Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Blood Culture Identification (BCID) Panel

TECHNICAL
::: NOTE

Laboratory Protocols for Use with a ZeptoMetrix NATtrol™ Verification Panel

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 BioFire BCID Panel performance on BioFire® FilmArray® Systems as required by CLIA. Two possible verification schemes, compatible with the BioFire BCID Panel, have been designed using non-clinical specimens. Each verification scheme provides positive and negative tests for each organism detected by the BioFire BCID 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 BCID 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 BCID Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BioFire Systems. The BioFire BCID Panel is capable of simultaneous detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BioFire BCID Panel assay 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 gram-positive bacteria, gram-negative bacteria, and yeast are identified using the BioFire BCID Panel: Enterococci, *Listeria monocytogenes*,

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BioFire Diagnostics, LLC
www.biofiredx.com

QS-339B-01

Staphylococci (including specific differentiation of *Staphylococcus aureus)*, Streptococci (with specific differentiation of *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*), *Acinetobacter baumannii*, *Enterobacteriaceae* (including specific differentiation of the *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus,* and *Serratia marcescens*), *Haemophilus influenzae*, *Neisseria meningitidis* (encapsulated), *Pseudomonas aeruginosa*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*.

The BioFire® FilmArray® Blood Culture Identification (BCID) Panel also contains assays for the detection of genetic determinants of resistance to methicillin (*mecA*), vancomycin (*vanA* and *vanB*), and carbapenems (bla_{KPC}) to aid in the identification of potentially antimicrobial resistant organisms in positive blood culture samples.

The complete intended use statement and additional information about the use of the BioFire® FilmArray® System can be found in the *BioFire® FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet.*

Performance Verification: Overview

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of BioFire BCID Panel performance and (2) a Blood Culture Media Protocol that evaluates BioFire BCID Panel performance when organisms are in a blood culture media sample matrix. These protocols are examples of procedures to assist your laboratory in developing a protocol for the verification of BioFire BCID Panel performance on BioFire Systems.

The performance verification protocols have been designed to take advantage of the multiplex nature of the BioFire BCID Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. Each procedure described below will generate multiple positive and negative results for each of the BioFire BCID Panel assays. The procedures were developed using a BCID Verification Panel available from ZeptoMetrix™ Corporation, Buffalo, NY (part number NATBCP-BIO).

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 verification protocol on each individual instrument, it is advised that test replicates are evenly distributed among the instruments or modules.

In addition to, or in place of, the verification protocol examples described here, a laboratory may choose to test clinical/patient samples to assess clinical sensitivity and sample matrix effects in its performance verification of the BioFire BCID Panel.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results	Expected Negative Results	Approximate Days of Testing[a]
Example 1: Simple protocol	5 or 6	4	4	16	4 per organism	12 per organism	4
Example 2: Blood Culture media protocol	5 or 6	4	4	16	4 per organism	12 per organism	4

[a] The approximate number of days for testing assumes a BioFire® FilmArray® System configured with one instrument/module.

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 2. Recommended materials for verification protocol

Material	Part Number
BioFire® FilmArray® Blood Culture Identification (BCID) Panel (30 tests per kit)	BioFire Diagnostics, LLC RFIT-ASY-0126
Blood culture media[a]	BD BACTEC™ Plus Aerobic/F Medium (with resin) BD, 442192 (or equivalent)
BioFire® FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet	BioFire Diagnostics, LLC (RFIT-PRT-0369)
BioFire® FilmArray® Blood Culture Identification Panel Quick Guide	BioFire Diagnostics, LLC (RFIT-PRT-0370)
Control Organism	ZeptoMetrix NATBCP-BIO[b]

[a] See Table 77 in the BioFire® FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet for other acceptable blood culture media/bottle types.

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

Performance Verification Protocol

Simple Protocol

The Simple Protocol utilizes samples prepared by pooling either 5 or 6 different organisms (ZeptoMetrix NATBCP-BIO). 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 BCID Verification Panel organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

The Simple Protocol can be followed to test a total of 16 pooled samples, providing 4 positive results and 12 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. The testing scheme can be modified to run more samples per day based on the number of instruments configured on the BioFire® FilmArray® System.

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.

Table 3. Proposed Organism Pooling Scheme

Organism	Approximate Organism Volume	Approximate Final Volume of Pool
Pool 1		
Candida albicans	0.2 mL	1.0 mL
Neisseria meningitidis	0.2 mL	
Staphylococcus aureus (MRSA)*	0.2 mL	
Streptococcus agalactiae	0.2 mL	
Streptococcus pyogenes	0.2 mL	
Pool 2		
Acinetobacter baumannii	0.2 mL	1.2 mL
Candida glabrata	0.2 mL	
Candida krusei	0.2 mL	
Enterobacter cloacae complex	0.2 mL	
Listeria monocytogenes	0.2 mL	
Staphylococcus epidermidis (MSSE)**	0.2 mL	
Pool 3		
Candida parapsilosis	0.2 mL	1.2 mL
Haemophilus influenzae	0.2 mL	
Enterococcus faecalis (VRE)	0.2 mL	
Klebsiella pneumoniae (KPC)	0.2 mL	
Serratia marcescens	0.2 mL	
Streptococcus pneumoniae	0.2 mL	
Pool 4		
Escherichia coli	0.2 mL	1.0 mL
Pseudomonas aeruginosa	0.2 mL	
Klebsiella oxytoca	0.2 mL	
Candida tropicalis	0.2 mL	
Proteus	0.2 mL	

*MRSA, methicillin resistant *S. aureus*
**MSSE, methicillin susceptible *S. epidermidis*

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. Refer to Figure 1 for the suggested workflow.

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 2 sample pools is based on testing 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments connected within a BioFire System.

Day 1

1. Prepare two sample pools (i.e. pools #1 and #2, in Table 3 above) from ZeptoMetrix NATBCP-BIO control material. Organism vials should be well mixed prior to preparing each pool.

    a. Use a transfer pipette to remove 0.2 mL material from the ZeptoMetrix organism vial (draw material to the second line of the transfer pipette) and transfer to a new vial or tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms into a single vial or tube (approximately 1.0 mL total volume for five organisms or 1.2 mL for six organisms).
    c. Cap and vortex prior to testing.

2. Test two samples from a single sample pool. The duplicate samples should be tested in a single day by different users.

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

3. Repeat Step 2 for the remaining sample pools (i.e. pool #2) to be tested that day.

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

Day 2

To evaluate day-to-day variation, test the remaining volume of the sample pools prepared on Day 1 by repeating Step 2 and 3 above.

Day 3

Prepare 2 new sample pools (i.e. pools #3 and #4) as described in Step 1. Test samples according to Step 2 and 3 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 and 3 above.

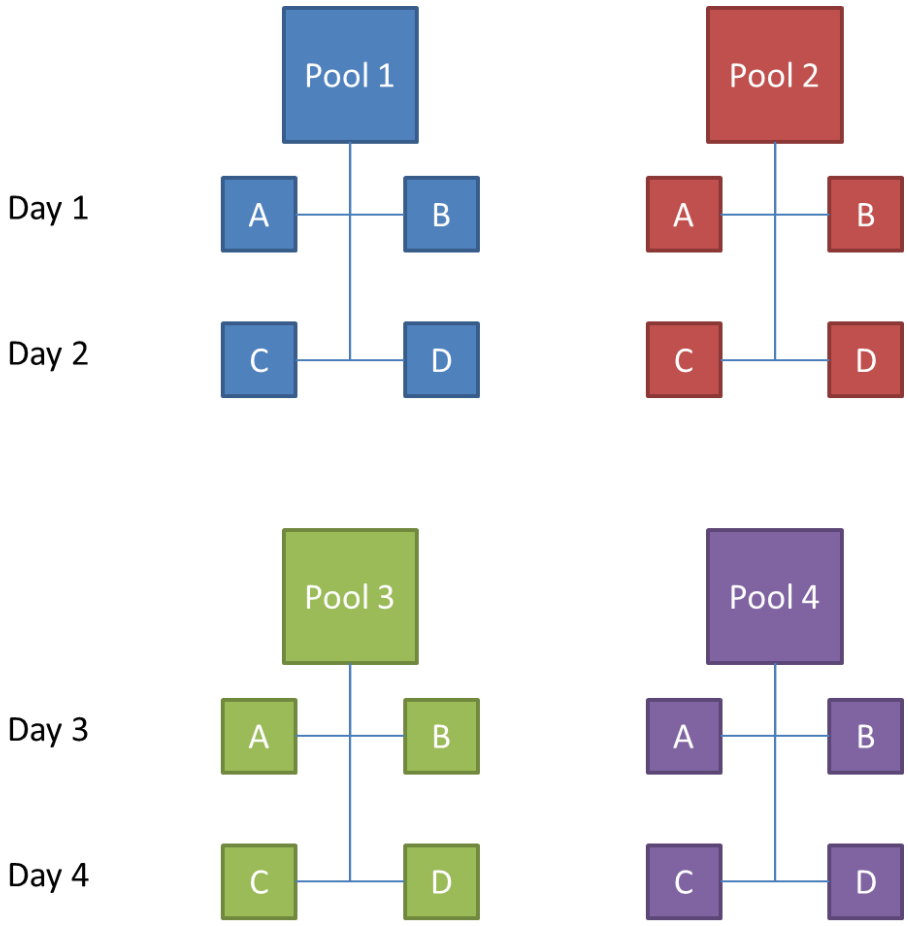

Figure 1. Workflow for Simple Protocol

Blood Culture Media Protocol Example

The Blood Culture Media Protocol evaluates BioFire® FilmArray® Blood Culture Identification (BCID) Panel performance in a blood culture sample matrix. Sample material is pooled and added to an equal volume of blood culture media matrix.

The Blood Culture Media Protocol can be followed to test a total of 16 pooled samples, providing 4 positive results and 12 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. The testing scheme can be modified to run more samples per day based on the number of instruments configured on the BioFire® FilmArray® System.

Blood culture media 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. To evaluate user-to-user variation, multiple laboratory technicians may perform testing.

Table 4. Proposed Blood Culture Media Sample Preparation Scheme

Organism	Approximate Organism Volume	Volume of Blood Culture Medium	Approximate Final Volume of Pool
Pool 1			
Candida albicans	0.2 mL	1.0 mL	2.0 mL
Neisseria meningitidis	0.2 mL		
Staphylococcus aureus (MRSA)*	0.2 mL		
Streptococcus agalactiae	0.2 mL		
Streptococcus pyogenes	0.2 mL		
Pool 2			
Acinetobacter baumannii	0.2 mL	1.2 mL	2.4 mL
Candida glabrata	0.2 mL		
Candida krusei	0.2 mL		
Enterobacter cloacae complex	0.2 mL		
Listeria monocytogenes	0.2 mL		
Staphylococcus epidermidis (MSSE)**	0.2 mL		
Pool 3			
Candida parapsilosis	0.2 mL	1.2 mL	2.4 mL
Haemophilus influenzae	0.2 mL		
Enterococcus faecalis (VRE)	0.2 mL		
Klebsiella pneumoniae (KPC)	0.2 mL		
Serratia marcescens	0.2 mL		
Streptococcus pneumoniae	0.2 mL		
Pool 4			
Escherichia coli	0.2 mL	1.0 mL	2.0 mL
Pseudomonas aeruginosa	0.2 mL		
Klebsiella oxytoca	0.2 mL		
Candida tropicalis	0.2 mL		
Proteus	0.2 mL		

*MRSA, methicillin resistant *S. aureus*

**MSSE, methicillin susceptible *S. epidermidis*

Blood Culture Protocol Example

The estimated total time for completion for the Blood Culture Protocol verification example is 4 days for a BioFire® FilmArray® System configured with 1 module. A proposed organism pooling scheme is presented above in Table 4. Refer to Figure 2 for the suggested workflow.

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 2 sample pools is based on testing 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments connected within a BioFire® FilmArray® System.

Day 1

1. Prepare two sample pools (i.e. pools #1 and #2, in Table 4 above) from ZeptoMetrix NATBCP-BIO control material and blood culture media. Organism vials should be well mixed prior to preparing each pool.

    a. Pipet 1.0 or 1.2 mL of blood culture media (as described in Table 4) into a sterile tube or vial.
    b. Use a transfer pipette to remove 0.2 mL material from the ZeptoMetrix organism vial (draw material to the second line of the transfer pipette) and transfer to the tube containing blood culture media.
    c. Repeat with the second (and subsequent) organisms to combine the appropriate organisms into a single vial or tube (approximately 2.0 mL total volume for five organisms or 2.4 mL for six organisms).
    d. Cap and vortex prior to testing.

2. Test two samples from a single sample pool. The duplicate samples should be tested in a single day by different users.

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

3. Repeat Step 2 for the remaining sample pools (i.e. pool #2) to be tested that day.

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

Day 2

To evaluate day-to-day variation, test the remaining volume of the sample pools prepared on Day 1 by repeating Step 2 and 3 above.

Day 3

Prepare 2 new sample pools (i.e. pools #3 and #4) as described in Step 1. Test samples according to Step 2 and 3 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 and 3 above.

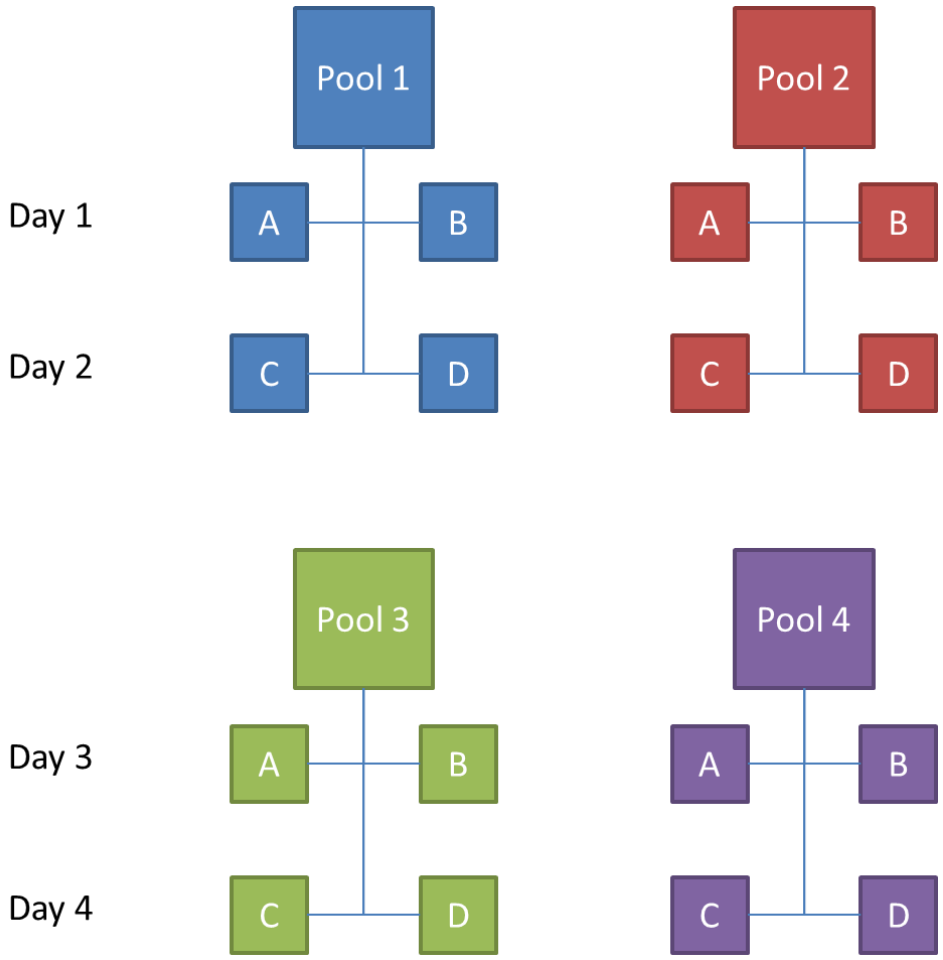

Figure 2. Workflow for Blood Culture Media Protocol

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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 2 mL, which is enough material to complete many tests 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® FilmArray® Blood Culture Identification (BCID) Panel. The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples should not be pooled or diluted.

2. Test the selected specimens/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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BioFire® FilmArray® Blood Culture Identification Verification Record

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System Serial # _____

BioFire® FilmArray® Blood Culture Identification (BCID) Panel, Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

Organism	System Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Acinetobacter baumannii		☐ Yes ☐ No					
Candida albicans		☐ Yes ☐ No					
Candida glabrata		☐ Yes ☐ No					
Candida krusei		☐ Yes ☐ No					
Candida parapsilosis		☐ Yes ☐ No					
Candida tropicalis		☐ Yes ☐ No					
Enterobacter cloacae		☐ Yes ☐ No					
Enterococcus faecalis (with *vanA/B* call)		☐ Yes ☐ No					
Escherichia coli		☐ Yes ☐ No					
Haemophilus influenzae		☐ Yes ☐ No					
Klebsiella oxytoca		☐ Yes ☐ No					
Klebsiella pneumoniae (with KPC call)		☐ Yes ☐ No					
Listeria monocytogenes		☐ Yes ☐ No					
Neisseria meningitidis		☐ Yes ☐ No					
Proteus mirabilis		☐ Yes ☐ No					
Pseudomonas aeruginosa		☐ Yes ☐ No					
Serratia marcescens		☐ Yes ☐ No					
Staphylococcus aureus (MRSA, with *mecA* call)		☐ Yes ☐ No					
Staphylococcus epidermidis (MSSE, no mecA call)		☐ Yes ☐ No					
Streptococcus agalactiae		☐ Yes ☐ No					
Streptococcus pneumoniae		☐ Yes ☐ No					
Streptococcus pyogenes		☐ Yes ☐ No					

Reviewed by: _____

Signature Date