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Mitigation of Nucleic Acid Contamination Present in Blood Culture Media Formulations with an Enhanced Molecular Diagnostic Test

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Background

Molecular diagnostic tests provide faster, more objective, and generally more sensitive and specific results as compared to standard culture methods. The BioFire® FilmArray® Blood Culture Identification (BCID) Panel tests positive blood culture (PBC) samples to identify key pathogens implicated in bloodstream infections. Studies have shown that sterile blood culture media can contain residual nucleic acid (NA) from a variety of bacteria likely introduced from raw materials or manufacturing processes. Recently the BioFire BCID Panel was affected by the presence of NA found to trigger *Proteus* spp as well as *Enterobacteriaceae* (reported as Enteric bacteria by the BioFire BCID2 Panel) detections from sterile blood culture bottles. This study evaluated the ability of a prototype BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel with algorithm and chemistry enhancements to mitigate false positive (FP) results caused by the presence of *Proteus* and *Enterobacteriaceae* (Enteric bacteria) NA in sterile blood culture bottles.

Methods

Sterile blood culture media bottles used during a 5-site prospective pilot study as well as during development studies at BioFire Diagnostics, LLC were tested with both BioFire BCID and BioFire BCID2 Panels. This included 40 unique media lots of 6 different formulations manufactured by Becton Dickinson (BD) and 20 unique media lots of 5 different formulations manufactured by bioMérieux (BMX). Contrived and residual clinical PBC with *Proteus* spp. were also assayed with both Panels for comparison.

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
*mec*A/C and MREJ
bla_{NDM}
$bla_{OXA-48-like}$
bla_{VIM}
*van*A/B

Composition of Blood Culture Media

The various blood culture media formulations utilized by many diagnostic laboratories are effective sterile growth mediums that enrich low levels of pathogens known to cause bloodstream infections to allow for identification and treatment. The evolution of highly sensitive molecular based tests, however, has led to an increase in false positive detections caused by residual levels of nucleic acid present in these medias. These potential false positives can confound results and impact patient care.

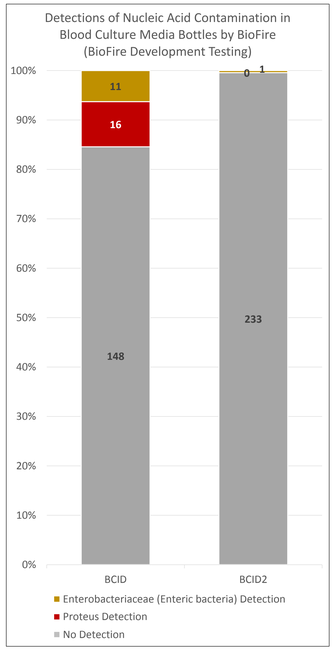
Many common ingredients in blood culture media are derived from biological sources: animal tissues extracts, plant extracts, yeast extracts, and carbon sources, in addition to proprietary components. These biologically sourced materials may contain nucleic acids and are also potential carriers of common organisms that various molecular based diagnostics may be designed to detect.

Other medias have also been shown to contain nucleic acid contamination that may impact molecular testing for example BHI and Cary Blair.

BioFire BCID & BCID2 Panel Detections of Nucleic Acid Contamination in Blood Culture Media Bottles (tested at BioFire)

Bottle Type/Formulation	Lot ID	*Proteus* NA Contamination Detected		*Enterobacteriaceae*¥ NA Contamination Detected†	
		BioFire BCID	BioFire BCID2	BioFire BCID	BioFire BCID2
BD BACTEC™ Plus Anaerobic medium	7088914	0/5	0/8	0/5	0/8
	8053748*	5/5	0/5	0/5	0/5
	7138842*	0/5	0/8	0/5	0/8
	7068851	0/5	0/8	0/5	0/8
	6344850	0/5	0/8	0/5	0/8
BD BACTEC™ Standard Anaerobic medium	8072510	4/5	0/5	0/5	0/5
BD BACTEC™ Plus Aerobic medium	7297958	0/5	0/8	0/5	0/8
	8072702	0/5	0/5	0/5	0/5
	8053836*	3/5	0/8	0/5	0/8
	7177857	0/5	0/8	0/5	0/8
	7254994	0/5	0/8	0/5	0/8
BD BACTEC™ Standard Aerobic medium	8072716	0/5	0/5	0/5	0/5
BD BACTEC™ Lytic Anaerobic medium	8065930	1/5	0/5	0/5	0/5
	8236621	0/5	0/5	0/5	0/5
BD BACTEC™ Peds Plus™ medium	8053868	0/5	0/5	0/5	0/5
BACT/ALERT® FA Plus	3048966	0/5	0/8	0/5	0/8
	3049628	0/5	0/8	0/5	0/8
	4050844	0/5	0/5	0/5	0/5
	4051302*	0/5	0/8	0/5	0/8
	4051712*	0/5	0/8	0/5	0/8
	4052546	0/5	0/5	4/5	1/5
	4052800	0/5	0/5	3/5	0/5
BACT/ALERT® FN Plus	3049094	0/5	0/8	0/5	0/8
	4050934	0/5	0/8	0/5	0/8
	4051920*	3/5	0/8	0/5	0/8
	4052704	0/5	0/4	1/5	0/4
BACT/ALERT® PF Plus	4050704	0/5	0/5	0/5	0/5
	4052718	0/5	0/5	3/5	0/5
BACT/ALERT® SA	1045865	0/5	0/8	0/5	0/8
	1048735	0/5	0/8	0/5	0/8
	1049104	0/5	0/8	0/5	0/8
	1050694	0/5	0/5	0/5	0/5
BACT/ALERT® SN	1050658	0/5	0/8	0/5	0/8
	1050818	0/5	0/5	0/5	0/5
	1051594	0/5	0/8	0/5	0/8

* Bottle lot used for additional testing to determine level of NA contamination
¥ The BioFire BCID Panel detections of *Enterobacteriaceae* are reported as Enteric Bacteria by the BioFire BCID2 Panel.
† Counts represented here do not include *Proteus* if/when *Proteus* is detected.

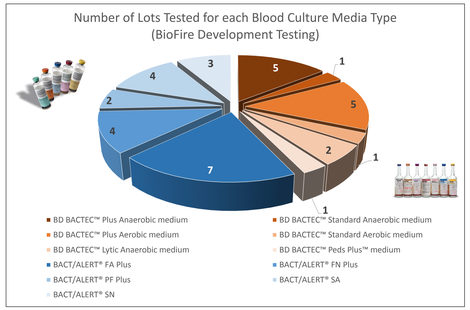


- **Overall results with the BioFire BCID Panel:** 16/175 (9%) *Proteus* and 11/175 (6%) *Enterobacteriaceae* detections; overall rate of 27/175 (15%).
- **Overall results with the BioFire BCID2 Panel:** 0/234 (0%) *Proteus* and 1/234 (0.4%) Enteric bacteria detections; overall rate of 0.4%.

- **BD media bottles** (15 lots of 6 formulations) contained detectable NA for *Proteus* at a rate of 13/75 (17%) with the BioFire BCID Panel.
- No detections in the BioFire BCID2 Panel.

- **BMX media bottles** (20 lots of 5 formulations) contained detectable NA for *Proteus* at a rate of 3/100 (3%) and 11/100 (11%) for *Enterobacteriaceae* with the BioFire BCID Panel.
- 1/135 (0.7%) Enteric bacteria with the BioFire BCID2 Panel.

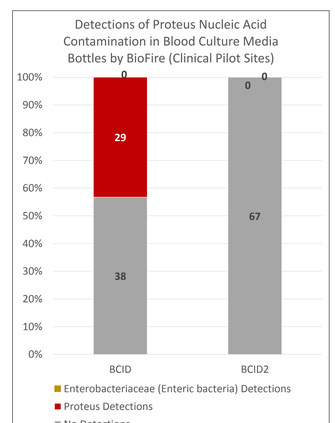
- Incubation of the blood culture media bottles tested resulted in no positive blood culture bottles.



BioFire BCID & BCID2 Panel Detections of Nucleic Acid Contamination in Blood Culture Media Bottles (clinical pilot sites)

Bottle Type	Lot ID	*Proteus* NA Contamination Detected		*Enterobacteriaceae*¥ NA Contamination Detected†	
		BioFire BCID	BioFire BCID2	BioFire BCID	BioFire BCID2
BD BACTEC™ Lytic Anaerobic medium	8024708	2/2	0/2	0/2	0/2
	8037618	2/3	0/3	0/3	0/3
	8065927	5/5	0/5	0/5	0/5
	8086905	0/2	0/2	0/2	0/2
	7353823	1/2	0/2	0/2	0/2
	8002695	4/6	0/6	0/6	0/6
	8037599	1/1	0/1	0/1	0/1
BD BACTEC™ Plus Aerobic medium	8002726	1/4	0/4	0/4	0/4
	8002731	1/1	0/1	0/1	0/1
	8011754	1/2	0/2	0/2	0/2
	8037649	0/1	0/1	0/1	0/1
	8037658	2/3	0/3	0/3	0/3
	8053767	1/6	0/6	0/6	0/6
	8053770	1/1	0/1	0/1	0/2
	8053772	1/1	0/1	0/1	0/2
	8086967	0/1	0/1	0/1	0/2
	8086981	0/2	0/2	0/2	0/3
	8114742	0/3	0/3	0/3	0/3
	8128547	0/5	0/5	0/5	0/5
	7361853	0/1	0/1	0/1	0/1
	8015962	2/2	0/2	0/2	0/2
BD BACTEC™ Plus Anaerobic medium	7332636	0/5	0/5	0/5	0/5
	7361828	4/4	0/4	0/4	0/4
	8053754	0/1	0/1	0/1	0/1
	8114710	0/3	0/3	0/3	0/3

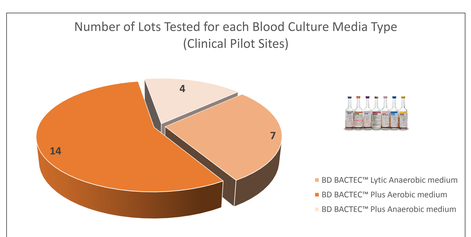
¥ The BioFire BCID Panel detections of *Enterobacteriaceae* are reported as Enteric Bacteria by the BioFire BCID2 Panel.
† Counts represented here do not include *Proteus* if/when *Proteus* is detected.



- **BD media bottles** (25 lots of 3 formulations) contained detectable NA for *Proteus* at a rate of 29/67 (43%) with the BioFire BCID Panel.
- No detections with the BioFire BCID2 Panel.

- **BMX media bottles** were not utilized by pilot sites; no data available.

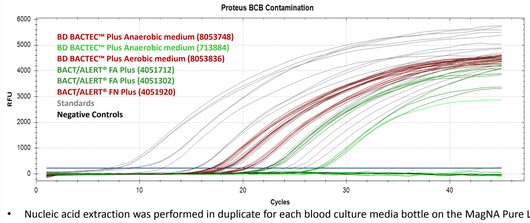
- Incubation of the blood culture media bottles tested resulted in no positive blood culture bottles.



This poster contains data regarding the BioFire BCID2 Panel which has not yet been reviewed or approved by regulatory agencies for in vitro diagnostic use.

Quantifying Proteus Nucleic Acid Present in Blood Culture Media Bottles

Blood culture media bottles that were positive for *Proteus* by BioFire were extracted and amplified using an independent PCR assay to determine the relative concentration of *Proteus* nucleic acid present in the media. Blood culture media bottles that were negative by BioFire were also tested for comparison.



- Nucleic acid extraction was performed in duplicate for each blood culture media bottle on the MagNA Pure LC 2.0 using a Total Nucleic Acid kit and High Performance protocol (200 µL input/100 µL eluate).
- Each eluate was amplified using an independent nested real-time PCR assay for *Proteus*.
- A freshly cultured stock of *Proteus mirabilis* (ATCC 35659) was quantified (OD600), serially diluted, extracted and amplified in parallel to provide a standard curve reference.

- Media bottles linked to false positive detections of *Proteus* in the BioFire BCID Panel were observed to have levels of nucleic acid corresponding to organism titers of 10^4 to 10^5 CFU/mL.
- Media bottles with no BioFire BCID Panel detections do contain low levels of *Proteus* nucleic acid.

- Previous culturing studies indicate that *Proteus* generally grows to a titer of ~$1x10^9$ CFU/mL in a positive blood culture media specimen.

Vendor & Bottle Type	Lot ID	FA BCID Detections (initial data)	Concentration (GE/mL)
BD BACTEC™ Plus Anaerobic medium	8053748	5/5	2.17E+05
			2.05E+05
	713884	0/5	Not Detected
			2.92E+02
BD BACTEC™ Plus Aerobic medium	8053836	3/5	1.00E+05
			1.16E+05
BACT/ALERT® FN Plus	4051920	3/5	2.79E+04
			2.95E+04
BACT/ALERT® FA Plus	4051712	0/5	6.37E+03
			7.49E+03
	4051302	0/5	2.98E+02
			3.49E+02

Results

- *Proteus* spp was detected in 16/175 (9%) of the additional bottles from development studies at BioFire Diagnostics with the BioFire BCID Panel compared to 0/234 detections with the BioFire BCID2 Panel.
- *Enterobacteriaceae* (Enteric bacteria) was detected in 11/175 (6%) in the additional bottles from development studies at BioFire Diagnostics with the BioFire BCID Panel as compared to 1/234 (0.4%) with the BioFire BCID2 Panel.

- *Proteus* spp was detected in 29/67 (43%) sterile media tested at pilot sites with the BioFire BCID Panel compared to 0/67 detections with the BioFire BCID2 Panel.
- No *Enterobacteriaceae* (Enteric bacteria) detections were observed at pilot sites.

- *Proteus* NA was present at levels ranging from to $1x10^2$ to $1x10^5$ GE/mL in sterile media; levels of $1x10^4$ to $1x10^5$ were linked to the detection of NA contamination.

- BioFire testing of contrived *Proteus* PBC samples correctly identified *Proteus* at 1,000-10,000-fold below PBC levels (~$1x10^9$ CFU/mL).
- The BioFire BCID2 Panel detected 8 *Proteus* true positive clinical samples confirmed by culture. Refer to ASM Microbe 2019 Poster #3661.

Conclusions

This study has demonstrated that the updated BioFire BCID2 Panel is less vulnerable to false positive detections of *Proteus* and *Enterobacteriaceae* caused by nucleic acid contamination observed in specific lots of sterile blood culture media bottles while retaining a high level of sensitivity that is capable of detecting true *Proteus* PBCs at levels several orders of magnitude below what may be expected in a true clinical sample. The unfortunate reality, however, is that raw materials used to manufacture media are derived from biological sources that have been shown to contain nucleic acid contamination which may continue to confound molecular diagnostics unless materials are screened for and qualified as nucleic acid free in the future.