



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® 2.0 and on BIOFIRE® FILMARRAY® TORCH Systems as required by CLIA. The method described generates 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 operators may test the same sample. In addition, testing patient samples for verification of 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:

Bacteria:

- *Escherichia coli* K1
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis* (encapsulated)





- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

Viruses:

- Cytomegalovirus
- Enterovirus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6
- Human parechovirus
- 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 Instruction Booklet*.

Performance Verification Overview

Examples of performance verification procedures are described for the BIOFIRE ME Panel. The protocol can be used with CSF or with artificial cerebrospinal fluid (aCSF) prepared using published recipes or from commercially available sources.



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

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 procedure described will generate multiple positive and negative detections for each of the BIOFIRE ME Panel assays. The procedures were developed using the NATtrol™ Meningitis/Encephalitis (ME) panel available from ZeptoMetrix, Buffalo, NY (part number NATMEP-BIO).

A BIOFIRE System is defined as all BIOFIRE® FILMARRAY® 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 module, it is advised that test replicates are evenly distributed among the modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

Clinical/patient specimens may be used in addition to, or in place of the verification schemes described here to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BIOFIRE ME Panel.



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



Table 1. Overview of Verification Protocol

Organisms per Pool	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 module.

Performance Verification: Materials

The following materials may be used to perform verification procedures:

Table 2. Recommended materials for the verification protocol

Material	Part Number
BIOFIRE® FIMARRAY® ME Panel Kit (30 tests)	BioFire Diagnostics, LLC: RFIT-ASY-0118
BIOFIRE® FIMARRAY® Meningitis /Encephalitis Panel Instruction Booklet	BioFire Diagnostics, LLC: RFIT-PRT-0276
BIOFIRE® FIMARRAY® Meningitis / Encephalitis Panel Quick Guide	BioFire Diagnostics, LLC: RFIT-PRT-0275
Control organism ^a	ZeptoMetrix: NATMEP-BIO
5 mL Sample Tubes	Various manufacturers
Disposable Transfer pipets, graduated	Avantor (formerly VWR): 612-4469 (or equivalent)
Artificial cerebrospinal fluid (aCSF)	Tocris Biosciences #3525 (or equivalent)

^aAny 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 (i.e. not the ZeptoMetrix NATMEP-BIO material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

Performance Verification: Protocol

The verification protocol evaluates the BIOFIRE ME Panel performance when sample material (ZeptoMetrix NATMEP-BIO) is pooled and combined with aCSF or clinical CSF and tested with the BIOFIRE ME Panel. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner. aCSF is commercially available (Tocris Biosciences Part # 3525 or equivalent) or may be prepared following published recipes.



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



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



Figures 1 and 2 (below) illustrate workflow schemes for testing 4 replicates per pool for 3 different 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 pooling scheme provides sufficient volume for testing more replicates if desired.

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 operators may perform testing.

Table 3. Proposed Organism Pooling Scheme

Control Organism	Approximate Organism Volume	Approximate Volume of aCSF or CSF	Approximate Final Volume of Pool
Pool 1			
<i>Escherichia coli</i> K1	0.30 mL	0.9 mL	2.4 mL
Cytomegalovirus	0.30 mL		
Echovirus type 11 (Enterovirus)	0.30 mL		
<i>Streptococcus pneumoniae</i>	0.30 mL		
Human herpesvirus 6 (HHV6)	0.30 mL		
Pool 2			
Herpes simplex virus 1 (HSV1)	0.30 mL	0.9 mL	2.1 mL
<i>Neisseria meningitidis</i>	0.30 mL		
<i>Streptococcus agalactiae</i>	0.30 mL		
<i>Cryptococcus gattii</i>	0.30 mL		
Pool 3			
<i>Haemophilus influenzae</i>	0.30 mL	0.9 mL	2.4 mL
Herpes simplex virus 2 (HSV2)	0.30 mL		
Varicella zoster virus (VZV)	0.30 mL		
<i>Listeria monocytogenes</i>	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 System 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 2); refer to Table 3 for the pooling scheme. Negativity of CSF may be confirmed by screening on the BIOFIRE ME Panel Prior to starting the verification study.
2. Prepare one sample pool (i.e., Pool 1) from ZeptoMetrix NATMEP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 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 5 mL tube.
 - b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The volume will be approximately 1.5 mL for Pools 1 and 3 and 1.2 mL for Pool 2.
 - c. Transfer 0.9 mL of aCSF or CSF to the tube containing the organism pool (step b). The total volume will be 2.4 mL for Pools 1 and 3 and 2.1 mL for Pool 2.
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 (Figure 1: Pool 1 replicates A and B). Ensure the pooled sample is well mixed prior to removing a sample for testing. The replicate samples should be tested in a single day by different operators.

Note: For each sample, follow instructions in the *BIOFIRE® FIMARRAY® Meningitis/Encephalitis (ME) Panel Instruction Booklet* or the *BIOFIRE® FIMARRAY 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., Pool 3, replicates C and D) by repeating Step 4 above.

 **Note:** A BIOFIRE ME Panel Verification Record is provided and may serve as a template for recording your results.

Figure 1. Verification Workflow

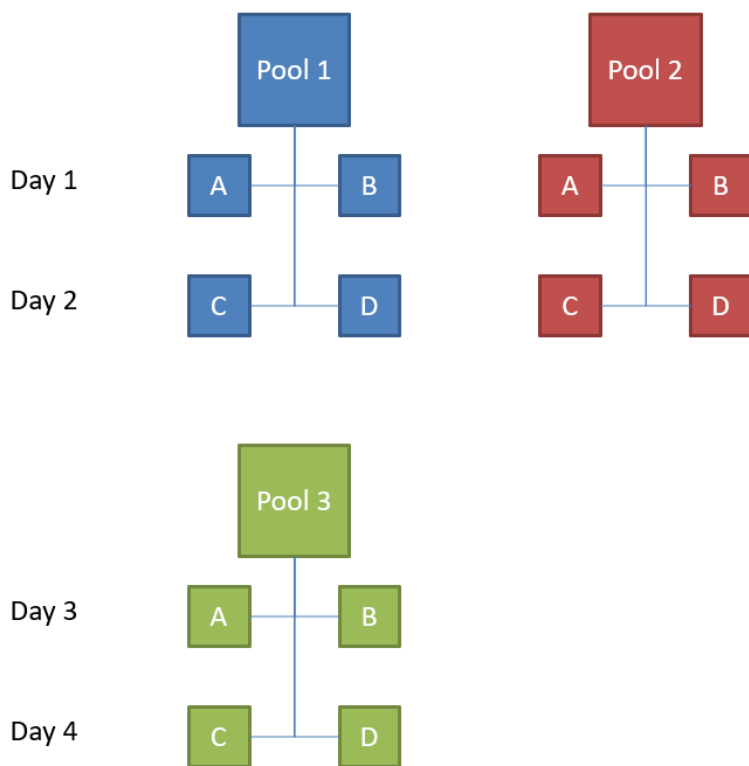




Figure 2. Example of Verification workflows for use with multiple BIOFIRE Modules

Verification with 2 modules	Module 1		Module 2	
	Day 1	Pool 1/ Operator 1	Pool 2/ Operator 2	Pool 1/ Operator 2
Day 2	Pool 1/ Operator 2	Pool 2/ Operator 1	Pool 1/ Operator 1	Pool 2/ Operator 2
Day 3	Pool 3 / Operator 1		Pool 3 / Operator 2	
Day 4		Pool 3 / Operator 2		Pool 3 / Operator 1

Verification with 4 modules	Module 1	Module 2	Module 3	Module 4
	Day 1	Pool 1/ Operator 1	Pool 1/ Operator 2	Pool 2/ Operator 1
Day 2	Pool 2/ Operator 2	Pool 2/ Operator 1	Pool 1/ Operator 2	Pool 1/ Operator 1
Day 3	Pool 3 / Operator 1	Pool 3 / Operator 2		
Day 4			Pool 3 / Operator 2	Pool 3 / Operator 1

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

Expanding the Protocols

The protocol described above can be expanded by increasing the number of test replicates 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 Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement module, the following protocol may serve as a guideline but should be verified by the Laboratory Director.

1. Select an appropriate number of 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 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 module and document the results.



Technical Support Contact Information

bioMérieux 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: biofiresupport@biomerieux.com
Phone: +1-801-736-6354, select Option 5

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BIOFIRE® FILMARRAY® Meningitis/ Encephalitis (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	<i>Escherichia coli</i> K1																	
	Cytomegalovirus																	
	Enterovirus																	
	<i>Streptococcus pneumoniae</i>																	
	Human herpesvirus 6																	
Pool 2	Herpes simplex virus 1																	
	<i>Neisseria meningitidis</i>																	
	<i>Streptococcus agalactiae</i>																	
	<i>Cryptococcus neoformans/gattii</i>																	
Pool 3	<i>Haemophilus influenzae</i>																	
	Herpes simplex virus 2																	
	Varicella zoster virus																	
	<i>Listeria monocytogenes</i>																	
	Human parechovirus																	

Reviewed by: _____
 Signature _____ Date _____

