



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

## Laboratory Protocols for Use with a ZeptoMetrix NATtrol™ Verification Panel

### Purpose

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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 non-clinical 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 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

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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*),  $\beta$ -lactams including penicillins, cephalosporins, monobactams, and carbapenems (*bla*<sub>CTX-M</sub>, *bla*<sub>IMP</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA48-like</sub>, *bla*<sub>VIM</sub>) 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,  $\beta$ -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

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the BIOFIRE BCID2 Panel using pooled control material from ZeptoMetrix and (2) a Blood Culture Media Protocol that evaluates pooled control material from ZeptoMetrix in the presence of blood culture media sample matrix. Both protocols evaluate the performance of each assay on the BIOFIRE BCID2 Panel.



**Note:** It is important to characterize clinical matrix specimens for BCID2 Panel targets by screening the specimen on the BIOFIRE BCID2 Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes detected on the Panel.

These protocols have 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 procedures described will generate multiple positive and negative detections for each of the Panel assays. The procedures were developed using the Blood Culture Identification 2 (BCID2) Panel available from ZeptoMetrix LLC, Buffalo, NY (part number NATBCP2-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 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 Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results <sup>a</sup>	Expected Negative Results	Approximate Days of Testing <sup>b</sup>
Example 1: Simple Protocol	6 or 7	5	4	20	≥4 per organism	12-16 per organism	4
Example 2: Blood Culture Media Protocol	6 or 7	5	4	20	≥4 per organism	12-16 per organism	4

<sup>a</sup> 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 multiple *K. pneumoniae* strains; therefore, the number of expected *K. pneumoniae* group positives would be 8 and the number of expected negatives would be 12.

<sup>b</sup> 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:

**Table 2.** Recommended materials for the verification protocols:

Material	Part Number
BIOFIRE® Blood Culture Identification 2 (BCID2) Panel	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 <sup>a</sup>	BACT/ALERT® FA PLUS, 410851 <sup>a</sup> (or equivalent)
Control Organism <sup>b</sup>	ZeptoMetrix: NATBCP2-BIO
2 mL or 5 mL Sample Tubes	Various manufacturers
Polystyrene tubes with cap (15 mL)	Avantor (VWR) #82050-278 (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)

<sup>a</sup> See Table 84 in the BIOFIRE® Blood Culture Identification 2 (BCID2) Panel Instructions for Use for other compatible blood culture bottle types.

<sup>b</sup> 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 (i.e. not the ZeptoMetrix NATBCP2-BIO material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.



The BIOFIRE BCID2 Panel contains assays for the detection of genetic determinants of resistance, including the mobilized colistin resistance gene 1 (*mcr-1*). The NATBCP2-BIO contains inactivated organisms and materials of human and animal origin. Safe practices suggest that the product be considered potentially infectious and to use universal precautions when handling. Refer to local laboratory guidelines for proper handling and disposal guidelines. 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](https://www.cdc.gov/hicpac/2007IP/2007ip_part3.htm)

Infection control guidelines:

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

[control/about/index.html?CDC\\_AAref\\_Val=https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4](https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html#a4)

## Performance Verification Protocols

### Simple Protocol

The Simple Protocol evaluates the BIOFIRE BCID2 Panel performance when control material (ZeptoMetrix NATBCP2-BIO) is pooled in the absence of clinical matrix. 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 control 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 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.

Pooled samples can be stored overnight (or up to 14 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 for Simple Protocol

Control Organism and Resistance Genes: NATBCP2-BIO	Approximate Organism Volume	Approximate Pool Volume
<b>Pool 1</b>		
<i>Acinetobacter baumannii</i> (307-0294)	0.2 mL	1.4 mL
<i>Candida albicans</i> (Z006)	0.2 mL	
<i>Enterococcus faecalis</i> (Z345, <i>vanB</i> )	0.2 mL	
<i>Enterococcus faecium</i> (Z347, <i>vanA</i> )	0.2 mL	
<i>Staphylococcus aureus</i> ( <i>mecA</i> and MREJ)	0.2 mL	
<i>Streptococcus agalactiae</i> (Z019)	0.2 mL	
<i>Streptococcus pyogenes</i> (Z018)	0.2 mL	





Pool 2		
<i>Candida glabrata</i> (Z007)	0.2 mL	1.4 mL
<i>Candida krusei</i> (Z009)	0.2 mL	
<i>Enterobacter cloacae</i> (Z101)	0.2 mL	
<i>Haemophilus influenzae</i> (MinnA)	0.2 mL	
<i>Klebsiella oxytoca</i> (Z115)	0.2 mL	
<i>Listeria monocytogenes</i> (serotype 1/2b)	0.2 mL	
<i>Staphylococcus epidermidis</i> (MSSE; HER 1292)	0.2 mL	
Pool 3		
<i>Bacteroides fragilis</i> (Z029)	0.2 mL	1.4 mL
<i>Candida parapsilosis</i> (Z011)	0.2 mL	
<i>Candida tropicalis</i> (Z012)	0.2 mL	
<i>Klebsiella pneumoniae</i> (KPC-2, KPC)	0.2 mL	
<i>Pseudomonas aeruginosa</i> (Z139, VIM)	0.2 mL	
<i>Serratia marcescens</i> (Z053)	0.2 mL	
<i>Streptococcus pneumoniae</i> (Z022)	0.2 mL	
Pool 4		
<i>Candida auris</i> (Z485)	0.2 mL	1.4 mL
<i>Escherichia coli</i> (Z521, mcr-1)	0.2 mL	
<i>Escherichia coli</i> (Z297, IMP)	0.2 mL	
<i>Klebsiella aerogenes</i> (Z052)	0.2 mL	
<i>Neisseria meningitidis</i> (serogroup A)	0.2 mL	
<i>Proteus mirabilis</i> (Z050)	0.2 mL	
<i>Stenotrophomonas maltophilia</i> (Z074)	0.2 mL	
Pool 5		
<i>Cryptococcus gattii</i> (Z156)	0.2 mL	1.2 mL
<i>Cryptococcus neoformans</i> (serotype A)	0.2 mL	
<i>Klebsiella pneumoniae</i> (Z138, CTX-M and OXA-48 like)	0.2 mL	
<i>Klebsiella pneumoniae</i> (Z460, CTX-M and NDM)	0.2 mL	
<i>Salmonella enterica typhimurium</i> (Z005)	0.2 mL	
<i>Staphylococcus lugdunensis</i> (Z097.)	0.2 mL	

## Simple Protocol Example

The estimated total time for completion for this Simple Protocol verification example is 4 days for a BIOFIRE System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3. 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 3 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.



**Note:** It is important to prepare only the number of sample pools that will be tested within 14 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).
2. Prepare sample pools from ZeptoMetrix NATBCP2-BIO control material. 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 2 mL tube. Alternatively, a 5mL tube may be used.
  - b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube (approximately 1.4 mL total volume for pools 1-4 and 1.2 mL for pool 5).
3. Repeat Step 2 for the remaining sample pools (i.e. Pool 2 and 3) to be prepared on Day 1.
4. Test 2 replicates from each sample pool (see Figure 1: Pool 1 replicates 1A and 1B, Pool 2 replicates 2A and 2B and Pool 3 replicates 3A and 3B). 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:** 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.

5. Refrigerate samples (2–8°C) for up to 14 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 above (i.e. Pool 1 replicates 1C and 1D, Pool 2 replicates 2C and 2D and Pool 3 replicates 3C and 3D).

## Day 3

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

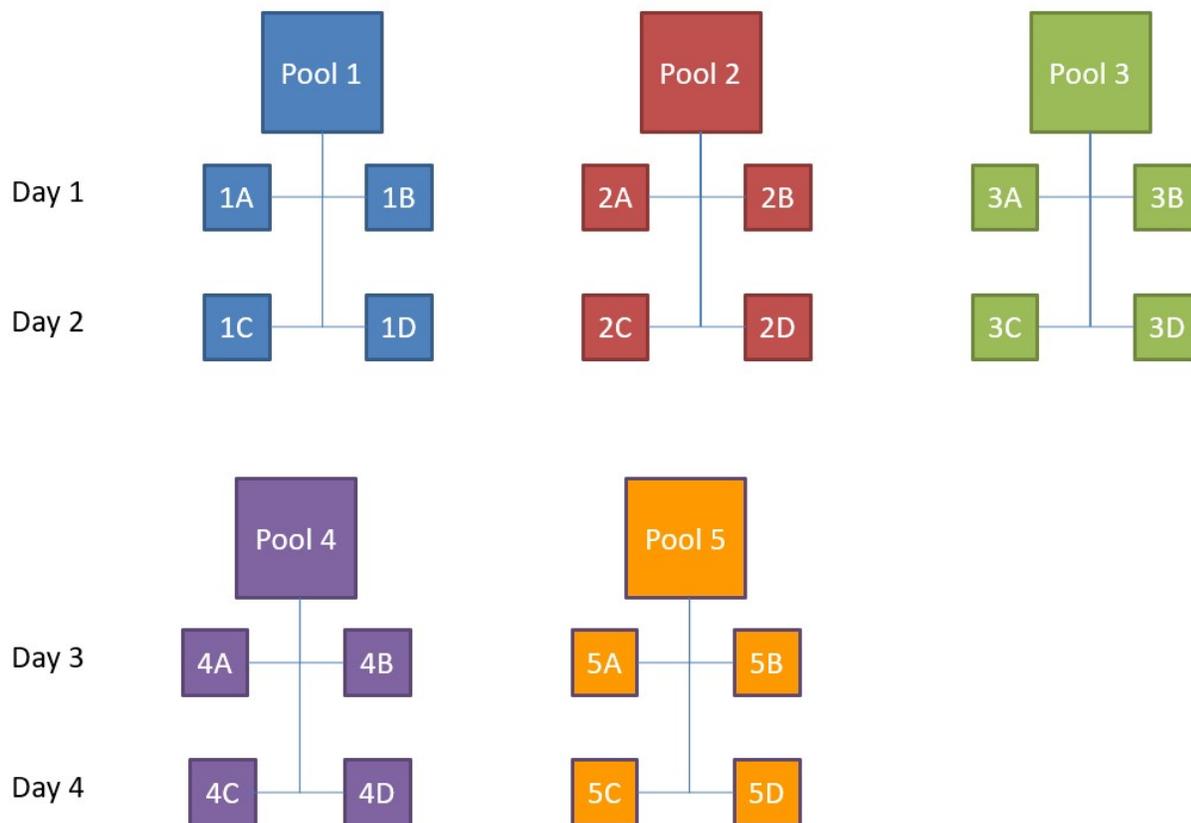


## Day 4

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

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

**Figure 1. Workflow for the Simplified or the Blood Culture Media Protocols**





**Figure 2. Example of a Verification Workflow 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	



## Blood Culture Media Protocol

The Blood Culture Media Protocol evaluates the BIOFIRE BCID2 Panel performance when sample material (ZeptoMetrix NATBCP2-BIO) is pooled and combined with blood culture media. The proposed organism pooling scheme (Table 4) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner. Blood culture media should be screened on the BIOFIRE BCID2 Panel to characterize the sample.

-  **Note:** It is important to characterize clinical matrix specimens/ blood culture media for BCID2 Panel targets by screening the specimen on the BIOFIRE BCID2 Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes detected on the BIOFIRE BCID2 Panel.
-  **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 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® System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 14 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

**Table 4.** Proposed Organism Pooling Scheme for the Blood Culture Media Sample Matrix Protocol

Control Organism and Resistance Genes: NATBCP2-BIO	Approximate Organism Volume	Volume of Blood Culture Medium	Approximate Final Pool Volume
<b>Pool 1</b>			
<i>Acinetobacter baumannii</i> (307-0294)	0.2 mL	1.4 mL	2.8 mL
<i>Candida albicans</i> (Z006)	0.2 mL		
<i>Enterococcus faecalis</i> (Z345, vanB)	0.2 mL		
<i>Enterococcus faecium</i> (Z347, vanA)	0.2 mL		
<i>Staphylococcus aureus</i> (mecA and MREJ)	0.2 mL		
<i>Streptococcus agalactiae</i> (Z019)	0.2 mL		
<i>Streptococcus pyogenes</i> (Z018)	0.2 mL		



Pool 2			
<i>Candida glabrata</i> (Z007)	0.2 mL	1.4 mL	2.8 mL
<i>Candida krusei</i> (Z009)	0.2 mL		
<i>Enterobacter cloacae</i> (Z101)	0.2 mL		
<i>Haemophilus influenzae</i> (MinnA)	0.2 mL		
<i>Klebsiella oxytoca</i> (Z115)	0.2 mL		
<i>Listeria monocytogenes</i> (serotype 1/2b)	0.2 mL		
<i>Staphylococcus epidermidis</i> (MSSE; HER 1292)	0.2 mL		
Pool 3			
<i>Bacteroides fragilis</i> (Z029)	0.2 mL	1.4 mL	2.8 mL
<i>Candida parapsilosis</i> (Z011)	0.2 mL		
<i>Candida tropicalis</i> (Z012)	0.2 mL		
<i>Klebsiella pneumoniae</i> (KPC-2, KPC)	0.2 mL		
<i>Pseudomonas aeruginosa</i> (Z139, VIM)	0.2 mL		
<i>Serratia marcescens</i> (Z053)	0.2 mL		
<i>Streptococcus pneumoniae</i> (Z022)	0.2 mL		
Pool 4			
<i>Candida auris</i> (Z485)	0.2 mL	1.4 mL	2.8 mL
<i>Escherichia coli</i> Z521 (Z521, <i>mcr-1</i> )	0.2 mL		
<i>Escherichia coli</i> Z297 (Z297, IMP)	0.2 mL		
<i>Klebsiella aerogenes</i> (Z052)	0.2 mL		
<i>Neisseria meningitidis</i> (serogroup A)	0.2 mL		
<i>Proteus mirabilis</i> (Z050)	0.2 mL		
<i>Stenotrophomonas maltophilia</i> (Z074)	0.2 mL		
Pool 5			
<i>Cryptococcus gattii</i> (Z156)	0.2 mL	1.2 mL	2.4 mL
<i>Cryptococcus neoformans</i> (serotype A)	0.2 mL		
<i>Klebsiella pneumoniae</i> ( Z138, CTX-M and OXA-48 like)	0.2 mL		
<i>Klebsiella pneumoniae</i> (Z460, CTX-M and NDM)	0.2 mL		
<i>Salmonella enterica typhimurium</i> (Z005)	0.2 mL		
<i>Staphylococcus lugdunensis</i> (Z097)	0.2 mL		

## Blood Culture Media Protocol Example

The estimated total time for completion for this Blood Culture Media Protocol verification example is 4 days for a BIOFIRE System configured with 1 module. 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.

**Note:** It is important to prepare only the number of sample pools that will be tested within 14 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.

Pooled samples may be stored overnight (or up to 14 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.

## Day 1

1. Organize materials needed (Table 2), refer to Table 4 for pooling scheme. Blood Culture media should be screened on the BIOFIRE BCID2 Panel prior to preparing pools. The optimal clinical matrix specimen will be negative for all analytes detected on the BIOFIRE BCID2 Panel.
2. Prepare sample pools from ZeptoMetrix NATBCP2-BIO control material. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for example organism pooling schemes and specific volumes for each pool.
  - a. Use a 10-mL syringe and an 18-gauge needle to remove approximately 8 mL of blood culture medium from a blood culture bottle and transfer it to a 15mL tube. Care should be taken to minimize transferring resin beads into the sample.
  - b. Use a serological pipette to add 1.2 or 1.4 mL of blood culture media (as described in Table 4) into a sterile 5 mL tube.
  - c. Transfer the entire contents of the ZeptoMetrix organism vial (approximately 0.2 mL) into the 5 mL tube containing blood culture media.
  - d. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube (approximately 2.8 mL total volume for pools 1-4 or 2.4 mL for pool 5).
3. Repeat Step 2 for the remaining sample pools (i.e. Pool 2 and 3) to be prepared on Day 1.
4. Test 2 replicates from each sample pool (see Figure 1: Pool 1 replicates 1A and 1B, Pool 2 replicates 2A and 2B and Pool 3 replicates 3A and 3B). 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:** 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.

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. Pool # 2 replicates A and B)
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.





 **Note:** The proposed organism pooling scheme (Table 4) 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 same sample pools prepared on Day 1 by repeating Step 4 above (i.e. Pool 1 replicates 1C and 1D).

## Day 3

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

## Day 4

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

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

## Expanding or Modifying the Protocols

The protocols 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 may use blood culture media as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960 Validation or Verification Studies - Specimen Selection.



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

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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

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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.



## BIOFIRE® Blood Culture Identification 2 (BCID2) Panel Verification Record

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

Module Serial #  
 Module Serial #  
 Module Serial #

Module Serial #  
 Module Serial #  
 Module Serial #

BIOFIRE BCID2 Panel Detection	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	# Operators	# Days	# Modules	Patient Samples?	
Pool 1 Organism and Resistance Genes	<i>Acinetobacter calcoaceticus-baumannii</i> complex																										
	<i>Candida albicans</i>																										
	<i>Enterococcus faecalis</i>	vanB																									
	<i>Enterococcus faecium</i>	vanA																									
	<i>Staphylococcus aureus</i>	mecA/C and MREJ (MRSA)																									
	<i>Streptococcus agalactiae</i>																										
	<i>Streptococcus pyogenes</i>																										
	mecA/C and MREJ (MRSA)																										
	vanA/B																										
	Pool 2	<i>Candida glabrata</i>																									
<i>Candida krusei</i>																											
Enterobacter cloacae complex																											
<i>Haemophilus influenzae</i>																											
<i>Klebsiella oxytoca</i>																											
<i>Listeria monocytogenes</i>																											
<i>Staphylococcus epidermidis</i>																											
Pool 3	<i>Bacteroides fragilis</i>																										
	<i>Candida parapsilosis</i>																										
	<i>Candida tropicalis</i>																										
	<i>Klebsiella pneumoniae</i> group	KPC																									
	<i>Pseudomonas aeruginosa</i>	VIM																									
	<i>Serratia marcescens</i>																										
	<i>Streptococcus pneumoniae</i>																										
	KPC																										
	VIM																										
	Pool 4	<i>Candida auris</i>																									
<i>Escherichia coli</i>		Z521 (mcr-1)																									
		Z297 (IMP)																									
<i>Klebsiella aerogenes</i>																											
<i>Neisseria meningitidis</i> (encapsulated)																											
<i>Proteus</i> spp.																											
<i>Stenotrophomonas maltophilia</i>																											
IMP																											
mcr-1																											
Pool 5	<i>Cryptococcus neoformans/gattii</i>																										
	<i>Klebsiella pneumoniae</i> group	Z138 (CTX-M and OXA-48 and Z460 (CTX-M and NDM)																									
		<i>Salmonella</i> spp.																									
	<i>Staphylococcus lugdunensis</i>																										
	CTX-M																										
NDM																											
OXA-48 like																											

Reviewed by: \_\_\_\_\_  
 Signature Date

