Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel

TECHNICAL ::: NOTE

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® FilmArray® Meningitis/Encephalitis (ME) Panel has been categorized by the FDA as a CLIA moderate complexity test.

This document provides an example verification procedure to assist your laboratory in developing a protocol for the verification of the BioFire ME Panel performance on BioFire® FilmArray® Systems as required by CLIA. This BioFire ME Panel verification scheme has been designed to generate positive and negative tests for each organism detected by the BioFire ME Panel using non-clinical specimens and may be easily modified or expanded to meet specific criteria. The procedure includes an evaluation of day-to-day and user-to-user variation when repeatedly testing the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the BioFire ME 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 ME Panel is a multiplexed nucleic acid test intended for use with BioFire® FilmArray® 1.5, BioFire® FilmArray® 2.0, or BioFire® FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple viral, yeast, and bacterial nucleic acid targets in cerebrospinal fluid (CSF) samples obtained from individuals suspected of meningitis and/or encephalitis. The following are identified using the BioFire ME Panel: *Escherichia coli* K1, *Haemophilus influenzae, Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Cryptococcus gattii/neoformans*, cytomegalovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, enterovirus, human parechovirus, and varicella zoster virus.

The complete intended use statement and additional information about the use of the BioFire System can be found in the *FilmArray Meningitis/Encephalitis (ME) Panel Instruction Booklet.*

FLM1-PRT-0165-04

Technical Note
BioFire Diagnostics, LLC
www.BioFireDX.com

QS-339B-02

Performance Verification: Overview

TECHNICAL ::: NOTE

The procedure described below will generate multiple positive and negative results for each of the organisms targeted by the BioFire® ME Panel. The procedures were developed using a panel available from ZeptoMetrix Corporation, Buffalo, NY (NATMEP-BIO).

An example procedure for performance verification is described below. The procedure can be performed without any further dilution of the reference material. The procedures can also be performed using samples prepared in an artificial cerebrospinal fluid (aCSF) background (published recipes or commercially available) or with cerebrospinal fluid (CSF) that has been verified as negative for BioFire ME Panel targets.

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 the test replicates are evenly distributed among the instruments and modules within a single instrument.

The procedures have been designed to take advantage of the multiplex nature of the BioFire ME Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run.

Clinical/patient specimens may be used in addition to, or in place of the verification schemes described here, in order to assess clinical sensitivity and sample matrix effects for verification of the BioFire ME Panel.

Table 1. Overview of 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]
4 or 5	3	4	12	4 per organism	8 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 approximate number of days for testing assumes a system configured with one instrument/module.

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 2. Materials needed for recommended protocol

Material	Part Number
BioFire® FilmArray® ME Panel Kit (30 tests)	Biofire Diagnostics, LLC RFIT-ASY-0118
Control organism	ZeptoMetrix NATMEP-BIO[a]
5mL sample tubes	VWR Part # 89497-740 (or equivalent)
Transfer pipettes	VWR Part # 13-711-43 (or equivalent)
Artificial cerebrospinal fluid (aCSF)	Tocris Biosciences #3525

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire ME Panel. However, when alternate organism sources are used, the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

Performance Verification: Protocol

The protocol can be followed to test a total of 12 pouches, providing 4 positive results and 8 negative results per organism. 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 instruments or modules configured on the BioFire® System.

The recommended protocol requires the preparation of 3 organism pools for testing, each containing up to 5 different control organisms (ZeptoMetrix NATMEP-BIO). If a larger volume of each pool is desired, organisms can be combined with an additional volume of artificial cerebrospinal fluid (aCSF) background (Tocris Biosciences Part # 3525 or equivalent) or follow published recipes for aCSF preparation) or with residual clinical CSF that has been verified as negative for BioFire® ME Panel targets.

The proposed pooling scheme (Table 3) should be followed to obtain the expected positive and negative results for each assay in a time and resource-efficient manner.

Note: Dilution of ZeptoMetrix ME Verification Panel organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Table 3. Recommended Organism Pooling Scheme

Control Organism	Control Organism Volume	Approximate Final Volume of Pool	Optional Addition of aCSF or CSF	
			Volume of aCSF or CSF	Approximate Final Volume of Pool
Pool 1				
Escherichia coli K1	0.30 mL	1.5 mL	0.9 mL	2.4 mL
Cytomegalovirus (CMV)	0.30 mL			
Echovirus type 11	0.30 mL			
Streptococcus pneumoniae	0.30 mL			
Human herpesvirus 6 (HHV-6)	0.30 mL			
Pool 2				
Herpes simplex virus 1 (HSV-1)	0.30 mL	1.2 mL	0.9 mL	2.1 mL
Neisseria meningitidis	0.30 mL			
Streptococcus agalactiae	0.30 mL			
Cryptococcus gattii	0.30 mL			
Pool 3				
Haemophilus influenzae	0.30 mL	1.5 mL	0.9 mL	2.4 mL
Herpes simplex virus 2 (HSV-2)	0.30 mL			
Varicella zoster virus (VZV)	0.30 mL			
Listeria monocytogenes	0.30 mL			
Human parechovirus (HPeV)	0.30 mL			

Protocol Example

The estimated total time to completion for this verification example is 4 days for systems configured with one instrument (Figure 1).

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Day 1

1. Prepare one organism pool (e.g. pool 1) from ZeptoMetrix NATMEP-BIO control material. An example organism pooling scheme is presented in Table 3.

   Note: It is important to prepare only the number of organism pools that will be tested within 3 days of preparation. The number of organism pools prepared and samples tested may be increased or decreased based on the laboratory's work schedule and the number of instruments connected within a BioFire® System.

   a. Transfer 0.3 mL of the ZeptoMetrix organism to a tube large enough (at least 3 mL) to hold the entire organism pool volume.

   b. Repeat step a. for each of the remaining organisms to combine the appropriate organisms for each pool into a single vial or tube (approximately 1.5 mL total volume). Vortex to mix well.

   c. **Optional:** transfer 0.9 mL of aCSF or CSF to the organism pool (approximately 2.4 mL total volume) and vortex to mix well.

   d. Proceed to Step 2 for testing. The organism pool may be stored refrigerated (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

2. Test 2 samples (e.g. samples A and B) from a single organism pool (e.g. pool 1). The duplicate samples should be tested in a single day by the same operator to evaluate run-to-run variation or different operators to evaluate operator-to-operator variation.

   Note: Follow instructions in the *FilmArray® Meningitis/Encephalitis (ME) Panel Instruction Booklet* or *FilmArray Meningitis/Encephalitis Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

3. Repeat steps 1 and 2 for another organism pool (e.g. pool 2) to be tested that day.

Day 2

To evaluate day-to-day variation, test the remaining samples (e.g. samples C and D) from the same organism pool(s) prepared on Day 1 by repeating Step 2 above.

Day 3

Prepare a new organism pool (e.g. pool 3) as described in Step 1. Test samples according to Step 2 (e.g. samples A and B) for the pool.

Day 4

To evaluate day-to-day variation, test the remaining samples (e.g. samples C and D) from the organism pool(s) prepared on Day 3 by repeating Step 2 above.

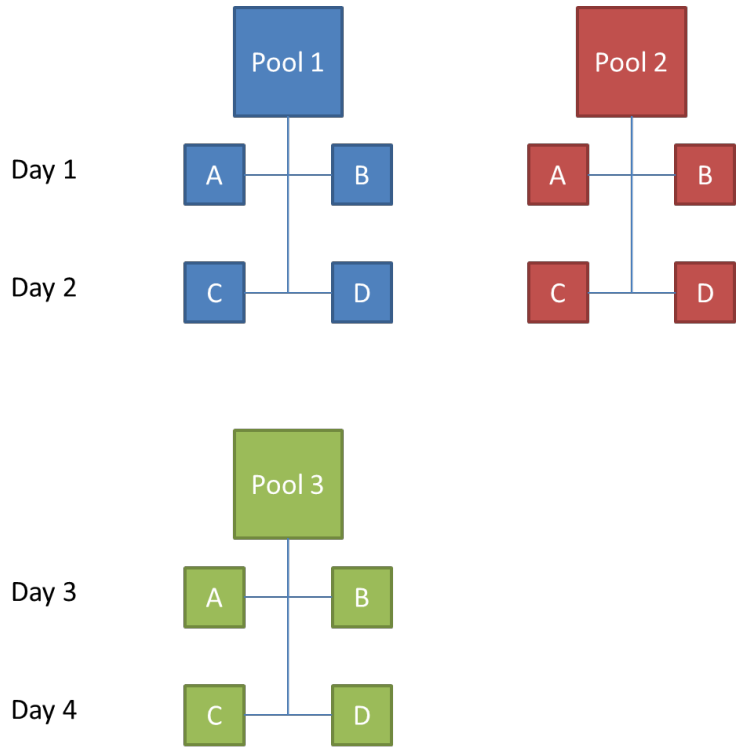

Figure 1. Protocol workflow

Expanding the protocols

The protocols described above can be expanded to increase the number of samples or tests for each of the organism pools. Additional testing (up to 11 tests per pool when using the additional volume of aCSF or CSF described in Table 3) may include more replicates per pool, more replicates per day (no more than 3 days per pool), more replicates per operator, and/or more instruments or modules per BioFire® System.

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® ME Panel. The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples or other stored samples should not be pooled or diluted prior to testing.

2. Select a set of controls that verify detection of all targets on the BioFire ME Panel.

3. Test the selected 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

BioFire® ME Panel Verification Record

Computer System Serial #: _____

BioFire ME Panel Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

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Organism	System Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Escherichia coli K1		☐ Yes ☐ No					
Cytomegalovirus (CMV)		☐ Yes ☐ No					
Enterovirus (EV)		☐ Yes ☐ No					
Streptococcus pneumoniae		☐ Yes ☐ No					
Human herpesvirus 6 (HHV-6)		☐ Yes ☐ No					
Herpes simplex virus 1 (HSV-1)		☐ Yes ☐ No					
Neisseria meningitidis		☐ Yes ☐ No					
Streptococcus agalactiae		☐ Yes ☐ No					
Cryptococcus gattii		☐ Yes ☐ No					
Haemophilus influenzae		☐ Yes ☐ No					
Herpes simplex virus 2 (HSV-2)		☐ Yes ☐ No					
Varicella zoster virus (VZV)		☐ Yes ☐ No					
Listeria monocytogenes		☐ Yes ☐ No					
Human parechovirus (HPeV)		☐ Yes ☐ No					

Reviewed by: _____
Signature Date