EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
BioFire Joint Infection (JI) Panel
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN200066

B Applicant

BioFire Diagnostics, LLC

C Proprietary and Established Names

BioFire Joint Infection (JI) Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QSN	Class II	21 CFR 866.3988 - Device to detect and identify microorganism nucleic acids and resistance markers from patients with suspected orthopedic infection	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for BioFire Joint Infection (JI) Panel

B Measurand:

Anaerococcus prevotii/vaginalis, Bacteroides fragilis, Candida spp., *Candida albicans, Citrobacter, Clostridium perfringens, Cutibacterium avidum/granulosum, Enterobacter cloacae* complex, *Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fingoldia magna, Haemophilus influenzae, Kingella kingae, Klebsiella aerogenes, Klebsiella pneumoniae* group, *Morganella morganii, Neisseria gonorrhoeae, Parvimonas micra, Peptoniphilus, Peptostreptococcus anaerobius, Proteus* spp., *Pseudomonas aeruginosa, Salmonella* spp., *Serratia marcescens,*

Staphylococcus aureus, Staphylococcus lugdunensis, Streptococcus spp., *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes,* CTX-M, IMP, KPC, NDM, OXA-48-like, VIM, *mec*A/C and MREJ.

C Type of Test:

Qualitative nucleic acid amplification assay

III Indications for Use:

A Indication(s) for Use:

The BioFire Joint Infection (JI) Panel is a multiplexed nucleic-acid-based, in vitro diagnostic test intended for use with BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems for the simultaneous qualitative detection and identification of multiple bacterial and yeast nucleic acids and select antimicrobial resistance genes from synovial fluid obtained from individuals suspected to have a joint infection.

The following organisms are identified using the BioFire JI Panel*: Anaerococcus prevotii/vaginalis, Bacteroides fragilis, Candida* spp.*, Candida albicans, Citrobacter, Clostridium perfringens, Cutibacterium avidum/granulosum, Enterobacter cloacae complex, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fingoldia magna, Haemophilus influenzae, Kingella kingae, Klebsiella aerogenes, Klebsiella pneumoniae group, Morganella morganii, Neisseria gonorrhoeae, Parvimonas micra, Peptoniphilus, Peptostreptococcus anaerobius, Proteus* spp.*, Pseudomonas aeruginosa, Salmonella* spp.*, Serratia marcescens, Staphylococcus aureus, Staphylococcus lugdunensis, Streptococcus* spp.*, Streptococcus agalactiae, Streptococcus pneumoniae, and Streptococcus pyogenes.*

The BioFire JI Panel contains assays for the detection of genetic determinants associated with *S. aureus* resistance to methicillin (mecA/C) in conjunction with the SCCmec right extremity junction (MREJ)), enterococcal resistance to vancomycin (*vanA* and *vanB*), and some mechanisms of gram-negative bacterial resistance ß-lactams including penicillins, cephalosporins, monobactams, and carbapenems ($bla_{\text{CTX-M}}$, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{\text{OXA-48-like}}$, bla_{VIM}). Detection of these genetic determinants can aid in the identification of potentially antimicrobial-resistant organisms in synovial fluid samples. The antimicrobial resistance gene or marker detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene assays do not indicate susceptibility, as multiple mechanisms of resistance to methicillin, vancomycin, and ß-lactams exist.

The BioFire JI Panel is indicated as an aid in the diagnosis of specific agents of joint infection and results should be used in conjunction with other clinical and laboratory findings. Negative results may be due to infection with pathogens that are not detected by this test, pathogens present below the limit of detection of the assay, or infection that may not be detected in a synovial fluid specimen. Positive results do not rule out co-infection with other organisms. The BioFire JI Panel is not intended to monitor treatment for joint infections.

Culture of synovial fluid is necessary to recover organisms for susceptibility testing and epidemiological typing, to identify organisms in the synovial fluid that are not detected by the BioFire JI Panel, and to further identify species in the genus, complex or group results.

B Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

C Special Instrument Requirements:

The BioFire JI Panel is performed on the FilmArray 2.0 or FilmArray Torch systems.

IV Device/System Characteristics:

A Device Description:

The BioFire Joint Infection (JI) Panel is designed to simultaneously identify 39 different bacteria, yeast, and select genetic determinants of antimicrobial resistance from synovial fluid specimens. The BioFire JI Panel is compatible with BioFire's PCR-based in vitro diagnostic BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems for infectious disease testing. A panel-specific software module (i.e., BioFire JI Panel pouch module software) is used to perform BioFire JI Panel testing on these systems.

A test is initiated by loading Hydration Solution into one port of the BioFire JI Panel pouch and the synovial fluid sample mixed with the provided Sample Buffer into the other port of the BioFire JI Panel pouch and placing it in a FilmArray instrument. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the BioFire Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

The FilmArray instrument contains a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically-controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the FilmArray performs a nested multiplex PCR that is executed in two stages. During the first stage, the FilmArray performs a single, large volume, highly

multiplexed reverse transcription PCR (rt-PCR) reaction. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green Plus, BioFire Diagnostics). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in singleplex fashion in each well of the array. At the end of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The FilmArray Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the panel.

Materials provided in each BioFire Joint Infection Panel kit:

Each kit contains sufficient reagents to test 30 samples (30-test kit; RFIT-ASY-0138):

- Individually-packaged BioFire JI Panel pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually-packaged Transfer Pipettes

Materials required but not provided:

- FilmArray system including:
  - FilmArray 2.0 or FilmArray Torch and accompanying software
  - FilmArray Pouch Loading Station
- 10% bleach solution

Interpretation of Results

When PCR2 is complete, the FilmArray instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate FilmArray Operator's Manual). The FilmArray Software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

Analysis of Melt Curves

The FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software

determines that the Tm of the curve is within the assay-specific Tm range, the melt curve is called positive. If the software determines that the Tm of the curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of Replicates

Once positive melt curves have been identified, the software evaluates the replicates for each assay to determine the assay result. For an assay to be called positive, two associated melt curves must be called positive, and both Tms must be similar. Assays that do not meet these criteria are called negative.

Organism and Antimicrobial Resistance Gene Interpretation

Each positive and negative assay result is interpreted by the FilmArray Software to provide results for the identification of specific bacteria and antimicrobial resistance (AMR) genes. For most analytes detected by the BioFire JI Panel, interpretations are based on the result of a single assay. However, results for the AMR genes require interpretation based on more than one assay result, as discussed in the relevant sections below.

Interpretations for Gram-positive Bacteria

The BioFire Joint Infection Panel provides a Detected or Not Detected result for most gram-positive bacteria based on one corresponding assay result. If the assay is positive, the test result will be Detected, and if the assay is negative, the test result will be Not Detected. Detection of organisms for which results are based on the interpretation of more than one assay are described below.

Cutibacterium avidum/granulosum

The BioFire JI Panel contains two assays (Cutibacterium1 and Cutibacterium 2) for the detection of these two *Curibacterium* species. A positive result for one or both assays will generate a *Cutibacterium avidum/granulosum* Detected test result. *Cutibacterium avidum/granulosum* will be reported as Not Detected when both assays are negative.

Staphylococcus aureus

The BioFire JI Panel contains two different assays (Saureus1 and Saureus2) for the detection of *Staphylococcus aureus*. The FilmArray Software interprets each of these assays independently (as described above) and if one or a combination of the assays is positive, the result will be *Staphylococcus aureus* Detected. If both assays are negative the result will be *Staphylococcus aureus* Not Detected.

Streptococcus spp.

The BioFire JI Panel contains four assays for the detection of *Streptococcus* species. Species-specific assays are included for the detection of *Streptococcus pyogenes* (Spyogenes), *Streptococcus agalactiae* (Sagalactiae), and *Streptococcus pneumoniae* (Spneumoniae). The fourth assay is a genus level assay (Streptococcus) designed to react with most Viridans group and other *Streptococcus* species that are not specifically identified by one of the other assays on the panel. The software integrates the results of all four *Streptococcus* assays into a *Streptococcus spp.* result as shown in the table below.

Table 1. *Streptococcus* Species Results Reporting

BioFire JI Panel Results	Streptococcus Assay	Sagalactiae Assay	Spneumoniae Assay	Spyogenes Assay	Description
Streptococcus spp Not Detected *Streptococcus agalactiae* Not Detected *Streptococcus pneumoniae* Not Detected *Streptococcus pyogenes* Not Detected	Negative	Negative	Negative	Negative	No *Streptococcus* species detected in the sample
Streptococcus spp Detected *Streptococcus agalactiae* Not Detected *Streptococcus pneumoniae* Not Detected *Streptococcus pyogenes* Not Detected	Positive	Negative	Negative	Negative	One or more *Streptococcus* species detected in the sample (not *S. agalactiae*, *S. pneumoniae*, or *S. pyogenes*)
Streptococcus spp Detected *Streptococcus agalactiae* Detected *Streptococcus pneumoniae* Not Detected *Streptococcus pyogenes* Not Detected	Any Result	Positive	Negative	Negative	*S. agalactiae* detected in the sample. Note: additional *Streptococcus* species (not *S. pneumoniae* or *S. pyogenes*) may also be in the sample.
Streptococcus spp Detected *Streptococcus agalactiae* Not Detected *Streptococcus pneumoniae* Detected *Streptococcus pyogenes* Not Detected	Any Result	Negative	Positive	Negative	*S. pneumoniae* detected in the sample. Note: additional *Streptococcus* species (not *S. agalactiae* or *S. pyogenes*) may also be in the sample.
Streptococcus spp Detected *Streptococcus agalactiae* Not Detected *Streptococcus pneumoniae* Not Detected *Streptococcus pyogenes* Detected	Any Result	Negative	Negative	Positive	*S. pyogenes* detected in the sample. Note: additional *Streptococcus* species (not *S. agalactiae* or *S. pneumoniae*) may also be in the sample.

Interpretations for Gram-negative Bacteria

The BioFire JI Panel contains assays for the specific detection of several gram-negative aerobic and anaerobic species associated with bone and joint infections. Species are identified individually (*Bacteroides fragilis, Escherichia coli, Haemophilus influenzae, Kingella kingae, Klebsiella aerogenes, Morganella morganii, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Serratia marcescens*), or as multi-species complex, group, or genus results (*Enterobacter cloacae complex, Klebsiella pneumoniae group, Citrobacter, Proteus spp., and Salmonella* spp.). Each species, complex, group, or genus result is reported as Detected or Not Detected based on an individual corresponding assay result. If the assay is positive, the result will be Detected; if the assay is negative, the result will be Not Detected.

Interpretations for Antimicrobial Resistance (AMR) Genes

The BioFire JI Panel contains assays for the specific detection of several genetic determinants of resistance to multiple classes of antibiotics found in select gram-positive bacteria (*mecA/C* and MREJ [MRSA] and *vanA/B*) or gram-negative bacteria (CTX-M, IMP, KPC, NDM, OXA-48-like, and VIM). Results for the AMR genes are not reported unless an applicable bacterium (Table 2) is also detected, therefore the results are based on multiple assays, as described below.

The results for each of the antimicrobial resistance genes will be listed as either:

- Detected – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are positive.
- Not Detected – when an applicable bacterium is detected AND the antimicrobial resistance gene assay(s) are negative.
- N/A – when all applicable bacteria are Not Detected, regardless of the result for the antimicrobial resistance gene assay(s).

Table 2: Antimicrobial Resistance (AMR) Genes and Applicable Organisms

AMR Gene Result	Applicable Bacteria
***mecA/C* and MREJ**	*Staphylococcus aureus*
vanA/B	*Enterococcus faecalis* *Enterococcus faecium*

AMR Gene Result	Applicable Bacteria
CTX-M **IMP** **KPC** **NDM** **VIM**	*Citrobacter* *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *Klebsiella pneumoniae* group *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Salmonella* spp. *Serratia marcescens*
OXA-48-like	*Citrobacter* *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *Klebsiella pneumoniae* group *Morganella morganii* *Proteus* spp. *Salmonella* spp. *Serratia marcescens*

Each AMR gene result is associated with a single corresponding assay except for the mecA/C and MREJ result, which is dependent on both the *mec*A/C assay and the MREJ assay. Detection of both *Staphylococcus aureus* and the *mec*A/C and MREJ markers is indicative of Methicillin Resistant *Staphylococcus Aureus (*MRSA).

Run Summary

The Run Summary section of the test report provides information about the sample and the run including: Sample ID, time and date of run, control results, and an overall summary of the test results. Control results are reported as Passed, Failed, or Invalid. The Table 3 below provides additional information for each of the possible control field results.

Table 3: Interpretation of Controls Field on the BioFire JI Panel Test Report

Control Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful.	None Report the results provided on the test report.
Failed	The run was completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2) failed.	Repeat the test using a new pouch. If the error persists, contact Customer Technical Support for further instruction.

Control Result	Explanation	Action
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note the Run Status field in the Run Details section of the report. Refer to the appropriate BioFire operator's manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another module, if available.

Result Summary

The Results Summary section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid. Possible results for each antimicrobial resistance gene are Detected, Not Detected, N/A, or Invalid. Table 4 below provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 4: Reporting of Results and Required Actions

Result	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were POSITIVE	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism were NEGATIVE	Report results.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	See Table 3 for instruction.
N/A (Antimicrobial Resistance Genes only)	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay(s) for the organism(s) associated with the antimicrobial resistance gene were NEGATIVE so the results of the antimicrobial resistance gene are not applicable to the test results.	Report results.

B Principle of Operation

The BioFire JI Panel pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple bacterial and/or fungal pathogens within a single synovial fluid specimen. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a FilmArray instrument, and starts a run. The entire run process takes about one hour. Additional detail can be found in the appropriate FilmArray Operator's Manual.

During a run, the FilmArray system:

- Lyses the sample by agitation (bead beading).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by:
    - First performing reverse transcription and a single, large volume, massively-multiplexed reaction (PCR1)
    - Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products
- Uses endpoint melting curve data to detect and generate a result for each target on the BioFire Joint Infection Panel array.

C Instrument Description Information

1. Instrument Name:

   FilmArray 2.0 or FilmArray Torch

2. Specimen Identification:

   Synovial fluid specimens

3. Specimen Sampling and Handling:

   Synovial fluid specimens should be tested as soon as possible after collection. If transport or storage is required, specimens can be held refrigerated for up to 7 days (2-8°C),

4. Calibration:

   N/A

5. Quality Control:

   See section VI.5 for information on internal and external controls.

V Standards/Guidance Documents Referenced:

- ISO 14971:2019 Medical devices – Applications of risk management to medical devices
- IEC 62366-1:2015, Medical device – Application of usability engineering to medical devices
- ISO 62304:2006, Medical device software – Software life-cycle processes – IEC 62304:2006, November 27, 2008
- ISO 15223-1:2016 Medical Devices – Symbols to be used with medical devie labels, labeling and information to be supplied – Part 1: General requirements
- ISO13485:2016/EN ISO 13485:2016, Medical devices – Quality Management System – Requirements for regulatory purposes
- ISO 20916:2019 In vitro diagnostic medical devices – Clinical performance studies using specimens from human subjects – Good study practice
- EN 13612:20002, Performance evaluation of in vitro diagnostic medical devices (European Commission)
- EN ISO 18113-1:2011, In vitro diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 1: Terms, definition, and general requirements
- EN ISO 18113-2:2011, In vitro diagnostic medical devices – Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use
- EN ISO 23640: 2015, In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

A multi-site reproducibility study of the BioFire JI Panel was performed with contrived synovial fluid samples over multiple days at three laboratory locations (sites) on a combination of FilmArray 2.0 and FilmArray Torch systems. Reproducibility represents the run-to-run variability of results under actual use conditions over time and is measured as agreement with the expected result. The study evaluated contrived samples containing a subset of representative organisms and AMR genes at two concentrations (and negative). The study incorporated potential variation introduced by site (three), day (five), operator (at least two per site), instrument module/system, and reagent kit lot (three). Negative results were obtained from samples that were not spiked with the organism or AMR gene.

Each of the three sites tested 20 replicates per sample and system for a total of 120 valid runs per sample and 480 valid runs overall.

A summary of results (percent (%) agreement with the expected Detected or Not Detected result) for each atypical bacterium and virus (by site and system) is provided Table 5 below.

Table 5: Reproducibility of BioFire Joint Infection Panel Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result		
			FilmArray 2.0	FilmArray Torch	All Sites [95% CI]
Gram Positive Bacteria					
Anaerococcus prevotii/vaginalis	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Clostridium perfringens	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Cutibacterium avidum/granulosum	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Enterococcus faecalis	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**238/240** 99.2%	**478/480 99.6%** [98.5%-99.9%]
Enterococcus faecium (ATCC 700221)	**Moderate Positive 3× LoD** 3.6E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 1.2E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Finegoldia magna	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Parvimonas micra	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Peptoniphilus	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Peptostreptococcus anaerobius (ATCC 27337)	**Moderate Positive 3× LoD** 4.8E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]

			Agreement with Expected Result		
	Low Positive 1× LoD 1.6E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Staphylococcus aureus (ATCC 43300)	**Moderate Positive 3× LoD** 1.3E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 4.2E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**119/120** 99.2%	**239/240 99.6%** [97.7%-99.9%]
Staphylococcus lugdunensis	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
***Streptococcus* spp.** (*Streptococcus pneumoniae;* ATCC 6303)	**Moderate Positive 3× LoD** 1.6E+03 copies/mL	**Detected**	**58/60** 96.7%	**60/60** 100%	**118/120 98.3%** [94.1%-99.8%]
	Low Positive 1× LoD 5.3E+02 copies/mL	**Detected**	**59/60** 98.3%	**59/60** 98.3%	**118/120 98.3%** [94.1%-99.8%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Streptococcus agalactiae	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Streptococcus pneumoniae (ATCC 6303)	**Moderate Positive 3× LoD** 1.6E+03 copies/mL	**Detected**	**58/60** 96.7%	**60/60** 100%	**118/120 98.3%** [94.1%-99.8%]
	Low Positive 1× LoD 5.3E+02 copies/mL	**Detected**	**59/60** 98.3%	**59/60** 98.3%	**118/120 98.3%** [94.1%-99.8%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Streptococcus pyogenes	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Gram Negative Bacteria					

			Agreement with Expected Result		
Bacteroides fragilis (ATCC 25285)	**Moderate Positive 3× LoD** 3.3E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 1.1E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Citrobacter (*Citrobacter freundii;* ATCC 8090)	**Moderate Positive 3× LoD** 1.4E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 4.7E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
***Enterobacter cloacae* complex**	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Eschericia coli AR-Bank#0150	**High Positive 30× LoD** 1.8E+05 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	High Positive 10× LoD 6.0E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Haemophilus influenzae ATCC 10211	**Moderate Positive 3× LoD** 2.1E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 6.9E+02 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Kingella kingae	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]

			Agreement with Expected Result		
Klebsiella aerogenes	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
***Klebsiella pneumoniae* group** (*Klebsiella pneumoniae;* AR-Bank#0097)	**Moderate Positive** **3× LoD** 4.8E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	Low Positive **1× LoD** 1.6E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** **100%** [98.5%-100%]
Morganella morganii	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
Neisseria gonorrhoeae ATCC 19424	**Moderate Positive** **3× LoD** 6.6E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	Low Positive **1× LoD** 2.2E+03 copies/mL	**Detected**	**59/60** 98.3%	**60/60** 100%	**119/120** **99.2%** [95.4%-99.9%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** **100%** [98.5%-100%]
***Proteus* spp.**	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
Pseudomonas aeruginosa	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
***Salmonella* spp.**	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
Serratia marcescens	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
Yeast					

			Agreement with Expected Result		
Candida (*Candida krusei*; ATCC 6258)	**Moderate Positive 3× LoD** 3.0E+03 CFU/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 1.0E+03 CFU/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**N/A**		
Candida albicans (ATCC 90028)	**Moderate Positive 3× LoD** 1.5E+03 CFU/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	Low Positive 1× LoD 5.0E+02 CFU/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120 100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
Antimicrobial Resistance Genes					

			Agreement with Expected Result		
CTX-M	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
IMP	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
KPC (*Klebsiella pneumoniae*; AR-Bank#0097)	**Moderate Positive** **3× LoD** 4.8E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	Low Positive **1× LoD** 1.6E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** **100%** [98.5%-100%]
***mecA/C* and MREJ (MRSA)** (Staphylococcus aureus; ATCC 43300)	**Moderate Positive** **3× LoD** 1.3E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	Low Positive **1× LoD** 4.2E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** **100%** [98.5%-100%]
NDM (E. coli; AR-Bank#0150)	**High Positive** **30× LoD** 1.8E+05 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	High Positive **10× LoD** 6.0E+04 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240** **100%** [98.5%-100%]
OXA-48-like	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480** **100%** [99.2%-100%]
vanA/B (*Enterococcus faecium;* ATCC 700221)	**Moderate Positive** **3× LoD** 3.6E+03 copies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]
	Low Positive **1× LoD** 1.2E+03 opies/mL	**Detected**	**60/60** 100%	**60/60** 100%	**120/120** **100%** [97.0%-100%]

			Agreement with Expected Result		
	None (No Analyte)	**Not Detected**	**120/120** 100%	**120/120** 100%	**240/240 100%** [98.5%-100%]
VIM	**None** (No Analyte)	**Not Detected**	**240/240** 100%	**240/240** 100%	**480/480 100%** [99.2%-100%]
Overall Agreement with Expected Results [95% Confidence Interval]			**18,468/18,480 99.94% [99.89-99.97]**		

2. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

   Analytical Reactivity

   Analytical reactivity of the BioFire JI Panel assays was evaluated using a combination of *in silico* analysis of sequences available in public databases and testing of over 350 different isolates representing various species, subspecies, strains, serotypes, AMR gene types, and other characterized variants. Each isolate was tested in triplicate at concentrations near LoD or the lowest reportable level for the analyte.

   Limitations on assay reactivity (observed and/or predicted by *in silico* analysis) with specific bacterial and yeast isolates or sequences and specific AMR gene types or sequences are noted (Table 6). Most limitations are associated with single-base sequence variants under one or more assay primers.

Table 6. Limitations on Analytical Reactivity of BioFire JI Panel Assays

Limitation	Analyte	Strain/Isolate Variant
Minor (Detected at ≤30X LoD)	*Anaerococcus prevottii/vaginalis*[a]	clinical isolates (private collection) with variant sequences[a]
	Enterobacter cloacae complex[b]	*Enterobacter hormaechei* ATCC 49162[b]
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa* ATCC 9027
Major (Detected at ≥100X LoD Or Not Detected)	*Candida albicans*[c]	'petite' strains (altered or no mitochondrial DNA)[c]
	Cutibacterium granulosum	clinical isolate (private collection) with variant sequence
	Enterobacter cloacae complex[b]	*Enterobacter asburiae* ATCC

		35953, ATCC35954, and ATCC 35957[b]
	Haemophilus influenzae	clinical isolate (private collection; USA 2012) with gene target deletion
	Klebsiella aerogenes	*Klebsiella (Enterobacter) aerogenes* ATCC 29751
	Neisseria gonorrhoeae	*Neisseria gonorrhoeae* NCTC 13817 (strain WHO-U)
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa* ATCC 25619
	Streptococcus pyogenes	clinical isolate (private collection; USA 2019) with gene target deletion or re-arrangement
	AMR Gene Types	
	CTX-M	CTX-M types 74, 75, 113, 151
	IMP	IMP types 31, 35, 46
	mecA/C and MREJ[d,e]	MREJ type xv[d], xviii[e], xix[e], xx[e]
	VIM	VIM types 7, 39, 45, 46, 61, 65, 67
	Rare or Non-relevant Species	
	Candida spp.	several *Candida* species; see **Error! Reference source not found.**
	Citrobacter	*Citrobacter almonaticus*, *C. farmeri*, *C.gillenii*, *C. rodentium*, *C. sedlakii*
	Peptoniphilus spp.	*Peptoniphilus coxii*, *P. duerdenii*, *P. ivorii*, *P. koenoeneniae*, *P. massiliensis*[f], *P. porci*, *P. olsenii*, *P. tyrelliase*
	Streptococcus spp.	*Streptococcus entericus*, *S.halitosis*, *S. hyovaginalis*, *S. pantholopis*

[a] Detection near LoD was impaired for four isolates of *A. vaginalis*. Sequencing revealed primer mismatches predicted to impair detection. Comparable sequence variants were observed in two *A. vaginalis* sequences retrieved from public databases. A limitation on reactivity is predicted for approximately 25% of *A. prevotii/vaginalis* sequences and isolates evaluated.

[b] Reactivity limitations observed or predicted for sequence variants identified for *E. hormaechei* ATCC 49162, *E. asburiae* ATCC 35953 (tested), ATCC 35954 (not tested), ATCC 35955 (not tested), and a small subset of database sequences for *E. cloacae*, *E. hormaechei*, *E. ludwigii* and *E. mori* with similar or less impactful variants under assay primers. Variant sequences with major or minor reactivity limitations represent less than 2% of sequences for ECC species.

[c] Petite strains of *Candida albicans* will not be detected by the Candida albicans-specific assay but will be amplified by the multi-species Candida assay and reported as *Candida* Detected.

[d] Sequence analysis predicts that approximately 40% of MREJ type xv-like sequences will not be detected due to a variant base at the 3' end of an assay primer.

[e] MREJ types xviii, xix and xx will not be detected. MREJ types xix and xx are described in association with methicillin-sensitive isolates, so the *mecA/C* and MREJ (MRSA) Not Detected result will be consistent with the methicillin-sensitive phenotype of isolates with these MREJ types.

[f] Not a validly published *Peptoniphilus* species.

Table 7: *Anaerococcus prevotii/vaginalis* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Anaerococcus	ATCC 9321	(b)(4)	1x	*Anaerococcus prevotii/vaginalis* Detected[a]

Organism	Isolate ID (Strain)	Test Concentration		Result
prevotii	(PC 1)			
	ATCC 14952 (M3)	(b)(4)	3x	
	CCUG 72601	(b)(4)	3x	
	VTK 400239	(b)(4)	3x	
Anaerococcus vaginalis	ATCC 51170 (GIFU 12669)	4.8E+04	1x	
	DSM 25446 (ph9)	(b)(4)	3x	
	GRE 1654021	(b)(4)	3x	
	GRE 1554051[a]	(b)(4)	30x	
	GRE 1653021	(b)(4)	10x	
	GRE 1757298[a]	(b)(4)	10x	
	VTK 401665[a]	(b)(4)	30x	
	VTK 401672[a]	(b)(4)	10x	

[a]Variant sequence with 3' base mismatch to a primer that may impair detection near LoD (10 to 30-fold). Variant sequences with minor detection impairment represent ~25% of total *A. prevotii/vaginalis* sequences evaluated.

Table 8: *Clostridium perfringens* Isolates Tested

Organism	Toxinotype	Isolate ID	Test Concentration		Result
			(copies/mL)	X LoD	
Clostridium perfringens	A	ATCC 13124 (S 107)	(b)(4)	1x	*Clostridium perfringens* Detected
		ATCC 27059 (814)		3x	
	C	ATCC 3628 (Strain 51)		3x	
	E	ATCC 8009		3x	
	-	ATCC 9081 (13942)		3x	

Table 9: *Cutibacterium avidum/granulosum* Isolates Tested

Organism	Isolate ID	Test Concentration		Result
		(copies/mL)	X LoD	
Cutibacterium avidum	ATCC 25577 (1689B, VPI 0179)	(b)(4)	1x	*Cutibacterium* Detected
	ATCC 49753 (VPI 0575)		3x	
	ATCC 49754 (VPI 0576)		10x	
	ATCC 49755 (VPI 0589)		3x	
	ATCC 49769 (VPI 0670)		10x	
Cutibacterium granulosum	ATCC 25564 (VPI 0507)		1x	*Cutibacterium* Detected
	ATCC 11829 (VPI 0210)		3x	
	ATCC 25746 (D-34)		3x	
	CCUB 14831 (Serovar 3)		3x	
	GRE 1554046		3x	
	(b)(4)		10x	
	GRE 1760015[a]		100x	*Cutibacterium* Not Detected

[a]Sequences with predicted impacts on reactivity by in silico analysis

Table 10: *Enterococcus faecalis* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	X LoD	
Enterococcus faecalis	ATCC 51299 (NJ-3)	5.0E+03	1x	*Enterococcus faecalis* Detected
	ATCC 19433 (Tissier)	(b)(4)	3x	
	ATCC 49533 (UWH 1936)		3x	
	ATCC 700802 (V583)		3x	
	ATCC BAA-2573 (bMx 0502240)		3x	
	JMI 12536		3x	

Table 11: *Enterococcus faecium* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Enterococcus faecium	ATCC 700221	1.2E+03	1x	*Enterococcus faecium* Detected
	ATCC 19434 (Grumbach serotype 11)	(b)(4)	3x	
	ATCC 27270 (X3)	(b)(4)	3x	
	ATCC 51858 (Vancomycin-dependent #4)	(b)(4)	3x	
	ATCC BAA-2318	(b)(4)	3x	
	JMI 475	(b)(4)	3x	

Table 12: *Finegoldia magna* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Reported Result
		(copies/mL)	X LoD	
Finegoldia magna	ATCC 15794 (2974)	(b)(4)	1x	*Finegoldia magna* Detected
	ATCC 14955 (BU)		3x	
	ATCC 29328 (WAL2508)		3x	
	ATCC 53516 (312)		3x	
	DSM 20362 (168)		3x	
	GRE 1556006		3x	

Table 13: *Parvimonas micra* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Parvimonas micra	ATCC 33270 (3024A)	4.8E+03	1x	*Parvimonas micra* Detected
	CCUG 56809	(b)(4)	3x	
	CCUG 57049		3x	
	GRE 1651163		3x	
	GRE 1757098		3x	

Table 14: *Peptoniphilus* spp. Isolates Tested

Species	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Peptoniphilus assacharolyticus	ATCC 14963 (BAI, UW 228)	4.0E+04	1x	*Peptoniphilus* Detected
	ATCC 29743 (WAL 3218)	(b)(4)	3x	
Peptoniphilus allenii[a]	ATCC BAA-1643 (WAL 1768N)		3x	
Peptoniphilus gorbachii	ATCC BAA-1383 (WAL 10418)]		3x	
Peptoniphilus grossensis[a]	DSM 25475 (ph5)		3x	

Peptoniphilus harei	ATCC BAA-601 (SBH 432)	(b)(4)	3x	
	DSM 10021 (SBH 064)		3x	
	GRE 1554070		3x	
Peptoniphilus indolicus	ATCC 29427 (R13)		3x	
	GRE 1556024		3x	
Peptoniphilus lacrimalis	ATCC 51171 (GIFU 7667)		3x	
	CCUG 47146		3x	
Peptoniphilus senegalensis	DSM 25694 (JC140)		3x	
Peptoniphilus tyrrelliae[b]	CCUG 59621 (RMA 19911)		High	
Peptoniphilus koenoeneniae[b]	ATCC BAA-1638 (WAL 20371)		High	
Peptoniphilus coxii	ATCC BAA-2106 (RMA 16757)		High	
Peptoniphilus duerdenii	ATCC BAA-1640 (WAL1998L)		High	*Peptoniphilus* Not Detected
Peptoniphilus ivorii	ATCC BAA-602 (SBH 093)		High	
Peptoniphilus massiliensis[a]	ATCC BAA-1641 (WAL 18041)		High	
Peptoniphilus olsenii	ATCC BAA-1384 (WAL 12922)		High	
Peptoniphilus porci	*In silico* prediction (not tested)			
Other *Peptoniphilus* species	Unknown Reactivity (no sequences/not tested)			

[a]Isolates tested were characterized by the culture collection as *P. allenii*, *P. grossensis*, and *P. massiliensis*, though none are currently validly published *Peptoniphilus* species.
[b]*Peptoniphilus koenoeneniae* and *Peptoniphilus tyrelliae* were detected at a concentration >100x LoD.

Table 15: *Peptostreptococcus anaerobius* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Peptostreptococcus anaerobius	ACC 27337 (A prevot 4372)	1.6E+04	1x	*Peptostreptococcus anaerobius* Detected
	ATCC 49031 (MSHD)	(b)(4)	3x	
	CCUG 37992		3x	
	CCUG 38379		3x	
	CCUG 46594 (GIFU 7800)		3x	

Table 16: *Staphylococcus aureus* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Staphylococcus aureus	ATCC BAA-2313 (M10/0148)	(b)(4)	1x	*S. aureus* Detected
	ATCC BAA-2312 (M10/0061)		3x	
	ATCC BAA-1700 (HFH-33798)		3x	
	ATCC BAA-1707 (MW2)		3x	
	ATCC BAA-1749 (96:308)		3x	
	ATCC BAA-1759 (N7129)		3x	

	ATCC BAA-1764 (7031)	(b)(4)	3x	
	ATCC BAA-1765 (102-04)		3x	
	NARSA NRS662 (CO-34)		3x	
	NARSA NRS683 (GA-298)		3x	
	NARSA NRS689 (GA-442)		3x	
	NARSA NRS691 (GA-62)		3x	
	NARSA NRS701 (MN-082)		3x	
	NARSA NRS705 (NY-12)		3x	
	NARSA NRS707 (NY-155)		3x	
	NARSA NRS745 (CA-629)		3x	
	BEI NR-46081 (HIP12899)		3x	
	GRE 0860042		3x	
	NARSA NRS648 (CA-347)		3x	
	SHSC Sun1		3x	
Staphylococcus aureus ssp. *aureus*	ATCC 4330 (F-182)	4.2E+03	1x	
	ATCC 12600	(b)(4)	3x	
	ATCC 14154		3x	
	ATCC 25923 (Seattle 1945)		3x	
	ATCC BAA-39		3x	
	ATCC BAA-42 (HDE288)		3x	
	ATCC BAA-44 (HPV107)		3x	
	ATCC BAA-1717 (TCH1516)		3x	
	ATCC BAA-1720 (MRSA252)		3x	
Staphylococcus aureus ssp. *anaerobius*	ATCC 35844 (MVF-7)		3x	

Table 17: *Staphylococcus lugdunensis* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Staphylococcus lugdunensis	ATCC 43809 (N860297)	2.6E+03	1x	*S. aureus* Detected
	ATCC 49576 (LRA 260.05.09)	(b)(4)	3x	
	ATCC 700582 (7829)		3x	
	NCTC 7990 (Kelly)		3x	
	ATCC 700328 (6733)		3x	

Table 18: Isolates *Streptococcus* spp. Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Streptococcus agalactiae	See *S. agalactiae* table			
Streptococcus dysgalactiae ssp. *dysgalactiae*	ATCC 43078 (NCDO 2023)	(b)(4)	3x	*Streptococcus spp.* Detected
Streptococcus dysgalactiae ssp. *equisimilis*	ATCC 8543 (LRA 06 11 76)		3x	
Streptococcus bovis/equinus	ATCC 33317 (Pearl 11 NCDO 597)		3x	
	ATCC 9812 (H 12 B)		3x	
Streptococcus gallolyticus ssp. *gallolyticus*	DSM 16831		3x	

Streptococcus gallolyticus ssp. *pasteurianus*	ATCC 7000338 (RG)	(b)(4)	3x
Streptococcus infantarius ssp. *infantarius*	ATCC BAA-102 (NCIMB 700599)		3x
Streptococcus anginosus	ATCC 33397 (Havill III R. Lancefield F68A)		3x
Streptococcus constellatus	ATCC 27513 (VPI 7712)		3x
Streptococcus intermedius	ATCC 27335 (VPI 3372A)		3x
Streptococcus salivarius	ATCC 13419 (C699 [S30D])		3x
Streptococcus salivarius ssp. *thermophilus*	ATCC 19258 (NCDO 573)		3x
Streptococcus vestibularis	ATCC 49124 (MM1)		3x
Streptococcus australis	ATCC 700641 (AI-1)		3x
Streptococcus sobrinus[a]	ATCC 33478 (SL1)		3x
Streptococcus mutans	ATCC25175 (IFO 13955)		1x
Streptococcus gordonii	ATCC 10558 (SK3)		3x
Streptococcus mitis	ATCC 49456 (NS 51: SK142)		3x
Streptococcus oralis[a]	ATCC 35037 (PB182; LVG/1)		3x
Streptococcus oralis ssp. *tigurinus*	DSM 24864 (AZ_3a)		3x
Streptococcus pneumoniae	See *S. pneumoniae* table		
Streptococcus pseudopneumoniae	ATCC BAA-960 (CDC-SS-1757)	(b)(4)	3x
Streptococcus pyogenes	See *S. pyogenes* table		
Streptococcus sanguinis	ATCC 10556 (DSS-10)	(b)(4)	3x
Streptococcus cristatus	ATCC 51100 (CR311)		3x
Streptococcus parasanguinis	ATCC 15912 (SS 898)		3x
Streptococcus peroris	ATCC 700780 (GTC 848)		3x
Streptococcus equi ssp. *equi*[b]	ATCC 33398 (C15)		3x
Streptococcus equi ssp. *zooepidemicus*[b]	ATCC 43079 (NCDO 1358)		3x
Streptococcus suis[a]	ATCC 43765 (735)		3x
Streptococcus sinensis	DSM 14990 (HKU4)		3x

[a]*In silico* analysis identified sequence variation that is predicted to impact reactivity in approximately 8% of *S. oralis* sequences evaluated and in approximately 2% of the *S. sobrinus, S suis*, and *S. uberis* sequences evaluated.
[b]Although the two isolates of *S. equi* tested were detected near LoD, in silico analysis predicts some impairment of detection for most (97%) *S. equi* sequences.

Table 19. *Streptococcus* species Isolates Predicted Reactivity (*In silico*)

Organism	Result
Streptococcus acidominimus	*Streptococcus spp.* Detected
Streptococcus azizii	
Streptococcus bovimastitidis	
Streptococcus caballi	
Streptococcus canis	
Streptococcus castoreus	
Streptococcus criceti	

Streptococcus cuniculi	
Streptococcus devriesei	
Streptococcus didelphis	
Streptococcus downei	
Streptococcus ferus	
Streptococcus halotolerans	
Streptococcus henryi	
Streptococcus himalayensis	
Streptococcus hongkongensis	
Streptococcus hyointestinalis	
Streptococcus ictaluri	
Streptococcus infantis	
Streptococcus iniae	
Streptococcus intermedius	
Streptococcus lactarius	
Streptococcus lutetiensis	
Streptococcus macacae	
Streptococcus marimammalium	
Streptococcus marmotae	
Streptococcus massiliensis	
Streptococcus merionis	
Streptococcus milleri	
Streptococcus minor[a]	
Streptococcus orisasini	
Streptococcus orisratti	
Streptococcus ovis	
Streptococcus parasuis	
Streptococcus parauberis	
Streptococcus penaeicida	
Streptococcus phocae	
Streptococcus pluranimalium	
Streptococcus plurextorum	
Streptococcus porci	
Streptococcus porcinus	
Streptococcus pseudoporcinus	
Streptococcus ratti	
Streptococcus respiraculi	
Streptococcus ruminantium	
Streptococcus thoraltensis	
Streptococcus troglodytae	
Streptococcus uberis[b]	
Streptococcus urinalis	
Streptococcus entericus	
Streptococcus halitosis	
Streptococcus hyovaginalis	
Streptococcus pantholopis	

[a]*In silico* analysis identified 1/2 (50%) *S. minor* sequences with sequence variation that is predicted to impact reactivity
[b]*In silico* analysis identified sequence variation that is predicted to impact reactivity in approximately 8% of *S. oralis* sequences evaluated and in approximately 2% of the S. *sobrinus, S suis*, and *S. uberis* sequences evaluated.

Table 20: *Streptococcus agalactiae* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Streptococcus agalactiae	ATCC 13813 (G19)	1.9E+04	1x	*Streptococcus agalactiae* Detected
	ATCC 12403 [D136C(3)]	(b)(4)	3x	
	ATCC BAA-2669 (5030-08)		3x	
	CI 2460		3x	
	ATCC BAA-611 (2603 V/R)		3x	
	ATCC 12386 (090R)		3x	

Table 21: *Streptococcus pneumoniae* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Streptococcus pneumoniae	ATCC 6303 (CIP 104225)	5.3E+02	1x	*Streptococcus pneumoniae* Detected
	ATCC 33400 (SV1)	(b)(4)	3x	
	ATCC 700672 (VH14)		3x	
	ATCC 700673 (19A-6 [HUN663])		3x	
	ATCC BAA-1409 (62076)		3x	
	ATCC BAA-341 (SPN1439-106)		3x	

Table 22: *Streptococcus pyogenes* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Streptococcus pyogenes	ATCC 19615 (Bruno)	(b)(4)	1x	*Streptococcus pyogenes* Detected
	ATCC 49399 (QC A62)		3x	
	ATCC 12344 (T1)		3x	
	ATCC 12348 (S43)		3x	
	ATCC 700294 (SF370;M1 GAS)		3x	
	ATCC BAA-947 (MGAS 5005)		3x	
	ATCC 12384 (C203)		3x	
	P-03-0543 804 ISO[a]		100x	*Streptococcus pyogenes* Not Detected

[a]Isolate was from a clinical specimen; a deletion in the gene target was identified that prevents amplification/detection

Table 23: *Bacteroides fragilis* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	

Bacteroides fragilis	ATCC 25285 (VPI 2553)	1.1E+03	1x	*Bacteroides fragilis* Detected
	ATCC BAA-2283 (2-1-56 FAA)	(b)(4)	3x	
	ATCC 29768 (12256/P8)		3x	
	ATCC 29771 (2044 [CDC 1261; M-488])		3x	
	ATCC 43937 (F1355 [WAL 78-189A])		3x	

Table 24: *Citrobacter* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Citrobacter braakii	ATCC 51113 (CDC 80-58)	(b)(4)	3x	*Citrobacter* Detected
Citrobacter europaeus	GRE 1953016		3x	
Citrobacter freundii	ATCC 8090 (ATCC 13316)	4.7E+03	1x	
	ATCC 43864 (LRA 117.03.76)	(b)(4)	3x	
	AR Bank #0116		3x	
	AR Bank #0157		3x	
	GRE 1062177		3x	
Citrobacter koseri	ATCC 27156 (CDC 3613-63)		3x	
	ATCC 27028 (14804)		3x	
Citrobacter murliniae	ATCC 51118 (CDC 2970-59)		3x	
Citrobacter werkmanii[a]	ATCC 51114 (CDC 0876-58)		3x	
Citrobacter youngae	ATCC 29935 (460-61)		3x	
Citrobacter amalonaticus	ATCC 25405 (9823)		High	*Citrobacter* Not Detected
Citrobacter farmeri	ATCC 51112 (CDC 2991-81)		High	
Citrobacter gillenii	ATCC 51117 (CDC 4693-86)		High	
Citrobacter rodentium	GRE 1654045		High	
Citrobacter sedlakii	ATCC 51494		High	
Citrobacter cronae	Unknown Reactivity (no sequence/not tested)			

[a]*In silico* analysis identified sequence variation that is predicted to impact reactivity in 4/6 (50%) *C. werkmanii* sequences

Table 25. Citrobacter Reactivity Predicted (*in silico*)

Organism	Result
Citrobacter pasteurii	*Citrobacter* Detected
Citrobacter portucalensis	

Table 26: *Enterobacter cloacae* complex Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Enterobacter asburiae[a]	GRE 1753006	(b)(4)	3x	*Enterobacter cloacae* Detected
	ATCC 35953 (CDC 1497-78)		100x	*Enterobacter cloacae*

				Not Detected
Enterobacter cloacae[a]	AR Bank #0154	1.3E+05	1x	*Enterobacter cloacae* Detected
	NCTC 13464	(b)(4)	3x	
Enterobacter cloacae ssp. *cloacae*[a]	ATCC 13047 (CDC 442-68)		3x	
	ATCC 222 (CDC 435)		3x	
Enterobacter cloacae ssp. *dissolvens*[a]	ATCC 23373D-5 gDNA (ICPB ED105)		3x	
Enterobacter hormaechei	ATCC BAA-2082		3x	
	ATCC 700323		3x	
Enterobacter hormaechei ssp. *hormaechei*	ATCC 49162		100x	
Enterobacter hormaechei ssp. *oharea*	CCUG 53905T		3x	
Enterobacter hormaechei ssp. *steigerwaltii*	CCUG 53904T		3x	
Enterobacter hormaechei ssp. *xiangfangensis*	DSM 46348		3x	
Enterobacter kobei	GRE 1753004		3x	
Enterobacter ludwigii[a]	DSM 16688 (EN-119)		3x	
	CCUG 23050		3x	
Enterobacter mori	DSM 26271 (R18-2)		3x	
Enterobacter roggenkampii	DSM 16690 (EN-117)		3x	

[a]*Enterobacter asburiae* isolate ATCC 35953 has sequence variation under assay primers that impairs detection at 100x LoD and lower. A similar impact on reactivity is predicted for 6/76 (7.9%) *Enterobacter asburiae* sequences evaluated and impaired amplification and detection is also predicted for a subset of *E. cloacae* (8/516, 1.6%) and *E. ludwigii* (2/25, 8.0%) sequences.

[b]*E. hormaechei* ssp. *hormaechei* isolate ATCC 49162 has sequence variation under assay primers that impairs detection at 10x LoD and lower. A similar impact on reactivity is predicted for 10/685 (1.4%) *E. hormaechei* and 1/8 (12.5%) *E. mori* sequences evaluated.

Table 27: *E. coli* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
E. coli	CDC AR Bank #0150	(b)(4)	1x	*E. coli* Detected
	CDC AR Bank #0137		3x	
	CDC AR Bank #0162		3x	
	CDC AR Bank #0086		3x	
	CDC AR Bank #0061		3x	
	ATCC 11775 (9001 U 5/41)		3x	
	GRE 1256018		3x	
	Zeptometrix 0801905 (Z136)		3x	

Table 28: *Haemophilus influenzae* Isolates Tested

Organism	Serotype	Biotype	Isolate ID (Strain)	Test Concentration		Result
				(copies/mL)	xLoD	
Haemophilus influenzae	b	I	ATCC 10211 (AMC 36-A-1 [572])	(b)(4)	1x	*Haemophilus influenzae*
	a		ATCC 9006 (AMC 36-A-3 [610, PCM 2436])		3x	

	c	-	ATCC 49699 (C 9007)	(b)(4)	3x	Detected
	d		ATCC 9008 (AMC 36-A-6 [611])		3x	
	e		ATCC 8142 (595 Murray Biotype IV: AMC 36-A-7 [595])		3x	
	f		ATCC 700223 (GA 1264)		3x	
	Non-typeable	II	ATCC 33391 (680 Biotype II)		3x	
		V	ATCC 51997 (INT 1 Biotype V)		3x	
	Unknown	-	BF Clinical Isolate 006433-PBC-1-0029-ISO-1[a]		High	*Haemophilus influenzae* Not Detected

[a]Isolate was obtained from a clinical specimen; a deletion in the gene target was identified that prevents amplification/detection.

Table 29: *Kingella kingae* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Kingella kingae	ATCC 23330 (4177/66)	3.4E+03	1x	*Kingella Kingae* Detected
	ATCC 23331	(b)(4)	3x	
	CCUG 63569		3x	
	CCUG 50167A		3x	
	CCUG 44801		3x	

Table 30: *Klebsiella aerogenes* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Klebsiella aerogenes	AR Bank #0074	7.5E+03	1x	*Kingella Kingae* Detected
	AR Bank #0062	(b)(4)	3x	
	AR Bank #0161		3x	
	ATCC 13048 (NCDC 819-56)		3x	
	ATCC 29751 (MULB-250)[a]		100x	*Kingella Kingae* Not Detected
	GRE 1254066		3x	*Kingella Kingae* Detected

[a]Klebsiella aerogenes isolate ATCC 29751 has sequence variation under the assay primers that impairs detection at 100x LoD and lower. A similar impact on reactivity is predicted for 9/193 (4.7%) *Klebsiella aerogenes* sequences evaluated.

Table 31: *Klebsiella pneumoniae* group Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Klebsiella pneumoniae	AR Bank #0097	1.6E+04	1x	*Klebsiella pneumoniae* Detected
	AR Bank #0079	(b)(4)	3x	
	AR Bank #0107		3x	
	AR Bank #0075		3x	

	JMI 766	(b)(4)	3x	
	AR Bank #0040		3x	
	AR Bank #0068		1.9x	
	AR Bank #0080		2.5x	
Klebsiella pneumoniae ssp. *ozaenae*	ATCC 11296		3x	
	AR Bank #0051		3x	
Klebsiella pneumoniae ssp. *pneumoniae*	ATCC 13883		3x	
Klebsiella pneumoniae ssp. *rhinosclermatis*	ATCC 13884 (R-70)		3x	
Klebsiella quasipneumoniae	DSM 28211		3x	
Klebsiella quasipneumoniae ssp. *similipneumoniae*	DSM 28212		3x	
Klebsiella variicola	ATCC BAA-830 (F2R9)		3x	

Table 32: *Morganella morganii* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Morganella morganii	AR Bank #0057	(b)(4)	3x	*Morganella morganii* Detected
Morganella morganii ssp. *morganii*	ATCC 25830 (M11)	2.2E+03	1x	
	ATCC 33791 (Potter)	(b)(4)	3x	
Morganella morganii ssp. *sibonii*	ATCC 49948 (CDC 9103-85)		3x	
	ATCC51207 (CDC 8246-91)		3x	

Table 33: *Neisseria gonorrhoeae* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Neisseria gonorrhoeae	ATCC 19424 (B 5025)	(b)(4)	1x	*Neisseria gonorrhoeae* Detected
	NCTC 6820 (Gono 4)		3x	
	ATCC 19088 (CH-6)		3x	
	ATCC 700825 (FA1090)		3x	
	Zeptometrix 0801482 (Z017)		3x	
	NCTC 13817[a]		High	*Neisseria gonorrhoeae* Not Detected

[a]Isolate (also described as WHO-U strain) carries an atypical variant of the gene target (suspected horizontal transfer with homologous gene in *N. meningitidis*) that is not amplified/detected by the assay.

Table 34: *Proteus* spp.[a] Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Proteus salimentorum	*In silico* prediction (not tested)			*Proteus* spp. Detected
Proteus columbae	*In silico* prediction (not tested)			
Proteus hauseri	ATCC 13315 (Lehmann)	(b)(4)	3x	
	ATCC 700826 (CDC 1732-80)		3x	
Proteus mirabilis	ATCC 35659 (LRA 08 01 73)	5.2E+03	1x	

	ATCC 29906 (CDC PR 14)	(b)(4)	3x	
	AR Bank #0156		10x	
	AR Bank #0159		10x	
	GRE1254053		3x	
Proteus penneri	ATCC 33519 (CDC 1808-73)		3x	
	ATCC 35197 (CDC 1655-67)		3x	
Proteus terrae	DSM 29910 (N5/687)		3x	
Proteus terrae ssp. cibarius	DSM 100173 (JS9)		3x	
Proteus vulgaris	ATCC 29905 (CDC PR1)		3x	
	ATCC 27973 (CDC 1787-64-SC1)		3x	

[a]The *Proteus* genus now also includes the species *P. cibi* and *P. faecis*. Reactivity with these species has not been evaluated.

Table 35: *Pseudomonas aeruginosa* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Pseudomonas aeruginosa	CDC AR Bank #0092	1.30E+04	1x	*Pseudomonas aeruginosa* Detected
	ATCC 27853 (Boston 41501)	(b)(4)	3x	
	CDC AR Bank #0090		3x	
	CDC AR Bank #0100		3x	
	CDC AR Bank #0054		3x	
	CUSM PS28		3x	
	NCTC 13437		3x	
	CDC AR Bank #0111		3x	
	CDC AR Bank #0064		3x	
	CDC AR Bank #0103		10x	
	ATCC 9027[a]		100x	
	ATCC 25619[b]		High	*Pseudomonas aeruginosa* Not Detected

[a]*Pseudomonas aeruginosa* isolate ATCC 9027 has sequence variation under assay primers that impairs detection at 10× LoD and lower. Detection was observed in all replicates at 100× LoD (~1.3E+06 copies/mL). Similar impacts on reactivity are predicted for approximately 50/1524 (3.3%) *P. aeruginosa* sequences evaluated.
[b]*Pseudomonas aeruginosa* isolate ATCC 25619 has sequence variation under assay primers that prevents amplification and detection.

Table 36: *Salmonella* spp. Isolates Tested

Organism	Isolate ID (Strain)	Serovar	Test Concentration		Result
			(copies/mL)	xLoD	
Salmonella bongori	SGSC 3100/SarC11 (RKS3041)	-	(b)(4)	3x	*Salmonella* spp. Detected
	NCTC 10946 (BR 1859 66:z41:-)	Brookfield		3x	
	ATCC 43975 (1224.72)	-		3x	
Salmonella enterica ssp. *arizonae*	ATCC 13314 (DC5.CIP 8230)	-		3x	
Salmonella enterica ssp. *diarizonae*	SGSC 3069 (RKS2979; SarC8)	-		3x	
Salmonella enterica ssp. *enterica*	CDC AR Bank#0407	Concord	1.6E+03	1x	
	ATCC 700720 (LT2)	Typhimirium	(b)(4)	3x	

	ATCC BAA-708	Enteritidis	(b)(4)	3x	
	SGSC 2210 (SARA30)	Heidelberg		3x	
	ATCC BAA-710 (G4639)	Montevideo		3x	
	CDC AR Bank #0127	Senftenberg		3x	
	ATCC 700931D-5 (Ty2)	Typhi		3x	
Salmonella enterica ssp. *houtenae*	SGSC 3074 (RKS3015)	-		3x	
Salmonella enterica ssp. *indica*	SGSC 3116 (RKS2995)	-		10x	
Salmonella enterica ssp. *salamae*	SGSC 3047 (RKS2993)	-		3x	

Table 37: *Serratia marcescens* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Serratia marcescens	API 1411137	(b)(4)	3x	*Serratia marcescens*. Detected
	API 1512393		3x	
	CDC AR Bank #0091		3x	
	JMI 697		3x	
Serratia marcescens ssp. *marcescens*	ATCC 13880 (BS 303)	1.1E+04	1x	
Serratia marcescens ssp. *sakuensis*	ATCC BAA-885 (KRED)	(b)(4)	3x	

Table 38: *Candida* spp. Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Candida albicans	See Table 41			
Candida dubliniensis	ATCC MYA-646 (CBS 7987)	(b)(4)	3x	*Candida* spp. Detected
	ATCC MYA-578 (H12)		3x	
Candida glabrata	ATCC 15545 (NRRL YB-4025)		3x	
	ATCC 2001 (CBS 138)		3x	
	CI-953		3x	
Candida krusei (*Issatchenkia orientalis*)	ATCC 6258	1.0E+03	1x	
	ATCC 28870 (CBS 2052)	(b)(4)	3x	
Candida orthopsilosis	ATCC 96139 (MCO457)		3x	
Candida parapsilosis	ATCC 28475 (CBS 2915)		3x	
	ATCC 22019 (CBS 604)		3x	
Candida tropicalis	ATCC 750		3x	
	ATCC 66029 (AmMS 227)		3x	
Candida metapsilosis	ATCC96143 (MCO429)		10x	
Candida sojae	NRRL Y-17909		10x	
Candida sphaerica	GRE 1951001		100x	
Candida inconspicua	ATCC 16783 (CBS 180)		100x	
Candida auris	AR Bank #0381		100x	*Candida* spp. Not Detected
	AR Bank #0385		100x	
	GRE 1756004		8000x	*Candida* spp. Detected

Candida lusitaniae	ATCC 42720 (45090)	(b)(4)	100x	*Candida* spp. Not Detected
	ATCC 34449 (IFO 1019)		8000x	*Candida* spp. Detected
Candida nivariensis	CCUG 56432		795,000x	
Candida Intermedia	ATCC 14439		8000x	
Candida kefyr	ATCC 204093		8000x	
Candida norvegensis	GRE 0856055		8000x	
Candida utilis	ATCC 22023		8000x	
Candida haemolunii[a]	AR Bank #0393		389,000x	
Candida viswanathii	ATCC 22981		430,000x	
Candida guilliermondii	ATCC 38290 (Tu 62304-2)		570,000x	*Candida* spp. Not Detected
Candida ciferrii	ATCC 584433 (CBS 5295)		8000x	
Candida colliculosa	ATCC 10662 (NRRL Y-866)		8000x	
Candida holmii	DSM 70627		8000x	
Candida lipolytica	ATCC 18944 (NRRL YB-423-12)		8000x	
Candida rugosa	ATCC 10571 (NRRL Y-1496)		8000x	
Candida thermophila	ATCC 58401		8000x	
Candida famata	ATCC 4144 (D.R. 1658 No. 14)		893,000x	

[a]Species may be detected at high concentration (>100x LoD).

Table 39: *Candida* spp. Predicted Reactivity (*In silico*)

Organism	Result
Candida duobushaemulonis	*Candida* spp. Not Detected
Candida fabianii (Cyberlindner fabianii)	
Candida fermentati (Myerozyma carribica)	
Candida jadinii	
Candida pelliculosa (Wickerhamomyces anomalus)	

Table 40: *Candida albicans* Isolates Tested

Organism	Isolate ID (Strain)	Test Concentration		Result
		(copies/mL)	xLoD	
Candida albicans	ATCC 90028 (NCCLS 11)	5.0E+02	1x	*Candida albicans* Detected
	ATCC 10231 (3147)	(b)(4)	3x	
	ATCC 11006		3x	
	ATCC 14053 (NIH 3172)		3x	
	ATCC 22972 (M 97)		3x	

The following tables describe the reactivity of the AMR genes assays with different AMR gene types in various host bacteria. Results are shown for the isolates tested as well as

predictions of reactivity with untested AMR gene types based on in silico analysis of sequences retrieved from public databases.

Table 41: Isolates Tested Containing the *bla*CTX-M gene and *In Silico* Predicted Reactivity for CTX-M Types

CTX-M Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
CTX-M-3	*E. coli*	NCTC 13452	(b)(4)	CTX-M Detected
	S. flexneri	AR Bank #0421		
CTX-M-14	*K. pneumoniae*	AR-Bank #079		
CTX-M-15	*C. freundii*	GRE 1062177		
	K. pneumoniae	AR Bank #0075		
	K. pneumoniae	AR-Bank #0040		
	M. morganii	AR-Bank #0057		
	S. enterica ssp. *enterica*	AR-Bank #0407		
CTX-M-22	*P. mirabilis*	GRE 1254053		
CTX-M-55	*E. coli*	AR Bank #0346		
CTX-M-2	*K. pneumoniae*	AR Bank #0107		
CTX-M-124	*K. ascorbate*[a]	AR Bank #0144		
CTX-M-8	*E. coli*	NCTC 13463		
CTX-M-9	*E. cloacae*	NCTC 13464		
CTX-M-25	*K. pneumoniae*	NCTC 13465		

***In Silico* Reactivity Predictions**[b]				
Detected		**Not Detected**	**Unknown Reactivity**	
CTX-M-1 – CTX-M-69	CTX-M-136 – CTX-M-139	CTX-M-74	CTX-M-70	CTX-M-140
CTX-M-71 – CTX-M-73	CTX-M-141 – CTX-M-142	CTX-M-75	CTX-M-119	CTX-M-143
CTX-M-76 – CTX-M-112	CTX-M-144	CTX-M-113	CTX-M-120	CTX-M-145
CTX-M-114 – CTX-M-117	CTX-M-146 – CTX-M-148	CTX-M-151	CTX-M-128	CTX-M-149
CTX-M-121 – CTX-M-127	CTX-M-150		CTX-M-133	CTX-M-153
CTX-M-129 – CTX-M-132	CTX-M-152		CTX-M-135	CTX-M-154
CTX-M-134	CTX-M-155 – CTX-M-229			

[a]Isolate was tested only to evaluate CTX-M assay reactivity, the species is not detected by the panel.
[b]A subset of CTX-M sequences (<1%) of various types have sequence variation under the assay primers that may impact detection.

Table 42: Isolates Tested Containing the *bla*IMP gene and *In Silico* Predicted Reactivity for IMP Types

IMP Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
IMP-1	*P. aeruginosa*	AR Bank #0103	(b)(4)	IMP Detected
IMP-4	*K. aerogenes*	AR-Bank #0161		

IMP Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
	K. pneumoniae	AR-Bank #0080	(b)(4)	
IMP-8	*E. cloacae*	AR Bank #0502		
	K. pneumoniae	GRE 1062084		
IMP-13	*K. pneumoniae*	Zeptometrix 0801904		
IMP-14	*P. aeruginosa*	AR Bank #0092		
***In Silico* Reactivity Predictions**				
Detected[a]		**Not Detected**	**Unknown Reactivity**	
IMP-1 – IMP-30	IMP-51 – IMP-56	IMP-31	IMP-36	
IMP-32 – IMP-34	IMP-58 – IMP-64	IMP-35	IMP-47	
IMP-37 – IMP-45	IMP-66 – IMP-84	IMP-46	IMP-50	
IMP-48 – IMP-49			IMP-57	
			IMP-65	

[a]Approximately 10% of IMP sequences of various types have mismatches to the assay primer(s) that may impact detection.

Table 43: Isolates Tested Containing the *bla*KPC gene and *In Silico* Predicted Reactivity for KPC Types[a]

KPC Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
KPC-2	*C. freundii*	AR Bank #0116	(b)(4)	KPC Detected
	P. aeruginosa	CUDM PS28		
	S. marcescens	JMI 697		
KPC-3	*K. pneumoniae*	AR Bank #0097		
	E. coli	AR Bank #0061		
KPC-4	*Klebsiella pneumoniae*	JMI 697		
KPC-5	*P. aeruginosa*	AR Bank #0090		
KPC-6	*P. mirabilis*	AR Bank #0155		
KPC-11	*K. pneumoniae*	AR Bank #0525		
Unknown	*E. hormaechei*	BAA-2082		

a In silico analyses predict reactivity with all KPC types (KPC-1 – KPC-46).

Table 44: Isolates Tested Containing *mecA/C*[a] and MREJ (MRSA) sequences and *In Silico* Predicted Reactivity for MREJ Types

Organism	Isolate ID (Strain)	SCCmec Type/ MREJ Type	Test concentration (copies/mL)	Result
Methicillin-resistant *Staphylococcus aureus* (MRSA)	NARSA NRS705 (NY-12)	SCCmec Type II	(b)(4)	*mecA/C* and MREJ (MRSA) Detected
	NARSA NRS701 (MN-082)			
	ATCC BAA-1717 (TCH1516)	SCCmec Type IV		
	NARSA NRS683 (GA-298)			
	NARSA NRS662 (CO-34)			
	NARSA NRS707 (NY-155)			
	ATCC BAA-1707 (MW2)			
	NARSA NRS691 (GA-62)			
	NARSA NRS648 (CA-347)	SCCmec Type II or IV		
	NARSA NRS689 (GA-442)	SCCmec Type IV		
	ATCC BAA-1700 (HFH-30137)			
	BEI NR-46081 (HIP12899)			
	ATCC 43300 (F182 Kansas)	SCCmec Type II		
	ATCC BAA-1720			
	NARSA NRS745 (CA-629)	SCCmec Type IV or V		
	ATCC BAA-2312	SCCmec Type XI		
	ATCC BAA-2313			
	ATCC BAA-38	MREJ Type i		
	NARSA NRS686			
	ATCC BAA-44	MREJ Type ii		
	ATCC BAA-42			
	ATCC BAA-39	MREJ Type iii		
	ATCC BAA-40	MREJ Type iv		
	GRE 1062264			
	ATCC BAA-2096	MREJ Type v		
	GRE 1055015	MREJ Type vi		
	GRE 0860042	MREJ Type vii		
	GRE 1052034	MREJ Type ix		
	GRE 1151100	MREJ Type xi		
	GRE 0960006	MREJ Type xii		
	GRE 1055017	MREJ Type xiii		
	GRE 0759163	MREJ Type xiv		
	GRE 1057114	MREJ Type xvii		
	GRE 1062373	MREJ Type xv[b]		*mecA/C* and MREJ (MRSA) Not Detected
	GRE 1062292	MREJ Type xviiii		
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	ATCC BAA-2421[c]	SCCmec Type II[c]		*mecA/C* and MREJ (MRSA) Detected
	Rennes 1060728 DAC	Empty SCCmec cassette		*mecA/C* and

Organism	Isolate ID (Strain)	SCCmec Type/ MREJ Type	Test concentration (copies/mL)	Result
	GRE 1062519	MREJ Type xix[d]	(b)(4)	MREJ (MRSA) Not Detected

In Silico Reactivity Predictions for MREJ Types				
Detected[e]			**Not Detected**	**Unknown Reactivity**
MREJ Type i	MREJ Type vii	MREJ Type xvi	MREJ Type xv[b]	MREJ Type viii
MREJ Type ii	MREJ Type ix	MREJ Type xvii	MREJ Type xviii	MREJ Type x
MREJ Type iii	MREJ Type xi	MREJ Type xxi	MREJ Type xix[d]	
MREJ Type iv	MREJ Type xii		MREJ Type xx[d]	
MREJ Type v	MREJ Type xiii			
MREJ Type vi	MREJ Type xiv			

[a]In silico analysis predicts that more than 99.9% of the mecA and mecC sequences evaluated will be detected.
[b]Approximately 40% of the MREJ type xv – like sequences evaluated have a sequence variation that is predicted to substantially impair or prevent detection by the MREJa assay. However, no limitations on detection are predicted for ~60% of MREJ type xv – like sequences evaluated. The prevalence of MREJ type xv, with or without the sequence variation, is currently unknown.
[c]Isolate carries a mecA gene variant that is amplified by the mecA/C assay but is nonfunctional. Reporting based on genotype will not match the phenotype.
[d]Isolates with MREJ Types xix and xx have been described as methicillin sensitive.
[e]Approximately 1% of MREJ sequences of various types have mismatches to the assay primer(s) that may impact detection.

Table 45: Isolates Tested Containing *bla*NDM gene and *In Silico* Predicted Reactivity for NDM Types

NDM Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
NDM-1	*C. freundii*	AR Bank #0157	(b)(4)	NDM Detected
	E. cloacae	AR-Bank #0038		
	M. morganii	AR-Bank #0057		
	P. mirabilis	AR Bank #0159		
	P. aeruginosa	AR Bank #0246		
	S. enterica	AR Bank #0127		
NDM-2	*A. baumanii*[a]	GRE 1153064		
NDM-5	*E. coli*	AR Bank #0150		
NDM-6		AR Bank #0137		
NDM-7	*K. pneumoniae*	AR Bank #0138		
		AR Bank #0068		

In Silico Reactivity Predictions			
Detected[b]		**Unknown Reactivity**	
NDM-1 – NDM-13	NDM-32	NDM-14	NDM-33-39

NDM Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
NDM-15 – NDM-23	NDM-40	NDM-24 – NDM-26		
NDM-27 – NDM-29		NDM-30 – NDM-31		

[a]Isolate was tested only to evaluate NDM assay reactivity, the species is not detected by the panel.
[b]Less than 1% of NDM sequences of various types have mismatches to the assay primer(s) that may impact detection.

Table 46: Isolates Tested Containing *bla*OXA-48-like gene and *In Silico* Predicted Reactivity for OXA-48-like Types

OXA-48-like Type	Organism	Isolate ID	Test concentration	Result
OXA-48	*K. aerogenes*	AR Bank #0074	(b)(4)	OXA-48-like Detected
OXA-48-like	*S. marcescens*	API 1411137		
OXA-162	*K. pneumoniae*	GRE 1355030		
OXA-181	*K. pneumoniae*	AR Bank #0051		
OXA-232	*K. pneumoniae*	AR Bank #0075		

***In Silico* Reactivity Predictions**					
Detected			**Not Detected[a,b,c]**		
OXA-48	OXA-244	OXA-515	OXA-54	OXA-439	OXA-551
OXA-48-like	OXA-245	OXA-519	OXA-163	OXA-517	OXA-552
OXA-162	OXA-252	OXA-546	OXA-247	OXA-535	OXA-553
OXA-181	OXA-370	OXA-547	OXA-405	OXA-538	OXA-567
OXA-199	OXA-484	OXA-566	OXA-416	OXA-548	OXA-731
OXA-204	OXA-505		OXA-436	OXA-549	
OXA-232	OXA-514		OXA-438	OXA-550	

[a]Non-OXA-48-like types (e.g., OXA-23-like, OXA-40/240like, OXA-51-like, OXA-58-like, OXA-143a-like and OXA-143-like) will not be detected
[b]OXA-48-like types with altered carbapenem hydrolysis activity will not be detected.
[c]OXA-48-like types with altered carbapenem hydrolysis activity will not be detected.

Table 47: Isolates Tested Containing *vanA/B* genes

***van* Type**	**Organism**	**Isolate ID**	**Test concentration (copies/mL)**	**Result**
vanA	*E. faecium*	ATCC 700221	1.2 E+03	*vanA/B* Detected
		JMI 475	3.6E+03	
		ATCC BAA-2318	3.6E+03	
	E. faecalis	JMI 12536	3.6E+03	
		ATCC BAA-2573	3.6E+03	
vanB	*E. faecium*	ATCC 51858	3.6E+03	
	E. faecalis	ATCC 700802	1.5E+04	
		ATCC 51575	1.5E+04	
		ATCC BAA-2365	1.5E+04	

van Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
		ATCC 51299	5.0E+03	

Table 48: Isolates Tested Containing *bla*VIM-like gene and *In Silico* Predicted Reactivity for VIM Types

VIM Type	Organism	Isolate ID	Test concentration (copies/mL)	Result
VIM-1	*E. cloacae*	AR Bank #0154	(b)(4)	VIM Detected
VIM-2	*P. aeruginosa*	AR Bank #0100		
VIM-4	*P. aeruginosa*	AR Bank #0054		
VIM-7	*E. coli*	GRE 1256018		
VIM-10	*P. aeruginosa*	NCTC 13437		
VIM-11	*P. aeruginosa*	AR Bank #0239		
VIM-27	*K. pneumoniae*	AR Bank #0040		

***In Silico* Reactivity Predictions**				
Detected[a]		**Not Detected**		**Unknown Reactivity**
VIM-1 – VIM-6	VIM-47 – VIM-60	VIM-7	VIM-61	VIM-21
VIM-8 – VIM-20	VIM-52 – VIM-64	VIM-39	VIM-65	VIM-22
VIM-23 – VIM-38	VIM-66	VIM-45	VIM-67	
VIM-40 – VIM-44		VIM-46		

[a]Approximately 3% of VIM sequences of various types have mismatches to assay primer(s) that may impact detection.

Exclusivity

The potential for non-specific amplification and detection (cross-reactivity) by the BioFire JI Panel assays was evaluated by in silico analysis of available sequences and by testing high concentrations of on-panel and off-panel organisms (and antimicrobial resistance genes). Each organism was tested in triplicate with most bacteria tested at a concentration >1.0E+08 CFU/mL and most yeast tested at a concentration >1.0E+06 CFU/mL. Off-panel fungi, viruses, and parasites were tested at the highest cultured concentration possible.

The on-panel and off-panel organisms tested are listed in Table 50 below. Testing included species and AMR genes that are genetically related to the species or AMR genes detected by the panel (same genus or otherwise related) as well as unrelated organisms that may be found in synovial fluid as pathogens or contaminants (e.g. skin microorganisms, viruses, etc.). All observed or predicted cross-reactivities are indicated. Erroneous results due to cross-reactivity with organisms that were not evaluated or due to cross-reactivity with emerging or novel sequences are also possible.

Table 49. Summary of Observed and Predicted Cross-Reactivity of BioFire JI Panel Assays

BioFire JI Panel Result	Cross-Reactive Organism
Anearococcus prevottii/vaginalis	*Anaerococcus degeneri*
	Anaerococcus hydrogenalis
	Anaerococcus lactolyticus
	Anaerococcus murdochii
	Anaerococcus nagyae
	Anaerococcus octavius
	Anaerococcus senegalensis
	Anaerococcus tetradius
Bacteroides fragilis	*Bacteroides xylanisolvens*
Clostridium perfringens	*Clostridium cadaveris*
	Clostridium fallax
Enterobacter cloacae complex	*Enterobacter bugandensis*[b]
	Enterobacter chengduensis[b]
Escherichia coli	*Escherichia albertii*
	Escherichia fergusonii
	Shigella boydii
	Shigella dysenteriae
	Shigella flexneri
	Shigella sonnei
Haemophilus influenzae	*Haemophilus aegyptius*
Kingella kingae	*Kingella negevensis*
Proteus spp.	*Cosenzaea* (*Proteus*) *myxofaciens*
Staphylococcus aureus[b] (and *mecA/C* and MREJ (MRSA))	*Staphylococcus argenteus*[b]
	Staphylococcus schweitzeri[b]
AMR Genes Derived from Similar Lineages	
CTX-M[c]	*ampC*, bla_{KLU}, bla_{OXY}, bla_{RAHN}
vanA/B	*vanM*

[a] *Enterobacter bugandensis* and *E. chengduensis* are recently identified species that are very closely-related to ECC species. Both are indicated as cross-reactive with the *Enterobacter cloacae* complex assay because their designation as ECC members is currently uncertain.

[b] *Staphylococcus aureus*, *S. argenteus* and *S. schweitzeri* are closely-related members of the *Staphylococcus aureus* complex.

[c] CTX-M cross-reactivity with ancestral bla_{KLU} genes and other related beta-lactamases is predicted to be inefficient and will only occur at high concentrations. The cross-reactive product will only be reported as CTX-M Detected if an applicable gram-negative bacterial species is also detected in the sample.

Table 50. On-Panel and Off-Panel Organisms Tested for Evaluation of BioFire JI Panel Analytical Specificity (Organisms detected or predicted to be detected at high concentration are shown in **bold**. Grey shading indicates cross-reactivity.)

ON PANEL			
Gram Positive Bacteria			
Anaerococcus vaginalis	***Peptoniphilus koenoeneniae***	***Streptococcus equinus***	***Streptococcus oligofermentans***
Clostridium perfringens	***Peptoniphilus lacrimalis***	***Streptococcus gallolyticus (ssp. gallolyticus & pasteruianus)***	***Streptococcus peroris***
Cutibacterium avidum	*Peptoniphilus massiliensis*[a]	***Streptococcus gordonii***	***Streptococcus pneumoniae***
Cutibacterium granulosum	***Peptoniphilus senegalensis***	***Streptococcus infantarius***	***Streptococcus psseudopneumoniae***
Enterococcus faecalis	***Peptoniphilus tyrelliae***	***Peptonipilus allenii***	***Streptococcus pyogenes***
Enterococcus faecium	***Streptococcu equis***	***Peptoniphilus harei***	***Streptococcus salivarius (ssp. salivarius & thermophilus)***
Finegoldia magna	***Streptococcus agalactiae***	***Peptoniphilus indolicus***	***Streptococcus vestibularis***
Parvimonas micra	***Streptococcus alactolyticus***	***Peptostreptococcus anaerobius***	***Staphylococcus argenteus***
Peptoniphilus asaccharolyticus	***Streptococcus anginosus***	*Peptoniphilus olsenii*[a]	***Staphylococcus aureus***
Peptoniphilus coxii[a]	***Streptococcus bovis***	***Streptococcus australis***	***Streptococcus oralis***
Peptoniphilus duerdenii[a]	***Streptococcus constellatus***	***Streptococcus intermedius***	***Streptococcus parasanguinis***
Peptoniphilus gorbachii	***Streptococcus cristatus***	***Streptococcus mitis***	***Streptococcus sanguinis***
Peptoniphilus grossensis	***Streptococcus downei***	***Streptococcus mutans***	***Streptococcus suis***
Peptoniphilus ivorii[a]	***Streptococcus dysgalactiae (ssp. dysgalactiae & equismilis)***	***Staphylococcus lugdunensis***	
Gram Negative Bacteria			
Bacteroides fragilis	*Citrobacter sedlakii*[b]	***Enterobacter mori***	***Proteus hauseri***
Citrobacter braakii	***Citrobacter werkmanii***	***Escherichia coli***	***Proteus mirabilis***
Citrobacter europaeus	***Citrobacter youngae***	***Haemophilus influenzae***	***Proteus penneri***
Citrobacter farmeri[b]	***Enterobacter asburiae***	***Kingella kingae***	***Proteus vulgaris***
Citrobacter freundii	***Enterobacter cloacae***	***Klebsiella aerogenes***	***Pseudomonas aeruginosa***
Citrobacter gillenii[b]	***Enterobacter hormaechei***	***Klebsiella pneumoniae***	***Salmonella bongori***
Citrobacter koseri	***Enterobacter kobei***	***Morganella morganii***	***Salmonella enterica***
Citrobacter murlinae	***Enterobacter ludwigii***	***Neisseria gonorrhoeae***	***Serratia marascens***
Citrobacter rodentium[b]			
Antimicrobial Resistance Genes			
mecA/C and MREJ (MRSA)	$bla_{\text{CTX-M}}$	bla_{KPC}	$Bla_{\text{OXA-48-like}}$
vanA/B	bla_{IMP}	bla_{NDM}	Bla_{VIM}
Yeast and Fungi			
Candida albicans	*Candida (Meyerozma) guilliermondii*[c,d]	***Candida metapsilosis***	***Candida sojae***
Candida auris[c]	*Candida holmii*[c]	*Candida nivariensis (Nakaseomyces nivariensisa)*	***Candida sphaerica (Kluyveromyces lactis)***[n]
Candida (Trichomonascus) ciferrii[c]	***Candida intermedia***[c]	***Candida (Pichia) norvegensis***[c]	*Candida thermophila*[c,e]

Candida colliculosa (Torulaspora delbrueckii)[c]	***Candida kefyr (Kluyveromyces marxianus)***[c]	***Candida orthopsilosis***	***Candida tropicalis***
Candida dubliniensis	***Candida krusei (Issatchenkia orientalis)***	***Candida parapsilosis***	***Candida utilis (Cyberlindnera jadinii)***[c]
Candida famata (Debaryomyces hansenii)[c]	*Candida (Yarrowia) lipolytica*[c]	*Candida (Diutina) rugosa*[c]	***Candida viswanathii***[c]
Candida glabrata (Nakaseomyces glabrataa)	***Candida (clavispora) lusitaniae***[c]		
OFF PANEL			
Gram Positive Bacteria			
Abaerococcus senegalensis[f]	***Clostridium fallax***[g]	*Enterococcus pseudoavium*	*Peptostreptococcus stomatis*
Actinomyces (Schaalia) odontolyticus	*Clostridium ramosum*	*Enterococcus saccharolyticus*	*Propionibacterium freudenreichii*
Actinomyces israelii	*Clostridium septicum*	*Filifactor alocis*	*Rhodococcus equi*
Actinomyces naeslundii	*Clostridium sordellii*	*Gallicola barnesae*	*Sarcina (Clostridium) ventriculi*
Aerococcus sanquinicola	*Clostridium sphenoides*	*Gemella haemolysans*	*Slackia heliotrinitrireducens*
Aerococcus urinae	*Clostridium sporogenes*	*Helcococcus kunzii*	***Staphylococcus argenteus***
Aidiproprionbacterium acidipropionici	*Clostridium tertium*	*Gemella morbllorum*	*Staphylococcus caprae*
Aidiproprionbacterium jensenii	*Clostridium tetani*	*Gamella sanguinis*	*Staphylococcus saprophyticus*
Anaerococcus degeneri[f]	*Corynebacterium diphtheriae*	*Gordonia bronchialis*	*Staphylococcus capitis*
Anaerococcus hydrogenalis[f]	*Corynebacterium jeikeium*	*Granulicatella adiacens*	*Staphylococcus carmosus*
Anaerococcus lactolyticus[f]	*Corynebacterium pseudodiphtheriticum*	*Lactobacillus casei*	*Staphylococcus cohnii*
Anaerococcus murdochii[f]	*Enterococcus hirae*	*Lactobacillus salivarius*	*Staphylococcus epidermidis*
Anaerococcus nagyae[f]	*Enterococcus raffinosus*	*Lactococcus garvieae*	*Staphylococcus equorun*
Anaerococcus octavius[f]	*Lactobacillus rhamnosus*	*Lactococcus lactis*	*Staphylococcus haemolyticus*
Anaerococcus pacaensis	*Enterococcus mundtii*	*Listeria monocytogenes*	*Staphylococcus pasteuri*
Anaerococcus tetradius[f]	*Cutibacterium (Proprionibacterium) acidifaciens*	*Lysinibacillus sphaericus*	*Staphylococcus hominis*
Atopobium parvulum	*Cutibacterium (Propionibacterium) acnes*	*Macrococcus caseolyticus*	*Staphylococcus intermedius*
Bifidobacterium bifidum	*Cutibacterium (Propionibacterium) namnetense*	*Micrococcus luteus*	*Staphylococcus lutrae*
Bifidobacterium dentium	*Corynebacterium striatum*	*Murdochiella asaccharolytica*	*Staphylococcus pseudointermedius*
Blautia producta	*Corynebacterium urealyticum*	*Mycobacterium kansasii*	*Staphylococcus saprophyticus*
Brevibacterium linens	*Enterococcus avium*	*Mycobacterium abscessus*	*Staphylococcus schleiferi*

Clostridioides (Clostridium) dificile	*Enterococcus casseliflavus*	*Peptococcus niger*	***Staphylococcus schweitzeri*[h]**
Clostridium botulinum	*Enterococcus cecorum*	*Nocardia brasiliensis*	*Staphylococcus warneri*
Clostridium butyricum	*Enterococcus durans*	*Mycobacterium marinum*	*Staphylococcus xylosus*
***Clostridium cadaveris*[g]**	*Enterococcus gallinarum*	*Mycobacterium tuberculosis*	*Vagococcus fluvialis*
Clostridium clostridioforme			
Gram Negative Bacteria			
Actinobacillus arthritidis	*Edwardsiella tarda*	*Massilia timonae*	*Pseudomonas otitidis*
Acidaminococcus fermentans	*Eikenella corrodens*	*Megasphaera elsdenii*	*Pseudomonas pertucinogena*
Acinetobacter nosocomialis	***Enterobacter bugandensis*[l]**	*Megasphaera indica*	*Pseudomonas protegens*
Acinetobacter schindleri	*Enterobacter cancerogenus*	*Megasphaera massiliensis*	*Pseudomonas putida*
Aeromonas hydrophila	***Enterobacter chengduensis*[l]**	*Moraxella catarrhalis*	*Pseudomonas stutzeri*
Aggregatibacter actinomycetemcomitans	***Escherichia albertii*[m]**	*Moraxella lacunata*	*Ralstonia pickettii*
Bacteroides dorei	*Escherichia coli*	*Neisseria cinerea*	*Raoultella ornithinolytica*
Bacteroides caccae	***Escherichia fergusonii*[m]**	*Neisseria flava*	*Raoultella planticola*
Bacteroides eggerthii	*Escherichia hermannii*	*Neisseria f;avescens*	*Serratia ficaria*
Bacteroidesforsytus	*Escherichia vulneris*	*Neisseria lactamica*	*Serratia fonticola*
Bacteroides helcogenes	*Fusobacterium nucleatum*	*Neisseria meningitidis*	*Serratia liquifaciens*
Bacteroides stercoris	***Haemophilus aegyptius*[n]**	*Neisseria mucosa*	*Serratia odorifera*
Bacteroides thetaiotaomicron	*Haemophilus ducreyi*	*Neisseria perflava*	*Serratia plymuthica*
Bacteroides uniformis	*Haemophilus haemolyticus*	*Neisseria sicca*	*Serratia proteamaculens*
Bacteroides vulgatis	*Haemophilus parahaemoluticus*	*Neisseria subflava*	*Serratia rubidaea*
***Bacteroides xylanisolvens*[i]**	*Haemophilus parainfluenzae*	*Pantoea agglomerans*	*Shewanella algae*
Bacteroides ovatus	*Haemophilus parasuis*	*Parabacteroides distasonis*	*Shewanella denitrificans*
Bordetella flabilis	*Haemophilus quentini*	*Parabacteroides merdae*	*Shewanella putrefaciens*
Borrelia burgdorferi	*Haemophilus sputorum*	*Pasteruella multocida*	***Shigella boydii*[m]**
Brucella abortus	*Hafnia alvei*	*Photorhabdus asymbiotica*	***Shigella dysenteriam*[i]**
Brucella melitensis	*Hafnia paralveis*	*Pluralibacter (Enterobacter(gergoviae*	***Shigella flexneri*[m]**
Brucella suis	*Kingella denitrificans*	*Porphyromonas gingivalis*	***Shigella sonnei*[m]**
Burkholderia mallei	***Kingella negevensis*[o]**	*Prevotella intermedia*	*Shimwellia blattae*
Burkholderia multivorans	*Kingella oralis*	*Prevotella melaninogenica*	*Stenotrophomonas maltophilia*
Burkholderia pseudomallei	*Klebsiella grimontii*	*Prevotella nigrescens*	*Stenotrophomonas rhizophila*
Campylobacter jejuni	*Klebsiella michiganensis*	*Providencia rettgeri*	*Trabulsiella guamensis*
Cedecea davisae	*Klebsiella oxytoca*	*Providencia stuartii*	*Veillonella atypica*
Citrobacter amalonaticus[j]	***Klebsiella quasipneumoniae***	*Pseudomonas alcaligenes*	*Veillonella dispar*
***Coszenaea (Proteus) myxofaciens*[k]**	***Klebsiella variicola***	*Pseudomonas chlororaphis*	*Veillonella parvula*

Cronobacter malonaticus	*Kluyvera intermedia*	*Pseudomonas fluorescens*	*Veillonella rogosae*
Cronobacter muytjensii	*Kosakonia (Enterobacter) sacchari*	*Pseudomonas luteola*	*Vibrio vulnificus*
Cronobacter sakazakii	*Lelliotia (Enterobacter) amnigena*	*Pseudomonas mendocina*	*Yersinia enterocolitica*
Cronobacter turicensis	*Lelliotia (Enterobacter) nimipressuralis*	*Pseudomonas nitroreducens*	
Cronobacter zurichensis (Siccibacter turicensis)	*Leclercia adecarboxylata*	*Pseudomonas oryzihabitans*	
Mycoplasma and Intracellular Bacteria			
Chlamydia trachomatis	*Mycoplasma fermentans*	*Mycoplasma hominis*	*Mycoplasma penetrans*
Mycoplasma arthritidis	*Mycoplasma genitalium*	*Mycoplasma orale*	*Ureaplasma urealyticum*
Antimicrobial Resistance Genes			
vanM[p]	***bla*$_{OXY}$[q]**	OmpC	SME
AmpC[q]	***bla*$_{RAHN}$[q]**	OmpK	SPM
***bla*$_{KLUA}$/*bla*$_{KLUC}$[q]**	CMY (II)	SHV	TEM
Yeast and Fungi			
Aspergillus candidus	*Coccidioides immitis*	*Histoplasma capsulatum*	*Talaromyces (Penecillium) marneffei*
Aspergillus clavatus	*Cryptococcus gattii*	*Malessexia fufur*	*Saccharomyces cerevisiae*
Aspergillus fumigatus	*Cryptococcus neoformans*	*Malassexia globose*	*Schizosaccharomyces pombe*
Aspergillus terreus	*Exophiala dermatitidis*	*Neosartorya fischeri*	*Sporothrix schenckii*
Blastomyces dematitidis	*Exophiala xenobiotica*	*Penicillium chrysogenum*	
Parasites			
Chryptosporidium parvum	*Entamoeba histolytica*		
Viruses			
Chikungunya Virus	Hepatitis C Virus (HCV)	Human T-cell Lymphotropic Virus (HTLV)	Rubella Virus
Dengue Virus	Herpes Simplex Virus 1 (HSV-1)	Parvovirus B19	Varicella Zoster Virus (VZV)
Epstein Barr Virus (EBV)	Herpes Simples Virus 2 (HSV-2)	Measles Virus	West Nile Virus
Hepatitis A virus (HAV)	Human Immunodeficiency Virus (HIV)	Mumps Virus	Zika Virus
Hepatitis B Virus (HBV)			

[a]The *Peptoniphilus* assay may not react with several *Peptoniphilus* species; see Analytical Reactivity section above.

[f]The *Citrobacter* assay may not react with several *Citrobacter* species; see Analytical Reactivity section above.

[c]Several of the *Candida* species detected at the high concentrations tested in this study may not be detected at lower concentrations; see the Analytical Reactivity section.

[d]*C. guilliermondii* is also classified as *Candida fermentatis*

[e]*C. thermophila* is also classified as *Candida (Hansenula) parapolymorpha.*

[f]Various *Anaerococcus* species are detected as *Anaerococcus prevotii/vaginalis* due to cross reactivity. The efficiency of the cross-reactivity varies by species.

[g]*Clostridium cadaveris* and *Clostridium fallax* are detected as *Clostridium perfringens* due to cross-reactivity. Sequence analysis predicts a similar risk of cross-reactivity at high concentrations for *C. baratii*, *C. disporicum* and *C. grantii*.

[h]*Staphylococcus argenteus* and *Staphylococcus schweitzeri* are detected as *Staphylococcus aureus* (all three species are part of the *S. aureus* complex) due to cross-reactivity. mecA/C and MREJ (MRSA) was also detected in the *S. argenteus* isolate.
[i]*Bacteroides xylanisolvens* is detected as Bacteroides fragilis due to cross-reactivity.
[j]The Citrobacter assay may not react with several Citrobacter species; see Analytical Reactivity section above.
[k]*Cosenzaea myxofaciens* (formerly *Proteus myxofaciens*) is detected as *Proteus* spp. due to cross-reactivity.
[l]*Enterobacter bugandensis* (tested) and *Enterobacter chengduensis* (not tested, in silico prediction only) are detected as *Enterobacter cloacae* complex due to cross-reactivity.
[m]*Escherichia albertii*, *Escherichia fergusonii* and *Shigella* species are detected as *Escherichia coli* due to cross-reactivity.
[n]*Haemophilus aegyptius* (formerly described as *H. influenzae* biogroup *aegyptius*) is detected as *Haemophilus influenzae* due to cross-reactivity.
[o]*Kingella negevensis* is detected as *Kingella kingae* due to cross-reactivity.
[p]*vanM* is detected as *vanA/B* (not tested, in silico prediction only) due to cross-reactivity
[q]The CTX-M assay cross-reacts weakly with the bla_{OXY} gene carried in an isolate of *Klebsiella michiganensis* (reported as N/A because and applicable bacterium is not detected by the panel). Based on sequence analysis, the CTX-M assay is predicted to cross-react weakly with the bla_{OXY} gene, bla_{RAHN} gene (found primarily in *Rahnella* and *Leminorella* species), bla_{KLU} genes (isolated primarily from *Kluyvera* species), and some variants of ampC (not observed when tested at high concentration in this study).

Interference Testing

Potentially interfering substances that could be present in synovial fluid specimens or that may be introduced during specimen collection and testing were evaluated for their effect on the BioFire JI Panel performance. Substances included endogenous substances that may be found in specimens at normal or elevated levels, various commensal or infectious microorganisms, medications, a variety of sample processing substances and substances used to clean, decontaminate, or disinfect work areas. The effect of interfering substances has only been evaluated for those listed in Table 51. Interference from substances that were not evaluated could lead to erroneous results.

For this study, contrived samples were prepared in synovial fluid (SF) matrix, with each sample containing multiple organisms (and AMR genes) at low levels (3× the limit of detection (LoD)). The subset of organisms included in the samples represent all organism types and AMR genes detected by the panel (aerobic and anaerobic gram-positive and gram-negative bacteria, including fastidious species, with AMR genes for methicillin-resistance and vancomycin resistance in the gram-positive bacteria and for extended-spectrum beta lactamase and carbapenemase activity in gram-negative bacteria, as well as Candida yeast species). Since the functions of the test that could be affected by interference from various substances would impact detection of the different types of organisms and AMR genes similarly, testing with a representative subset is effective for evaluating interference for the full panel. Testing was performed with analytes at concentrations near LoD in order to identify the effects of even low-level interference on analyte detection.

Each contrived sample was tested first as a 'no substance' or 'no interference' positive control followed by testing of the same sample after addition of a substance or microorganism. Substances were added to the contrived samples (or to negative SF, to test the impact of substance alone) at concentrations equal to or greater than the levels expected in clinical SF specimens, and microorganisms were added at the highest possible concentration to evaluate the 'worst case' scenario for interference. Control and test samples were tested in triplicate with three reagent lots.

Table 51: Evaluation of Potentially Interfering Substances on the BioFire JI Panel

Substance	Concentration Tested	Testing Outcome
Endogenous Substances		
Blood	30% v/v	No Interference
Cholesterol	4 mg/mL	No Interference
C-Reactive Protein	0.17 mg/mL	No Interference
Fibronectin	3 mg/mL	No Interference
Lactate	5.7 mg/mL	No Interference
Monosodium urate/Uric Acid	0.235 mg/mL	No Interference
Calcium Phosphate	16 mg/mL	No Interference
Calcium Oxalate	7.9 µg/mL	No Interference
Bilirubin	0.4 mg/mL	No Interference
White Blood Cells	3.0E+07 cells/mL	No Interference
Rheumatoid Factor	1,800 IU/mL	No Interference
Type II Collagen	10.1 µg/mL	No Interference
Exogenous Substances		
Acetaminophen	156 µg/mL	No Interference
Salicylic Acid	28.6 µg/mL	No Interference
Ibuprofen	219 µg/mL	No Interference
Capsaicin Cream (0.1% capsaicin)	0.5% (m/v)	No Interference
Salicylate Cream (30% methyl salicylate)	0.5% (m/v)	No Interference
Camphor Balm (11% camphor)	0.5% (m/v)	No Interference
Arnica Gel (7% *Arnica montana*)	1.0% v/v	No Interference
Nystatin	5000 Units/mL	No Interference
Fluconazole	25.5 µL	No Interference
Mupirocin	1.5 µg/mL	No Interference
Ceftriaxone	840 µg/mL	No Interference
Vancomycin	120 µg/mL	No Interference
Clindamycin	51 µg/mL	No Interference
Triple antibiotic ointment (10000 U polymyxin B, 3.5 mg neomycin, 500 U bacitracin)	0.5% (m/v)	No Interference
Hydrocortisone	8.3 mg/mL	No Interference
Hyaluronic acid	16 mg/mL	No Interference
Lidocaine	23 mg/mL	No Interference
Cobalt Ions	20 µg/mL	No Interference
Chromium Ions	50 µg/mL	No Interference
Ultra-High Molecular Weight Polyethylene	1 mg/mL	No interference

Substance	Concentration Tested	Testing Outcome
Polymethyl methacrylate Bone cement	1% m/v	No Interference
Iohexol	250 mg/mL	No Interference
Competitive Microorganisms		
Streptococcus pyogenes	7.56E+08 CFU/mL	No Interference
Eschericia coli	8.1E+08 CFU/mL	No Interference
Finegoldia magna	8.8E+07 CFU/mL	No Interference
Candida albicans	7.9E+07 CFU/mL	No Interference
Cutibacterium acnes	1.1E+07 cells/mL	No Interference
Staphylococcus epidermidis	8.8E+08 CFU/mL	No Interference
Cornebacterium striatum	7.8E+08 CFU/mL	No Interference
Cryprococcus neoformans	1.0E+07 CFU/mL	No Interference
Parvovirus B19	7.0E+04 IU/mL	No Interference
Chikungunya virus	2.2E+07 genomic equivalents/mL	No Interference
Disinfection/Cleaning Substances		
Reagent Alcohol	1.0% v/v	No Interference
Povidone-iodine	1.0% v/v	No Interference
Bleach	1.0% v/v (600 ppm chlorine)	No Interference
Sample Processing Materials		
K2-EDTA anticoagulant	0.99 µg/mL	No Interference

Notably, bleach is a strong oxidizer capable of damaging nucleic acids. When bleach has been evaluated for interference in other sample types associated with different BioFire FilmArray panels (e.g. nasopharyngeal swab in transport medium, cerebrospinal fluid (CSF), blood culture, stool in Cary Blair transport medium), the oxidizing effects of this disinfectant on results has varied based on the properties of the sample type, the concentration of bleach, and the amount of time the bleach was incubated with the sample. In some cases, such as with CSF, the damaging effect of bleach on the organisms and nucleic acids in the sample were apparent at low bleach concentration and short incubation times. In other cases, no impact on analyte detection was observed, even after 24-hour incubation or bleach concentration up to 5% (3000 ppm). In this study, bleach was tested at 1% (600 ppm) in SF following 15-minute and 24-hour incubation times and no effects on low-level analyte amplification and detection were observed. Nevertheless, to maintain sample integrity, caution should be taken to prevent the direct mixing of bleach with SF samples prior to testing.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Process Controls

   Two process controls are included in each pouch:

RNA Process Control: The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive RNA Process Control result indicates that all steps carried out in the BioFire JI Panel pouch were successful.

PCR2 Control: The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that 2nd stage PCR was successful.

Both control assays must be positive for the test run to pass. If either control fails, the Controls field of the test report will display "Failed" and all results will be listed as "Invalid". If the controls fail, the sample should be retested using a new pouch.

External Controls

External controls should be used in accordance with laboratory protocols and the appropriate accrediting organization requirements, as applicable. Previously characterized positive samples or negative samples spiked with well-characterized organisms can be used as external positive controls.

Specimen Stability

Diagnostic testing of SF specimens is intended to be performed as soon as possible after collection of the specimen, however, transport to the testing lab and subsequent storage of a specimen is sometimes required. A study was performed to confirm that accurate BioFire JI Panel test results can be obtained from SF samples stored refrigerated for up to seven days. Two contrived samples were prepared in a pooled SF matrix, with each sample containing a representative subset of organisms at a concentration near (3×) the established limit of detection (LoD) (as well as 'native' analytes present in the pooled SF matrix at an unknown concentration). The subset of spiked organisms included aerobic and anaerobic gram-positive and gram-negative bacteria (some harboring antimicrobial resistance (AMR) genes), as well as two species of Candida yeast.

Each contrived sample was tested in ten replicates immediately after preparation (D0, no storage) and the remaining sample volume was stored under standard refrigerated conditions for subsequent testing. Ten replicates were tested for each sample after one (D1), three (D3), five (D5) and seven (D7) days of refrigerated storage. Prolonged room temperature storage of SF prior to testing with the BioFire JI Panel will not be recommended and was not evaluated.

Detection of organisms and AMR genes was observed in 90.0 - 100% of the no storage control (D0) sample replicates tested and there was no trend toward inaccurate test results associated with sample storage over time.

Table 52: BioFire Joint Panel Results for Stored Synovial Fluid Specimens[a]

Analyte	Strain	Analyte Detection				
		No Storage (D0)	Day 1 (D1)	Day 3 (D3)	Day 5 (D5)	Day 7 (D7)
Gram Positive Bacteria						
Clostridium perfringens	ATCC 13124	(b)(4)				
Enterococcus faecium	ATCC 700221					
vanA/B						
Staphylococcus aureus	ATCC43300					
mecA/C and MREJ						
Streptococcus spp.	ATCC 6303					
Streptococcus pneumoniae						
Gram Negative Bacteria						
Bacteroides fragilis	ATCC 25285	(b)(4)				
Escherichia coli	AR-BANK #0150					
NDM						
Haemophilus influenzae	ATCC 10211					
Kingella kingae	ATCC 23330					
Klebsiella pneumoniae	AR-BANK #0097					
KPC						
Neisseria gonorrhoeae	ATCC 19424					
Candida	ATCC 90028					
Candida albicans						
Candida (Candida krusei)	ATCC 6258					

[a]Additional analytes (*E. faecalis*, *S. aureus*, mecA/C and MREJ (MRSA) and *S. lugdunensis*) present in the pooled synovial fluid matrix were also detected.

[b]An extra replicate of Sample 2 was tested due to a suspected pouch anomaly/error. Unexpected Not Detected results were reported for [b] analytes (b)(4) in replicate 7 of Sample 2 stored for three days (D3).

Analysis of the Cp values across the evaluated analytes did not indicate a negative trend across the entire period of the stability study.

Fresh vs. Frozen Study

In order to utilize frozen clinical respiratory samples in the evaluation of BioFire JI Panel, an analytical study was conducted to demonstrate that preservation of samples by freezing at ≤-70°C does not affect the accuracy of test results compared to freshly collected or freshly prepared samples.

Testing was performed on a representative panel of 30 paired fresh and frozen contrived specimens that were prepared by co-spiking each specimen with two organisms. Organisms were spiked into residual clinical synovial fluid specimens that were previously screened and found to be negative for the spiked analytes. The tested analytes included gram-positive, gram-negative, aerobic, and anaerobic bacteria (*Streptococcus pyogenes, Anaerococcus prevotii, Enterococcus faecium, Bacteroides fragilis, and Klebsiella pneumoniae*). Select gram-negative and gram-positive bacteria in the testing pool also have the antimicrobial resistance (AMR) genes CTX-M, OXA-48-like, and vanA. Additionally, this study included one yeast (*Candida krusei*). Ten (10) negative (unspiked) samples were randomized with the spiked specimens to facilitate specimen blinding and to monitor BioFire JI Panel control performance.

Each analyte was spiked into ten specimens. The majority of the contrived specimens (six for each analyte) were spiked at 2 × the limit of detection (LoD), with the remaining four specimens spiked at levels that spanned the detection range of each assay (10-1000 × LoD). AMR genes were tested at the host organism spike concentrations. All contrived specimens were split into two aliquots and the aliquots were coded and randomized so that personnel performing the testing were blinded to the expected results. One aliquot was tested fresh (without a freeze-thaw) on the BioFire JI Panel; the second aliquot was tested after a single freeze-thaw event (frozen for at least 24 hours at ≤ -70ºC).

Additionally, 25 clinical specimens (collected during the prospective study) were tested at BioFire (by users without knowledge of the expected results) from a frozen aliquot, and the results were compared to the results that were obtained when the specimens were tested fresh at the clinical study sites.

For contrived specimens, BioFire JI Panel results demonstrated 100% concordance for all evaluated analytes when tested fresh or frozen. For clinical specimens, BioFire JI Panel results demonstrated 100% concordance for most evaluated analytes when tested fresh or frozen. One exception was a missed detection for *Pseudomonas aeruginosa*, which was present in the specimen at a level near the LoD of the assay.

6. Detection Limit:

A limit of detection (LoD) was established for bacteria and yeast detected by the BioFire JI Panel. LoD was estimated by testing serial dilutions of contrived samples containing known concentrations of organisms in pooled synovial fluid matrix. Confirmation of LoD was achieved by testing at least 10 replicates each on both the FilmArray 2.0 and FilmArray Torch systems (20 replicates total dilution). LoD concentration was confirmed when the analyte was detected in at least 95% of the replicates tested.

The confirmed LoD for each bacterium or yeast detected by the panel is listed in Table 53. The LoD concentration is based on quantification of each culture in viable units ($TCID_{50}$/mL or CFU/mL) and a corresponding molecular LoD concentration (copies/mL) is provided based on quantitative real-time or digital PCR.

Table 53: Summary of Limit of Detection (LoD) for BioFire JI Panel Bacteria and Yeast

Analyte	Isolate Strain/Serotype/ Source ID	LoD Concentration[a]	
		Viable Units	Molecular (DNA)
Gram Positive Bacteria			
Anaerococcus prevotii/vaginalis	ATCC 9321	(b)(4)	4.8E+04 copies/mL
Clostridium perfringens	ATCC 13124		1.3E+03 copies/mL
	ATCC 8009		1.4E+03 copies/mL
Cutibacterium avidum	ATCC 25577		5.0E+04 copies/mL
Cutibacterium granulosum	ATCC 25564		5.0E+04 copies/mL
Enterococcus faecalis (vanA/B)	ATCC 51299		5.0E+03 copies/mL
Enterococcus faecium (vanA/B)	ATCC 700221		1.2E+03 copies/mL
Finegoldia magna	ATCC 15794		3.1E+05 copies/mL
Parvimonas micra	ATCC 33270		4.8E+03 copies/mL
Peptoniphilus assacharolyticus	ATCC 14963		4.0E+04 copies/mL
Peptostreptococcus anaerobius	ATCC 27337		1.6E+04 copies/mL
Staphylococcus aureus (mecA/C and MREJ) (MRSA)	ATCC 43300		4.2E+03 copies/mL
	ATCC BAA-2313		4.2E+03 copies/mL
Staphylococcus lugdunensis	ATCC 43809		2.6E+03 copies/mL
Streptococcus mutans	ATCC 25175		2.5E+05 copies/mL
Streptococcus agalactiae	ATCC 13813		1.9E+04 copies/mL
Streptococcus pneumoniae	ATCC 6303		5.3E+02 copies/mL
Streptococcus pyogenes	ATCC 49399		8.9E+03 copies/mL
Gram Negative Bacteria			

	Isolate	LoD Concentration[a]	
Bacteroides fragilis	ATCC 25285	(b)(4)	1.1E+03 copies/mL
Citrobacter	ATCC 8090		4.7E+03 copies/mL
***Enterobacter cloacae* complex (VIM)**	AR Bank #0154		1.3E+05 copies/mL
***Escherichia coli* (NDM)**	AR Bank #0150		6.0E+03 copies/mL
Haemophilus influenzae	ATCC 10211		6.9E+02 copies/mL
Kingella kingae	ATCC 23330		3.4E+03 copies/mL
***Klebsiella aerogenes* (OXA-48-like)**	AR Bank #0074		7.5E+03 copies/mL
***Klebsiella pneumoniae* group (KPC)**	AR Bank		1.6E+04 copies/mL
Morganela morganii	ATCC 25830		2.2E+03 copies/mL
Neisseria gonorrhoeae	ATCC 19424		2.2E+03 copies/mL
***Proteus* spp.**	ATCC 35659		5.2E+03 copies/mL
***Pseudomonas aeruginosa* (IMP)**	AR Bank #0092		1.3E+04 copies/mL
***Salmonella* spp. (CTX-M)**	AR Bank #0407		1.6E+03 copies/mL
Serratia marcescens	ATCC 13880		1.1E+04 copies/mL
Yeast			
***Candida* spp.**	ATCC 6258	1.0E+03 CFU/mL	-
Candida albicans	ATCC 90028	5.0E+02 CFU/mL	-

For resistance genes, data were collected to demonstrate detection of each AMR gene at a concentration corresponding to the lowest LoD of the applicable bacteria. LoD estimate dilution series data and/or LoD confirmation testing data for host organisms were used to demonstrate detection at the applicable bacterial LoD concentrations. Data from this testing establish that amplification and positive assay results for the AMR genes are observed at a host concentration that is the same as or similar to the lowest LoD of the applicable bacteria.

Table 54: AMR Gene Detection at Lowest Applicable LoD

BioFire JI AMR Target	Applicable Organism	LoD Concentration
		Molecular (DNA)
CTX-M	*Salmonella enterica*	1.6E+03 copies/mL
IMP	*Pseudomonas aeruginosa*	1.3E+03 copies/mL
KPC	*Klebsiella pneumoniae*	1.6E+03 copies/mL

BioFire JI AMR	Applicable	LoD Concentration
***mecA/C* and MREJ**	*Staphylococcus aureus*	4.2E+03 copies/mL
NDM	*Escherichia coli*	6.0E+03 copies/mL
	Morganella morganii	2.2E+03 copies/mL
	Salmonella enterica	1.6E+03 copies/mL
OXA-48-like	*Klebsiella aerogenes*	7.5E+02 copies/mL
vanA/B	*Enterococcus faecium*	1.2E+03 copies/mL
	Enterococcus faecalis	1.0E+03 copies/mL
VIM	ATCC 15794	2.6E+03 copies/mL

7. Assay Cut-Off:

The BioFire Joint Infection Panel is part of BioFire Diagnostics' (BFDX) FilmArray system. The FilmArray system is designed to interpret the test data and automatically report the test results to the operator. The FilmArray system uses the results of the Melt Detector to determine each test result. The Melt Detector is part of the FilmArray Analysis Software and assigns a positive or negative result to each reaction on the array through analysis of the melt data collected during the test. These positive and negative results are combined in the FilmArray Analysis Software (using the replicate, assay and interpretation rules) to report the presence or absence of each pathogen in the panel.

The purpose of this study was to validate the use of this Melt Detector with current optimization parameters with the BioFire Bone and Joint Panel. To evaluate the Melt Detector performance, the observed sensitivity and specificity rates for the individual melt curves and assay calls are reported. These sensitivity and specificity rates are determined by comparing the FilmArray test results obtained from well-characterized samples, collected as part of the clinical evaluation and analytic testing of the Bone and Joint Panel, to expert annotation. Annotations (positive and negative calls) for all melt curves and assay calls were determined by the sponsor.

For individual melt curves, the observed sensitivity and specificity, as compared to expert annotation, of the Melt Detector is 99.59% and 99.98%, respectively. For the Analysis Software, the observed sensitivity and specificity, as compared to the expert annotation, of the assay calls are 99.49% for sensitivity and 99.97% specificity. The validation results met the predefined acceptance criteria of >95% accuracy as compared to expert annotation.

8. Carry-Over:

A formal carry-over study in support of this regulatory submission for the BioFire JI Panel was not performed, since carry-over studies with high positive samples followed by negative samples have been performed for other FDA-cleared FilmArray Panels (i.e., FilmArray RP, BCID, and GI Panels) for both the FilmArray 2.0 and the FilmArray Torch systems, and no carry-over has been observed.

B Comparison Studies:

9. Method Comparison:

   Not Applicable.

10. Matrix Comparison:

    Not applicable.

C Clinical Studies:

11. Clinical Sensitivity:
    The clinical performance of the BioFire Joint Infection Panel was established during a multi-center study conducted at thirteen geographically distinct study sites in the U.S. and in Europe over approximately two years from May 2018 to March 2020. A total of 1591 synovial fluid specimens were acquired for the prospective clinical study. A total of 47 synovial fluid specimens were excluded from the final data analysis. The most common reasons for specimen exclusion was the specimen was found to not meet the inclusion criteria after the specimen had been enrolled, a valid JI Panel test result was not obtained, or the study site was unable to complete the Case Report Form (CRF). The final data set consisted of 1544 specimens, of which 771 (49.9%) were frozen before testing. No difference in performance between fresh and frozen specimens was observed when results were compared, therefore the data from both specimen types have been combined for all analyses.

Table 55. Overall and Per Site Demographic Analysis for Synovial Fluid Specimens

All specimens were evaluated with the BioFire Joint Infection Panel at clinical study sites.

		Overall	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13
Sex	Male	878 (56.9%)	184 (52.3%	46 (52.3%)	108 (53.7%)	65 (73.0%)	60 (58.8%)	45 (51.7%)	108 (54.8%)	39 (59.1%)	88 (60.3%)	88 (64.7%)	10 (58.8%)	27 (55.1%)	10 (71.4%)
	Female	666 (43.1%)	168 (47.7%)	42 (47.7%)	93 (46.3%)	24 (27.0%)	42 (41.2%)	42 (47.7%)	89 (45.2%)	27 (40.9%)	58 (39.7%)	48 (35.3%)	7 (41.2%)	22 (44.9%)	4 (28.6%)
Age	≤ 90 days	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.9%)	0 (0%)	0 (0%)
	91 days - 4 years	22 (1.4%)	1 (0.3%)	0 (0%)	2 (1.0%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	88 (64.7%)	3 (17.6%)	11 (22.4%)	3 (21.4%)
	5 - 15 years	75 (4.9%)	8 (2.3%)	0 (0%)	3 (1.5%)	0 (0%)	3 (2.9%)	2 (2.3%)	1 (0.5%)	1 (1.5%)	0 (0%)	48 (35.3%)	13 (76.5%)	13 (76.5%)	8 (57.1%)
	16 - 25 years	35 (2.3%)	8 (2.3%)	1 (1.1%)	3 (1.5%)	3 (3.4%)	2 (2.0%)	0 (0%)	2 (1.0%)	0 (0%)	3 (2.1%)	6 (4.4%)	0 (0%)	0 (0%)	3 (21.4%)
	26 - 64 years	774 (50.1%)	200 (56.8%)	43 (48.9%)	80 (39.8%)	71 (79.8%)	38 (37.3%)	27 (31.0%)	101 (51.3%)	37 (56.1%)	99 (67.8%)	76 (55.9%)	0 (0%)	0 (0%)	0 (0%)
	≥ 65 years	637 (41.3%)	135 (38.4%)	44 (50.0%)	113 (56.2%)	15 (16.9%)	58 (56.9)	58 (66.7%)	93 (47.2%)	28 (42.4%)	44 (30.1%)	49 (36/0%)	0 (0%)	0 (0%)	0 (0%)
Total		**846**	**352**	**88**	**201**	**89**	**102**	**87**	**197**	**66**	**146**	**136**	**17**	**49**	**14**

Refrigerated specimen aliquots were sent to a central reference laboratory for quantitative reference culture (qRefCx) and frozen specimen aliquots were also sent to BioFire for evaluation by polymerase chain reaction (PCR)/sequencing-based comparator methods.

The reference methods used in this study were as follows:

Bacterial analytes and yeast analytes were compared to SoC culture to evaluate sensitivity and specificity. These analytes were also evaluated by comparison to a single PCR assay for the organism of interest followed by a quantitative molecular assay that included sequencing (tMol). For specimens with an applicable bacteria detected by FilmArray, AMR genes were compared to a single PCR assay (from the specimen) followed by sequencing. A separate PCR was also performed on cultured isolates at BioFire. Standard manual and automated phenotypic AST of appropriate cultured isolates was performed at the study sites as SOC testing. A specimen was considered to be positive for an analyte if bi-directional sequencing data meeting pre- defined quality acceptance criteria matched organism-specific sequences deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) with acceptable E-values. When two PCR comparator assays were used, any specimen that tested negative by both of the comparator assays was considered Negative.

Positive Percent Agreement (PPA) or Sensitivity for each analyte was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the BioFire Joint Infection Panel and the comparator method had a positive result for this specific analyte, and false negative (FN) indicates that the BioFire Joint Infection Panel result was negative while the comparator result was positive. Negative Percent Agreement (NPA) or Specificity was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the BioFire Joint Infection Panel and the comparator method had negative results, and a false positive (FP) indicates that the BioFire Joint Infection Panel result was positive but the comparator result was negative.

The exact binomial two-sided 95% confidence interval was calculated. Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioFire Joint Infection Panel results to the comparator method results were further investigated. For discrepancies between the Bone Joint Infection Panel and reference culture for bacterial and yeast analytes, discrepant samples were first examined by an independent molecular assay performed directly on the specimen in an attempt to observe the analyte of interest. If this did not resolve the discrepancy, the study site was queried to ensure that the CRF accurately reflected the source documents. And if these methods still did not resolve the discrepancy, results of additional laboratory testing were considered. Results from the discrepancy testing did not change the final performance estimates. The prospective clinical study results are summarized in Table 56 below:

Table 56: BioFire Joint Infection Panel Prospective Clinical Performance Summary

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
Gram Positive Bacteria						
Anaerococcus prevotii/vaginalis	1/1	100	-	1543/1543	100	99.8-100%
Clostridium perfringens	0/0	-	-	1544/1544	100	99.8-100%
Cutibacterium avidum/granulosum	0/0	-	-	1544/1544	100	99.8-100%
Enterococcus faecalis[a]	10/10	100	72.2-100%	1529/1534	99.7	99.2-99.9%
Enterococcus faecium[b]	1/1	100	-	1541/1543	99.9	99.5-100%
Finegoldia magna[c]	3/3	100	43.9-100%	1540/1541	99.9	99.6-100%
Parvimonas micra[d]	0/1	0	-	1543/1543	100	99.8-100%
Peptoniphilus[e]	1/1	100	-	1542/1543	99.9	99.6-100%
Peptostreptococcus anaerobius[f]	0/0	-	-	1541/1544	99.8	99.4-99.9%
Staphylococcus aureus[g]	98/105	93.3	86.9-96.7%	1417/1439	98.5	97.7-99.0%
Staphylococcus lugdunensis[h]	2/2	100	34.2-100%	1539/1542	99.8	99.4-99.9%
***Streptococcus* spp.**[i]	38/44	86.4	73.3-93.6%	1488/1500	99.2	98.6-99.5%
Streptococcus agalactiae[j]	10/11	90.9	62.3-98.4%	1532/1533	99.9	99.6-100%
Streptococcus pneumoniae	3/3	100	43.9-100%	1541/1541	100	99.8-100%
Streptococcus pyogenes[k]	11/12	91.7	64.6-98.5%	1532/1532	100	99.7-100%
Gram Negative Bacteria						
Bacteroides fragilis[l]	0/0	-	-	1543/1544	99.9	99.6-100%
Citrobacter	2/2	100	34.2-100%	1542/1542	100	99.8-100%
***Enterobacter cloacae* complex**[m]	2/4	50	15-85.0%	1538/1540	99.9	99.5-100%
Escherichia coli[n]	14/14	100	78.5-100%	1529/1530	99.9	99.6-100%
Haemophilus influenzae[o]	1/1	100	-	1542/1543	99.9	99.6-100%
Kingella kingae[p]	1/1	100	-	1537/1543	99.6	99.2-99.8%
Klebsiella aerogenes	0/0	-	-	1544/1544	100	99.8-100%
***Klebsiella pneumoniae* group**[q]	4/5	80.0	37.6-96.4%	1538/1539	99.9	99.6-100%
Morganella morganii[r]	1/1	100	-	1541/1543	99.9	99.5-100%
Neisseria gonorrhoeae[s]	2/2	100	34.2-100%	1539/1542	99.8	99.4-99.9%
***Proteus* spp.**[t]	4/4	100	51.0-100%	1536/1540	99.7	99.3-99.9%
Pseudomonas aeruginosa[u]	2/2	100	34.2-100%	1539/1542	99.8	99.4-99.9%
***Salmonella* spp.**	0/0	-	-	1544/1544	100	99.8-100%
Serratia marcescens[v]	2/2	100	34.2-100%	1541/1542	99.9	99.6-100%
Yeast						
Candida[w]	4/7	57.1	25.0-84.2%	1536/1537	99.9	99.6-100%
Candida albicans[x]	3/5	60.0	23.1-88.2%	1539/1539	100	99.8-100%

[a] *E. faecalis* was detected in all five FP specimens using an additional comparator method
[b] *E. faecium* was detected in both FP specimens using an additional molecular method
[c] *F. magna* was detected in the single FP specimen using an additional molecular method
[d] *P. micra* was detected in the single FN specimen using an additional molecular method
[e] *P.* was detected in the single FP specimen using an additional molecular method
[f] *P. anaerobius* was detected in all three FP specimens using an additional molecular method
[g] *S. aureus* was detected in 5/7 FN specimens using an additional molecular method; molecular testing of one of the remaining two FN specimens and its isolate identified it as *S. argenteus*. *S. aureus* was detected in 19/22 FP specimens using an additional molecular method.
[h] *S. lugdunensis* was detected in all three FP specimens using an additional molecular method
[i] *Streptococcus* spp. was detected in 4/7 FN specimens and in all 12 FP specimens using an additional molecular method
[j] *S. agalactiae* was detected in the single FN specimen and in the single FP specimen using an additional molecular method
[k] The single FN specimen was negative for *S. pygogenes* when tested by additional molecular methods
[l] *B. fragilis* was detected in the single FP specimen using an additional molecular method
[m] *E. cloacae* complex was detected in 1/2 FN specimens using an additional molecular method
[n] *E. coli* was detected in the single FP specimen using an additional molecular method
[o] *H. influenzae* was detected in the single FP specimen using an additional molecular method
[p] *K. kingae* was detected in all six FP specimens using an additional molecular methods
[q] *K. pneumoniae* group was detected in the single FN specimen and in the single FP specimen using an additional molecular method
[r] *M. morganii* was detected in both FP specimens using an additional molecular method
[s] *N. gonorrhoeae* was detected in all three FP specimens using an additional molecular method
[t] *Proteus* spp. was detected in all four FP specimens using an additional molecular method
[u] *P. aeruginosa* was detected in all three FP specimens using an additional molecular method
[v] *S. marcescens* was detected in the single FP specimen using an additional molecular method
[w] *Candida* was detected in 2/3 FN specimens and in the single FP specimen using an additional molecular method
[x] Candida albicans was detected in 1/2 FN specimens using an additional molecular method

BioFire JI Panel Genus and Group level organism assay performance is stratified by species for *Anaerococcus prevotii/vaginalis*, *Peptoniphilus*, *Streptococcus* spp., *Citrobacter*, *Enterobacter cloacae* complex, *Klebsiella pneumoniae* group, *Proteus* spp., and *Candida* in Table 50. Note: multiple organisms from a group may be detected in a single specimen, therefore the 'Total' values in these tables may not match the performance values presented above, which are reported per specimen.

Table 57. Sensitivity of the BioFire JI Panel Species Inclusive Assays Stratified by Species

Species	BioFire JI Panel Sensitivity
Anaerococcus prevotii/vaginalis	
A. vaginalis	1/1 (100%)
***Peptoniphilus* spp.**	
P. asaccharolyticus	1/1 (100%)
***Streptococcus* spp.**	
S. agalactiae	10/11 (90.9%)
S. anginosus	1/1 (100%)
S. anginosus group	1/1 (100%)
S. constellatus	1/1 (100%)
S. dysgalactiae	7/8 (85.7%)
S. gallolyticus	0/1 (0%)

S. gordonii	2/2 (100%)
S. mitis	1/1 (100%)
S. oralis	1/1 (100%)
S. pneumoniae + *S. pyogenes*	1/1 (100%)
S. pneumoniae	2/2 (100%)
S. pyogenes	10/11 (90.9%)
S. salivarius/vestibularis group	1/1 (100%)
Viridans streptococci	1/2 (50%)
Total *Streptococcus* species	38/44 (86.4%)
***Citrobacter* spp.**	
C. freundii	1/1 (100%)
C. koseri	1/1 (100%)
Total *Citrobacter* species	2/2 (100%)
***Enterobacter coacae* complex**	
E. cloacae	1/2 (50%)
E. cloacae complex	1/2 (50%)
Total *E. cloacae* species	2/4 (50%)
***Klebsiella pneumoniae* group**	
K. pneumoniae	4/5 (80%)
***Proteus* spp.**	
P. mirabilis	4/4 (100%)
***Candida* spp.**	
C. albicans	3/5 (60.0%)
C. parapsilosis	1/2 (50%)
Total *Candida* species	4/7 (57.1%)

Antimicrobial Resistance Genes

AMR gene results are reported only when one or more applicable bacteria that may carry the gene are also detected in the sample. If no applicable bacteria are detected, the AMR gene results are reported as Not Applicable (N/A). The results are summarized for each AMR gene in Table 51.

Table 58: BioFire Joint Infection Panel Prospective Clinical Performance Summary – AMR Genes

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
CTX-M	5/5	100	56.6-100%	33/33	100	89.6-100%
IMP	0/0	-	-	38/38	100	90.8-100%
KPC	0/0	-	-	40/40	100	91.2-100%
mecA/C and MREJ (MRSA)	19/19	100	83.2-100%	90/94[a]	95.7	89.6-98.3%
NDM	0/0	-	-	40/40	100	91.2-100%

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
OXA-48-like	1/1	100	-	33/33	100	89.6-100%
vanA/B	3/3	100	43.9-100%	14/14	100	78.5-100%
VIM	0/0	-	-	38/38	100	90.8-100%

[a] Three out of the four FP specimens evidence of mecA/C AMR gene was observed in the isolates using independent molecular method with sequencing. Additionally, SOC testing confirmed an AST phenotype of methicillin resistant for all three isolates. The mecA/c and MREJ AMR genes were found in the remaining FP specimen using independent molecular methods.

AMR gene results are reported only when one or more applicable bacteria that may carry the gene are also detected in the sample. If no applicable bacteria are detected, the AMR gene results are reported as Not Applicable (N/A). The results are summarized for each AMR gene in Table 59 through Table 74. Note: the 'Performance Summary' tables below do not include specimens for which an applicable bacteria was not reported (i.e. the AMR gene was reported as N/A); these specimens are instead accounted for in the 'Distribution of Clinical Specimens' tables below.

Table 59: Distribution of *mecA/C* and MREJ in Clinical Specimens

S. aureus *mecA/C* and MREJ		SoC: S. aureus PCR/seq: *mecA/C*			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	15	3	5	23
	Org+ / Res-	0	73	17	90
	Org -	0	7	1393	1400
	Total	15	83	1415	1513[a]
Performance			Agreement	%	95%CI
Org+ / Res+			15/15	100%	79.6-100%
Org+ / Res-			73/83	88.0%	79.2-93.3%
Org -			1393/1415	98.4%	97.7-99.0%%
Interpretation			PPA	NPA	Prevalence
MRSA			(b)(4)	(b)(4)	(b)(4)
MSSA					
S. aureus					

[a]Thirty-one (31) specimens excluded from molecular analysis for mecA/C and MREJ (MRSA) due to volume constraints either initially or following a failure during comparator testing

Table 60. Stratification of mecA/C and MREJ by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	N	Positive Percent Agreement		Negative Percent Agreement	
		%	95% CI	%	95% CI
Staphylococcus aureus	98	100% (19/19)	83.2-100%	95.7% (90/94)	89.6-98.3%

Table 61. Distribution of VIM in Clinical Specimens

VIM	SoC: Applicable Bacteria PCR/seq: VIM

		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	0	0	0	0
	Org+ / Res-	0	28[a]	10	38
	Org -	0	3	1472	1475
	Total	0	31	1482	1513[b]
Performance			Agreement	%	95%CI
Org+ / Res+			0/0	-	-
Org+ / Res-			28/31	90.3	75.1-96.7%
Org -			1472/1482	99.3%	98.8-99.6%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.
[b]Thirty-one (31) specimens were excluded from molecular analysis for VIM due to volume constraints either initially or following a failure during comparator testing

Table 62. Stratification of VIM Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	- (0/0)	-	100% (38/38[a])	90.8-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	- (0/0)	-	100% (15/15)	79.6-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	- (0/0)	-	100% (5/5)	56.6-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (4/4)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (2/2)	34.2-100%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

Table 63. Distribution of CTX-M in Clinical Specimens

CTX-M		SoC: Applicable Bacteria PCR/seq: CTX-M			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	5	0	0	0
	Org+ / Res-	0	23[a]	10	33
	Org -	0	3	1472	1475

	Total	0	26	1482	1513[b]
Performance			**Agreement**	**%**	**95%CI**
Org+ / Res+			5/5	100%	56.6-100%
Org+ / Res-			23/26	88.5%	71.0-96.0%
Org -			1472/1482	99.3%	98.8-99.6%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.
[b]Thirty-one (31) specimens excluded from tMol analysis for CTX-M due to volume constraints either initially or following a failure during comparator testing

Table 64. Stratification of CTX-M Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	**Positive Percent Agreement**		**Negative Percent Agreement**	
	%	**95% CI**	**%**	**95% CI**
Overall (any applicable bacteria Detected)	100 (5/5)	56.6-100%	100% (33/33[a])	89.6-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	100 (2/2)	34.2-100%	100% (13/13)	77.2-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	100 (3/3)	43.9-100%	100% (2/2)	34.2-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (4/4)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (2/2)	34.2-100%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

Table 65. Distribution of IMP in Clinical Specimens

IMP		**SoC: Applicable Bacteria PCR/seq: IMP**			
		Org+ / Res+	**Org+ / Res-**	**Org -**	**Total**
JI Panel Result	**Org+ / Res+**	0	0	0	0
	Org+ / Res-	0	28[a]	10	38
	Org -	0	3	1472	1475
	Total	0	31	1482	1513[b]
Performance			**Agreement**	**%**	**95%CI**
Org+ / Res+			0/0	-	-

Org+ / Res-	28/31	90.3%	75.1-96.7%
Org -	1472/1482	99.3%	98.8-99.6%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.
[b]Thirty-one (31) specimens excluded from molecular analysis for IMP due to volume constraints either initially or following a failure during comparator testing

Table 66. Stratification of IMP Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	- (0/0)	-	100% (38/38[a])	90.8-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	- (0/0)	-	100% (15/15)	79.6-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	- (0/0)	-	100% (5/5)	56.6-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (4/4)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (2/2)	34.2-100%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

Table 67. Distribution of OXA-48-like in Clinical Specimens

OXA-48-like		SoC: Applicable Bacteria PCR/seq: OXA-48-like			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	1	0	0	1
	Org+ / Res-	0	25[a]	8	33
	Org -	0	3	1476	1479
	Total	1	28	1484	1513[b]
Performance			Agreement	%	95%CI
Org+ / Res+			1/1	100%	-
Org+ / Res-			25/28	89.3%	72.8-96.3%
Org -			1476/1484	99.5%	98.9-99.7%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

[b]Thirty-one (31) specimens excluded from molecular analysis for OXA-48-like due to volume constraints either initially or following a failure during comparator testing.

Table 68. Stratification of OXA-48-like Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	- (0/0)	-	100% (33/33[a])	89.6-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	- (0/0)	-	100% (15/15)	79.6-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	100 (1/1)	-	100% (4/4)	51.0-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (4/4)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (2/2)	34.2-100%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

Table 69. Distribution of *vanA/B* in Clinical Specimens

vanA/B		SoC: Applicable Bacteria PCR/seq: *vanA/B*			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	1	0	2	3
	Org+ / Res-	0	9	5	14
	Org -	0	0	1496	1496
	Total	1	9	1503	1513[a]
Performance			Agreement	%	95%CI
Org+ / Res+			1/1	100%	-
Org+ / Res-			9/9	100%	70.1-100%
Org -			1496/1503	99.5%	99.0-99.8%

[a]Thirty-one specimens excluded from molecular analysis for *vanA/B* due to volume constraints either initially or following a failure during comparator testing

Table 70. Stratification of *vanA/B* Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	100 (3/3)	43.9-100	100% (14/14)	78.5-100%
Enterococcus faecalis	- (0/0)	-	100% (14/14)	78.5-100%
Enterobacter cloacae complex	100 (3/3)	43.9-100	- (0/0)	-

Table 71. Distribution of NDM in Clinical Specimens

NDM		SoC: Applicable Bacteria PCR/seq: NDM			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	0	0	0	0
	Org+ / Res-	0	29[a]	11	40
	Org -	0	3	1488	1491
	Total	0	32	1499	1531[b]
Performance			Agreement	%	95%CI
Org+ / Res+			0/0	0%	-
Org+ / Res-			29/32	93.5%	75.8-96.8%
Org -			1488/1499	99.3%	98.7-99.6%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

[b]Thirteen (13) specimens were excluded from molecular analysis for NDM due to volume constraints during comparator testing

Table 72. Stratification of NDM Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	- (0/0)	-	100% (40/40)[a]	91.2-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	- (0/0)	-	100% (15/15)	79.6-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	- (0/0)	-	100% (5/5)	56.6-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (4/4)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (2/2)	34.2-100%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

Table 73. Distribution of KPC in Clinical Specimens

KPC		SoC: Applicable Bacteria PCR/seq: KPC			
		Org+ / Res+	Org+ / Res-	Org -	Total
JI Panel Result	Org+ / Res+	0	0	0	0
	Org+ / Res-	0	29[a]	11	40
	Org -	0	2	1488	1491
	Total	0	31	1499	1531[b]
Performance			Agreement	%	95%CI
Org+ / Res+			0/0	0%	-
Org+ / Res-			29/31	93.5%	79.3-98.2%
Org -			1488/1499	99.3%	98.7-99.6%

[a]Two specimens had co-detection of *Escherichia coli* with *Proteus* spp.; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii*; one specimen had co-detection of *Klebsiella pneumoniae* group with *Morganella morganii* and *Proteus* spp.

[b]Thirteen (13) specimens were excluded from molecular analysis for KPC due to volume constraints during comparator testing

Table 74. Stratification of KPC Clinical Performance by Applicable Host Organism

Applicable Bacteria Result (JI Panel)	Positive Percent Agreement		Negative Percent Agreement	
	%	95% CI	%	95% CI
Overall (any applicable bacteria Detected)	- (0/0)	-	100% (40/40)[a]	91.2-100%
Citrobacter	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	- (0/0)	-	100% (4/4)	51.0-100%
Escherichia coli	- (0/0)	-	100% (15/15)	79.6-100%
Klebsiella aerogenes	- (0/0)	-	- (0/0)	-
Klebsiella pneumoniae group	- (0/0)	-	100% (5/5)	56.6-100%
Morganella morganii	- (0/0)	-	100% (3/3)	43.9-100%
Proteus spp.	- (0/0)	-	100% (8/8)	67.6-100%
Pseudomonas aeruginosa	- (0/0)	-	100% (5/5)	51.0-100%
Salmonella spp.	- (0/0)	-	- (0/0)	-
Serratia marcescens	- (0/0)	-	100% (3/3)	34.2-100%

The BioFire JI Panel AMR gene reporting in the specimen was also compared to phenotypic antimicrobial susceptibility testing (AST) methods performed on organism isolates recovered from those specimens. The results presented in Table 75 through Table 78 are only for specimens with concordant (true positive) results, and are further stratified by each applicable host organism recovered from that specimen. Note that antimicrobial resistance, particularly extended-spectrum β-lactamase (ESBL) activity and carbapenem resistance, may be due to mechanisms other than the presence of the AMR genes detected by the BioFire JI Panel; conversely, detection of these genes may not always indicate an antimicrobial resistance phenotype. Additionally, discordant results between *mecA/C* and MREJ (MRSA) detection in a SF specimen by the BioFire JI Panel and the observed methicillin (oxacillin/cefoxitin) resistance of cultured *Staphylococcus aureus* isolates may be due to polymicrobial *Staphylococcus aureus* cultures containing a mixture of resistant and sensitive organisms.

Table 75. CTX-M Performance (compared to phenotypic AST methods for ESBL activity on cultured isolate(s) from SF specimens)

Organism Identified by SOC and Detected by BioFire JI Panel	N		Positive Percent Agreement		Negative Percent Agreement	
	ESBL	Non-ESBL	%	95% CI	%	95% CI
Overall *(any applicable bacteria Detected)*	7	24	71.4% (5/7)	35.9-91.8%	100% (24/24)	86.2-100%
Citrobacter	0	2	- (0/0)	-	100% (2/2)	34.2-100%
Enterobacter cloacae complex	0	2	- (0/0)	-	100% (2/2)	34.2-100%
Escherichia coli	2	12	100% (2/2)	34.2-100%	100 (12/12)	75.8-100%
Klebsiella aerogenes	0	0	- (0/0)	- (0/0)	- (0/0)	- (0/0)
Klebsiella pneumoniae group	4	0	75.0% (3/4)	30.1-95.4%	- (0/0)	-
Morganella morganii	0	1	- (0/0)	-	100% (1/1)	-
Proteus spp.	1	3	0 (0/1)	-	100% (3/3)	43.9-100%
Pseudomonas aeruginosa	0	2	- (0/0)	-	100% (2/2)	34.2-100%
Salmonella spp.	0	0	- (0/0)	- (0/0)	- (0/0)	- (0/0)
Serratia marcescens	0	2	- (0/0)	-	100% (2/2)	34.2-100%

Table 76. Carbapenem Resistance Genes Performance (as compared to phenotypic AST methods for carbapenem resistance on cultured isolate(s) from SF specimens).

Organism Identified by SOC and Detected by JI Panel	N		IMP		KPC		NDM		OXA-48-like		VIM		*Overall* (any resistance gene)	
	R	S	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
Overall *(any applicable bacteria Detected)*	1	30	0% (0/1)	100% (30/30)	0% (0/1)	100% (30/30)	0% (0/1)	100% (30/30)	100% (1/1)	100% (28/28)	0% (0/1)	100% (30/30)	100% (1/1)	100% (30/30)
Citrobacter	0	2	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)
Enterobacter cloacae complex	0	2	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)
Escherichia coli	2	12	- (0/0)	100% (14/14)	- (0/0)	100% (14/14)	- (0/0)	100% (14/14)	- (0/0)	100% (14/14)	- (0/0)	100% (14/14)	- (0/0)	100% (14/14)
Klebsiella aerogenes	0	0	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)
Klebsiella pneumoniae group	4	0	0% (0/1)	100% (3/3)	0% (0/1)	100% (3/3)	0% (0/1)	100% (3/3)	100% (1/1)	100% (3/3)	0% (0/1)	100% (3/3)	100% (1/1)	100% (3/3)
Morganella morganii	0	1	- (0/0)	100% (1/1)	- (0/0)	100% (1/1)	- (0/0)	100% (1/1)	- (0/0)	100% (1/1)	- (0/0)	100% (1/1)	- (0/0)	100% (1/1)
Proteus spp.	1	3	- (0/0)	100% (4/4)	- (0/0)	100% (4/4)	- (0/0)	100% (4/4)	- (0/0)	100% (4/4)	- (0/0)	100% (4/4)	- (0/0)	100% (4/4)
Pseudomonas aeruginosa	0	2	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	N/A	N/A	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)
Salmonella spp.	0	0	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)	- (0/0)
Serratia marcescens	0	2	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)	- (0/0)	100% (2/2)

Table 77. mecA/C and MREJ (MRSA) Performance (compared to phenotypic AST methods for methicillin (oxacillin/cefotoxitin) resistance on cultured isolates from SF specimens.

Organism Identified by SOC and Detected by BioFire JI Panel	N		Positive Percent Agreement		Negative Percent Agreement	
	R	S	%	95% CI	%	95% CI
Staphylococcus aureus	22	76	81.8 (18/22)	61.5-92.7%	100% (76/76)	95.2-100%

Table 78. *vanA/B* Performance (as compared to phenotypic AST methods for vancomycin resistance on cultured isolates from SF specimens.

Organism Identified by SOC and Detected by BioFire JI Panel	N		Positive Percent Agreement		Negative Percent Agreement	
	R	S	%	95% CI	%	95% CI
Overall *(any applicable bacteria Detected)*	0	11	- (0/0)	-	90.9% (10/11)	62.3-98.4%
Enterococcus faecalis	0	10	- (0/0)	-	100% (10/10)	72.2-100%
Enterococcus faecium	0	1	- (0/0)	-	100% (0/1)	-

Archived Specimen Study

Many analytes on the BioFire Joint Infection (JI) Panel were of low prevalence during the prospective study and were not encountered in large enough numbers to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective synovial fluid specimens was performed at BioFire.

A total of 134 frozen archived specimens were obtained from external laboratories for testing in this evaluation; 107 specimens were expected to contain a single analyte of interest, 14 specimens were expected to contain two analytes of interest, and 13 specimens were expected to be negative for all analytes of interest. Twenty-five (25) specimens were excluded from performance analysis due to low volume (23), because they were found to be the wrong specimen type (1), or because they were discovered to be a duplicated specimen. The remaining 97 expected positives and 12 expected negatives were further analyzed.

Prior to testing with the BioFire JI Panel, the composition/integrity of the laboratory-identified analytes in archived specimens was first confirmed with confirmatory molecular methods. Confirmation testing verified the presence of 93 out of 109 expected analytes (93/109; 85.3%) in a total of 88 of the 97 expected positive specimens. Specimens with unconfirmed (or unexpected) analytes were excluded from performance calculations for that particular analyte.

Table 79: BioFire Joint Infection Panel Performance Summary for Confirmed Archived Specimens

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
Gram Positive Bacteria						
Cutibacterium avidum/granulosum	3/3	100	43.9-100%	4/4	100	51.0-100%
Enterococcus faecalis	8/8	100	67.6-100%	92/92	100	96.0-100%
Enterococcus faeciu	1/1	100	-	100/100	100	96.3-100%
Staphylococcus lugdunensis	8/8	100	67.6-100%	94/94	100	96.1-100%
Streptococcus agalactiae[j]	15/16	93.8	71.7-98.9%	81/81	100	95.5-100%
Streptococcus pneumoniae	1/1	100	-	101/101	100	96.3-100%
Streptococcus pyogenes	3/3	100	43.9-100%	98/98	100	96.2-100%
Gram Negative Bacteria						

Analyte	Sensitivity/PPA			Specificity/NPA		
	TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
***Enterobacter cloacae* complex**	8/9	88.9	56.5-98.0	86/86	100	95.7-100%
Escherichia coli	9/9	100	70.1-100%	91/91	100	95.9-100%
Haemophilus influenzae	1/1	100	-	6/6	100	61.0-100%
Kingella kingae	1/1	100	-	100/100	100	96.3-100%
Klebsiella aerogenes	1/1	100	-	101/101	100	96.3-100%
***Klebsiella pneumoniae* group**	3/3	100	43.9-100%	98/98	100	96.2-100%
Neisseria gonorrhoeae	2/2	100	34.2-100%	100/100	100	96.3-100%
***Proteus* spp.**	3/3	100	43.9-100%	97/97	100	96.2-100%
Pseudomonas aeruginosa	13/13	100	77.2-100%	87/87	100	95.8-100%
***Salmonella* spp.**	3/3	100	43.9-100%	98/98	100	96.2-100%
Serratia marcescens	2/2	100	34.2-100%	99/99	100	96.3-100%
Yeast						
Candida	2/2	100	34.2-100%	100/100	100	96.3-100%
Candida albicans	1/1	100	-	101/101	100	96.3-100%
AMR Genes						
CTX-M	3/3	100	43.9-100%	32/32	100	89.3-100%

Contrived Specimen Testing

Some analytes were of insufficient prevalence in the prospective and archived specimen evaluations to adequately demonstrate system performance. Therefore, contrived clinical specimens were created to evaluate the performance of the BioFire JI Panel assays for these rare analytes. Note that results for mecA/C and MREJ (MRSA) was not rare in the prospective study, but was included in this study for the evaluation of the rare antimicrobial gene mecC. Contrived specimens (N=1235) were spiked using residual clinical samples that were pre-screened and characterized as negative for the analytes of interest. Specimens were spiked with a variety of different isolates/strains for each organism at concentrations that spanned the detection range of each assay such that approximately 50% of specimens were spiked at a near-LoD test level (i.e. within ~2-fold of the assay LoD). Due to changes in the methods used for organism quantification over the course of the study, specimens were also spiked with analytes at levels below the established LoD for each assay.

Different isolates of organisms were used from those used in analytical testing when possible. Samples positive for one analyte served as negatives for other analytes. Eighty-one (81) negative (unspiked) samples were also randomized with the spiked specimens to facilitate specimen blinding.

The results of the 1235 specimens tested in this study are summarized in Table 80 below

Table 80. BioFire Joint Infection Panel Performance Summary for Contrived Specimen Testing

Analyte	Level Tested	Sensitivity/PPA			Specificity/NPA		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
Gram Positive Bacteria							
Anaerococcus prevotii/vaginalis[a,b]	≥ LoD	83/93	89.2	81.3-94.1%	1125/1125	100	99.7-100%
	Overall	83/95	87.4	79.2-92.6%			
Clostridium perfringens[c]	≥ LoD	92/102	90.2	82.9-94.6%	1101/1101	100	99.7-100%
	Overall	113/134	84.3	77.2-89.5%			
Cutibacterium avidum/granulosum[d]	≥ LoD	74/82	90.2	81.9-95.0%	1128/1128	100	99.7-100%
	Overall	80/107	74.8	65.8-82.0%			
Enterococcus faecalis	≥ LoD	51/51	100	93.0-100%	1182/1182	100	99.5-100%
	Overall	53/53	100	93.2-100			
Enterococcus faecium[e]	≥ LoD	63/65	96.9	89.5-99.2%	1169/1170[f]	99.9	99.5-100%
	Overall	63/65	96.9	89.5-99.2%			
Finegoldia magna[g]	≥ LoD	78/87	89.7	92.9-100%	1142/1142	100	99.7-100%
	Overall	82/93	88.2	80.1-93.3%			
Parvimonas micra[h]	≥ LoD	52/57	91.2	81.1-96.2%	1158/1158	100	99.7-100%
	Overall	54/77	70.1	59.2-79.2%			
Peptoniphilus[i]	≥ LoD	56/61	91.8	82.2-96.4%	1173/1173	100	99.7-100%
	Overall	57/62	91.9	82.5-96.5%			
Peptostreptococcus anaerobius[j]	≥ LoD	91/91	100	95.9-100%	1135/1135	100	99.7-100%
	Overall	98/100	98.0	93.0-99.4%			
Staphylococcus lugdunensis[k]	≥ LoD	46/48	95.8	86.0-98.8%	1184/1185[f]	99.9	99.5-100%
	Overall	48/50	96.0	86.5-98.9%			
Streptococcus agalactiae	≥ LoD	58/58	100	93.8-100%	1175/1175	100	99.7-100%
	Overall	59/59	100	93.9-100%			
Streptococcus pneumoniae[l]	≥ LoD	70/76	92.1	83.8-96.3%	1152/1157[f]	99.6	99.0-99.8%
	Overall	70/78	89.7	81.0-94.7%			
Streptococcus pyogenes[m]	≥ LoD	64/65	98.5	91.8-99.7%	1170/1170	100	99.7-100%
	Overall	64/65	98.5	91.8-99.7%			
Gram Negative Bacteria							
Bacteroides fragilis[n]	≥ LoD	95/95	100	96.1-100%	1125/1125	100	99.7-100%
	Overall	98/100	98.0	93.0-99.4%			
Citrobacter[o]	≥ LoD	67/69	97.1	90.0-99.2%	1165/1165	100	99.7-100%
	Overall	67/70	95.7	88.1-98.5%			
***Enterobacter cloacae* complex**	≥ LoD	48/48	100	92.6-100%	1185/1185	100	99.7-100%
	Overall	50/50	100	92.9-100%			
Escherichia coli	≥ LoD	75/75	100	95.1-100%	1158/1158	100	99.7-100%
	Overall	75/75	100	95.1-100%			
Haemophilus influenzae[p]	≥ LoD	52/53	98.1	90.1-99.7%	1180/1180	100	99.7-100%
	Overall	53/55	96.4	87.7-99.0			
Kingella kingae	≥ LoD	48/48	100	92.6-100%	1185/1185	100	99.5-100%
	Overall	50/50	100	92.9-100%			
Klebsiella aerogenes[q]	≥ LoD	97/97	100	96.2-100%	1135/1135	100	99.7-100%
	Overall	99/100	99.0	94.6-99.8%			
***Klebsiella pneumoniae* group**	≥ LoD	93/93	100	96.0-100%	1141/1141	100	99.7-100%
	Overall	94/94	100	96.1-100%			
Morganella morganii[r]	≥ LoD	59/63	93.7	84.8-97.5%	1171/1171	100	99.7-100%
	Overall	59/64	92.2	83.0-96.6%			
Neisseria gonorrhoeae[s]	≥ LoD	46/48	95.8	86.0-98.8%	1178/1179[f]	99.9	99.5-100%

Analyte	Level Tested	Sensitivity/PPA			Specificity/NPA		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
	Overall	47/50	94.0	83.0-96.6%			
***Proteus* spp.**[t]	≥ LoD	52/52	100	93.1-100%	1182/1182	100	99.7-100%
	Overall	52/53	98.1	90.1-99.7%			
Pseudomonas aeruginosa[u]	≥ LoD	117/119	98.3	94.1-99.5%	1105/1105	100	99.7-100%
	Overall	121/125	96.8	92.1-98.7%			
***Salmonella* spp.**[v]	≥ LoD	57/60	100	92.9-100%	1173/1173	100	99.7-100%
	Overall	59/62	95.2	86.7-98.3%			
Serratia marcescens[w]	≥ LoD	53/54	98.1	90.2-99.7%	1179/1179	100	99.7-100%
	Overall	54/56	96.4	87.9-99.0%			
Yeast							
Candida[x]	≥ LoD	102/105	97.1	91.9-99.0%	1126/1126	100	99.7-100%
	Overall	105/109	96.3	90.9-98.6%			
Candida albicans[y]	≥ LoD	50/51	98.0	89.7-99.7%	1182/1182	100	99.5-100%
	Overall	52/53	98.1	90.1-99.7%			

[a]Sequence variation in *A. vaginalis* isolates result in impaired detection near the LoD of the assay, See Table 52.
[b]Ten *Anaerococcus prevotii/vaginalis* FN were observed at or above LoD and two FN were observed below LoD.
[c]Ten *Clostridium perfringens* FN were observed at or above LoD and 11 FN were observed below LoD.
[d]Eight *Cutibacterium avidum/granulosum* FN were observed at or above LoD and 19 FN were observed below LoD.
[e]Both *Enterococcus faecium* FN were observed at or above LoD.
[f]FP results due to background contamination in the matrix used for spiking.
[g]Nine *Finegoldia magna* FN were observed at or above LoD and two FN were observed below LoD.
[h]Five *Parvimonas micra* FN were observed at or above LoD and 18 FN were observed below LoD.
[i]Five *Peptoniphilus* FN were observed at or above LoD.
[j]Both *Peptostreptococcus anaerobius* FN were observed below LoD.
[k]Both *Staphylococcus lugdunensis* FN were observed at or above LoD.
[l]Six *Streptococcus pneumoniae* FN were observed at or above LoD and two FN were observed below LoD.
[m]The *Streptococcus pyogenes* FN was observed above LoD.
[n]Both *Bacteroides fragilis* FN were observed below LoD.
[o]Two *Citrobacter* FN were observed at or above LoD and one FN was observed below LoD.
[p]One *Haemophilus influenzae* FN was observed above LoD and one FN was observed below LoD.
[q]The *Klebsiella aerogenes* FN was observed below LoD.
[r]Four *Morganella morganii* FN were observed at or above LoD and one FN was observed below LoD.
[s]Two *Neisseria gonorrhoeae* FN were observed at or above LoD and one FN was observed below LoD.
[t]The *Proteus* spp. FN was observed below LoD.
[u]Two *Pseudomonas aeruginosa* FN were observed at or above LoD and two FN were observed below LoD.
[v]Three *Salmonella* spp. FN were observed at or above LoD.
[w]One *Serratia marcescens* FN was observed at or above LoD and one FN was observed below LoD.
[x]Three *Candida* FN were observed at or above LoD and one FN was observed below LoD.
[y]The *Candida albicans* FN was observed above LoD.

Table 81. BioFire Joint Infection Panel Performance Summary for Contrived Specimen Testing – AMR Genes

Analyte	Level Tested	Sensitivity/PPA			Specificity/NPA		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
CTX-M[a]	≥ LoD	149/150	99.3	96.3-100%	544/544	100	99.3-100%
	Overall	152/153	99.3	96.4-99.9%			

Analyte	Level Tested	Sensitivity/PPA			Specificity/NPA		
		TP/ (TP + FN)	%	95%CI	TN/ (TN + FP)	%	95%CI
IMP	≥ LoD	90/90	100	95.9-100%	603/603	100	99.4-100%
	Overall	93/93	100	96.0-100%			
KPC	≥ LoD	77/77	100	95.2-100%	618/618	100	99.4-100%
	Overall	79/79	100	95.4-100%			
mecA/C and MREJ (MRSA)[b,c]	≥ LoD	48/48	100	92.6-100%	46/53[d]	86.8	75.2-93.5%
	Overall	49/49	100	92.7-100%			
NDM[e]	≥ LoD	66/67	98.5	92.0-99.7%	629/629	100	99.4-100%
	Overall	66/68	97.1	89.9-99.2%			
OXA-48-like	≥ LoD	64/64	100	94.3-100%	532/532	100	99.3-100%
	Overall	65/65	100	94.4-100%			
vanA/B	≥ LoD	96/96	100	96.2-100%	18/19[d]	94.7	75.4-99.1%
	Overall	98/98	100	96.2-100%			
VIM	≥ LoD	79/79	100	95.4-100%	614/614	100	99.4-100%
	Overall	83/83	100	95.6-100%			

[z]The CTX-M FN was observed above the host organism's LoD.
[b]Results were reported as N/A for the resistance marker because the host organism was reported as Not Detected.
[c]Two different strains of *Staphylococcus aureus* containing *mecC* were used for spiking 50 contrived specimens.
[d]FP results due to background contamination in the matrix used for spiking.
[e]One NDM FN was observed at or above the host organism's LoD and one FN was observed below the host organism's LoD.

12. Clinical Specificity:

See Clinical Sensitivity section above.

13. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

In the prospective clinical evaluation of the BioFire JI Panel, 1544 synovial fluid (SF) specimens were collected and tested at 13 study sites across the United States and Europe over approximately two years (May 2018 to March 2020). Expected values (as determined by the BioFire JI Panel) are stratified by enrollment site in Table 82.

Table 82. Expected Value (EV) as Determined by BioFire JI Panel: Summary by Site for SF Specimens Collected During the BioFire JI Panel Prospective

BioFire JI Panel Result	Overall (N=1544)		Site 1 (N=352)		Site 2 (N=88)		Site 3 (N=201)		Site 4 (N=89)		Site 5 (N=102)		Site 6 (N=87)		Site 7 (N=197)		Site 8 (N=66)		Site 9 (N=146)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Gram Positive Bacteria																				
Anaerococcus prevotii/vaginalis	1	0.1%	0	0%	0	0%	1	0.5%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Clostridium perfringens	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Cutibacterium avidum/granulosum	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Enterococcus faecalis	15	1.0%	4	1.1%	0	0%	6	3.0%	0	0%	0	0%	1	1.1%	1	0.5%	1	1.5%	0	0%
Enterococcus faecium	3	0.2%	1	0.3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1.5%	0	0%
Finegoldia magna	4	0.3%	0	0%	0	0%	2	1.0%	0	0%	0	0%	0	0%	1	0.5%	1	1.5%	0	0%
Parvimonas micra	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Peptoniphilus	2	0.1%	0	0%	0	0%	1	0.5%	0	0%	0	0%	1	1.1%	0	0%	0	0%	0	0%
Peptostreptococcus anaerobius	3	0.2%	1	0.3%	0	0%	1	0.5%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Staphylococcus aureus	120	7.8%	19	5.4%	8	9.1%	16	8.0%	4	4.5%	13	12.7%	20	23.0%	6	3.0%	3	4.5%	12	8.2%
Staphylococcus lugdunensis	5	0.3%	0	0%	0	0%	3	1.5%	0	0%	0	0%	1	1.1%	0	0%	0	0%	1	0.7%
Streptococcus spp.	50	3.2%	6	1.7%	6	6.8%	3	1.5%	3	3.4%	7	6.9%	9	10.3%	4	2.0%	3	4.5%	4	2.7%
Streptococcus agalactiae	11	0.7%	0	0%	2	2.3%	1	0.5%	1	1.1%	0	0%	1	1.1%	2	1.0%	1	1.5%	2	1.4%
Streptococcus pneumoniae	3	0.2%	1	0.3%	0	0%	0	0%	0	0%	1	1.0%	1	1.1%	0	0%	0	0%	0	0%
Streptococcus pyogenes	11	0.7%	2	0.6%	1	1.1%	0	0%	2	2.2%	0	0%	1	1.1%	0	0%	2	3.0%	0	0%
Gram Negative Bacteria																				
Bacteroides fragilis	1	0.1%	1	0.3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Citrobacter	2	0.1%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1.1%	1	0.5%	0	0%	0	0%
Enterobacter cloacae complex	4	0.3%	1	0.3%	0	0%	0	0%	0	0%	0	0%	1	1.1%	1	0.5%	0	0%	1	0.7%
Escherichia coli	15	1.0%	3	0.9%	1	1.1%	4	2.0%	0	0%	1	1.0%	5	5.7%	0	0%	0	0%	1	0.7%
Haemophilus influenzae	2	0.1%	1	0.3%	0	0%	0	0%	0	0%	0	0%	1	1.1%	0	0%	0	0%	0	0%
Kingella kingae	7	0.5%	1	0.3%	0	0%	1	0.5%	0	0%	1	1.0%	0	0%	0	0%	0	0%	0	0%

BioFire JI Panel Result	Overall (N=1544)		Site 1 (N=352)		Site 2 (N=88)		Site 3 (N=201)		Site 4 (N=89)		Site 5 (N=102)		Site 6 (N=87)		Site 7 (N=197)		Site 8 (N=66)		Site 9 (N=146)	
	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV	#	EV
Klebsiella aerogenes	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Klebsiella pneumoniae group	5	0.3%	1	0.3%	0	0%	1	0.5 %	0	0%	1	1.0 %	3	3.4%	0	0%	0	0%	0	0%
Morganella morganii	3	0.2%	0	0%	0	0%	1	0.5 %	0	0%	0	0%	2	2.3%	0	0%	0	0%	0	0%
Neisseria gonorrhoeae	5	0.3%	2	0.6%	0	0%	0	0%	2	2.2%	0	0%	1	1.1%	0	0%	0	0%	0	0%
Proteus spp.	8	0.5%	1	0.3%	0	0%	5	2.5 %	0	0%	0	0%	2	2.3%	0	0%	0	0%	0	0%
Pseudomonas aeruginosa	5	0.3%	1	0.3%	0	0%	2	1.0 %	0	0%	0	0%	0	0%	1	0.5 %	0	0%	1	0.7%
Salmonella spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Serratia marcescens	3	0.2%	1	0.3%	0	0%	0	0%	0	0%	0	0%	1	1.1%	0	0%	0	0%	1	0.7%
AMR Genes																				
CTX-M	5	0.3%	0	0%	0	0%	0	0%	0	0%	1	1.0 %	4	4.6%	0	0%	0	0%	0	0%
IMP	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
KPC	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
mecA/C and MREJ (MRSA)	23	1.5%	4	1.1%	3	3.4%	0	0%	0	0%	0	0%	4	4.6%	0	0%	1	1.5 %	6	4.1%
NDM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
OXA-48-like	1	0.1%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1.1%	0	0%	0	0%	0	0%
vanA/B	3	0.2%	1	0.3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1.5 %	1	0.7%
VIM	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Yeast																				
Candida	5	0.3%	0	0%	1	1.1%	3	1.5 %	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.7%
Candida albicans	3	0.2%	0	0%	0	0%	2	1.0 %	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.7%

BioFire JI Panel Result	Site 10 (N=136)		Site 11 (N=17)		Site 12 (N=49)		Site 13 (N=14)	
	#	EV	#	EV	#	EV	#	EV
Anaerococcus prevotii/vaginalis	0	0%	0	0%	0	0%	0	0%
Clostridium perfringens	0	0%	0	0%	0	0%	0	0%
Cutibacterium avidum/granulosum	0	0%	0	0%	0	0%	0	0%
Enterococcus faecalis	0	0%	0	0%	0	0%	0	0%
Enterococcus faecium	0	0%	0	0%	0	0%	0	0%
Finegoldia magna	0	0%	0	0%	0	0%	0	0%
Parvimonas micra	0	0%	0	0%	0	0%	0	0%
Peptoniphilus	0	0%	0	0%	0	0%	0	0%
Peptostreptococcus anaerobius	0	0%	0	0%	0	0%	0	0%
Staphylococcus aureus	4	2.9%	3	17.6 %	9	18.4 %	3	21.4 %
Staphylococcus lugdunensis	0	0%	0	0%	0	0%	0	0%
Streptococcus spp.	2	1.5%	1	5.9%	1	2.0 %	1	7.1%
Streptococcus agalactiae	1	0.7%	0	0%	0	0%	0	0%
Streptococcus pneumoniae	0	0%	0	0%	0	0%	0	0%
Streptococcus pyogenes	0	0%	1	5.9%	1	2.0 %	1	7.1%
Bacteroides fragilis	0	0%	0	0%	0	0%	0	0%
Citrobacter	0	0%	0	0%	0	0%	0	0%
Enterobacter cloacae complex	0	0%	0	0%	0	0%	0	0%
Escherichia coli	0	0%	0	0%	0	0%	0	0%
Haemophilus influenzae	0	0%	0	0%	0	0%	0	0%
Kingella kingae	0	0%	0	0%	4	8.2 %	0	0%
Klebsiella aerogenes	0	0%	0	0%	0	0%	0	0%
Klebsiella pneumoniae group	0	0%	0	0%	0	0%	0	0%
Morganella morganii	0	0%	0	0%	0	0%	0	0%

BioFire JI Panel Result	Site 10 (N=136)		Site 11 (N=17)		Site 12 (N=49)		Site 13 (N=14)	
	#	EV	#	EV	#	EV	#	EV
Neisseria gonorrhoeae	0	0%	0	0%	0	0%	0	0%
Proteus spp.	0	0%	0	0%	0	0%	0	0%
Pseudomonas aeruginosa	0	0%	0	0%	0	0%	0	0%
Salmonella spp.	0	0%	0	0%	0	0%	0	0%
Serratia marcescens	0	0%	0	0%	0	0%	0	0%
CTX-M	0	0%	0	0%	0	0%	0	0%
IMP	0	0%	0	0%	0	0%	0	0%
KPC	0	0%	0	0%	0	0%	0	0%
mecA/C and MREJ (MRSA)	2	1.5%	0	0%	2	4.1 %	1	7.1%
NDM	0	0%	0	0%	0	0%	0	0%
OXA-48-like	0	0%	0	0%	0	0%	0	0%
vanA/B	0	0%	0	0%	0	0%	0	0%
VIM	0	0%	0	0%	0	0%	0	0%
Candida	0	0%	0	0%	0	0%	0	0%
Candida albicans	0	0%	0	0%	0	0%	0	0%

In addition, the observed multiple detections (as determined by the BioFire JI Panel) during the prospective clinical evaluation are presented in Table 15. At least one organism was detected in a total of 242 SF specimens (15.7% positivity rate; 242/1544). Polymicrobial detections of up to seven organisms were observed.

Table 83: Expected Values Multiple Detections as Determined by the BioFire JI Panel for the BioFIre JI Panel Clinical Evaluation (May 2018 – March 2020)

BioFire JI Panel Organism Result	Expected Value (as Determined by Testing of 1544 Prospective SF Specimens)	
	Number Detected and Reported	% of Total (% of Positives)
Detected (at least one result)	242	15.7% (100%)
One analyte result	226	14.6% (93.4%)
Two analyte results	12	0.8% (5.0%)
Three analyte results	2	0.1% (0.8%)
More than three organism results	2[a]	0.1% (0.8%)

[a]One specimen had six organisms and one specimen had seven organisms observed

The BioFire JI Panel reported a total of 16 specimens with discernible detection of multiple organisms (1.0% of all specimens, 16/1544; and 6.6% of positive specimens, 16/242). The different types of co-detections (categorized by gram stain classification) as reported by the BioFire JI Panel are presented in Table 98 below. The resulting co-detection analyte combinations are presented in Table 99. This table also indicates the number of specimens with false positive (FP) results for each co-detection combination, as well as the specific analytes that were discrepant. FP results were determined by comparison only to the primary comparator method (e.g., standard of care (SOC) culture for organisms, and molecular comparator for the antimicrobial resistance (AMR) genes, irrespective of host organism SOC culture results).

Table 84: Expected Values (Co-detection Types as Determined by the BioFIre JI Panel for the BioFire JI Panel Prospective Clinical Evaluation

BioFire JI Panel Co-Detection Type	Positive Specimens (N =16)	
	#	EV
Gram Positive + Gram Positive	4	25.0%
Gram Positive + Gram Negative	9	56.3%
Gram Positive + Yeast	0	0%
Gram Negative + Gram Negative	2	12.5%
Gram Negative + Yeast`	1	6.3%
Gram Positive + Gram Negative + Yeast	0	0%

Table 85: Co-detection Combinations as Determined by the BioFire JI Panel, Prospective Study

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6	Analyte 7	AMR Gene(s)	Total Specimens with Co-Detection Combination	# Specimens with False Positive Co-Detections[a]	False Positive Analyte(s)
E. faecalis	*F. magna*	*K. pneumoniae group*	*M. morganii*	*Peptoniphilus*	*Proteus* spp.	*P. anaerobius*	-	1	1	*F. magna, M. morganii, Peptoniphilus, P. anaerobius*
E. cloacae complex	*E. faecalis*	*H. influenzae*	*K. Kingae*	*Streptococcus* spp., *S. pneumoniae*	*Streptococcus* spp., *S. pyogenes*	-	-	1	1	*H. influenzae, K. kingae*
A. prevotii/vaginalis	*F. magna*	*Streptococcus* spp.	-	-	-	-	-	1	0	-
E. coli	*E. faecium*	*Proteus* spp.	-	-	-	-	*vanA/B*	1	1	*E. faecium, Proteus* spp.
B. fragilis	*P. anaerobius*	-	-	-	-	-	-	1	1	*B. fragilis, P. anaerobius*
Candida, C. albicans	*E. cloacae* complex	-	-	-	-	-	-	1	1	*E. cloacae* complex
E. faecalis	*Proteus* spp.	-	-	-	-	-	-	1	0	-
E. faecalis	*S. aureus*	-	-	-	-	-	*mecA/C* and MREJ (MRSA)	1	0	-
E. coli	*Proteus* spp.	-	-	-	-	-	-	1	0	-
E. coli	*S. aureus*	-	-	-	-	-	CTX-M, *mecA/C*, and MREJ (MRSA)	1	1	*S. aureus*
K. kingae	*S. aureus*	-	-	-	-	-	-	1	1	*K. kingae, S. aureus*
K. pneumoniae group	*M. morganii*	-	-	-	-	-	-	1	1	*K. pneumoniae* group, *M. morganii*
Proteus spp.	*S. lugdunensis*	-	-	-	-	-	-	1	1	*Proteus* spp., *S. lungdunensis*
S. marcescens	*S. aureus*	-	-	-	-	-	-	1	1	*S. marcescens*
S. aureus	*Streptococcus* spp., *S. agalactiae*	-	-	-	-	-	-	1	0	-
S. aureus	*Streptococcus* spp., *S. pyogenes*	-	-	-	-	-	-	1	0	-
							Total Co-Detections	**16**	**11**	**21/43[a]**
							Total Double Detections	**12**	**8**	**13/24**
							Total Quintuple Detections	**2**	**1**	**2/6**
							Total Sextuple Detections	**1**	**1**	**2/6**

[a] Determined by comparison to SOC culture for organisms, and molecular methods for AMR genes, irrespective of host organism SOC culture results

[b] Of the 21 discrepant analytes (out of 43 total analytes), 20 (95.2%) were confirmed as being present in the specimen during discrepancy investigation: 2/20 (10.0%) were identified from additional laboratory testing performed as SOC and 18/20 (90.0%) were observed using an independent molecular method (b)(4) were detected by qMol, (b)(4) were detected using an additional molecular method, and the remaining (b)(4) were identified in SOC culture.

F Other Supportive Performance Characteristics Data:

Not applicable.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Identified Risks to Health	Mitigation Measures
Risk of false test results leading to improper patient management	Use of certain specimen collection devices identified in special control (1). Certain labeling information identified in special control (2), including limitations, warnings, device descriptions, explanation of procedures, and performance information identified in special controls (3)(iii) and (3)(iv). Certain design verification and validation identified in special control (3), including documentation of certain analytical studies and clinical studies and device descriptions.
Failure to correctly interpret test results leading to misdiagnosis and associated risk of false test results	Certain labeling information identified in special control (2), including limitations, warnings, device descriptions, explanation of procedures, and performance information identified in special controls (3)(iii) and (3)(iv). Certain design verification and validation identified in special control (3), including documentation of certain analytical studies and clinical studies and device descriptions.
Failure to correctly operate the device leading to false test results or incorrect interpretation of test results	Use of certain specimen collection devices identified in special control (1). Certain labeling information identified in special control (2), including limitations, warnings, device descriptions, explanation of procedures, and performance information identified in special controls (3)(iii) and (3)(iv). Certain design verification and validation identified in special control (3), including documentation of certain analytical studies and clinical studies and device descriptions.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The benefit of the assay is aiding in the accurate diagnosis of specific agents of joint infections in conjunction with other clinical and laboratory findings. The BioFire Joint Infection Panel

simultaneously detects and identifies nucleic acids from multiple different pathogens as well as eight antimicrobial resistance genes in a platform that provides results in about an hour which is a significant improvement over standard microbiological methods. Aiding in the diagnosis of specific agents of joint infection and identification of genes associated with antimicrobial resistant strains may identify patients for which treatment may be appropriate, including, but not limited to, antibiotic therapy and revision surgery, if the infection is tied to a prosthetic joint. Appropriate treatment of prosthetic joint infection can lead to alleviation of symptoms associated with infection and restoration of joint function.

B Summary of the Assessment of Risk:

The risks associated with the device, when used as intended, are those related to the risk of false test results, the failure to correctly interpret test results, and failure to correctly operate the device.

The risk of a false positive test result is improper patient management, including inappropriate administration of unnecessary antibiotics or anti-fungal medications. Inappropriate administration of antibiotics is associated with toxicity, allergic reactions, and other adverse outcomes including secondary infections such as *C. difficile* colitis.

The risk of a false negative test result is delayed identification of the cause of the disease in the patient, which could lead to improper patient management, including administration of unnecessary treatment and/or delay or discontinuation of appropriate treatment. An undiagnosed infection or delayed diagnosis could result in increased morbidity and mortality.

Failure to correctly operate the device can lead to false test results. Failure to correctly interpret test results can lead to erroneous results (i.e., false positives, false negatives), with the same risks discussed above.

C Patient Perspectives:

Not applicable.

D Summary of the Assessment of Benefit-Risk:

General controls are insufficient to mitigate the risks associated with the device. However, the clinical benefits outweigh the risks for the proposed assay, considering the mitigations of the risks provided by the special controls established for this device, as well as general controls. The required special controls will help ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. The clinical performance observed in the clinical trial suggests that errors will be uncommon and that the assay will provide substantial benefits to patients in the diagnosis of joint infection and when used in conjunction with other clinical and diagnostic findings.

The risk of false test results (both positive and negative) is mitigated by the intended use clearly stating that the assay results are intended to be used with other clinical and laboratory findings which include standard of care culture to identify organisms and antimicrobial susceptibility testing. The risk of false results is also mitigated by the inclusion of performance characteristics from analytical and clinical studies in the labeling.

Risks of failure to correctly interpret the test results are mitigated through the inclusion in the labeling of a detailed description of what the device detects, the specimen type for which testing is indicated, the type of results provided to the user in the intended use statement, as well as a detailed explanation of the interpretation of results. Finally, the risk of failure to correctly operate the device is mitigated by the inclusion of detailed directions for use in the package insert, such that the operator can successfully use the instrument.

The clinical performance observed in the clinical studies suggests that errors will be uncommon and that the assay will provide substantial benefits to patients in the diagnosis of joint infection when used in conjunction with other clinical and diagnostic findings.

Given the combination of the device's indications for use, labeling, the required general controls, and the special controls established for this device, the benefits outweigh the risks.

X Conclusion:

The De Novo request is granted and the device is classified under the following:

Product Code(s): QSN
Device Type: Device to detect and identify microorganism nucleic acids and resistance markers from patients with suspected orthopedic infection
Class: II
Regulation:21 CFR 866.3988