Protocol for Laboratory Verification of Performance of the BioFire® Blood Culture Identification 2 (BCID2) Panel

TECHNICAL
::: NOTE

A Laboratory Protocol for Use with Live Organisms

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.

This document provides an example of a verification procedure to assist your laboratory in developing a protocol for the verification of the BioFire BCID2 Panel performance on BioFire® FilmArray® Systems as required by CLIA. A verification scheme, compatible with the BioFire BCID2 Panel, has been designed using nonclinical specimens. This scheme provides positive and negative tests for each organism detected by the BioFire BCID2 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 technicians may test the same sample. In addition, testing patient samples for verification of the performance of the BioFire BCID2 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 BCID2 Panel is a multiplexed nucleic acid test intended for use with BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems for the simultaneous qualitative detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants

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BFR0000-6592-01
QS-339B-02

associated with antimicrobial resistance. The BioFire BCID2 Panel test is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results. The following organism types and subtypes are identified using the BioFire BCID2 Panel:

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Gram Positive Bacteria		
Enterococcus faecalis	*Staphylococcus* spp.	*Streptococcus* spp.
Enterococcus faecium	*Staphylococcus aureus*	*Streptococcus agalactiae* (Group B)
Listeria monocytogenes	*Staphylococcus epidermidis*	*Streptococcus pneumoniae*
	Staphylococcus lugdunensis	*Streptococcus pyogenes* (Group A)
Gram Negative Bacteria		
Acinetobacter calcoaceticus-baumannii complex		*Enterobacterales*
Bacteroides fragilis		*Enterobacter cloacae* complex
Haemophilus influenzae		*Escherichia coli*
Neisseria meningitidis (encapsulated)		*Klebsiella aerogenes*
Pseudomonas aeruginosa		*Klebsiella oxytoca*
Stenotrophomonas maltophilia		*Klebsiella pneumoniae* group
		Proteus spp.
		Salmonella spp.
		Serratia marcescens
Yeast		
Candida albicans	*Candida krusei*	*Cryptococcus neoformans/gattii*
Candida auris	*Candida parapsilosis*	
Candida glabrata	*Candida tropicalis*	

The BioFire BCID2 Panel contains assays for the detection of genetic determinants associated with resistance to methicillin (*mecA/C* and *mecA/C* in conjunction with MREJ), vancomycin (*vanA* and *vanB*), ß-lactams including penicillins, cephalosporins, monobactams, and carbapenems ($bla_{\text{CTX-M}}$, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{\text{OXA48-like}}$, bla_{VIM}) to aid in the identification of potentially antimicrobial-resistant organisms in positive blood culture samples. In addition, the panel includes an assay for the detection of the mobilized genetic determinant *mcr-1*, an emerging marker of public health importance. 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 and marker assays do not indicate susceptibility, as multiple mechanisms of resistance to methicillin, vancomycin, ß-lactams, and colistin exist.

Antimicrobial Resistance Genes				
CTX-M	KPC	*mecA/C*	NDM	*vanA/B*
IMP	*mcr-1*	*mecA/C* and MREJ (MRSA)	OXA-48-like	VIM

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The complete intended use statement and additional information about the use of the BioFire System can be found in the *BioFire® Blood Culture Identification 2 (BCID2) Panel Instructions for Use.*

Performance Verification: Overview

The verification procedure described here may be used to evaluate the performance of each assay on the BioFire BCID2 Panel. The performance verification protocol has been designed to take advantage of the multiplex nature of the BioFire BCID2 Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedure described below will generate multiple positive and negative detections for each of the BioFire BCID2 assays. The procedures were developed using organism strains available from Microbiologics®, Saint Cloud, MN. Table 3 provides a list of part numbers.

A BioFire® System is defined as all BioFire® FilmArray® Instruments or 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 instrument, it is advised that test replicates are evenly distributed among the instruments or 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 scheme described here in order to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BioFire BCID2 Panel.

Table 1. Overview of Verification Protocol

Verification Protocol	Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[b]	Expected Negative Results	Approximate Days of Testing[c]
Simple Protocol[d]	1, 8, or 9	5	4	20	≥4 per organism	4-16 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 expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification. The proposed verification procedure recommends two *E. coli* strains; therefore the number of expected *E. coli* positives would be 8 and the number of expected negatives would be 12.

[c] The approximate number of days for testing assumes a system configured with one instrument/module and does not include time to grow microbial cultures.

[d]This simple protocol may be easily expanded to increase the number of pouches tested on one instrument/module or for the verification of multiple instruments/modules.

Note: The BioFire BCID2 Panel contains assays for the detection of genetic determinants of resistance, including colistin (*mcr-1*). *mcr-1*, the mobilized colistin resistance gene, confers plasmid-borne resistance to colistin, a last-resort drug for some

multidrug-resistant infections. Laboratories conducting verification of performance of the BioFire BCID2 Panel may choose to not verify the *mcr-1* colistin resistance interpretation on the BioFire BCID2 Panel. In this case, verification can be performed by preparing Pools 1 - 4 as described in Table 4. If a lab chooses to validate the *mcr-1* colistin resistance detection, the organism conferring the resistance (*Escherichia coli* CDC AR-0346) should not be pooled with any other organisms to reduce the risk of inter-species transfer of antibiotic resistance genes. **Proper decontamination and disposal procedures must be strictly followed**. More information can be found in the *BioFire® Blood Culture Identification 2 (BCID2) Panel Instructions for Use* and on the Center for Disease Control and Preventions (CDC) website:
Standard and Contact Precautions:
https://www.cdc.gov/hicpac/2007IP/2007ip_part3.htm
Infection control guidelines:
https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4

Performance Verification: Materials

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

Table 2. Recommended materials for the verification protocol:

Material	Part Number
BioFire® Blood Culture Identification 2 (BCID2) Panel (30-test kit)	BioFire Diagnostics, LLC RFIT-ASY-0147
BioFire® Blood Culture Identification 2 (BCID2) Panel Instructions for Use	BioFire Diagnostics, LLC RFIT-PRT-0841
BioFire® Blood Culture Identification 2 (BCID2) Panel Quick Guide	BioFire Diagnostics, LLC RFIT-PRT-0867
Blood culture media	BACT/ALERT® FA PLUS , 410851, 442192[a] (or equivalent)
Human Whole Blood	BioIVT, HUMANWBACDAUZN (or equivalent, with anticoagulant)
McFarland Turbidity Standard	Fisher Scientific, R20411 (or equivalent)
Phosphate Buffered Saline, pH 7.4	Hardy Diagnostics, R201 (or equivalent)
Polystyrene tubes with cap (15 mL or 50 mL conical)	VWR, 82050-278 or 82050-350 (or equivalent)
10-mL syringe and 18 gauge needle	VWR, 75846-756 and BD-305196 (or equivalent)
Serological pipette, 5 mL	VWR, 82050-478 (or equivalent)
Disposable Transfer pipets, graduated	VWR, 414004-024 (or equivalent)

[a] SeeTable 84 in the BioFire® Blood Culture Identification 2 (BCID2) Panel Instructions for Use for other compatible blood culture bottle types.

Note: It is important to use pathogen free human whole blood for the verification procedure. The blood may be screened on the BioFire BCID2 Panel prior to starting the verification procedure. The optimal human whole blood specimen will be negative for all analytes tested on the BioFire BCID2 Panel.

Table 3. Recommended organism strain and source for verification protocol

Organism	Microbiologics® Catalog Number[a]
Acinetobacter baumannii ATCC® 19606™ KWIK-STIK	0357P
Bacteroides fragilis ATCC® 25285™ KWIK-STIK	0320P
Candida albicans ATCC® 10231™ KWIK-STIK	0443P
Candida auris CDC B11903 KWIK-STIK	01256P
Candida glabrata ATCC® 15126™ KWIK-STIK	0737P
Candida krusei ATCC® 14243™ KWIK-STIK	0809P
Candida parapsilosis ATCC® 22019™ KWIK-STIK	0726P
Candida tropicalis ATCC® 1369™ KWIK-STIK	01036P
Cryptococcus gattii ATCC® MYA-4560™ KWIK-STIK	01051P
Cryptococus neoformans serotype A ATCC® 66031™ KWIK-STIK	0985P
Enterobacter cloacae subsp.cloacae ATCC® 13047™ KWIK-STIK	0323P
Enterococcus faecalis ATCC® 51299™ KWIK-STIK	0959P[b]
Enterococcus faecium NCTC 12204 KWIK-STIK	01143P[c]
Escherichia coli NCTC 13476 KWIK-STIK	01136P[d]
Escherichia coli CDC AR-0346 KWIK-STIK	01259P[e]
Haemophilus influenzae ATCC® 10211™ KWIK-STIK	0441P
Klebsiella aerogenes ATCC® 35029™ KWIK-STIK	0399P
Klebsiella oxytoca ATCC® 13182™ KWIK-STIK	0530P
Klebsiella pneumoniae NCTC 13443 KWIK-STIK	01145P[f]
Klebsiella pneumoniae ATCC® BAA-1705™ KWIK-STIK	01005P[g]
Klebsiella pneumoniae CDC AR-0039 KWIK-STIK	01241P[h]
Klebsiella pneumoniae NCTC 13440 KWIK-STIK	01112P[i]
Listeria monocytogenes ATCC® 19111™ KWIK-STIK	0277P
Neisseria meningitidis ATCC® 13077™ KWIK-STIK	0453P
Proteus mirabilis ATCC® 35659™ KWIK-STIK	0944P
Pseudomonas aeruginosa ATCC® 27853™ KWIK-STIK	0353P
Salmonella enterica subsp. *arizonae* ATCC® 13314™ KWIK-STIK	0901P
Serratia marcescens ATCC® 13880™ KWIK-STIK	0247P
Staphylococcus aureus subsp. *aureus* ATCC® 33591™ KWIK-STIK	0496P[j]
Staphylococcus epidermidis ATCC® 12228™ KWIK-STIK	0371P
Staphylococcus lugdunensis ATCC® 49576™ KWIK-STIK	0217P
Stenotrophomonas maltophilia ATCC® 17666™ KWIK-STIK	0759P
Streptococcus agalactiae ATCC® 12386™ KWIK-STIK	0439P
Streptococcus pneumoniae ATCC® 10015™ KWIK-STIK	0865P
Streptococcus pyogenes ATCC® 19615™ KWIK-STIK	0385P
Blood Culture Identification 2 (BCID2) Verification Panel (Live Culture)	5258P[k]

[a] Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire BCID2 Panel. However, when alternate organism sources are used, the sample volumes or pooling schemes suggested in the examples below may need to be adjusted. Alternate organism strains may not provide the same results for antimicrobial resistance genes as those suggested here.

[b] This strain of *E. faecalis* (ATCC® 51299) carries the *vanB* gene (vancomycin resistance) .

[c] This strain of *E. faecium*(NCTC 12204) carries the *vanA* gene (vancomycin resistance).

[d] This strain of *E. coli* (NCTC 13476) carries the IMP gene (ß-lactam resistance).

[e] This strain of *E. coli* (CDC AR-0346) carries the *mcr-1* gene (colistin resistance) and the CTX-M gene (ß-lactam resistance).

[f] This strain of *K. pneumoniae* (NCTC 13443) carries the CTX-M and NDM-1 genes (ß-lactam resistance)

[g] This strain of *K. pneumoniae* (ATCC® BAA-1705) carries the KPC gene (ß-lactam resistance).

[h] This strain of *K. pneumoniae* (CDC AR-0039) carries the CTX-M and OXA-48 like genes (ß-lactam resistance).

[i] This strain of *K. pneumoniae* (NCTC 13440) carries the VIM-1 gene (ß-lactam resistance).

[j] This strain of *S. aureus* subsp. *aureus* (ATCC® 33591) carries the *mecA* and MREJ (MRSA) genes (methicillin resistance).

[k] This is a bundled part number containing all the organisms listed above.

Note: Extreme care should be used in properly handling and disposing of organisms containing antibiotic resistance genes. Follow your institution's guidelines for proper handling and disposal of pathogens or refer to the CDC Infection control guidelines: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4

Performance Verification Protocol

The protocol described below evaluates the BioFire BCID2 Panel performance by pooling together up to 9 different organism suspensions in a simulated blood culture matrix. The pooling scheme described in Table 4 should be followed to obtain the expected number of positive and negative results for each organism in a time and resource-efficient manner.

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

The protocol and workflow schemes (Figures 1 and 2) illustrate testing 4 replicates per pool for 5 pools over multiple days. This produces a total of 20 verification sample test runs and provides at least 4 positive results and as many as 16 negative results per assay. Some organisms, such as *Klebsiella pneumoniae*, are represented multiple times. This is done to ensure all antimicrobial resistance genes are represented in the verification protocol.

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.

Table 4. Proposed Organism Pooling Scheme

Organism and Resistance Genes	Microbiologics Part No.	Organism Volume	Blood Culture Medium Volume	Human Whole Blood Volume	Approximate Pool Volume
Pool 1					
Candida albicans	0443P	0.1 mL	8 mL	3 mL	12 mL
Candida krusei	0809P	0.1 mL			
Klebsiella pneumoniae (CTX-M, NDM)	01145P	0.1 mL			
Listeria monocytogenes	0277P	0.1 mL			
Neisseria meningitidis	0453P	0.1 mL			
Pseudomonas aeruginosa	0353P	0.1 mL			
Staphylococcus aureus (mecA and MREJ)	0496P	0.1 mL			
Streptococcus agalactiae	0439P	0.1 mL			
Streptococcus pyogenes	0385P	0.1 mL			
Pool 2					
Acinetobacter baumannii	0357P	0.1 mL	8 mL	3 mL	12 mL
Candida glabrata	0737P	0.1 mL			
Candida parapsilosis	0726P	0.2 mL			
Candida tropicalis	01036P	0.4 mL			
Escherichia coli (IMP)	01136P	0.2 mL			
Klebsiella oxytoca	0530P	0.1 mL			
Klebsiella pneumoniae (KPC)	1005P	0.1 mL			
Staphylococcus epidermidis	0371P	0.4 mL			
Pool 3					
Candida auris	01256P	0.2 mL	8 mL	3 mL	12 mL
Cryptococcus gattii	01051P	0.1 mL			
Enterococcus faecalis (vanB)	0959P	0.2 mL			
Haemophilus influenzae	0411P	0.1 mL			
Klebsiella pneumoniae (VIM)	01112P	0.1 mL			
Proteus mirabilis	0944P	0.2 mL			
Serratia marcescens	0247P	0.1 mL			
Staphylococcus lugdunensis	0217P	0.1 mL			
Streptococcus pneumoniae	0865P	0.1 mL			
Pool 4					
Klebsiella pneumoniae (CTX-M, OXA-48 like)	01241P	0.1 mL	8 mL	3 mL	12 mL
Bacteroides fragilis	0320P	0.1 mL			
Cryptococcus neoformans	0985P	0.1 mL			
Enterobacter cloacae	0323P	0.1 mL			
Enterococcus faecium (vanA)	01143P	0.1 mL			
Klebsiella aerogenes	0399P	0.1 mL			
Salmonella enterica typhimurium	0901P	0.1 mL			
Stenotrophomonas maltophilia	0759P	0.1 mL			
Pool 5					
Escherichia coli (CTX-M, *mcr-1*)	01259P	0.2 mL	8 mL	3 mL	11 mL

Note: *E.coli* (CTX-M. *mcr-1)* should not be pooled and should be properly disposed of to avoid possible transfer of the resistance gene to other bacteria. Follow your institution's guidelines for proper handling and disposal of pathogens or refer to the CDC Infection control guidelines: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4

Protocol Example

The estimated total time for completion for this verification example is 4 days for a BioFire System configured with 1 module, not including the time required to grow microbial cultures.

A proposed organism pooling scheme is presented above in Table 4. Figure 1 illustrates a simplified workflow schematic. The number of samples tested per day should be determined by the individual laboratory. The protocol can be modified to run more samples per day (or fewer) based upon the number of modules in the BioFire System. The proposed organism pooling scheme in Table 4 provides sufficient volume for testing more replicates, if desired. Figure 2 provides an examples of user-to-user, day-to-day, and module-to-module testing for labs with multiple BioFire Modules.

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

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

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The suggestion to prepare 3 sample pools is based on testing up to 6 pouches per day. The number of samples 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). Human whole blood should be screened in the BioFire BCID2 Panel in order to characterize the sample prior to preparing pools. The optimal human whole blood specimen will be negative for all organisms tested on the BioFire BCID2 Panel.

2. Obtain a pure culture of each organism that has been streaked for isolation on agar media appropriate for the organism. It is recommended to use agar plate cultures that are less than 1 week old. See Microbiologics product insert for use of KWIK-STIK cultures.

3. Prepare a suspension of each organism equivalent to McFarland turbidity standard 1.0 using approximately 3 mL of phosphate buffered saline (PBS), pH 7.4.

4. Prepare three sample pools according to the organism pooling scheme presented in Table 4. The sample pool preparation worksheet in the Appendix may be useful to ensure all components are added to each pool.

    a. Use a serological pipette to add 3 mL of human whole blood to a 15 mL conical bottom tube. Alternatively, a 50 mL tube may be used.
    b. Use a 10-mL syringe and an 18 gauge needle to remove 8 mL of blood culture medium from a blood culture bottle and add it to the conical tube containing whole blood. Care should be taken to minimize transferring resin beads into the sample.
    c. Use a disposable transfer pipette to add the appropriate volume (Table 4) of organism suspension to the blood-medium mixture.
    d. Repeat for the remaining organisms in the pool to combine the appropriate organisms into a single tube.
    e. Ensure the pooled sample is effectively mixed prior to removing a sample for testing.

5. Prepare and test two replicates from a single sample pool (i.e. Figure 1: Pool #1 replicates A and B). The replicate samples should be tested in a single day by different users.

    Note: For each sample, follow instructions in the *BioFire® Blood Culture Identification 2 (BCID2) Panel Instruction Booklet* and *BioFire® Blood Culture Identification 2 (BCID2) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

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

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

Day 2

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

Day 3

Prepare 2 new sample pools (i.e. pools #4 and #5) as described in Steps 3 to 4. Test replicates as described in Step 5 above.

Day 4

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

Note: Extreme care should be used in properly handling and disposing of organisms containing antibiotic resistance genes. Follow your institution's guidelines for proper handling and disposal of pathogens or refer to the CDC Infection control guidelines: https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4

Figure 1. Simplified Workflow for Protocol

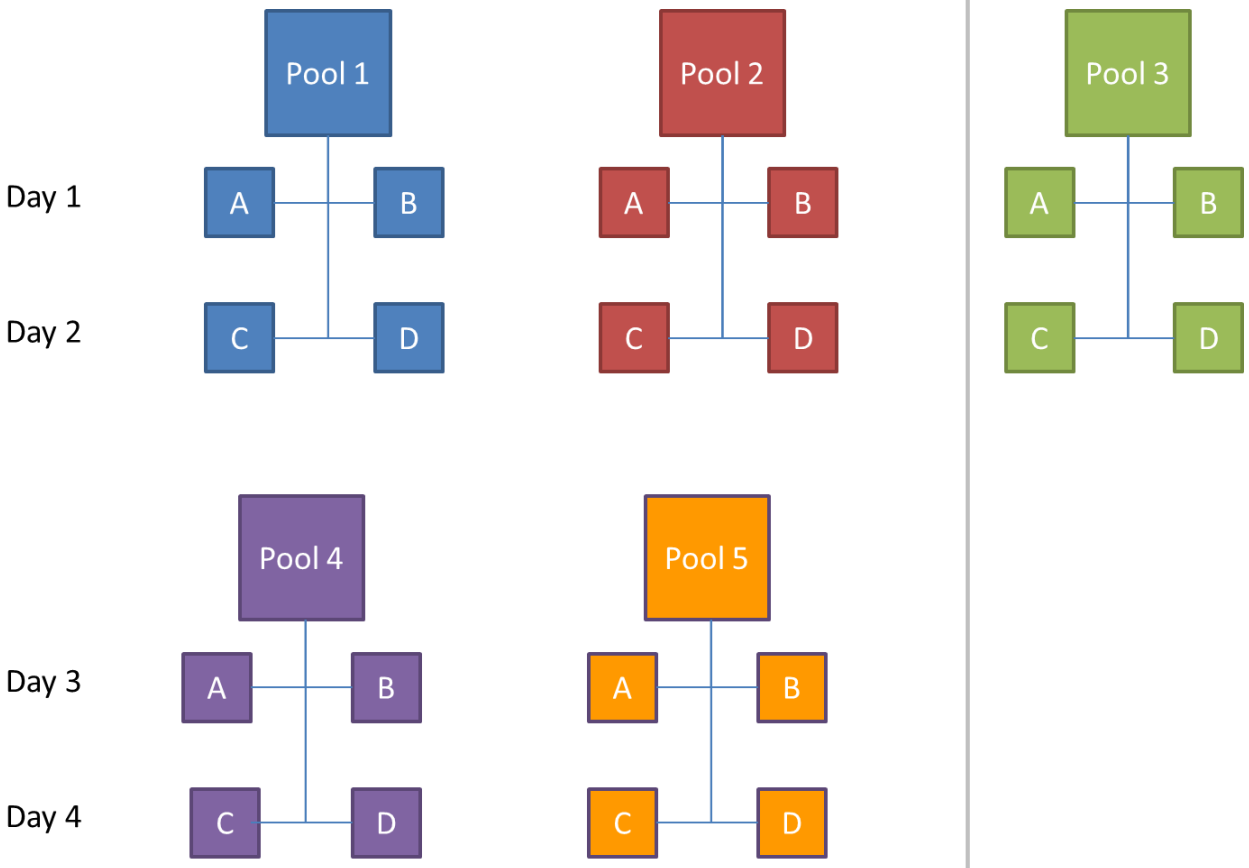

Figure 2. Example of a Verification workflow for use with multiple BioFire Modules

2 modules	Module 1			Module 2		
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 3 / User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 3/ User 2
Day 2	Pool 1/ User 2	Pool 2/ User 1	Pool 3/ User 2	Pool 1/ User 1	Pool 2/ User 2	Pool 3 / User1
Day 3	Pool 4 / User 1	Pool 5 / User 2		Pool 4 / User 2	Pool 5 / User 1	
Day 4	Pool 4 / User 2	Pool 5 / User 1		Pool 4 / User 1	Pool 5 / User 2	

4 modules	Module 1		Module 2		Module 3		Module 4	
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1	Pool 3 / User 1		Pool 3/ User 2	
Day 2	Pool 3 / User1		Pool 3/ User 2		Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1
Day 3	Pool 4 / User 1		Pool 4 / User 2		Pool 5 / User 2		Pool 5 / User 1	
Day 4	Pool 5 / User 1		Pool 5 / User 2		Pool 4 / User 1		Pool 4 / User 2	

6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2	Pool 3 / User 1	Pool 3/ User 2
Day 2	Pool 3/ User 2	Pool 3 / User1	Pool 1/ User 2	Pool 1/ User 1	Pool 2/ User 2	Pool 2/ User 1
Day 3			Pool 4 / User 1	Pool 4 / User 2	Pool 5 / User 1	Pool 5 / User 2
Day 4	Pool 5 / User 1	Pool 4 / User 2	Pool 5 / User 2		Pool 4 / User 1	

Expanding the protocols

The protocol described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains

approximately 12 mL, which is enough material to complete many replicates for each pool.

Verification of Loaner, Repaired, and Permanent Replacement Instruments

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument, 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 BCID2 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 BCID2 Panel.

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

Technical Support Contact Information

BioFire 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: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5

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Appendix

Sample Pool Preparation Worksheet

Organism and Resistance Genes	Organism Volume		Blood Culture Medium Volume	Human Whole Blood Volume	Approximate Pool Volume
Pool 1					
Candida albicans	☐	0.1 mL	☐ 8 mL	☐ 3 mL	~12 mL
Candida krusei	☐	0.1 mL			
Klebsiella pneumoniae (CTX-M, NDM)	☐	0.1 mL			
Listeria monocytogenes	☐	0.1 mL			
Neisseria meningitidis	☐	0.1 mL			
Pseudomonas aeruginosa	☐	0.1 mL			
Staphylococcus aureus (mecA and MREJ)	☐	0.1 mL			
Streptococcus agalactiae	☐	0.1 mL			
Streptococcus pyogenes	☐	0.1 mL			
Pool 2					
Acinetobacter baumannii	☐	0.1 mL	☐ 8 mL	☐ 3 mL	~12 mL
Candida glabrata	☐	0.1 mL			
Candida parapsilosis	☐	0.2 mL			
Candida tropicalis	☐	0.4 mL			
Escherichia coli (IMP)	☐	0.2 mL			
Klebsiella oxytoca	☐	0.1 mL			
Klebsiella pneumoniae (KPC)	☐	0.1 mL			
Staphylococcus epidermidis	☐	0.4 mL			
Pool 3					
Candida auris	☐	0.2 mL	☐ 8 mL	☐ 3 mL	~12 mL
Cryptococcus gattii	☐	0.1 mL			
Enterococcus faecalis (vanB)	☐	0.2 mL			
Haemophilus influenzae	☐	0.1 mL			
Klebsiella pneumoniae (VIM)	☐	0.1 mL			
Proteus mirabilis	☐	0.2 mL			
Serratia marcescens	☐	0.1 mL			
Staphylococcus lugdunensis	☐	0.1 mL			
Streptococcus pneumoniae	☐	0.1 mL			
Pool 4					
Klebsiella pneumoniae (CTX-M, OXA-48 like)	☐	0.1 mL	☐ 8 mL	☐ 3 mL	12 mL
Bacteroides fragilis	☐	0.1 mL			
Cryptococcus neoformans	☐	0.1 mL			
Enterobacter cloacae	☐	0.1 mL			
Enterococcus faecium (vanA)	☐	0.1 mL			
Klebsiella aerogenes	☐	0.1 mL			
Salmonella enterica typhimurium	☐	0.1 mL			
Stenotrophomonas maltophilia	☐	0.1 mL			
Pool 5					
Escherichia coli (CTX-M, *mcr-1*)	☐	0.2 mL	☐ 8 mL	☐ 3 mL	11 mL

BioFire® Blood Culture Identification 2 (BCID2) Panel Verification Record-Page 1

BioFire® Blood Culture Identification 2 (BCID2) Panel	**Module Serial #**	**Module Serial #**
Kit Part #	**Module Serial #**	**Module Serial #**
Lot #	**Module Serial #**	**Module Serial #**

	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 albicans*																										
	Candida krusei																										
	Klebsiella pneumoniae (CTX-M, NDM)																										
	Listeria monocytogenes																										
	Neisseria meningitidis (encapsulated)																										
	Pseudomonas aeruginosa																										
	Staphylococcus aureus (mecA and MREJ)																										
	Streptococcus agalactiae																										
	Streptococcus pyogenes																										
	CTX-M																										
	mecA/C and MREJ (MRSA)																										
	NDM																										
Pool 2	*Acinetobacter baumannii*																										
	Candida glabrata																										
	Candida parapsilosis																										
	Candida tropicalis																										
	Escherichia coli (IMP)																										
	Klebsiella oxytoca																										
	Klebsiella pneumoniae (KPC)																										
	Staphylococcus epidermidis																										
	KPC																										
	IMP																										

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	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 3	*Candida auris*																										
	Cryptococcus gattii																										
	Enterococcus faecalis (vanB)																										
	Haemophilus influenzae																										
	Klebsiella pneumoniae (VIM)																										
	Proteus mirabilis																										
	Serratia marcescens																										
	Staphylococcus lugdunensis																										
	Streptococcus pneumoniae																										
	vanA/B																										
	VIM																										
Pool 4	*Klebsiella pneumoniae* (CTX-M, OXA-48 like)																										
	Bacteroides fragilis																										
	Cryptococcus neoformans																										
	Enterobacter cloacae																										
	Enterococcus faecium (vanA)																										
	Klebsiella aerogenes																										
	Salmonella sp.																										
	Stenotrophomonas maltophilia																										
	CTX-M																										
	OXA-48 like																										
	vanA/B																										
Pool 5	*Escherichia coli* (CTX-M, *mcr-1*)																										
	CTX-M																										
	mcr-1																										

Reviewed by: _____

Signature **Date**