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

Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

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. 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 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® FilmArray® Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BioFire® FilmArray® Systems. The BioFire ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The following organisms are identified using the BioFire ME Panel:

Table 1. Bacteria, Viruses, and Yeast Detected by the BioFire ME Panel

Bacteria	
Escherichia coli K1	*Neisseria meningitidis* (encapsulated)
Haemophilus influenzae	*Streptococcus agalactiae*
Listeria monocytogenes	*Streptococcus pneumoniae*

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
FLM1-PRT-0165-05

QS-339F-01

Viruses	
Cytomegalovirus	Enterovirus
Human herpesvirus 6	Herpes simplex virus 1
Human parechovirus	Herpes simplex virus 2
Varicella zoster virus	
Yeast	
Cryptococcus neoformans/gattii	

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

Performance Verification Overview

The performance verification procedure described may be used to evaluate the performance of each assay on the BioFire ME Panel. 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).

Note: It is important to characterize CSF specimens for ME Panel targets by screening the specimen on the BioFire ME Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BioFire ME Panel.

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 or module, it is advised that the test replicates are evenly distributed among the instruments or modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

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. The procedures described below will generate multiple positive and negative detections for each of the BioFire ME Panel assays. The procedures were developed using a panel available from ZeptoMetrix LLC, Buffalo, NY (NATMEP-BIO).

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.

Note: The laboratory should only perform the verification with analytes that will be reported using the BioFire ME Panel in their laboratory setting.

Table 2. Overview of Verification Protocol

Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	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 BioFire® system configured with one instrument/module.

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 3. Recommended materials for the verification protocol

Material	Part Number
BioFire® FilmArray® ME Panel Kit (30 tests)	BioFire Diagnostics, LLC RFIT-ASY-0118
BioFire® FilmArray® Meningitis /Encephalitis Panel Instructions for Use	BioFire Diagnostics, LLC RFIT-PRT-0276
BioFire® FilmArray® Meningitis / Encephalitis Panel Quick Guide	BioFire Diagnostics, LLC RFIT-PRT-0275
Control organism[a]	ZeptoMetrix NATMEP-BIO
2 mL or 5 mL Sample Tubes	Various manufacturers
Disposable Transfer pipets, graduated	VWR, 414004-024 (or equivalent)
Artificial cerebrospinal fluid (aCSF)	Tocris Biosciences #3525 (or equivalent)

[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). Organisms may be combined with an additional volume of artificial cerebrospinal fluid (aCSF) background or with residual clinical CSF that has been verified as negative for BioFire® ME Panel targets. aCSF is commercially available (Tocris Biosciences Part # 3525 or equivalent) or may be prepared following published recipes.

Note: It is important to characterize CSF specimens for ME Panel targets by screening the specimen on the BioFire ME Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BioFire ME Panel.

Figures 1 and 2 (below) illustrate protocol and workflow schemes for testing 4 replicates per pool for 3 pools over multiple days. This produces a total of 12 verification sample test runs and provides 4 positive results and 8 negative results per assay. 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 modules in the BioFire® System. The proposed pooling scheme (Table 4) should be followed to obtain the expected positive and negative results for each assay in a time and resource-efficient manner.

Pooled samples may 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.

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

Table 4. Recommended Organism Pooling Scheme

Control Organism	Approximate Organism Volume	Approximate Pool Volume	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	0.30 mL			
Echovirus type 11 (Enterovirus)	0.30 mL			
Streptococcus pneumoniae	0.30 mL			
Human herpesvirus 6 (HHV6)	0.30 mL			
Pool 2				
Herpes simplex virus 1 (HSV1)	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 (HSV2)	0.30 mL			
Varicella zoster virus (VZV)	0.30 mL			
Listeria monocytogenes	0.30 mL			
Parechovirus Type 3	0.30 mL			

Protocol Example

The estimated total time to completion for this verification example is 4 days for a BioFire® FilmArray® System configured with configured with one module.

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

Day 1

1. Organize materials needed (Table 3).

2. Prepare two sample pools (i.e. Pools 1 and 2) from ZeptoMetrix NATMEP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for example organism pooling schemes and specific volumes for each pool.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 2 mL or 5 mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.2 or 1.5 mL.
    c. **Optional:** transfer 0.9 mL of aCSF or CSF to the organism pool, total volume will be 2.1 or 2.4 mL.
    d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pool (i.e. Pool 2) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (i.e. Figure 1: Pool 1 replicates A and B). The replicate samples should be tested in a single day by different operators.

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

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. Pool 2 replicates A and B)

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

Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more replicates if desired.

Day 2

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 (i.e. replicates C and D from Pools 1 and 2) by repeating Step 4 above.

Day 3

Prepare a new organism pool (i.e. pool 3) as described in Day 1, Step 2. Test replicates as described in Step 4 above.

Day 4

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 3 (i.e.replicates C and D from Pool 3) by repeating Step 4 above.

Figure 1. Workflow for Verification

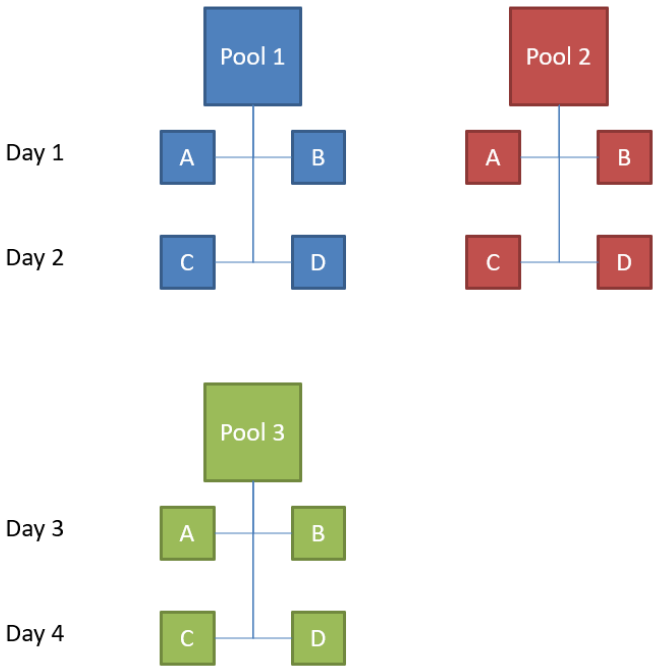

Figure 2. Example of a Verification workflow for use with multiple BioFire Modules

Verification with 2 modules	Module 1		Module 2	
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1
Day 2	Pool 1/ User 2	Pool 2/ User 1	Pool 1/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1		Pool 3/ User 2	
Day 4		Pool 3/ User 2		Pool 3 / User1

Verification with 4 modules	Module 1	Module 2	Module 3	Module 4
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 2	Pool 2/ User 2	Pool 2/ User 1	Pool 1/ User 2	Pool 1/ User 1
Day 3	Pool 3 / User 1	Pool 3/ User 2		
Day 4			Pool 3/ User 2	Pool 3 / User1

Verification with 6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2		
Day 2			Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 3/ User 2				
Day 4					Pool 3 / User1	Pool 3/ User 2

Expanding the Protocols

The protocols described above can be expanded to increase the number of tests for each of the organism pools. Each organism pool contains sufficient volume for testing additional replicates. The verification study may use CSF and aCSF in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960.

Verification of Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or module, 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 or module 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

BioFire® FilmArray® Meningitis/ Encephalitis (ME) Panel Verification Record

Kit Part # _____ **Module Serial #** _____ **Module Serial #** _____

Lot # _____ **Module Serial #** _____ **Module Serial #** _____

	Organism	Replicate Testing- Record Organism Detections												Summary					
		1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D	3-A	3-B	3-C	3-D	# Positives	# Negatives	# Operators	# Days	# Modules	Patient Samples?
Pool 1	*Escherichia coli* K1																		
	Cytomegalovirus																		
	Enterovirus																		
	Streptococcus pneumoniae																		
	Human herpesvirus 6																		
Pool 2	Herpes simplex virus 1																		
	Neisseria meningitidis																		
	Streptococcus agalactiae																		
	Cryptococcus neoformans/gattii																		
Pool 3	*Haemophilus influenzae*																		
	Herpes simplex virus 2																		
	Varicella zoster virus																		
	Listeria monocytogenes																		
	Human parechovirus																		

Reviewed by: _____

Signature **Date**

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