Preclinical Assessment of a Fully Integrated Real-Time PCR System for the Multiplexed Detection of Central Nervous System Pathogens

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REVISED ABSTRACT

Background: Meningitis and encephalitis are neurological emergencies that can result in serious morbidity and mortality. Determining an infectious etiology of meningoencephalitis (ME) is essential for patient management, but making a microbiologic diagnosis is often difficult and time-consuming. We evaluated FilmArray™ technology (BioFire Diagnostics, LLC; Salt Lake City, UT) for the multiplexed molecular detection of viral pathogens in cerebrospinal fluid (CSF) as compared to single-plex laboratory developed tests (LDTs).

Materials and Methods: The FilmArray (FA) system integrates specimen preparation, nucleic amplification and detection via melt curve analysis into one complete process with results in about an hour. A preclinical assessment of the research use only (RUO), FilmArray Meningitis/Encephalitis (FA ME) system that targets 8 viral, 7 bacterial and 2 fungal central nervous system pathogens was performed at ARUP Laboratories using residual CSF. FA ME testing was performed as per the manufacturer's instructions with a focus on the viral components of the assay only. Discrepancies were resolved with repeat molecular testing.

Results: A total of 132 previously tested CSF specimens were retrieved from frozen storage, de-identified, and included in the study. Individual assay identifications are shown in the **2 x 2 Tables**. Overall, 95.5% (127/133) of LDT positive results were also detected by the FA. Eight of these specimens had additional organisms detected by FA that were not originally tested for at ARUP; these included EBV (n=6), HSV1 and HSV2. There was 100% (50/50) agreement between LTD negative results and the FA. However, the FA detected 3 additional viruses (EBV, HHV6 and VZV [n=2]) in 4 specimens not previously analyzed as a part of clinical care. Six specimens also had bacteria or fungi detected by FA ME. Bacterial and/or fungal cultures were not available for confirmation; therefore, additional laboratory developed PCR assays followed by Sanger sequencing were used for resolution testing. One *S. pneumoniae,* one *Cryptococcus neoformans,* and one *S. agalactiae* identification were confirmed.

Conclusions: Excellent positive (95.5%) and negative (100%) agreement was observed between our LDTs and the RUO FA ME system. As expected, the LDTs were slightly more sensitive than the FA, but the multiplexed FA detected more viruses overall and was faster to perform. EBV was the most common organism identified as a part of dual infections. The clinical significance of detecting endogenous, potentially latent herpes viruses in CSF is uncertain and will require clinicians to interpret the results in the context of the patient. Future laboratory work will be aimed at confirming the FA only positive results.

METHODS

De-identified, residual CSF samples that previously tested positive for a viral pathogen with our laboratory developed PCR tests (LDT) were included in the retrospective study if there was enough volume for both FA ME and discrepancy resolution testing. Each CSF sample was split into two aliquots, one for FA ME testing and the other for possible discrepancy resolution testing if needed. For the FA ME analysis, a RUO version of the panel was used. CSF was diluted 1:4 with FA Sample Buffer and injected into a single use FA ME pouch. Testing was performed on the current commercially available FA instrument with RUO software. Nucleic acid extraction, purification, amplification, and results analysis are automated within the FA system. LDT results were compared directly to FA ME testing.

Discrepant analysis was performed by either repeat LDT testing (viral discrepant specimens) or with PCR and sequence analysis of extracted DNA (bacterial/fungal discrepant specimens).

The FA ME panel detects the following organisms:

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

Fungi
Cryptococcus neoformans
Cryptococcus gattii

Bacteria
Escherichia coli
Haemophilus influenzae
Listeria monocytogenes
Neisseria meningitidis
Streptococcus agalactiae
Streptococcus pneumoniae

Acknowledgments: BioFire Diagnostics donated the RUO reagents and FilmArray instrument

RESULTS

LDT vs FA ME Test Data - comparison based on ARUP LDT results

Organism	LDT positive	FA ME positive
CMV[1]	7	5
EBV[1]	9	8
HSV	40	39
Enterovirus	37	36
HHV6	11	10
Parechovirus	0	0
VZV	29	29
Total	133	127

[1] 1 CSF specimen was LDT positive for both CMV and EBV

Other Organisms Detected by FA ME

Sample ID	Other organisms Detected by FA ME	Sequencing Result
012937-01-0035	*S. pneumoniae*	Positive for *S. pneumoniae*
012937-01-0041	*S. agalactiae*	PCR negative
012937-01-0274	*S. pneumoniae*	PCR negative
012937-01-0274	*S. agalactiae*	PCR negative
012937-01-0395	*S. agalactiae*	Positive for *S. agalactiae*
012937-01-0361	*Cryptococcus neoformans*	Positive for *Cryptococcus neoformans*

LDT vs FA ME test data including FA only positive results – comparisons after discrepant analysis,

EBV		ARUP +	ARUP -
FA ME	+	14	4
	-	1	160
		Sensitivity	**Specificity**
		93%	98%

EV		ARUP +	ARUP -
FA ME	+	36	0
	-	1	142
		Sensitivity	**Specificity**
		97%	100%

CMV		ARUP +	ARUP -
FA ME	+	4	0
	-	3*	172
		Sensitivity	**Specificity**
		57%	100%

*These detections were low titer (based on C_t values).

HHV6		ARUP +	ARUP -
FA ME	+	11	1
	-	1	166
		Sensitivity	**Specificity**
		92%	99%

HSV1		ARUP +	ARUP -
FA ME	+	10	1
	-	1	167
		Sensitivity	**Specificity**
		91%	99%

VZV		ARUP +	ARUP -
FA ME	+	31	0
	-	0	148
		Sensitivity	**Specificity**
		100%	100%

HSV2		ARUP +	ARUP -
FA ME	+	28	0
	-	0	151
		Sensitivity	**Specificity**
		100%	100%

HPeV		ARUP +	ARUP -
FA ME	+	0	0
	-	0	179
		Sensitivity	**Specificity**
		-	100%

CONCLUSIONS

- Excellent positive (95.5%) and negative (100%) agreement was observed between our LDTs and the FA ME system.
- LDTs were slightly more sensitive than the FA, but the multiplexed FA detected more viruses overall and was faster to perform.
- EBV was the most common organism identified as a part of dual infections.
- The clinical significance of detecting endogenous, potentially latent herpes viruses in CSF is uncertain and will require clinicians to interpret the results in the context of the patient.
- Future laboratory work will be aimed at confirming the FA only positive results.