

Verification of Performance of the 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 FilmArray ME Panel on FilmArray® Systems. The procedure can also be used for other applications at the laboratory director's discretion, e.g. lab personnel training, lot-to-lot change testing, verification of loaner and repaired instruments.

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 FilmArray ME Panel should be done under the guidance of the Laboratory Director, but is not described here.

FilmArray Intended Use

The FilmArray ME Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray Systems. The FilmArray 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 FilmArray ME Panel: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae*, *Streptococcus pneumoniae*, cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parechovirus, varicella zoster virus, *Cryptococcus neoformans/gattii*.

Refer to the *FilmArray Meningitis/Encephalitis (ME) Panel Instruction Booklet* for the complete intended use statement and additional information about the use of the FilmArray System.

Verification Procedure: Overview

The verification procedures (i.e. simplified and expanded) have been designed to take advantage of the multiplex nature of the FilmArray® Meningitis/Encephalitis (ME) Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures were developed using a panel available from Qnostics Ltd., UK (Part number: MEEP01-C).

This FilmArray ME Panel verification scheme generates positive and negative tests for each organism detected by the FilmArray ME Panel. The procedures described below may be easily modified or expanded to meet specific criteria.

A simplified procedure for performance verification is described below. An expanded procedure is also described in case the laboratory director chooses to expand the verification protocol to cover more instruments, evaluate day-to-day and/or operator-to-operator variability. Both procedures are based on preparing 3 different pools of organisms to cover all 14 analytes from the FilmArray ME Panel. The simplified protocol will only require 3 pouches, the expanded protocol will require 12 pouches (Table 1).

Table 1. Overview of Expanded and Simplified Verification Protocols

| Organisms per Pool | Number of Sample Pools | Protocol | Replicates per Sample Pool | Pouches Required | Expected Positive Results | Expected Negative Results | Approximate Days of Testing ^a |
|--------------------|------------------------|------------|----------------------------|------------------|---------------------------|---------------------------|--|
| 4 or 5 | 3 | Simplified | 1 | 3 | 1 per organism | 2 per organism | 1 |
| | | Expanded | 4 | 12 | 4 per organism | 8 per organism | 2 |

^a The approximate number of days for testing assumes a system configured with one or three instruments depending on the protocol considered.

Verification Procedure: Recommended Materials

Table 2 lists materials recommended to perform verification procedures.

Table 2. Recommended Materials for Protocols

| Material | Part Number |
|--|--|
| FilmArray ME Panel Kit (30 tests) | Biofire Diagnostics, LLC RFIT-ASY-0118 |
| Control Organism | Qnostics Ltd, UK ^(a) ; MEEP01-C |
| Biohit mLine Single Channel Manual Pipette 100-1000 µl | BioHit Part # 725070(or equivalent) |
| 1.5 mL Microfuge tubes | Eppendorf®, 0030 120.086 (European) |

^aAny 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.

Verification Procedure: 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 recommended protocol requires the preparation of 3 organism pools for testing, each containing up to 5 different control organisms (Qnostics Ltd, MEEP01-C; see Table 3 for a detailed description of each pool).

TECHNICAL ::: NOTE

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.

TECHNICAL ::: NOTE

Table 3. Recommended Organism Pooling Scheme

| Control Organism | Control Organism Volume | 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 | 0.25 mL | |
| <i>Streptococcus pneumoniae</i> | 0.25 mL | |
| Human Herpesvirus 6 (HHV-6) | 0.25 mL | |
| POOL 2 | | |
| Herpes simplex virus 1 (HSV-1) | 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 (HSV-2) | 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 systems configured with one instrument (Figure 1).

Step 1 Prepare Organism Pool

Prepare the three organism pools (e.g. pool 1-3) from Qnostics control material.



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

- a. Transfer **0.25 mL** of the Qnostics organism to a tube large enough (at least 1.5 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 1.0 – 1.25 mL total volume). Cap tube and vortex to mix well.

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

Step 2 FilmArray Testing

- a. Test 1 pouch from a single organism pool (e.g. pool 1). Refer to the *FilmArray® Meningitis/Encephalitis (ME) Panel Instruction Booklet* or *FilmArray Meningitis/Encephalitis Panel Quick Guide* for pouch procedure details.



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

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

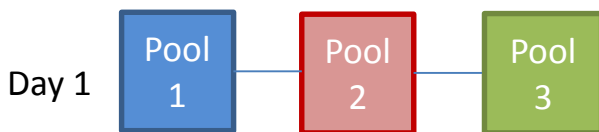


Figure 1. Simplified Protocol Workflow

Verification Procedure: Expanded Protocol

The protocol described above can be expanded to increase the number of tests for each of the organism pools and to include more variables (e.g. day, operator, instruments). The protocol can be followed to test a total of 12 pouches, providing 4 positive results and 8 negative results per organism (Table 1).

Example of an advanced verification protocol is provided below (Figure 2).

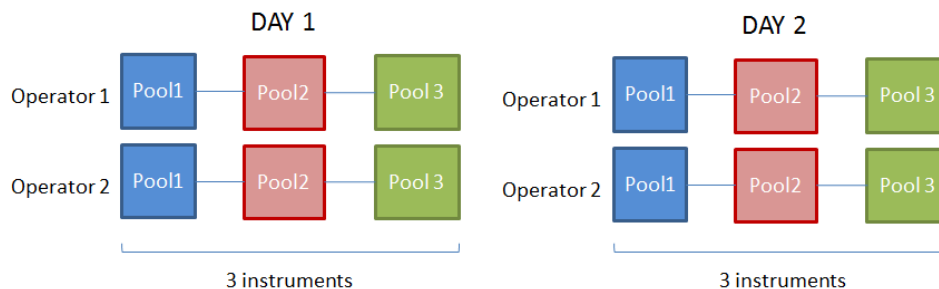


Figure 2. Expanded Protocol workflow (example).

Expanded Protocol Example

The estimated total time to completion for this verification example is 2 days for systems configured with at least 3 instruments (Figure 2).

On day 1, each Operator will test all 3 pools as described in the simplified protocol. Testing is repeated on day 2. The laboratory Director may choose to increase the number of operators, or in contrast limit the testing to one operator.

TECHNICAL
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Contact and Legal Information

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Information

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TECHNICAL
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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 #: _____

**TECHNICAL
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| Organism | Instrument System # | Was the Organism Detected? | No. Positive | No. Negative | No. Days Tested | No. Users |
|---------------------------------------|---------------------|---|--------------|--------------|-----------------|-----------|
| <i>Escherichia coli</i> K1 | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Cytomegalovirus (CMV) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Enterovirus | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Streptococcus pneumoniae</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Human Herpesvirus 6 (HHV-6) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Herpes simplex virus 1 (HSV-1) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Neisseria meningitidis</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Streptococcus agalactiae</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Cryptococcus neoformans/gattii</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Haemophilus influenzae</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Herpes simplex virus 2 (HSV-2) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Varicella zoster virus (VZV) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| <i>Listeria monocytogenes</i> | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |
| Human parechovirus (HPeV) | | <input type="checkbox"/> Yes <input type="checkbox"/> No | | | | |

Reviewed by: _____
Signature Date