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Original Article

Usefulness of the FilmArray meningitis/encephalitis (M/E) panel for the diagnosis of infectious meningitis and encephalitis in Taiwan

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Abstract *Background/purpose:* Early recognition of causative pathogens is critical for the appropriate management of central nervous system infection and improved outcomes. The BioFire® FilmArray® Meningitis/Encephalitis Panel (BioFire® ME Panel, BioFire Diagnostics) is the first U.S. Food and Drug Administration (FDA)-approved multiplex PCR assay that allows the rapid detection of 14 pathogens, including bacteria ($n = 6$), viruses ($n = 7$), and fungi ($n = 1$), from cerebrospinal fluid (CSF). The performance of the panel is expected to be dependent on the epidemiology of M/E in different geographical regions.
Methods: In this preliminary study, we used the BioFire® ME Panel in 42 subjects who presented to the emergency department with symptoms of M/E in our hospital. The results were compared to conventional culture, antigen detection, PCR, and various laboratory findings.
Results: The panel detected six positive samples, of which five were viral and one bacterial. We observed an overall agreement rate of 88% between the BioFire® ME Panel results and the conventional methods. There were no false-positive findings, but five discordant results were observed for enterovirus, herpes simplex virus type 1, *Escherichia coli*, and *Cryptococcus* species.

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Conclusions: The BioFire® ME Panel performed equivalently to the traditional PCR methods for virus detection, and better than bacterial cultures. This revolutionary system represents a paradigm shift in the diagnosis of M/E and may aid in the rapid identification of community-acquired M/E. However, the usefulness of this tool is limited in regions with a high prevalence of infectious M/E caused by microorganisms not included in the panel.

Introduction

Infectious meningitis and encephalitis (M/E) can be caused by various organisms, including viruses, bacteria, fungi, parasites or, in rare situations, prions.[1] The cardinal clinical symptoms and signs are mainly indistinguishable regardless of the inciting pathogen.[2] Laboratory findings may provide M/E clues but are often nonspecific.[2] The early identification of pathogens is of paramount importance to enable prompt therapy for a better outcome.[3] Whereas viral ME is usually benign and self-limiting, bacterial M/E and certain viruses that cause encephalitis, for example, herpes simplex virus (HSV), can lead to catastrophic damage.[4] In the past, routine cerebrospinal fluid (CSF) studies rarely identified specific pathogens.[5] Over 90% of all patients presenting with community-acquired M/E have negative Gram-stain results.[6,7] Microbiological cultures have long been recognized as the gold standard for definitively diagnosing bacterial causes, but have posed a diagnostic dilemma due to their low yield rate and time-consuming methods.[8]

In the modern era, the diagnosis of viral infections has been revolutionized by the establishment molecular diagnostics, which has now become a standard methodology.[2] An epidemiologic study on negative CSF Gram-stains reported the highest diagnostic yield using molecular diagnostics (24.2%).[6] In contrast, the yield of blood cultures for the diagnosis of bacterial M/E was 10.3% and that of CSF cultures was only 4%. More than 50% of patients remained undiagnosed after one month.[6]

Viruses account for most cases of M/E. Enteroviruses and HSV-2 are the major etiologic agents of aseptic M/E in adults, as observed in a study in Finland.[9] Other studies in the United States reported that the primary causative pathogens were enterovirus, followed by unknown pathogens and bacterial M/E in both children and adults.[10,11] Pathogens vary across different age groups, seasons and geographic locations; however, there has been little discussion about the epidemiology of nonepidemic aseptic M/E in Taiwan. A single center study in southern Taiwan identified *Cryptococcus neoformans* and *Mycobacterium tuberculosis* as the leading causative pathogens of M/E and found Japanese encephalitis and herpesviruses in encephalitic patients by routine CSF workup. In addition, they were able to determine 31 cases of unknown pathogens using Mass Tag PCR and 16S rRNA PCR; Epstein–Barr virus (EBV) accounted for the majority of the identified pathogens, followed by *Escherichia coli*.[12]

Despite a substantial decrease in bacterial M/E, particularly in children after the introduction of vaccination program against *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis*, the prevalent pathogens have remained consistent.[13–16] *S. pneumoniae*, *N. meningitidis* and *H. influenzae* are the leading causes of bacterial M/E in the western world.[13,15] However, the prevalent organism of community-acquired M/E among adults in Taiwan is *Klebsiella pneumoniae*.[17–21]

The BioFire FilmArray® (BioFire Diagnostics, a bioMérieux Company, Salt Lake City, UT, USA) is an automated nested multiplex PCR system that enables the simultaneous detection of a wide range of pathogens using a small amount of sample with a rapid turnaround time.[22] The BioFire® ME Panel provides a comprehensive test that covers the 14 most common pathogens of community-acquired M/E. The BioFire® ME Panel was the first nucleic acid-based CSF test approved by the U.S. Food and Drug Administration (FDA) in October 2015 and the Taiwan FDA in March 2017.[23] Due to the different etiological epidemiology of M/E in different geographical regions, the performance of the panel might vary. Here, we evaluated the performance of the BioFire® ME Panel for the diagnosis of M/E and compared the results with those of standard-of-care testing ordered by attending physicians from a medical center in Northern Taiwan.

Methods

Study subjects

This prospective study was conducted at the National Taiwan University Hospital (NTUH), a 2500-bed tertiary care center in Northern Taiwan. Between May 1, 2018, and August 20, 2018, patients that presented to the emergency department of NTUH with clinical signs and symptoms (fever, headache, nausea, vomiting, nuchal rigidity, altered mental status, and other neurological symptoms) and abnormal routine CSF evaluations (pleocytosis and high protein level [>60 mg/dL]) indicating possible M/E were enrolled. A total of 42 subjects with possible M/E for whom the physician ordered a lumbar puncture for CSF analysis were enrolled. All patients' medical records were retrospectively reviewed for immune status, clinical presentation, laboratory data, administered therapies and discharge diagnoses. The Institutional Review Board of the National Taiwan University Hospital (201808032RINB) approved this study.

Conventional assays

Routine tests included CSF cell counts and differential counts, glucose and total protein analyses, and bacterial

culture. Additional tests, such as cryptococcal antigen testing, CSF fungal cultures, viral cultures, serological tests, and polymerase chain reaction (PCR) testing for specific viruses, were also performed when necessary. Bacterial M/E was diagnosed by the presence of bacteria in the blood and/or CSF cultures. Viral M/E was defined by positive culture or PCR results from CSF samples. Neurosyphilis was defined as a CSF white blood cell (WBC) count of $\geq$20 cells/mL or by a reactive CSF venereal disease research laboratory (VDRL) test, a rapid plasma reagin (RPR) test (ASI RPR card test, Arlington Scientific Inc., UT, USA), or a *Treponema pallidum* hemagglutination assay (FTI-SERODIA-TPPA; Fujirebio Taiwan Inc., Taoyuan, Taiwan).[24] Cryptococcal M/E was diagnosed by positive cultures from blood and/or CSF samples or a positive cryptococcal antigen test (IMMY, Inc., Norman, OK, USA). Leptospiral M/E was diagnosed either by a 4-fold or greater increase in or seroconversion of specific antibodies against *Leptospira* spp. using a microscopic agglutination test (MAT) or antibodies against a specific *Leptospira* serovar using a cutoff titer >1:100.[25]

BioFire® ME panel assay

All patients' residual CSF samples were analyzed with the BioFire® ME Panel according to the manufacturer's instructions. Briefly, uncentrifuged fresh CSF (200 μL) was loaded into the pouch after injecting the hydration solution. It was then inserted into the FilmArray® system and the results for fourteen pathogens, including six bacteria (*S. pneumoniae*, *N. meningitidis*, *Streptococcus agalactiae*, *H. influenzae*, *Listeria monocytogenes*, and *E. coli K1*), seven viruses (herpes simplex viruses types 1 and 2 [HSV-1 and HSV-2], human herpesvirus type 6, cytomegalovirus [CMV], enterovirus, human parechovirus, and varicella zoster virus [VZV]) and fungi (*Cryptococcus neoformans/gattii*) were obtained in approximately 1 h.

Data analysis

The BioFire® ME Panel results were compared to conventional and additional tests ordered by the attending physicians. The results of the BioFire® ME Panel were considered concordant when they were in agreement with those of conventional and additional tests. Pathogens that were not detected by BioFire® ME Panel owing to limited database coverage were considered concordant as well. The results of the BioFire® ME Panel were considered discordant, with either false-negative or false-positive findings when the results did not agree with the results of the routine and additional tests and clinical diagnosis. Sensitivity and specificity were not calculated because of the small sample size.

Results

During the study period, 42 patients admitted to the emergency department of NTUH were suspected to have M/E. Among the 42 patients, 25 were male and 40 were adults. The median age was 40 years old, ranging from one month to 74 years old. Of the 42 CSF specimens analyzed by the BioFire® ME Panel, 35 (83.3%) were negative, six (14.3%) were positive, and one was invalid. Table 1 summarizes the performance of the BioFire® ME Panel for the diagnosis of M/E among the 42 patients. Overall, there were 37 concordant samples and five discordant results, with an overall agreement of 88% (Table 1). Among the six positive samples, there was one HSV-1, three HSV-2, one VZV and one *S. agalactiae* (Table 2). These results were in agreement with the results of bacterial culture, serology or PCR testing for viruses. There were no false-positive results in our study.

Of the 35 negative samples identified by the BioFire® ME Panel, no pathogens were identified by PCR or microbiological cultures in 25 patients. The 25 patients included 6 with a discharge diagnosis of aseptic M/E with an unknown pathogen and 19 with noninfectious causes. Serology and PCR testing were able to detect additional pathogens in five of the 35 negative BioFire® ME Panel samples. These five causative pathogens, including Japanese encephalitis virus (n = 2), adenovirus (n = 1), *Leptospira* species, and *T. pallidum* (n = 1), were not included in the M/E panel database. Table 3 details the clinical and microbiological characteristics of the five patients. There were five discordant results in which conventional methods identified these pathogens, yet the BioFire® ME Panel reported false-negative results. These pathogens included one HSV-1, one enterovirus, one *E. coli*, and two cryptococci (Table 4).

Table 1 Summary of the performance of the FilmArray® meningitis/encephalitis (M/E) panel for the diagnosis of M/E among 42 patients who were admitted to the emergency department of the National Taiwan University Hospital from 1st May 2018 to 20th August 2018.

Pathogen detected by the indicated methods			No. of patients	% of patients
Interpretation	FilmArray M/E panel	Comparator methods (culture/serology/PCR)		
Concordant				
	Positive	Positive	6	88
	Negative	Positive (pathogens not in the FilmArray M/E panel list)	5	
	Negative	Negative	26	
Discordant				
False-negative	Negative	Positive (pathogens in the FilmArray M/E panel list)	5	12

Table 2 Six patients with meningitis/encephalitis (M/E) caused by pathogens detected by both the FilmArray® M/E panel and comparator serological and molecular methods.

No.	FilmArray	Clinical diagnosis	Age/gender	CSF culture	RBC $\times 10^9$/uL	WBC $\times 10^9$/uL	Lymphocyte/ neutrophil	Total protein mg/dL	Glucose mg/dL	CSF/blood glucose	Serology/molecular method (specimen)
1	HSV-2	HSV M/E	27 yr/F	Negative	36	389	99/1	170.6	50	0.51	HSV IgM+, HSV-1 IgG+, HSV-2 IgG− (serum)
2	VZV	Ramsay Hunt syndrome with VZV M/E	40 yr/M	Negative	4	252	249/3	42.3	49	−	VZV PCR+ (CSF) VZV IgG+, IgM− (serum)
3	HSV-2	HSV M/E	27 yr/F	Negative	1	340	290/50	83.4	51	0.49	HSV PCR+ (CSF)
4	HSV-2	HSV M/E	28 yr/M	Negative	2	629	100/0	122.9	46	0.36	HSV PCR+ (CSF) HSV-1 IgG−, HSV-2 IgG−, HSV IgM+ (serum)
5	HSV-1	HSV M/E	21 yr/M	Negative	111	226	223/3	62.9	69	0.56	HSV PCR+ (CSF)
6	*S. agalactiae*	*S. agalactiae* sepsis	29 d/M	Negative	1	1	0/1	113.4	64	0.79	*S. agalactiae* (blood)

Table 3 Five patients with M/E diagnosed by serological and comparative molecular methods due to pathogens that were not included in the FilmArray® meningitis/encephalitis (M/E) panel.

No.	Clinical diagnosis	Age/gender	CSF bacterial culture	CSF viral culture	RBC (10^9/uL)	WBC (10^9/uL)	No. (10^9/uL) of lymphocyte/ neutrophil	Total protein mg/dL	Glucose mg/dL	Serology/molecular method (specimen)
1	Japanese encephalitis	53 yr/F	Negative	Negative	4000	208	185/23	106.3	59	JEV IgM+ (CSF)
2	Japanese encephalitis	24 yr/M	Negative	Negative	0	656	42/58	126.8	80	JEV IgM+, IgG+ (CSF)
3	Adenovirus encephalitis	55 yr/M	Negative	Negative	582	4	2/2	76.8	−	Adenovirus PCR+ (CSF)
4	Leptospirosis	62 yr/M	Negative	Negative	55	0	0/0	25.9	69	MAT+ 200× (serum), *Leptospira* serovar Shermani
5	Neurosyphilis	35 yr/M	Negative	Negative	1296	26	26/0	47.1	67	VDRL 1:8 (CSF), VDRL 1:1024 (serum)

JEV, Japanese encephalitis virus; VDRL, venereal disease research laboratory; MAT, microscopic agglutination test.

Table 4 Five patients with M/E diagnosed by serological and comparative molecular methods but negative by FilmArray M/E panel tests due to pathogens that were included in the FilmArray® meningitis/encephalitis (M/E) panel.

No.	Clinical diagnosis	Age/gender	CSF bacterial culture	CSF viral culture	RBC (10^9/uL)	WBC (10^9/uL)	No. (10^9/uL) of lymphocyte/ neutrophil	Total protein mg/dL	Glucose mg/dL	CSF/blood glucose ratio	Serology/molecular method/culture (specimen)
1	HSV M/E	63 yr/F	Negative	Negative	84	144	144/0	162.7	43	–	HSV DNA+ (CSF)
2	Systemic enterovirus	42 yr/F	Negative	Negative	243	0	0/0	26.8	54	0.56	Enterovirus PCR+ (throat)
3	*E. coli* M/E	1 mo/M	*E. coli*	–	297	18	11/7	86.1	54	0.61	*E. coli* (urine) Blood culture: negative
4	Cryptococcosis	45 yr/M	Negative	Negative	1350	118	117/1	289.6	48	–	Cryp. Ag 1:16 (CSF); Cryp. Ag 1:512 (serum)
5	Cryptococcosis	54 yr/M	Negative	–	0	30	26/4	86.4	75	0.73	Cryp. Ag 1:2 (CSF)

–, not performed; HSV, herpes simplex virus.
Cryp. Ag, cryptococcal antigen.

Discussion

Consistent with previous reports,[26–28] the overall positivity rate observed with the BioFire® ME Panel in our study was 14.3%. According to local epidemiology data, the BioFire® ME Panel may reach approximately 45%–56.5% coverage for the most prevalent agents of community-acquired M/E among adults in Taiwan but only 12.3%–22.5% coverage for both community and nosocomial bacterial M/E (Table 5). Our analysis showed that all five positive samples were of the herpesviruses family, and the results were concordant with PCR or serology testing. All patients' CSF analyses showed pleocytosis with lymphocyte predominance and substantial elevation of CSF protein. Contrary to a previous study in which normal CSF to blood glucose was observed in viral M/E patients and a lower ratio ($\leq$0.5) indicated bacterial M/E,[5,29] our results showed two patients with hypoglycorrhachia (low CSF glucose). All patients received prompt anti-viral treatment and recuperated without significant neurologic sequelae except the fifth patient who presented with medial temporal hemorrhage on CT scan resulting in postencephalitic epilepsy and permanent intellectual disability. The premature newborn baby who tested positive *for S. agalactiae* presented with neonatal sepsis on his 29th day of life. Blood culture was also positive for *S. agalactiae*, but rapid screening using the latex agglutination method and CSF bacteria culture failed to identify the pathogen. Maternal *S. agalactiae* status was unknown as the screening test is usually ordered at gestational age 35–37 weeks, and he was born prematurely at 32 weeks.

Of the five samples showing discordant results, two samples were positive by the latex agglutination test for a cryptococcal antigen but negative by fungal culture. These two patients had a low cryptococcal antigen titer (1:2 and 1:16, respectively). One patient was referred from another hospital and was treated with an antifungal agent prior to lumbar puncture. Another patient was HIV-positive and had recurrent cryptococcal M/E with repeated treatment during the past two years. A study from the Mayo clinic reported 24 cases of false negative results (positive agreement 57.8%) for *C. neoformans*/*C. gattii* when comparing the BioFire® ME Panel results to antigen testing by latex agglutination.[30] However, another report from Uganda demonstrated 100% sensitivity of the BioFire® ME Panel assay for newly diagnosed cryptococcus M/E in an HIV-infected population.[31] Several studies have shown a persistent detectable cryptococcal antigen despite negative culture, smear and BioFire® ME Panel results.[22,26,30] While cryptococcal antigens are highly sensitive for the diagnosis of M/E, they may remain detectable after treatment and cannot be used as an index of cure.[32] Nevertheless, the BioFire® ME Panel package insert, and a case report of kidney transplant patients on the calcineurin inhibitor tacrolimus, suggest the possibility of false-negative results for Cryptococci.[33] Furthermore, a previous epidemiological survey in Taiwan demonstrated that among 219 isolates analyzed, 95.9% were *C. neoformans* and 4.1% were *Cryptococcus gattii* (4.1%). The predominant genotype was VNI (n = 206), followed by VGII (n = 6), VNII (n = 4), and VGI (n = 3).[34] Biofire® ME panel has been verified to detect

Table 5 Proportion of pathogens included in the FilmArray meningitis/encephalitis (M/E) panel and other main pathogens causing M/E in adults in Taiwan. Columns highlighted in gray are listed as targets of the FilmArray® M/E panel.

Authors	Hsu et al.[12]	Fang et al.[17]	Lu et al.[18]	Chang et al.[19]	Lai et al.[12]	Lien et al.[21]
Patient population (no. of patients)	Adults (212)	Adults (48)	Adults (202)	Adults (181)	Adults (261)	Adults (157)
Pathogens studied	Virus, bacteria, Fungus	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
No. of patients with known etiology or monomicrobial etiology	108	36	180	165	221	145
Acquisition	Community-acquired	Community-acquired	Community and nosocomial	Spontaneous and postneurosurgical	Community and nosocomial	Spontaneous and postneurosurgical
Year of study	2006–2008	1993–1998	1986–1999	1999–2005	2000–2010	2006–2015
FilmArray® coverage	56.5%[a]	45%[b]	17.8%[b]	12.3%[b]	22.5%[b]	12.4%[b]
Viruses						
Enterovirus	5.6% (n = 6)	–	–	–	–	–
Herpes simplex virus type 1	4.6% (n = 5)	–	–	–	–	–
Herpes simplex virus type 2	0.9% (n = 1)	–	–	–	–	–
Cytomegalovirus	0.9% (n = 1)	–	–	–	–	–
Varicella-zoster virus	2.8% (n = 3)	–	–	–	–	–
Human herpesvirus type 6	–	–	–	–	–	–
Human parechovirus	–	–	–	–	–	–
Epstein–Barr virus	14.8% (n = 16)	–	–	–	–	–
Japanese encephalitis	2.8% (n = 3)	–	–	–	–	–
Bacteria						
Streptococcus pneumoniae	1.9% (n = 2)	28% (n = 10)	10.5% (n = 19)	3.3% (n = 6)	10% (n = 12)	4.8% (n = 7)
Neisseria meningitidis	0%	6% (n = 2)	1.7% (n = 3)	1.2% (n = 2)	0%	0%
Haemophilus influenzae	0%	0%	0.6% (n = 1)	0%	0%	0%
Listeria monocytogenes	1.9% (n = 2)	11% (n = 4)	0%	1.2% (n = 2)	2% (n = 3)	4.1% (n = 6)
Streptococcus agalactiae	0%	0%	0%	0%	1.5% (n = 2)	1.4% (n = 2)
Escherichia coli	4.6% (n = 5)	0%	5% (n = 9)	6.6% (n = 12)	9% (n = 11)	2.1% (n = 3)
Klebsiella pneumoniae	6.5% (n = 7)	33% (n = 12)	31.7% (n = 57)	23% (n = 42)	36% (n = 47)	13.1% (n = 19)
Staphylococcus aureus	0%	6% (n = 2)	8.3% (n = 15)	10.9% (n = 19)	24% (n = 31)	12.4% (n = 18)
Pseudomonas aeruginosa	0%	6% (n = 2)	11.1% (n = 20)	5.5% (n = 10)	7% (n = 11)	6.2% (n = 9)
Other *Streptococcus* spp.	1.9% (n = 2)	6% (n = 2)	7.2% (n = 13)	4.4% (n = 8)	8% (n = 11)	4.1% (n = 6)
Mycobacterium tuberculosis	17.6% (n = 19)	–	–	–	–	–
Fungi						
Cryptococcus neoformans/C. gattii	33.3% (n = 36)	–	–	–	–	–

[a] Percentage of pathogens detected in the study included in the list of 14 targets of the FilmArray® M/E panel.
[b] Percentage of bacterial pathogens detected in the study included in the list of six bacterial targets of the FilmArray® M/E panel.
–, No data.

all these specific genotypes when tested at concentration 300 CFU/mL according to the evaluation designation summary by FDA (Den150013).[35] Therefore, the false-negative results could be resulted from pretreatment with antifungals, a low burden of disease and a high PCR crossing threshold.[22]

The other three samples with discrepant results were HSV-1 in a 64-year-old woman, enterovirus in a 42-year-old woman, and *E. coli* in a one-month-old infant. A previous study reported two HSV-1 false negative results; additional research claimed that the dropouts were due to sample centrifugation, which deviates from the testing protocol.[27,36] Another report of false-negative results, when compared to quantitative real-time PCR testing, was due to samples with low nucleic acid levels (late average Ct = 38 on PCR-based laboratory developed test).[22] In our study, we used fresh, unspun CSF samples with a threshold cycle of 30.62 on an in-house multiplex PCR test using human endogenous retrovirus 3 as an internal control. The undetected enterovirus sample was from a patient who presented with fever, headache and hemorrhagic rash over her lower extremities. CSF analysis did not show pleocytosis, biochemistry data were within normal limits, and viral culture was not informative. PCR of CSF for enterovirus was not performed, but in-house pan-enterovirus real-time RT-PCR assay was positive from throat swab samples. False-negative enterovirus results were reported in two studies despite a high sensitivity and specificity at 95.7% and 99.5%, respectively.[26,36] Biofire® ME panel has been verified to detect various enteroviruses, including enteroviruses 68, 70, and 71; coxsackieviruses A6, A9, A10, A16, A17, A21, A24; and B1–B5; and echoviruses 6, 9, and 18; at a range of concentration of 5–150 median tissue culture infective dose per mL ($TCID_{50}$/mL).[35] The discrepancy of results between Biofire® ME assay and in-house pan-enterovirus real-time RT-PCR assay might be attributed to the different specimens (CSF vs. throat swab) tested and less coverage of enterovirues targets in the Biofire® ME panel.

For the discrepant *E. coli* result, upon chart review, the one-month-old infant presented with fever and EEElethargy and received a lumbar puncture as a routine workup for newborn sepsis, but the CSF sample was collected after administration of an empiric antibiotic. Both the urine and CSF culture were positive for *E. coli*, while the blood culture was negative. The pretreatment with antibiotics might have reduced the bacterial load and lowered the sensitivity. Likewise, the BioFire® ME Panel only detects the *E. coli* K1 capsular type, which accounts for 80% of *E. coli* M/E in newborns.[37] This narrowed specificity for *E. coli* was an important design consideration to minimize cross-contamination between *E. coli* nucleic acid and common PCR reagents.[26,38]

The BioFire® ME Panel failed to detect five pathogens that were not included in the M/E panel. Our institute is a tertiary referral hospital with a complex patient population with multiple comorbidities. A large proportion of patients are immunocompromised from diseases or conditions such as cancer, solid organ or hematopoietic stem cell transplantation, cirrhosis, dialysis, and immunodeficiency. These patients are susceptible to various pathogens that are not part of the M/E panel. For example, one post-allogeneic hematopoietic stem cell transplantation patient was diagnosed with adenovirus encephalitis where detection was not possible using the M/E panel. Likewise, *M. tuberculosis*, which is also not included in the panel, remains a major public health challenge in Taiwan where incidence ranges from 62 to 75 per 100,000 population and the mortality rate is 3.3–5.7 per 100,000 population.[39,40] Extrapulmonary tuberculosis represented 10–15% of the newly diagnosed tuberculosis cases, of which tuberculous M/E was the most difficult to diagnose and the most severe form of infection.[39,40] Although the actual incidences of the other four M/E pathogens (Japanese encephalitis virus, *Toxoplasma gondii*, *Leptospira* species, and *T. pallidum*) that were not detected by the BioFire® ME Panel in this study were not well known, several reports have described the clinical features of patients with M/E caused by these microorganisms.[12,41–43] A previous study during 2006–2008 showed that the proportion of Japanese encephalitis among patients with M/E in Taiwan was 2.8%.[12] Among 30 laboratory-confirmed cases of *T. gondii* disease revealed from a seroepidemiologic study during 2009–2010 in Taiwan, four (13.3%) presented with central nervous system disease.[41] As for leptospiral M/E, among the 57 laboratory and clinical confirmed cases of leptospirosis, six (11%) had meningitis.[42] A recent clinical study on 157 HIV-infected patient with syphilis found during 2000–2009 in a medical center in middle Taiwan, 14 (8.9%) of them were diagnosed with neurophilis.[43]

In summary, we report the performance of the BioFire® ME Panel in 42 patients who presented clinically with CNS infection. We observed an overall positivity rate of 14.3% with perfect agreement for the detection of HSV, VZV, and *S. agalactiae* between conventional PCR, bacterial culture, BioFire® ME Panel, and multiplex RT-PCR results. A negative result does not preclude CNS infection. The BioFire® ME Panel is indicated as an adjunctive diagnostic tool, and it should not be used as the sole basis to guide medical decisions. The main advantage of using the BioFire® ME Panel is the potential for faster diagnosis with only a small amount of CSF. However, the coverage was not ideal, specifically, as some of the prevalent pathogens are not covered by the panel in Taiwan. A modification of target organisms in an updated BioFire® ME Panel, or a specific regional BioFire® ME Panel, to meet the Taiwan need is crucial. In the interim, BioFire® ME Panel in conjunction with other more single robust tests to detect local prevalent specific pathogens not on the panel but clinically suspicious in presentation, eg. tuberculosis, would be necessary.

Conflict of interest

We declare no conflicts of interest.

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