Protocols for Laboratory Verification of Performance of the BIOFIRE® Joint Infection (JI) Panel

Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

Purpose

The Clinical Laboratory Improvement Amendments (CLIA), passed in 1988, establishes quality standards for all laboratory testing to ensure the accuracy and reliability of patient test results, regardless of where the test is performed. The CLIA regulations include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA. The BIOFIRE® Joint Infection (JI) Panel has been categorized by the FDA as a CLIA moderate complexity test.

This document provides examples of verification procedures to assist your laboratory in developing a protocol for the verification of the BIOFIRE JI Panel performance on BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® Torch Systems. Verification schemes compatible with the BIOFIRE JI Panel have been designed using nonclinical specimens. The methods described provide positive and negative tests for each organism detected by the BIOFIRE JI Panel and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each sample on two separate days. To evaluate user-to-user variation, multiple laboratory operators may test the same sample. In addition, testing patient samples for verification of the performance of the BIOFIRE JI Panel should be done under the guidance of the Laboratory Director but is not described here.

As per the CLIA regulation, the Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for CLIA and applicable laboratory accrediting agencies.

BIOFIRE Intended 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:

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
BFR0000-9503-02

QS-339P-01

Gram Positive Bacteria		
Anaerococcus prevotii/vaginalis	*Finegoldia magna*	*Streptococcus* spp.
Clostridium perfringens	*Parvimonas micra*	*Streptococcus agalactiae*
Cutibacterium avidum/granulosum	*Peptoniphilus*	*Streptococcus pneumoniae*
Enterococcus faecalis	*Peptostreptococcus anaerobius*	*Streptococcus pyogenes*
Enterococcus faecium	*Staphylococcus aureus*	
	Staphylococcus lugdunensis	
Gram Negative Bacteria		
Bacteroides fragilis	*Kingella kingae*	*Proteus* spp.
Citrobacter	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*
Enterobacter cloacae complex	*Klebsiella pneumoniae* group	*Salmonella* spp.
Escherichia coli	*Morganella morganii*	*Serratia marcescens*
Haemophilus influenzae	*Neisseria gonorrhoeae*	
Yeast		
Candida *Candida albicans*		

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 to ß-lactams including penicillins, cephalosporins, monobactams, and carbapenems (bla_{CTX-M}, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, 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.

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

The complete intended use statement and additional information about the use of the BIOFIRE System can be found in the *BIOFIRE® Joint Infection (JI) Panel Instructions for Use.*

Performance Verification: Overview

Two different examples of performance verification procedures are described: (1) a Synthetic Matrix Protocol for the verification of the BIOFIRE JI Panel performance in a synthetic background (Negative) provided with the ZeptoMetrix control organisms and (2) a Clinical Matrix Protocol that evaluates the performance of each assay on the BIOFIRE JI Panel in a clinical specimen matrix of synovial fluid.

Note: It is important to characterize clinical specimens for Joint Infection Panel targets by screening the synovial fluid specimen on the BIOFIRE JI Panel prior to starting the verification procedure. The optimal specimen will be negative for all analytes tested on the BIOFIRE JI Panel.

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The procedures have been designed to take advantage of the multiplex nature of the BIOFIRE JI Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described will generate multiple positive and negative detections for each of the BIOFIRE JI Panel assays. The procedures were developed using a NATtrol™ Joint Infection Verification Panel available from ZeptoMetrix LLC, Buffalo, NY (part number NATJIP-BIO).

A BIOFIRE System is defined as all BIOFIRE® FILMARRAY® Modules that are connected to and controlled by a single computer system. If the laboratory director chooses not to perform the entire verification protocol on each individual module, it is advised that test replicates are evenly distributed among the modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here in order to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BIOFIRE JI Panel.

Note: The laboratory should only perform the verification study with analytes that will be reported using the BIOFIRE JI Panel in their laboratory setting.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	Expected Negative Results	Approximate Days of Testing[b]
Synthetic Matrix Protocol	6 or 7	5	4	20	4 per organism	16 per organism	4
Clinical Matrix Protocol	6 or 7	5	4	20	4 per organism	16 per organism	4

[a] Depending on the material used for verification, pooling of organisms may not be appropriate and the values in the table may need to be modified.
[b] The approximate number of days for testing assumes a BIOFIRE® system configured with one module.

Performance Verification: Materials

The following materials may be used to perform the verification procedure:

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BioFire Diagnostics, LLC
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Table 2. Recommended materials for the verification protocols

Material	Part Number
BIOFIRE® Joint Infection (JI) Panel (30-test kit)	BIOFIRE Diagnostics, LLC: RFIT-ASY-0138
BIOFIRE® Joint Infection (JI) Panel Instructions for Use	BIOFIRE Diagnostics, LLC: RFIT-PRT-0690
BIOFIRE® Joint Infection (JI) Panel Quick Guide	BIOFIRE Diagnostics, LLC: RFIT-PRT-0710
Control Organism[a]	ZeptoMetrix: NATJIP-BIO
Synovial fluid[b]	Discovery Life Science, 920000.01 Diagnostic Remnant Samples Synovial Fluid or Lee BioSolutions, 991-42-P Synovial Fluid from Pooled Human Donors
5 mL Sample Tubes	Various manufacturers
1 mL Wide bore pipette tips (for pipetting synovial fluid)	Various manufacturers
1.5 mL Disposable Transfer pipets, graduated	Avantor (formerly VWR): 612-4469 (or equivalent)

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the BIOFIRE JI panel. However, when alternate organism sources are used (i.e. not the ZeptoMetrix NATJIP-BIO material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

[b]For use with the Clinical Matrix protocol; synovial fluid from various clinical or commercial sources may be used. The vendors listed may not be inclusive. The optimal specimen will be negative for all analytes tested on the BIOFIRE Joint Infection Panel.

Performance Verification Protocols

Synthetic Matrix Protocol

The Synthetic Matrix Protocol evaluates the BIOFIRE JI Panel performance when sample material (ZeptoMetrix NATJIP-BIO) is pooled and combined with an equal volume of synthetic matrix/negative provided in the control panel. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

Note: Dilution of ZeptoMetrix organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Figures 1 and 2 (below) illustrate workflow schemes for testing 4 replicates per pool for 5 different pools over multiple days. This produces a total of 20 verification sample test runs and provides 4 positive results and 16 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more samples per day based on the number of modules in the BIOFIRE® System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples may be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation. To evaluate user-to-user variation, multiple laboratory operators may perform testing.

Table 3. Proposed Organism Pooling Scheme

Organism and Resistance Genes	Approximate Organism Volume	Approximate Volume of Synthetic or Clinical Matrix	Approximate Pool Volume
Pool 1			
Candida albicans	0.2 mL	1.4 mL	2.8 mL
Enterococcus faecalis (vanB)	0.2 mL		
Enterococcus faecium (vanA)	0.2 mL		
Finegoldia magna	0.2 mL		
Morganella morganii	0.2 mL		
Streptococcus agalactiae	0.2 mL		
Streptococcus pyogenes	0.2 mL		
Pool 2			
Enterobacter cloacae	0.2 mL	1.2 mL	2.4 mL
Haemophilus influenzae	0.2 mL		
Peptoniphilus asaccharolyticus	0.2 mL		
Peptostreptococcus anaerobius	0.2 mL		
Serratia marcescens	0.2 mL		
Staphylococcus aureus (mecA/C + MREJ)	0.2 mL		
Pool 3			
Bacteroides fragilis	0.2 mL	1.4 mL	2.8 mL
Citrobacter freundi	0.2 mL		
Klebsiella aerogenes	0.2 mL		
Neisseria gonorrhoeae	0.2 mL		
Parvimonas micra	0.2 mL		
Pseudomonas aeruginosa (VIM)	0.2 mL		
Streptococcus pneumoniae	0.2 mL		
Pool 4			
Anaerococcus prevotii (recombinant in *E.coli*)	0.2 mL	1.2 mL	2.4 mL
Cutibacterium avidum (recombinant in *E.coli*)	0.2 mL		
Cutibacterium granulosum (recombinant in *E.coli*)	0.2 mL		
Escherichia coli (IMP)	0.2 mL		
Kingella kingae	0.2 mL		
Proteus mirabilis	0.2 mL		
Pool 5			
Clostridium perfringens	0.2 mL	1.2 mL	2.4 mL
Klebsiella pneumoniae KPC2 *(KPC)*	0.2 mL		
Klebsiella pneumoniae Z138 (CTX-M, OXA)	0.2 mL		
Klebsiella pneumoniae Z460 (CTX-M, NDM)	0.2 mL		
Salmonella enterica typhimurium	0.2 mL		
Staphylococcus lugdunensis	0.2 mL		

Synthetic Matrix Protocol Example

The estimated total time for completion for this verification example is 4 days for a BIOFIRE® FILMARRAY® System configured with 1 module.

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The number of polols prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BIOFIRE® System.

Day 1

1. Organize materials needed (Table 2); refer to Table 3 for pooling scheme.

2. Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATJIP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.

    a. Transfer the entire contents of the ZeptoMetrix organism vial (approximately 0.2 mL) into a 5 mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The volume will be approximately 1.2 mL for Pools 2, 4, and 5 or 1.4 mL for Pools 1 and 3.
    c. Add Negative/Synthetic Matrix (1.2 or 1.4 mL as described in Table 3) to the tube containing the organism pool (step b). The total volume will be approximately 2.4 to 2.8 mL.
3. Repeat Step 2 for the remaining sample pools (i.e. Pools 2 and 3) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (Figure 1: Pool 1, replicates A and B). Ensure the pooled sample is well mixed prior to removing a sample for testing. The replicate samples should be tested in a single day by different operators.

Note: For each sample, follow instructions in the *BIOFIRE® Joint Infection (JI) Panel Instructions for Use* and the *BIOFIRE® Joint Infection (JI) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (i.e. Pool 2, replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.

Day 2

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 by repeating Step 4 and 5 above (i.e. Pool 1, replicates C and D).

Day 3

Prepare 2 new sample pools (i.e. Pools 4 and 5) as described in Day 1, Steps 2 and 3. Test replicates as described in Steps 4 ,5 and 6.

Day 4

To evaluate day-to-day variation, test replicates from the pools prepared on Day 3 by repeating Step 4 and 5 above (i.e. Pool 4, replicates C and D).

Note: A BIOFIRE Joint Infection Verification Record is provided and may serve as a template for recording your results.

Figure 1. Workflow for the Synthetic and the Clinical Matrix Protocols

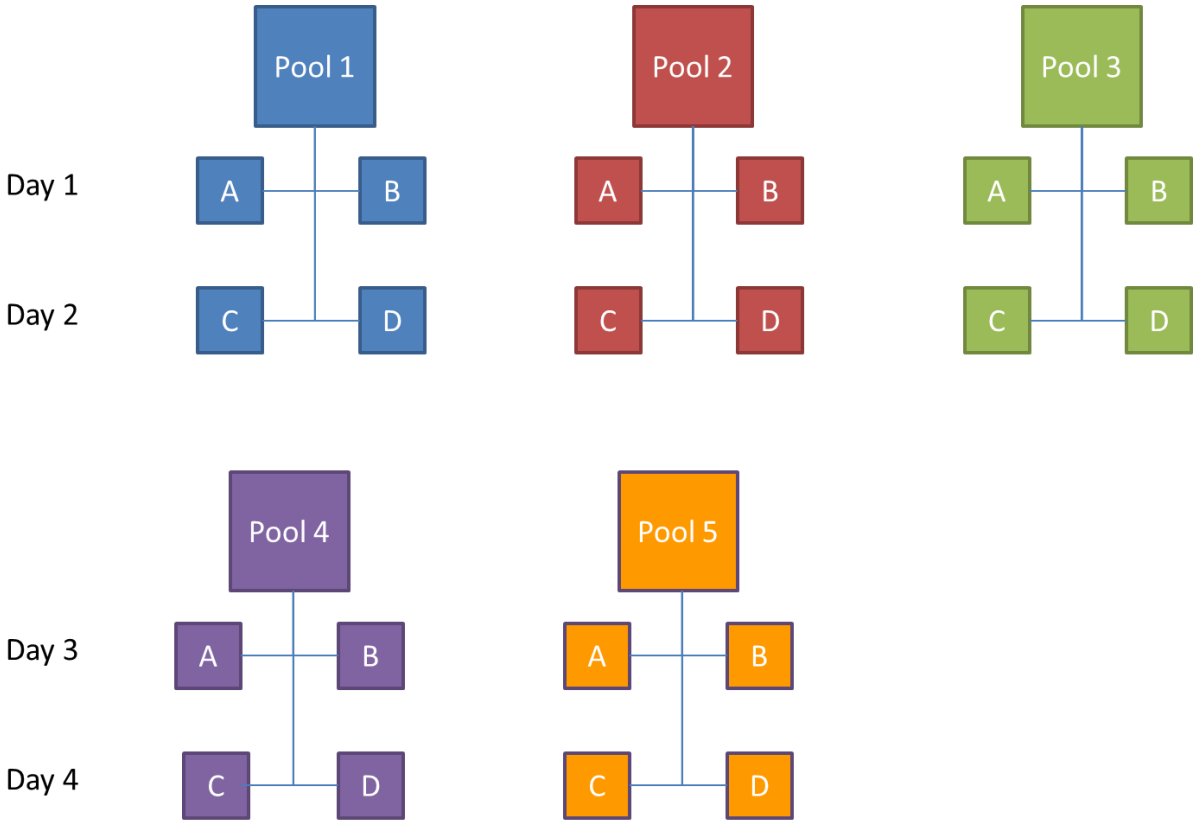

Figure 2. Example of Verification Workflows for use with Multiple BIOFIRE modules.

2 modules	Module 1			Module 2		
Day 1	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator 1	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3/ Operator 2
Day 2	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3/ Operator 2	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator 1
Day 3	Pool 4 / Operator 1	Pool 5 / Operator 2		Pool 4 / Operator 2	Pool 5 / Operator 1	
Day 4	Pool 4 / Operator 2	Pool 5 / Operator 1		Pool 4 / Operator 1	Pool 5 / Operator 2	

4 modules	Module 1		Module 2		Module 3		Module 4	
Day 1	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 3 / Operator 1		Pool 3/ Operator 2	
Day 2	Pool 3 / Operator1		Pool 3/ Operator 2		Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 1/ Operator 2	Pool 2/ Operator 1
Day 3	Pool 4 / Operator 1		Pool 4 / Operator 2		Pool 5 / Operator 2		Pool 5 / Operator 1	
Day 4	Pool 5 / Operator 1		Pool 5 / Operator 2		Pool 4 / Operator 1		Pool 4 / Operator 2	

6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ Operator 1	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 2/ Operator 2	Pool 3 / Operator 1	Pool 3/ Operator 2
Day 2	Pool 3/ Operator 2	Pool 3 / Operator1	Pool 1/ Operator 2	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 2/ Operator 1
Day 3			Pool 4 / Operator 1	Pool 4 / Operator 2	Pool 5 / Operator 1	Pool 5 / Operator 2
Day 4	Pool 5 / Operator 1	Pool 4 / Operator 2	Pool 5 / Operator 2		Pool 4 / Operator 1	

Clinical Matrix Protocol

The Clinical Matrix Protocol evaluates the BIOFIRE JI Panel performance when sample material (ZeptoMetrix NATJIP-BIO) is pooled and combined with synovial fluid. Multiple synovial fluid specimens may be pooled to meet the volumes described in Table 3. Clinical specimens should be screened on the BIOFIRE JI Panel to characterize the sample.

Note: It is important to characterize Synovial Fluid clinical matrix specimens for JI Panel targets by screening the specimen on the BIOFIRE JI Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BIOFIRE JI Panel.

Control material (ZeptoMetrix NATJIP-BIO) is pooled and added to an equal volume of clinical matrix (synovial fluid). The proposed organism pooling scheme (Table 3) should be followed to obtain the expected results for each assay

in a time and resource-efficient manner. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated.

Note: Dilution of ZeptoMetrix control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Figures 1 and 2 (above) illustrate workflow schemes for testing 4 replicates per pool for 5 different pools over multiple days. This produces a total of 20 verification sample test runs and provides 4 positive results and 16 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more samples per day based on the number of modules in the BIOFIRE System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Clinical Matrix Protocol Example

The estimated total time for completion for the Clinical Matrix Protocol verification example is 4 days for a BIOFIRE System configured with 1 module.

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The number of pools prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BIOFIRE System.

Day 1

1. Organize materials needed (Table 2); refer to Table 3 for pooling scheme. Clinical specimens should be screened in the BIOFIRE JI Panel in order to characterize the sample prior to preparing pools.

2. Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATJIP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.
    a. Transfer the entire contents of the ZeptoMetrix organism vial (approximately 0.2 mL) into a 5 mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube, the volume will be approximately 1.2 mL for Pools 2, 4, and 5 or 1.4 mL for Pools 1 and 3.
    c. Add Synovial Fluid/Clinical Matrix (1.2 or 1.4 mL as described in Table 3) to the tube containing the organism pool (step b). Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated; the use of a wide bore pipette tip may be useful. The total volume will be approximately 2.4 to 2.8 mL.

3. Repeat Step 2 for the remaining sample pools (i.e. Pools 2 and 3) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates A and B). Ensure the pooled sample is well mixed prior to removing the sample. The replicate samples should be tested in a single day by different operators.

Note: For each sample, follow instructions in the *BIOFIRE® Joint Infection (JI) Panel Instructions for Use* and the *BIOFIRE® Joint Infection (JI) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (i.e. Pool 2, replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.

Day 2

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 by repeating Steps 4 and 5 above (i.e. replicates C and D from Pools 1, 2 and 3).

Day 3

Prepare 2 new sample pools (i.e. Pools 4 and 5) as described in Day 1, Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the pools prepared on Day 3 by repeating Steps 4 and 5 above (i.e. Pool 3 replicates C and D).

Note: A BIOFIRE JI Verification Record is provided and may serve as a template for recording your results.

Expanding the Protocols

The protocols described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains sufficient volume for testing additional replicates. The verification study may use synovial fluid as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960.

Verification of Loaner, Repaired, and Permanent Replacement Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement module, the following protocol may serve as a guideline but should be verified by the Laboratory Director.

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the BIOFIRE JI Panel. The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples should not be pooled or diluted.

2. Select a set of controls that verify detection of all targets on the BIOFIRE JI Panel.

3. Test the selected samples on the loaner, repaired, or permanent replacement module and document the results.

Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the BIOFIRE Technical Support team for assistance.

BIOFIRE Technical Support
Email: biofiresupport@biomerieux.com
Phone: +1-801-736-6354, select Option 5

BIOFIRE Joint Infection (JI) Panel Verification Record

BIOFIRE® Joint Infection (JI) Panel | **Module Serial #** | **Module Serial #** | **Module Serial #**

Kit Part # | **Module Serial #** | **Module Serial #** | **Module Serial #**

Lot #

	Organism and Resistance Genes		Replicate Testing																				Summary					
			1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D	3-A	3-B	3-C	3-D	4-A	4-B	4-C	4-D	5-A	5-B	5-C	5-D	# Positives	# Negatives	# Users	# Days	# Modules	Patient Samples?
Pool 1	*Candida*																											
	Candida albicans																											
	Enterococcus faecalis	*vanB*																										
	Enterococcus faecium	*vanA*																										
	Finegoldia magna																											
	Morganella morganii																											
	Streptococcus spp.																											
	Streptococcus agalactiae																											
	Streptococcus pyogenes																											
	vanA/B																											
Pool 2	*Enterobacter cloacae* complex																											
	Haemophilus influenzae																											
	Peptoniphilus																											
	Peptostreptococcus anaerobius																											
	Serratia marcescens																											
	Staphylococcus aureus	*mecA/C + MREJ*																										
	mecA/C + MREJ																											
Pool 3	*Bacteroides fragilis*																											
	Citrobacter																											
	Klebsiella aerogenes																											
	Neisseria gonorrhoeae																											
	Parvimonas micra																											
	Pseudomonas aeruginosa	VIM																										
	Streptococcus spp.																											
	Streptococcus pneumoniae																											
	VIM																											
Pool 4	*Anaerococcus prevotii/vaginalis*																											
	Cutibacterium avidum/granulosum																											
	Escherichia coli	IMP																										
	Kingella kingae																											
	Proteus spp.																											
	IMP																											
Pool 5	*Clostridium perfringens*																											
	Klebsiella pneumoniae group	KPC-2 (KPC)																										
		Z138 (CTX-M and OXA-48 like)																										
		Z460 (CTX-M & NDM)																										
	Salmonella spp.																											
	Staphylococcus lugdunensis																											
	CTX-M																											
	KPC																											
	NDM																											
	OXA-48 like																											

Reviewed by:

Signature **Date**