Viral Detections in Cerebrospinal Fluid Patient Samples with the FilmArray Meningitis / Encephalitis System

B. Baarda[1], E. Lo[1], S. Bellamkonda[1], T. Lunt[2], J. Cloud[1], K. Hanson[2], R. Crisp[1], and A. Hemmert[1]

[1]BioFire Diagnostics, LLC, Salt Lake City, UT, [2]ARUP, Salt Lake City, UT

ID 1305

INTRODUCTION AND BACKGROUND

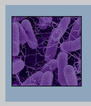
Viral infections of the meninges (meningitis) or brain tissue (encephalitis) are potentially life-threatening conditions. No etiological diagnosis is made in the majority of aseptic meningitis and viral encephalitis cases. Patients may be unnecessarily treated with antibiotics and kept in intensive care units until a bacterial infection can be ruled out. Currently, laboratory gold standard PCR testing for aseptic meningitis and viral encephalitis is limited to technical "in-house" developed and validated assays or time-consuming reference laboratory testing. A rapid, accurate, comprehensive diagnostic system would facilitate better patient care through improved antibiotic stewardship and reduced hospital stays. BioFire Diagnostics is developing a Meningitis / Encephalitis (ME) panel for use in the FilmArray™ (FA) system. This comprehensive panel requires only 200 µL of cerebrospinal fluid (CSF) and 2 minutes of hands-on time to test for 16 different bacteria, viruses, and fungi causing ME. Test results are returned in approximately 1 hour.

MATERIALS AND METHODS

Archived patient CSF samples were included in the study based on positive detection by in-house developed PCR assays from ARUP. Each sample was split into multiple aliquots for FA ME and quantitative real-time PCR (qPCR) testing. For the qPCR assays, nucleic acids were extracted using the QIAcube (QIAGEN, Germany) DNA Blood and Body Fluid extraction protocol. Extracted nucleic acids were tested using commercially purchased qPCR assays from genesig (PrimerDesign, UK) on a Bio-Rad CFX96 Touch™ (Bio-Rad, Hercules, CA) real-time PCR system according to the manufacturer's instructions. For FA ME testing, a research use only (RUO) version of the panel was used. CSF was diluted 1:4 with FA Sample Buffer and injected into a FA ME pouch. Each sample was tested in a single-use FA ME pouch in a FA instrument. Nucleic acid extraction, purification, amplification, and results analysis are automated within the FA system.

THE FILMARRAY MENINGITIS / ENCEPHALITIS (ME) PANEL

Simultaneous detection of 16 targets:



Bacteria

- *Escherichia coli*
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

Fungi

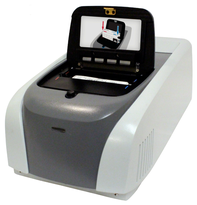
- *Cryptococcus neoformans*
- *Cryptococcus gattii*

Viruses

- Cytomegalovirus
- Enterovirus
- Epstein-Barr virus
- Herpes Simplex virus, Type 1
- Herpes Simplex virus, Type 2
- Human Herpesvirus 6
- Human Parechovirus
- Varicella zoster virus

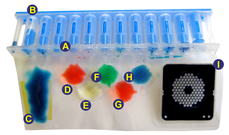
The FilmArray System

The FilmArray is a lab-in-a-pouch medium-scale fluid manipulation test performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion.



The FilmArray ME pouch has a fitment (B) containing freeze-dried reagents and plungers that plunge liquids to the film portion of the pouch. This portion consists of stations for cell lysis (C), magnetic-bead based nucleic acid purification (D & E), first-stage multiplex PCR (F & G) and an array of 102, second-stage nested PCRs (I).

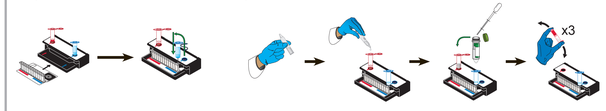
PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second-stage PCR product is detected in a melting analysis using a fluorescent double-stranded DNA binding dye, LCGreen®.



A. Fitment with freeze-dried reagents
B. Plungers- deliver reagents to blisters
C. Sample lysis and bead collection
D. Wash station
E. Magnetic bead collection blister
F. Elution Station
G. Multiplex Outer PCR blister
H. Dilution blister
I. Inner Nested PCR array

Sample Processing and Pouch Loading Instruction

Step 1 Step 2



Testing requires minimal pre-processing of specimens. Cerebrospinal fluid and FA Sample Buffer are combined in a novel filter-injection vial (FAIV) and then loaded into the FA ME pouch. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run.

RESULTS

Detection results for the FA ME system were compared to the specific qPCR results (Table 1). No qPCR assay was available for Enterovirus; therefore, FA ME detection was compared to the in-house developed PCR result from ARUP. The FA ME system exhibited similar sensitivity to the qPCR assays. Based on the qPCR results, DNA viruses were present across a range of 10^2 – 10^7 copies/mL. Further, the FA ME system detected multiple dual-infections that were not previously identified in the archived sample set. Each dual-infection was confirmed using the qPCR assays. These data demonstrate that the FA ME panel exhibits reliable results across a broad range of viral titers.

Table 1. FA ME Panel Viral Detections and Quantifications

Virus	FA ME Detection vs. qPCR Results	Min. Quantification (copies/mL)	Max. Quantification (copies/mL)
Cytomegalovirus (CMV)	9/9 (100%)	$5.00x10^3$	$1.77x10^6$
Enterovirus (EV)	46/50 (92%)[a]	N/A	N/A
Epstein-Barr virus (EBV)	13/13 (100%)	$1.00x10^3$	$3.00x10^4$
Human Herpesvirus 6 (HHV6)	16/18 (89%)	$5.00x10^3$	$2.32x10^5$
Herpes Simplex virus - Type 1 (HSV1)	17/18 (94%)	$1.31x10^3$	$1.85x10^7$
Herpes Simplex virus - Type 2 (HSV2)	32/32 (100%)	$2.00x10^3$	$3.30x10^7$
Varicella zoster virus (VZV)	38/40 (95%)	$6.60x10^2$	$8.52x10^6$

[a] FA ME Detection vs. ARUP developed PCR Assay

Figure 1. Boxplots of Viral Quantifications with genesig Assays

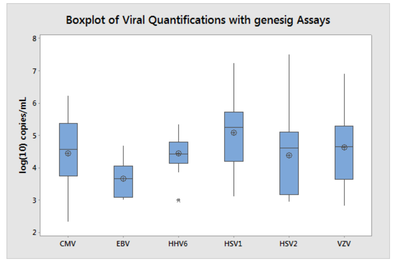
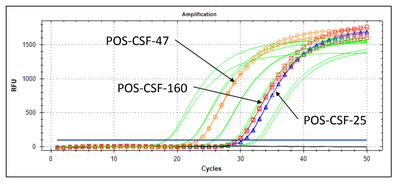


Figure 2. VZV Quantification of Archived CSF Samples



Green Curves: Template Standards

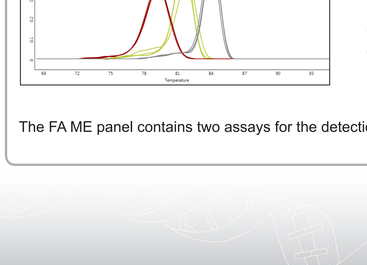
Table 2. VZV Quantification Results of Archived CSF Samples

Sample ID	Source Result	Source Cp	FA ME Results	genesig Result	genesig Cp	Quantification (copies/mL)
POS-CSF-25	VZV	31.1	VZV	VZV	30.49	$8.36x10^4$
POS-CSF-47	VZV	24.4	VZV	VZV	23.33	$8.52x10^5$
POS-CSF-160	VZV	28.8	VZV	VZV	29.57	$1.51x10^5$

Cp: Crossing Point

Figure 3. CMV/HSV1 Co-detection in POS-CSF-109

The ability of the ME panel to detect multiple pathogens in a single sample highlights the clinical utility of a comprehensive panel for the diagnosis of aseptic meningitis and viral encephalitis. In the CSF samples presented in Figures 3 and 5, only one viral pathogen was identified by traditional testing methods. However, when tested on the FA ME system, multiple pathogens were detected in both samples.

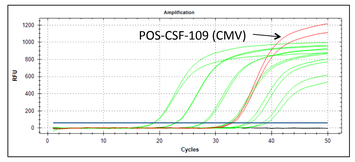


DETECTIONS:

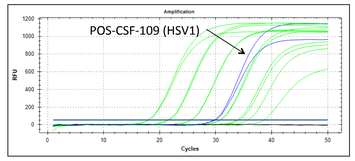
ARUP: CMV
FA ME: CMV and HSV1

CMV and HSV1 detections confirmed by independent qPCR assays (genesig).

Figure 4. Viral Quantifications of POS-CSF-109



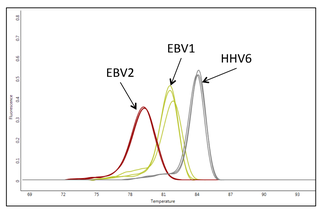
Green Curves: Template Standards



Green Curves: Template Standards

Virus	Quantification (copies/mL)
CMV	$8.96x10^4$
HSV1	$1.93x10^5$

Figure 5. HHV6/EBV Co-detection in POS-CSF-233



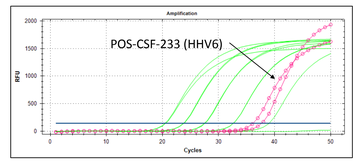
DETECTIONS:

ARUP: HHV6
FA ME: HHV6 and EBV

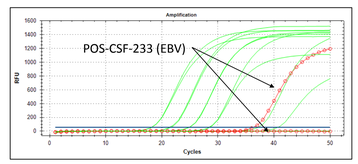
HHV6 and EBV detections confirmed by independent qPCR assays (genesig).

The FA ME panel contains two assays for the detection of Epstein-Barr virus.

Figure 6. Viral Quantifications of POS-CSF-233



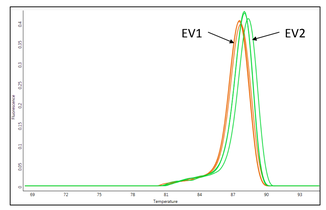
Green Curves: Template Standards



Green Curves: Template Standards

Virus	Quantification (copies/mL)
HHV6	$2.74x10^4$
EBV	$1.81x10^4$

Figure 7. Enterovirus Detection in POS-CSF-203



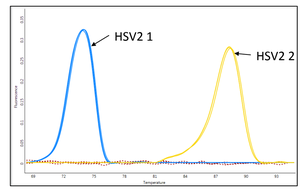
DETECTIONS:

ARUP: EV
FA ME: EV

No genesig qPCR assay was available for Enterovirus. The FA ME results match those of the in-house ARUP EV assay.

The FA ME panel contains two assays for the detection of Enterovirus.

Figure 8. HSV2 Detection in POS-CSF-135



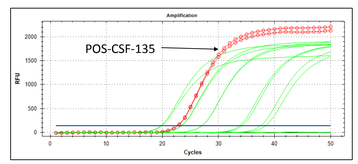
DETECTIONS:

ARUP: HSV
FA ME: HSV2

HSV was not differentiated by ARUP. HSV2 detection confirmed by independent qPCR assays (genesig).

The FA ME panel contains two assays for the detection of Herpes Simplex virus – Type 2.

Figure 9. HSV2 Quantification of POS-CSF-135



Green Curves: Template Standards

Virus	Quantification (copies/mL)
HSV2	$3.30x10^7$

CONCLUSION

The FilmArray Meningitis / Encephalitis system was able to rapidly and accurately detect viruses across a broad range of concentrations, which could greatly improve medical management of cases of aseptic meningitis and viral encephalitis.

The FA ME system has not been evaluated by the FDA for In Vitro Diagnostic use.

BIOFIRE™
A BIOMÉRIEUX COMPANY

Presented at the CVS Conference, April 2014

CONTACT INFORMATION
Andrew Hemmert
BioFire Diagnostics
Andrew.Hemmert@BioFireDX.com