:15:00	4.9E+08	√*	√
	BacT/ALERT SN	7:40:48	1.1E+08	√*	√
	BacT/ALERT FA	NR	8.5E+08	--	√
	BacT/ALERT FA+	9:49:12	4.8E+08	√*	√
	BacT/ALERT FN	12:18:20	1.2E+09	--	√
	BacT/ALERT FN+	11:36:54	2.5E+08	√*	√
	Bactec aerobic F	14:58:07	5.9E+08	√*	√
	Bactec Anaerobic F	16:42:09	6.2E+08	√*	√
E. faecium	BacT/ALERT SA	11:52:00	1.4E+09	√*	√
	BacT/ALERT SN	11:32:00	1.6E+09	√*	√
	BacT/ALERT FA	19:25:00	6.0E+07	--	√
	BacT/ALERT FA+	11:02:00	9.1E+08	√*	√
	BacT/ALERT FN	14:04:20	2.6E+08	--	√
	BacT/ALERT FN+	11:02:00	1.3E+09	√*	√
	Bactec aerobic F	15:24:43	3.2E+08	√*	√
	Bactec Anaerobic F	18:09:00	1.3E+08	√*	√
L. monocytogenes	BacT/ALERT SA	17:56:00	6.6E+08	√	√
	BacT/ALERT SN	17:51:00	3.2E+08	√	√
	BacT/ALERT FA+	17:21:00	3.8E+08	√	√
	BacT/ALERT FN+	17:41:00	2.1E+08	√	√
	Bactec aerobic F	16:36:00	3.3E+08	√	√
	Bactec Anaerobic F	21:46:00	1.4E+09	√	√
S. agalactiae	BacT/ALERT SN	9:01:08	1.6E+09	√	√
	BacT/ALERT FA+	10:30:27	1.3E+09	√	√
	BacT/ALERT FN+	9:35:42	1.4E+09	√	√
S. aureus	BacT/ALERT SA	12:44:42	1.6E+08	√	√
	BacT/ALERT FA+	15:13:21	3.4E+07	√	√
	BacT/ALERT FN+	12:07:40	1.7E+08	√	√
	Bactec aerobic F	17:42:18	5.6E+07	--	√
S. epidermidis	BacT/ALERT SA	18:03:53	9.1E+07	√**	√
	BacT/ALERT SN	15:37:02	8.5E+07	√**	√
	BacT/ALERT FN+	16:59:54	3.1E+07	√**	√
S. lugdunensis	BacT/ALERT SA	22:28:12	--	√**	√
	Bactec aerobic F	16:03:34	3.1E+08	√**	√
	Bactec Anaerobic F	15:25:00	1.0E+08	√**	√
S. pneumoniae	BacT/ALERT SA	16:36:00	9.9E+08	√	√
	BacT/ALERT SN	15:32:00	1.1E+09	√	√
	BacT/ALERT FA+	14:11:30	3.6E+08	√	√
	BacT/ALERT FN+	15:47:00	6.4E+08	√	√
	Bactec aerobic F	12:35:00	5.8E+08	√	√
	Bactec Anaerobic F	16:30:00	2.9E+08	√	√
S. pyogenes	BacT/ALERT SA	12:26:00	1.2E+09	√	√
	BacT/ALERT SN	10:51:00	6.0E+08	√	√
	BacT/ALERT FA	36:49:50	2.3E+07	--	√
	BacT/ALERT FA+	11:01:38	3.8E+08	√	√
	BacT/ALERT FN	14:37:00	1.7E+08	--	√
	BacT/ALERT FN+	10:39:02	9.9E+08	√	√
	Bactec aerobic F	21:45:25	4.6E+07	--	√

* Enterococcus reported by BCID ** Staphylococcus reported by BCID

FilmArray Detections of Fungal PBCs

Organism	Blood Culture Media	Avg. TTD (hh:mm:ss)	Avg. Titer in PBC	Detections BCID	Detections BCID2
C. albicans	BacT/ALERT SA	16:35:48	2E+07	√	√
	BacT/ALERT FA+	16:03:55	6E+06	√	√
	Bactec aerobic F	16:11:22	6E+06	√	√
C. auris	BacT/ALERT SA	20:48:43	4E+07	N/A	√
	BacT/ALERT FA+	20:20:46	5E+07		√
	Bactec aerobic F	20:08:53	4E+07		√
C. gattii	BacT/ALERT SA	35:03:31	5E+06	N/A	√
	BacT/ALERT FA+	54:04:18	3E+06		√
	Bactec aerobic F	45:56:07	8E+05		√
C. glabrata	BacT/ALERT SA	38:29:49	7E+06	√	√
	BacT/ALERT FA+	35:36:27	1E+07	√	√
	Bactec aerobic F	27:17:00	8E+06	√	√
C. krusei	BacT/ALERT SA	16:51:21	9E+06	√	√
	BacT/ALERT FA+	14:59:47	1E+07	√	√
	Bactec aerobic F	13:33:19	2E+07	√	√
C. neoformans	Bactec aerobic F	41:29:00	5E+05	N/A	√
C. parapsilosis	BacT/ALERT SA	22:26:35	1E+07	√	√
	BacT/ALERT FA+	22:58:55	1E+07	√	√
	Bactec aerobic F	20:44:33	1E+07	√	√
C. tropicalis	BacT/ALERT SA	14:51:00	2E+07	√	√
	BacT/ALERT FA+	14:03:30	1E+07	√	√
	Bactec aerobic F	13:38:25	1E+07	√	√

Key	
N/A	No Assay Available
√	Detected
✗	Undetected
--	Not Tested

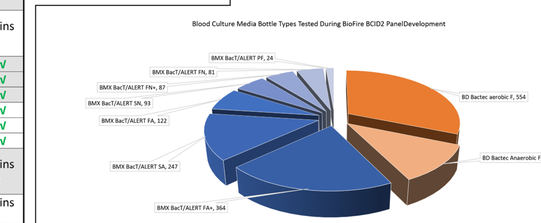
Polymicrobial Results

Polymicrobial PBCs (31) showed equivalent performance from 0 to 24 hours beyond when the blood culture media bottles were identified as positive. For the organisms and associated antibiotic resistance markers (ARM) expected to be present in the polymicrobial PBCs, BioFire BCID Panel positivity was 153/154 while BioFire BCID2 Panel was 271/272. Based on analysis of TTDs, it was concluded that slow growing organisms likely had reduced titers when seeded with fast growing organisms but were still detected by both BioFire BCID Panels at a rate of 99%.

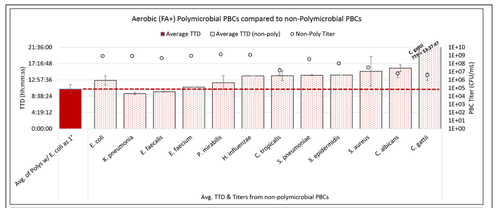
Detections of Aerobic (BacT/ALERT FA+) Polymicrobial PBCs over time in BioFire BCID & BCID2 Panel Pouches

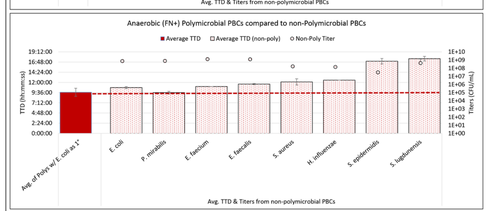
Bottle TTD (hh:mm)	Post TTD Time Point (h)	1° Organism	1° BCID	1° BCID2	1° ARM	1° BCID	1° BCID2	2° Organism	2° BCID	2° BCID2	2° ARM	2° BCID	2° BCID2	3° Organism	3° BCID	3° BCID2
10:54	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*C. tropicalis*	√	√	Organism contains no resistance			*C. gattii*	N/A	√
	24		√	√			√ √		√	√					N/A	√
9:05	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*H. influenzae*	√	√	Organism contains no resistance			*S. pneumoniae*	√	√
	24		√	√			√ √		√	√					√	√
10:11	0	*E. coli*	√	√	IMP	N/A	√	*P. mirabilis*	√	√	Organism contains no resistance			*S. agalactiae*	√	√
	24		√	√			√		√	√					√	√
11:22	0	*E. coli*	√	√	IMP	N/A	√	*S. aureus*	√	√	mec*	√	√	*C. albicans*	√	√
	24		√	√			√		√	√		√	√		√	√
11:52	0	*E. coli*	√	√	IMP	N/A	√	*E. faecalis*	√**	√	vanA/B	√	√	*C. parapsilosis*	√	√
	24		--	√			√		--	√		--	√		-	√
9:05	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*H. influenzae*	√	√	Organism contains no resistance			*S. pneumoniae*	√	√
10:01	0	*K. oxytoca*	√	√	Organism contains no resistance			*P. aeruginosa*	√	√	VIM	N/A	√	*S. pyogenes*	√	√
	24		√	√					√	√			√		√	√
12:17	0	*K. aerogenes*	√‡	√	CTX-M	N/A	√	*S. lugdunensis*	√¥	√	Organism contains no resistance			*C. krusei*	√	√
	24		--	√			√		--	√					-	√
12:15	0	*E. cloacae*	√	√	CTX-M, OXA-48	N/A	√ √	*L. monocytogenes*	√	√	Organism contains no resistance			*C. glabrata*	√	√
12:15	0	*E. coli*	√	√	IMP	N/A	√	*C. albicans*	√	√	Organism contains no resistance			No 3rd organism Seeded		
	8		√	√			√		√	√						
	24		√	√			√		√	√						
10:15	0	*E. coli*	√	√	IMP	N/A	√	*H. influenzae*	√	√	Organism contains no resistance					
	8		√	√			√		√	√						
	24		√	√			√		√	√						
9:35	0	*E. coli*	√	√	IMP	N/A	√	*K. pneumonia*	√	√	KPC	√	√			
	8		√	√			√		√	√		√	√			
	24		√	√			√		√	√		√	√			
10:55	0	*E. coli*	√	√	IMP	N/A	√	*S. aureus*	√	√	mec*	√	√			
	8		√	√			√		√	√		√	√			
	24		√	√			√		√	√		√	√			
12:35	0	*E. coli*	√	√	IMP	N/A	√	*S. pneumoniae*	√	√	Organism contains no resistance					
	8		√	√			√		√	√						
	24		√	√			√		√	√						
12:44	0	*E. coli*	√	√	IMP	N/A	√	*C. gattii*	N/A	√	Organism contains no resistance					
	24		√	√			√			√						
10:31	0	*E. coli*	√	√	IMP	N/A	√	*E. faecium*	√**	√	Organism contains no resistance					
	24		√	√			√		√**	√						
9:05	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*S. epidermidis*	√	√	Organism contains no resistance					
10:05	0	*N. meningiditis*	√	√	Organism contains no resistance			*S. marcesens*	√	√	Organism contains no resistance					

* BCID calls mec, BCID2 calls mecA/C & MREJ ** Enterococcus reported by BCID ‡ Enterobacteriaceae reported by BCID ¥ Staphylococcus reported by BCID



Development efforts for BioFire BCID2 Panel resulted in 1774 contrived PBC specimens of 109 different organism strains, using as many as 9 different blood culture media types from Becton Dickinson and BioMérieux over the span of approximately 12 months. The data presented in this poster has been curated from these 1774 PBCs.





Shown above are TTDs of aerobic and anaerobic polymicrobial PBCs containing *E. coli* as compared to the TTDs & titers observed for the individual organisms. Titers of slower growing organisms are expected to be lower due to the presence of *E. coli* and faster TTDs; BioFire BCID & BCID2 Panel detections remain robust despite reduced titers.

Detections of Anaerobic (BacT/ALERT FN+) Polymicrobial PBCs over time in BioFire BCID & BCID2 Panel Pouches

Bottle TTD (hh:mm)	Post TTD Time Point (h)	1° Organism	1° BCID	1° BCID2	1° ARM	1° BCID	1° BCID2	2° Organism	2° BCID	2° BCID2	2° ARM	2° BCID	2° BCID2	3° Organism	3° BCID	3° BCID2
8:45	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*H. influenzae*	√	√	Organism contains no resistance			*S. pneumoniae*	√	√
	24		√	√			√ √		√	√					√	√
9:11	0	*E. coli*	√	√	IMP	N/A	√	*P. mirabilis*	√	√	Organism contains no resistance			*S. agalactiae*	√	√
	24		√	√			√		√	√					√	√
8:45	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*H. influenzae*	√	√	Organism contains no resistance			*S. pneumoniae*	√	√
9:41	0	*K. oxytoca*	√	√	Organism contains no resistance			*P. aeruginosa*	√	√	VIM	N/A	√	*S. pyogenes*	√	√
	24		√	√					√	√			√		√	√
10:25	0	*E. cloacae*	√	√	CTX-M, OXA-48	N/A	√ √	*L. monocytogenes*	√	√	Organism contains no resistance			*C. glabrata*	√	√
10:42	0	*E. coli*	√	√	IMP	N/A	√	*S. aureus*	√	√	mec*	√	√	No 3rd organism seeded		
	24		√	√			√		√	√		√	√			
10:21	0	*E. coli*	√	√	IMP	N/A	√	*E. faecium*	√**	√	Organism contains no resistance					
	24	*E. coli*	√	√	IMP		√	*E. faecium*	√**	√						
9:05	0	*E. coli*	√	√	CTX-M, MCR-1	N/A	√ √	*S. epidermidis*	✗	✗	Organism contains no resistance					
10:57	0	*E. coli*	√	√	IMP	N/A	√	*E. faecalis*	√**	√	vanA/B	√	√			
	24		--	√			√		--	√		--	√			
9:55	0	*N. meningiditis*	√	√	Organism contains no resistance			*S. marcesens*	√	√	Organism contains no resistance					
10:17	0	*K. aerogenes*	√‡	√	CTX-M	N/A	√	*S. lugdunensis*	√¥	√	Organism contains no resistance					
	24		--	√			√		--	√						

* BCID calls mec, BCID2 calls mecA/C & MREJ ** Enterococcus reported by BCID ‡ Enterobacteriaceae reported by BCID ¥ Staphylococcus reported by BCID

The BioFire BCID2 Panel has not yet been evaluated by the FDA or other regulatory agencies for in vitro diagnostic use.