Guidelines for Laboratory Verification of Performance of the FilmArray® BCID System

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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 FilmArray Blood Culture Identification (BCID) system performance required by CLIA. A simple scheme, compatible with the FilmArray BCID system, has been designed. This scheme provides positive and negative tests for each organism detected by the FilmArray BCID system and may be easily modified or expanded to meet specific criteria. This simple scheme was designed so that a trained laboratory technician could test at least 6 FilmArray pouches per day on each FilmArray instrument to be verified. 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, patient samples can be tested. 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 FilmArray Blood Culture Identification (BCID) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray Instrument. The FilmArray BCID Panel is capable of simultaneous detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BCID Panel test is performed directly on positive blood culture samples that demonstrate the presence of organisms as determined by Gram stain.

The following gram-positive bacteria, gram-negative bacteria, and yeast are identified using the FilmArray 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 FilmArray BCID Panel also contains assays for the detection of genetic determinants of resistance to methicillin (*mecA*), vancomycin (*vanA* and *vanB*),

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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 FilmArray system can be found in the *FilmArray Blood Culture Identification (BCID) Panel Instruction Booklet*

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Performance Verification: Overview

The procedure described below will generate multiple positive and negative results for each of the FilmArray BCID assays. The procedures were developed using organism strains available from Microbiologics®, Saint Cloud, MN (part numbers listed in materials section).

A simple procedure has been designed to take advantage of the multiplex nature of the FilmArray BCID Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run.

In addition to, or in place of, verification schemes described here; a laboratory may chose to test clinical/patient samples to assess clinical sensitivity and sample matrix effects in its performance verification of the FilmArray BCID Panel.

Table 1. Overview of Verification Protocol

Verification Protocol	Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results	Expected Negative Results	Approximate Days of Testing[b]
Simple protocol[c] (one instrument)	6, 7, or 9	3	4	12	4 per organism	8 per organism	2

[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] Not including time to grow microbial cultures.
[c] This simple protocol may be easily expanded to increase the number of pouches tested on one instrument or for the verification of multiple instruments.

Performance Verification: Materials

The following materials may be needed to perform verification procedures:

Material	Part Number
FilmArray BCID Panel (30 tests per kit)	BioFire Diagnostics, Inc., RFIT-ASY-0114
BD BACTEC™ Plus Aerobic/F Medium (with resin)	BD, 442192
Human Whole Blood with EDTA (pathogen free)	Bioreclamation LLC, HMWBEDTA2 (or equivalent, with anticoagulant)
McFarland Turbidity Standard, 1.0	Fisher Scientific, R20411 (or equivalent)
Phosphate Buffered Saline, pH 7.4	Sigma, P3813 (or equivalent)
Polystyrene tube with cap (14 mL, 16 x 100 mm, round-bottom)	VWR, 82050-246 (or equivalent)
Polypropylene centrifuge tube with flat cap (50 mL, sterile)	VWR, 89004-364 (or equivalent)

Organism	Microbiologics Catalog Number[a]
Acinetobacter baumannii ATCC® 19606™ KWIK-STIK	0357P
Candida albicans ATCC® 10231™ Lab-Elite	0443-CRM
Candida glabrata ATCC® 15126™ KWIK-STIK	0737P
Candida krusei ATCC® 14243™ KWIK-STIK	0809P
Candida parapsilosis ATCC® 22019™ KWIK-STIK	0726P
Candida tropicalis ATCC® 1369™ KWIK-STIK	01036P
Enterobacter cloacae subsp. cloacae ATCC® 13047™ Lab-Elite	0323-CRM
Enterococcus faecalis ATCC® 51299™ KWIK-STIK	0959P[b]
Escherichia coli ATCC® 11229™ Lab-Elite	0681-CRM
Haemophilus influenzae ATCC® 10211™ KWIK-STIK	0441P
Klebsiella oxytoca ATCC® 13182™ KWIK-STIK	0530P
Klebsiella pneumoniae ATCC® BAA-1705™ KWIK-STIK	01005P[c]
Listeria monocytogenes ATCC® 19111™ KWIK-STIK	0277P
Neisseria meningitidis ATCC® 13077™ KWIK-STIK	0453P
Proteus mirabilis ATCC® 35659™ Lab-Elite	0944-CRM
Pseudomonas aeruginosa ATCC® 27853™ KWIK-STIK	0353P
Serratia marcescens ATCC® 13880™ KWIK-STIK	0247P
Staphylococcus aureus subsp. *aureus* ATCC® 33591™ Lab-Elite	0496-CRM[d]
Staphylococcus epidermidis ATCC® 12228™ Lab-Elite	0371-CRM
Streptococcus agalactiae ATCC® 12386™ KWIK-STIK	0439P
Streptococcus pneumoniae ATCC® 10015™ KWIK-STIK	0865P
Streptococcus pyogenes ATCC® 19615™ Lab-Elite	0385-CRM

[a] Any appropriate source of organism may be used for verification of any or all of the assays in the FilmArray BCID 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 *K. pneumoniae* (ATCC® BAA-1705) carries the bla_{KPC} gene (carbapenems resistance).
[d] This strain of *S. aureus* subsp. *aureus* (ATCC® 33591) carries the *mecA* gene (methicillin resistance).

Performance Verification: Organism Pooling

The protocol described below utilizes samples prepared by pooling together up to 9 different organism suspensions in a simulated blood culture matrix background.

The pooling scheme (Table 2) was designed so that both positive and negative target results can be obtained 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.

Table 2. Example Organism Pooling Scheme

Organism	Organism Volume	Human Whole Blood	BD Culture Medium	Approximate Final Volume of Pool
Pool 1				
Candida albicans	0.1 mL	3 mL	8 mL	~ 12 mL
Candida krusei	0.1 mL			
Streptococcus agalactiae	0.1 mL			
Neisseria meningitidis	0.1 mL			
Pseudomonas aeruginosa	0.1 mL			
Staphylococcus aureus (MRSA)*	0.1 mL			
Streptococcus pyogenes	0.1 mL			
Pool 2				
Enterococcus faecalis	0.2 mL	3 mL	8 mL	~ 12 mL
Staphylococcus epidermidis (MSSE)**	0.4 mL			
Acinetobacter baumannii	0.1 mL			
Candida glabrata	0.1 mL			
Candida tropicalis	0.2 mL			
Enterobacter cloacae	0.3 mL			
Klebsiella oxytoca	0.1 mL			
Listeria monocytogenes	0.1 mL			
Escherichia coli	0.1 mL			
Pool 3				
Candida parapsilosis	0.1 mL	3 mL	8 mL	~ 12 mL
Klebsiella pneumoniae	0.1 mL			
Proteus mirabilis	0.1 mL			
Serratia marcescens	0.1 mL			
Haemophilus influenzae	0.1 mL			
Streptococcus pneumoniae	0.1 mL			

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

Example Protocol: Protocol for the Verification of One Instrument

In this example, a total of 12 pouches are tested, providing 4 positive results and 8 negative results per organism. This example assumes six tests can be completed per day on an instrument (about an hour per test). The actual number should be determined by the individual laboratory. The estimated total time to completion for this verification example is 2 days (not including time to grow microbial cultures).

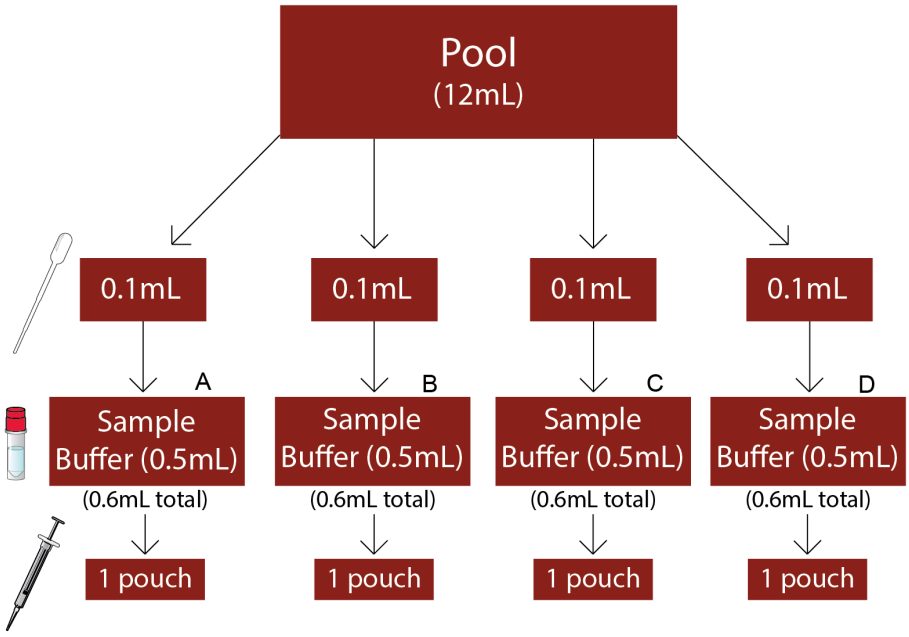

Figure 1. Simple protocol workflow. Each sample pool has enough material to assess day-to-day and user-to-user variability. Workflow can be repeated for each sample pool until all testing is complete.

Day 1

1. Obtain a pure culture of each organism that has been streaked for isolation on agar media appropriate for the organism. See Microbiologics product insert for use of KWIK-STIK cultures.

   Note: It is recommended to use agar plate cultures that are less than 1 week old.

   a. 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.

2. Prepare three sample pools according to the organism pooling scheme presented in Table 2.

Note: The sample pool preparation worksheet in Addendum 2 can assist in the set-up to ensure all components are added to each pool.

   a. Use a pipette to transfer the appropriate volume (Table 2) of organism suspension to a 50 mL conical bottom tube.
   b. Repeat for the remaining organisms in the pool to combine the appropriate organisms into a single 50 mL tube.
   c. Use a pipette to add the appropriate volume of human whole blood to the pooled organism according to Table 2.

   Note: It is recommended to use blood that has been prescreened as negative for FilmArray BCID pathogens.

   d. Use a pipette to add the appropriate volume of BD BACTEC™ Plus Aerobic/F Medium to the pool according to Table 2. Do not pipette resin beads into sample.

e. Ensure the pooled sample is effectively mixed by vortexing prior to removing a sample for testing.

Note: It is important to prepare only the number of sample pools that will be tested within 2 days. The suggestion to prepare 3 sample pools is based on an average workday, testing 6 pouches per day using one instrument. The number of samples prepared may be increased or decreased based on the laboratory's work schedule.

Note: Store samples at refrigeration temperature (2–8°C) for up to 2 days for the evaluation of day-to-day variation.

3. Prepare and test two samples (i.e. A and B, see Figure 1) from a single sample pool (i.e. Pool #1). The duplicate samples should be prepared consecutively and tested in a single day. For each sample:
    a. Follow instructions in the *FilmArray Blood Culture Identification (BCID) Panel Instruction Booklet* for pouch preparation and pouch hydration.
    b. Prepare Sample Mix: Use the Transfer Pipette provided with the FilmArray BCID kit and draw from sample pool to the first line of the pipette (approximately 0.1 mL). Add sample to the red-capped Sample Buffer vial and gently pipette up and down to mix.
    c. Follow instructions in the *FilmArray Blood Culture Identification (BCID) Panel Instruction Booklet* for sample loading and FilmArray BCID testing.

4. Repeat Step 3 for the remaining sample pools (pools #2 and #3) to be tested that day.

Day 2

To evaluate day-to-day variation, test the remaining samples (i.e. samples C and D) from the same sample pools prepared on Day 1 by repeating Step 3 above.

Note: Please see Addendum 1 for a detailed day-to-day protocol for Example 1: Protocol for verification of one instrument

Verification of Loaner and Repaired Instruments

If it becomes necessary to verify the performance of a loaner or repaired instrument, the following protocol may serve as a guideline.

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the FilmArray 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 or repaired instrument and document the results.

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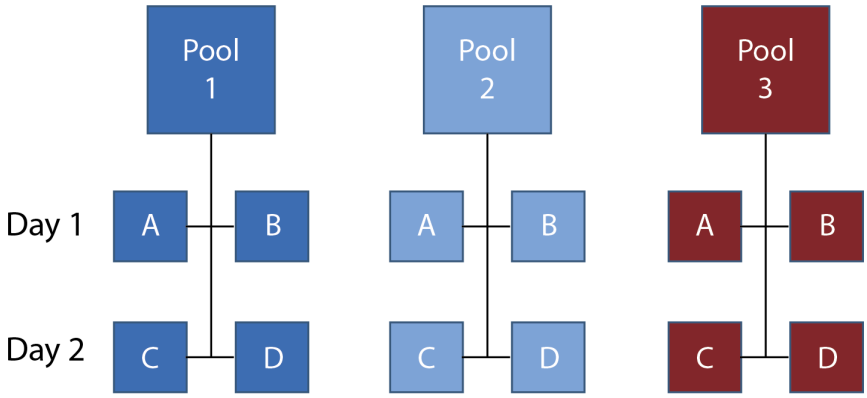
Addendum 1:

Example 1. Protocol for verification of one instrument.



Addendum 2:

Sample Pool Preparation Worksheet

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Organism	Organism Volume	Human Whole Blood Volume	BD Culture Medium Volume	Approximate Final Volume of Pool
Pool 1				
Candida albicans	☐ 0.1 mL	☐ 3 mL	☐ 8 mL	~ 12 mL
Candida krusei	☐ 0.1 mL			
Streptococcus agalactiae	☐ 0.1 mL			
Neisseria meningitidis	☐ 0.1 mL			
Pseudomonas aeruginosa	☐ 0.1 mL			
Staphylococcus aureus (MRSA)*	☐ 0.1 mL			
Streptococcus pyogenes	☐ 0.1 mL			
Pool 2				
Enterococcus faecalis	☐ 0.2 mL	☐ 3 mL	☐ 8 mL	~ 12 mL
Staphylococcus epidermidis (MSSE)**	☐ 0.4 mL			
Acinetobacter baumannii	☐ 0.1 mL			
Candida glabrata	☐ 0.1 mL			
Candida tropicalis	☐ 0.2 mL			
Enterobacter cloacae	☐ 0.3 mL			
Klebsiella oxytoca	☐ 0.1 mL			
Listeria monocytogenes	☐ 0.1 mL			
Escherichia coli	☐ 0.1 mL			
Pool 3				
Candida parapsilosis	☐ 0.1 mL	☐ 3 mL	☐ 8 mL	~ 12 mL
Klebsiella pneumoniae	☐ 0.1 mL			
Proteus mirabilis	☐ 0.1 mL			
Serratia marcescens	☐ 0.1 mL			
Haemophilus influenzae	☐ 0.1 mL			
Streptococcus pneumoniae	☐ 0.1 mL			

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

FilmArray® Instrument Verification Record

Instrument Serial #: _____

FilmArray BCID Panel, Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

Organism	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