



Verification of Performance of the BIOFIRE® FILMARRAY® Meningitis/Encephalitis (ME) Panel

Laboratory Guidelines – for Qnostics Materials

Purpose

This document provides examples of verification procedures to assist your laboratory in developing a protocol for performance verification of the BIOFIRE® FILMARRAY® Meningitis/Encephalitis (ME) Panel on BIOFIRE® FILMARRAY® 2.0 and on BIOFIRE® FILMARRAY® TORCH Systems. The methods described 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 protocols can also be used for other applications at the laboratory director's discretion, e.g., lab personnel training, lot-to-lot pouch testing, verification of loaner and repaired instruments. 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.

The Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for applicable laboratory accrediting agencies. 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.

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. These protocols 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 a control panel available from Qnostics Ltd., UK (Part number: MEEP01-C).

A simplified procedure for performance verification is described below. An expanded procedure is also described for evaluation of multiple modules, day-to-day variation, and operator-to-operator variation. Both procedures require preparation of 3 different pools of organisms to provide detections of all organisms reported by the BIOFIRE ME Panel. The simplified protocol requires 3 pouches, the expanded protocol requires 12 pouches (Table 1).

Table 1. Overview of Expanded and Simplified Verification Protocols

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
Simplified Protocol	4 or 5	3	1	3	1 per organism	2 per organism	1
Expanded Protocol	4 or 5	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 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 protocols

Material	Part Number
BIOFIRE® FILMARRAY® Meningitis/ Encephalitis Panel Kit (30 tests)	BioFire Diagnostics, LLC: RFIT-ASY-0118
BIOFIRE® FILMARRAY® Meningitis / Encephalitis Panel Instructions Booklet	BioFire Diagnostics, LLC: RFIT-PRT-0276
BIOFIRE® FILMARRAY® Meningitis / Encephalitis Panel Quick Guide	BioFire Diagnostics, LLC: RFIT-PRT-0275
Control organism ^a	Qnostics Ltd, UK: MEEP01-C
1.5 mL Microfuge tubes	Various manufacturers
Disposable Transfer pipets, graduated	VWR: 414004-024 (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, the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.

Performance Verification: Simplified Protocol

The simplified protocol tests a total of 3 pouches, providing 1 positive result and 2 negative results per organism (Table 1). The number of samples tested per day should be determined by the individual laboratory.

The protocol describes the preparation of 3 organism pools for testing, each containing up to 5 different control organisms (Qnostics Ltd, MEEP01-C). The proposed pooling scheme (Table 3), in line with Qnostics product format, i.e., 3 boxes of biological material, should be followed to obtain the expected positive and negative results for each assay in a time and resource-efficient manner.



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

Table 3. Proposed Organism Pooling Scheme

Control Organism	Approximate Volume of Control Organism	Approximate Final Volume of Pool
Pool 1		
<i>Escherichia coli</i> K1	0.25 mL	1.25 mL
Cytomegalovirus (CMV)	0.25 mL	
Coxsackie B3 (Enterovirus)	0.25 mL	
<i>Streptococcus pneumoniae</i>	0.25 mL	
Human herpesvirus 6 (HHV6)	0.25 mL	



Pool 2		
Herpes simplex virus 1 (HSV1)	0.25 mL	1.0 mL
<i>Neisseria meningitidis</i>	0.25 mL	
<i>Streptococcus agalactiae</i>	0.25 mL	
<i>Cryptococcus gattii</i>	0.25 mL	
Pool 3		
<i>Haemophilus influenzae</i>	0.25 mL	1.25 mL
Herpes simplex virus 2 (HSV2)	0.25 mL	
Varicella zoster virus (VZV)	0.25 mL	
<i>Listeria monocytogenes</i>	0.25 mL	
Human parechovirus (HPEV)	0.25 mL	

Simplified Protocol Example

The estimated total time to completion for this verification example is 1 day for a BIOFIRE System configured with one module.



Note: It is important to prepare only the number of organism pools that will be tested within 2 days of preparation.

Step 1: Prepare Organism Pools

Prepare the three organism pools (e.g. pool 1-3) from Qnostics control material. Organism vials should be mixed prior to preparing each pool. Refer to Table 3 for organism pooling schemes and specific volumes for each pool.

- a. Transfer 0.25 mL of the control organism to a tube large enough (at least 1.5 mL) to hold the entire organism pool volume.
- 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 1.25 mL for Pools 1 and 3 and 1.0 mL for Pool 2.
- c. Ensure the pooled sample is well mixed prior to removing a sample for testing.



Note: The organism pool may be stored refrigerated (2–8°C) for up to 2 days.

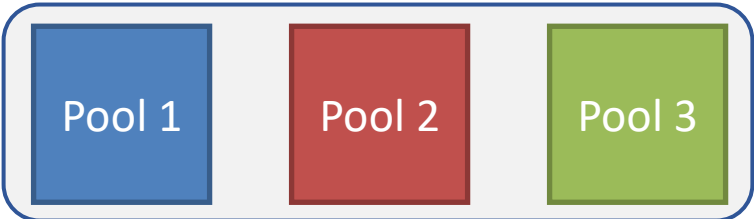
Step 2: BIOFIRE® FILMARRAY® Testing

- a. Test one replicate from a single sample pool (e.g. Pool 1). 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.
- b. Repeat steps 1 and 2 for testing a single replicate from Pools 2 and 3.



Note: Use clean gloves and other Personal Protective Equipment (PPE) when handling pouches and samples. Only prepare one BIOFIRE ME pouch one at a time and change gloves between samples and pouches.

Figure 1. Simplified Protocol Workflow



Performance Verification: Expanded Protocol

The protocol described above provides sufficient volume for testing more replicates if desired. For example- testing 4 test replicates per pool (described in Table 1) for a total of 12 pouches provides 4 positive results and 8 negative results per organism. Day-to-day variation may be evaluated by testing pool replicates on two separate days. To evaluate user-to-user variation, multiple laboratory operators may test the same pools. Test replicates may be evenly distributed among the modules to evaluate multiple modules.

Examples of an expanded verification protocol is provided in Figure 2 and workflows for testing multiple BIOFIRE Modules is described in Figure 3.

Figure 2. Expanded Verification Workflow

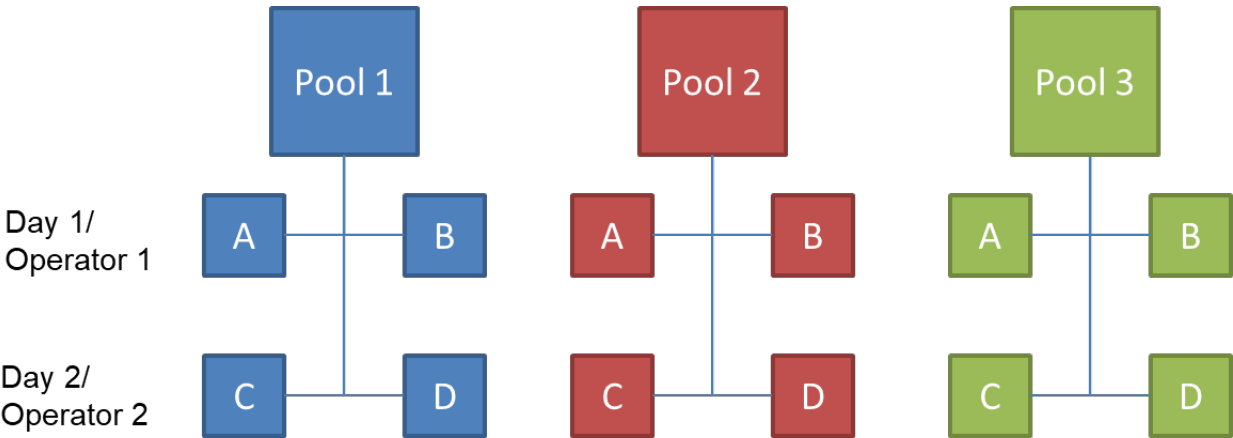




Figure 3. Example of Verification workflows for use with multiple BIOFIRE Modules

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

Expanded Protocol Example

The expanded verification protocol can be completed in one or more days as needed. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run fewer or more samples per day based on the number of modules in the BIOFIRE System.

Multiple operators may test replicates of the same pools to evaluate operator-to-operator variation. Test replicates from each of the pools can be tested on multiple days to evaluate day-to-day variation. Figure 3 (above) describes testing multiple modules with two operators.

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 _____

