Analytical Validation of the BioFire® Bone and Joint Infection (BJI) Panel: Identification of Bacteria, Yeast, and Antimicrobial Resistance Genes from Synovial Fluid

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Introduction

The BioFire Bone and Joint Infection (BJI) Panel is a sample-to-answer test for the qualitative detection of nearly 40 different bacteria, yeast, and antimicrobial resistance (AMR) genes in synovial fluid (SF)(Table 1). The panel aims to improve on current culture-based diagnostics, particularly for detection of anaerobes (e.g. *Finegoldia magna*, *Kingella kingae*, *Parvimonas micra*, *Peptoniphilus* species, and others) in about an hour. Analytical studies were conducted to establish performance characteristics of the panel, including limit of detection (LoD), analytical reactivity and specificity, interference, and reproducibility. In addition, the recommended SF specimen storage conditions were validated.

Antimicrobial Resistance Genes

CTX-M	IMP	KPC
mecA/C and MREJ (MRSA)	NDM	OXA-*48*-like
vanA/B	VIM	

Gram Positive Bacteria

Anaerococcus prevotii/vaginalis	*Clostridium perfringens*
Cutibacterium avidum/granulosum	*Enterococcus faecalis*
Enterococcus faecium	*Finegoldia magna*
Parvimonas micra	*Peptoniphilus*
Peptostreptococcus anaerobius	*Staphylococcus aureus*
Staphylococcus lugdunensis	*Streptococcus* spp.
Streptococcus agalactiae	*Streptococcus pneumoniae*
Streptococcus pyogenes	

Gram Negative Bacteria

Bacteroides fragilis	*Citrobacter*
Enterobacter cloacae complex	*Escherichia coli*
Haemophilus influenzae	*Kingella kingae*
Klebsiella aerogenes	*Klebsiella pneumoniae* group
Morganella morganii	*Neisseria gonorrhoeae*
Proteus spp.	*Pseudomonas aeruginosa*
Salmonella spp.	*Serratia marcescens*

Yeast

Candida	*Candida albicans*

Table 1. BioFire Bone and Joint Infection Panel Analytes

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Limit of Detection

LoD for each analyte was estimated from serial dilutions and confirmed with ≥95% detection in a minimum of 20 replicates prepared and tested at a known concentration in synovial fluid sample matrix (Table 2).

- LoD for gram-positive and gram-negative **bacteria** ranged from: 1.0E+02 CFU/mL - 5.0E+04 CFU/mL (5.3E+02 copies/mL -3.1E+05 copies/mL)
- LoD for **yeast** ranged from: 5.0E+02 CFU/mL - 1.0E+03 CFU/mL (copies/mL not determined (ND) for yeast)
- Each **AMR gene** was confirmed to be reliably detected at the lowest LoD of the applicable host bacteria

Detection near LoD was achieved in samples stored refrigerated for up to 7 days.

Analytical Reactivity (Inclusivity)

Analytical reactivity of each panel assay was assessed via a combination of *in silico* analysis of sequences available in public databases and testing near LoD. Over 350 isolates were prepared and tested in pooled human synovial fluid.

The diversity of isolates tested included:

- Species, subspecies, and serotypes
- AMR gene types
- Clinical isolates with known genetic variants (deletions and polymorphisms in the gene targeted by the panel assay)

Testing and sequence analysis demonstrated that with the exception of rare sequence variants:

- Single-species assays are reactive with relevant subspecies and serotypes
- Multi-species assays are reactive with the most prevalent and clinically relevant species and subspecies within the indicated genus, complex, or group.
- AMR genes are reactive with nearly all characterized AMR gene types

Assay	LoD (CFU/mL)	LoD (copies/mL)	Assay	LoD (CFU/mL)	LoD (copies/mL)
Gram Positive Bacteria			**Gram Negative Bacteria**		
Anaerococcus prevotii/vaginalis	1.0E+04	4.8E+04	***Bacteroides fragilis***	1.0E+03	1.1E+03
			Citrobacter	1.0E+03	4.7E+03
Clostridium perfringens	5.0E+02	1.3E+03	***Enterobacter cloacae* complex**	5.0E+04	1.3E+05
Cutibacterium avidum/granulosum	3.4E+04	5.0E+04	***Escherichia coli***	5.0E+02	6.0E+03
			Haemophilus influenzae	5.0E+02	6.9E+02
Enterococcus faecalis	2.1E+03	5.0E+03	***Kingella kingae***	1.0E+03	3.4E+03
Enterococcus faecium	1.0E+03	1.2E+03	***Klebsiella aerogenes***	5.0E+03	7.5E+03
Finegoldia magna	1.0E+04	3.1E+05	***Klebsiella pneumoniae* group**	1.0E+04	1.6E+04
Parvimonas micra	1.0E+03	4.8E+03	***Morganella morganii***	1.0E+03	2.2E+03
Peptoniphilus	1.1E+04	4.0E+04	***Neisseria gonorrhoeae***	1.0E+02	2.2E+03
Peptostreptococcus anaerobius	1.0E+04	1.6E+04	***Proteus* spp.**	1.0E+03	5.2E+03
Staphylococcus aureus	1.0E+02	4.2E+03	***Pseudomonas aeruginosa***	1.0E+04	1.3E+04
Staphylococcus lugdunensis	1.0E+03	2.6E+03	***Salmonella* spp.**	5.0E+02	1.6E+03
***Streptococcus* spp.**	1.0E+05	2.5E+05	***Serratia marcescens***	5.0E+03	1.1E+04
Streptococcus agalactiae	1.0E+04	1.9E+04	**Yeast**		
Streptococcus pneumoniae	1.0E+02	5.3E+02	***Candida***	1.0E+03	ND
Streptococcus pyogenes	5.0E+03	8.9E+03	***Candida albicans***	5.0E+02	ND

Table 2. Limit of Detection (LoD) for Bone and Joint Infection Analytes

Precision (Reproducibility)

The precision (reproducibility) of detection was assessed in a multi-variable study that evaluated: concentration (negative, low positive, and moderate or high positive), test site (3), test day (5), operator (2 per site), instrument/system (BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch) and kit lot (3). Positive samples included a representative subset of gram-positive and gram-negative bacteria, *Candida* yeast, and AMR genes.

For positive samples:

- Agreement with the expected detected result was 98.3% - 100%

For negative samples:

- Agreement with the expected not detected result was 99.6% - 100%

Overall, in 420 replicate tests, panel agreement with the expected detection result was 99.9% between runs, days, sites, operators, systems, and lots.

Analytical Specificity (Exclusivity)

Over 420 microorganisms were tested at high titer (>10^8 CFU/mL for bacteria, >10^6 CFU/mL for yeast, and >10^5 units/mL for viruses and parasites) to evaluate assay specificity. Isolate testing included all on-panel species as well as off-panel phylogenetic near-neighbors and other commensal, pathogenic, or environmental species (including viruses and parasites) that could be present in synovial fluid.

Few instances of cross reactivity and false-positive results were observed. Most non-specific interactions are between closely-related species, such as:

- *Anaerococcus prevotii/vaginalis*: other *Anaerococcus* species
- *Bacteroides fragilis: Bacteroides xylanisolvens*
- *Clostridium perfringens*: *Clostridium cadaveris* and *C. fallax*
- *Escherichia coli: Escherichia albertii, E. fergusonii*, and *Shigella* species
- *Staphylococcus aureus: Staphylococcus argenteus* and *S. schweitzeri*

Interference

Thirty-eight substances, including endogenous, exogenous, technique specific substances, and ten potentially competing microorganisms were evaluated at high concentration in contrived multi-analyte samples prepared in synovial fluid. No interference was observed for samples containing the substances and microorganisms listed in Table 3.

Substance Tested			
Endogenous	**Exogenous**		**Competing Microorganisms**
Blood	Acetaminophen	Clindamycin	*Streptococcus pyogenes*
Cholesterol	Salicylic Acid	Triple antibiotic ointment	*Escherichia coli*
C-reactive protein	Ibuprofen	Hydrocortisone	*Finegoldia magna*
Fibronectin	Capsaicin Cream	Hyaluronic acid	*Candida albicans*
Lactate	Salicylate Cream	Lidocaine	*Cutibacterium acnes*
Monosodium urate/ Uric Acid	Camphor Balm	Cobalt Ions	*Staphylococcus epidermidis*
Calcium phosphate	Arnica Gel	Chromium ions	*Corynebacterium striatum*
Calcium oxalate	Nystatin	Ultra-High M.W. Polyethylene	*Cryptococcus neoformans*
Bilirubin	Fluconazole	Polymethyl Methacrylate Bone	Parvovirus B19
White Blood Cells	Mupirocin	Cement	Chikungunya virus
Rheumatoid Factor	Ceftriaxone	Iohexol	
Type II collagen	Vancomycin		
	Technique Specific		
	Ethanol	Povidone-iodine	
	Bleach	K2-EDTA	

Table 3. Substances Evaluated for Interference with BJI Panel

Conclusions

The BioFire BJI Panel is a robust, accurate, and easy-to-use multiplex PCR test capable of detecting many relevant aerobic and anaerobic bacteria, yeast, and AMR genes in synovial fluid specimens. Rapid and reliable molecular detection of BJI analytes, particularly for challenging anaerobic species may aid in the timely diagnosis and effective management of bone and joint infections.

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