



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

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. The BIOFIRE® Blood Culture Identification 2 (BCID2) Panel has been categorized by the FDA as a CLIA moderate complexity test.

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® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems as required by CLIA. A verification scheme, compatible with the BIOFIRE BCID2 Panel, has been designed using non-clinical specimens. This method described 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 operators may test the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on 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.

Intended Use

The BIOFIRE® Blood Culture Identification 2 (BCID2) Panel is a multiplexed nucleic acid 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 genetic determinants 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:





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

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*), β -lactams including penicillins, cephalosporins, monobactams, and carbapenems (*bla*_{CTX-M}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{NDM}, *bla*_{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, β -lactams, and colistin exist.

Antimicrobial Resistance Genes				
CTX-M	KPC	<i>mecA/C</i>	NDM	<i>vanA/B</i>
IMP	<i>mcr-1</i>	<i>mecA/C</i> 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® 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 Panel 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 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 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.



Note: The laboratory should only perform the verification study with analytes that will be reported using the BIOFIRE BCID2 Panel in their laboratory setting.

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
	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 module and does not include time to grow microbial cultures. The number of testing days can be increased or decreased, as needed.



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/infection-control/about/index.html?CDC_AAref_Val=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 kit	BioFire Diagnostics, LLC: RFIT-ASY-0147 (30 tests) or RFIT-ASY-0148 (6 tests)
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 ^a	BACT/ALERT® FA PLUS 410851 (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)	Avantor (VWR) #82050-278 or 82050-350 (or equivalent)
10-mL syringe and 18-gauge needle	BD-309695 and BD-305196 (or equivalent)
Serological pipette, 5 mL	Avantor (VWR) # 82050-478 (or equivalent)
Disposable Transfer pipets, graduated	Avantor (VWR) 414004-024 (or equivalent)

^a See Table 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
<i>Acinetobacter baumannii</i> ATCC® 19606™ KWIK-STIK	0357P
<i>Bacteroides fragilis</i> ATCC® 25285™ KWIK-STIK	0320P
<i>Candida albicans</i> ATCC® 10231™ KWIK-STIK	0443P
<i>Candida auris</i> CDC B11903 KWIK-STIK	01256P
<i>Candida glabrata</i> ATCC® 15126™ KWIK-STIK	0737P
<i>Candida krusei</i> ATCC® 14243™ KWIK-STIK	0809P
<i>Candida parapsilosis</i> ATCC® 22019™ KWIK-STIK	0726P
<i>Candida tropicalis</i> ATCC® 1369™ KWIK-STIK	01036P
<i>Cryptococcus gattii</i> ATCC® MYA-4560™ KWIK-STIK	01051P
<i>Cryptococcus neoformans</i> serotype A ATCC® 66031™ KWIK-STIK	0985P
<i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC® 13047™ KWIK-STIK	0323P
<i>Enterococcus faecalis</i> ATCC® 51299™ KWIK-STIK	0959P ^b
<i>Enterococcus faecium</i> NCTC 12204 KWIK-STIK	01143P ^c
<i>Escherichia coli</i> NCTC 13476 KWIK-STIK	01136P ^d
<i>Escherichia coli</i> CDC AR-0346 KWIK-STIK	01259P ^e
<i>Haemophilus influenzae</i> ATCC® 10211™ KWIK-STIK	0441P
<i>Klebsiella aerogenes</i> ATCC® 35029™ KWIK-STIK	0399P
<i>Klebsiella oxytoca</i> ATCC® 13182™ KWIK-STIK	0530P
<i>Klebsiella pneumoniae</i> derived from ATCC® BAA-2146™ KWIK-STIK	01060P ^f
<i>Klebsiella pneumoniae</i> ATCC® BAA-1705™ KWIK-STIK	01005P ^g
<i>Klebsiella pneumoniae</i> derived from NCTC 13442 KWIK-STIK	01241P ^h
<i>Klebsiella pneumoniae</i> derived from NCTC 13439 KWIK-STIK	01245P ⁱ
<i>Listeria monocytogenes</i> ATCC® 19111™ KWIK-STIK	0277P
<i>Neisseria meningitidis</i> ATCC® 13077™ KWIK-STIK	0453P
<i>Proteus mirabilis</i> ATCC® 35659™ KWIK-STIK	0944P
<i>Pseudomonas aeruginosa</i> ATCC® 27853™ KWIK-STIK	0353P
<i>Salmonella enterica</i> subsp. <i>arizonae</i> ATCC® 13314™ KWIK-STIK	0901P
<i>Serratia marcescens</i> ATCC® 13880™ KWIK-STIK	0247P
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC® 33591™ KWIK-STIK	0496P ^j
<i>Staphylococcus epidermidis</i> ATCC® 12228™ KWIK-STIK	0371P
<i>Staphylococcus lugdunensis</i> ATCC® 49576™ KWIK-STIK	0217P
<i>Stenotrophomonas maltophilia</i> ATCC® 17666™ KWIK-STIK	0759P
<i>Streptococcus agalactiae</i> ATCC® 12386™ KWIK-STIK	0439P
<i>Streptococcus pneumoniae</i> ATCC® 10015™ KWIK-STIK	0865P
<i>Streptococcus pyogenes</i> ATCC® 19615™ KWIK-STIK	0385P

^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 (β-lactam resistance).

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

^f This strain of *K. pneumoniae* (ATCC® BAA-2146) carries the NDM-1 genes (β-lactam resistance).

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

^h This strain of *K. pneumoniae* (NCTC 13442) carries the OXA-48 like genes (β-lactam resistance).

ⁱ This strain of *K. pneumoniae* (NCTC 13439) carries the VIM-1 gene (β-lactam resistance).

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



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/infection-control/about/index.html?CDC_AAref_Val=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.

Figures 1 and 2 (below) illustrate protocol and workflow schemes for 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® FILMARRAY® 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 (NDM)	01060P	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			





<i>Klebsiella oxytoca</i>	0530P	0.1 mL			
<i>Klebsiella pneumoniae</i> (KPC)	1005P	0.1 mL			
<i>Staphylococcus epidermidis</i>	0371P	0.4 mL			
Pool 3					
<i>Candida auris</i>	01256P	0.2 mL	8 mL	3 mL	12 mL
<i>Cryptococcus gattii</i>	01051P	0.1 mL			
<i>Enterococcus faecalis</i> (vanB)	0959P	0.2 mL			
<i>Haemophilus influenzae</i>	0411P	0.1 mL			
<i>Klebsiella pneumoniae</i> (VIM)	01245P	0.1 mL			
<i>Proteus mirabilis</i>	0944P	0.2 mL			
<i>Serratia marcescens</i>	0247P	0.1 mL			
<i>Staphylococcus lugdunensis</i>	0217P	0.1 mL			
<i>Streptococcus pneumoniae</i>	0865P	0.1 mL			
Pool 4					
<i>Klebsiella pneumoniae</i> (OXA-48 like)	01148P	0.1 mL	8 mL	3 mL	12 mL
<i>Bacteroides fragilis</i>	0320P	0.1 mL			
<i>Cryptococcus neoformans</i>	0985P	0.1 mL			
<i>Enterobacter cloacae</i>	0323P	0.1 mL			
<i>Enterococcus faecium</i> (vanA)	01143P	0.1 mL			
<i>Klebsiella aerogenes</i>	0399P	0.1 mL			
<i>Salmonella enterica typhimurium</i>	0901P	0.1 mL			
<i>Stenotrophomonas maltophilia</i>	0759P	0.1 mL			
Pool 5					
<i>Escherichia coli</i> (CTX-M, <i>mcr-1</i>)	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/infection-control/about/index.html?CDC_AAref_Val=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® FILMARRAY® 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 example of user-to-user, day-to-day, and module-to-module testing for labs with multiple BIOFIRE Modules.

To evaluate day-to-day variation: pooled organisms may be stored overnight or up to 2 days at refrigeration temperature (2–8°C). To evaluate operator-to-operator variation, multiple laboratory technicians/ staff may perform testing.



Note: It is important to prepare only the number of sample pools that will be tested within 2 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 modified based on the laboratory's work schedule and number of modules connected within a BIOFIRE FILMARRAY System.

Day 1

1. Organize materials needed (Table 2). Human whole blood should be screened with the BIOFIRE Blood Culture Identification 2 (BCID2) Panel in order to characterize the sample prior to preparing pools.
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 Microbiology 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 one sample pool (i.e. Pool 1) 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.
5. Test two replicates from each sample pool (refer to Figure 1: replicates A and B for Pools 1, 2 and 3). Ensure the pooled sample is well mixed prior to removing a sample for testing. Replicate samples A and B should be tested in a single day by different operators. Refer to Figure 2 for suggested workflows depending upon the module configuration in the verification study.



Note: 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. 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 additional replicates from the pools prepared on Day 1 by repeating Step 5 above (i.e. replicates C and D for Pools 1, 2 and 3).





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 from the pools prepared on Day 3 (i.e. Pool 4, replicates 4C and 4D and Pool 5, replicates 5C and 5D) by repeating Step 5.



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/infection-control/about/index.html?CDC_AAref_Val=https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4.

Figure 1. Verification Protocol Testing Workflow

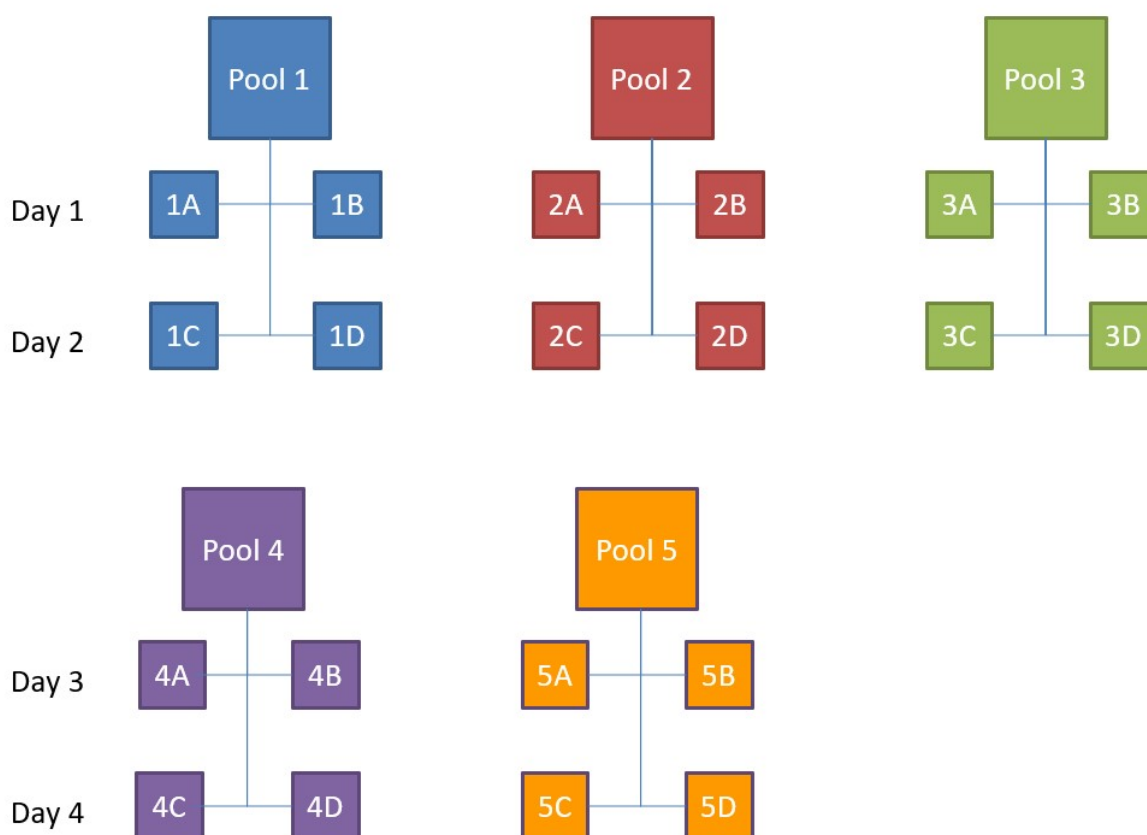




Figure 2. Example of a Verification Workflows for use with Multiple BIOFIRE Modules

Verification with 2 modules						
Testing Day	Module 1			Module 2		
Day 1	Pool 1A/ Operator 1	Pool 2B/ Operator 2	Pool 3A / Operator 1	Pool 1B/ Operator 2	Pool 2A/ Operator 1	Pool 3B/ Operator 2
Day 2	Pool 1D/ Operator 2	Pool 2C/ Operator 1	Pool 3D/ Operator 2	Pool 1C/ Operator 1	Pool 2D/ Operator 2	Pool 3C / Operator1
Day 3	Pool 4 A/ Operator 1	Pool 5 B/ Operator 2		Pool 4 B/ Operator 2	Pool 5A / Operator 1	
Day 4	Pool 4 D/ Operator 2	Pool 5C / Operator 1		Pool 4C / Operator 1	Pool 5D / Operator 2	

Verification with 4 modules								
Testing Day	Module 1		Module 2		Module 3		Module 4	
Day 1	Pool 1A/ Operator 1	Pool 2B/ Operator 2	Pool 1B/ Operator 2	Pool 2A/ Operator 1	Pool 3A / Operator 1		Pool 3B/ Operator 2	
Day 2	Pool 3C / Operator1		Pool 3D/ Operator 2		Pool 1C/ Operator 1	Pool 2D/ Operator 2	Pool 1D/ Operator 2	Pool 2C/ Operator 1
Day 3	Pool 4 A/ Operator 1		Pool 4 B/ Operator 2		Pool 5 B/ Operator 2		Pool 5A / Operator 1	
Day 4	Pool 5C / Operator 1		Pool 5D / Operator 2		Pool 4C / Operator 1		Pool 4 D/ Operator 2	

Verification with 6 modules						
6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1A/ Operator 1	Pool 1B/ Operator 2	Pool 2A/ Operator 1	Pool 2B/ Operator 2	Pool 3A / Operator 1	Pool 3B/ Operator 2
Day 2	Pool 3D/ Operator 2	Pool 3C / Operator1	Pool 1C/ Operator 1	Pool 1D/ Operator 2	Pool 2D/ Operator 2	Pool 2C/ Operator 1
Day 3			Pool 4 A/ Operator 1	Pool 4 B/ Operator 2	Pool 5A / Operator 1	Pool 5 B/ Operator 2
Day 4	Pool 5C / Operator 1	Pool 4 D/ Operator 2	Pool 5D / Operator 2		Pool 4C / Operator 1	



Expanding or Modifying the Protocols

The protocol described above can be expanded by increasing the number of test replicates from each of the organism pools. Each organism pool contains sufficient volume for testing additional replicates. The verification study uses human whole blood and blood culture media as clinical matrix in the pools. Reference CAP accreditation checklist requirements: MIC.64960 Validation or Verification Studies - Specimen Selection.

Verification of Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or 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® Blood Culture Identification 2 (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 or module and document the results.

Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have questions or concerns about this process, please contact your local bioMérieux representative or your authorized distributor.

*All product names, trademarks and registered trademarks are property of their respective owners.





Appendix

Sample Pool Preparation Worksheet

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

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

Reviewed by: _____

Signature Date