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

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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 verification procedure to assist your laboratory in developing a protocol for the verification of the FilmArray ME Panel performance required by CLIA. This FilmArray ME Panel verification scheme has been designed to generate positive and negative tests for each organism detected by the FilmArray ME Panel 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, patient samples can be tested for verification or to evaluate matrix effects on the performance of the FilmArray ME Panel.

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 ME Panel is a multiplexed nucleic acid test intended for use with FilmArray 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 FilmArray 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, Varicella zoster virus.

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

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Technical Note
BioFire Diagnostics, LLC
www.BioFireDX.com

QS-339B-01

Performance Verification: Overview

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The procedure described below will generate multiple positive and negative results for each of the organisms targeted by the FilmArray 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 ME Panel targets.

A FilmArray system is defined as all FilmArray instruments 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.

The procedures have been designed to take advantage of the multiplex nature of the FilmArray ME 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 choose to test clinical/patient specimens to assess clinical sensitivity and sample matrix effects for verification of the FilmArray 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.

Performance Verification: Materials

The following materials may be needed to perform verification procedures:

Table 2. Materials needed for recommended protocol

Material	Part Number
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)

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the FilmArray 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 in the FilmArray 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 (available commercially from 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 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 A				
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 B				
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 C				
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 A) 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 FilmArray 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 1 and 2) from a single organism pool (e.g. pool A). 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 ME Panel Instruction Booklet* or *ME 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 B) to be tested that day.

Day 2

To evaluate day-to-day variation, test the remaining samples (e.g. samples 3 and 4) 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 C) as described in Step 1. Test samples according to Step 2 (e.g. samples 1 and 2) for the pool.

Day 4

To evaluate day-to-day variation, test the remaining samples (e.g. samples 3 and 4) from the organism pool(s) prepared on Day 3 by repeating Step 2 above.

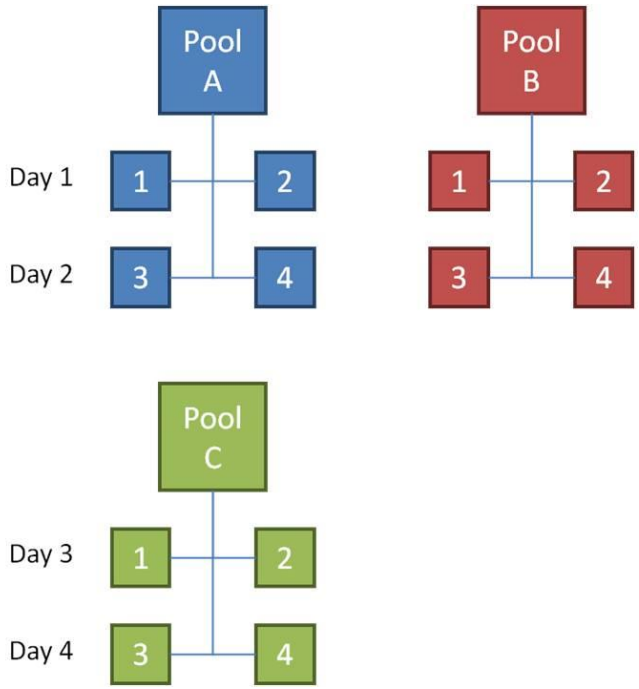

Figure 1. Protocol workflow

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 per FilmArray system.

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 ME Panel. The Laboratory Director should determine the appropriate number of samples to test. Three to six samples may be sufficient.

   Note: Proficiency samples or other stored samples should not be pooled or diluted prior to testing.

2. Test the selected samples on the loaner or repaired 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 FilmArray Technical Support team for assistance.

BioFire Technical Support
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5 and then Option 1

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FilmArray Meningitis/Encephalitis (ME) Panel Verification Record

Computer System Serial #: _____

FilmArray ME Panel kit Part #: _____ Lot #: _____

Organism/sample Source and Lot #: _____

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Organism	Instrument 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		☐ 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