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

TECHNICAL ::: NOTE

Laboratory Protocols for Use with Microbiologics® Helix Elite™ Molecular Standards

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.

FilmArray 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 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.

FLM1-PRT-0246-01

Technical Note
BioFire Diagnostics, LLC
www.BioFireDX.com

QS-339B-01

The following gram-positive bacteria, gram-negative bacteria, and yeast are identified using the BioFire® FilmArray® Blood Culture Identification (BCID) Panel: Enterococci, *Listeria monocytogenes*, 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 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 (See Table 1): (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 Helix Elite™ Molecular Standards available from Microbiologics®, Saint Cloud, MN (part number 8201).

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	6, 7, or 9	3	4	12	4 per organism	8 per organism	2
Example 2: Blood culture media protocol	6, 7, or 9	3	4	12	4 per organism	8 per organism	2

[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 protocols

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) , 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 and Hydration Buffer	Microbiologics® Helix Elite™ Molecular Standards 8201[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. This protocol is specific for the Microbiologics 8201 material. Use of alternate organisms may require a different protocol. Refer to the BioFire website for a complete list of verification protocols.

Simple Protocol

The Simple Protocol utilizes lyophilized pooled samples from Microbiologics® (Helix Elite™ Molecular Standards #8201). The material should be rehydrated as described in Table 3 to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

Note: Dilution of Microbiologics Helix Elite standards 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 12 pooled samples, providing 4 positive results and 8 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.

Rehydrated 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 3. Simple Protocol Rehydration Scheme

Organism	Hydration Buffer	Approximate Final Volume of Pool
Control 1		
Candida albicans	1.2 mL	1.2 mL
Candida krusei		
Streptococcus agalactiae		
Neisseria meningitidis		
Pseudomonas aeruginosa		
Staphylococcus aureus (MRSA)*		
Streptococcus pyogenes		
Control 2		
Enterococcus faecalis	1.2 mL	1.2 mL
Staphylococcus epidermidis (MSSE)**		
Acinetobacter baumannii		
Candida glabrata		
Candida tropicalis		
Enterobacter cloacae		
Klebsiella oxytoca		
Listeria monocytogenes		
Escherichia coli		

Control 3		
Candida parapsilosis	1.2 mL	1.2 mL
Klebsiella pneumoniae		
Proteus mirabilis		
Serratia marcescens		
Haemophilus influenzae		
Streptococcus pneumoniae		

*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 2 days for a BioFire® FilmArray® System configured with 1 module. Rehydration instructions are described 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.

Day 1

1. Rehydrate the control materials as described in Table 3 using the Hydration buffer provided by Microbiologics.

2. Cap and vortex prior to testing.

3. Test two samples from a single sample control 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 Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

4. Repeat Step 2 for the remaining controls be tested that day.

5. Refrigerate rehydrated controls (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 control pools prepared on Day 1 by repeating Steps 2 through 5 above.

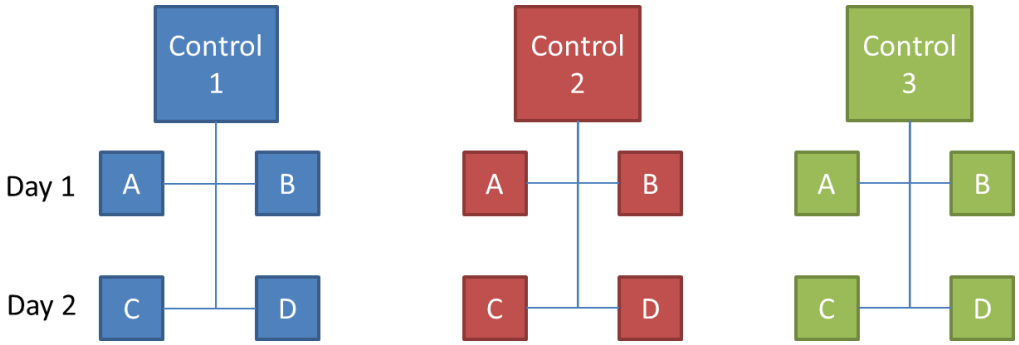


Figure 1. Workflow for Simple Protocol

The protocol can be followed to test a total of 12 pooled samples, providing 4 positive results and 8 negative results per organism. This example demonstrates how the verification can be completed by performing six tests per day. This testing scheme can be modified to run more samples per day based on the number of instruments in the BioFire® FilmArray® System. The number of samples tested per day should be determined by the individual laboratory.

Blood Culture Media Protocol

The Blood Culture Media Protocol evaluates BioFire® FilmArray® Blood Culture Identification (BCID) Panel performance in a blood culture sample matrix using lyophilized pooled organisms from Microbiologics® (Helix Elite™ Molecular Standards #8201). The control material is hydrated with blood culture media as described in Table 4.

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

The Blood Culture Media Protocol can be followed to test a total of 12 pooled samples, providing 4 positive results and 8 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 System.

Rehydrated 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. Blood Culture Media Protocol Rehydration Scheme

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

*MRSA, methicillin resistant *S. aureus*.

**MSSE, methicillin susceptible *S. epidermidis.*

Blood Culture Media Protocol Example

The estimated total time for completion for the Blood Culture Protocol verification example is 2 days for a BioFire® FilmArray® System configured with 1 module. Rehydration instructions are described 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.

Day 1

1. Rehydrate the control materials from Microbiologics #8201 as described in Table 4 using blood culture media. See Table 77 in the BioFire® FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet for acceptable blood culture media/bottle types.

2. Cap and vortex prior to testing.

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

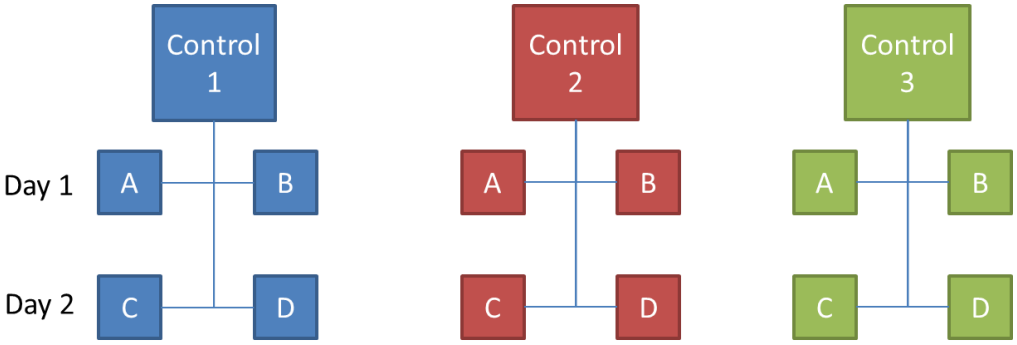
Note: For each sample, follow instructions in the *BioFire® FilmArray® Blood Culture Identification (BCID) Panel Instruction Booklet* and *BioFire® FilmArray® BCID Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

4. Repeat Step 2 for the remaining controls be tested that day.

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

Day 2

To evaluate day-to-day or instrument-instrument variation, test the remaining



volume of the control pools prepared on Day 1 by repeating Steps 2 through 5 above.

Figure 2. Workflow for Blood Culture Media Protocol

The protocol can be followed to test a total of 12 pooled samples, providing 4 positive results and 8 negative results per organism. This example demonstrates how the verification can be completed by performing six tests per day. This testing scheme can be modified to run more samples per day based on the number of instruments in the BioFire® FilmArray® System. The number of samples tested per day should be determined by the individual laboratory.

Expanding the protocols

The protocols described here may be expanded but may require additional control materials.

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

TECHNICAL
::: NOTE

BioFire® FilmArray® Blood Culture Identification Verification Record

TECHNICAL ::: NOTE

System Serial # _____

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

Organism/Sample Source and Lot #: _____

Organism Detection	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